BME Design-Fall 2024 - Althys Cao
Complete Notebook

PDF Version generated by

JULIA SALITA

on

Dec 17, 2024 @10:16 PM CST

Table of Contents

Project Information 2
Team contact Information 2
Project description 3
Team activities 4
Client Meetings 4
Introduction_9/13/2024 4
Required Trainings 6
Timeline Plan & Background_9/16/2024 7
Cell Line Decision Advice_9/23/2024 9
Tissue Culture Cell Passaging_10/11/2024 12
Midsemester Review_10/18/2024 17
Spheroid Formation Protocols_10/25/2024 21
Timeline & Passaging_11/1/2024 24
Materials & qPCR_11/8/2024 27
BioSpa_11/22/2024 30
Advisor Meetings 33
Advisor Introduction_9_6_2024 33
Advisor Meeting2_9/13/2023 35
Advisor Meeting 3_9/20/2024 36
Advisor Meeting 4_9/25/2024 37
Advisor Meeting 5_10/10/2024 38
Advisor Meeting 6_10/18/2024 39
Advisor Meeting 7_11/5/2024 40
Advisor Meeting 8_11/22/2024 41
Advisor Meeting 9_12/13/2024 42
Materials and Expenses 43
Materials used in cell passaging 10/14/2024 43
Spheroid Materials_Preliminary List_11/2/2024 48
Polyhema and Methylcellulose Receipt 49
Fabrication 50
Design Process 50
Hess Lab Protocols 50
Hess Lab Cell Line Maintenance 50
A549 Cell Line Maintenance 51
CryoFLEX Protocol_10/11/2024 53
Spheroid Formation Protocols 55
Spheroid Protocols Master Document_10/30/2024 55
Spheroid Protocols Matrix_11/2/2024 56
Draft 1_Final Protocols_11/4/2024 58
Cell Seeding/Spheroid Formation Protocol_11/18/2024 61
Other Protocols 66
CytoFLEX Protocol_11/21/2024 66
Lab Work 67
10/14/2024 WIMR Lab Visit- Cell Passage 1 67
A549 Photos_80% Confluency_10/18/2024 69
Benchling Data Update_11/11/2024 71

Benchling Data Update_12/11/2024 74
Testing and Results 76
BioTek Imaging_11/22/2024 76
Spheroid Images_11/22/2024 79
Accutase Dissociation of Spheroids_11/22/2024 89
Percent Cell Viability After Spheroid Formation_11/22/2024 92
ImageJ Analysis Protocol_11/25/2024 95
Project Files 97
Progress Reports 97
Progress Reports (PDF) 97
Primary Deliverables 103
PDS_9/19/2024 103
Design Matrix_9/27/2024 105
Preliminary Presentation 10/04/2024 106
Preliminary Report_10/14/2024 107
Show and Tell 108
Show and Tell Presentation_10/31/2024 108
BPAG Materials Sheet 109
BPAG Excel Sheet 109
Final Materials Table_12/12/2024 110
Final Deliverables 112
Final Poster_12/6/2024 112
Final Poster Supplemental Figures_12/6/2024 114
Final Report_12/11/2024 115
Emily Rhine 116
Research Notes 116
Client Provided Articles 116
CRISPR screen on growth in spheroids_9_10_2024 116
CRISPRi_9_10_2024 118
γH2AX _9_10_2024 120
Background 121
Client Questions_9_8_2024 121
Role of ECM in Cancer_9_7_2024 122
What to Know About CRISPR Screens_9_10_2024 124
Generation of cancer spheroids_9_15_2024 126
TP53_Genetic Mutation_9/27/2024 128
Alexa Fluor 647_10/25/2024 131
Spheroid Size Threshold_ImageJ Analysis_11/25/2024 132
Safety Information 133
Biosafety Level 2_9_15_2024 133
Lentivirus_9_15_2024 135
Biosafety Guide to CRISPR_9_15_2024 137
Human Cancer Cell Lines_Additional Safety Considerations_10/9/2024 139
Standards & Patents 140
Tissue Engineering Standard_9_12_2024 140
Cancer Spheroid Patent_9_12_2024 142
Other Relevant Sources 143
Design Ideas 144
Cell Line 144
Lung Cancer Cell Lines_9/19/2024 144
Rudimentary Cell Line Comparison_9/19/2024_Sigma Aldrich 147
Sigma Aldrich 150
PC-9_Human adenocarcinoma_9/19/2024 150
LC-2/AD_Human lung adenocarcinoma_9/23/2024 152
LUDLU-1_Human Caucasian lung squamous cell carcinoma_9/23/2024 154
NCI-H322_Human Caucasian bronchioalveolar carcinoma_9/23/2024 156
OVCAR-5_9/27/2024 158
ATCC 160
ATCC_Possible Cell Lines_9/20/2024 160
H1314_9.23.2024 162
ThermoFisher Scientific 163

Preliminary TFS Website Search_9/23/2024 ... 164
NCI-H23_9/23/2024 ... 166
Spheroid Formation ... 168
3D Cell Culture Models_9_7_2024 ... 168
Spheroid Formation_Matrigel_9/27/2024 ... 170
SOX2_A549_10/23/2024 ... 172
Scalable Hydrogel Dish Spheroid Formation_A549_10/23/2024 ... 174
Protocols Matrix_Spheroids_11/2/2024 ... 177
Design Matrix Research ... 179
Cell Maintainance ... 182
Condensed Passaging Protocol_10/21/2024 ... 182
Training Documentation ... 183
Hess Lab Training Requirements_9/10/2024 ... 183
HIPAA 24-25 Training ... 185
Miscellaneous Documentation ... 186
Show and Tell Presentation_10/30/2024 ... 186
Preliminary Materials Sheet_11/2/2024 ... 187
Final Poster Section_11/22/2024 ... 188
Future Work ... 189
SOX2_Stemness Gene_12/17/2024 ... 189
Goals and Experiments for Spring 2025_12/17/2024 ... 191
γ-H2AX_12/17/2024 ... 194
Althys Cao ... 196
Research Notes ... 196
Background Research ... 196
2024/09/09 - Cancer Cell Line ... 196
2024/09/17 - ECM-Free and ECM-based Cancer Spheroid Formation and CRISPR-Cas9 Screening Protocol ... 198
2024/09/18 - Standards Research ... 200
Cancer Cell Line Research ... 202
2024/09/23 - Drug Sensitivity and DNA Damage Inducing Drugs ... 202
2024/09/23,24 - Cancer Cell Line Research ... 204
Design Ideas ... 206
2024/09/26 - Low Attachment Plates Method / Treated Tissue Culture Plates Method ... 206
2024/09/26 - Hanging Drop Method ... 208
2024/09/26 - Matrigel Method ... 210
2024/10/28 - PolyHEMA Plate Formation Protocol ... 211
2024/10/28 - Additional Spheroid Formation Protocols using Low Attachment Plates ... 212
Ana Martinez ... 214
Research Notes ... 214
Biology and Physiology ... 214
2024/09/09 - Spheroids as a Type of 3D Cell Culture ... 214
2024/09/10 - What is CRISPR Screening? ... 217
2024/10/11 - Stemness Markers in A549 ... 219
Design Ideas ... 222
Cell Lines ... 222
2024/09/22 - Common Mutations in Lung Cancer Cell Lines ... 222
2024/09/22 - Relevant Lung Cancer Cell Lines ... 225
2024/09/22 - Relevant Ovarian Cancer Cell Lines ... 227
2014/09/23 Relevant Lung Cancer Cell Lines (cont.) ... 229
Spheroid Formation Methods ... 230
2024/09/09 - Spheroids as a Type of 3D Cell Culture - Copy ... 230
2024/12/17 - Spheroid Sizes ... 233
Protocols ... 236
2024/10/11 - Spheroid Formation Protocol ... 236
2024/10/11 - High-Throughput CRISPR Screen Protocol ... 238
2024/10/11 - qPCR Testing Protocol ... 244
2024/11/15 - polyHEMA stock protocol ... 247
2024/11/15 - Methylcellulose stock protocol ... 248
2024/11/15 - Live/Dead Flow Cytometry Assay Protocol ... 249
2024/12/12 - BME Design CytoFLEX Operation Protocol ... 250
2024/12/12 - Hess Lab General CytoFLEX Operation Protocol ... 251

Data 255
A549 Cell Line Maintenance 255
2024/11/15 - A549 Cell Line Maintenance Data 255
Miscellaneous 258
2024/11/15 - Tong Lecture Notes 258
Julia Salita 259
Research Notes 259
Biology and Physiology 259
Client articles 259
CRISPR Screening in Cancer Spheroids (article 1) 259
What is CRISPR screening 260
Spheroids as a Type of Three-Dimensional Cell Cultures—Examples of Methods of Preparation and the Most Important Application 263
colorectal carcinoma spheroids 264
Mutation Research 265
Understanding qPCR 266
Spheroid Formation Protocols 267
Lab work 270
10/14/2024 WIMR Lab Visit- Cell Passage 1 - Copy 270
Passage Records 272
Jayson O'Halloran 275
Research Notes 275
Biology and Physiology 275
2024/09/09 Introductory Research I 275
2024/09/10 Spheroid Research II 277
2024/09/11 Initial γH2AX Research III 280
2024/09/15 Cell Line Research IV 282
2024/09/18 Types of Cancer Cell Lines Research V 284
2024/09/21 A549 Cell Line Research VI 288
2024/09/23 OVCAR-5 Cell Line Research VII 291
2024/09/26 NCI-H23 Cell Line Research VIII 293
2024/10/01 Matrigel Research IX 295
2024/10/03 Hanging Drop Method Research X 298
2024/10/07 Treated Tissue Culture Plates Research XI 301
Competing Designs 303
ATCC Lung Cancer Cell Line 303
Ovarian cancer cell line I PA-1 304
Ovarian cancer cell line II SK-OV-3 305
Ovarian cancer cell line III Caov-3 306
Colon cancer cell line I CACO-2 307
Colon cancer cell line II COLO 205 308
Colon cancer cell line III COLO 741 309
Kidney cancer cell line A-498 310
BPAG 311
9/27/2024 BPAG Slides 311
2014/11/03-Entry guidelines 312
2014/11/03-Template 313

Team contact Information

Emily Rhine - Sep 10, 2024, 3:01 PM CDT

Last Name	First Name	Role	E-mail	Phone	Office Room/Building
Campagnola	Paul	Advisor	pcampagnola@wisc.edu		
Schwartz	Carley	Client	cischwartz@wisc.edu		
Cao	Althys	Leader	nvcao@wisc.edu	(616) 469-0243	N/A
Martinez	Ana	Communicator	almartinez4@wisc.edu	(262) 751-1037	N/A
Rhine	Emily	BSAC	erhine@wisc.edu	(763) 321-0407	N/A
Salita	Julia	BWIG	jsalita@wisc.edu	(612)-990-4423	N/A
O'Halloran	Jayson	BPAG	ohalloran2@wisc.edu	715-529-5317	N/A

Project description

Emily Rhine - Sep 10, 2024, 3:0

Course Number: BME 400

Project Name: CRISPRi screening in cancer spheroids to investigate factors in genome stability

Short Name: CRISPRi Screening in Cancer Spheroids

Project description/problem statement:

Previous CRISPR screening in 2D monolayers has provided extensive knowledge on cancer drivers and therapeutic susceptibilities, but it can fail to accurately identify facto to the 3D environment of in vivo tumors, including genes that regulate genome stability/DNA damage. Therefore, we want to develop a cell culture method that recapitulates environment and is compatible with CRISPR screening to identify sources of DNA damage affected by the tumor environment compared to 2D culture.

The main objectives of the BME team would involve: selecting the cell line for the screen, creating and optimizing a spheroid formation protocol, and developing a protocol γH2AX. Longer term goals could involve: investigating how biomaterial properties affect DNA damage, conducting the screen collaboratively with members of the Hess lab downstream analysis.

About the client:

Ms. Carley Schwartz
Biomolecular Chemistry and Center for Human Genomics and Precision Medicine
Biochemistry
cischwartz@wisc.edu

Alternate Contact:
Dr. Gaelen Hess
Department of Biomolecular Chemistry and Center for Human Genomics and Precision Medicine
UW School of Medicine and Public Health
ghess3@wisc.edu

Relevant Journal Articles and Websites:
CRISPR screen on growth in spheroids:
https://www.nature.com/articles/s41586-020-2099-x#Sec11
H2AX:
https://www.sciencedirect.com/science/article/pii/S0887233312001567#:~:text=Histone%20H2AX%20is%20rapidly%20phosphorylated,in%20in%20vitro%20mechanistic%
CRISPR Screening: https://www.idtdna.com/pages/education/decoded/article/overview-what-is-crispr-screening
https://www.nature.com/articles/s41580-022-00571-x
CRISPR interference (CRISPRi)
https://en.wikipedia.org/wiki/CRISPR_interference#:~:text=The%20technology%20uses%20a%20catalytically,sgRNA)%20to%20the%20genomic%20locus.
Genomic Instability: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/genome-instability
γH2AX screening: https://www.cell.com/molecular-cell/fulltext/S1097-2765(23)00472-0
γH2AX screening: https://www.cell.com/molecular-cell/fulltext/S1097-2765(23)00518-X
ECM and DNA Repair: https://www.science.org/doi/full/10.1126/sciadv.abb2630

Introduction_9/13/2024

Emily Rhine - Sep 13, 2024, 2:36 PM CDT

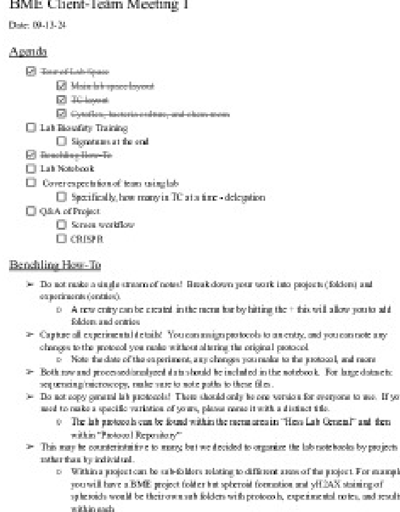
Title: Client Meeting1_ Lab Space Introduction
Date: 9/13/2024
Present/Content By: Julia, Ana, Althys, and Emily
Goals: Better understand the scope of this course and the Project in terms of the lab space and client requirements.
Content:

- See attached document

Conclusions/Action Items:

- Complete PDS with new information and client requirements.
- Continue Individual research:
    - Meet with the team to touch base about the PDS and the research we have individually conducted.
- Meet with Advisor and Jayson to catch them up with the new information.

Emily Rhine - Sep 13, 2024, 2:37 PM CDT



BME Client-Team Meeting 1

Date: 09-13-24

Agenda

- [x] ~~Tour of Lab Space~~
    - [x] ~~Main lab space layout~~
    - [x] ~~TC layout~~
    - [x] ~~Cytoflex, bacteria culture, and chem room~~
- [ ] Lab Biosafety Training
    - [ ] Signatures at the end
- [x] ~~Benchling How-To~~
- [ ] Lab Notebook
- [ ] Cover expectation of team using lab
    - [ ] Specifically, how many in TC at a time - delegation
- [ ] Q&A of Project
    - [ ] Screen workflow
    - [ ] CRISPR

Benchling How-To

- Do not make a single stream of notes! Break down your work into projects (folders) and experiments (entries).
    - A new entry can be created in the menu bar by hitting the + this will allow you to add folders and entries
- Capture all experimental details! You can assign protocols to an entry, and you can note any changes to the protocol you make without altering the original protocol.
    - Note the date of the experiment, any changes you make to the protocol, and more
- Both raw and processed/analyzed data should be included in the notebook. For large datasets: sequencing/microscopy, make sure to note paths to these files.
- Do not copy general lab protocols! There should only be one version for everyone to use. If you need to make a specific variation of yours, please name it with a distinct title.
    - The lab protocols can be found within the menu area in "Hess Lab General" and then within "Protocol Repository"
- This may be counterintuitive to many, but we decided to organize the lab notebooks by projects rather than by individual.
    - Within a project can be sub-folders relating to different areas of the project. For example, you will have a BME project folder but spheroid formation and γH2AX staining of spheroids would be their own sub folders with protocols, experimental notes, and results within each

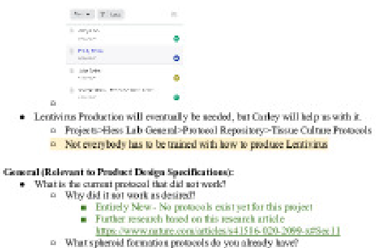
Download

BME_Client-Team_Meeting_09-13.pdf (230 kB)

Emily Rhine - Sep 13, 2024, 4:30 PM CDT

Title: Client Meeting1_ Lab Space Introduction
Date: 9/13/2024
Present/Content By: Julia, Ana, Althys, and Emily
Goals: Better understand the scope of this course and the Project in terms of the lab space and client requirements.
Content:

- Lab tour
    - Text her do not email her
- See
    - [illegible]
- Biosafety
- Set up biweekly meetings with Gaden and Carley?
- Benchling
    - "Within a project can be sub-folders relating to different areas of the project. For example, you will have a BME project folder but spheroid formation and γH2AX staining of spheroids would be their own sub folders with protocols, experimental notes, and results within each"



- Lentivirus Production will eventually be needed, but Carley will help us with it.
  - Projects>Hess Lab General>Protocol Repository>Tissue Culture Protocols
  - Not everybody has to be trained with how to produce Lentivirus

General (Relevant to Product Design Specifications):

- What is the current protocol that did not work?
  - Why did it not work as desired?
    - Entirely New - No protocols exist yet for this project
    - Further research based on this research article https://www.nature.com/articles/s41516-020-2099-x#Sec11
  - What spheroid formation protocols do you already have?

[Download](#)

Client_Meeting1_Lab_Space_Introduction_9_13_2024.pdf (127 kB)

Required Trainings

Emily Rhine - Oct 11, 2024, 3:13 PM CDT

General:

2020-21 HIPAA Privacy & Security Training

SMPH: SMPH: Creating a Respectful and Welcoming Learning Environment (2020)

Preventing Sexual Harassment and Sexual Violence at UW-Madison

Cybersecurity Training (https://it.wisc.edu/it-projects/cybersecurity-awareness-training/#started)

Research-Based:

-Biosafety Required Training

-Chemical Safety: The OSHA Lab Standard

-Chemical Safety: Cryogen Safety Training

-Chemical Safety: Fume Hood Safety Training

-Chemical Safety: Hazard Communication

-Disposing of Hazardous Chemicals

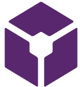
-Biosafety 102: Bloodborne Pathogens for Laboratory and Research

-Biosafety 205: Bio HazMat Shipping Training (Both Campus Transport of Biologicals & Dry Ice Training Path and Dangerous Goods Training Paths)

-Biosafety 105: Biosafety Cabinet Use

-Biosafety 106: Autoclave Use

-Biosafety 107: Centrifuge Safety



Timeline Plan & Background_9/16/2024

Emily Rhine - Sep 16, 2024, 11:29 AM CDT

Title: Client Meeting2_ Timeline Planning
Date: 9/16/2024
Present/Content By: Entire team (zoom)
Goals: Meet with Dr. Hess and Carley to set a general project timeline for the next 3 weeks.
Content:

- Ask Gaelen:
  - What cell type?
    - Available in lab: things to consider
      - Cells that grow well/fast
      - if there is evidence of screening in previous papers → good choice
        - Able to engineer to get library of guide RNAs
      - Growth rate
      - Does DNA damage plays a major role?
        - Lots of cancers are treated with platinum therapy → generate DNA damage
        - Choosing one of these might be more relevant
        - More of a secondary spec
    - Recommend: come up with a list of multiple, can also ask other labs about getting them
    - Which cancer?
      - For starting point Gaelen has no preference
        - Think about DNA damage and what treatments are used
      - Cell line used in the paper H23
      - Others: lung, ovarian, colorectal (not as much) → depends on how easy they are to work with
      - Stick to human if given the choice, but not against mouse/other mammal
  - Scale
    - Need 100-150 million cells per replicate for a screen
    - Used poly hema-covered 10cm diameter plates
      - Can be done in 96 plate or 24 well
      - Check to see if there is a media/create a media that preserves cellular damage of yH2AX for screening with larger scale culture.
  - Viscosity
    - Used methyl cellulose percentage
    - Small difference in percentage made a large difference in scale of spheroids made/their size
    - Works well with non-small cell line
  - **How to confirm cell type**
    - Check for markers for spheroid (via qPCR)
    -
  - **Current protocol:** There is not a current protocol, this is an entirely new project in the lab. Gaelen has previously worked with people that followed the CRISPR screens in cancer spheroids paper's protocol but we do not have the experience ourselves
    - The 3D screening paper - the protocol is optimized for H23 cell line and "seem to work better" with other cell lines
      - May need to change cellular percentages. "Not one size fits all"
  - With this, we are most familiar with the one in the paper and it has served as inspiration for our screening idea - getting the spheroids made, optimized, and compatible with the staining we do is where we need y'all
- Size of the spheroid depends on the number of cells we use to create them which would need to be determined via optimization of protocol

    - Furthermore, γH2AX staining optimization may also impact the size of the spheroid and how many replicates we will need to achieve optimization
    - Overall, there probably isn't a set number for right now how many you'll need to make but there is likely one we could determine with Gaelen that would be necessary for coverage during the screen
- Set up biweekly meetings with Gaelen and Carley?
    - Early on maybe weekly
    - 1:30 on Fridays is good for Dr. Hess, except for 2nd friday of the month (has faculty meeting)
- High throughput CRISPR screening for gRNA to check if each gene affects a specific process such as genome instability (damage)
    - Both helps treatments and helps diagnosis
- Motivation for spheroid system
    - Spheroids are a better representation of the 3D tumor environment (compared to standard 2D culture) → better mimics tumor biology,
        - Different genes present that affect proliferation in 2D vs. 3D
- Gamma-H2AX: histone protein, gets phosphorylated when there is DNA damage, used as a proxy to read out how much damage is in cell → how much genome stability there is
    - If there is a perturbation = is there more or less damage?
- Our goal:
    - On a high scale, stain the cells for gamma-H2AX and optimize both spheroid culture (seeding density, viscosity) and a screen for differences between a 2D and 3D environment. This will help Hess lab eventually complete a genome widescreen.
        - Increase viscosity by methyl cellulose
        - There is also no "idea" how hard/easy it is to stain the cells for H2AX
        - Also do not know if staining affects DNA damage or not
            - Understand how staining and DNA damage screening are related to find the best protocol
    - Important parts of spheroids specs
        - Seeding density → starting amount affect how much the spheroids grow
        - Viscosity → if not viscous enough, would just get all spheroids combined into a giant spheroid
        - Can check certain markers indicative of spheroids by qPCR
            - Mostly will be visual markers: size, good growth during a couple days, etc
            - Spheroid: outer and inner core.
                - Inner core can get depleted of nutrients and oxygen if spheroid too big

Conclusions/ Action Items:

- Gaelen (preferred to be called this) meeting
    - Weekly at the start → Biweekly later
    - 2nd Friday will always be a department meeting, so that will not work.
- Using new information, update the PDS.
- Continue research into cell lines to prepare for the design matrix next week.
- Send out PDS with progress report to advisor and client by Thursday 5:00pm.

Cell Line Decision Advice_9/23/2024

Emily Rhine - Sep 23, 2024, 5:56 PM CDT

Title: Client Meeting3_ Design Review
Date: 9/23/2024
Present/Content By: Entire team (zoom)
Goals: Meet with Dr. Hess to review the PDS and other potential design characteristics to be considered before a design matrix is made.
Content:

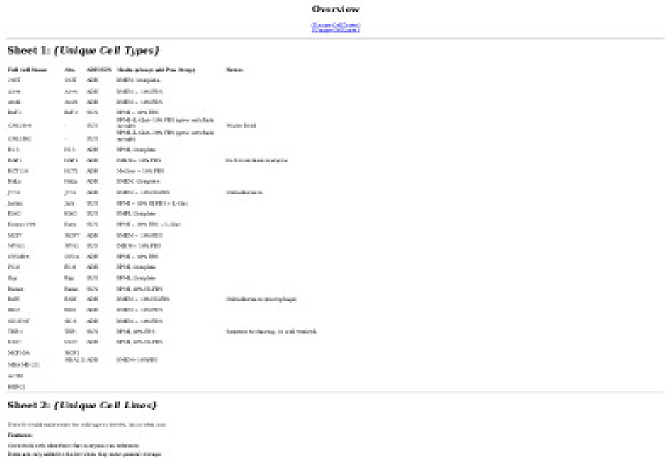
- Ask if there is anything in the PDS they would like to discuss
  - Double check all the current criteria in the design matrix if possible - anything else you want to add?
    - May be worth clarifying seeding density → "only 1 sgRNA per cell"
      - Shouldn't matter because will seed in 2D, then make spheroids in 3D
      - Therefore seeding density is important to be able to get sufficiently sized/amount of spheroids, NOT for only 1 sgRNA/cell
- What kind of cell lines does the lab have? Any database of all cell lines that the team can access?
  - Cell Line Ideal Criteria (recall)
    - Want cell line to grow at least fairly well
      - Double rate ~72 hours = harder for experiments vs. ~24 hours
    - Size of cells → often helpful to have smaller cells
      - Can pack more cells per spheroid
      - More of a "luxury" → less important
    - Able to infect with lentivirus
    - Treated with platinum-core drugs
      - Cisplatin, etc → more common
      - There are also drugs that also damage dsDNA without platinum core (etoposide, etc)
      - DNA damage to dsDNA is also how gammaH2AX works
      - Reasoning: if treated with these, likely that there is already a DNA damage baseline to deal with
  - Hess Lab has a database → 60+ cell lines (mostly lung cancer)
      - Lab nearby has a lot of ovarian cancer cell lines
- Ask where they prefer to buy their cell lines
  - ATCC?
  - Sigma Aldrich?
  - Thermofisher Scientific?
  - If in the worst case we will need a $5000 vial of cell, what will be your approach to it? (like can we ask around different labs or what's going on)
  - Likely from another lab
    - Try to avoid labs that require lots of paperwork
    - Try to come up with multiple options → as backups
- Do you have the related antibodies for mutations:
  - **TP53**
    - Be careful → may be harder to work with if +
  - CDKN2A
  - KEAP1
  - EGFR
  - KRAS

    - Can find a lot on depmap (CCLE database) → did CRISPR screens on 2D, will say certain cell lines have certain mutations
    - Can PCR and sequence for these instead
- Is looking at drug sensitivity z-score for DNA damage-inducing drugs a good option when looking at cancer cell line?
    - If yes, which kind of DNA damage do you think is appropriate, *except for the drugs with platinum cores that you mentioned in previous meeting* (some induce DNA damage by poisoning topoisomerase or inhibiting various enzymes for DNA synthesis, some block DNA metabolism etc. - are they appropriate or not).
    - Cisplatin and bleomycin are a common one to use as DNA damage drugs, but essentially look at studies used with the drug as there is already a base of DNA damage (if used with gamma-H2AX staining, even better)
- Do you recommend we select a primary cell line? (vs. continuous)
    - Continuous
- Should we prioritize an **adherent** cell line or a **suspension** cell line?
    - Adherent likely → usually suspension cell lines don't form "true", tumor-like spheroids (instead they just naturally clump together but not spheroids)
- ECM-based or ECM-free approach (the paper you mentioned used ECM-free). How much importance do you place on ECM-cell interactions?
    - Matrigel
        - Costly → especially because we are doing this at a large scale
        - Made from animal → batch-to-batch effect/variability because each container is not necessarily from the same animal
            - Like FBS (fetal bovine serum): ordered from a large scale to keep things stable/ no batch-to-batch variability
        - Do not know how important cell-to-ECM interactions are
            - Another question is whether that paper determines whether the cell lines can make ECM on their own
    - ECM-free
        - Don't run into above effects
        - Can fine-tune system much more easily

- Timeline for cell culture and spheroid formation
    - Wait for Carley to get back to start tissue culture for chosen cell line
    - Can even practice with non-chosen cell line for tissue culture and flow cytometer ****Carley will be back in lab next week Monday (will be back in Madison this coming weekend)

Conclusions/ Action Items:

- Review cell lines that Hess lab currently has to select one
    - Dr. Hess will send
- Complete design matrix with updated information
- Meet with advisor to discuss design matrix and preliminary presentation



Overview

Sheet 1: (Unique Cell Types)

Sheet 2: (Unique Cell Lines)

Download

Hess_Lab_Cell_Line_Database.xlsx (13.5 kB)

Tissue Culture Cell Passaging_10/11/2024

Emily Rhine - Oct 12, 2024, 7:30 PM CDT

Title: Tissue Culture Introduction_Cell Passaging

Date: 10/11/2024

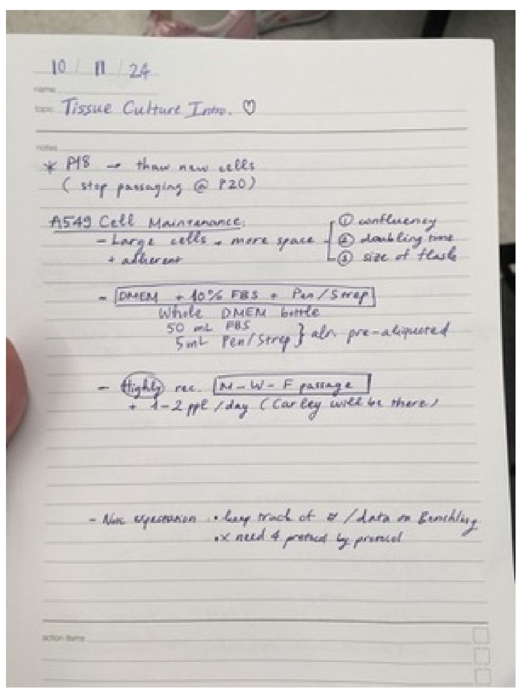
Content:

- see images attached below

Conclusions & Action Items:

- Create regular MWF schedule for passaging cells for the next month
- Establish a standard passaging time
    - A549 are between 22-40 hours and we need to know where our cells fall in that range
- See "Cell Line Maintenance" folder in Benchling

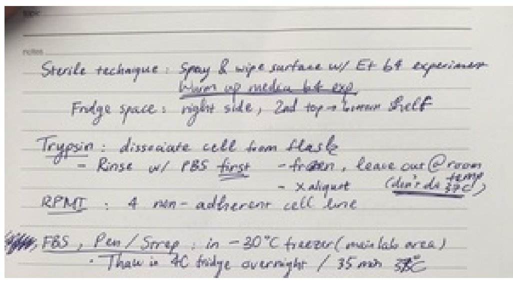
Emily Rhine - Oct 11, 2024, 2:56 PM CDT

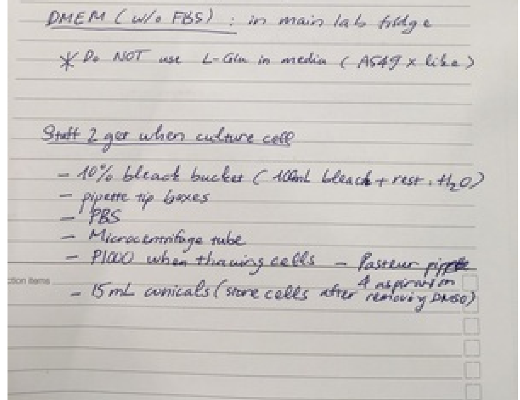

10 / 11 / 24
topic: Tissue Culture Intro. ♡
notes
* P18 → thaw new cells
(stop passaging @ P20)
A549 Cell Maintenance:
- Large cells → more space
+ adherent
① confluency
② doubling time
③ size of flask
- DMEM + 10% FBS + Pen/Strep
Whole DMEM bottle
50 mL FBS
5mL Pen/Strep } alr pre-aliquoted
- Highly rec. M-W-F passage
+ 1-2 ppl / day (Carley will be there)
- Non expectation: • keep track of # / data on Benchling
• X need 4 protocol by protocol
action items

Download

32499FB2-5954-406F-8148-E9E4B8F1F99A.jpg (36.4 kB)

Emily Rhine - Oct 11, 2024, 2:57 PM CDT


notes
Sterile technique: Spray & wipe surface w/ Et 64 experiment
Warm up media b4 exp
Fridge space: right side, 2nd top → lower shelf
Trypsin: dissociate cell from flask
- Rinse w/ PBS first
- frozen, leave out @ room temp (don't do 37°C)
- X aliquot
RPMI: 4 non-adherent cell line
FBS, Pen/Strep: in -30°C freezer (main lab area)
• Thaw in 4C fridge overnight / 35 min 37°C



DMEM (w/o FBS) : in main lab fridge

* Do NOT use L-Glu in media (A549 x like)

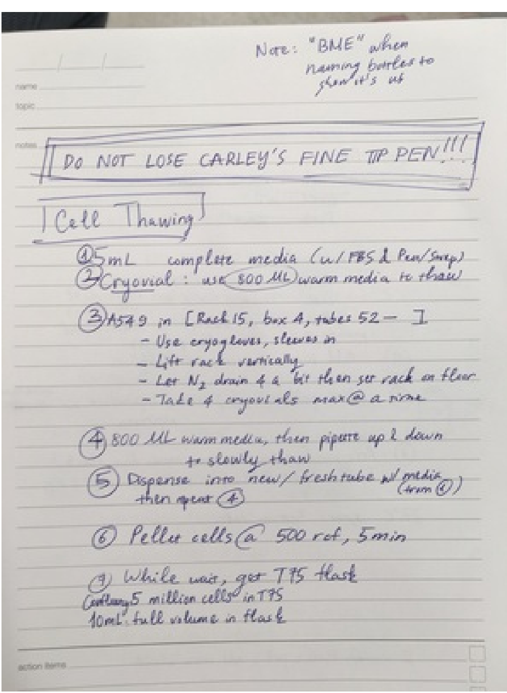
Stuff 2 get when culture cell

- 10% bleach bucket (100mL bleach + rest: H_2O)
- pipette tip boxes
- PBS
- Microcentrifuge tube
- P1000 when thawing cells - Pasteur pipette 4 aspiration
- 15mL conicals (store cells after removing DMSO)

Download

50CC6932-3EB0-49B0-94EA-9E95B7534A56.jpg (141 kB)

Emily Rhine - Oct 11, 2024, 2:57 PM CDT



Note: "BME" when naming bottles to show it's us

DO NOT LOSE CARLEY'S FINE TIP PEN!!!

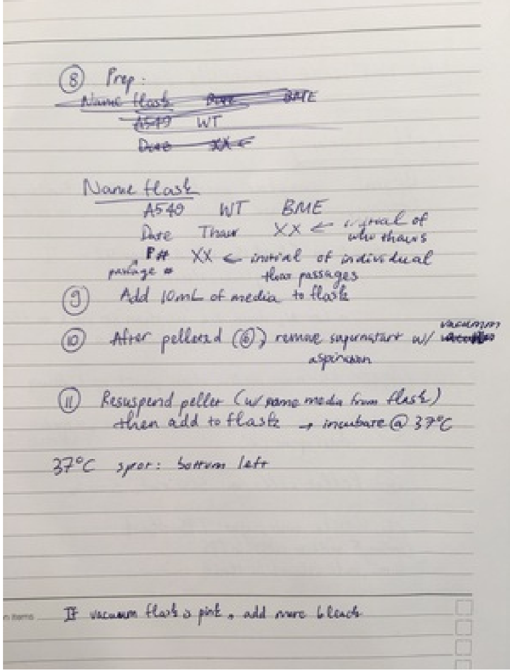
Cell Thawing

1. 5mL complete media (w/ FBS & Pen/Strep)
2. Cryovial: use 800 μL warm media to thaw
3. A549 in [Rack 15, box 4, tubes 52 -]
   - Use cryogloves, sleeves in
   - Lift rack vertically
   - Let N_2 drain 4 a bit then set rack on floor
   - Take 4 cryovials max @ a time
4. 800 μL warm media, then pipette up & down to slowly thaw
5. Dispense into new/fresh tube w/ media (from 1) then repeat 4
6. Pellet cells @ 500 rcf, 5min
7. While wait, get T75 flask
Carley: 5 million cells in T75
10mL: full volume in flask

Download

E2C0F52E-30A4-4247-9E27-7FE970A86B99.jpg (402 kB)

Emily Rhine - Oct 11, 2024, 2:57 PM CDT


8) Prep:
~~Name flask ~~ ~~DATE~~
~~A549 WT~~
~~Date XX~~

Name flask
A549 WT BME
Date Thaw XX ← initial of who thaws
P# XX ← initial of individual
passage # flask passages
9) Add 10mL of media to flask
10) After pelleted (8) remove supernatant w/ vacuum aspiration
11) Resuspend pellet (w/ same media from flask) then add to flask → incubate @ 37°C

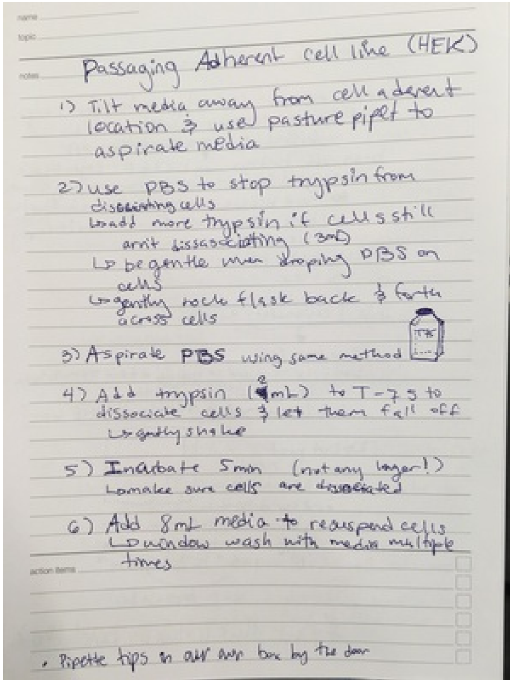
37°C spot: bottom left

If vacuum flask is pink, add more bleach

[Download]

CFE10400-558B-4C6E-94E9-044041E76C3E.jpg (359 kB)

Emily Rhine - Oct 11, 2024, 2:57 PM CDT

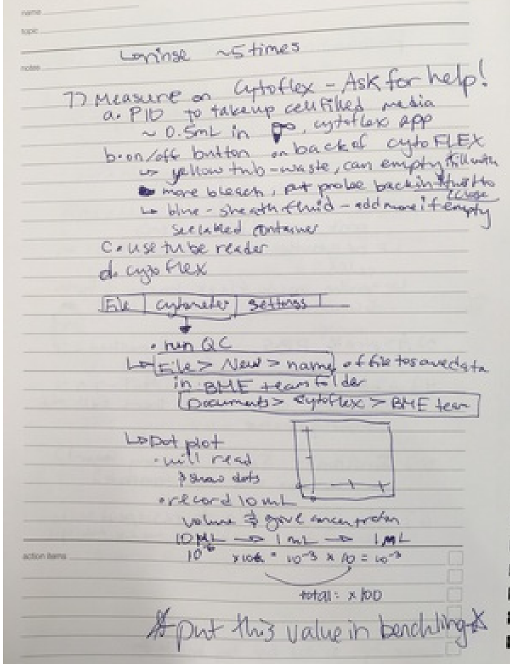

Passaging Adherent cell line (HEK)
1) Tilt media away from cell adherent location & use pasture pipet to aspirate media
2) use PBS to stop trypsin from dissociating cells
↳ add more trypsin if cells still aren't dissassociating (3mL)
↳ be gentle when droping PBS on cells
↳ gently rock flask back & forth across cells
3) Aspirate PBS using same method
4) Add trypsin (2mL) to T-75 to dissociate cells & let them fall off
↳ gently shake
5) Incubate 5min (not any longer!)
↳ make sure cells are dissociated
6) Add 8mL media to reauspend cells
↳ window wash with media multiple times

• Pipette tips in our own box by the door

[Download]

ADCBB43F-BEDC-467C-B274-D497782CD9EC.jpg (470 kB)

Emily Rhine - Oct 11, 2024, 2:58 PM CDT



name
topic
notes

↳ rinse ~5 times

7) Measure on Cytoflex - Ask for help!
a. P1000 to takeup cell filled media
~ 0.5mL in [tube], cytoflex app
b. on/off button on back of cytoFLEX
↳ yellow tub - waste, can empty fill with more bleach, put probe back in the 1% tube
↳ blue - sheath fluid - add more if empty
secluded container
c. use tube reader
d. cytoflex

File | cytometer | settings

• run QC
↳ File > New > name of file to save data in BME team folder
Documents > cytoflex > BME team

↳ Dot plot
• will read & show dots
• record 10 mL
volume & give concentration
10 mL → 1 mL → 1 mL
10 x10^4 = 10^-3 x 10 = 10^-3

action items

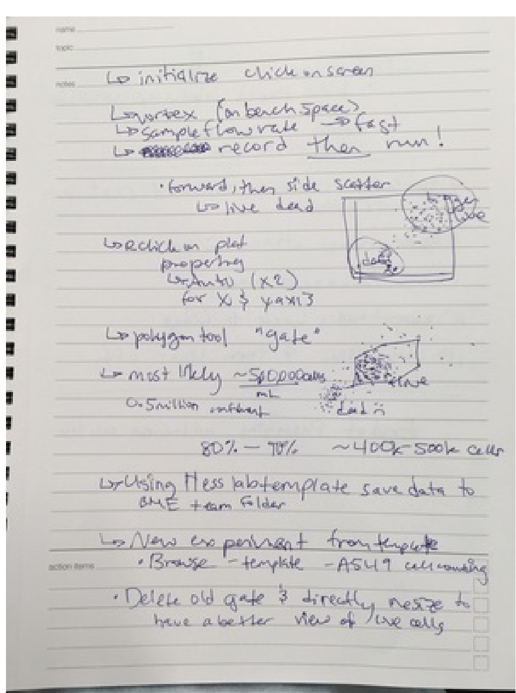
total: x 100

* put this value in benchling *

[Download](#)

47F3E40A-27AE-4A40-A7A3-1CD9C6E51D3B.jpg (455 kB)

Emily Rhine - Oct 11, 2024, 2:58 PM CDT



name
topic
notes

↳ initialize click on screen

↳ vortex (on bench space)
↳ sample flow rate → fast
↳ record then run!

• forward, then side scatter
↳ live dead

↳ Rclick on plot
properties
↳ auto (x2)
for x & y axis

↳ polygon tool "gate"

↳ most likely ~500,000 cells/mL
0.5 million in total

live
dead

80% - 90% ~400k - 500k cells

↳ Using Hess lab template save data to BME team folder

↳ New experiment from template
• Browse - template - A549 cell counting

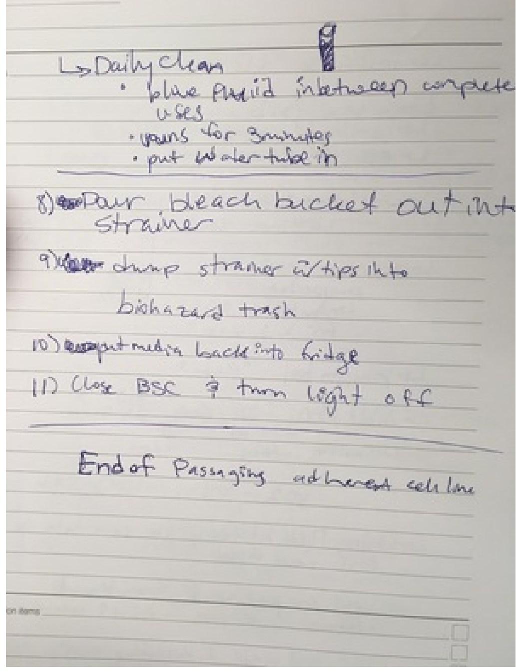
action items

• Delete old gate & directly resize to have a better view of live cells

[Download](#)

2BCEDA15-F6C3-416C-845A-2988B4C35644.jpg (414 kB)

Emily Rhine - Oct 11, 2024, 2:58 PM CDT



Download

4980C2C1-5D69-4D9A-9541-DD939EB7CAD4.jpg (383 kB)

Midsemester Review_10/18/2024

Emily Rhine - Oct 18, 2024, 7:03 PM CDT

Title: Client Meeting_Midsemester Review
Date: 10/18/2024

Content:

- Main goal: Get spheroid working and gamma H2AX staining
- A548 Passaging
    - Doubling speed ~ 20 hours
        - Confirm up to passage 6 or 7 to give us more TC information for cell maintenance
    - Using DMEM now (more nutrient rich)
- Methylcellulose levels alter gamma H2AX presentation
    - (Unperturbed)
    - Are there differences?
        - Screen at both?
- Timeline
    - Next 3 weeks
        - Passage cells
            - Establish doubling time
            - Start selecting for faster passaging cells
        - Set spheroid goals
            - Start small scale
            - Define which parameters to look for when determine spheroid success (such as qPCR - what to "scan" for)
        - Set and adjust protocols
            - Note: there are questions about how different culture conditions (such as % methylcellulose) affects baseline of gamma-H2AX even before screening
    - 6 Weeks
        - Every member comfortable passaging
        - Spheroid protocol
            - Based on personal research and previous paper given by Hess lab
                - Do we just copy exact from literature or figure something else - very much depends
            - Include all possible materials
            - Ask Carley to order
        - Attempt protocols and optimize
            - Optimize through trial and error - do not attempt to tweak variables yet (such as change methylcellulose concentration)
            - Define spheroid success, parameters? How to test it? (for example, size, qPCR markers, live cells)
        - Characterize cells via repeats and alterations
            - CRISPRi cells
                - WT A549s
                - A549 with CRISRPi to add gRNA and look for specific mutation
                    - See loss in staining if specific proteins are selected to be removed with gRNA

Loading [MathJax]/extensions/Safe.js

- Quohan? Experiment: KRAS comparison knockdown with WT (can also show ideal seeding density)
  - His end goal "Model better captures KRAS model"
  - Optimized for non-small lung cancer cell lines
- Markers → qPCR

1. 1st attempt -
   a. What went well? What didn't? How does it line up with original parameter for success
   b. Define different parameters such as seeding density, methylcellulose concentration
2. Try again
   a. Try sub optimal just to see comparison of properties
3. Can also do a pseudo-screen with one RNA just to see the results

- If the spheroid has been perfected → gamma H2AX stain
- Look into ordering:
  - PolyHEMA plates
  - Other research
- Move forward to other areas of the project
  - Passage constantly to seed spheroids
- Next semester
  - Gamma H2AX staining
  - 3D screen

- General information
  - CRISPR screen will take a long time (weeks)
  - CRISPR screen requires
    - Spheroid protocol (scaling)
    - Gamma H2AX staining
  - 2D screen seems pointless to Gaelen
  - 3D screen
    - $2000 to sequence
    - Install library
  - Does 3D affect A549 characteristics
    - Incucyte
    - Plate assay - intensity based staining to show what fraction of live cells
      - Viability dye (over growth)
    - Growth assay
      - Disassociate cells
        - t=0 or t=?
      - Bright fluorescence
        - Optimize fluorescence integration
  - Find pre existing research and confirm/ brainstorm with Gaelen and Carley
    - 2-3 protocols and notice differences to see what variable to alter
- Report Feedback
  - Written feedback
  - Overarching
    - Wide view that can be narrowed down
    - Narrow motivation
    - Logic DNA damage → optimization
      - Optimize walkthrough and link experiments

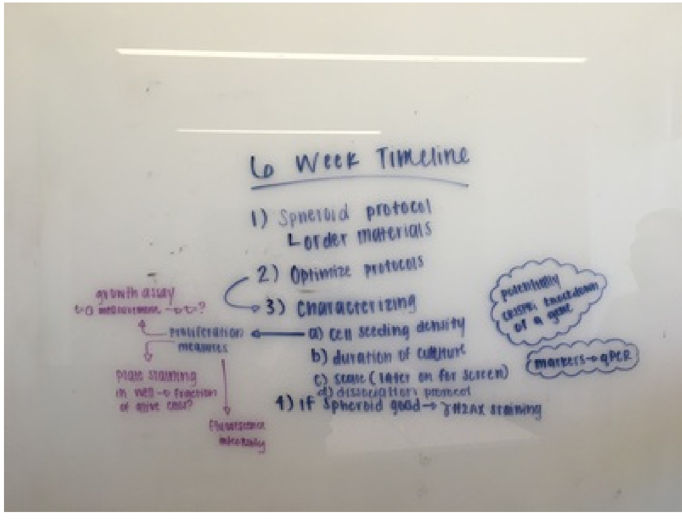
Loading [MathJax]/extensions/Safe.js

- Show and tell
  - Cell imaging (phase)
  - Citation? Brightfield imaging
    - Austin
    - Grace (40x flat cell imaging)
    - Ethan in alternate lab (organoids)

Conclusions & Action Items:

- Main goal: Get spheroid working and gamma H2AX staining
- See timeline goals above
  - Protocols and next steps
  - Meet with Carley again in 1-2 weeks to contextualize biology of genetics
- Received report feedback
  - Carley will send feedback soon
- Show and tell
  - Cell imaging

Emily Rhine - Oct 21, 2024, 11:32 AM CDT



Download

D70C9512-4F2C-46EE-AC1B-136D844781C1.jpg (160 kB)

Emily Rhine - Oct 25, 2024, 12:49 PM CDT

Gaelen Notes

Clarification and emphasis are needed for why DNA damage/genome instability is such an important characteristic of tumors.

I think they understand this on the whole, but I'm not sure they have fully internalized the biological question. Based on reading their report, I am unsure if they understand where DNA damage comes from. They at times suggest it is from DNA damaging drugs or even Cas9 and not necessarily more endogenous sources, which is what we are after.

For future literature searches, it may be worth considering what types of pathways/genes are often missed between 2D/3D culture models (i.e., is there a reason why DNA damage might be more/less susceptible to these 2D/3D issues?)

Loading [MathJax]/extensions/Safe.js

How is yH2AX staining or intracellular staining in spheroids performed? Given the proposed goal, I felt this was missing from the report.

Based on where they are on the project, I think getting spheroids up and running at all would be good for the semester, so perhaps this may be a focus for next semester.

Have staining protocols been used more commonly with one of the different fabrication methods?

Small specific tidbits:

Page 8: "and 21 days for spheroid maturation", not necessarily since yH2AX is the readout. Just need to culture enough cells to stain

Page 13: Transfection vs Transduction (this was misused)

Page 7: They should define Z-score by how it is calculated. I assume it is the difference from mean normalized by st deviation. However, they say that a z-score of +/-1 is considered significant, which would not be true by my definition.

Page 20: 0.45nM filter is 0.45 um

Loading [MathJax]/extensions/Safe.js

Spheroid Formation Protocols_10/25/2024

Emily Rhine - Oct 25, 2024, 1:28 PM CDT

Title: Spheroid formation protocol review
Date: 10/24/2024
Content:
Agenda

- Spheroid formation protocols
- Spheroids needed for screen
    - Wells needed?
    - Cells needed?
- Cell density needed for spheroid
- Materials to be ordered?
- Some parameters/considerations:
    - What stemness markers? Size of spheroids?
    - Centrifugation yes or no? Any additional stuff (like methylcellulose, agar)
    - PolyHEMA plates or hydrogel casted plates

Meeting Notes:

- Genome Stability
    - Different environmental factors = different genome stability
    - UV radiation may cause an increase in random mutation
        - dsDNA break
        - ssDNA mutation - ex: point mutation/frameshift
        - Chromosomal mutation
    - Our cells are always undergoing DNA damage (bc of error prone DNA replication process etc.) → to fix it: genes involved in DNA repair (exp, P53) and thus genome stability (others: telomere stability, chromosome translocations, etc)
        - P53 mutation does not cause cancer, P53 helps fix cellular damage so loss of P53 means that genes are less regulated
        - How to discover new genes that help contribute to DNA stability: screening (+ added 3D complexity with this project)
    - Genome wide screen
        - For example, knockdown expression of gene 1 which repairs DNA damage → DNA stability decreases
        - How do we measure the extent of decreasing DNA stability - γ-H2AX staining
        - γ-H2AX
            - Marker on histone due to phosphorylation (happens when changes in DNA damage occur)
            - The inverse could also be true: if genome stability increases → decrease in γ-H2AX
            - Therefore, can notice both high and low γ-H2AX populations
                - Take top 20% (γ-H2AX enriched for DNA repair) and bottom 20% of γ-H2AX population (endogenous expression meaning: within the cell and not from external DNA expression such as plasmids)
                    - Why 20%: there are both targeting and non-targeting control guide RNAs in genome-wide screening → 20% will capture negative controls

- γ-H2AX staining process (basic procedure - as if don't have spheroids)
    ***Takes 4-4.5 hours

1. Infect cells with lentivirus
    a. Will introduce plasmid with guideRNA, which will target some gene that is involved in gamma-H2AX/DNA damage
        i. The plasmid also has fluorescence: mCherry (positive)
            1. Therefore, associate mCherry+ cells with guideRNA infection into cell
    b. Will not get 100% infection → ideal so we can have controls (with no plasmid aka no vector and no mCherry - negative)
    c. **Wait 3-5 days so that DNA damage caused by lentivirus infection itself will be "healed" → only downstream data and can be ignored**
2. Gather population of cells (3-5 million)
3. Fix cells
    a. Immobilize and kill cells
    b. This allows us to stain, image, sort, and other downstream steps
    c. Dissociate cells from spheroid here?
4. Permeabilize cells
    a. Makes holes in cells so "stuff" can get in them (because we are doing an intracellular staining)
    b. Dissociate cells from spheroid here?
5. Antibody 1
    a. In our case, this will be gamma-H2AX
    b. We actually have a primary-conjugated antibody (already has fluorescence so do not need a secondary antibody)
        i. Our fluorophore will be APC - Alexa fluorophore 647
    c. Dissociate cells from spheroid here?
6. Flow Cytometry
    a. Will have graph: mCherry vs. FSC
    b. In each population, will have some cells positive for mCherry and some negative for mCherry (control, not lentivirally infected)
    c. Will "gate" mCherry+ cells and average their gamma-H2AX expression
        i. Two more subplots:
            1. mCherry+ only with APC gamma-H2AX
                a. Expected to see increase in APC gamma-H2AX
                b. On plot, "peak" will be shifted higher/to the right on x-axis (APC gamma-H2AX)
            2. mCherry- only with APC gamma-H2AX
                a. Expect to see decrease in APC-gamma-H2AX (because no lentiviral infection = gene was not knocked down = no increase in DNA damage)
                b. On plot, "peak" will be shifted lower/to the left on x-axis (APC gamma-H2AX)

NOTE: will need to refer to current spheroid CRISPR screening gamma-H2AX staining protocols to see the process (do we dissociate spheroids before fixing, after fixing, or after permeabilizing?)

- **Possible: if earlier dissociation, may be losing some of DNA markers)**
- **Our job: find a "starting point" for when to dissociate based on literature, then test staining at alternative disassociation points and compare whether there are differences in results**

- Stemness Markers

- Discuss next week/ when it becomes relevant

Conclusions & Action Items:

- Identify key factors in genome stability
- Show and tell Nov.1 12:00-2:00 pm
- Continue cell passaging
- Continue research on γ-H2AX staining with 3D protocols

Timeline & Passaging_11/1/2024

Emily Rhine - Nov 07, 2024, 4:54 PM CST

Title: Timeline & Passaging

Date:11/1/2024

Content By: Emily & Carley

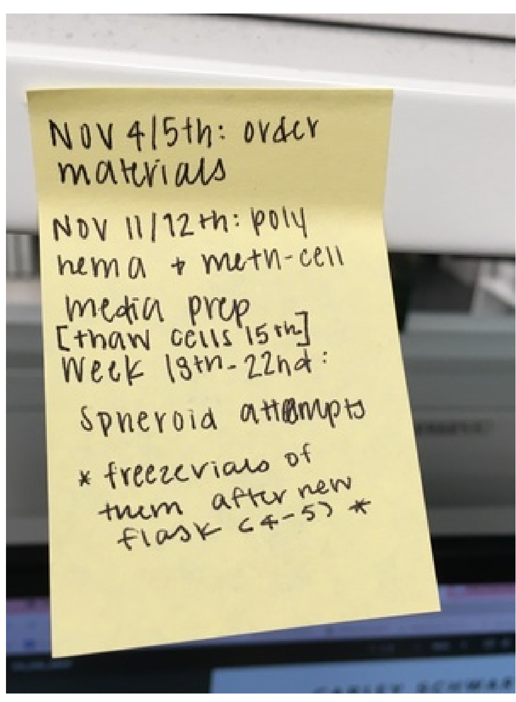
Content:

See images below

Conclusions/ Action Items:

- Follow up with team regarding new information
- Begin materials matrix and BPAG materials sheet

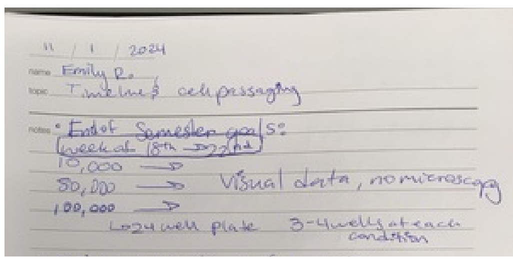
Emily Rhine - Nov 07, 2024, 4:55 PM CST

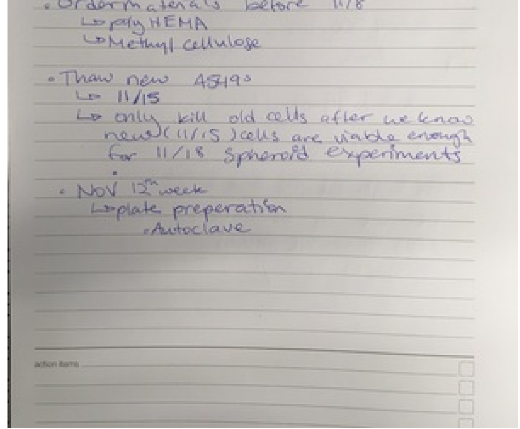


Download

89ECB445-1C0C-4DD4-B593-59746C3F9931.jpg (350 kB)

Emily Rhine - Nov 01, 2024, 8:23 PM CDT





• Order materials before 11/8
↳ poly HEMA
↳ Methyl cellulose

• Thaw new A549s
↳ 11/15
↳ only kill old cells after we know new (11/15) cells are viable enough for 11/18 Spheroid experiments

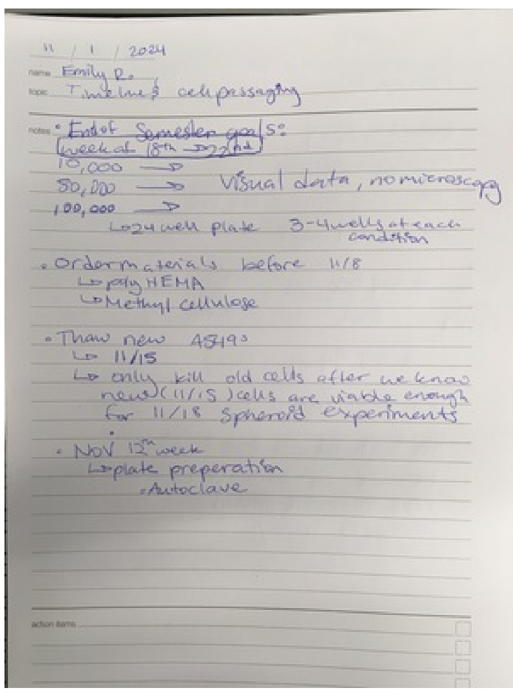
• Nov 12th week
↳ plate preperation
• Autoclave

action items

[Download](#)

6735BE38-8754-4779-A27E-B474D8273718.jpg (385 kB)

Emily Rhine - Nov 01, 2024, 8:24 PM CDT



11 / 1 / 2024
name Emily R.
topic Timelines & cell passaging

notes • End of Semester goals:
week of 18th → 22nd
10,000 →
50,000 → Visual data, no microscopy
100,000 →
↳ 24 well plate 3-4 wells at each condition

• Order materials before 11/8
↳ poly HEMA
↳ Methyl cellulose

• Thaw new A549s
↳ 11/15
↳ only kill old cells after we know new (11/15) cells are viable enough for 11/18 Spheroid experiments

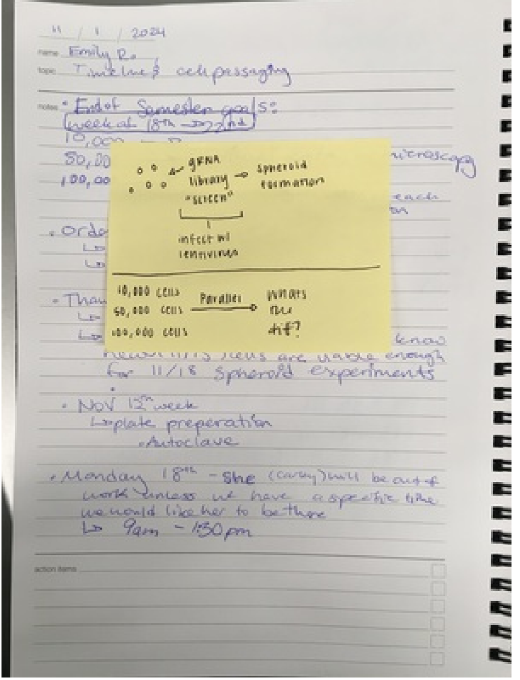
• Nov 12th week
↳ plate preperation
• Autoclave

action items

[Download](#)

720F07C8-00F4-4A16-8FB7-4B2961A02B3A.jpg (385 kB)

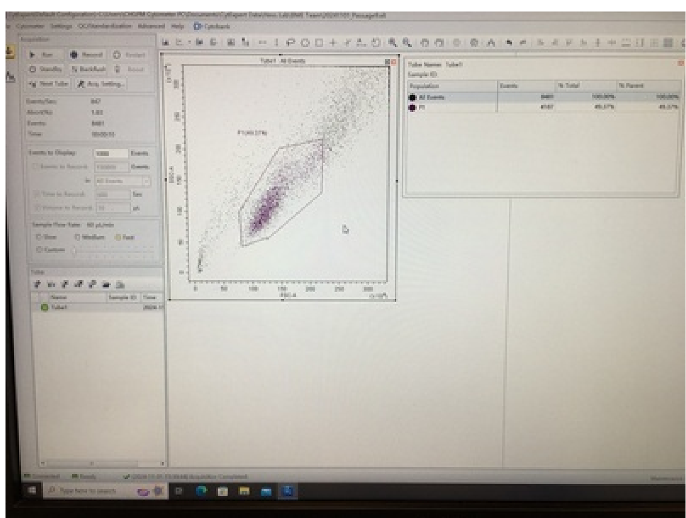
Emily Rhine - Nov 01, 2024, 8:24 PM CDT



[Download](#)

8F1B9841-8C49-45F6-A625-E969D80B7517.jpg (392 kB)

Emily Rhine - Nov 01, 2024, 8:24 PM CDT



[Download](#)

440D73AA-B9C0-412B-BC64-C0E514A08A24.jpg (420 kB)

Materials & qPCR_11/8/2024

Emily Rhine - Nov 10, 2024, 11:57 PM CST

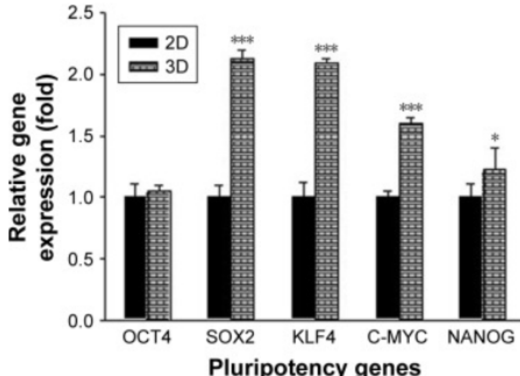
Title: Materials and qPCR
Date: 11/8/2024
Content by: Emily & Jayson
Content:
Agenda:

- What materials have been ordered
- qPCR
- Spheroid formation protocol finalization of steps
- PolyHEMA preparation detailed steps

Notes:

- qPCR
  - How much is each gene expressed (specific DNA sequence)
    - In this case, we know that there is a difference in SOX2 expression between 3D and 2D cultures
  - RT-qPCR (reverse transcription qPCR) - what we will be using
  - Steps:
    - Extract RNA from cells. 2 protocols in the lab:
      - Qiagen based protocol - what we will use, not the other one: RNAquick → due to Qiagen ripper
      - Using oligo dT to pull out everything that has a PolyA tail (aka RNA) → synthesized into cDNA
    - cDNA synthesis
    - Use cDNA to run qPCR
      - 2 methods
        - Sybr Green - will NOT use for our case
          - Sybr Green: amplified target sequences (via 2 opposite-direction primers) will bind and glow → more amplification, more glow. NONSPECIFIC
        - Taqman qPCR - we will use
          - Have primers for amplification
          - A probe in the middle of the target sequence, complimentary to the target sequence
            - Probe is attached with a fluorescent tag, attached with a quencher (when “inactive”, quencher inhibits probe from fluorescing
          - When Taqman enzyme comes through to start amplifying the target sequence → “break” probe so fluorescent can glow (quencher is no longer suppressing)
          - SPECIFIC Therefore you can do both control and test in the same well (use a different probe with different fluorescence)

        - Lab already has GADPH Taqman control
        - For SOX2, would order:
            - Forward primer
            - Reverse primer
              *Primers should be exonic because cDNA spans exon-to-exon (no introns!)
            - Taqman probe
            - SOX2 only has 1 exon, very popular gene so we have existing primers and probe (via thermoFisher link Carley sends)
- Other considerations include:
    - Target sequence specificity, exonic vs intronic, and conserved regions in the gene.
    - GC content around 50% (natively match with genome)
    - Primer design such as Amplicon Size: The ideal amplicon size for qPCR is between 70 and 150 base pairs. Shorter amplicons enhance reaction efficiency and are better suited for quantitative detection. Melting Temperature (Tm): Primer Tm should be around 58–60°C, with both primers in a pair having closely matched Tm (within 1–2°C) to ensure they bind optimally at the same annealing temperature. Secondary Structure Avoidance: Ensure primers do not form hairpins, self-dimers, or hetero-dimers, as these reduce efficiency and specificity.
    - Probe design: Length: A probe length of around 18–30 nucleotides is typical. Short probes with balanced GC content provide efficient binding without excessive stability. GC Content: Aim for 40–60% GC content to ensure the probe binds stably without secondary structure formation. Melting Temperature (Tm): The probe’s Tm should be 5–10°C higher than the primers (usually 68–70°C) so that it remains bound during the annealing phase. A higher Tm enhances probe specificity and stability. Avoid G at 5’ End: A guanine (G) at the 5’ end of the probe can quench fluorescence of certain dyes, reducing sensitivity. Adjust the sequence if possible. *Will need to adjust thermalcycler steps to reflect SOX2’s primers. Also in the next step we need a fluorescence labeler, looking at FSC/SSC staining.
    - To validate and optimize qPCR probe designs, specificity checks, such as BLAST analysis can be conducted to ensure the probe does not bind to unintended sequences. In silico testing, the standard curve generation is then used to confirm the probe’s range and efficiency, ideally between 90–110%. Probes should be stored in dark conditions at -20°C to maintain stability and prevent degradation from repeated freeze-thaw cycles
            - .Data given: CT value (cycle threshold) - the number of amplification cycles required for a fluorescent signal to surpass a threshold level
                - Low CT = less cycles required to pass threshold (therefore high amount of starting material (sequence)
                - High CT = low starting material, longer to reach threshold [MORE MATH (delta delta CT)]
        - GADPH (Glyceraldehyde 3-phosphate dehydrogenase) as control
- U-bottom plates
    - Expensive
    - Han paper uses flat bottom
    - Would need to transfer to flat bottom for imaging, creates extra steps
- Flat bottom
    - 24 well and 96 well available
    - Scale values via imaging spheroids
    - Live/dead cell measurement
        - Cells at beginning
        - Similar size and cell density
        - Measure cells at beginning
        - Can’t separate spheroids formed in these wells
- Water being used to dissolve methylcellulose works

    - Will not mess with osmolarity by dilution with DI water
    - Is 4 days too much to expose the cell to?
        - Expose them after formation?
        - Ana will ask Kreeger lab member that works with polyhema spheroids
    - Dissolve in media or PBS instead?
    - Research other protocols?
- FSC/SSC in flow cytometry rather than cytotoxicity stain
- Stemness gene
    - SOX2 - does this tie into gene stability (GAELEN's goal) - We treat it as a + control to show change of gene expression in 2D versus 3D
        - Human Gene SOX2
        - GADPH (Glyceraldehyde 3-phosphate dehydrogenase) as control
        - 

        - SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine
        - Quantitative real-time reverse transcription PCR(qRT-PCR)

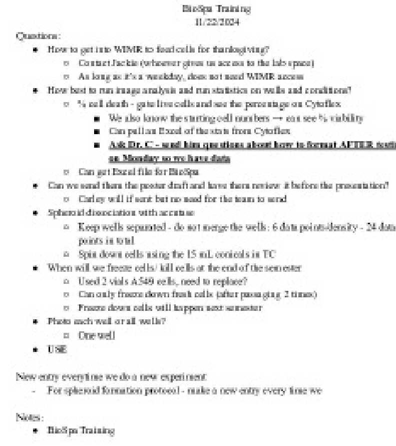
          Total RNA was extracted from cultured cells using the PureLink™ RNA Mini Kit (Thermo Fisher Scientific), and first-strand cDNA was synthesized using oligo-dT primers and M-MLV reverse transcriptase (Thermo Fisher Scientific). Real-time qPCR reactions were performed in triplicate in a final volume of 20 μL containing SYBR Premix Ex Taq II (Takara, Shiga, Japan), 10 ng of cDNA, and 20 pmol of each primer. Real-time qPCR was performed using a 7500HT fast real-time PCR system (Thermo Fisher Scientific) with the following conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control in each reaction. To verify specific amplification, melting curve analysis was performed (55°C–95°C, 0.5°C/s). Quantification of relative expression was performed by the ΔΔCT method. Genes and their primers are shown in Table S2. Expression of each mRNA was normalized to that of GAPDH in the same sample.
    - KLF4

Conclusions/Action items:

- SOX2 selected as + control to show change of gene expression in 2D versus 3D
- Reviewed Taqman PCR techniques and biological process explanation
- Begin PolyHEMA plate preparation next week Monday 11/11
- https://genome.ucsc.edu/ is super helpful for genetics information

BioSpa_11/22/2024

Emily Rhine - Nov 22, 2024, 7:19 PM CST



BioSpa Training
11/22/2024

Questions:

- How to get into WIMR to feed cells for thanksgiving?
  - Contact Jackie (whoever gives us access to the lab space)
  - As long as it's a weekday, does not need WIMR access
- How best to run image analysis and run statistics on wells and conditions?
  - % cell death - gate live cells and see the percentage on Cytoflex
    - We also know the starting cell numbers → can see % viability
    - Can pull an Excel of the stats from Cytoflex
    - **Ask Dr. C - send him questions about how to format AFTER testing on Monday so we have data**
  - Can get Excel file for BioSpa
- Can we send them the poster draft and have them review it before the presentation?
  - Carley will if sent but no need for the team to send
- Spheroid dissociation with accutase
  - Keep wells separated - do not merge the wells: 6 data points/density - 24 data points in total
  - Spin down cells using the 15 mL conicals in TC
- When will we freeze cells/ kill cells at the end of the semester
  - Used 2 vials A549 cells, need to replace?
  - Can only freeze down fresh cells (after passaging 2 times)
  - Freeze down cells will happen next semester
- Photo each well or all wells?
  - One well
- USE

New entry everytime we do a new experiment

- For spheroid formation protocol - make a new entry every time we

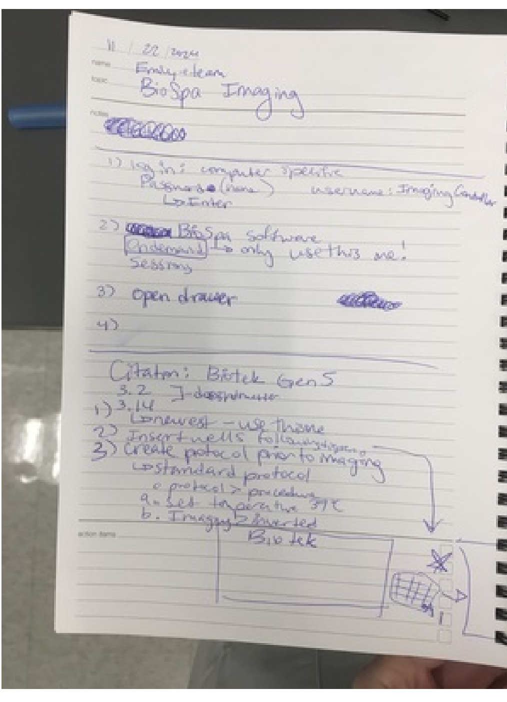
Notes:

- BioSpa Training

Download

BioSpa_Training_11_22_2024.pdf (79 kB)

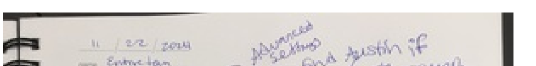
Emily Rhine - Nov 22, 2024, 7:07 PM CST

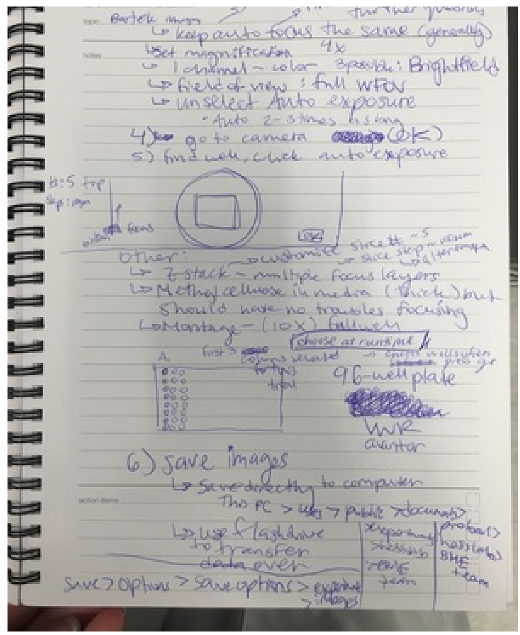


Download

8F06C3B7-1C4D-4B5B-9867-3F1907368F1D.jpg (391 kB)

Emily Rhine - Nov 22, 2024, 3:13 PM CST

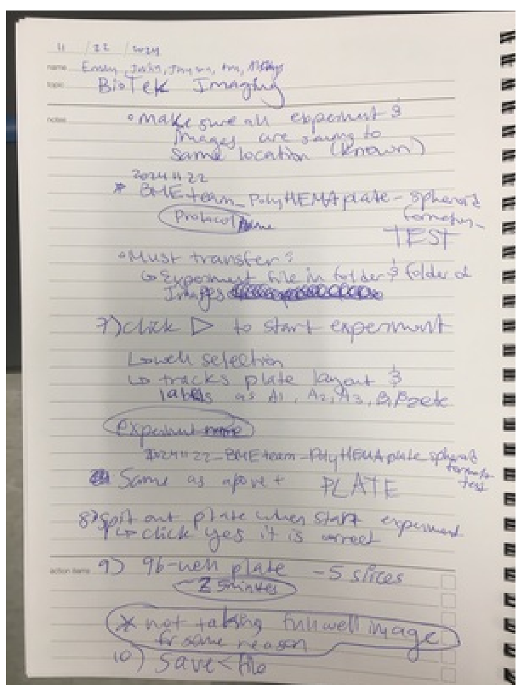




Download

501C1DB6-F395-4BC5-A4C0-47567E89CA44.jpg (522 kB)

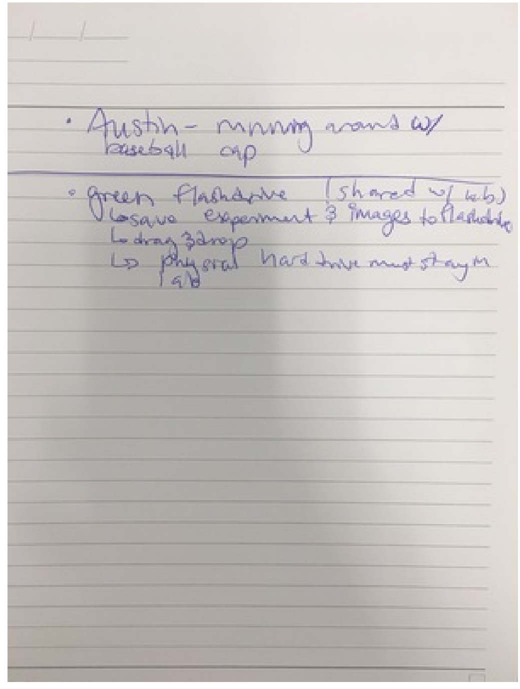
Emily Rhine - Nov 22, 2024, 7:07 PM CST



Download

531D1F9C-5F87-4733-888A-3E203608E642.jpg (450 kB)

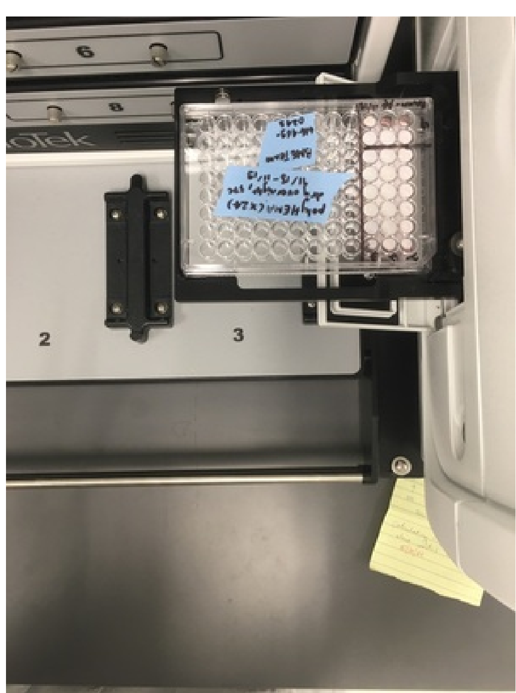
Emily Rhine - Nov 22, 2024, 7:07 PM CST



Download

48284C6B-0690-4D0E-9F37-5A491E320843.jpg (302 kB)

Emily Rhine - Nov 22, 2024, 3:13 PM CST



Download

3AC8C89F-F03F-4E01-BE58-8D3C87EA652D.jpg (385 kB)

Advisor Introduction_9_6_2024

Emily Rhine - Sep 06, 2024, 1:22 PM CDT

Title: Advisor Introduction & Team Meeting 1
Date: 9/6/2024
Present/ Content by: Entire team
Content:

- Advisor Meeting time
    - 12:05 pm
    - 9/13/2024 - Meeting time will be either Thursday night or Friday at 11:30 am before class
    - The Code Breaker
        - CRISPR
- Team expectations & role definitions
    - Team Leader: Responsible for organizing weekly progress reports, team goals and team meetings.
        - Althys - Team leader
        - Jayson - Team Co-leader
    - Communications: Primarily responsible for communications with the client and other professional contacts, as well as distributing progress reports.
        - Ana Martinez - Communicator
    - BSAC (Biomedical Student Advisory Committee): provides feedback to faculty about the design courses and curriculum and is chaired by an elected student. BSAC members also serve as peer advisors and mentors to the freshman.
        - Emily Rhine - BSAC
    - BWIG (Biomedical Web Implementation Group): is responsible for the team's website and the overall website is overseen by the BWIG chair.
        - Julia Salita - BWIG
    - BPAG (Biomedical Purchasing and Accounting Group): is responsible for ensuring that all necessary materials are acquired and for maintaining all financial records for the team.
        - Jayson - BPAG
- Progress Report
    - Split up
        - Brief status update:
        - Difficulties or advice requests:
        - Current design:
        - Materials and expenses:
        - Major team goals for the next week:
    - Individual
        - Next week’s individual goals:
        - Previous week’s goals and accomplishments:
- Email Client
- Client Question Document

Conclusion:

- Redefine problem statement after the team better understands the project.
- Set up a meeting with the client to get questions answered.
- Continue individual research and brainstorming.

Action Items:

- First progress report due
- Use a meaningful file name (i.e. team_catchphrase-progress_report-1)
- Team Leader: Prepare the progress report and establish goals with your team
- BWIG: Post to the website
- Communicator: Email the file website link to your advisor and client, cc your team

Advisor Meeting2_9/13/2023

Emily Rhine - Sep 13, 2024, 4:29 PM CDT

Title: Advisor Meeting 2

Date: 9/13/2024

Present/ Content by: Julia, Althys, Ana, and Emily

Content:

- Add team photo to website
- Since so much of this project depends on what we design the experiment to be, the PDS may be more vague than a typical PDS.
    - Ex: Size and porosity of spheroids will be up to us depending on what cell line we choose
- Gave a recap of the client meeting
    - CRISPR
    - γH2AX - quantify DNA
- BME 400 expectations:
    - Cannot work in BSL-2
    - No human cells
    - Our project scope is not within the scope of this course it is closer to undergraduate research
- Put down all team and individual entries into LabArchives
- Weekly progress report with advisor cc'd
- He said he is chill about deadlines
    - Quality work over rushed work
- Prepare for a plan B

Conclusions & Action Items:

- Potential meeting next Monday or Tuesday to make sure the client, advisor, and team are all on the same page.
- Make sure all research is uploaded to individual notebook
- Can send PDS to advisor to have him look it over before we submit it now

Advisor Meeting 3_9/20/2024

Emily Rhine - Sep 20, 2024, 7:25 PM CDT

Title: Advisor Meeting 3_PDS Review

Date: 9/20/2024

Present/ Content by: Entire Team

Content:

- Research must be conducted in Hess lab only
- This week was mostly working on the PDS
- Discussed design matrix introduction and potential categories to rank the spheroids and cell lines by
- OVCAR 433 forms spheroids really well if we choose ovarian over lung
- Hanging drop used previously however this will be hard to scale up
    - Will look into scaling up
- Planning on mating with Gaelen Monday 5:30 to discuss PDS and design matrix
- Discussed the team researching cell lines individually and discussing it for the matrix next week
- We should be well within budget assuming Hess lab already has a lot of the materials we will need for the project

Conclusions & Action Items:

- Advisor gone next week Thursday and Friday
    - Meet next week Wednesday 9/25 10:00 am in ECB
- Continue work on design matrix

Advisor Meeting 4_9/25/2024

Emily Rhine - Oct 12, 2024, 7:37 PM CDT

Title: Advisor Meeting 4

Date: 9/25/2024

Present: Entire Team (-Emily)

Content By: Emily

Content:

- Send advisor presentation slides by 10/2 to review before actual presentation
- See time and location of presentation in image attached below
- Discussed Matrix

Conclusions & Action Items:

- Catch Emily up with advisor meeting notes
- Meet with team this week (9/27) to divide up presentation slides
- Meet up with team Friday morning to practice presenting

Emily Rhine - Sep 24, 2024, 7:41 PM CDT

BME 400: 3024 Engineering Hall

Time	Project	Advisor
12:05 pm	CRISPRi screening in cancer spheroids to investigate factors in genome stability	Prof. Paul Campagnola

Loading [MathJax]/extensions/Safe.js

Advisor Meeting 5_10/10/2024

Emily Rhine - Oct 12, 2024, 7:38 PM CDT

Title: Advisor Meeting Notes
Date: 10/11/2024
Present: Entire team
Content By: Emily & Ana
Content:

- Presentation Feedback
  - Background was long, some part may have been unnecessary
    - Make more focused for final presentation
    - Explain the gap within the first minute!
  - Define problem statement right away - reduce clutter
  - Dr. Block's comments were very positive
  - Project motivation was unclear
    - Stress 3D importance over 2D application!
      - Focal adhesions, other structures, etc are not the same in 2D as in 3D
  - By December know what markers to look for in qPCR
- Update with timeline
  - Going to WIMR after this to meet with client and start tissue culture
  - PolyHEMA or ultra low attachment plates
    - Reuse or not?
  - A549 thaw cells and begin passaging
  - qPCR protocol – got from Hess Lab
    - Likely will use their primers they already have
    - They don't have specific ideas for markers because they may change across cell lines
      - Research on A549 tends to focus on SOX2 and OCT4
        - Plan to order these BEFORE break!
- Notebook and prelim report update
  - Sunday night or monday morning is fine for submitting notebook and prelim report
  - Upload to LabArchives and website (PDF)
- Advisor
  - Create testing plan
  - Create viable timeline for project for fall and spring
  - Plan for ordering supplies
- Show and Tell
  - Advice for BME 200/300
- Final Presentation
  - Imaging of spheroids
  - video if time
  - Spheroid model - jello?
- 15 years ago NIH
  - Select agent need permission form NIH director
  - Bloodborne pathogens

Conclusion & Action Items:

- Sunday night or Monday morning is fine for submitting notebook and prelim report
- Catch up with client at 1 pm today
- Blood borne pathogens course completes

Advisor Meeting 6_10/18/2024

Emily Rhine - Oct 18, 2024, 12:25 PM CDT

Title: Advisor Meeting 6
Date: 10/18/2024
Present: Entire team (-Julia)
Content:

- Show and tell **-** it is okay to bring in photos and jello
- Established MWF passing schedule
  - ~20 hour doubling time
- A549
  - Cells are really large, so confluency is hard to see with a microscope
  - 3 mL trypsin due to large cells
- Better establish timeline
  - How to prepare for CRISPR screening
- Good endpoint for this semester (end of November)
  - Bulk up cells to ~100 million cells
  - Start CRISPR screen before the end of the semester
- Presentation, Report, and notebook review
  - Report **-** he thinks “it was incredibly well done”
    - He doesn't have anything else to add
  - Paper
    - Final report → Article published?
      - Talk to client
    - 1 month → 1 year turn around for publication
      - ~3 month turn around expected

Conclusions & action items:

- Good endpoint for this semester (end of November)
  - Bulk up cells to ~100 million cells
  - Start CRISPR screen before the end of the semester
- Freeze down cells for over month break

Advisor Meeting 7_11/5/2024

Emily Rhine - Nov 05, 2024, 4:18 PM CST

Title: Advisor Meeting: Project Timeline and Materials
Date: 11/5/2024
Present: Entire team
Content By: Emily
Content:
Agenda:

- Expectations for end of semester goals
    - Nothing "novel to present"
    - Attempt 1 spheroid protocol
        - 3 different cell densities
    - According to the client they do not want us to get to CRISPR or staining yet, our only goal is attempting spheroid formation protocols.
- Numerical testing data?
    - Spheroid size on confocal
    - Spheroid size on phase contrast or brightfield
    - Live/death (dissociate spheroids, do flow cytometry on single cells)
    - qPCR substitute: SOX2?
        - Stemness markers
        - Will client agree?

Meeting Notes:

- CRISPR → not our job/project
    - Client will take care of it
    - We just prepare for the screen
- Experiment conditions
    - No tagging or fluorescence
    - Just image and check size
- Why we can't use hanging drop
    - Scale
    - Reproducibility - variation in size
- Timeline
    - 11/11 - PolyHEMA plates, new cells to thaw and passage
    - 11/18 - Make spheroids
    - 11/25 - Dissociate cells and work on imaging/ project
        - live cell/dead cell data
    - Semester ends unprecedentedly early
- Lack of novelty in first semester is okay

Conclusions/Action Items:

- Meet with client soon
- See timeline above
- Prepare for end of semester final deliverables

Advisor Meeting 8_11/22/2024

Emily Rhine - Nov 22, 2024, 12:17 PM CST

Title: Advisor Meeting
Date: 11/22/2024
Present: Entire team
Content:

- Current issues
  - Plates made incorrectly
  - Cells seeded at wrong density
  - Leave in TC hood to dry
- Keep cells after Thanksgiving
  - Make sure we have WIMR access
- Testing Plans
  - BioSpa Imaging
  - Image J Analysis
- Preparation for final poster/final report

Conclusions/Action Items:

- Poster Draft - send to Dr. C by Tuesday
- Report/Notebook/etc by 12/17 (Tuesday – one week after actual due date)

Advisor Meeting 9_12/13/2024

Emily Rhine - Dec 16, 2024, 11:49 PM CST

Title: Advisor Meeting
Date: 12/13/2024
Present: Julia, Althys, Jayson
Content to discuss:

- Progress: spheroid formation protocol kinda finalized, took images and analyzed size and number of spheroids formed
- Next semester:
  - Thaw new cryovial of cells for passaging, freeze down new vials
  - % viability, another round of spheroid formation protocol with different methylcellulose concentration (if possible) + testings (% viability, imaging), live/dead staining (if possible), qPCR SOX2 (if possible), gammaH2AX staining protocol
- Feedback about poster presentation
  - Did not really explain some stuff (why lung cell line, why SOX2)
- Next Semester Logistics
  - Prelim presentation - just to Dr. C in normal meeting time
  - Instead of show and tell ourselves, we act as advisors to 200/300 students for their show and tell
  - Final report - journal-style
    - Good to get started EARLY in the semester
      - Ex: background/intro can get started early

Conclusions/Action Items:

- Create timeline over winter break for next semester
- Finish final report
- View and respond to poster and team feedback

Materials used in cell passaging 10/14/2024

JULIA SALITA - Oct 14, 2024, 12:20 PM CDT

Title: Cell Passaging Materials- Media (D-MEM), Trypsin, FBS, and PBS

Date: 10/14/2024

Content by: Julia Salita

Present: Julia Salita and Jayson O'Halloran

Goals: To document specifically what we are using to passage cells in (media, trypsin, and FBS)

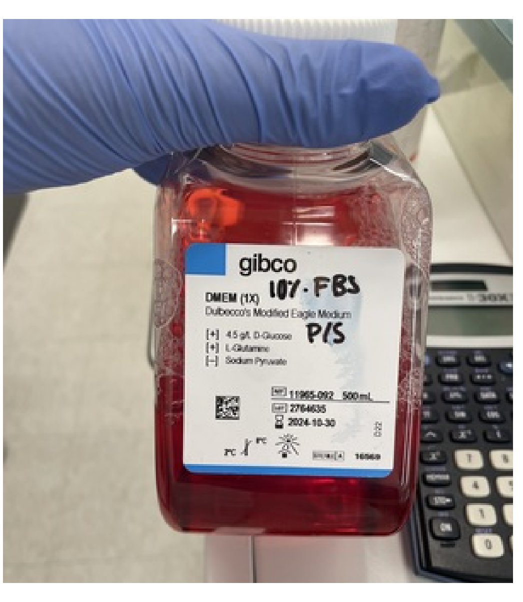
Content:

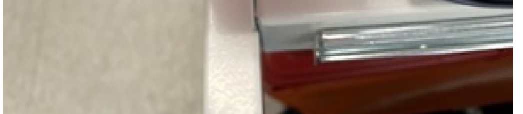
1. D-MEM (1x) Delbecco's Modified Eagle Medium:
    1. Brand: gibco
    2. Volume: 500 mL
    3. Content added (by us): 10% FBS (fetal bovine serum), P/S
2. Trypsin 0.05% (1x):
    1. Brand: cytiva
    2. Volume: 125 mL
3. Fetal Bovine Serum, Value FBS:
    1. Brand: gibco
    2. Volume: 500 mL
4. PBS pH 7.4 (1x):
    1. Brand: gibco
    2. Volume: 500 mL
5. See images for more details

Conclusions/action items:

1. Get an image of the Penicillin/streptomycin used
2. Maybe see if we can get the containers cost as well

JULIA SALITA - Oct 14, 2024, 12:21 PM CDT

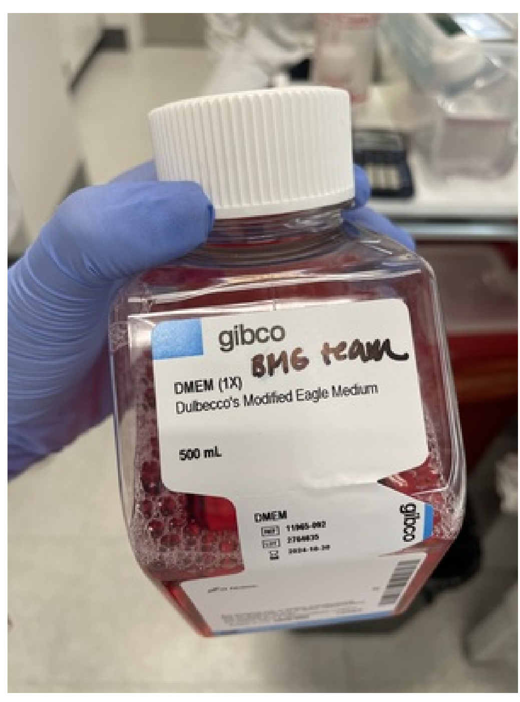




Download

IMG_2642.jpg (2.51 MB) Images of DMEM used

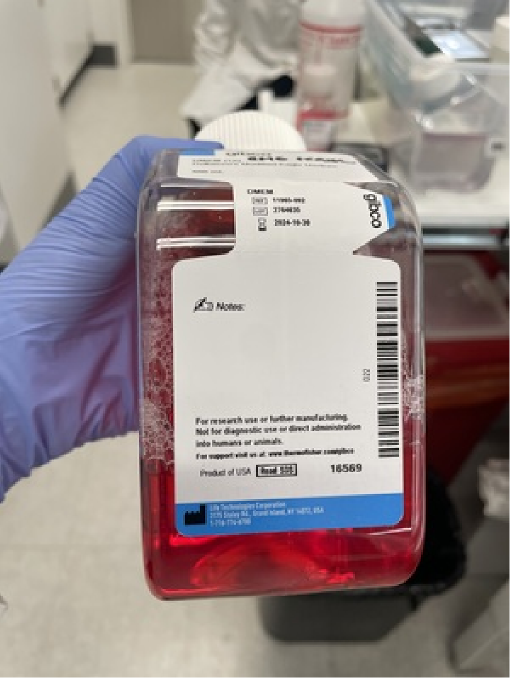
JULIA SALITA - Oct 14, 2024, 12:21 PM CDT



Download

IMG_2641.jpg (2.52 MB) Images of DMEM used

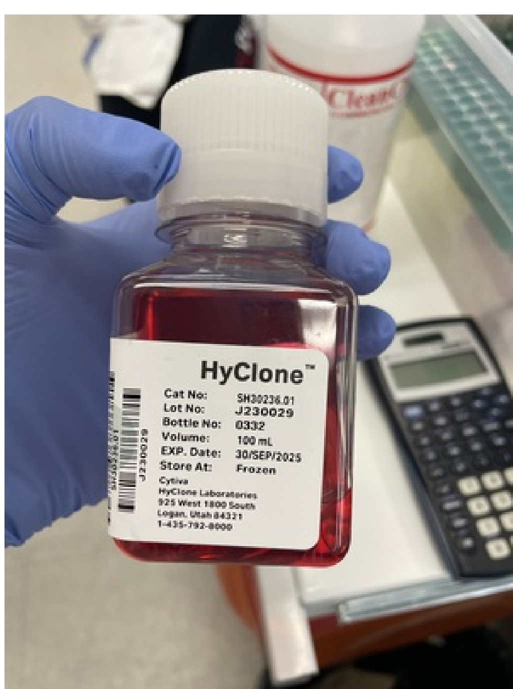
JULIA SALITA - Oct 14, 2024, 12:21 PM CDT



Download

IMG_2640.jpg (2.06 MB) Images of DMEM used

JULIA SALITA - Oct 14, 2024, 12:23 PM CDT



Download

IMG_2639.jpg (2.3 MB) Images of the Trypsin used

JULIA SALITA - Oct 14, 2024, 12:23 PM CDT

Download

IMG_2638.jpg (2.42 MB) Images of the Trypsin used

JULIA SALITA - Oct 14, 2024, 12:24 PM CDT

Download

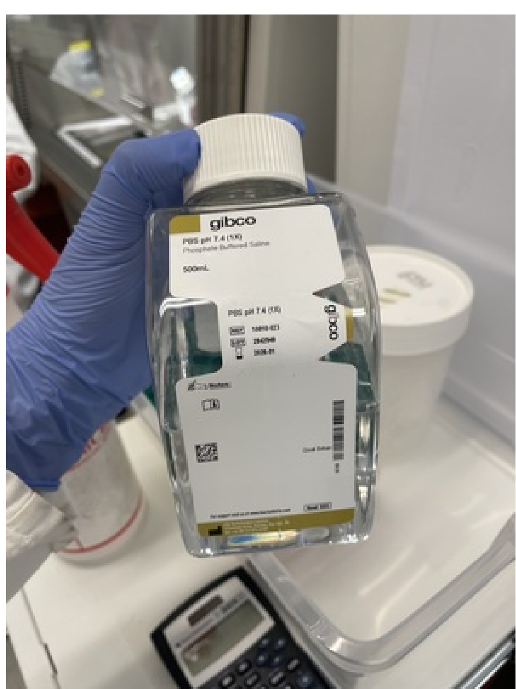
IMG_2643.jpg (2.93 MB) Image of the FBS used

JULIA SALITA - Oct 14, 2024, 12:25 PM CDT

Download

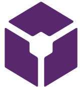
IMG_2637.jpg (2.41 MB) Images of the PBS used

JULIA SALITA - Oct 14, 2024, 12:25 PM CDT



Download

IMG_2636.jpg (1.89 MB) Images of the PBS used



Spheroid Materials_Preliminary List_11/2/2024

Emily Rhine - Nov 02, 2024, 6:07 PM CDT

Title: Preliminary Spheroid Materials Sheet

Date:11/2/2024

Content by: Emily

Content:

Emily Rhine - Nov 02, 2024, 6:06 PM CDT

Item	Amount	Vendor	Cost	Associated Protocol	Link
Methylcellulose	[illegible]	Sigma-Aldrich	[illegible]	1,2,3,4	[illegible]
Poly-HEMA (P3932)	10 g	Sigma-Aldrich	[illegible]	1,2,3,4	[illegible]
RPMI 1640 growth medium		Sigma-Aldrich		1,2	[illegible]

Download

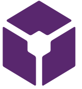
BPAG_Expense_Sheet_Materials_-_Sheet1.pdf (51.7 kB)

Emily Rhine - Nov 02, 2024, 6:07 PM CDT

Conclusions/Action Items:

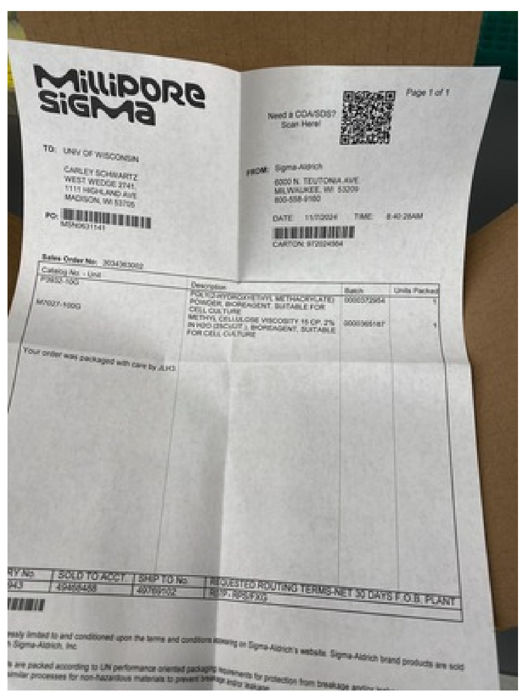
- Meet with team to discuss this first materials draft
- Get materials approved and ordered by the client
- Add materials as needed
- Materials based on those listed in "Spheroid Protocols Matrix" entry

Loading [MathJax]/extensions/Safe.js



Polyhema and Methylcellulose Receipt

JAYSON O'HALLORAN - Nov 15, 2024, 6:16 PM CST



MilliporeSigma

Page 1 of 1

Need a COA/SDS? Scan Here!

TO: UNIV OF WISCONSIN
CARLEY SCHWARTZ
WEST WEDGE 2741,
1111 HIGHLAND AVE
MADISON, WI 53705

PO: MSN0631141

FROM: Sigma-Aldrich
6000 N. TEUTONIA AVE
MILWAUKEE, WI 53209
800-558-9160

DATE: 11/7/2024 TIME: 8:40:28AM

CARTON: 972024564

Sales Order No: 3034363002

Catalog No. - Unit	Description	Batch	Units Packed
P3932-10G	POLY(2-HYDROXYETHYL METHACRYLATE) POWDER, BIOREAGENT, SUITABLE FOR CELL CULTURE	0000372954	1
M7027-100G	METHYL CELLULOSE VISCOSITY 15 CP, 2% IN H2O (25C)(LIT.), BIOREAGENT, SUITABLE FOR CELL CULTURE	0000365187	1

Your order was packaged with care by JLH3

SOLD TO ACCT.	SHIP TO No.	REQUESTED ROUTING TERMS-NET 30 DAYS F.O.B. PLANT
[illegible]	[illegible]	[illegible]

Download

753111689.JPG (434 kB)

Hess Lab Cell Line Maintenance

Emily Rhine - Oct 05, 2024, 7:36 PM CDT

Title: Hess Lab Cell Line Maintenance
Date: 10/5/2024
Content By: Hess Labs
Content:
Passaging Protocol [1]:

1. This assumes passaging in a T75 flask, if using a T150, double the volumes)
2. Remove all media with Pasteur pipette and vacuum
3. Add 2ml of PBS to remove remaining media and remove with vacuum
4. Add 2mL trypsin and wait five min at 37C
5. Add 6mL media to neutralize trypsin while mixing to remove cells from the bottom of the flask (at least 3x the volume of trypsin used)
6. Wash the flask with the cells in media, remove and add to a 15ml conical tube
7. Return enough media to the flask so cells will be confluent when next checked
8. Add additional media (eg. 10 ml)
9. The media just needs to be enough to cover the cells, provide nutrients and won't dry out. Adding more media doesn't change the confluence of the cells.

Thawing Protocol [1]:

1. Prepare 10mL of the appropriate media in a 15ml tube.
2. Using a P1000 slowly mix the media in the cryovial to thaw the pellet of cells.
3. Set the pipette to something below 1000uL, maybe 900uL to avoid clogging the filter while resuspending
4. Once thawed, add to the remaining media and spin down at 1400 rpm for 5 min.
5. Resuspend the cells in fresh media and add to a flask to incubate for typically 3 days before passaging further.

References
[1] “Passaging Cells · Benchling.” Accessed: Oct. 05, 2024. [Online].
Available: https://benchling.com/uw_hesslab/f/lib_jUyOwxBE-tissue-culture-protocols/prt_PWYXyJOG-passaging-cells/edit

Conclusions/ Action Items:

- Add additional Hess Lab protocols to notebook and preliminary report as needed
- Cell feeding should occur every other day
- Cell passaging should occur 1-2 times a week

A549 Cell Line Maintenance

Emily Rhine - Oct 05, 2024, 7:42 PM CDT

Title: Hess Lab Cell Line Maintenance
Date: 10/5/2024
Content By: Hess Labs (Carley Schwartz)
Content:

- The A549 cell line is a human alveolar basal epithelial cell line
    - They are adherent, squamous, and have an epithelial like apearance and are a larger cell line so they take up more volume
    - They are a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells
- Media: DMEM + 10% FBS + P/S
- The doubling time ranges between 20-40 hours (we will need to keep track of this)
- They are good for 20 passages

Passaging Schedule

For adherent cell lines while media is important to ensure your cells get proper nutrients confluency is really based on the surface area of the flask or dish they are forming their monolayer on. A T75 flask is 75cm^2 and a T50 is 150cm^2 and based on the area, cell size, and how fast your cells double you can determine how often you need to passage to ensure they don't get overgrown. You typically want to keep them around/under 80% confluent.

T75 Example Cell Values

	Flask	Surface Area	Seeding Density (million)	Cells at Confluency (million)	PBS Volume	Trypsin Volume	Resuspension Volume	Final Volume
1	T-75	75	1	5	2	2	8 (needs to be x3 the amount of trypisin to neutralize it)	10
2			On the cytoflex 100% confluency with this cell line would be .5 million cells per mL			You may need 3 since these cells can be a bit more adherent than others		

If they double between 20-28 hours:

- You will do a Monday, Wednesday, Friday passaging schedule
    - How this works: on Monday your cells are at confluency in 10 mL of media, the next time you will be in lab is Wednesday so you need to cut your cells back. Between Monday and Wednesday your cells will double twice (x2 x2). Thus, you need to cut them back to a point where by Wednesday (48 hours later) they won't be overgrown but won't be too low either. After going through the process of PBS, trypisin, and resuspending the cells in media to get a volume of 10 mL you will keep 2 mL of this volume and bleach the remaining 8 mL. The way this works is if you have roughly 1 million cells in 1 mL of media and you keep 2 mL you're saving 1 million cells, this will double to 2, and then double again to 4 million which roughly puts you at 80% confluency. On Friday though you won't be in till Monday so you will need to save only 1 mL of cell solution volume.

- This math is not perfect and passaging does not need to be perfect, but for the health of your cells it is important to be roughly on track. This is will also allow you to begin to recognize how well or poorly your cells are growing.

If they double between 30-48 hours:

- You will do a Monday, Wednesday, Friday passage schedule but will be cutting them back less
    - How this works: On Monday your cells are at confluency in 10 mL of media, the next time you will be in lab is Wednesday so you need to cut your cells back. Between Monday and Wednesday your cells will double once. Thus, you need to cut them back to a point where by Wednesday (48 hours later) they won't be overgrown but won't be too low either. After going through the process of PBS, trypisin, and resuspending the cells in media to get a volume of 10 mL you will keep 4 mL of this volume and bleach the remaining 6 mL. The way this works is if you have roughly .5 million cells in 1 mL of media and you keep 4 mL you're saving 2 million cells, this will double to 4 which roughly puts you at 80% confluency. On Friday though you won't be in till Monday so you will need to save only 2 mL of cell solution volume.

LN2 A549 Storage

	Rack Number	Box Number	Cell Type	Position	Box Name
1	Rack 15	4	A549 WT	52	Hess Stocks 4
2	Rack 15	4	A549 WT	53	Hess Stocks 4
3	Rack 15	4	A549 WT	54	Hess Stocks 4
4	Rack 15	4	A549 WT	55	Hess Stocks 4
5	Rack 15	4	A549 WT	56	Hess Stocks 4
6	Rack 15	4	A549 WT	57	Hess Stocks 4
7	Rack 15	4	A549 WT	58	Hess Stocks 4
8	Rack 15	4	A549 WT	59	Hess Stocks 4

References

[1] “A549 Cell Line Maintenance · Benchling.” Accessed: Oct. 05, 2024. [Online]. Available: https://benchling.com/uw_hesslab/f/lib_tD5E8G7L-cell-line-maintenance/etr_5Skd74TL-a549-cell-line-maintenance/edit

Conclusion & Action Items:

- Meet with team to discuss how to split who comes into WIMR when
- Go into WIMR to meet with Carley to thaw and begin passaging A549 cells
- Update Preliminary report with relevant information

CryoFLEX Protocol_10/11/2024

Emily Rhine - Oct 12, 2024, 7:45 PM CDT

Title: CryoFLEX Protocol

Date: 10/11/2024

Content By: Emily & Carley Schwartz

Content:

Cytoflex Equipment Overview:

- On the back of the machine is on the switch

- To the left is two container
  - Sheath: this is the fluid that is fed through the machine when running cell samples, there is a larger container labeled sheath fluid to refill it when the alarm goes off
  - Waste: This is where your ran samples end up, these will include cells so when you need to empty it you put in some bleach and water

Software Use:

- CytExpert is the application that allows you to analyze your samples

- From the Start page, select New Experiment or New Experiment from Template to start a new experiment, or Open Experiment to continue an existing experiment.
  - NOTE Templates contain information on hardware and software settings including channels used, gain settings, flow rate, and stop criteria.
- If a tube has not been created, use the Tube toolbar buttons to create a new tube. To modify a tube's property, highlight the tube name then select located on the bottom, left section of the Acquisition screen.
- Familiarize yourself with the toolbar.
- Create the desired histograms or dot plots using the Plots tool
  - Fluorescence key
- Set the desired sample flow rate, then select on the Acquisition panel to start the lasers and fluidics.
- Load a sample tube into the sample port, then select to start acquisition and display data.
  - Forward scatter (FSC) that detects scatter along the path of the laser, and side scatter (SSC) which measures scatter at a ninety-degree angle relative to the laser
    - This really just means the size of the cells moving through
    - Dead cells are smaller than alive ones
- Use the Threshold tool or select to set discrimination to eliminate undesired populations on the plot.

- Use the Scale and Gain tools or select to move the population displayed on the plot to the desired location.
- Once done - Follow the Daily Clean procedures, which consist of: Run FlowClean cleaning fluid for 3 minutes. Run DI water for 3 minutes.

QC:

- Select Start QC in the QC menu. The Acquisition screen is now replaced by the QC screen.

- Ensure that the QC fluorosphere lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, import the lot-specific target value file. Refer to Importing Lot-Specific Target Values in Chapter 4,

Instrument Quality Control of the CytoFLEX Flow Cytometer Instructions for Use.

- QC beads are found in the hess lab fridge outside
- QC must be passed daily before use

References

[1] “Cytoflex Basics · Benchling.” Accessed: Oct. 12, 2024. [Online].
Available: https://benchling.com/uw_hesslab/f/lib_tD5E8G7L-cell-line-maintenance/etr_syJbLT7n-cytoflex-basics/edit

Conclusions & Action Items:

- More detailed instructions: https://med.nyu.edu/research/scientific-cores-shared-resources/sites/default/files/cytoflex-quick-start-guide.pdf
- Entire team learned how to operate machine 10/11/2024
  - DO NOT OPERATE ALONE
  - VERY EXPENSIVE SHARED MACHINE

Spheroid Protocols Master Document_10/30/2024

Emily Rhine - Nov 11, 2024, 1:38 PM CST

Title: Spheroid Protocols Master Document

Date:10/30/2024

Content by: Entire team

Goal: Research options for spheroid protocols to present to Carley and determine what materials to order

Content:

See PDF below

Emily Rhine - Nov 11, 2024, 1:35 PM CST

Plate Formation Protocol

1. Poly-HEMA plate coating
   a. First protocol: from: "In vitro differentiation of human embryonic stem cells to hemogenic endothelium and blood progenitors via embryoid body formation." Accessed: Oct. 29, 2024. [Online]. Available: https://star-protocols.cell.com/protocols/499
      i. Poly-HEMA solution is made by dissolving 6 g of Poly-HEMA in 500 mL of 95% ethanol (25 mL of tissue culture grade H2O in 475 mL of biological grade ethanol) for 10–12 h on a heated stirrer at 30°C.
      ii. The solution is sterilized using a 0.2 µm filter (Nalgene) and kept at 20°C until needed.
      iii. 10 cm tissue culture dishes are coated with 4 mL of Poly-HEMA solution and the dishes are allowed to dry completely for 24 h at 40°C in a ventilated oven. Similarly, 6-well plates are coated with 1 mL of Poly-HEMA solution per well. Plates and dishes are kept with their lids on during the drying step in the oven to maintain sterility.
      iv. Coated dishes and plates can be sealed with parafilm and stored at 20°C for 3 months.
   b. Second protocol: from: C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, "SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine," OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.
      i. A total 1.3 g of poly-HEMA (Sigma-Aldrich Co.) was dissolved in 33 mL of 99% ethanol, and the solution was mixed overnight at 37°C. Fifty microliters or 3.2 mL of the poly-HEMA stock solution was added to 96-well plates and 10 cm dishes, respectively, in the tissue culture hood, and plates and dishes were swirled using a plate rotator for 10 minutes. Plates were left to dry overnight and then washed with PBS immediately before use.
2. Hydrogel casting: from: F. C. Valdoz et al., "An Improved Scalable Hydrogel Dish for Spheroid Culture," Life, vol. 11, no. 6, p. 517, Jun. 2021, doi: 10.3390/life11060517.
   a. Molecular biology grade agarose powder (Fisher Scientific, Hampton, NH, USA) was dissolved in the corresponding volume of DMEM/F12 50:50 mix (Difco, Detroit, MI, USA) depending on the desired concentration and then boiled using a standard microwave oven until dissolved. Inside a biosafety cabinet, boiled agarose solution was poured over the silicone mold and left to solidify for 10 to 15 min at room temperature. Once solidified, hydrogel plates were transferred into appropriate TC dishes. The hydrogel dishes were then UV irradiated for at least 30 min before use. For storage, approximately 3 mL of basic culture media (i.e., serum-free DMEM/F12) was added to each TC dish, and each dish was sealed with Parafilm to maintain hydrogel hydration. The hydrogel plates were stored in a 4 °C fridge for up to a maximum of 2 weeks.

Spheroid Formation Protocol:

Download

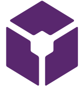
Spheroid_Formation_Protocols_-_Master_Document.pdf (267 kB)

Emily Rhine - Nov 11, 2024, 1:42 PM CST

Conclusions & Action Items:

- Present findings to classmates and advisor at show and tell
- Narrow down to only feasible protocols
  - Discuss at next team/client meeting
- Generate materials list from best protocols
  - Order materials ASAP

Loading [MathJax]/extensions/Safe.js



Spheroid Protocols Matrix_11/2/2024

Emily Rhine - Nov 02, 2024, 6:11 PM CDT

Title: Protocols Matrix Spheroids

Date:11/2/2024

Content by: Emily

Content:

- https://docs.google.com/document/d/1V0KqotLmFkym9m775Kiu9r5dyKeXrehgmuUmdLO44a0/edit?tab=t.0
- https://docs.google.com/spreadsheets/d/1S-YuXcOUBqRxixd6mI4gAO7uC6ZT2_2mvwn8vR7HT0M/edit?gid=0#gid=0

Emily Rhine - Nov 02, 2024, 6:10 PM CDT

Design matrix for Spheroid Protocols

Design Criteria (weight)	1. K. Han et al.		2. F. R. Fernandes et al.		3. N. Abu-[illegible] et al.		4. J. C. [illegible] et al.	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
Cost (25)	medium	20/25	low	25/25	high	N/A	medium	N/A
Time* (25)	Overnight*	25/25	24 hours	20/25	~2–10 days	N/A	Mold = 1 day Hydrogel ~2 hours	N/A
Scalability (20) [illegible]	[illegible]	20/20	[illegible]	20/20	[illegible]	N/A	[illegible]	N/A
Complexity (15) steps#	3 Main steps	15/15	3 Main steps	15/15	high	N/A	high	N/A
Required machinery & materials (15)	[illegible]	15/15	[illegible]	15/15	[illegible]	N/A	[illegible]	N/A
Total Score (100)	85		85					

*time does not include the plate preparation using PolyHEMA. [illegible]

Download

Protocols_Matrix.pdf (72.8 kB)

Emily Rhine - Nov 02, 2024, 6:10 PM CDT

Conclusions/Action Items:

- Meet with team to discuss this first materials draft
- Use materials list to draft a order sheet to send the client
- Order materials by 11/5 at the latest!!
- Consider other protocols if needed
  - Poly-HEMA plate coating:
    - C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.
    - “In vitro differentiation of human embryonic stem cells to hemogenic endothelium and blood progenitors via embryoid body formation.” Accessed: Oct. 29, 2024. [Online]. Available: https://star-protocols.cell.com/protocols/499
  - Other spheroid protocols:
    - T. Stiff, S. Bayraktar, P. Dama, J. Stebbing, and L. Castellano, “CRISPR screens in 3D tumourspheres identified miR-4787-3p as a transcriptional start site miRNA essential for

Loading [MathJax]/extensions/Safe.js

breast tumour-initiating cell growth," Commun. Biol., vol. 7, no. 1, pp. 1–11, Jul. 2024, doi: 10.1038/s42003-024-06555-1.

- L. Carroll, B. Tiwari, J. Curtin, and J. Wanigasekara, "U-251MG Spheroid generation using low attachment plate method protocol," May 2021, Accessed: Oct. 29, 2024. [Online]. Available: https://www.protocols.io/view/u-251mg-spheroid-generation-using-low-attachment-p-bszmnf46
- "Corning® Spheroid Microplates - Spheroid Formation Protocol." Accessed: Oct. 29, 2024. [Online]. Available: https://www.corning.com/catalog/cls/documents/protocols/CLS-AN-308.pdf
- Y. T. Phung, D. Barbone, V. C. Broaddus, and M. Ho, "Rapid Generation of In Vitro Multicellular Spheroids for the Study of Monoclonal Antibody Therapy," Journal of Cancer, vol. 2, p. 507, Oct. 2011, doi: 10.7150/jca.2.507.

Loading [MathJax]/extensions/Safe.js

Draft 1_Final Protocols_11/4/2024

Title: Team Meeting Finalize Protocol
Date: 11/4/2024
Present: Entire team
Content By: Althys
Content:

See: Protocols Matrix

Timeline:
- Week of 11.4: finalize stuff to order, then order stuff
  - Can we do SOX2 (SOX2 paper already has primer sequence and protocols)
  - If Carley doesn't want to order new primers - maybe GAPDH
- Week of 11.11: thaw new "batch" of A549 and start doing passaging, start doing PolyHEMA plates
- Week of 11.18: start doing spheroid protocols, take pictures (cannot do fluorescence tag), start poster
  - Will not have time to tweak different concentrations of methylcellulose, we will change cell densities - make the differences obvious (ie log10)
- Week of 11.25: Thanksgiving! Tentatively meet on Monday to work on the poster if the team agrees (will NOT meet anymore this week afterwards - take a break!)

Protocol:

- Each well in a U-bottom 96-well plate is 0.36 cm^2
  - In Han's protocol: 500uL/cm^2 → will need 180uL/well (max volume per well: 300 uL)
  - Han's protocol: 20,000 cells/cm^2 → 150,000 cells/cm^2 to find peak 30% cell death

	Case 1	Case 2	Case 3	Case 4	X
Cell density (cells/cm^2)	25,000	50,000	75,000	150,000	X
# of cells in one well in a 96-well plate (0.36cm^2/well)	9,000 cells	18,000 cells	27,000 cells	54,000 cells	X
Cell suspension density (180uL / well)	50 cells/µL	100 cells/µL	150 cells/µL	300 cells/µL	X
6 trials - Total number of cells	54,000 cells	108,000 cells	162,000 cells	324,000 cells	Total # of cells needed = 648,000 cells

- Total number of RPMI 1640 media needed for (6 trials/case * 4 cases) = 24 cases: 4320 µL
- Amount of methylcellulose needed (0.75% w/v): 32.4 µg

PolyHEMA Coating Protocol

A total 1.3 g of poly-HEMA (Sigma-Aldrich Co.) was dissolved in 33 mL of 99% ethanol, and the solution was mixed overnight at 37°C. Fifty microliters or 3.2 mL of the 96-well plates and 10 cm dishes, respectively, in the tissue culture hood, and plates and dishes were swirled using a plate rotator for 10 minutes. Plates were left to dry over immediately before use.

Spheroid Formation Protocol

- Make 2% w/v methylcellulose stock
  - Methyl Cellulose (2% (w/v) in $diH_2 0$) – Sigma Aldrich, Cat: M0512
    - Add 2g of Methylcellulose to 100 ml of $diH_2 0$ and mix the solution at 80ºC on a hot plate with a stir bar until the solution is homogeneous. Subseque solution using the Liquid 2 or "L2" setting on the autoclave to sterilize. Following this, move the solution to the walk-in fridge where the solution wi You can store the 2% Methylcellulose solution at 4ºC for up to a year.
- To make 10 mL of RPMI 1640 0.75% methylcellulose (for spheroid formation), mix 6.25 mL of RPMI 1640 and 3.75 mL of 2% w/v methylcellulose stock.

Find % cell death to determine spheroid viability:

- 3D spheroids were then split every 3~4 days.
- Spheroids were then centrifuged at 800g for 15 min and media/PBS was removed from the spheroid pellets.
- Accutase (Innovative Cell Technologies, #AT104) was added to the pellets to dissociate the spheroids into single cells. We used 10 ml of accutase per 100 million c
  for about 30 min until spheroids were fully dissociated into single cells.

Materials ordering sheet

Items	Purpose	Amount	Vendor	Link
U-bottom 24-well plate	Standard plates used per Han et al protocol	1	ThermoFischer	https://www.thermofisher.com/orde
PolyHEMA (P3932) (10g)	For coating the plates	1	SigmaAldrich	https://www.sigmaaldrich.com/US/
RPMI 1640 growth medium (500ml) (SKU R8758-500ML)	Media used in Han *et al* protocol to form spheroids	1	SigmaAldrich	https://tinyurl.com/5n82r5rs
Methylcellulose (100g)	For spheroid formation	1	SigmaAldrich	https://www.sigmaaldrich.com/US/ srsltid=AfmBOopOye1Rkb7P8Hk eRtIZ_pKEkSdXPykT0SFDa
Primer AGCTACAGCATGATGCAGGA (25 nmole)	Forward SOX2 primer sequence for qPCR (Ref: https://pmc.ncbi.nlm.nih.gov/articles/PMC6163012/#SD2-ott-11-6197)	1	Integrated DNA Technologies	https://www.idtdna.com/pages/proc oligos/custom-dna-oligos
Primer GGTCATGGAGTTGTACTGCA (25 nmole)	Reverse SOX2 primer sequence for qPCR (Ref: https://pmc.ncbi.nlm.nih.gov/articles/PMC6163012/#SD2-ott-11-6197)	1	Integrated DNA Technologies	https://www.idtdna.com/pages/proc oligos/custom-dna-oligos
Accutase (100 mL)	For spheroid dissociation to find % cell death, gentler than Trypsin	1	ThermoFischer	https://www.thermofisher.com/orde
Total	X	X	X	X

Title: Team Meeting Finalize Protocol
Date: 11/4/2024
Present: Entire team
Content By: Althys
Content:

See: Protocols Matrix

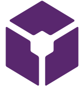
Timeline:
- Week of 11.4: finalize stuff to order, then order stuff
  - Can we do SOX2 (SOX2 paper already has primer sequence and protocols)
  - If Carley doesn't want to order new primers - maybe GAPDH
- Week of 11.11: thaw new "batch" of A549 and start doing passaging, start doing PolyHEMA plates
- Week of 11.18: start doing spheroid protocols, take pictures (cannot do fluorescence tag), start poster
  - Will not have time to tweak different concentrations of methylcellulose, we will change cell densities - make the differences obvious (ie log10)
- Week of 11.25: Thanksgiving! Tentatively meet on Monday to work on the poster if the team agrees (will NOT meet anymore this week afterwards - take a break!)

Protocol:
- Each well in a U-bottom 96-well plate is 0.36 cm^2
  - In Han's protocol: 500uL/cm^2 → will need 180uL/well (max volume per well: 300 uL)
  - Han's protocol: 20,000 cells/cm^2 → 150,000 cells/cm^2 to find peak 30% cell death

	Case 1	Case 2	Case 3	Case 4	X
Cell density (cells/cm^2)	25,000	50,000	75,000	150,000	X
# of cells in one well in a 96-well plate (0.36cm^2/well)	9,000 cells	18,000 cells	27,000 cells	54,000 cells	X
Cell suspension density	50 cells/µL	100 cells/µL	150 cells/µL	300 cells/µL	X

Download

Team_Meeting_Finalize_Protocol_11_04_2024.pdf (137 kB)



Cell Seeding/Spheroid Formation Protocol_11/18/2024

Althys Cao - Dec 17, 2024, 9:18 PM CST

Most recent protocol

Althys Cao - Dec 17, 2024, 9:17 PM CST

Title: Cell Seeding/Spheroid Formation Protocol

Date: 12/15/2024

Goals: Establish cell seeding protocol for spheroid formation.

Content By: Althys

Content:

NOTE: THIS PROTOCOL IS USED TO SEED CELLS IN A 96-WELL FLAT-BOTTOM PLATE. This protocol is currently the most full-fledged and can be used to seed at 4 different densities: 25k, 50k, 75k, 150k cells/cm^2.

1. Collect cells from that day's passage and obtain cell concentration using the CytoFLEX to obtain cell concentration (cells/mL).
2. Obtain a 1.5 mL cell solution of 500,000 cells/mL (750,000 cells in total)
    a. Volume needed to obtain 750,000 cells: 750,000 (cells) / cell concentration from CytoFLEX (Step 1, cells/mL)
    b. Obtain the volume calculated from the T75 flask and transfer into a 15 mL conical tube.
    c. Spin down the the tube at 200g for 5 minutes, then remove supernatant to collect cells using vacuum filter
    d. Resuspend cells in 1.5 mL of serum-free DMEM.
3. Prepare master mix of cell solutions for spheroid formation for 4 different densities. The master mix can be used for 6 wells in a 96-well plate and can be scaled to adjust for different numbers of wells if needed.
    a. Density 1: 25,000 cells/cm^2
        i. Into a new 1.5 mL eppendorf tube, add:
            1. 100 µL of 500,000 cells/mL cell solution (Step 2)
            2. 525 µL of serum-free DMEM
            3. 375 µL of 2% methylcellulose stock
        ii. Mix gently by pipetting up and down, make sure the solution is well-mixed
    b. Density 2: 50,000 cells/cm^2
        i. Into a new 1.5 mL eppendorf tube, add:
            1. 200 µL of 500,000 cells/mL cell solution (Step 2)
            2. 425 µL of serum-free DMEM
            3. 375 µL of 2% methylcellulose stock
        ii. Mix gently by pipetting up and down, make sure the solution is well-mixed
    c. Density 3: 75,000 cells/cm^2
        i. Into a new 1.5 mL eppendorf tube, add:
            1. 300 µL of 500,000 cells/mL cell solution (Step 2)
            2. 325 µL of serum-free DMEM
            3. 375 µL of 2% methylcellulose stock
        ii. Mix gently by pipetting up and down, make sure the solution is well-mixed
    d. Density 4: 150,000 cells/cm^2
        i. Into a new 1.5 mL eppendorf tube, add:
            1. 600 µL of 500,000 cells/mL cell solution (Step 2)

1. [illegible] cell solution (Step 2)
2. 25 µL of serum-free DMEM
3. 375 µL of 2% methylcellulose stock

ii. Mix gently by pipetting up and down, make sure the solution is well-mixed

4. Aliquot the prepared master mix into each well: add 160 µL of cell mixture into each well.
5. Grow spheroids in 37C, 5% CO2 incubator. Spheroids are split every 3-4 days.

References

[1] K. Han *et al.*, “CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities,” *Nature*, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

Action items:

- This protocol will be used next semester to seed cells at different densities. The biggest updated in this protocol from the previous version (used for trial 2, 11/22/2024, see below) is that all full DMEM will be removed so serum-free DMEM is used instead. This makes sure that there is no trace of FBS in the cellular media that can interfere with the spheroid formation process.
- This protocol will be rewritten for varying methylcellulose concentration.
- This protocol will be fully finalized once the optimal cell seeding density and methylcellulose concentration are found.

Althys Cao - Dec 17, 2024, 9:13 PM CST

Older versions of the protocol used for this semester + Notes

Emily Rhine - Nov 25, 2024, 11:22 AM CST

Title: Cell Seeding/Spheroid Formation Protocol

Date: 11/28/2024

Goals: Establish cell seeding protocol for spheroid formation.

Content By: Althys

Content:

NOTE: THIS PROTOCOL IS USED TO SEED CELLS IN A 96-WELL FLAT-BOTTOM PLATE. SEE FULL PROTOCOL AT THE END

- Diameter of one well of a flat-bottom 96-well plate: 6.35 mm → Surface area: 0.32 cm2
- Each well in a **flat-bottom 96-well plate** is 0.32 cm^2
- In Han’s protocol: 500uL/cm^2 → will need 160 µL/well (max volume per well: 300 uL)
- Han’s protocol: 20,000 cells/cm^2 → 150,000 cells/cm^2 to find peak 30% cell death
- 160 µL of total media (0.75% methylcellulose + cells) PER WELL:
- x µL of 2% methylcellulose stock:
- x µL * 2% = 160 µL * 0.75% → x = 60 µL of 2% methylcellulose stock
- Add additional 100 µL of cell media to reach 160 µL of total media and desired cellular concentration.

Emily Rhine - Nov 25, 2024, 11:22 AM CST

Table 1 - Number of Cells per Well

	A	B	C	D	E	F
1		Density 1	Density 2	Density 3	Density 4	X
2	Final cell density (cells/cm2)	25000	50000	75000	150000	X
3	# of cells in one well in a 96-well plate	8,000 cells	16,000 cells	24,000 cells	48,000 cells	X
4	Cell concentration at each well (cells/µL) (160 µL per well)	50 cells/µL	100 cells/µL	150 cells/µL	300 cells/µL	X
5	Volume of 500,000 cells/mL needed (µL)	16 µL	32 µL	48 µL	96 µL	X
6	6 trials - Total number of cells	48,000 cells	96,000 cells	144,000 cells	288,000 cells	Total # of cells needed = 576,000 cells

Table 2 - Relevant Protocol Values

	A	B	C	D	E	F
1		Cellular Concentration (cells/mL)	Volume needed from passage to get a total of 750,000 cells (mL)	Do we need to add extra serum-free DMEM to reach final volume of 1.5 mL? If yes, how much mL should be added?	Do we need to spin down cells and resuspend in 1.5 mL of serum-free DMEM?	
2		**ADD CELLULAR CONCENTRATION FROM CYTOFLEX (CELLS/ML), NOT TOTAL NUMBER OF CELLS**	#VALUE!	#VALUE!	#VALUE!	
3	11/19/2024	~~3350000~~	~~0.223880597~~	~~1.276119403~~	NO	**NOTE: Put in incorrect value (put in total number of cells instead of cell concentration), scrap this experiment**
4	11/22/2024	298500	2.5125628141	NO	YES	

Emily Rhine - Nov 25, 2024, 11:27 AM CST

6 wells for each concentration → 960 µL of total media is needed for each concentration, round up to **1000 µL of total media (methylcellulose + cells) for each concentration** to account for pipetting error

- Amount of 2% methylcellulose stock: x µL * 2% = 1000 µL * 0.75% → x = **375 µL of 2% methylcellulose stock**
- Amount of 500,000 cells/mL (or 500 cells/µL) solution needed: cell concentration of a well (Table 1, row 4) * 1000 µL / (500 cells/µL). Amount of extra serum-free DMEM needed = 1000 µL - amount of 500,000 cells/mL solution - amount of 2% methylcellulose solution
- **Density 1: 25,000 cells/cm^2:**
    - 50 cells/µL * 1000 µL / (500 cells/µL) = **100 µL of 500,000 cells/mL**

    - 1000 µL - 100 µL - 375 µL = **525 µL of serum-free DMEM**
- **Density 2: 50,000 cells/cm^2**
    - 100 cells/µL * 1000 µL / (500 cells/µL) = **200 µL of 500,000 cells/mL**
    - 1000 µL - 200 µL - 375 µL = **425 µL of serum-free DMEM**
- **Density 3: 75,000 cells/cm^2**
    - 150 cells/µL * 1000 µL / (500 cells/µL) = **300 µL of 500,000 cells/mL**
    - 1000 µL - 300 µL - 375 µL = **325 µL of serum-free DMEM**
- **Density 4: 150,000 cells/cm^2**
    - 300 cells/µL * 1000 µL / (500 cells/µL) = **600 µL of 500,000 cells/mL**
    - 1000 µL - 600 µL - 375 µL = **25 µL of serum-free DMEM**

FULL PROTOCOL:

1) Collect cells from that day's passage, run Cytoflex to obtain cell concentration, **write in CELL CONCENTRATION (# OF CELLS/ML), NOT TOTAL NUMBER OF CELLS in *Table 2 - Relevant Protocol Values, Column B***
2) Obtain 1.5 mL cell solution of 500,000 cells/mL (750,000 cells in total)
3)Volume needed to get from the passage to obtain a total of 750,000 cells in total is in Column C.
4)Column C = 750,000 / Cellular Concentration (A2)
4)If Column C value is less than 1.5 mL: "Extract" Column C volume of cells (mL) from the T75 flask into a 15 mL conical tube (1.5 mL eppendorf should be sufficient too).
5)Add C2 value of serum-free DMEM (mL) to tube.
6)If Column C value is more than 1.5 mL:
"Extract" Column C volume of cells (mL) from the T75 flask into a 15 mL conical tube
7)Spin down tube at 200 x g for 5 min, remove supernatant using vacuum filter
8)Resuspend pellet in 1.5 mL of serum-free DMEM, pipette up and down to mix the cell mixture
9)If Column C value is equal to 1.5 mL:
Lucky you, just need to "extract" Column C volume of cells (mL) from the T75 flask into a 15 mL conical tube
10)Prepare full spheroid formation cell solutions for concentrations 1-4. Aliquot into each well.
- Density 1: 25,000 cells/cm^2: Into a new 1.5 mL eppendorf tube, add 100 µL of 500,000 cells/mL cell solution + 525 µL of serum free DMEM + 375 µL of 2% methylcellulose stock. Mix gently by pipetting up and down, make sure the solution is well-mixed. Add 160 µL of cell mixture into each well
- Density 2: 50,000 cells/cm^2: Into a new 1.5 mL eppendorf tube, add 200 µL of 500,000 cells/mL cell solution + 425 µL of serum free DMEM + 375 µL of 2% methylcellulose stock. Mix gently by pipetting up and down, make sure the solution is well-mixed. Add 160 µL of cell mixture into each well
- Density 3: 75,000 cells/cm^2: Into a new 1.5 mL eppendorf tube, add 300 µL of 500,000 cells/mL cell solution + 325 µL of serum free DMEM + 375 µL of 2% methylcellulose stock. Mix gently by pipetting up and down, make sure the solution is well-mixed. Add 160 µL of cell mixture into each well
- Density 4: 150,000 cells/cm^2: Into a new 1.5 mL eppendorf tube, add 600 µL of 500,000 cells/mL cell solution + 25 µL of serum free DMEM + 375 µL of 2% methylcellulose stock. Mix gently by pipetting up and down, make sure the solution is well-mixed. Add 160 µL of cell mixture into each well

11) Grow spheroids in 37C, 5% CO2 incubator. Spheroids are split every 3-4 days

Althys Cao - Dec 17, 2024, 9:12 PM CST

Conclusions/Action Items:

- Trial 1: 11/18/2024
    - In this trial, there were a few things that were miscalculated along the way, including: polyHEMA plates were not left to dry correctly (before seeding, there was still polyHEMA solution left in the wells), full

DMEM was used instead of serum-free DMEM which can prevent spheroid formation, and not enough cells were added.

- Trial 2: 11/22/2024
  - Mistakes from trial 1 were taken into account and fixed --> spheroids formed!
- Updated protocol to make it clearer: 2 times
  - First time: for trial 2 11/22/2024
  - Second time: 12/15/2024
- Added protocol to appendix of final report

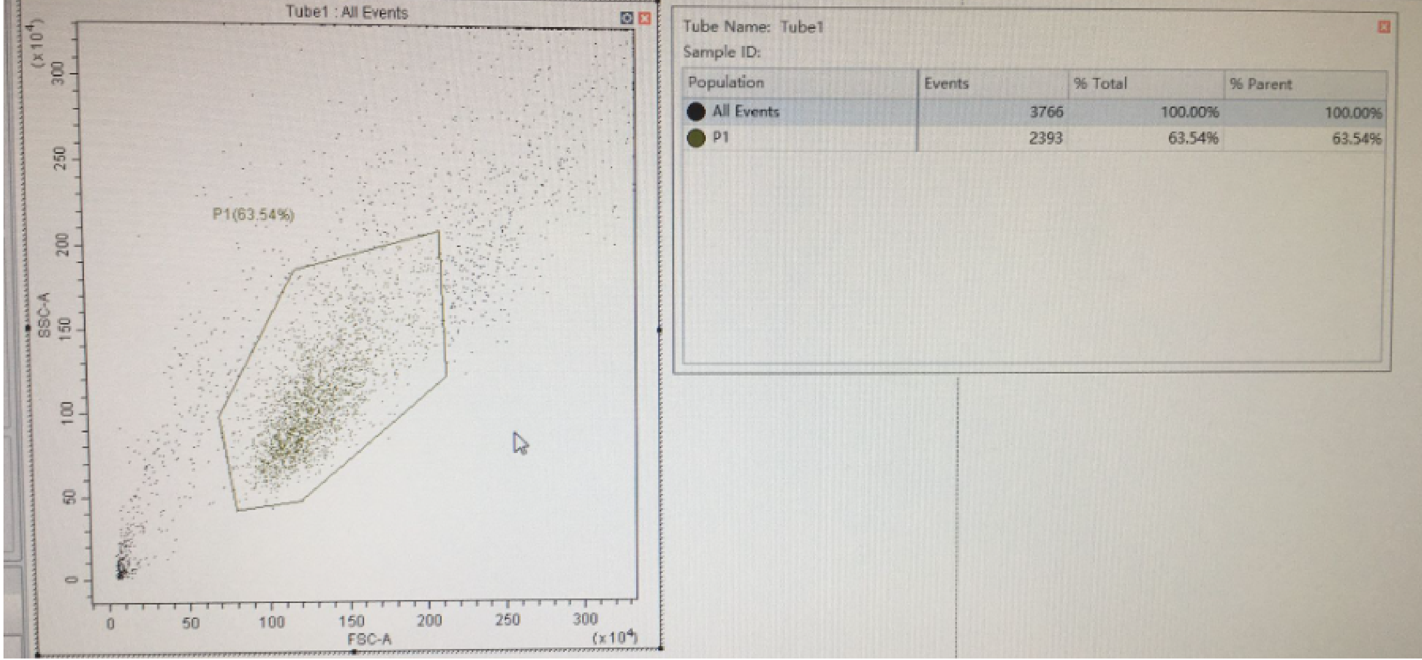
CytoFLEX Protocol_11/21/2024

Emily Rhine - Nov 21, 2024, 9:41 PM CST

Title: CytoFLEX Protocol
Date: 11/21/2024
Content by: Emily
Content:

Once the CytoFLEX its corresponding computer have been turned on, the following steps must be followed:

1. In the top left click Cytometer > Daily clean
    a. Load Blue tube and press run (3 minutes)
    b. Load DI water and press run (1 minute)
2. To create a new experiment click file> new> from template>Browse
    a. Click into BME Team cell counting file on computer drive Documents>CytoFLEX>BME Team
3. Name file after current date and passage number
4. Vortex sample
5. Uncap sample in 1 mL Eppendorf container and place in CytoFLEX
6. Select fast in settings on left side
7. Click record
8. Once a dot plot of the events has been created right click on the plot>properties
    a. Auto set x-axis
    b. Auto set y-axis
9. Right click on graph and select polygon tool
10. Gate the cells in a oblong hexagon fashion trying to bin all relevant events (live cells)
11. Save file
12. Rerun daily clean (step 1)



Conclusions/Action Items:

- See client meeting notes entry 10/11/2024 for original comprehensive protocol
- Add protocol to appendix of Final Report

10/14/2024 WIMR Lab Visit- Cell Passage 1

JULIA SALITA - Oct 14, 2024, 12:05 PM CDT

Title: Cell Passage 1

Date: 10/14/2024

Content by: Julia Salita

Present: Julia Salita and Jayson O'Halloran

Goals: To correctly passage our cells, get a better understanding of how to passage cells, learn to use the required equipment, and understand why each step of passaging cells is important.

Content:

1. See file below for in lab notes
2. Start as normal: look at your cells get, everything ready, then aspirate the current media, use 2mL of PBS to wash the cells GENTLY, aspirate the PBS out, **you're supposed to add 2mL of trypsin but we needed 3mL**, then let sit in the incubator for about 3 minutes, then add 7 mL of D-MEM, and mix up and down with the serological pipet making sure to wash the back of the flask, take about 0.5 mL and put it in a small centrifuge tube and close it, then go to they cytoflex
3. Fill in table: Run the cytoflex and get the # of alive cells (# of events in the shape you make) and multiply by 100. This will give you cells per mL. We got 5309 which means there is 530,900 cells in 1mL which in 10 mL is 5,309,000 cells total in flask.
   1. We were at about 110% confluency (100% = 5,000,000 cells) with 5,309,000 cells.
4. Then we used a doubling time calculator (https://www.cellseeker.org/cellcalc/growth-rate-and-doubling-time/) to calculate that they had a doubling time of 28.2 hr.
   1. We cut back the cells to 1,000,000 cells, so we left a volume of 1.9 mL of the ~10mL solution of the current flask and added back in 8.1 mL of D-MEM for anew total of 10mL.
5. Then record the passage and put the cells back in the incubator.

Conclusions/action items:

1. Passage cells again on Wednesday October 16th.

JULIA SALITA - Oct 14, 2024, 11:48 AM CDT

10/14/24

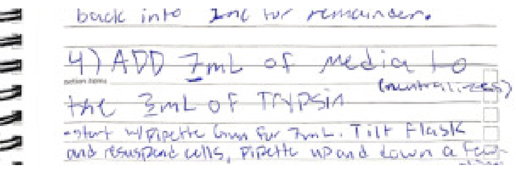
Cell Passage 1

Passage

1) Remove previous media using aspirator. ~~dispose~~ of

2) Add 2mL of PBS (front side) gently using pipette gun, back side. After, remove using aspirator (Put tips in bleach)

3) Add 2mL of Trypsin to flask, back side to dissociate. Incubate for 5 min, after 2-3 lightly shake and check to see dissociation. Add 1mL of Trypsin if not fully diss. Put


back into 1mL for remainder.
4) ADD 7mL of media to the 3mL of TRYPSIN (neutralizes)
- start w/ pipette Gun for 7mL. Tilt Flask and resuspend cells, pipette up and down a few

Download

CamScanner_10-14-2024_.pdf (1.19 MB)

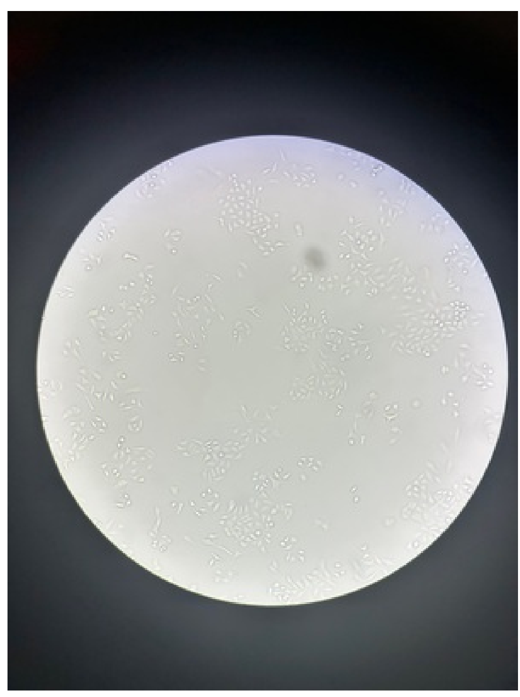
A549 Photos_80% Confluency_10/18/2024

Emily Rhine - Oct 21, 2024, 11:38 AM CDT

A549 Confluency Tracker

	Date	Cytoflex Confluency	Cells per mL	Total cells in solution	Doubling time	Cut Back To
1	10/11/24	N/A	N/A	1000000	Thaw	
2	10/14/24	5309	530900	5309000	28.2	1000000
3	10/16/24	5094	509400	5094000	20.4	750000
4	10/18/24	1956	195600	1956000	36.2	1200000

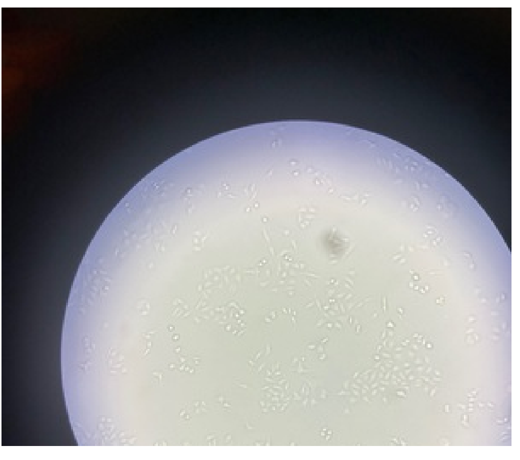
Emily Rhine - Oct 21, 2024, 11:30 AM CDT

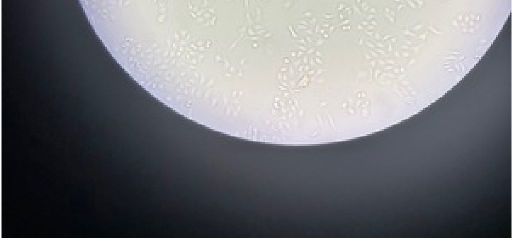


Download

62316050-A3B2-431D-8994-97DBA925F49A.jpg (276 kB)

Emily Rhine - Oct 21, 2024, 11:31 AM CDT





Download

F2597C76-2819-4C29-9843-A57D51ACEAC4.jpg (317 kB)

Emily Rhine - Oct 21, 2024, 11:31 AM CDT

Download

5BA984BF-67FC-49F5-8605-5A0BC2B772E4.jpg (303 kB)

Benchling Data Update_11/11/2024

Emily Rhine - Nov 11, 2024, 1:48 PM CST

Title: Benchling Data Update

Date: 11/11/2024

Content By: Entire Team

Content:

See notes below

https://benchling.com/uw_hesslab/f/lib_tD5E8G7L-cell-line-maintenance/etr_5Skd74TL-a549-cell-line-maintenance/edit

Emily Rhine - Nov 11, 2024, 1:47 PM CST

- The A549 cell line is a human alveolar basal epithelial cell line
  - They are adherent, squamous, and have an epithelial like apearance and are a larger cell line so they take up more volume
  - They are a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells
- Media: DMEM + 10% FBS + P/S
- The doubling time ranges between 20-40 hours (we will need to keep track of this)
- They are good for 20 passages

If they double between 20-28 hours:

- You will do a Monday, Wednesday, Friday passaging schedule
  - How this works: on Monday your cells are at confluency in 10 mL of media, the next time you will be in lab is Wednesday so you need to cut your cells back. Between Monday and Wednesday your cells will double twice (x2 x2). Thus, you need to cut them back to a point where by Wednesday (48 hours later) they won't be overgrown but won't be too low either. After going through the process of PBS, trypisin, and resuspending the cells in media to get a volume of 10 mL you will keep 2 mL of this volume and bleach the remaining 8 mL. The way this works is if you have roughly 1 million cells in 1 mL of media and you keep 2 mL you're saving 1 million cells, this will double to 2, and then double again to 4 million which roughly puts you at 80% confluency. On Friday though you won't be in till Monday so you will need to save only 1 mL of cell solution volume.
    - This math is not perfect and passaging does not need to be perfect, but for the health of your cells it is important to be roughly on track. This is will also allow you to begin to recognize how well or poorly your cells are growing.
    - To start, you will use the cytoflex to count the cells everytime to determine how many you have and how much you need to cut them back by based on the average growing rate

Loading [MathJax]/extensions/Safe.js

T75 Example Cell Values

	Flask	Surface Area	Seeding Density (million)	Cells at Confluency (million)	PBS Volume	Trypsin Volume	Resuspension Volume	Final Volume
1	T-75	75	1	5	2	3mL	7 mL	10
2			On the cytoflex 100% confluency with this cell line would be .5 million cells per mL			You may need 3 since these cells can be a bit more adherent than others	8 (needs to be x3 the amount of trypisin to neutralize it)	

A549 Confluency Tracker

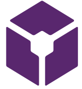
	Date	Passage Number	Cytoflex Confluency	Cells per mL	Total cells in solution	Doubling time	Cut Back To
1	10/11/24	0 (thaw)	N/A	N/A	1000000	Thaw	
2	10/14/24	1	5309	530900	5309000	28.2	1000000
3	10/16/24	2	5094	509400	5094000	20.4	750000
4	10/18/24	3	1956	195600	1956000	36.2	1200000
5	10/21/2024	4	2393	239300	2393000	72.3	2000000
6	10/23/2024	5	9156	915600	9156000	21.9	1500000
7	10/25/2024	6	3147	314700	3147000	44.9	1750000
8	10/28/2024	7	3708	370800	3708000	54.0	1875000
9	10/30/2024	8	8804	880400	8804000	21.1	1750000
10	11/1/2024	9	4187	418700	4187000	42.9	1650000
11	11/4/2024	10	4506	450600	4506000	46.9	1750000
12	11/6/2024	11	8117	811700	8117000	21.7	1700000
13	11/8/2024	12	4208	420800	4208000	35.7	1650000
14	11/11/2024	13	3305	330500	3305000	68.4	1725000

Emily Rhine - Nov 11, 2024, 1:52 PM CST

Conclusion/Action Items:

- Continue passaging using material amounts listed above
- Thaw new cells before passage 18
- Kill cells after 20 passages

Loading [MathJax]/extensions/Safe.js



Benchling Data Update_12/11/2024

Emily Rhine - Dec 11, 2024, 12:16 AM CST

A549 Confluency Tracker

	Date	Passage Number	Cytoflex Confluency	Cells per mL	Total cells in solution	Doubling time	Cut Back To
1	10/11/24	0 (thaw)	N/A	N/A	1000000	Thaw	
2	10/14/24	1	5309	530900	5309000	28.2	1000000
3	10/16/24	2	5094	509400	5094000	20.4	750000
4	10/18/24	3	1956	195600	1956000	36.2	1200000
5	10/21/2024	4	2393	239300	2393000	72.3	2000000
6	10/23/2024	5	9156	915600	9156000	21.9	1500000
7	10/25/2024	6	3147	314700	3147000	44.9	1750000
8	10/28/2024	7	3708	370800	3708000	54.0	1875000
9	10/30/2024	8	8804	880400	8804000	21.1	1750000
10	11/1/2024	9	4187	418700	4187000	42.9	1650000
11	11/4/2024	10	4506	450600	4506000	46.9	1750000
12	11/6/2024	11	8117	811700	8117000	21.7	1700000
13	11/8/2024	12	4208	420800	4208000	35.7	1650000
14	11/11/2024	13	3305	330500	3305000	68.4	1725000
15	11/13/2024	14	8124	812400	8124000	21.5	1710000
16	11/15/2024	15	4973	497300	4973000	33.1	1650000

Vial 1 of A549s Bleached at passage 15 once viability of Vial 2 cells were confirmed (11/18)

Emily Rhine - Dec 11, 2024, 12:18 AM CST

A549 Confluency Tracker1

	Date	Passage Number	Cytoflex Confluency	Cells per mL	Total cells in solution	Doubling time	Cut Back To	mL to Keep	
1	11/15/2024	0 (thaw)	N/A	N/A	1000000	Thaw	1000000		
2	11/18/2024	1	9296	929600	9296000	22.4	1750000	1.9	
3	11/19/2024	2	3535	353500	3535000	23.7	1750000	5.0	
4	11/20/2024	3	3426	342600	3426000	22.7	1500000	4.4	
5	11/22/2024	4	2985	298500	2985000	50.4	1600000	5.4	
6	11/25/2024	5	3934	393400	3934000	55.5	1700000	4.3	

Vial 2 A529s Bleached after failed experiment and unable to passage cells further due to broken CytoFlex (11/25).

Emily Rhine - Dec 11, 2024, 12:18 AM CST

Loading [MathJax]/extensions/Safe.js

LN2 A549 Storage

	Rack Number	Box Number	Cell Type	Position	Box Name
1	Rack 15	4	A549 WT	52	Hess Stocks 4
2	Rack 15	4	A549 WT	53	Hess Stocks 4
3	Rack 15	4	A549 WT	54	Hess Stocks 4
4	Rack 15	4	A549 WT	55	Hess Stocks 4
5	Rack 15	4	A549 WT	56	Hess Stocks 4
6	Rack 15	4	A549 WT	57	Hess Stocks 4
7	Rack 15	4	A549 WT	58	Hess Stocks 4
8	Rack 15	4	A549 WT	59	Hess Stocks 4

Loading [MathJax]/extensions/Safe.js

BioTek Imaging_11/22/2024

Emily Rhine - Dec 11, 2024, 12:08 AM CST

Title: BioTek Imaging

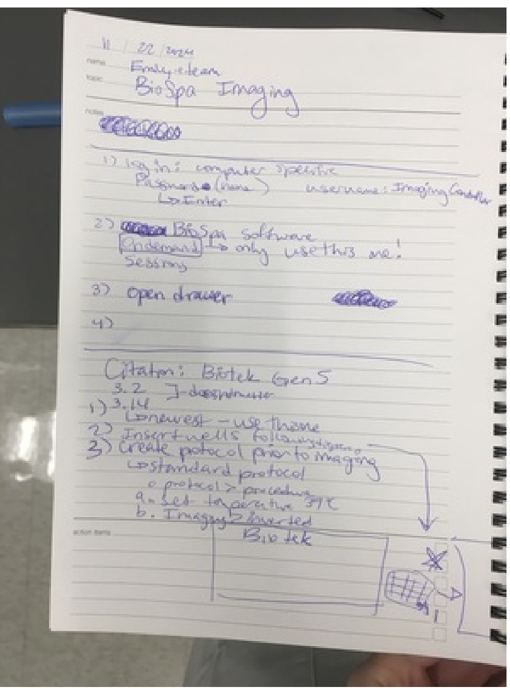
Date: 11/25/2024

Present: Emily, Althys, & Ana

Content:

- Followed steps outlined by Austin in "BioSpa_11/22/2024" Client meeting notes entry
- 5 z-stacks per well 24 wells (6 per condition with 4 conditions)
- See extensive notes below and "Spheroid Images" entry

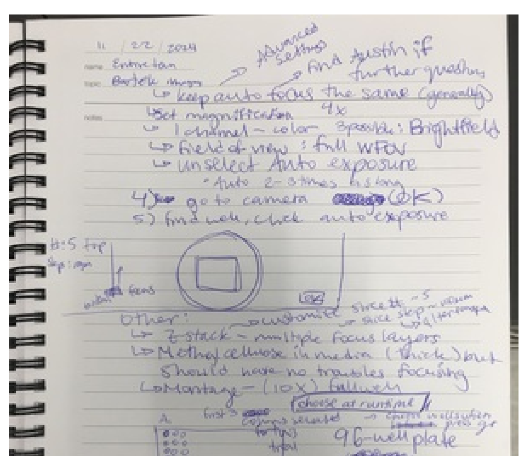
Emily Rhine - Dec 11, 2024, 12:09 AM CST



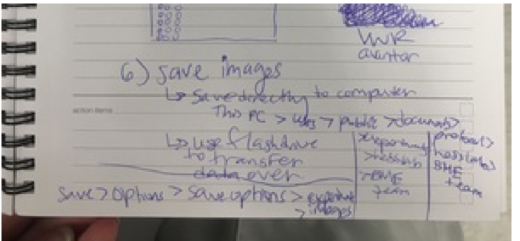
Download

8F06C3B7-1C4D-4B5B-9867-3F1907368F1D.jpg (391 kB)

Emily Rhine - Dec 11, 2024, 12:04 AM CST



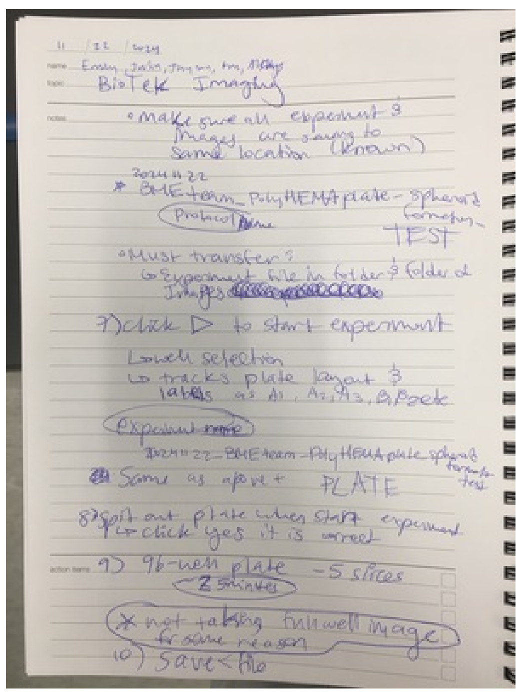
Loading [MathJax]/extensions/Safe.js



Download

501C1DB6-F395-4BC5-A4C0-47567E89CA44.jpg (522 kB)

Emily Rhine - Dec 11, 2024, 12:04 AM CST

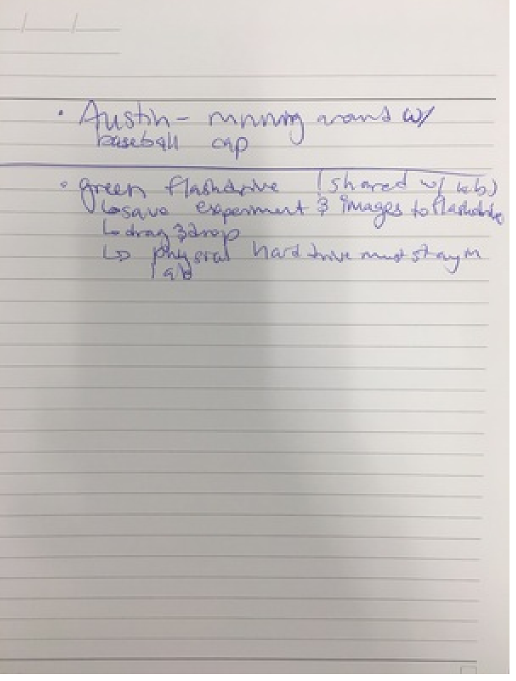


Download

531D1F9C-5F87-4733-888A-3E203608E642.jpg (450 kB)

Emily Rhine - Dec 11, 2024, 12:04 AM CST

Loading [MathJax]/extensions/Safe.js



• Austin - running around w/ baseball cap

• green flashdrive (shared w/ lab)
↳ save experiment & images to flashdrive
↳ drag & drop
↳ physical hard drive must stay in lab

Download

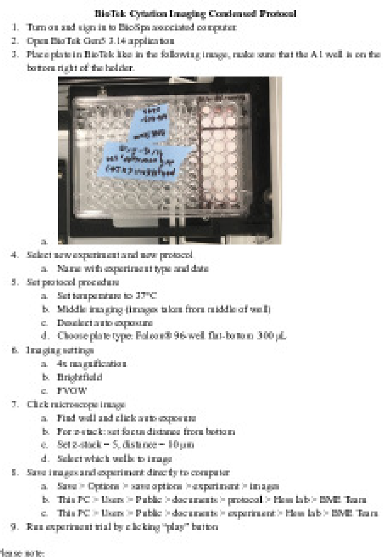
48284C6B-0690-4D0E-9F37-5A491E320843.jpg (302 kB)

Emily Rhine - Dec 11, 2024, 12:11 AM CST

Conclusions/Action Items:

- Flashdrive of images saved from BioTek Cytation to Ana's computer then uploaded into the shared Google Drive
- Image Analysis set to take place later today (11/25) by Julia and Jayson

Emily Rhine - Dec 12, 2024, 5:11 PM CST



BioTek Cytation Imaging Condensed Protocol

1. Turn on and sign in to BioSpa associated computer
2. Open BioTek Gen5 3.14 application
3. Place plate in BioTek like in the following image, make sure that the A1 well is on the bottom right of the holder.
   a.
4. Select new experiment and new protocol
   a. Name with experiment type and date
5. Set protocol procedure
   a. Set temperature to 37°C
   b. Middle imaging (images taken from middle of well)
   c. Deselect auto exposure
   d. Choose plate type: Falcon® 96-well flat-bottom 300 µL
6. Imaging settings
   a. 4x magnification
   b. Brightfield
   c. FVOW
7. Click microscope image
   a. Find well and click auto exposure
   b. For z-stack: set focus distance from bottom
   c. Set z-stack = 5, distance = 10 µm
   d. Select which wells to image
8. Save images and experiment directly to computer
   a. Save > Options > save options > experiment > images
   b. This PC > Users > Public > documents > protocol > Hess lab > BME Team
   c. This PC > Users > Public > documents > experiment > Hess lab > BME Team
9. Run experiment trial by clicking "play" button

Please note:

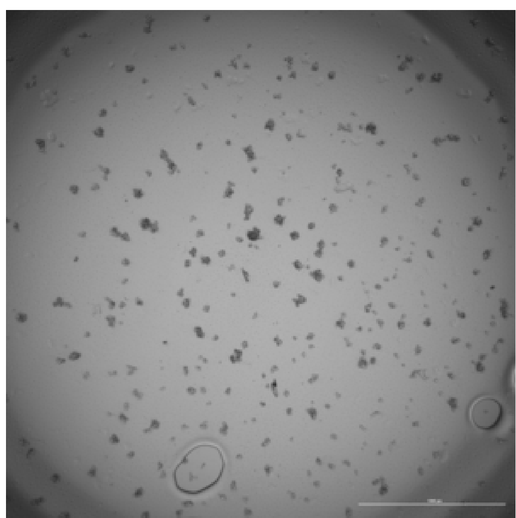
Download

BioTek_Cytation_Imaging_Condensed_Protocol_12_12_2024.pdf (1.57 MB)

Loading [MathJax]/extensions/Safe.js

Spheroid Images_11/22/2024

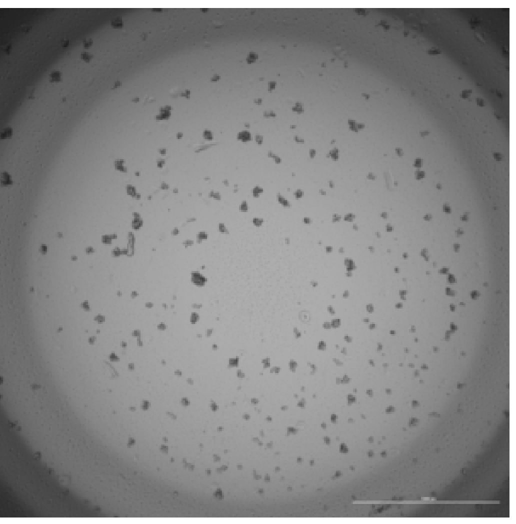
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_A1_1Z3_001.png (3.93 MB)

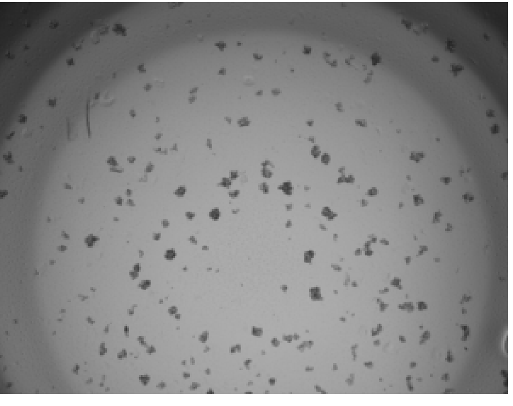
Emily Rhine - Nov 25, 2024, 6:23 PM CST

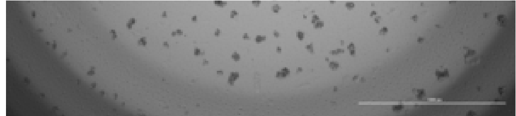


Download

Bright_Field_A2_1Z2_001.png (3.96 MB)

Emily Rhine - Nov 25, 2024, 6:23 PM CST

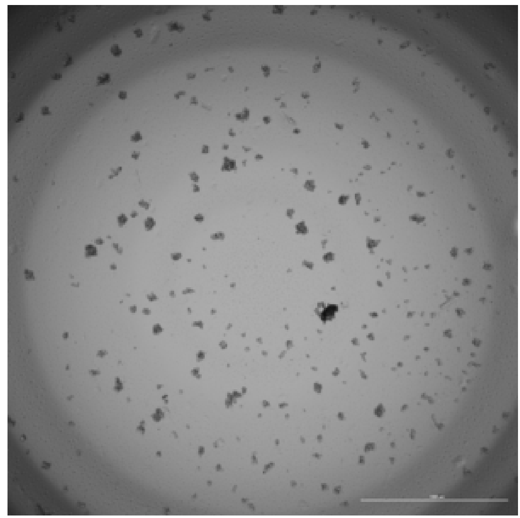




Download

Bright_Field_A3_1Z3_001.png (4.03 MB)

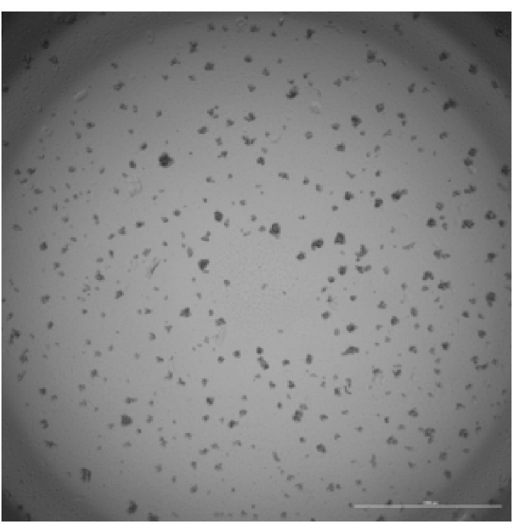
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_B1_1Z2_001.png (3.96 MB)

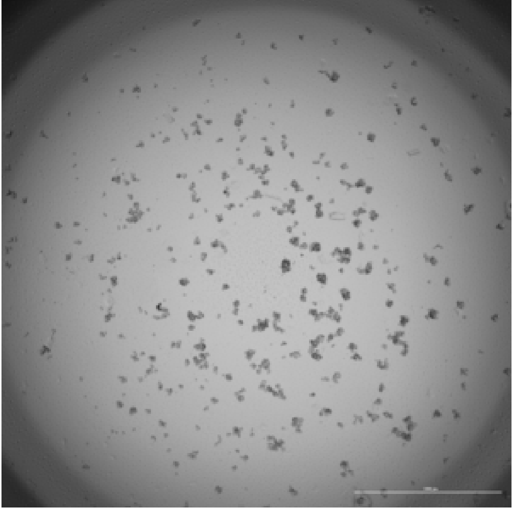
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_B2_1Z2_001.png (4 MB)

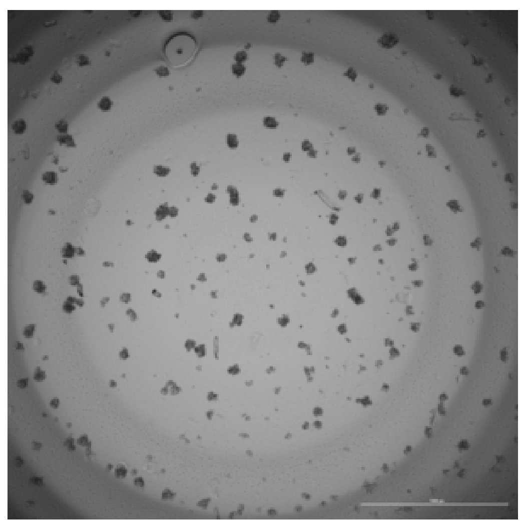
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_B3_1Z2_001.png (4.11 MB)

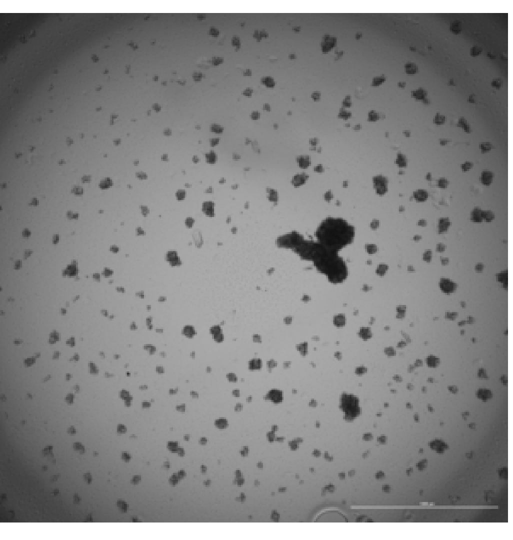
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_C1_1Z2_001.png (4.04 MB)

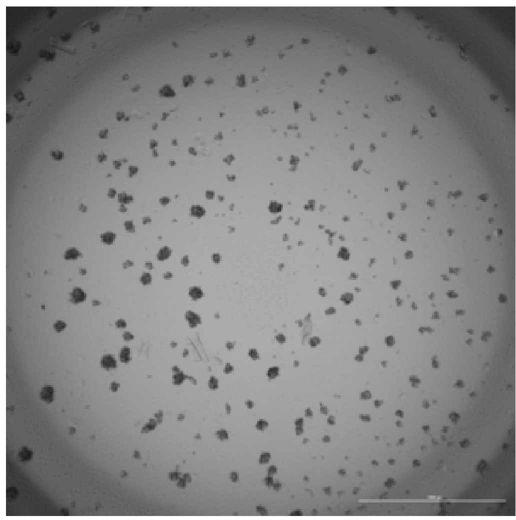
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_C3_1Z2_001.png (4.06 MB)

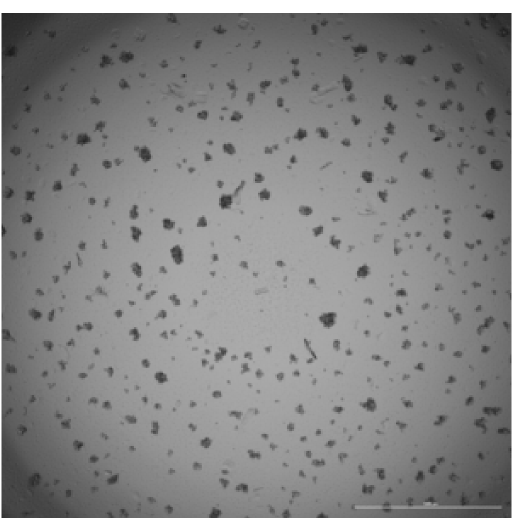
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_D1_1Z2_001.png (4.03 MB)

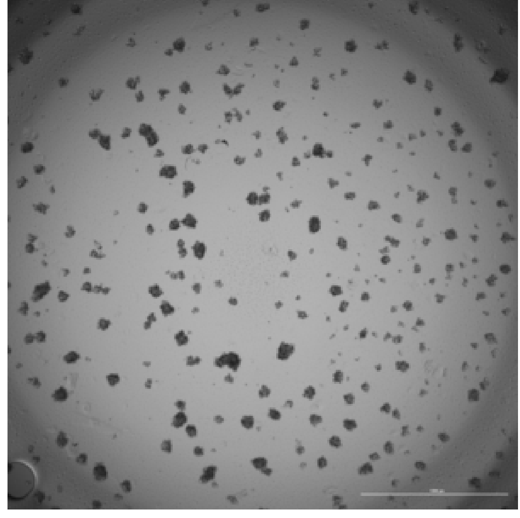
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_D2_1Z2_001.png (4.1 MB)

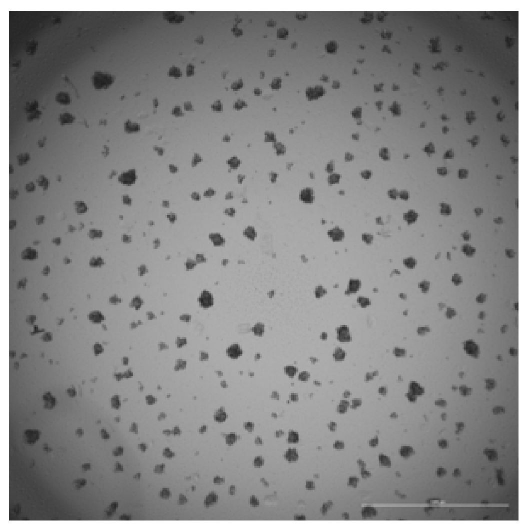
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_E1_1Z2_001.png (4.12 MB)

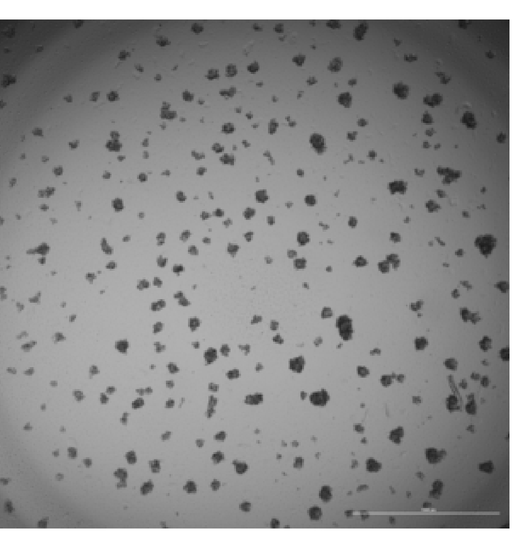
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_E2_1Z2_001.png (4.14 MB)

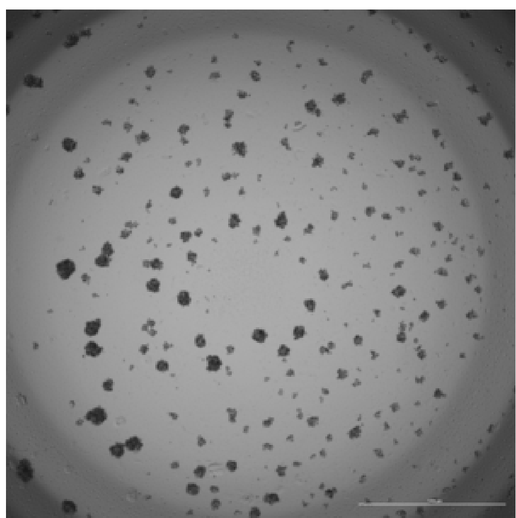
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_E3_1Z2_001.png (4.11 MB)

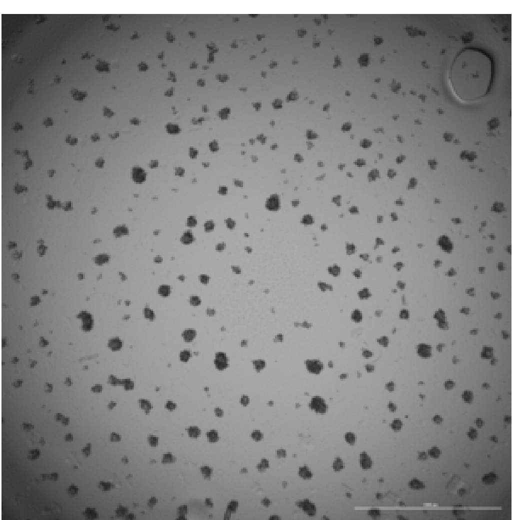
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_F1_1Z3_001.png (4.05 MB)

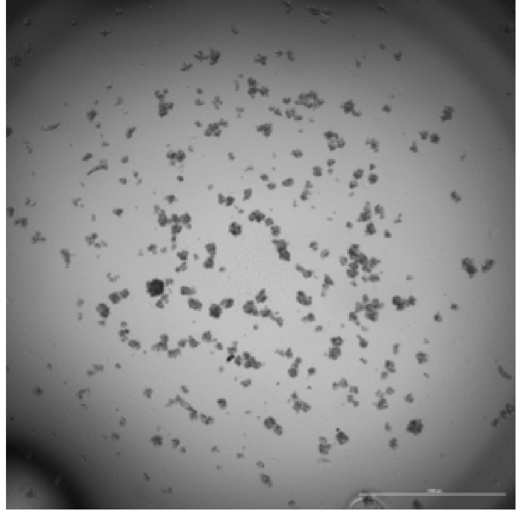
Emily Rhine - Nov 25, 2024, 6:23 PM CST



Download

Bright_Field_F2_1Z2_001.png (4.13 MB)

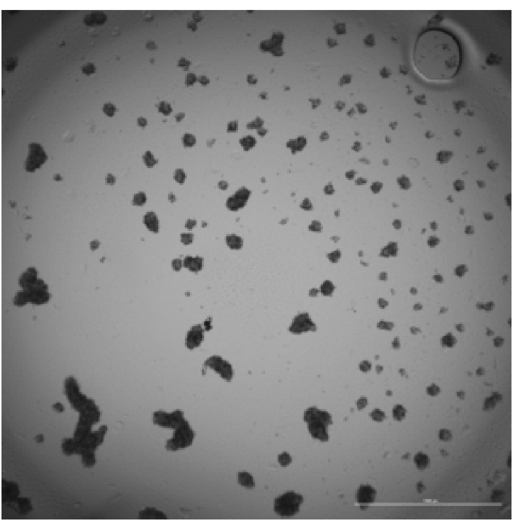
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_F3_1Z2_001.png (4.14 MB)

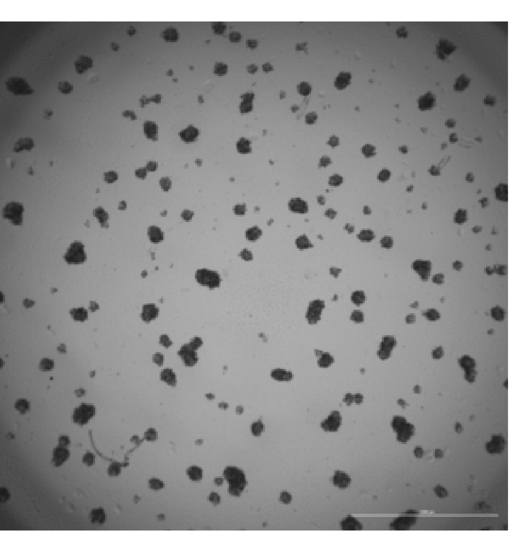
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_G1_1Z2_001.png (4.07 MB)

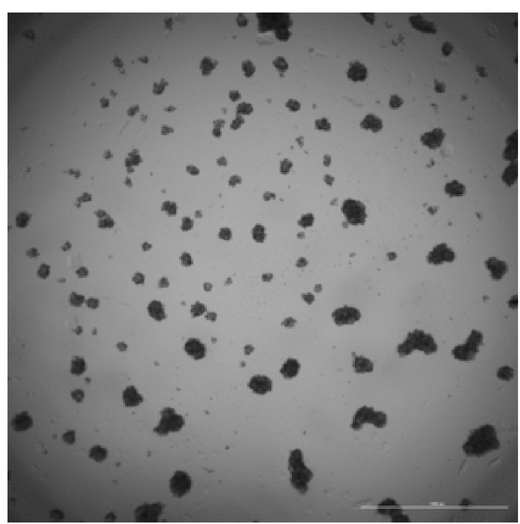
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_G2_1Z2_001.png (4.09 MB)

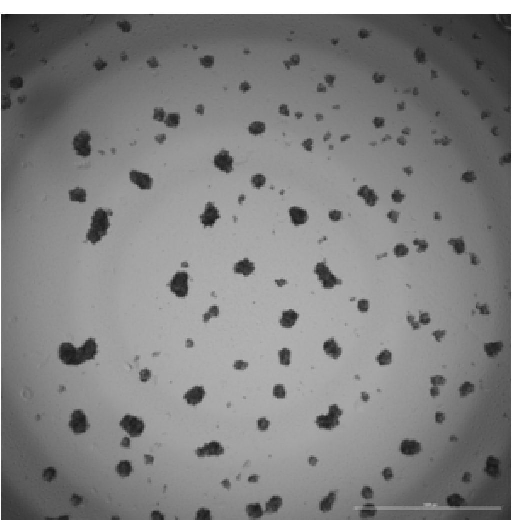
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_G3_1Z2_001.png (4.08 MB)

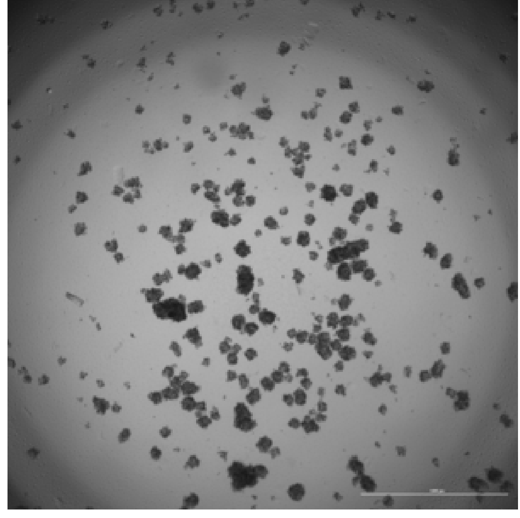
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_H1_1Z3_001.png (4.07 MB)

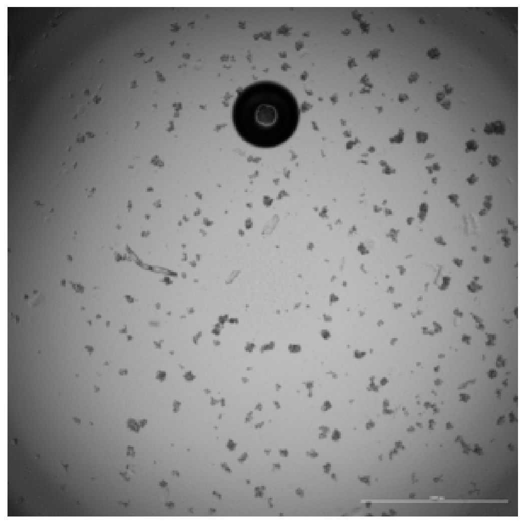
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_H3_1Z3_001.png (4.22 MB)

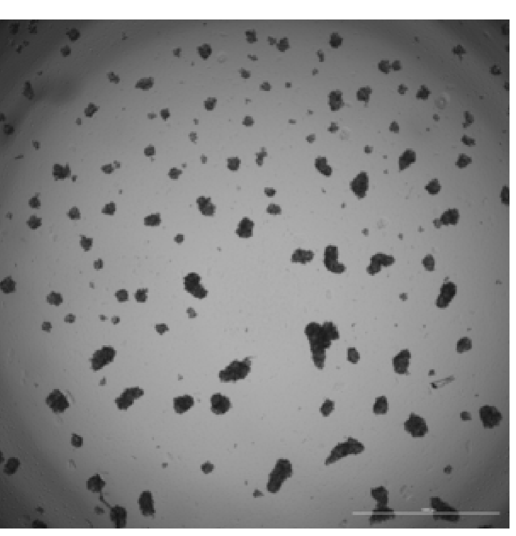
Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_D3_1Z2_001.png (4.12 MB)

Emily Rhine - Nov 25, 2024, 6:24 PM CST



Download

Bright_Field_H2_1Z3_001.png (4.03 MB)

Accutase Dissociation of Spheroids_11/22/2024

Althys Cao - Dec 17, 2024, 9:24 PM CST

Latest protocol

Althys Cao - Dec 17, 2024, 9:27 PM CST

Title: Accutase Dissociation of Spheroids into Single Cells

Date: 12/15/2024

Goals: Establish protocol for spheroid dissociation.

Content By: Althys

Content:

Materials

- Eppendorf tubes
- P200, P1000 pipettes, 15 mL serological pipets
- Accutase
- Full DMEM (DMEM + 10% FBS + 1% Pen/Strep)
- Formed spheroids (see Cell Seeding/Spheroid Formation Protocol entry in Team Activities/Fabrication/Design Process/Spheroid Formation Protocols)

Protocols:

1. Prepare 24 eppendorf 1.5 mL tubes (for dissociation of 24 wells). Label them accordingly.
2. Transfer the media (with cancer spheroids inside) from the wells into the according eppendorf 1.5 mL tubes using a 15 mL serological pipet.
3. To each eppendorf 1.5 mL tube, add 480 µL of PBS.
4. Pellet the spheroids via centrifugation at 800g, 15 min, then remove the supernatant using a pasteur pipet.
5. Resuspend the pellets in 150 µL Accutase using a P200 pipette. Thoroughly mix the solution by pipetting up and down 10 times using a P200 pipette set to 100 µL.
6. Incubate the tubes in 37°C for 10 minutes.
7. Pipette the solution in each tube up and down 10 times using a P200 pipette set to 100 µL.
8. Add 850 µL of DMEM so that the final volume reaches 1 mL.

References:

[1] K. Han *et al.*, “CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities,” *Nature*, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

[2] S. Honeder *et al.*, “Adipose Triglyceride Lipase Loss Promotes a Metabolic Switch in A549 Non–Small Cell Lung Cancer Cell Spheroids,” *Molecular & Cellular Proteomics*, vol. 20, pp. 100095–100095, Jan. 2021, doi: https://doi.org/10.1016/j.mcpro.2021.100095.

Conclusions: This should be the most updated protocol for next semester and there should be no changes to it, unless something did not go according to plan during experiments.

Loading [MathJax]/extensions/Safe.js

Althys Cao - Dec 17, 2024, 9:19 PM CST

Older version of protocol + Notes

Althys Cao - Dec 17, 2024, 9:20 PM CST

Title: Accutase Dissociation of Spheroids

Date: 11/22/2024

Goals: Establish accutase dissociation of spheroids protocol.

Content By: Althys

Content:

Used Han et al protocol:

"Spheroids were then split every 3–4 days. To passage cells, cancer spheroids were collected in methylcellulose media and diluted with PBS (~3 medium volumes) to reduce viscosity of the medium before centrifugation. Spheroids were then centrifuged at 800g for 15 min and medium and PBS was removed from the spheroid pellets. Accutase (Innovative Cell Technologies, no. AT104) was added to the pellets to dissociate the spheroids into single cells. We used 10 ml of accutase per 100 million cells in spheroids and incubated them for about 30 min until spheroids were fully dissociated into single cells."

Protocol:

1. Prepare 24 eppendorf 1.5 mL tubes (for dissociation of 24 wells). Label them accordingly.
2. Transfer the media (with cancer spheroids inside) from the wells into the according eppendorf 1.5 mL tubes using a 15 mL serological pipet.
3. To each eppendorf 1.5 mL tube, add 480 µL of PBS (3x the volume of PBS is added to cellular media, there is 160 µL of media in each well).
4. Pellet the spheroids via centrifugation at 800g, 15 min, then remove the supernatant using a pasteur pipet
5. Resuspend the pellets in Accutase, make sure to thoroughly resuspend in by pipetting up and down
6. Density 1 (8000 cells/well): resuspend in 0.8 µL (yes it's that little volume) Accutase for each eppendorf tube
7. Density 2 (16,000 cells/well): resuspend in 1.6 µL Accutase for each eppendorf tube
8. Density 3 (24,000 cells/well): resuspend in 2.4 µL Accutase for each eppendorf tube
9. Density 4 (48,000 cells/well): resuspend in 4.8 µL Accutase for each eppendorf tube
    1. Althys' note: the calculated Accutase volume based on the protocol used by Han et al is pretty low so I searched up some other protocols and this is what I got:
    2. https://pmc.ncbi.nlm.nih.gov/articles/PMC8214150/
10. Add 150 µL of Accutase, then for Step 6 incubate in 37C for 10 min
11. Note that this protocol uses spheroids made of 10,000 cells per 100 µL of media
    1. https://www.sciencedirect.com/science/article/pii/S0940960217301413?via%3Dihub
12. Add 300 µL of Accutase, make sure to thoroughly resuspend it by pipetting up and down 10 times using a P200 pipette.
13. For Step 6, incubate the spheroids for 10 minutes then resuspend it again for 10 times
14. Note that this protocol uses both 10,000 and 50,000-cell spheroids, so I am more inclined towards this one
15. Incubate the eppendorf tubes in 37C incubator for 30 minutes
16. If use different protocols - check incubation time
17. Dilute the dissociated cells in DMEM:
18. Density 1: resuspend in 999.2 µL DMEM (final volume: 1 mL)
19. Density 2: resuspend in 998.4 µL DMEM (final volume: 1 mL)
20. Density 3: resuspend in 997.6 µL DMEM (final volume: 1 mL)

Loading [MathJax]/extensions/Safe.js

21. Density 4: resuspend in 995.2 µL DMEM (final volume: 1 mL)
    1. Althys' note: Honestly because the Accutase solution is so so so low, you can probably get away with adding 1 mL to all 4 densities.
    2. Althys' EXTRA NOTES: if decide to use different amount of Accutate, add DMEM so that the final volume reaches 1 mL
22. Run the cytoflex, draw polygon and note down the Cytoflex number in the table below - Column C
23. If use tube reader, just insert eppendorf 1.5 mL tube
24. If use plate reader, mix the cellular suspension from Step 7 thoroughly (use vortex if possible) then transfer to a 96-well plate (one well per eppendorf tube, you should finish with 24 wells in total)

Conclusions/Action Items:

- Protocol shared with Carley on Benchling 11/22
    - https://benchling.com/uw_hesslab/f/lib_9oa99j6P-accutase-dissociation/etr_68iruynX-accutase-dissociation-of-spheroids-protocol/edit
- Apply protocol via validation testing 11/25/2024
- Record result and make any changes necessary

Emily Rhine - Dec 11, 2024, 12:04 AM CST

- Protocol unable to be tested on 11/25 due to broken CytoFLEX
- Cells were bleached and the experiment put on hold until next semester

Loading [MathJax]/extensions/Safe.js

Percent Cell Viability After Spheroid Formation_11/22/2024

Althys Cao - Dec 17, 2024, 9:45 PM CST

Latest protocol

Althys Cao - Dec 17, 2024, 9:48 PM CST

Title: Determination of Percent Cell Viability after Spheroid Formation

Date: 12/15/2024

Goals: Establish protocol for percent cell viability.

Content By: Althys

Content:

Materials

- Eppendorf tubes of 1 mL dissociated cells from spheroids (see Accutase Dissociation of Spheroids entry, from Team Activities/Testing and Results)
- Cytoflex machine

Protocols:

1. Run the Cytoflex for each tube.
2. Gate (/draw polygon) areas where there are alive cells, record the number shown on the screen.
    a. Cell concentration = number shown on screen * 10,000 (cells/mL)
    b. Total number of live cells = cell concentration (cells/mL) * 1 mL (volume in eppendorf tube)
3. Gate (/draw polygon) areas where there are dead cells (smaller scatter than live cells), record the number shown on the screen.
    a. Cell concentration = number shown on screen * 10,000 (cells/mL)
    b. Total number of dead cells = cell concentration (cells/mL) * 1 mL (volume in eppendorf tube)
4. Calculate percent viability: total number of live cells / (total number of live cells + total number of dead cells) * 100%

Conclusions: This should be the most updated protocol for next semester and there should be no changes to it, unless something did not go according to plan during experiments. In the previous protocol (see below), percent cell viability is determined by ratio of live cells to starting number of cells when seeding. This protocol was not applicable because cell proliferation was not taken into account).

Althys Cao - Dec 17, 2024, 9:31 PM CST

Older version of protocol + Notes

Althys Cao - Dec 17, 2024, 9:51 PM CST

Title: Determination of Percent Cell Viability after Spheroid Formation (version 1)

Date: 11/22/2024

Goals: Establish percent cell viability determination protocol.

Content By: Althys

Content:

Run the cytoflex, draw polygon and note down the Cytoflex number in the table below - **Column C**
If use tube reader, just insert eppendorf 1.5 mL tube
If use plate reader, mix the cellular suspension from Step 7 thoroughly (use vortex if possible) then transfer to a 96-well plate (one well per eppendorf tube, you should finish with 24 wells in total)

	Starting cell number	Cytoflex number	Cellular concentration (cells/mL)	Total number of alive cells	% alive cells
Density 1 - Well 1	8000		= cytoflex number * 10,000	= cellular concentration * 1 mL	= total number of alive cells/starting cell number
Density 1 - Well 2	8000				
Density 1 - Well 3	8000				
Density 1 - Well 4	8000				
Density 1 - Well 5	8000				
Density 1 - Well 6	8000				
Density 2 - Well 1	16000				
Density 2 - Well 2	16000				
Density 2 - Well 3	16000				
Density 2 - Well 4	16000				
Density 2 - Well 5	16000				
Density 2 - Well 6	16000				

Density 3 - Well 1	24000				
Density 3 - Well 2	24000				
Density 3 - Well 3	24000				
Density 3 - Well 4	24000				
Density 3 - Well 5	24000				
Density 3 - Well 6	24000				
Density 4 - Well 1	48000				
Density 4 - Well 2	48000				
Density 4 - Well 3	48000				
Density 4 - Well 4	48000				
Density 4 - Well 5	48000				
Density 4 - Well 6	48000				

Conclusions/Action Items:

- Protocol shared with Carley on Benchling 11/22
  - https://benchling.com/uw_hesslab/f/lib_9oa99j6P-accutase-dissociation/etr_68iruynX-accutase-dissociation-of-spheroids-protocol/edit
- Apply protocol via validation testing 11/25/2024
- Record result and make any changes necessary

Althys Cao - Dec 17, 2024, 9:31 PM CST

- Protocol unable to be tested on 11/25 due to broken CytoFLEX
- Cells were bleached and the experiment put on hold until next semester

ImageJ Analysis Protocol_11/25/2024

Emily Rhine - Dec 12, 2024, 3:28 PM CST

Title: ImageJ Analysis Protocol (abbreviated)

Date: 11/25/2024

Content by: Julia, Ana, Emily, and Jayson

Content:

Out of 120 images taken, 5 z-stack slices per 6 wells for 4 conditions, take the most in-focus images ("Spheroid Images" entry) complete the following steps:

1. Open ImageJ application and upload desired image
2. Set scale bar using the scale bar on the image
3. Go to image → type → 16 bit
4. Threshold image
5. Go to process → binary → watershed
6. Trace around well circle to isolate spheroids as the only thing being analyzed
7. Go to analyze particles → set interval to 4147-infinity, select show outlines from drop down, check “display results” and “exclude on edges” boxes, clear results boxes, and click OK
    - 4147 μm² taken from measurement of spheroid with ≥20 cells which is deemed the benchmark to accurately model the tumor micro environment [1].
    - See entry "Spheroid Size Threshold_ImageJ Analysis_11/25/2024" in Emily Rhine folder
8. Save measurements to an excel sheet
9. Record data results to seperate excel file by combine measurement averages
10. In excel compiled sheet and run 4 way ANOVA test in excel
11. Generate a suitable plot for this comparison (see example below)

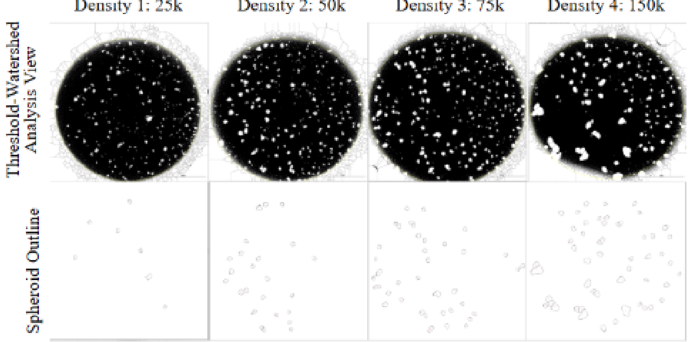


Figure 8: ImageJ analysis of spheroids across seeding densities 1-4 (25k, 50k, 75k, and 150k cells/cm², respectively). Images in top row are in threshold-watershed view and images in bottom row are in spheroid outline view.

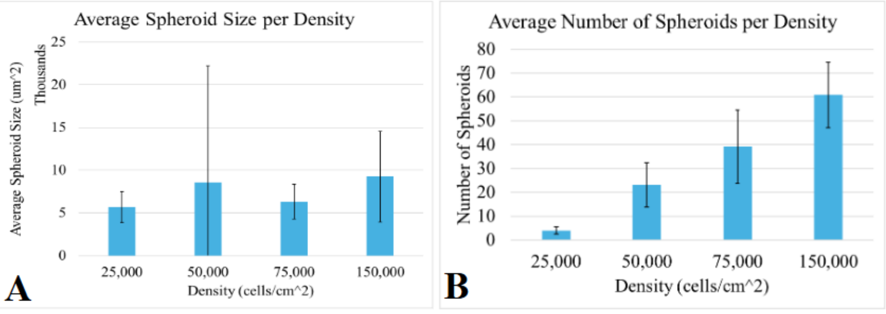


Figure 9: Spheroid analysis across seeding densities 1-4 (25k, 50k, 75k, and 150k cells/cm²). **A:** Average spheroid size (in μm²) across seeding densities (in cells/cm²). **B:** Average number of spheroids across seeding densities (in cells/cm²). Error bars in **A** and **B** display standard deviation.

References

[1] G. Razian, Y. Yu, and M. Ungrin, “Production of Large Numbers of Size-controlled Tumor Spheroids Using Microwell Plates,” J Vis Exp, no. 81, p. 50665, Nov. 2013, doi: 10.3791/50665.

Conclusions/Action Items:

- Add reduced protocol to poster and final report
- Add full protocol to appendix of final report
- Update ImageJ analysis, statistical comparison, and other testing protocols as needed

Progress Reports (PDF)

Emily Rhine - Sep 16, 2024, 11:42 AM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 1

Reporting Period: September 6th, 2024 - September 12th, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) amartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- First meeting with client scheduled in-person at 9/13 at WIMR, which will also include a tour of the Hess Lab.
- Group started training required to join the Hess Lab.

Difficulties / advice requests: N/A for week 2

Current design: N/A for week 2

Materials and expenses: N/A for week 2

Major team goals for the next week:

1. Finish PDS
2. Finalize expectations for project with client

Download

Week_1_Progress_Report_9_12_24.pdf (104 kB)

Emily Rhine - Sep 19, 2024, 4:38 PM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 2

Reporting Period: September 13th, 2024 - September 19th, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) amartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Currently working on required trainings
- Started more in-depth research to understand PDS requirements
- Finished first draft of PDS

Difficulties / advice requests: N/A for week 3

Current design: N/A for week 3

Materials and expenses: N/A for week 3

Major team goals for the next week:

1. Assign the proposed spheroid protocols a rank to determine which method will be used.
2. Assign proposed cell lines a rank to determine which line will be used.

Download

Week_2_Progress_Report_9_19_24.pdf (162 kB)

Emily Rhine - Sep 30, 2024, 9:19 AM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 3

Reporting Period: September 20th, 2024 - September 26th, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team came up with 3 cell lines to use (all already available in Hess Lab) and 3 spheroid formation protocols.
- Team generated two design matrices and rated the designs based on different criteria and narrowed down to the most viable cell line and protocol.

Difficulties / advice requests: The team does not have difficulties for week 4. However, we would appreciate feedback from our client and advisor for our design matrix so we can make appropriate changes in time for our preliminary presentation next Friday.

Current design: As will be outlined in the design matrices (will be sent out shortly), the team has agreed on a winning cell line and a spheroid formation protocol. The cell line selected was A549 and the spheroid formation protocol will involve the use of treated tissue culture plates for low attachment.

Download

Progress_Report_3_9_26_24.pdf (159 kB)

Emily Rhine - Oct 05, 2024, 7:26 PM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 4

Reporting Period: September 27th, 2024 - October 3, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- The team is prepared to propose our preliminary final design to the client and advisor in the preliminary design presentation
- Team worked on preliminary presentation.

Difficulties / advice requests: The team does not have difficulties for week 5. However, we would appreciate feedback from our client advisor on our preliminary presentation so we can make appropriate changes in time for Friday's presentation and next week's preliminary report.

Current design: As will be outlined in the design matrices and the preliminary presentation (will be sent out shortly), the team has agreed on a winning cell line and a spheroid formation

Download

Progress_Report_4_10_3_24.pdf (158 kB)

Emily Rhine - Oct 10, 2024, 7:14 PM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 5

Reporting Period: October 4, 2024 - October 10, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) acao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team presented preliminary presentation
- Team started establishing times to go into labs to start working on cell cultures

Difficulties / advice requests: The team does not have difficulties for week 6. However, we would appreciate feedback from our client advisor on our preliminary report (will be sent by end of week) so we can implement appropriate changes that will inform our work with cell culture, spheroid formation, etc. in the coming weeks. Additionally, the team would appreciate feedback regarding what type of treated tissue culture plates (ultra-low attachment vs. polyHEMA-coated) are preferred or more readily available at the Hess Lab.

Download

Progress_Report_5_10_10_24.pdf (164 kB)

Emily Rhine - Oct 21, 2024, 11:36 AM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 6

Reporting Period: October 11, 2024 - October 17, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) acao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team started passaging A549 cell line to determine our cell line's doubling time.

Difficulties / advice requests:

- Review the possibility of running another cell line.

Download

Progress_Report_6_10_17_24.pdf (171 kB)

Emily Rhine - Oct 25, 2024, 11:52 AM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 7

Reporting Period: October 11, 2024 - October 23, 2024

Client: Carley Schwartz cschwartz@wisc.edu
Dr. Gaelen Haas ghaas3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team continued passaging A549 cells to determine our cell line's approximate doubling time. Team researched additional spheroid formation protocols to finalize the protocol we will begin using and materials required.

Difficulties / advice requests:

- Review the possibility of running another cell line.
- Current cell line has highly varying doubling time (20-72 hours), which we believe might be due to A549 cells preferring a higher confluency (more "neighbor" cells) rather than lower.

Download

Progress_Report_7_10_24_24.pdf (173 kB)

Emily Rhine - Oct 31, 2024, 9:20 PM CDT

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 8

Reporting Period: October 23, 2024 - October 31, 2024

Client: Carley Schwartz cschwartz@wisc.edu
Dr. Gaelen Haas ghaas3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) mcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team finished passaging 7 and 8.
- Team compiled a list of protocols to make low-attachment plates and form spheroids to send to the clients.

Difficulties / advice requests:

- A549 cell line has highly varying doubling time (20-72 hours), which we believe might be due to A549 cells preferring a higher confluency (more "neighbor" cells) rather than lower. We are currently fine-tuning the volume of cells we passage to find a more consistent doubling time.

Download

Progress_Report_8_10_31_24.pdf (167 kB)

Emily Rhine - Nov 07, 2024, 4:50 PM CST

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 9

Reporting Period: November 1, 2024 - November 7, 2024

Client:	Carley Schwartz	cischwartz@wisc.edu
	Dr. Gaelen Hess	ghess3@wisc.edu
Advisor:	Paul Campagnola	pcampagnola@wisc.edu
Team:	Althys Cao (Leader)	[illegible]@wisc.edu
	Ana Martinez (Communicator)	almartinez4@wisc.edu
	Emily Rhine (BSAC)	erhine@wisc.edu
	Julia Salita (BWIG)	jsalita@wisc.edu
	Jayson O'Halloran (BPAG)	ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Continue to passage cells/thaw new cells
- Work with client to finalize materials sheet this week
- In the process of finalizing plate making, spheroid formation and testing protocols

Difficulties / advice requests:

- Optimizing spheroid formation with correct size and media.
- Waiting for materials to be ordered/arrive.

Current design:

Download

Progress_Report_9_11_7_24.pdf (166 kB)

Emily Rhine - Nov 19, 2024, 1:04 PM CST

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 10

Reporting Period: November 8, 2024 - November 14, 2024

Client:	Carley Schwartz	cischwartz@wisc.edu
	Dr. Gaelen Hess	ghess3@wisc.edu
Advisor:	Paul Campagnola	pcampagnola@wisc.edu
Team:	Althys Cao (Leader)	[illegible]@wisc.edu
	Ana Martinez (Communicator)	almartinez4@wisc.edu
	Emily Rhine (BSAC)	erhine@wisc.edu
	Julia Salita (BWIG)	jsalita@wisc.edu
	Jayson O'Halloran (BPAG)	ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team continued to passage cells and will thaw a new vial on Friday.
- Spheroid formation materials (Methylcellulose, poly-HEMA) have arrived to Hess Lab.
- Team will make Methylcellulose and poly-HEMA stock solutions this Friday.
- Team will start making poly-HEMA coated plates starting next week after making stock solutions

Difficulties / advice requests:

- Optimizing Accutase volume needed for spheroid live/dead flow cytometry assay.

Download

Progress_Report_10_11_14_24.pdf (181 kB)

Emily Rhine - Nov 21, 2024, 9:45 PM CST

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 11

Reporting Period: November 15, 2024 - November 21, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) arcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team continued to passage cells.
- Team made Methylcellulose and poly-HEMA stock solutions.
- Team coated poly-HEMA plates and seeded cells for spheroid size experiment (Trial 1) and will re-do this with optimized cell concentrations on Friday.

Difficulties / advice requests:

- Getting familiar with microscope we will use for imaging spheroids on Monday.
- First spheroid size experiment had to be repeated because poly-HEMA plates were not properly dried and cell concentrations seeded onto each well were prepared incorrectly.
- Confirm with team Accutase volume needed for spheroid live/dead flow cytometry assay on Monday.

Download

Progress_Report_11_11_21_24_1_.pdf (186 kB)

Emily Rhine - Dec 05, 2024, 10:17 PM CST

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 12

Reporting Period: November 22, 2024 - December 5, 2024

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) arcao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Made new PolyHEMA-coated plates
- 2nd trial of cell seeding for spheroid formation
- Received training to use the BioTek Cytation
- Took pictures of spheroids using BioTek Cytation to determine spheroid number and size
- Final poster presentation

Difficulties / advice requests:

- Cytoflex machine in Hess Lab broke and maintenance took too long for team to complete %live/dead cytometry assay

Download

Progress_Report_12_12_5_24.pdf (182 kB)

PDS_9/19/2024

Emily Rhine - Sep 19, 2024, 4:37 PM CDT

Title: PDS

Date: 9/19/2024

Content:

See attached PDF below.

Conclusions/Action Items:

- Send out PDS with progress report to client and advisor
- Upload PDS to canvas and design website page
- Update PDS as needed throughout project

Emily Rhine - Oct 10, 2024, 7:12 PM CDT

CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

BME 400

September 19, 2024

Section 305

Product Design Specifications

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) ancao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Function: Although previous CRISPRi screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPRi screening in order to identify sources of DNA mutations in the tumor environment. On a high scale, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that acts as a sensitive marker for DNA damage.

Client requirements:

1. The team must choose an appropriate human cell line for the project, ensuring to maximize cell spheroid viability.
2. The team must develop a spheroid formation protocol for the chosen cell type or line that optimizes seeding density and viscosity.
3. The team must optimize the cancer spheroid characteristics and their formation protocols for CRISPR screens.
4. By May 2025, the team must develop a protocol to stain for γH2AX: an indicator for the location of maximal damage due to DNA double-strand breaks (DSBs).
5. By May 2025, the team must perform high throughput genome-wide CRISPR screening to check for the effects of different genes on the amount of γH2AX detected.
6. Future steps post-design project include performing an analysis to determine the

Download

CRISPRi_Screening_in_Cancer_Spheroids_Product-Design-Specifications__9_19_2024_1_.pdf (173 kB)

Emily Rhine - Sep 19, 2024, 4:38 PM CDT

CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

BME 400

September 19, 2023

Section 305

Product Design Specifications

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) ancao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu

Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Function: Although previous CRISPRi screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPRi screening in order to identify sources of DNA mutations in the tumor environment. On a high scale, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that acts as a sensitive marker for DNA damage.

Client requirements:

1. The team must choose an appropriate human cell line for the project, ensuring to maximize cell spheroid viability.
2. The team must develop a spheroid formation protocol for the chosen cell type or line that optimizes seeding density and viscosity.
3. The team must optimize the cancer spheroid characteristics and their formation protocols for CRISPR screens.
4. By May 2025, the team must develop a protocol to stain for γH2AX: an indicator for the location of maximal damage due to DNA double-strand breaks (DSBs).
5. By May 2025, the team must perform high throughput genome-wide CRISPR screening to check for the effects of different genes on the amount of γH2AX detected.
6. Future steps post-design project include performing an analysis to determine the

Download

CRISPRi_Screening_in_Cancer_Spheroids_Product-Design-Specifications__9_19_2024.pdf (173 kB)

Design Matrix_9/27/2024

Emily Rhine - Sep 27, 2024, 11:26 PM CDT

CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

BME 400

September 27, 2023

Section 305

Design Matrix

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess ghess3@wisc.edu

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) aocao@wisc.edu
Ana Martinez (Communicator) almartinez4@wisc.edu
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita (BWIG) jsalita@wisc.edu
Jayson O'Halloran (BPAG) ohalloran2@wisc.edu

Table 1. Design matrix for Cell Line.

Design Criteria (weight)	NCI-H23		A549		OVCAR-5	
	Score X/5	Weighted score	Score X/5	Weighted score	Score X/5	Weighted score
Viability (20)	4	16	5	20	3	12
Adhesion (20)	3	12	4	16	4	16
Reproduction Speed (20)	3	12	5	20	4	16
Drug Sensitivity (15)	4	12	5	15	3	9
Genetic Mutation (15)	5	15	3	9	2	6
Ease of Procurement (10)	5	10	5	10	5	10
Total Score (100)	77		90		69	

Download

CRISPRi_Screening_in_Cancer_Spheroids_Design_Matrix.pdf (156 kB)

Preliminary Presentation 10/04/2024

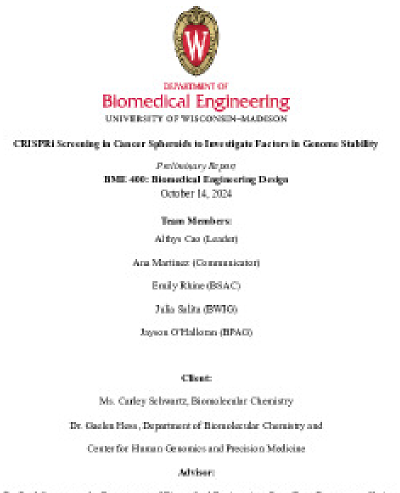
JULIA SALITA - Oct 04, 2024, 9:40 AM CDT

Download

CRISPRi_Screening_in_Cancer_Spheroids_Preliminary_Presentation_10_4_2024_1_.pdf (1.63 MB)

Preliminary Report_10/14/2024

Emily Rhine - Oct 15, 2024, 1:47 PM CDT



DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

Preliminary Report
BME 400: Biomedical Engineering Design
October 14, 2024

Team Members:

Althys Cao (Leader)

Ana Martinez (Communicator)

Emily Rhine (BSAC)

Julia Salita (BWIG)

Jayson O'Halloran (BPAG)

Client:

Ms. Carley Schwartz, Biomolecular Chemistry

Dr. Gaelen Hess, Department of Biomolecular Chemistry and

Center for Human Genomics and Precision Medicine

Advisor:

Dr. Paul Campagnola, Department of Biomedical Engineering, Peter Tong Department Chair

Download

CRISPRi_Screening_in_Cancer_Spheroids_Preliminary_Report_10_14_2024.pdf (1.76 MB)

Show and Tell Presentation_10/31/2024

Emily Rhine - Oct 31, 2024, 4:50 PM CDT

Download

Show_and_Tell_11_1_2024_1_.pdf (4.48 MB)

BPAG Excel Sheet

JAYSON O'HALLORAN - Nov 14, 2024, 12:01 PM CST

Download

BPAG_Expense_Sheet_Materials_-_Sheet1.pdf (47.6 kB)

Final Materials Table_12/12/2024

Emily Rhine - Dec 16, 2024, 11:54 PM CST

Title: Final Materials Table

Date: 12/13/2024

Content by: Althys, Emily

Content:

- Taken from final report

Emily Rhine - Dec 16, 2024, 11:51 PM CST

Materials listed are required for culturing and passaging A549 cells and are already available in Hess Lab and thus require no purchasing. One 1 mL cryovial of A549 can be used up to 20 passages and 3 passages will be performed for each week of the project. In total, two cryovials of A549 were used, and a total of 20 passages (15 for the first cryovial and 5 for the second) were performed.

Table 1. Materials List.

Material	Part Specification	Quantity	Price
Already available in Hess Lab, requires no purchasing			
T-75 Flasks	Catalog #156800 - Thermo Fisher Scientific	1 for up to 20 passages	$381.65 / case of 100 [75]
A549	CCL-185 - ATCC	N/A	$555 / vial [28]
DMEM (high glucose, no glutamine)	Catalog #11960069 - Thermo Fisher Scientific	1x500 mL bottle 10 mL / passage (used in solution with Pen/Strep and FBS)	$242.65 / 10 x 500 mL [76]
Penicillin-streptomycin (Pen/Strep)	Catalog #15070063 - Thermo Fisher Scientific	1% in solution with DMEM and FBS	$23.65 / 100 mL of 5,000 U/mL [77]
Fetal Bovine Serum (FBS),Value	Catalog #A5256701 - Thermo Fisher Scientific	10% in solution with DMEM and Pen/Strep	$314.37 / 500 mL [78]
PBS, pH 7.4	Catalog #10010072 - Thermo Fisher Scientific	2 mL / passage	$244.00 / 6 x 1000 mL [79]
Trypsin-EDTA (0.25%)	Catalog #25200114 - Thermo Fisher Scientific	3 mL / passage	$315 / 20 x 100 mL [69]
VWR® Flat-bottom 96-well polystyrene assay plates	Catalog #76446-962 - VWR	2 plates	$494.24 / 100 plates [80]
Ethanol	Catalog #BP28184 - Fisher Scientific	33 mL	$1,595.00/ (4 x 4L) [81]
Requires purchasing			

Loading [MathJax]/extensions/Safe.js

PolyHEMA	Catalog P3932-10G - SigmaAldrich	1.3 g	$238 / 10 g [82]* 1.3 g = $30.94
Methylcellulose	Catalog M0512-100G - SigmaAldrich	2 g	$51.40 / 100 g [83] * 2g = $1.03
Accutase	Catalog A1110501 - ThermoFisher	Has not been used	$60.65 / 100 mL [84]
		Total:	$92.62

Emily Rhine - Dec 16, 2024, 11:53 PM CST

References (taken from final report)

[28] "A549 - CCL-185 | ATCC." Accessed: Sep. 25, 2024. [Online]. Available: https://www.atcc.org/products/ccl-185

[75] "T75 Flasks - US." Accessed: Oct. 13, 2024. [Online]. Available: https://www.thermofisher.com/us/en/home/life-science/cell-culture/cell-culture-plastics/cell-culture-flasks/t75-flasks.html
[76] "DMEM, high glucose, no glutamine." Accessed: Oct. 13, 2024. [Online]. Available: https://www.thermofisher.com/order/catalog/product/11960044
[77] "Penicillin-Streptomycin (5,000 U/mL)." Accessed: Oct. 13, 2024. [Online]. Available: https://www.thermofisher.com/order/catalog/product/15070063
[78] "Fetal Bovine Serum, Value (formerly USDA-approved in North America or qualified, Brazil in other regions)." Accessed: Oct. 13, 2024. [Online]. Available: https://www.thermofisher.com/order/catalog/product/A5256701
[79] "PBS, pH 7.4." Accessed: Oct. 13, 2024. [Online]. Available: https://www.thermofisher.com/order/catalog/product/10010023
[80] "VWR® Polystyrene Assay Plates, 96- and 384-Well," VWR. Accessed: Dec. 12, 2024. [Online]. Available: https://us.vwr.com/store/product/42378328/vwr-polystyrene-assay-plates-96-and-384-well
[81] "Ethanol, Absolute (200 Proof), Molecular Biology Grade, Fisher BioReagents, Quantity: 100 mL | Fisher Scientific." Accessed: Dec. 12, 2024. [Online]. Available: https://www.fishersci.com/shop/products/ethanol-absolute-200-proof-molecular-biology-grade-fisher-bioreagents-3/BP28184
[82] "Poly(2-hydroxyethyl methacrylate) BioReagent, powder, cell culture mammalian 25249-16-5." Accessed: Dec. 12, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/product/sigma/p3932
[83] "Methyl cellulose viscosity: 4,000 cP 9004-67-5." Accessed: Dec. 12, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/product/sigma/m0512
[84] "StemPro™ Accutase™ Cell Dissociation Reagent." Accessed: Dec. 12, 2024. [Online]. Available: https://www.thermofisher.com/order/catalog/product/A1110501

Loading [MathJax]/extensions/Safe.js

Final Poster_12/6/2024

Emily Rhine - Dec 10, 2024, 11:44 PM CST

Title: Final Poster

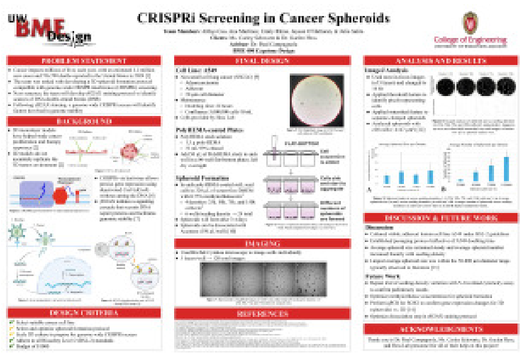
Date: 12/4-12/6

Present: Entire team

Content By: Emily

Content:

Emily Rhine - Dec 05, 2024, 10:11 PM CST



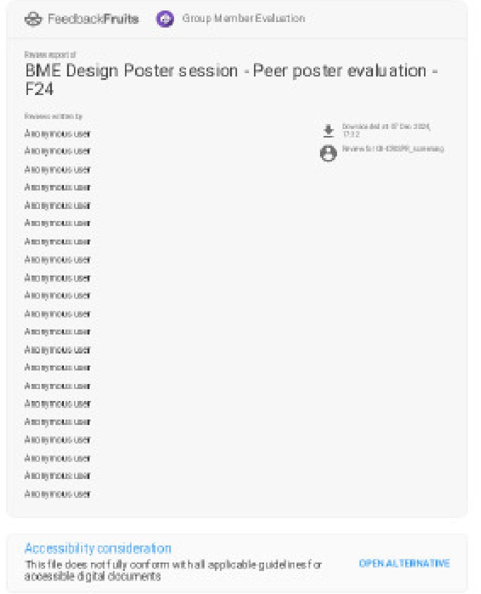
Download

CRISPRi_Screening_in_Cancer_Spheroids_Final_Poster_12_11_2024.pptx.pdf (4.26 MB)

Emily Rhine - Nov 19, 2024, 1:01 PM CST

https://docs.google.com/presentation/d/1e2kUBM1243vdwKiIt_wWo1ssHczuEipB/edit#slide=id.p1

Emily Rhine - Dec 10, 2024, 11:38 PM CST



Loading [MathJax]/extensions/Safe.js

Download

BME_Design_Poster_session_-_Peer_poster_evaluation_-_F24_-_Emily_Rhine_-_received_feedback.pdf (142 kB)

Emily Rhine - Dec 10, 2024, 11:43 PM CST

- Sent poster to Advisor, Dr.C and Received feedback on 12/3
- Printed poster 12/4
- Presented poster 12/6 and received feedback

Conclusion/ Action Items:

- Remember to be mindful the background and education level of your audience before giving a presentation/ elevator pitch
- Prepare to submit other final deliverables
  - Final report
  - Outreach activity draft
  - Client Review form
  - LabArchives Update
- All deliverables either due to canvas by 12/11 11:59pm or to Dr.C 12/17 11:59pm

Loading [MathJax]/extensions/Safe.js

Final Poster Supplemental Figures_12/6/2024

Emily Rhine - Dec 17, 2024, 7:53 PM CST

Supplemental Figure 1: 10x Brightfield image of A549 Passage 3 cells taken at ~50% confluency.

Download

Poster_Presentation_Photos_12_6_2024.pdf (3.25 MB)

Emily Rhine - Dec 17, 2024, 7:53 PM CST

Final Report_12/11/2024

Emily Rhine - Nov 19, 2024, 1:00 PM CST

Emily Rhine - Nov 19, 2024, 1:00 PM CST

https://docs.google.com/document/d/1-rnBC9nkIu-B9p5m5cqrJOWUzRYetvPrYIclSFG4m_4/edit?tab=t.0

CRISPR screen on growth in spheroids_9_10_2024

Emily Rhine - Sep 15, 2024, 7:41 PM CDT

Title: CRISPR screen on growth in spheroids

Date: 9/10/2024

Content by/Present: Emily

Goals: Review media provided by the client to better understand the project and its motivation.

Content:

Link: CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities

Facts and Information of Interest:

- "**CRISPR phenotypes in 3D more accurately recapitulated those of *in vivo* tumors**, and genes with differential sensitivities between 2D and 3D conditions were highly enriched for genes that are mutated in lung cancers." [1]
- "Notably, we found that carboxypeptidase D is responsible for removal of a C-terminal RKRR motif2 from the α-chain of the insulin-like growth factor 1 receptor that is critical for receptor activity. Carboxypeptidase D expression correlates with patient outcomes in patients with lung cancer, and **loss of carboxypeptidase D reduced tumor growth**." [1]
- Motivation: "Despite the large increase in the catalogue of mutations observed across diverse cancer types (the 'long tail')1, it is **frequently unclear which mutations are functional cancer drivers.** Therefore, it is a central challenge to scalably investigate these genes in relevant cancer models to assign causality and identify cancer-specific vulnerabilities." [1]
- **Competing Methods:**
  - "Genetically engineered mouse models recapitulate tumor growth and microenvironment, but are limited by scalability, time and cost." [1]
  - "Xenograft-based models are limited in scale, and can be difficult to manipulate in vitro." [1]
  - "Genome-scale investigation of cancer growth and drug sensitivity has largely relied on in vitro 2D cell culture, which lacks many features of disease, such as hypoxia, altered cell–cell contacts and rewired metabolism." [1]
  - "In vitro organoid models alleviate some of these concerns, but are much less scalable." [1]
- These researchers used **"non-small-cell lung carcinoma"** for their cell lines. [1]
- Methods
  - "These 10 cell lines were transduced with a spCas9 lentiviral vector with a blasticidin selection marker (Addgene no. 52962), and selected with blasticidin (10 μg ml−1). Single-cell clones of these selected cell lines were individually tested for their Cas9-cutting efficiency by lentiviral infection with pMCB30639, a self-GFP-cutting reporter that has both GFP and an sgRNA against GFP on the same backbone. Single clones with high Cas9-cutting efficiency were established and used in the CRISPR screens and other biological assays." [1]
- "To culture lung cancer cells as 3D spheroids at genome scale, we used either pre-treated ultra-low attachment plates (Corning, no. 3261) or polyhema (Sigma, no. P3932) coated tissue culture plates. " [1]

References

[1] K. Han et al., "CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities," Nature, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

Loading [MathJax]/extensions/Safe.js

Conclusions/action items:

Article Summary:

This article was incredibly helpful in understanding what our team will be doing for the next year. Most notably, the description of how CRISPR-Cas9 was used in conjunction with these lung cancer spheroids in the methods section was enlightening.

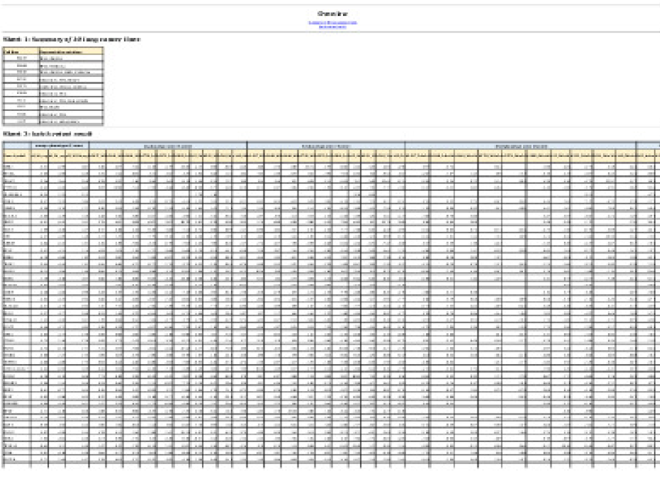
Action Items:

- Add "Competing Methods" bullet point to PDS document and add link for future reference.
- Continue reviewing the literature provided by the client to better understand the background and motivation for this project.
- Ask the client if they use the same method as outlined in the paper [CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities] they gave us for reference.

Emily Rhine - Sep 19, 2024, 7:31 PM CDT

Cell lines	Representative mutations
H1437	TP53, CDKN2A
H1568	TP53, SMARCA4
H1650	TP53, CDKN2A, EGFR, SMARCA4
H1792	KRAS G12C, TP53, KEAP1
H1975	EGFR, TP53, PIK3CA, CDKN2A
H2009	KRAS G12A, TP53
H23	KRAS G12C, TP53, LKB1, KEAP1
H322	TP53, KEAP1
H358	KRAS G12C, TP53
A549	KRAS G12S, LKB1, KEAP1

Emily Rhine - Sep 19, 2024, 7:31 PM CDT



Download

41586_2020_2099_MOESM7_ESM.xlsx (473 kB)

Loading [MathJax]/extensions/Safe.js

CRISPRi_9_10_2024

Emily Rhine - Sep 30, 2024, 11:24 AM CDT

Title: CRISPRi

Date: 9/10/2024, 9/30/2024

Content by/Present: Emily

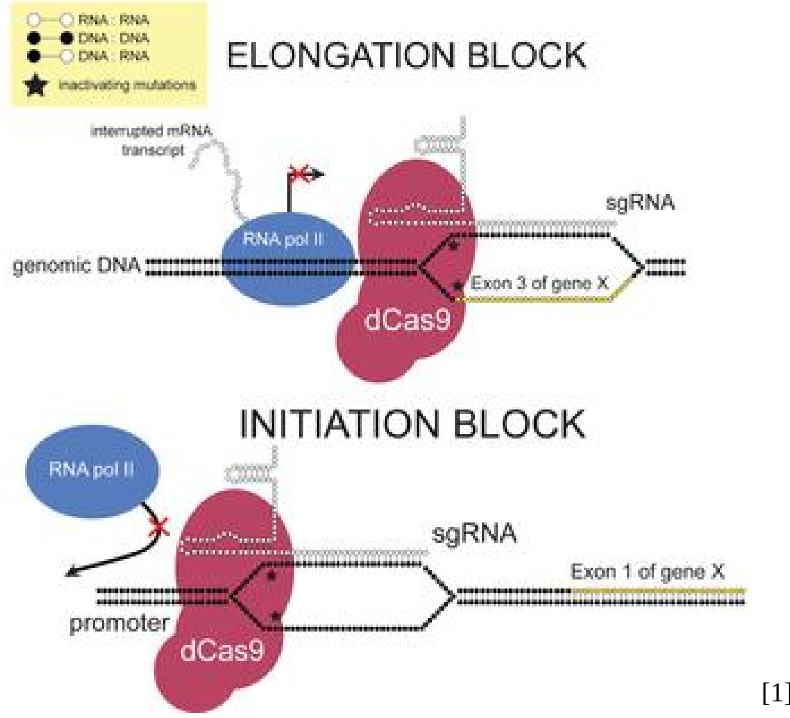
Goals: Review media provided by the client to better understand the project and its motivation.

Content:

Link: CRISPR interference

Facts and Information of Interest:

- Genetic screening
  - "The technology uses a catalytically dead Cas9 (usually denoted as dCas9) protein that lacks endonuclease activity to regulate genes in an RNA-guided manner. Targeting specificity is determined by complementary base-pairing of a single guide RNA (sgRNA) to the genomic locus. sgRNA is a chimeric noncoding RNA that can be subdivided into three regions: a 20 nt base-pairing sequence, a 42 nt dCas9-binding hairpin and a 40 nt terminator" [1]
- Advantages
  - "CRISPRi can silence a target gene of interest up to 99.9% repression. The strength of the repression can also be tuned by changing the amount of complementarity between the guide RNA and the target. Contrary to inducible promoters, partial repression by CRISPRi does not add transcriptional noise to the target's expression. Since the repression level is encoded in a DNA sequence, various expression levels can be grown in competition and identified by sequencing." [1]
- Limitations
  - "Sequence specificity to target loci is only 14 nt long (12 nt of sgRNA and 2nt of the PAM), which can recur around 11 times in a human genome. Repression is inversely correlated with the distance of the target site from the transcription start site. Genome-wide computational predictions or selection of Cas9 homologs with a longer PAM may reduce nonspecific targeting." [1]
  - "Sequence-specific toxicity has been reported in eukaryotes, with some sequences in the PAM-proximal region causing a large fitness burden. This phenomenon, called the "bad seed effect", is still unexplained but can be reduced by optimizing the expression level of dCas9." [1]

- 

[1]

References

[1] “CRISPR interference,” *Wikipedia*. Mar. 22, 2024. Accessed: Sep. 10, 2024. [Online]. Available: https://en.wikipedia.org/w/index.php?title=CRISPR_interference&oldid=1214981619

Conclusions & Action Items:

- Although CRISPR is a great tool, its limitations must be taken into account when conducting research and planning experiments.
- This is good background information for the Preliminary Design Presentation and Preliminary Report.
  - Great graphic!
- The team is looking at a sequence specificity of 18-30 nucleotides for the guide RNA to "latch" onto (according to Carley - see client meeting notes 1).

γH2AX _9_10_2024

Emily Rhine - Sep 10,

Title: γH2AX as a novel endpoint to detect DNA damage

Date: 9/10/2024

Content by/Present: Emily

Goals: Review media provided by the client to better understand the project and its motivation.

Content:

Link: γH2AX as a novel endpoint to detect DNA damage: Applications for the assessment of the in vitro genotoxicity of cigarette smoke

Facts and Information of Interest:

- "DNA frameshifts, double-strand breaks (DSBs), etc. DSBs are one of the most deleterious lesions since they affect both strands of the DNA helix. This lesion can le death by triggering apoptosis but if the lesion fails to repair or it is repaired incorrectly, DNA information can be compromised leading to mutation and ultimately ca heritable damage" [1]
- "The mechanism of γH2AX elimination has not been fully unravelled. There are multiple phosphatases involved in γH2AX dephosphorylation. Dephosphorylation c directly on the chromatin or could happen after the histone has been displaced from the nucleosomes. Both mechanisms could potentially occur simultaneously, inde the location of the γH2AX in the foci."
- "Other mechanisms mentioned by Bao involve histone chaperone proteins in the process of γH2AX elimination. Experiments carried out by Keogh and colleagues su the loss of γH2AX could be triggered not only by DSB repair but also by the activation of steps that precede DSB repair. However, some of their results seem to indi γH2AX loss is not mediated by single-stranded DNA resection, one of the cellular responses to DSBs." [1]

References

[1] C. Garcia-Canton, A. Anadón, and C. Meredith, "γH2AX as a novel endpoint to detect DNA damage: Applications for the assessment of the in vitro genotoxicity of cig Toxicology in Vitro, vol. 26, no. 7, pp. 1075–1086, Oct. 2012, doi: 10.1016/j.tiv.2012.06.006.

Conclusions/action items:

Article Summary:

Action Items:

- For more information review:
  - https://journals.sagepub.com/doi/10.1177/1010428317695931#:~:text=gamma%2DH2AX%20(gH2AX)%20is,to%20cancer%20initiation%20and%20progres

Client Questions_9_8_2024

Emily Rhine - Sep 10, 2024, 12:53 PM CDT

Title: Individual Brainstorming Session for Client Questions

Date: 9/8/2024

Goals: Prepare for next team meeting by drafting a list of potential questions for the client meeting

Content:

Project:

General (PDS Relevant)

- What is the current protocol that did not work?
    - Why did it not work as desired?
- What is the expected frequency and duration of use for our project?
- In what conditions should the device be stored at?
    - 37C in an incubator? Will you provide this or should we figure that out?
    - Assume all conditions are comparable to inside the human body?

Specific

- What kind of cells do you use?
    - Will we acquire them from you or another source?
- What type and stage of cancer are we trying to model using our spheroids?
- Is there a desired spheroid size?
- What qualifies as sufficiently accurate to an *in vivo* (3D) environment?
    - What traits/variables would you like us to consider most?

Client:

- What are you hoping to see at the end of the semester? At the end of the year?
- Since we cannot use the TE lab in ECB 1002 due to Biosafety level 1 regulations, what lab space are you expecting us to use?

Conclusions/ Action Items:

- Review PDS requirements to generate more questions to research or ask during the next client meeting.
- Compare list of questions with the team at the next team meeting.
- Continue preliminary project research.

Role of ECM in Cancer_9_7_2024

Emily Rhine - Sep 08, 2024, 12:11 AM CDT

Title: Role of ECM in Cancer

Date: 9/7/2024

Content by/Present: Emily

Goals: Gain a better understanding of specific cancer characteristics in tissue, cells, and ECM to follow up information found in "3D Cell Culture Models" notebook entry.

Content:

Search Engine: Google

Query: "Is cancer typically in the ECM"

Link: The Functional Role of Extracellular Matrix Proteins in Cancer

Facts and Information of Interest:

- "Extracellular matrix plays a critical role in the development and progression of cancer. The extracellular matrix of the tumor is very different from the matrix of the normal tissue." [1]
- "Mainly fibroblasts produce and regulate matrix remodeling, but in cancer, the tumor matrix also originates from cancer cells. We describe the mechanisms of how the protein composition and structure of the extracellular matrix changes during cancer progression and how abnormal matrix deregulates the behavior of stromal cells and influences cancer progression." [1]
- "Remodeling of the ECM, driven by proteolytic enzymes (such as matrix metalloproteinases) [2], by enzymes that control the modification and cross-linking of extracellular matrix proteins (such as lysyloxidases (LOX)) [3], results in increased stiffness and altered ECM composition." [1]
- "Collagens are the major proteins of the ECM." [1]
- "Compared to stromal cells, cancer cells produce less amount of ECM proteins (<10% in pancreatic cancer) but it was found that a number of cancer-cell–derived proteins can promote tumorigenesis and metastasis and correlate with poor patient survival." [1]
- "Laminins are also involved in cancer progression, including invasion, migration, angiogenesis, metastasis and drug resistance." [1]
- "ECM, and especially cancerized ECM, can be characterized not only by the protein composition and spatial organization but also by such a parameter as stiffness. As it was mentioned in the review, ECM remodeling, driven by proteolytic enzymes and cross-linking enzymes results in increased stiffness of ECM surrounding the tumor. Sometimes conventional cell and molecular biology methods are not enough to characterize complex physico-chemical properties of ECM in cancer.High-resolution microscopy techniques, for instance, second harmonic generation (SHG) or coherent anti-Stokes Raman scattering (CARS), are successfully used to quantify tissue structural changes during cancer progression. It was also reported about the combined use of SHG microscopy and mass spectrometry." [1]
- "Engineering approaches can be used to examine the effects of tumor-associated alterations in the ECM or ECM composition. To explore the molecular mechanisms of tumor progression and metastasis, 3D cancer models can be used for the imitation of key steps of cancer dissemination (invasion, intravasation and angiogenesis). The decellularized matrix allows a comprehensive study of the ECM role in the regulation of cancer cell behavior." [1]

References

[1] N. V. Popova and M. Jücker, “The Functional Role of Extracellular Matrix Proteins in Cancer,” Cancers (Basel), vol. 14, no. 1, p. 238, Jan. 2022, doi: 10.3390/cancers14010238.

Conclusions/action items:

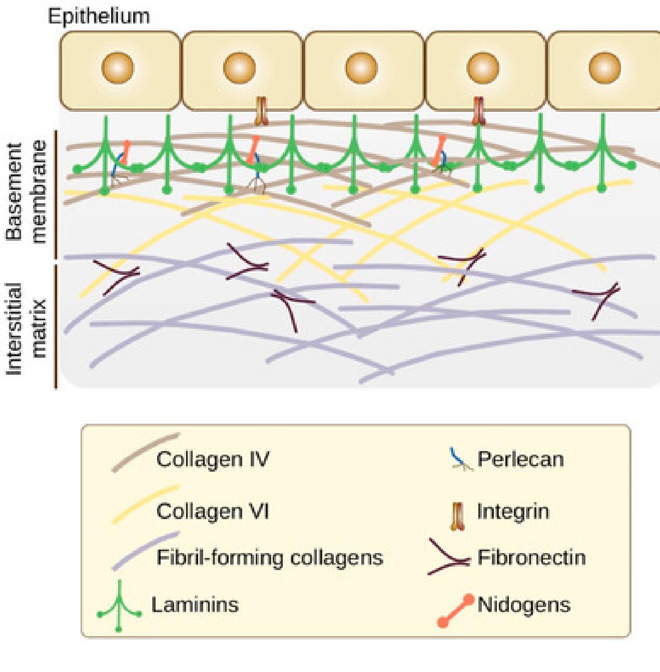
Article Summary:

ECM proteins play a key role in the health of the ECM and the cell in general. When the cell's ECM is effected by a tumor, collagen, laminin, and fibronectin quantity are altering the stiffness of the ECM. Further quantification of the role of each ECM protein in cancer development and metastasis can be observed using knockdown protocols in mice. Additionally, it was suggested that the decellularized matrix may allow a more comprehensive study of the ECM role in the regulation of cancer cell behavior. [1] Most of the information contained in this article was a good review and helped better prepare for 3D modeling cancer spheroids.

Action Items:

- Continue research into CRISPRi technology and techniques to help better understand the project the team has undertaken.
- Begin research into various cell types and eventually narrow down a cell type to propose using for the project.
- Continue brainstorming questions to ask the client in our first meeting.

Emily Rhine - Sep 08, 2024, 12:14 AM CDT



Download

ECM_Example_Diagram.jpg (120 kB)

What to Know About CRISPR Screens_9_10_2024

Emily Rhine - Sep 10, 2024, 3:06 PM CDT

Title: What to Know About CRISPR Screens

Date: 9/10/2024

Content by/Present: Emily

Goals: Gain a better understanding of specific cancer characteristics in tissue, cells, and ECM to follow up information found in "3D Cell Culture Models" notebook entry.

Content:

Search Engine: Google

Query: "CRISPRi Screening in Cancer Spheroids"

Link: What to Know About CRISPR Screens and Bulk Spheroid Production.

Facts and Information of Interest:

- "2D cancer models — the classic cells in a dish — and animal models of cancer have long histories, but both have limitations in terms of their relevance to human cancer and their ability to replicate all the features of diseases." [1]
- "For example, cells on the surface of a spheroid experience different microenvironments than those on the interior, effectively modeling the gradients of oxygen, drug, and nutrient availability in a real tumor." [1]
- "Researchers are combining CRISPR editing and spheroid cultures to perform genome-wide screens in cancer cells. This combination offers cancer researchers the chance to undertake a genome-scale investigation of the effects of gene knockouts or gene modulation on the 3D growth of cells in a more physiologically relevant model system, allowing the discovery of new cancer drivers that may not have a detectable effect in 2D cultures." [1]
- "A research group at Stanford performed genome-wide CRISPR screens on lung adenocarcinoma cells grown in both 2D monolayers and 3D spheroids. They found that compared to 2D screening, the 3D spheroid screen turned up many more growth-promoting genes that are often mutated in cancers. The team used the spheroid screen to identify the gene CPD as a 3D cancer driver and as a potential drug target and prognostic marker." [1]

References

[1] “What to Know About CRISPR Screens and Bulk Spheroid Production.” Accessed: Sep. 10, 2024. [Online]. Available: https://www.corning.com/worldwide/en/products/life-sciences/resources/stories/the-cutting-edge/what-to-know-about-crispr-screens-and-bulk-spheroid-production.html

Conclusions/action items:

Article Summary:

Since 2D models such as tissue culture plastic dishes have limited biological relevance, it is essential that a new, better way of modeling cancer be developed. These spheroids are fairly novel and have many potential uses for better replicating various tissue characteristics and *in vivo* environment. When paired with CRISPR-Cas9 technology, researchers have opened the door to a different approach to modeling cancer and treating it using gene manipulation.

Action Items:

- Ask the client of there is a specific stage or type of cancer our spheroids should be designed to replicate.

- Ask the client if the target environment/ patient is mice or humans.
- Continue research into how to screen to find tumor-specific factors that regulate genome stability.

Generation of cancer spheroids_9_15_2024

Emily Rhine - Sep 15, 2024, 6:57 PM CDT

Title: Generation of cancer spheroids

Date: 9/15/2024

Content by/Present: Emily

Goals: Gain a better understanding of cancer spheroid formulation, tips, and tricks to include in the PDS.

Content:

Search Engine: Google

Query: "Are cancer spheroids translucent?"

Link: PDF Attached

Facts and Information of Interest:

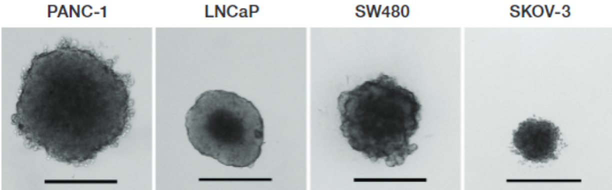
- " An ideal spheroid is translucent with a defined boundary and minimal dark core." [1]
- "Cell viability should be >90%" [1]
- 

Figure 1. Spheroids generated from cancer cell lines on a Nunclon Sphera plate. PANC-1: pancreatic cancer; LNCaP: prostate cancer; SW480: colorectal cancer; SKOV-3: ovarian cancer. A total of 5,000 cells were seeded in each case. Scale bar: 500 µm. [1]

Reference

[1] Thermo Fisher Scientific, "generation-cancer-spheroids-tips-tricks-application-note.pdf," Generation of cancer spheroids—tips and tricks. Accessed: Sep. 15, 2024. [Online]. Available: https://assets.thermofisher.com/TFS-Assets/BID/Application-Notes/generation-cancer-spheroids-tips-tricks-application-note.pdf

Conclusions/action items:

- Since the spheroids should have an overall appearance that will accurately replicate the 3D tumor microenvironment, the spheroids must be: translucent, porous, uniformly spherical, and have a stiffness analogous to the lung cancer cell line selected.
- Update PDS with relevant information.
- Add source to team Zotero for future reference.
- Continue research for "Appearance" section of the PDS.

Emily Rhine - Sep 15, 2024, 5:48 PM CDT

Introduction

Tumor cells grown as spheroids offer an intermediate complexity between cancer cells grown in 2D monolayers and in vivo tumors. This potentiates their use as model systems to study tumor progression as well as to perform high-throughput screening of cytotoxic therapies, including chemotherapies and cell-based treatments.

Cancer spheroids are formed when cells are allowed to grow in suspension, as a result of which they aggregate, either on their own or with the aid of extracellular matrices. There are two factors critical in limiting variation in high-throughput assays with cancer spheroids. First, it is essential to have one spheroid per well in a multiwell plate, to reduce variability in readouts. Second, it is important that spheroids be of uniform shape and size—otherwise there can be variability between experiments. In our lab, we have tested spheroid generation conditions in nine human cell lines belonging to six cancer types. To summarize the results, we have compiled a general workflow and a few tips and tricks that would help in high-throughput generation of uniform and reproducible spheroids in Thermo Scientific™ Nunclon™ Sphera™ multiwell plates. The tips are specific to the cell type tested but can also be referred to for troubleshooting spheroid generation in other cell types.

General workflow

1. On the day of experiment, dissociate cells using **Gibco™ TrypLE™ Express Enzyme** and then neutralize the enzyme using 4 volumes of complete medium (medium will vary depending on cell line chosen).
2. Count cells using the **Invitrogen™ Countess™ II FL Automated Cell Counter**. Cell viability should be >90%.
3. Dilute the suspension at a ratio of 1:10–1:20 in complete medium or a medium containing required additives. Seed the required number of cells in respective wells of **Nunclon Sphera 96-well plates** using **Thermo Scientific™ Finnpipette™ F2 Multichannel Pipettes**.
4. Centrifuge plates at the required speed (250–450 x g) for 5–10 min at room temperature or 4°C, based on the use of additive (e.g., for **Gibco™ Geltrex™ matrix** addition, 4°C is necessary, and for collagen I, a temperature below 15°C is required).
5. Change the medium as necessary until spheroids are ready. Add the medium slowly along the side of the wells without touching the spheroids.

Download

generation-cancer-spheroids-tips-tricks-application-note.pdf (2.86 MB)

TP53_Genetic Mutation_9/27/2024

Emily Rhine - Sep 30, 2024, 9:38 AM CDT

Title: Tumor Protein 53

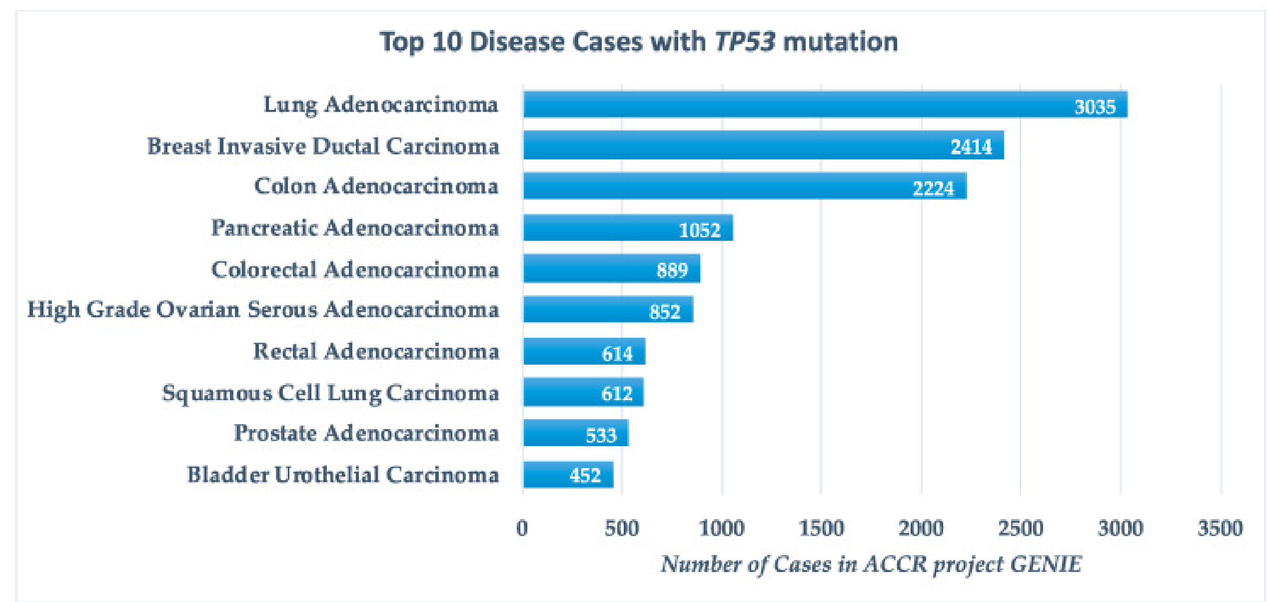
Date: 9/30/2024

Goal: Better understand TP53 and its key role in cell selection for the design matrix.

Content:

Link 1: Therapeutic Editing of the TP53 Gene: Is CRISPR/Cas9 an Option?

Key Facts & Information:

- **"The TP53 gene encodes the transcription factor and oncosuppressor p53 protein that regulates a multitude of intracellular metabolic pathways involved in DNA damage repair, cell cycle arrest, apoptosis, and senescence. In many cases, alterations (e.g., mutations of the TP53 gene) negatively affect these pathways resulting in tumor development. Recent advances in genome manipulation technologies, CRISPR/Cas9, in particular, brought us closer to therapeutic gene editing for the treatment of cancer and hereditary diseases.** Genome-editing therapies for blood disorders, blindness, and cancer are currently being evaluated in clinical trials. Eventually CRISPR/Cas9 technology is expected to target TP53 as the most mutated gene in all types of cancers. A majority of TP53 mutations are missense which brings immense opportunities for the CRISPR/Cas9 system that has been successfully used for correcting single nucleotides in various models, both in vitro and in vivo. In this review, we highlight the recent clinical applications of CRISPR/Cas9 technology for therapeutic genome editing and discuss its perspectives for editing TP53 and regulating transcription of p53 pathway genes." [1]
- **"The TP53 gene encodes the p53 protein, a well-known tumour suppressor involved in various regulatory pathways including cell cycle arrest, apoptosis, senescence and DNA repair. The TP53 is the most frequently mutated gene in human cancer with mutations found in about half of all cancer cases. The Genomics Evidence Neoplasia Information Exchange (GENIE) project by the American Association for Cancer Research (AACR) reports TP53 mutations in 32.8% of cancers, primarily lung adenocarcinoma, breast invasive ductal carcinoma and colon adenocarcinoma."** [1]
- 

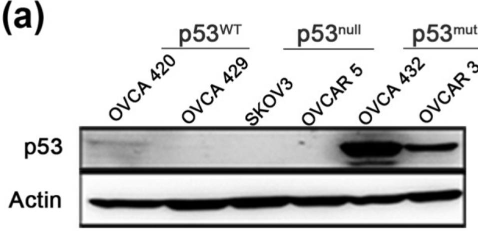
  [1]

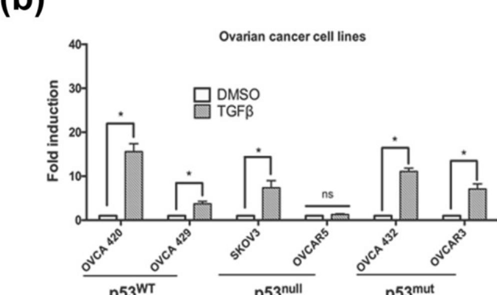
Loading [MathJax]/extensions/Safe.js

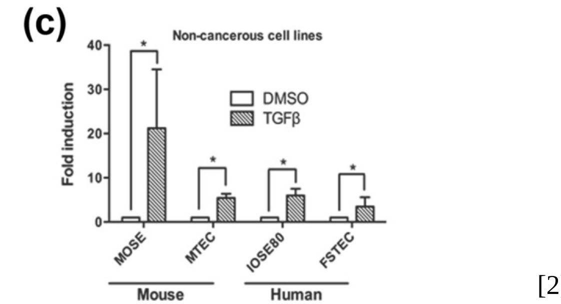
Link 2: Mutation or Loss of p53 Differentially Modifies TGFβ Action in Ovarian Cancer

Facts & Information:

- "To better understand the role of p53 in ovarian cancer, six known ovarian cancer cell lines were analyzed for p53 expression (Fig. 1a). OVCA 420 and 429 cells express low levels of p53 protein, consistent with reports that they have wild-type p53 [32]. Due to these low levels, cisplatin treatment was used to induce and confirm p53 expression in OVCA 429 (Fig. S1). SKOV3 and **OVCAR5 did not show any p53 protein expression, as is consistent with the previous finding that classified them as p53 null.**" [2]





- 

[2]

References

[1] R. Mirgayazova et al., “Therapeutic Editing of the TP53 Gene: Is CRISPR/Cas9 an Option?,” Genes (Basel), vol. 11, no. 6, p. 704, Jun. 2020, doi: 10.3390/genes11060704.

[2] E. Ó hAinmhire, S. M. Quartuccio, W. Cheng, R. A. Ahmed, S. M. King, and J. E. Burdette, “Mutation or Loss of p53 Differentially Modifies TGFβ Action in Ovarian Cancer,” PLoS One, vol. 9, no. 2, p. e89553, Feb. 2014, doi: 10.1371/journal.pone.0089553.

Conclusions & Action Items:

- In conclusion, TP53 is most common in lung adenocarcinoma, but is found in about half of all cancer cases. Due to the importance of this tumor protein in creating a broader application of our research to various other cancer types, OVCAR-5 ranked poorly because it is TP53 null.
- See team "Design Matrix" entry for more on TP53 in the cell lines we are considering for the matrix

Loading [MathJax]/extensions/Safe.js

- See "Lung Cancer Cell Lines" entry for more on TP53

Emily Rhine - Sep 27, 2024, 4:25 PM CDT

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7349023/

Information:

- "The TP53 gene encodes the transcription factor and oncosuppressor p53 protein that regulates a multitude of intracellular metabolic pathways involved in DNA damage repair, cell cycle arrest, apoptosis, and senescence. In many cases, alterations (e.g., mutations of the TP53 gene) negatively affect these pathways resulting in tumor development. Recent advances in genome manipulation technologies, CRISPR/Cas9, in particular, brought us closer to therapeutic gene editing for the treatment of cancer and hereditary diseases. Genome-editing therapies for blood disorders, blindness, and cancer are currently being evaluated in clinical trials. Eventually CRISPR/Cas9 technology is expected to target TP53 as the most mutated gene in all types of cancers. A majority of TP53 mutations are missense which brings immense opportunities for the CRISPR/Cas9 system that has been successfully used for correcting single nucleotides in various models, both in vitro and in vivo. In this review, we highlight the recent clinical applications of CRISPR/Cas9 technology for therapeutic genome editing and discuss its perspectives for editing TP53 and regulating transcription of p53 pathway genes."
- "The TP53 gene encodes the p53 protein, a well-known tumour suppressor involved in various regulatory pathways including cell cycle arrest, apoptosis, senescence and DNA repair. The TP53 is the most frequently mutated gene in human cancer with mutations found in about half of all cancer cases. The Genomics Evidence Neoplasia Information Exchange (GENIE) project by the American Association for Cancer Research (AACR) reports TP53 mutations in 32.8% of cancers, primarily lung adenocarcinoma, breast invasive ductal carcinoma and colon adenocarcinoma (Figure 1)."

Emily Rhine - Sep 27, 2024, 4:38 PM CDT

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3930740/

Loading [MathJax]/extensions/Safe.js

Alexa Fluor 647_10/25/2024

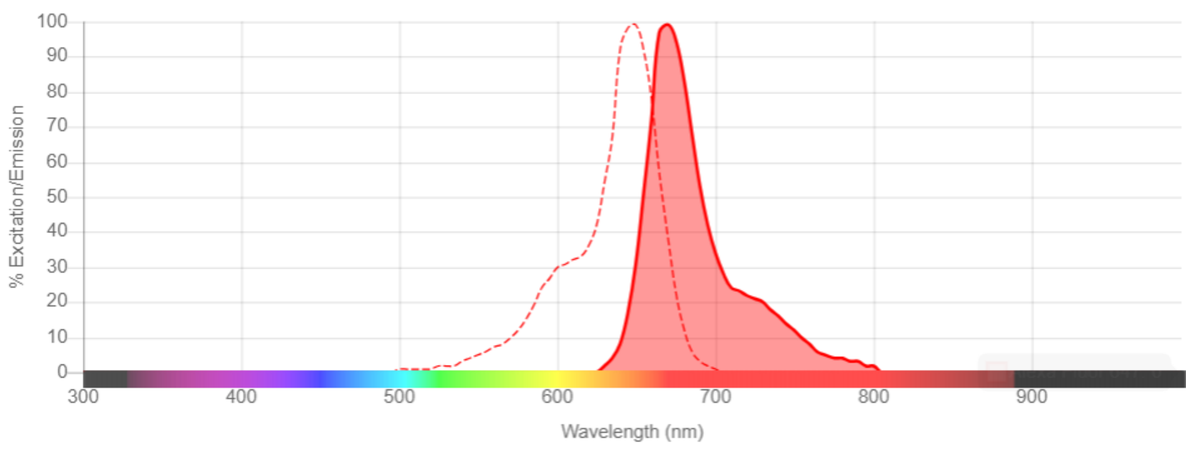
Emily Rhine - Oct 25, 2024, 1:14 PM CDT

Link: Alexa Fluor 647 Dye Profile

About Alexa Fluor 647

Alexa Fluor™ 647 (AF647, Alexa 647) has an excitation peak at 650 nm and an emission peak at 665 nm, and is spectrally similar to Cy®5 (GE Healthcare), iFluor® 647 (ATT Bioquest), and DyLight™ 650 (Thermo Fisher Scientific). Alexa 647 is commonly used for flow cytometry, microscopy, super-resolution microscopy applications. It is very bright, photostable, and pH insensitive, all of which contribute to sensitive detection while using this dye.

Spectra



Thermo Fisher Scientific™

628-640	660/20	650	665	239000	0.33	800	4,029	554	1	4
Laser Line	Common Filter	Excitation Max	Emission Max	Extinction Coefficient	Quantun Efficiency ()	Molecular Weight	Antibodies	Secondary	Conjugation Kits	Services

Spheroid Size Threshold_ImageJ Analysis_11/25/2024

Emily Rhine - Nov 29, 2024, 3:10 PM CST

Title: Spheroid Size Threshold for ImageJ Analysis

Date: 11/25/2024

Goal: Establish an industry standard for spheroid classification.

Content:

Summary: NIH classifies spheroids as having an average of as little as 20 cells/ spheroid [1].

Quotes & Facts:

- "Routine production of spheroids from an average of as little as 20 cells each is straightforward" [1].
- "The larger microwell size is not fully tapered, therefore attempts to form spheroids from small numbers of cells may result in multiple smaller spheroids in each microwell" [1].
- Propose some of these as alternate protocols for spheroid formation if our protocol need further optimization.

References

[1] G. Razian, Y. Yu, and M. Ungrin, “Production of Large Numbers of Size-controlled Tumor Spheroids Using Microwell Plates,” J Vis Exp, no. 81, p. 50665, Nov. 2013, doi: 10.3791/50665.

Conclusions/Action Items:

- Update team with relevant information
- Use information to quantification of spheroids in imageJ across 4 seeding densities
- Set the smallest spheroid with >20 cells as the minimal area threshold to classify as a spheroid for our data analysis

Biosafety Level 2_9_15_2024

Emily Rhine - Sep 15, 2024, 7:11 PM CDT

Title: Biosafety Level 2 Research

Date: 9/15/2024

Content by/Present: Emily

Goals: Gain a better understanding of BSL-2 to include in the "safety" section of the PDS.

Content:

Search Engine: Google

Query: "Biosafety Level 2 Standards"

Link: PDF Attached

Facts and Information of Interest:

- Biosafety Level 2 (BSL-2) is necessary for work with agents associated with human tissue and disease including the human lung cancer cell line which the team will be using [1]. The following universal precautions for BSL-2 are used to reduce the risk of bloodborne pathogens:
  - Laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures [1].
  - Access to the laboratory is restricted when work is being conducted [1].
  - All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment [1].
- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are to be placed in a container with a cover that prevents leakage during handling, processing, and storage [2].
- Although the team does not anticipate the need to remove our cells or cancer spheroids from Hess Lab, the following BSL-2 guideline must be followed.
  - Biological agents prepared for transport must be placed in a secondary leak-proof, unbreakable carrier. Carriers must have the biohazard label, including the identity of the agent, affixed to the outer surface of the transport container [2].

Reference

[1] CDC, “Biosafety in Microbiological and Biomedical Laboratories—6th Edition,” p. 37, Jun. 2020, [Online]. Available: https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

[2] NIH, “Laboratory Biosafety Manual BSL-2 and BSL 2/3.” Accessed: Sep. 12, 2024. [Online]. Available: https://ors.od.nih.gov/sr/dohs/Documents/bsl-2-lab-safety-manual.pdf

Conclusions/action items:

- I already have BSL-2 certification because of my previous lab experience.
- Update PDS with relevant information.
- Add source to team Zotero for future reference.
- Continue research for "Safety" section of the PDS.

Emily Rhine - Sep 15, 2024, 11:34 AM CDT

Laboratory Biosafety Manual
BSL-2 and BSL 2/3

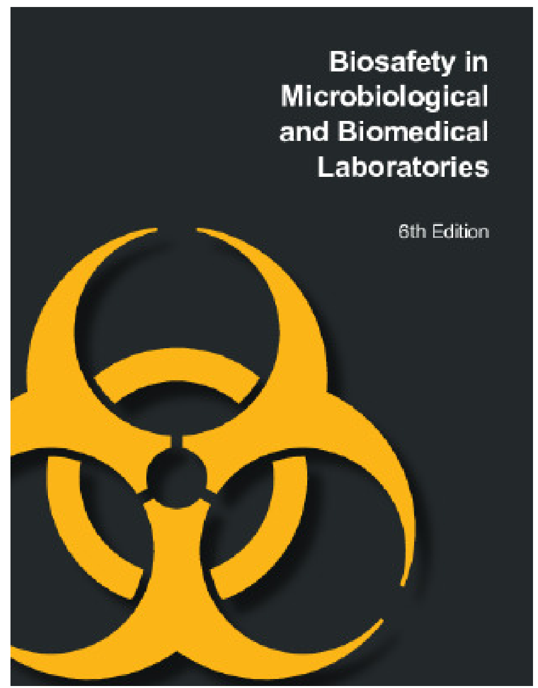
[Principal Investigator]
[Title]
[Institute/Department]
[Laboratory Address/Location]

Prepared by:
[Name/Date]

Download

bsl-2-lab-safety-manual.pdf (328 kB)

Emily Rhine - Sep 15, 2024, 7:10 PM CDT



Download

Biosafety_in_Microbiological_and_Biomedical_Laboratories_6th_Edition.pdf (823 kB) BSL-2 Page 37

Lentivirus_9_15_2024

Emily Rhine - Sep 15, 2024, 7:32 PM CDT

Title: Lentivirus Biosafety Research

Date: 9/15/2024

Content by/Present: Emily

Goals: Gain a better understanding of Lentivirus and necessary safety precautions to include in the "safety" section of the PDS.

Content:

Search Engine: Google

Query: "Biosafety precautions for working wit Lentivirus"

Link: PDF Attached

Summary:

A risk assessment and containment preparations for research with lentiviral vectors should consider (1) the nature of the vector system, (2) transgene insert, and (3) type of manipulations involved [1]. Either BL-2 or enhanced BL-2 will be appropriate for these such experiments. The major risks to be considered for research with HIV-1 based lentivirus vectors are (1) potential for generation of replication-competent lentivirus (RCL) and (2) potential for oncogenesis [1]. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector [1].

Reference

[1] NIH, "Lenti_Containment_Guidance.pdf," Major RAC Concerns. Accessed: Sep. 15, 2024. [Online]. Available: https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf

Conclusions/action items:

- See entry for "Biosafety Level 2" for comprehensive Lentivirus safety precautions.
  - Further research Lentivirus and how it will be used in our project.
- Update PDS with relevant information.
- Continue research for "Safety" section of the PDS.

Emily Rhine - Sep 15, 2024, 11:33 AM CDT

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting ([illegible]), the RAC offered the following findings and recommendations.

Risks of lentivirus vectors: The major risks to be considered for research with HIV-1 based lentivirus vectors are
- potential for generation of replication-competent lentivirus (RCL), and
- potential for oncogenesis.

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

General criteria for risk assessment of lentivirus vectors: Decisions about containment should take into account a range of parameters/considerations including:
- the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
- the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
- the vector titer and the total amount of vector,
- the inherent biological containment of the animal host, if relevant,
- negative RCL testing (see section below)

General containment considerations: Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L).

The appropriate containment level for specific lentivirus vector research is, of course, determined following a complete risk assessment and local IBC review. The following sections discuss some considerations which should form an important part of the biosafety assessment for research involving

lentivirus vectors.

Potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors: The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are

- the number of recombination events necessary to reassemble a replication competent virus genome and

Download

Lenti_Containment_Guidance.pdf (284 kB)

Biosafety Guide to CRISPR_9_15_2024

Emily Rhine - Sep 15, 2024, 7:40 PM CDT

Title: Biosafety Guide to CRISPR

Date: 9/15/2024

Content by/Present: Emily

Goals: Gain a better understanding of the biosafety guidelines for CRISPR for "safety" section of the PDS.

Content:

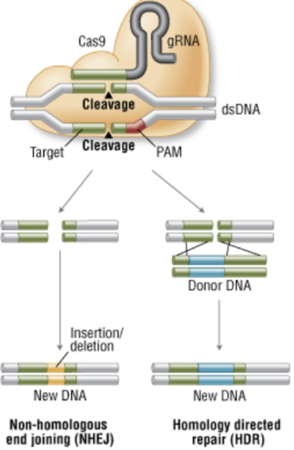
Search Engine: Google

Query: "Biosafety precautions for CRISPR"

Link: PDF Attached

Facts and Information of Interest:

- If genome editing leads to the inactivation of tumor suppressors or activation of oncogenes in human cells, then oncogenesis becomes a possibility in the event of exposure [3]. This is a concern when:
  - Genome editing components are delivered by a method that can enter human cells (e.g., viral vectors that can infect human cells).
  - All components required for genome editing are likely to be introduced in a single exposure event, such as a needle stick or mucosal splash.
  - Genome editing is designed to target human oncogenes/tumor suppressors or can target human oncogenes/tumor suppressors due to homology.



- *From NEB Expressions Issue I, 2014* [1]

Reference

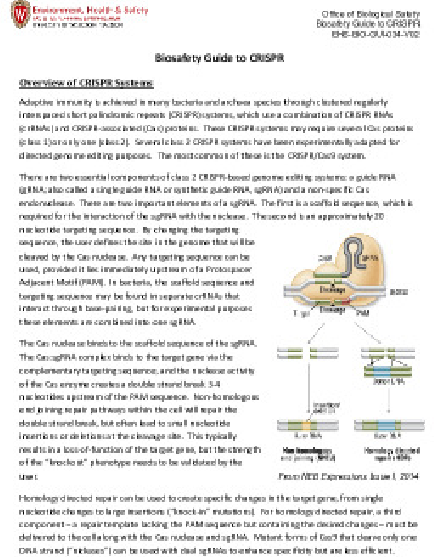
[3] University of Wisconsin-Madison Office of Biological Safety, “Biosafety Guide to CRISPR.” Accessed: Sep. 12, 2024. [Online]. Available: https://ehs.wisc.edu/wp-content/uploads/sites/1408/2022/04/EHS-BIO-GUI-034-V02.pdf

Conclusions/action items:

- Since we will be forming with cancerous cells the possibility of oncogenesis is not inherently an issue. The only issue that may arise from such CRISPR-Cas 9 editing is if the double break results in deletion of a sizable chunk of genetic information (nucleotide sequence).

- Add relevant information to "Safety" section of the PDS.
- See "CRISPRi" entry for more specific information.

Emily Rhine - Sep 15, 2024, 11:35 AM CDT



Environment, Health & Safety

Office of Biological Safety
Biosafety Guide to CRISPR
EHS-BIO-GUI-034-V02

Biosafety Guide to CRISPR

Overview of CRISPR Systems

Adaptive immunity is achieved in many bacteria and archaea species through clustered regularly interspaced short palindromic repeats (CRISPR) systems, which use a combination of CRISPR RNAs (crRNAs) and CRISPR-associated (Cas) proteins. These CRISPR systems may require several Cas proteins (class 1) or only one (class 2). Several class 2 CRISPR systems have been experimentally adapted for directed genome editing purposes. The most common of these is the CRISPR/Cas9 system.

There are two essential components of class 2 CRISPR-based genome editing systems: a guide RNA (gRNA; also called a single guide RNA or synthetic guide RNA, sgRNA) and a non-specific Cas endonuclease. There are two important elements of a sgRNA. The first is a scaffold sequence, which is required for the interaction of the sgRNA with the nuclease. The second is an approximately 20 nucleotide targeting sequence. By changing the targeting sequence, the user defines the site in the genome that will be cleaved by the Cas nuclease. Any targeting sequence can be used, provided it lies immediately upstream of a Protospacer Adjacent Motif (PAM). In bacteria, the scaffold sequence and targeting sequence may be found in separate crRNAs that interact through base-pairing, but for experimental purposes these elements are combined into one sgRNA.

From NEB Expressions Issue I, 2014

The Cas nuclease binds to the scaffold sequence of the sgRNA. The Cas:sgRNA complex binds to the target gene via the complementary targeting sequence, and the nuclease activity of the Cas enzyme creates a double strand break 3-4 nucleotides upstream of the PAM sequence. Non-homologous end joining repair pathways within the cell will repair the double strand break, but often lead to small nucleotide insertions or deletions at the cleavage site. This typically results in a loss-of-function of the target gene, but the strength of the "knockout" phenotype needs to be validated by the user.

Homology directed repair can be used to create specific changes in the target gene, from single nucleotide changes to large insertions ("knock-in" mutations). For homology directed repair, a third component – a repair template lacking the PAM sequence but containing the desired changes – must be delivered to the cell along with the Cas nuclease and sgRNA. Mutant forms of Cas9 that cleave only one DNA strand ("nickases") can be used with dual sgRNAs to enhance specificity but are less efficient.

Page 1 of 3

Download

EHS-BIO-GUI-034-V02.pdf (280 kB)

Human Cancer Cell Lines_Additional Safety Considerations_10/9/2024

Emily Rhine - Oct 10, 2024, 7:22 PM CDT

Title: Human Cancer Cell Lines

Date: 10/10/2024

Content:

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4580382/

Relevant Information:

- "The guidelines for using cell lines in biomedical research, published recently in BJC (Geraghty et al, 2014), include a pioneering safety warning about human-to-human cancer transmission through cancer cells, a route that we will call extracorporeal metastasis (XM). Because XM has been tacitly dismissed as implausible in everyday laboratory practice, if considered at all, we emphasise the warning by reviewing the underlying evidence and preventive measures." [1]
- "even organs free of overt cancerous tumours can still transmit cancer" [1]
- **"The risk of XM does not seem to apply to blood transfusion from donors who previously had cancer, at least to immunocompetent recipients, perhaps because cancer cells do not survive or adhere to the plastic containers during processing and storage of blood" [1]**
- "Such accidents—**pricking yourself with a needle or scalpel previously exposed to cancer cells—are not common**, but by no means extraordinarily rare in the operating room or the laboratory, implying that these two reported cases of transmission may be exceptional only in that XM was noticed, documented and communicated to warn the broader biomedical community." [1]
    - "In one reported case, a sarcoma was transmitted from a patient to the surgeon who pricked his hand during surgery (Gartner et al, 1996). The transmission was noticed and documented only because the pathologist who examined the patient's tumour also happened to examine the surgeon's tumour and noticed that their histopathology was remarkably similar, which prompted the investigation (Gartner et al, 1996). A similar accident occurred in a laboratory at the National Institutes of Health (USA), when a healthy young woman accidentally pricked her hand with a needle 'that had been previously used to draw up a suspension of a human colonic adenocarcinoma cell line' (Gugel and Sanders, 1986). The wound was superficial, but 2 weeks later it produced a nodule formed by the adenocarcinoma cell line. Remarkably, the nodule showed no signs of inflammation (Gugel and Sanders, 1986), highlighting the ability of cancer cells to avoid immune surveillance." [1]

Reference

[1] Y. Lazebnik and G. E. Parris, "Comment on: 'Guidelines for the use of cell lines in biomedical research': human-to-human cancer transmission as a laboratory safety concern," *Br J Cancer*, vol. 112, no. 12, pp. 1976–1977, Jun. 2015, doi: 10.1038/bjc.2014.656.

Conclusions Action Items:

- It is incredibly important that we adhere to BSL-2 regulations to limit the potential of becoming a new host for our selected cancer cells.
- We must also be extremely cautions when working with lentivirus!

Tissue Engineering Standard_9_12_2024

Emily Rhine - Sep 12, 2024, 9:20 PM CDT

Title: Tissue Engineering Standard

Date: 9/12/2024

Goals: Better understand any standards our team must consider in the design and fabrication of our prototype.

Content:

Google Query: Tissue Engineering Standard

Link: ISO/TS 21560:2020 "General requirements of tissue-engineered medical products"

Summary:

"This document specifies general requirements for tissue-engineered medical products (TEMPs), which are used in regenerative medicine. With regard to safety, this document outlines requirements for materials, manufacture, quality control, and unintentional biological effects elicited by TEMPs. This document does not address requirements for clinical trials and efficacy." [1]

1. This document is not applicable to tissue-engineered products used for diagnosis, ex-vivo testing or extracorporeal treatments of patients (e.g. dialysis with TEMP components). [1]
2. TEMPs containing viable xenogenic cells, genetically modified cells, or cells derived from abnormal cells or tissues (e.g. cancerous tissues) are also excluded from the scope. [1]
3. The combination of TEMPs with medical devices, with the exception of scaffolds comprised of synthetic and/or naturally-derived (e.g. animal sourced) materials, is also excluded from the scope. [1]

[1]

Summary:

After comparing this standard with the scope of our project, I have determined that we do not have to comply with it for the following reasons:

- We will not be doing human, *in vivo*, testing, implantation, or experimentation.
- Our tissue-engineered product will primarily be used for diagnosis

References

[1]14:00-17:00, “ISO/TS 21560:2020,” ISO. Accessed: Sep. 13, 2024. [Online]. Available: https://www.iso.org/standard/71086.html

Conclusions/Action Items:

- Although this standard may not currently apply, future iterations of this project may require a check-in to ensure the project complies if it falls under this standard.
- Continue research into applicable standards and patents to better understand the scope of our project and potential IP space.
- Sign into Benchling and prepare for client meeting by reviewing more literature.

Cancer Spheroid Patent_9_12_2024

Emily Rhine - Sep 12, 2024, 9:32 PM CDT

Title: Cancer Spheroid Patent

Date: 9/12/2024

Goals: Better understand various uses and patents associated with 3D cancer spheroids.

Content:

Google Query: "Cancer Spheroid Patent"

Link: US11098369B2: Methods for evaluating tumor cell spheroids using 3D microfluidic cell culture device

Summary:

- "Provided herein are methods for evaluating tumor cell spheroids in a three-dimensional microfluidic device by determining changes in the relative levels of live cells and dead cells in aliquots cultured under different conditions. Methods described herein allow ex vivo recapitulation of the tumor microenvironment such that the in vivo effectiveness of a test compound in treating tumor tissue may be predicted."
- The first three steps under the claim may be applicable to our project:
    - A method for evaluating primary patient-derived tumor cell spheroids in a three-dimensional microfluidic device, the method comprising:
        1. obtaining primary patient-derived tumor cell spheroids from an enzyme treated tumor sample,
        2. suspending a first aliquot of the primary patient-derived tumor cell spheroids in a biocompatible gel,
        3. suspending a second aliquot of the primary patient-derived tumor cell spheroids in a biocompatible gel,

References

[1] D. Barbie, R. W. Jenkins, C. P. Paweletz, E. Ivanova, and A. Aref, “Methods for evaluating tumor cell spheroids using 3D microfluidic cell culture device,” US11098369B2, Aug. 24, 2021 Accessed: Sep. 13, 2024. [Online]. Available: https://patents.google.com/patent/US11098369B2/en

Conclusions/Action Items:

- Continue research into applicable standards and patents to better understand the scope of our project and potential IP space.
- Reviewing the first three steps of the "method for evaluating primary patient-derived tumor cell spheroids" may be useful when we are determining our spheroid protocol.
- Add to "competing devices" section of PDS

Other Relevant Sources

Emily Rhine - Sep 15, 2024, 7:43 PM CDT

9/15/2024

- Authenticated Lung Cancer Cell Lines for Cancer Research
- A Reliable Flow-Based Method for the Accurate Measure of Mass Density, Size and Weight of Live 3D Tumor Spheroids
- Lung Adenocarcinoma

Emily Rhine - Oct 10, 2024, 7:23 PM CDT

10/10/2024

- Comment on: ‘Guidelines for the use of cell lines in biomedical research': human-to-human cancer transmission as a laboratory safety concern

Loading [MathJax]/extensions/Safe.js

Lung Cancer Cell Lines_9/19/2024

Emily Rhine - Sep 20, 2024, 12:08 PM CDT

Title: Lung Cancer Cell Lines

Date: 9/7/2024

Content by/Present: Emily

Goals: Research lung cancer cell lines to propose for the design matrix.

Content:

- Lung Cancer Types
NSCLC (Frequency: 85%)	SCLC (Frequency: 15%)
Squamous cell carcinoma • Arises from the proximal airway; more closely correlated with smoking than other NSCLC • Origin: Bronchial epithelium cells that line airways **Adenocarcinomas** • Arise from the distal airway; dominant subtype in nonsmoking patients • Origin: mucus-producing glands and cells	**Small-cell lung cancer** • Arises from neuroendocrine cells due to heavy carcinogen exposure. • Origin: Tracheal basal cell progenitors, pulmonary neuroendocrine cells[2]
Cell lines: PC9, LO68, LUDLU-1, COR-L105, SKLU1, SKMES1, NCI-H727, LC-2/AD, NCIH358, ChaGo-K-1, MOR/CPR, MOR/0.4R, MOR/0.2R, NCIH-322	Cell lines: H69, H69V, CORL-47, COR-L51, COR-L88, DMS53, DMS79, DMS454, COR-L311, COR L303, COR-L95, NCI-H69/CPR, NCI-H69/LX4, NCI-H69/LX10 , NCI-H69/LX20, SHP-77, NCI-H510A, DMS92, DMS153, COR-L279, DMS 273

      [1]
- Ideal Cell Lines
    - Good viability
    - High spheroid adhesion
    - Fast Reproduction Speed
    - DNA Damage Treated
    - Specific Genetic Mutations: "Genetic predisposition amplifies the risk of lung cancer, and lung adenocarcinoma is more associated than other histotypes with genetic factors." [1]

Mutated gene	Cell lines
TP53	PC9, NCIH-322, LC-2/AD, DMS 273, NCI-H69/CPR, COR-L279, NCI-H727, SHP-77, DMS153, LUDLU-1
LRP1B	NCIH-322, DMS 273, CALU 1, COR-L279, NCI-H727, SHP-77, DMS153, CORL-47, DMS 79, DMS 454
KRAS	A549, CALU 1, CORL23, NCI-H727, SHP-77, SKLU1, MOR/CPR, NCIH358
KEAP1	A549, SHP-77, LUDLU-1, MOR/CPR, DMS 454
KMT2C	A549, CORL23, COR-L47, SKLU1, COR-L105, DMS 454, SKMES1, NCIH358
FAT4	A549, NCI-H727, COR-L47, ChaGo-K-1
CDKN2A	PC9, CORL23
EGFR	PC9, SK MES 1
FAT2	NCI-H358

        - **Table 1.** Lung cancer cell lines with specific mutations [1]
- Non-small Human Lung Cancer Cell Line Options:
    - PC9

Loading [MathJax]/extensions/Safe.js

        - "PC-9, a lung adenocarcinoma cell line employed to study effects of small molecule inhibitors" [1]
        - Mutations for TP53, CDKN2A, and EGFR
    - LUDLU-1
        - Human Caucasian lung squamous cell carcinoma
            - Product no. 92012463
        - Genetic Mutations for TP53 & KEAP1
    - COR-L105
        - Human Caucasian lung adenocarcinoma
            - Product no. 92031918
        - Mutated KMT2C gene
    - SKLU1
        - Human Caucasian lung adenocarcinoma
            - Product no. 93120835
        - Mutations for KRAS & KMT2C
    - SKMES1
        - Human Caucasian lung squamous carcinoma
            - Product no. 93120837
        - Mutation for KMT2C
    - LC-2/AD
        - Human lung adenocarcinoma
            - Product no. 94072247
        - Mutation for TP53
    - NCIH-322
        - Human Caucasian bronchioalveolar carcinoma
            - Product no. 95111734
        - Mutations for TP53 and LRP1B
        - "Effect of ganetespib/erlotinib therapy was evaluated in non-small cell lung cancer (NCI-H322) xenograft tumors" [1]
    - NCI-H727
        - Human lung non-small cell carcinoma
            - Product no. 94060303
        - Mutations for TP53, LRP1B, KRAS, and FAT4
    - NCIH358
        - Human Caucasian bronchioalveolar carcinoma
            - "A complete homozygous deletion of the p53 gene and therefore a lack of p53 protein has been reported." [2]
            - Product no. 95111733
        - Mutation for FAT2
    - ChaGo-K-1
        - Human lung bronchus carcinoma
            - Product no. 96020948
        - Mutation for FAT4
    - MOR/CPR
        - Human lung adenocarcinoma, drug-resistant
            - Product no. 96042333
        - Mutations for KRAS and KEAP1
    - MOR/0.4R
        - Human lung adenocarcinoma, drug-resistant
            - Product no. 96042334
    - MOR/0.2R

Loading [MathJax]/extensions/Safe.js

- Human lung adenocarcinoma, drug-resistant
  - Product no. 96042335
- LO68
  - Human malignant mesothelioma
    - Product no. 10092311

Table 1. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [1].

Cell lines	Representative mutations
PC9	TP53, CDKN2A, and EGFR
LUDLU-1	TP53 & KEAP1
SKLU1	KRAS & KMT2C
NCIH-322	TP53 and LRP1B
COR-L105	KMT2C
LC-2/AD	TP53
NCI-H727	TP53, LRP1B, KRAS, and FAT4

References

[1] "Authenticated Lung Cancer Cell Lines." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[2] "NCI-H358 95111733 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: http://www.sigmaaldrich.com/

Conclusions/action items:

- Separate possible cell lines into various cancer sub-groups and organize based on mutation.
  - Research the cancer type and mutation type to see what is best for our project
- Continue cell line research in order to come up with the top 3 potential cell lines to put in the design matrix.

Emily Rhine - Sep 19, 2024, 4:43 PM CDT

https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

Loading [MathJax]/extensions/Safe.js

Rudimentary Cell Line Comparison_9/19/2024_Sigma Aldrich

Emily Rhine - Sep 27, 2024, 4:14 PM CDT

Title: Rudimentary Cell Line Comparison

Date: 9/19/2024

Content:

Ideal Cell Lines have the following traits:

- Good viability
- High spheroid adhesion
- Fast Reproduction Speed
- DNA Damage Treated
- Specific Genetic Mutations

Table 1: An excel file describing 10 lung cancer cell lines with their representative background mutations, and results from CRISPR batch-retest screens in these lines [1].

Cell lines	Representative mutations
H1437	TP53, CDKN2A
H1568	TP53, SMARCA4
H1650	TP53, CDKN2A, EGFR, SMARCA4
H1792	KRAS G12C, TP53, KEAP1
H1975	EGFR, TP53, PIK3CA, CDKN2A
H2009	KRAS G12A, TP53
H23	KRAS G12C, TP53, LKB1, KEAP1
H322	TP53, KEAP1
H358	KRAS G12C, TP53
A549	KRAS G12S, LKB1, KEAP1

Table 2. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [2].

Cell lines	Representative mutations
PC9	TP53, CDKN2A, and EGFR
LUDLU-1	TP53, KEAP1
SKLU1	KRAS, KMT2C*
NCIH-322	TP53, LRP1B
COR-L105	KMT2C*
LC-2/AD	TP53
NCI-H727	TP53, LRP1B, KRAS, and FAT4*

* KMT2C and FAT4 were genetic mutations not included in the study our project is based off of, so I rejected them from the potential cell list.

Additional Gene Mutation Content:

- KMT2C **-** lysine (K)-specific methyltransferase 2C: "This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation." [3]
- TP53 **-** tumor protein p53: "This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons." [4]
- KRAS **-** Kirsten rat sarcoma viral oncogene homolog: "This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region." [5]
- CDKN2A **-** cyclin-dependent kinase inhibitor 2A: "This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene." [6]
- KEAP1 **-** kelch-like ECH-associated protein 1: "This gene encodes a protein containing KELCH-1 like domains, as well as a BTB/POZ domain. Kelch-like ECH-associated protein 1 interacts with NF-E2-related factor 2 in a redox-sensitive manner and the dissociation of the proteins in the cytoplasm is followed by transportation of NF-E2-related factor 2 to the nucleus. This interaction results in the expression of the catalytic subunit of gamma-glutamylcysteine synthetase. Two alternatively spliced transcript variants encoding the same isoform have been found for this gene." [7]
- EGFR **-** epidermal growth factor receptor: "The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer. Multiple alternatively spliced transcript variants that encode different protein isoforms have been found for this gene." [8]

References

[1] K. Han et al., “CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities,” Nature, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

[2] "Authenticated Lung Cancer Cell Lines." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[3] "KMT2C lysine (K)-specific methyltransferase 2C HALR MLL3 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/kmt2c

[4] "TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp53

[5] "KRAS Kirsten rat sarcoma viral oncogene homolog C-K-RAS CFC2 K-RAS2A K-RAS2B K-RAS4A K-RAS4B KI-RAS KRAS1 KRAS2 NS NS3 RASK2 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/kras

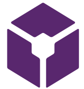
[6] "CDKN2A cyclin-dependent kinase inhibitor 2A ARF CDK4I CDKN2 CMM2 INK4 INK4A MLM MTS-1 MTS1 P14 P14ARF P16 P16-INK4A P16INK4 P16INK4A P19 P19ARF TP16 | Sigma-Aldrich." Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/cdkn2a

[7] "KEAP1 kelch-like ECH-associated protein 1 INrf2 KLHL19 | Sigma-Aldrich." Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/keap1

[8] "EGFR epidermal growth factor receptor ERBB ERBB1 HER1 PIG61 mENA | Sigma-Aldrich." Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/egfr

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry
- I propose the following 4 cell lines be considered for the design matrix:
  - PC9
  - LUDLU-1
  - NCIH-322
  - LC-2/AD
- Continue research on the selected 4 cell lines to ensure they have characteristics from the "Ideal Cell Line Traits List" as seen above.



PC-9_Human adenocarcinoma_9/19/2024

Emily Rhine - Sep 20, 2024, 7:44 PM CDT

Title: PC-9

Date: 9/20/2024

Content:

Ideal Cell Lines have the following traits:

- Good viability
- High spheroid adhesion
- Fast Reproduction Speed
- DNA Damage Treated
- Specific Genetic Mutations

Table 1. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [1].

Cell lines	Representative mutations
PC9	TP53, CDKN2A, and EGFR

Additional Gene Mutation Content:

- TP53 - tumor protein p53: "This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons." [2]
- CDKN2A - cyclin-dependent kinase inhibitor 2A: "This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene." [3]
- EGFR - epidermal growth factor receptor: "The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer. Multiple alternatively spliced transcript variants that encode different protein isoforms have been found for this gene." [4]

Loading [MathJax]/extensions/Safe.js

[5]

References

[1] “Authenticated Lung Cancer Cell Lines.” Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[2] “TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich.” Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp534

[3] “CDKN2A cyclin-dependent kinase inhibitor 2A ARF CDK4I CDKN2 CMM2 INK4 INK4A MLM MTS-1 MTS1 P14 P14ARF P16 P16-INK4A P16INK4 P16INK4A P19 P19ARF TP16 | Sigma-Aldrich.” Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/cdkn2a

[4] “EGFR epidermal growth factor receptor ERBB ERBB1 HER1 PIG61 mENA | Sigma-Aldrich.” Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/egfr

[5] “Authenticated PC-9 Cell Line Sigma Aldrich.” Accessed: Sep. 20, 2024. [Online]. Available: http://www.sigmaaldrich.com/

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry & "Rudimentary Cell Line Comparison" entry
- Continue individual cell line research to find 1 cell line to recommend to the team
- Compare 4 selected cell lines after all have been researched and evaluated.

Emily Rhine - Sep 19, 2024, 8:11 PM CDT

https://www.sigmaaldrich.com/US/en/product/sigma/cb_90071810

Loading [MathJax]/extensions/Safe.js

LC-2/AD_Human lung adenocarcinoma_9/23/2024

Emily Rhine - Sep 23, 2024, 11:05 AM CDT

Title: LC-2AD

Date: 9/23/2024

Content:

LC-2AD

- "Established from the pleural effusion of a pulmonary adenocarcinoma in a 51 year-old Japanese female. LC-2/ad cells exhibit an epithelial appearance and a tendency to form small domes. Immunocytochemically the cells are positive for CEA and cytokeratins, including cytokeratin No.18. LC-2/ad produces at least two functionally active trypsin inhibitors together with several proteinases in vitro." [1]

Table 1. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [2].

Cell lines	Representative mutations
LC-2/AD	TP53

PROPERTIES

biological source	human lung
growth mode	Adherent
karyotype	Modal No 53-56
morphology	Epithelial
products	alpha-1-anti-trypsin
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	cancer
shipped in	dry ice
storage temp.	−196°C

[1]

Additional Gene Mutation Content:

- TP53 - tumor protein p53: "This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons." [3]

References

Loading [MathJax]/extensions/Safe.js

[1] “Authenticated MRC-5 PD30 Cell Line Sigma Aldrich.” Accessed: Sep. 23, 2024. [Online]. Available: http://www.sigmaaldrich.com/

[2] “Authenticated Lung Cancer Cell Lines.” Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[3] “TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich.” Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp534

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry & "Rudimentary Cell Line Comparison" entry
- Continue individual cell line research to find 1 cell line to recommend to the team
- Compare 4 selected cell lines after all have been researched and evaluated.

Emily Rhine - Sep 19, 2024, 8:13 PM CDT

https://www.sigmaaldrich.com/US/en/product/sigma/cb_94072247

Loading [MathJax]/extensions/Safe.js

LUDLU-1_Human Caucasian lung squamous cell carcinoma_9/23/2024

Emily Rhine - Sep 23, 2024, 11:08 AM CDT

Title: LUDlU-1

Date: 9/23/2024

Content:

LUDLU-1

- "Derived from a lung squamous cell carcinoma of a 72 year old Caucasian male. The B-lymphoblastoid cell line AGLCL (Sigma Catalogue number. 89120566) was derived from the same patient. Cells grow as large swollen aggregates, which will detach and eventually grow in suspension." [1]

Table 1. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [2].

Cell lines	Representative mutations
LUDLU-1	TP53, KEAP1

PROPERTIES

biological source	human lung
growth mode	Adherent
karyotype	Not specified
morphology	Epithelial
products	Not specified
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	cancer
shipped in	dry ice
storage temp.	−196°C

[1]

Additional Gene Mutation Content:

- TP53 - tumor protein p53: "This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons." [3]
- KEAP1 - kelch-like ECH-associated protein 1: "This gene encodes a protein containing KELCH-1 like domains, as well as a BTB/POZ domain. Kelch-like ECH-associated protein 1 interacts with NF-E2-related factor 2 in a redox-sensitive manner and the dissociation of the proteins in the cytoplasm is followed by transportation of NF-E2-related factor 2 to the nucleus. This interaction results in the expression of the catalytic subunit of gamma-glutamylcysteine synthetase. Two alternatively spliced transcript variants encoding the same isoform have been found for this gene." [4]

Loading [MathJax]/extensions/Safe.js

References

[1] "LUDLU-1 92012463 | Sigma-Aldrich." Accessed: Sep. 23, 2024. [Online]. Available: http://www.sigmaaldrich.com/

[2] "Authenticated Lung Cancer Cell Lines." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[3] "TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp534

[4] "KEAP1 kelch-like ECH-associated protein 1 INrf2 KLHL19 | Sigma-Aldrich." Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/keap1

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry & "Rudimentary Cell Line Comparison" entry
- Continue individual cell line research to find 1 cell line to recommend to the team
- Compare 4 selected cell lines after all have been researched and evaluated.

Emily Rhine - Sep 19, 2024, 8:13 PM CDT

https://www.sigmaaldrich.com/US/en/product/sigma/cb_92012463

Loading [MathJax]/extensions/Safe.js

NCI-H322_Human Caucasian bronchioalveolar carcinoma_9/23/2024

Emily Rhine - Sep 23, 2024, 10:58 AM CDT

Title: NCl-H322

Date: 9/23/2024

Content:

NCIH-322

- Human Caucasian bronchioalveolar carcinoma
  - Product no. 95111734
- Mutations for TP53 and LRP1B
- "Effect of ganetespib/erlotinib therapy was evaluated in non-small cell lung cancer (NCI-H322) xenograft tumors" [1]

Table 1. Sigma Aldrich reduced list of potential non-small-cell lung cancer (NSCLC) cell lines [1].

Cell lines	Representative mutations
NCIH-322	TP53, LRP1B

PROPERTIES

biological source	human lung (cervical node metastasis)
description	Human Caucasian bronchioalveolar carcinoma
growth mode	Adherent
karyotype	Not specified
morphology	Not specified
products	Not specified
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	metastasis
shipped in	dry ice

[2]

Additional Gene Mutation Content:

- TP53 - tumor protein p53: "This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons." [3]
- LRP1B - low density lipoprotein receptor-related protein 1B: " LRP1B belongs to the low density lipoprotein (LDL) receptor gene family. These receptors play a wide variety of roles in normal cell function and development due to their interactions with multiple ligands." [4]

References

Loading [MathJax]/extensions/Safe.js

[1] "Authenticated Lung Cancer Cell Lines." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

[2] "Authenticated NCI-H322 Cell Line Sigma Aldrich." Accessed: Sep. 23, 2024. [Online]. Available: http://www.sigmaaldrich.com/

[3] "TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp534

[4] "LRP1B low density lipoprotein receptor-related protein 1B LRP-DIT LRPDIT | Sigma-Aldrich." Accessed: Sep. 23, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/lrp1b

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry & "Rudimentary Cell Line Comparison" entry
- Continue individual cell line research to find 1 cell line to recommend to the team
- Compare 4 selected cell lines after all have been researched and evaluated.

Emily Rhine - Sep 23, 2024, 9:50 AM CDT

https://www.sigmaaldrich.com/US/en/product/sigma/cb_95111734

Loading [MathJax]/extensions/Safe.js

OVCAR-5_9/27/2024

Emily Rhine - Sep 30, 2024, 11:05 AM CDT

Title: OVCAR-5

Date:9/27/2024

Goals: Better understand OVCAR-5 and its key properties to rank it correctly in the team's design matrix.

Content:

Link: OVCAR-5 Human Cancer Cell Line (see additional PDF below)

Facts & Information:

- "Ovarian carcinoma is a prevalent worldwide disease, with over 200,000 new cases diagnosed per year (1). Metastatic gastrointestinal cancer frequently presents as advanced ovarian carcinoma (2). The availability of cellular models that recapitulate the spectrum of forms of ovarian cancer are essential to understanding drug resistance, cellular physiology, and advancing new options for treatment." [1]
- "The OVCAR-5 cell line was established from ascites fluid from a non-treated patient with an advanced-stage ovarian tumor (3). The OVCAR-5 cell line has recently been classified as originating from the gastrointestinal tract, not of ovarian origin (4). OVCAR-5 cells harbor a homozygous Gly12Val mutation in the KRAS oncogene and are characterized by migration/invasion ability as well as exhibiting tumorigenicity in nude mice (5). OVCAR-5 cells overexpress claudin-4, a marker that plays a role in cancer malignancy (6). OVCAR-5 cells exhibit aggressive growth and invasion and represent a valuable model for metastatic gastrointestinal carcinoma." [1]
- Mutation Summary:
    - Homozygous Gly12Val mutation in the KRAS oncogene
    - OVCAR-5 cells overexpress claudin-4, a marker that plays a role in cancer malignancy.
    - EGFR -OVCAR-5 cells exhibit aggressive growth and invasion and represent a valuable model for metastatic gastrointestinal carcinoma.
- Only let OVCAR5 get to "~80-85% confluence" before passaging [1]

References

[1] "OVCAR-5 Human Cancer Cell Line | SCC259." Accessed: Sep. 27, 2024. [Online]. Available: https://www.emdmillipore.com/US/en/product/OVCAR-5-Human-Cancer-Cell-Line,MM_NF-SCC259?ReferrerURL=https%3A%2F%2Fwww.google.com%2F

Conclusions & Action Items:

- OVCAR-5 expresses several gene mutations common to cancer and would be helpful for modeling a larger population of ovarian cancer.
- However, OVCAR-5 does not express TP53 see Background folder > "TP53_Genetic Muation" entry
- See team "Design Matrix" entry for more information of OVCAR-5

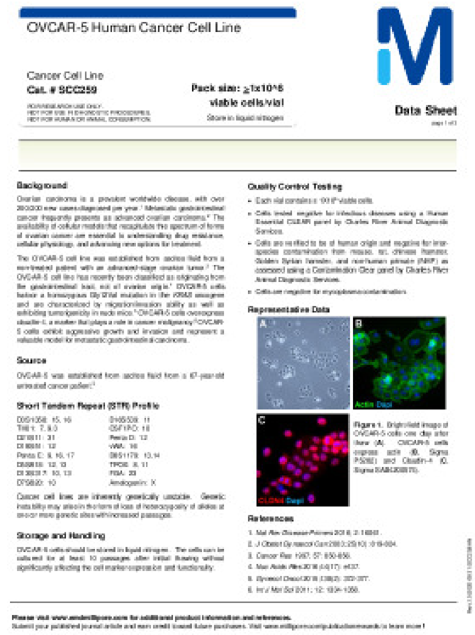
Loading [MathJax]/extensions/Safe.js

Emily Rhine - Sep 27, 2024, 5:43 PM CDT

OVCAR-5 Human Cancer Cell Line

https://www.emdmillipore.com/US/en/product/OVCAR-5-Human-Cancer-Cell-Line,MM_NF-SCC259?ReferrerURL=https%3A%2F%2Fwww.google.com%2F

Emily Rhine - Sep 27, 2024, 5:43 PM CDT



OVCAR-5 Human Cancer Cell Line

Cancer Cell Line
Cat. # SCC259

Pack size: ≥1x10^6 viable cells/vial

Data Sheet

Background

Source

Short Tandem Repeat (STR) Profile

Storage and Handling

Quality Control Testing

Representative Data

References

Download

scc259ds.pdf (211 kB)

Emily Rhine - Sep 30, 2024, 9:51 AM CDT

Loading [MathJax]/extensions/Safe.js

ATCC_Possible Cell Lines_9/20/2024

Emily Rhine - Sep 23, 2024, 11:29 AM CDT

Title: ATCC Cell Lines

Date: 9/23/2024

Content by/Present: Emily

Goals: Research lung cancer cell lines specifically from ATCC to propose for the design matrix.

Content:

Link: https://www.atcc.org/cell-products/human-cells#t=productTab&numberOfResults=24&f:Productcategory=[Human%20cells]&f:Productapplication=[3D%20cell%20culture]&f:Biosafetylevel=[BSL%202]&f:Tissue=[Lung]&f:Disease=[Carcinoma]

From the ATCC website I narrowed down my search by only looking for:

- Human cells
- Lung cells
- Carcinoma cells
- BSL-2
- Used for 3D cell culture

After inputting these limitations only 4 options were available from this website (see screenshot below) [1].

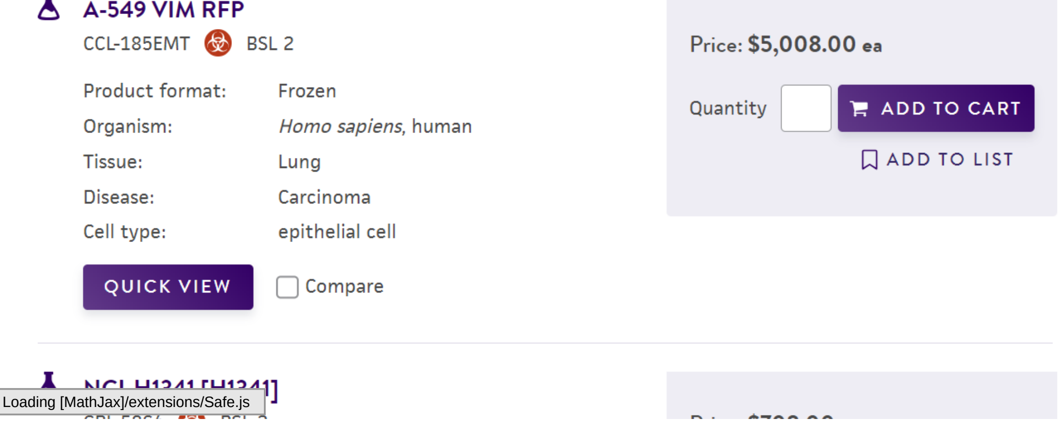
References

[1] "Human Cells." Accessed: Sep. 23, 2024. [Online]. Available: https://www.atcc.org/cell-products/human-cells

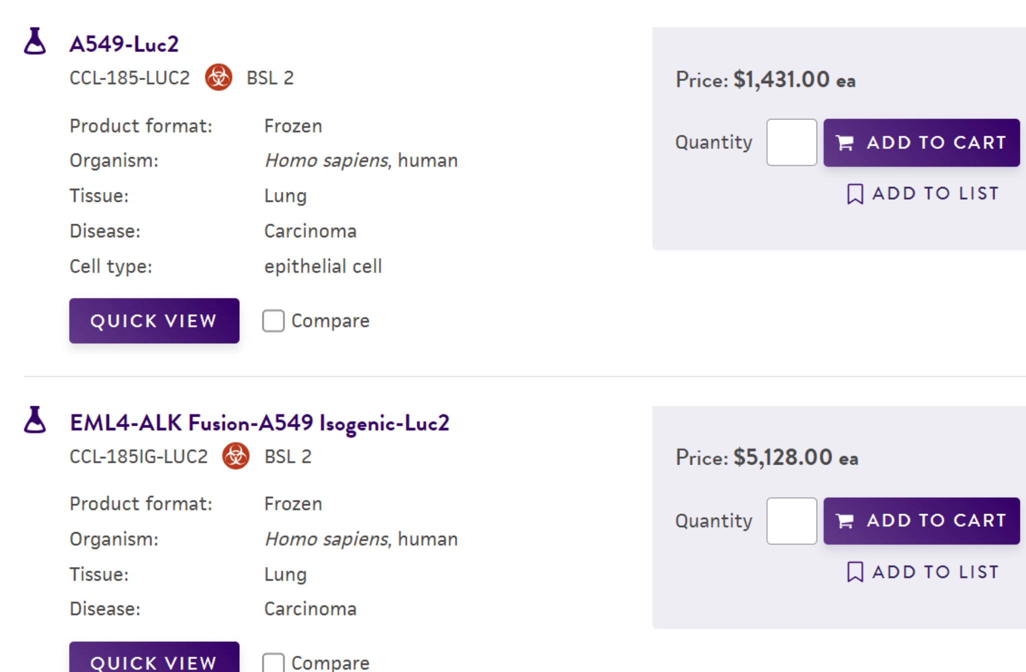
Conclusions/Action Items:

- Continue research on these 4 specific cell lines to check their mutations, viability, adhesion, and replication speed.
- Most of these cells are far too expensive
- Ask the client if he has a preferred business to buy cells from
- **Of the 4 possible cell lines found only 1 falls within the client's specified budget: H1341**

Emily Rhine - Sep 23, 2024, 11:10 AM CDT



Loading [MathJax]/extensions/Safe.js



Loading [MathJax]/extensions/Safe.js

H1314_9.23.2024

Emily Rhine - Sep 23, 2024, 11:29 AM CDT

Title: NCI-H1341 [H1341]

Date: 9/23/2024

Content:

- "NCI-H1341 [H1341] is a cell line that was isolated from the lungs of a non-smoker female with carcinoma. This product can be used for cancer research." [1]

Product category	Human cells
Organism	*Homo sapiens*, human
Morphology	floating aggregates of round cells with a few attached cells.
Tissue	Lung
Disease	Carcinoma; Small cell lung cancer
Applications	3D cell culture Cancer research
Product format	Frozen

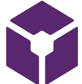
  - [1]
  - **Gene mutation information not given**

References

[1] “NCI-H1341 [H1341] - CRL-5864 | ATCC.” Accessed: Sep. 23, 2024. [Online]. Available: https://www.atcc.org/products/crl-5864

Conclusion/Action Items:

- See "Lung Cancer Cell Lines" entry & "Rudimentary Cell Line Comparison" entry
- Continue individual cell line research to find 1 cell line to recommend to the team
- Compare 4 selected cell lines after all have been researched and evaluated.
- **This cell line was disregarded due to its small cell lung cancer classification.**



Preliminary TFS Website Search_9/23/2024

Emily Rhine - Sep 23, 2024, 12:39 PM CDT

Title: TFS Lung Cancer Cell Lines

Date: 9/23/2024

Content by/Present: Emily

Goals: Research lung cancer cell lines to propose for the design matrix.

Content:

- Narrowed down possible cell lines
    - Human
    - Lung

Complete list from criteria above:

- H187 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-291.html
    - small cell lung cancer cells

- H441 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-294.html
- H1299 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-15.html
    - TP53
    - non-small
- Hi299 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-173.html
- HMVEC-1 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-579.html
- A594 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/a/cell-lines-detail-58.html
- IMR-90 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/i/cell-lines-detail-246.html
    - Human Embryonic Lung Fibroblasts
- NCI-H358 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-368.html
- NCI-H23 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-401.html
- NCI-H460 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-613.html
- NHBE- Normal Human Bronchial Epithelial
- wit49 wilms tumor -
    - "The WiT49 cell line was derived from a primary lung metastasis of an aggressive Wilms tumor.2 WiT49 cells were maintained in 1:1 high-glucose Dulbecco's modified Eagle's medium/ nutrient mixture F-12, 10% fetal calf serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. Transfections were done using LipofectAMINE Plus . Stably transfected cells were selected with 1 mg/ml G418 (Life Technologies, Inc.) and maintained in 0.6 mg/ml G418" [2]

References

[1] "Cell Line Information and Supporting Products - US." Accessed: Sep. 23, 2024. [Online]. Available: https://www.thermofisher.com/us/en/home/technical-resources/cell-lines.html

[2] "wit49 wilms tumor Cells - US." Accessed: Sep. 23, 2024. [Online]. Available: https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/w/cell-lines-detail-622.html

Conclusions/Action Items:

- See "Rudimentary Cell line comparison" entry
- See "ATCC Possible Cell lines" entry
- See "Lung Cancer Cell Lines" entry
- The following cell lines I will continue research for:
    - H441 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-294.html
    - H1299 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-15.html
        - TP53
        - non-small
    - NCI-H358 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-368.html
    - NCI-H23 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-401.html
    - NCI-H460 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-613.html
    - Hi299 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-173.html
    - HMVEC-1 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/h/cell-lines-detail-579.html
    - A594 - https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/a/cell-lines-detail-58.html

NCI-H23_9/23/2024

Emily Rhine - Sep 23, 2024, 2:22 PM CDT

Title: HCI-H23

Date: 9/23/2024

Goal:

Content:

Link: NCI-H23 & NCI-H23 [H23]

nci-h23 Cells

Organism Type	Human	This is a human lung cancer cell line.
Tissues	Lung	
Phenotype	Adherent	
Primary	no	
Application	Stable Cell Transfection	

[1]

Product category	Human cells
Organism	*Homo sapiens*, human
Morphology	epithelial
Tissue	Lung
Disease	Adenocarcinoma; Non-small cell lung cancer
Applications	3D cell culture Cancer research High-throughput screening Toxicology
Product format	Frozen

[2]

Derivation	This line was derived from a lung cancer obtained from a patient prior to therapy.
Age	51 years
Ethnicity	Black
Gender	Male
Clinical data	This line was derived from a lung cancer obtained from a patient prior to therapy.
Oncogene	myc+; src+; abl+; erb+; ras+; sis-
Genes expressed	myc+; src+; abl+; erb+; ras+; sis-
Comments	The cells carry the K-ras 12 mutation, and there is a mutation in codon 246 (ATC -> ATG, isoleucine -> methionine) of the p53 gene. There is expression of C-myc, L-myc, v-src, v-abl, v-erb B, c-raf 1, Ha-ras, Ki-ras and N-ras RNAs. The cells express heterogeneous mRNA expression for PDGF A and B chain, transforming growth factor alpha and beta and the epidermal growth factor receptor (EGFR). NCI-H23 exhibits a high degree of c-myc DNA amplification (20-fold) but no detectable amplification of c-myc RNA. The cells stain positive for keratins 5+8 and 18 and vimentin but are negative for neurofilament. NCI-H23 cells are L-dopa decarboxylase-negative. They have a reported colony forming efficiency of 9.7% in soft agarose.

[2]

References

[1] "nci-h23 Cells - US." Accessed: Sep. 23, 2024. [Online]. Available: https://www.thermofisher.com/us/en/home/technical-resources/cell-lines/n/cell-lines-detail-401.html

[2] "NCI-H23 [H23] - CRL-5800 | ATCC." Accessed: Sep. 23, 2024. [Online]. Available: https://www.atcc.org/products/crl-5800

Conclusions/Action Items:

- Preform a master comparison of the top 3 cell lines I have selected.
- Present my findings to the team this week before the design matrix is due.
- Ask client additional questions in client meeting today at 5:30 pm.

3D Cell Culture Models_9_7_2024

Emily Rhine - Sep 19, 2024, 4:39 PM CDT

Title: 3D Cell Culture Models

Date: 9/7/2024

Content by/Present: Emily

Goals: Gain a better understanding of spheroid formation protocols to prepare for 3D cell culture modeling.

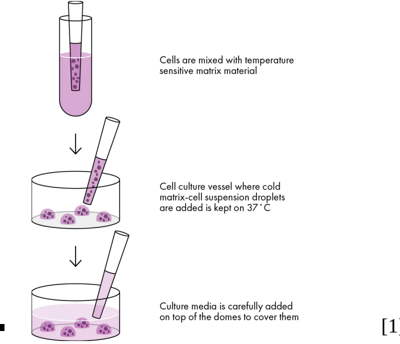
Content:

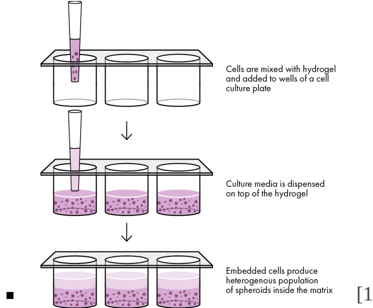
Search Engine: Google

Query: "spheroid formation protocol"

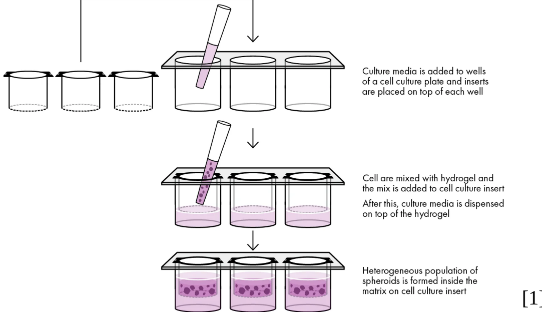
Link: https://www.upmbiomedicals.com/resource-center/learning-center/what-is-3d-cell-culture/3d-cell-culture-models/?gad_source=1&gclid=Cj0KCQjw8--2BhCHARIsAF_w1gzPbnVQ_Jhr0anijC5DEERjPGNX3cwTIAbnqTkeVgPk8Awxfti0V2saAneEEALw_wcB

Facts and Information of Interest:

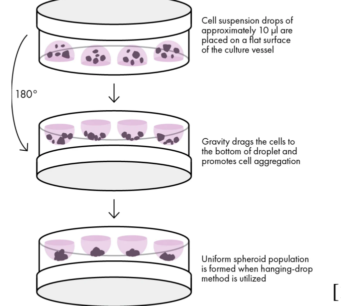
- There is a wide range of techniques for creating advanced 3D cell culture models, but most techniques are either scaffold-based or scaffold-free. [1]
- Scaffold based 3D cell culture models
  - Dome culture
    - Cons: Very temperature sensitive and contains high batch-to-batch variability. [1]
    - 

[1]
  - Seeding cells on top of hydrogel
    - "Hydrogel can also be used as an underlay material for cell suspension and therefore cells can grow on top of the gel. This is suitable, for example, with endothelial and epithelial cells, **which are not surrounded by extracellular matrix in physiological conditions**." [1]
  - Cells embedded in hydrogel
    - "In long-term 3D cell culture, animal-derived hydrogels that contain collagen and hyaluronic acid can be degraded enzymatically by cultured cells, causing a structural change over time." [1]
    - 

[1]
  - Cell culture inserts
    - Pros: The pore size of the membrane can be altered. [1]
    - Cons: "When compared to other 3D cell culture models the visualization with normal bright-field microscope has been detected to be difficult." [1]
    - "**Moreover, cells can be embedded in hydrogel which provides a cell-matrix interaction and a tissue-mimicking stiffness for the cells, simulating more in vivo like environment**." [1]

        - 

[1]

- Scaffold-based: These techniques typically lack cell-matrix interaction.
    - For **the hanging drop technique**, a commercial hanging drop plate would be ideal.
        - Pros: Typically the spheroids formed are uniform and the size can be adjusted by changing the cell seeding density. [1]
        - Cons: However, changing the cell culture media without disturbing or discarding the spheroids is a difficult procedure. [1] In addition, the technique can be very tedious if a large quantity of spheroids is desired. [1]
        - 

[1]
- Scaffold-free
    - **Ultra-Low Attachment plates** are made using liquid overlay techniques where the bottom of a cell culture dish is coated with a non-adhesive material, which prevents cell adhesion and protein absorption. [1]
        - Pros: N/A
        - Cons: In ULA-plate the spheroids float in suspension and are not physically in a fixed position. [1] In live cell imaging, this can cause difficulties to obtain and maintain optical focus when small movements can get spheroids on the move. [1] The biological relevance of ULA cell culture plates should be considered because this method lacks tissue-like stiffness and moreover cell-matrix interaction. [1] However, biological relevance in flat bottom ULA cell culture can be increased applying hydrogel. [1]

References

[1] "3D Cell Culture Models | Learning Center," 3D Cell Culture Models | Learning Center | UPM Biomedicals. Accessed: Sep. 08, 2024. [Online]. Available: https://www.upmbiomedicals.com/resource-center/learning-center/what-is-3d-cell-culture/3d-cell-culture-models/

Conclusions/action items:

Article Summary:

From the research conducted, I would heavily recommend either **the hanging drop technique** or **cell culture inserts** as detailed above. In Kreeger Lab group meetings, I have heard that some members have experienced success with the **the hanging drop technique.** However, more research must be done to ensure that the spheroids produced have the best biological relevance and 3D modeling accuracy as possible (within the scope of this course).

Action Items:

- Look more into specific cancer characteristics of tissue, cells, and ECM.
    - See next entry and its associated link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8750014/
- Continue more research into the hanging drop technique and what cells can be easily seeded into these spheroids.
- Create a list of "deal breaker contra" for each technique considered: the hanging drop technique or cell culture inserts.

Spheroid Formation_Matrigel_9/27/2024

Emily Rhine - Sep 30, 2024, 9:50 AM CDT

Title: Spheroid Formation Using Matrigel

Date: 9/27/2024

Goal: Better understand the reason why Dr. Hess recommends not using Matrigel.

Content:

Link 1: Insights into spheroids formation in cellulose nanofibrils and Matrigel hydrogels using AFM-based techniques

Facts & Information:

- "Monolayer cell culture is a useful in vitro testing platform to monitor biological events and drug development. However, **two-dimensional (2D) culture systems incompletely reflect the intricate physicochemical cellular microenvironment of tissues. In vivo, cells exist in a three-dimensional (3D) microenvironment with cell–cell and cell–matrix interactions and complex nutrient transport dynamics"** [1]
- "**Matrigel is an animal-based basement membrane extract derived from murine Engelbreth-Holm-Swarm sarcoma cells. It is widely used as a matrix in 3D culture for a range of cell types**. It is a complex and variable protein mixture which contains approximately 60 % laminins, 30 % collagens and 8 % entactin. It also contains heparan sulphate proteoglycan (perlecan), TGF-β, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, tissue plasminogen activator, and other growth factors which occur naturally in the EHS tumour. For stem cell culture, it is commonly used as an attachment substrate to provide cell adhesion sites. Compared to synthetic matrixes, Matrigel provides a more physiologically relevant microenvironment. However, **due to its animal origin, there is a batch-to-batch variation that can hinder reproducibility.**" [1]
- “approximately 100 µL of diluted Matrigel solution was added per cm2 of culture surface and allowed to gel at room temperature for 1 h” [1]

Link 2: https://www.sigmaaldrich.com/US/en/substance/corningmatrigelbasementmembranematrix1234598765

Facts & Information:

- $380.00- 10mL-10,000uL - Corning® Matrigel® Basement Membrane Matrix -CLS354234-1EA [2]

References:

[1] R. Teixeira Polez, N. Huynh, C. S. Pridgeon, J. J. Valle-Delgado, R. Harjumäki, and M. Österberg, “Insights into spheroids formation in cellulose nanofibrils and Matrigel hydrogels using AFM-based techniques,” Materials Today Bio, vol. 26, p. 101065, Jun. 2024, doi: 10.1016/j.mtbio.2024.101065.

[2] “Corning® Matrigel® Basement Membrane Matrix -.” Accessed: Sep. 27, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/substance/corningmatrigelbasementmembranematrix1234598765

Conclusions & Action Items:

Loading [MathJax]/extensions/Safe.js

- **Matrigel is rather expensive for high through-put experiments because it is approximately $3.80 for 100 µL undiluted Matrigel.**
- See team "Design Matrix" entry form more information on Matrigel
- Dr. Hess does not recommend Matrigel and now his reasoning is understood.

Emily Rhine - Sep 27, 2024, 5:02 PM CDT

Matrigel is rather expensive

- Approximately $3.80 for 100 µL undiluted Matrigel
    - $380.00- 10mL-10,000uL - Corning® Matrigel® Basement Membrane Matrix -CLS354234-1EA
        - https://www.sigmaaldrich.com/US/en/substance/corningmatrigelbasementmembranematrix1234598765
    - “approximately 100 µL of diluted Matrigel solution was added per cm2 of culture surface and allowed to gel at room temperature for 1 h,”
        - https://www.sciencedirect.com/science/article/pii/S2590006424001248

Emily Rhine - Sep 30, 2024, 9:39 AM CDT

https://www.sciencedirect.com/science/article/pii/S2590006424001248

Loading [MathJax]/extensions/Safe.js

SOX2_A549_10/23/2024

Emily Rhine - Oct 23, 2024, 2:14 PM CDT

Title: Alternate Protocols for Seeded A549 Spheroids on PolyHEMA

Date:10/23/2024

Content By: Emily

Content:

Google Query: "spheroid formation of a549 on poly hema coated plates protocol"

Link: SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine

Summary:

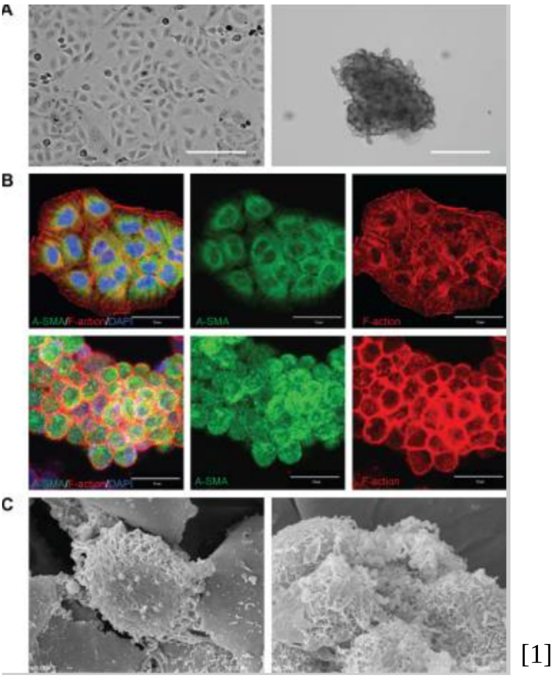
Poly-HEMA hydrogel coating [1]

A total 1.3 g of poly-HEMA (Sigma-Aldrich Co.) was dissolved in 33 mL of 99% ethanol, and the solution was mixed overnight at 37°C. Fifty microliters or 3.2 mL of the poly-HEMA stock solution was added to 96-well plates and 10 cm dishes, respectively, in the tissue culture hood, and plates and dishes were swirled using a plate rotator for 10 minutes. Plates were left to dry overnight and then washed with PBS immediately before use.

Growth behavior of human NSCLC A549 cell spheroids [1]

The architecture of a tissue and its functions are regulated by proper cell–cell and cell–ECM adhesions.36,37 During cancer progression toward malignancy, metastatic cancer cells would be exposed to periodic cell–substratum detachment. To first examine the role of cell–substratum detachment on cancer tissue architecture, we cultured A549 cells in poly-HEMA hydrogel substratum, which is superhydrophilic and prevents cell attachment.34,35 Following seeding on poly-HEMA coated substratum, A549 cells floated and aggregated to form spheroids with a compact organization, but the surface was quite rough, with a bulging presentation of cells on the periphery (Figure 1A). Cytoskeletal architecture was determined using phalloidin staining. Immunocytochemistry showed the appearance of actomyosin cortex in 3D aggregates, whose functions include cell–cell adhesion, physical barrier to hydrostatic pressure, and amoeboid-type cell motility (Figure 1B).38 Interestingly, cell aggregates displayed membrane protrusions and invaginations, which are known to lead to basement membrane degradation and amoeboid-type cell motility (Figure 1C).39,40 These results collectively indicate that A549 cells in suspension form 3D multicellular spheroidal structures, which may mimic the metastatic organization in vivo. Additional investigation using transmission electron microscopy (TEM) to include the accessible surfaces and gaps of cell contact could be useful to elucidate the underlying mechanisms of this cytoskeleton-mediated change in growth behavior of A549 cell spheroid. In addition, given recent interests on molecular linkages between the topographic configuration of cell contact and cellular processes such as cell–cell adhesion, cell migration, tumor cell invasion, and cytokinesis, it will be interesting to investigate the mechanisms by which the change in actin cytoskeleton architecture in 3D culture influences such links.

Loading [MathJax]/extensions/Safe.js



[1]

"Representative images of NSCLC A549 cells showing intercellular and intracellular architecture in a normal culture dish (2D, left panel) and in poly-HEMA-coated dishes (3D, right panel). Images were taken 72 hours following culturing. (A) Phase-contrast micrograph showing the morphology of human NSCLC A549 cells grown in 2D (left) and 3D (right) cultures. Original magnification: ×1,000. Scale bar: 200 µm. (B) Representative fluorescence microscopic images of NSCLC A549 cells grown in 2D (left) and 3D (right) cultures. Cells were stained with an antibody targeting α-SMA (green) and rhodamine-conjugated phalloidin (F-actin, red), while nuclei were counterstained blue with DAPI. Cells were imaged using confocal fluorescence microscopy. The image on the left is a merged image of DAPI, α-SMA, and phalloidin staining. Photographs are representative of n = 3 experiments. Original magnification: ×1,500. Scale bar: 50 µm. (C) Representative SEM images of NSCLC A549 cells grown in 2D (left) and 3D (right) cultures. Photographs are representative of n = 3 experiments." [1]

References

[1] C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.

Conclusions/Action items:

- Propose this plate preparation protocol to the team and client at Friday (10/25) meeting
- Continue research on spheroid formation protocols

Emily Rhine - Oct 23, 2024, 1:52 PM CDT

Loading [MathJax]/extensions/Safe.js

Scalable Hydrogel Dish Spheroid Formation_A549_10/23/2024

Emily Rhine - Oct 23, 2024, 2:28 PM CDT

Title: Scalable Hydrogel Dish Spheroid Formation

Date:10/23/2024

Content By: Emily

Content:

Google Query: "spheroid formation of a549 on poly hema coated plates protocol"

Link: An Improved Scalable Hydrogel Dish for Spheroid Culture

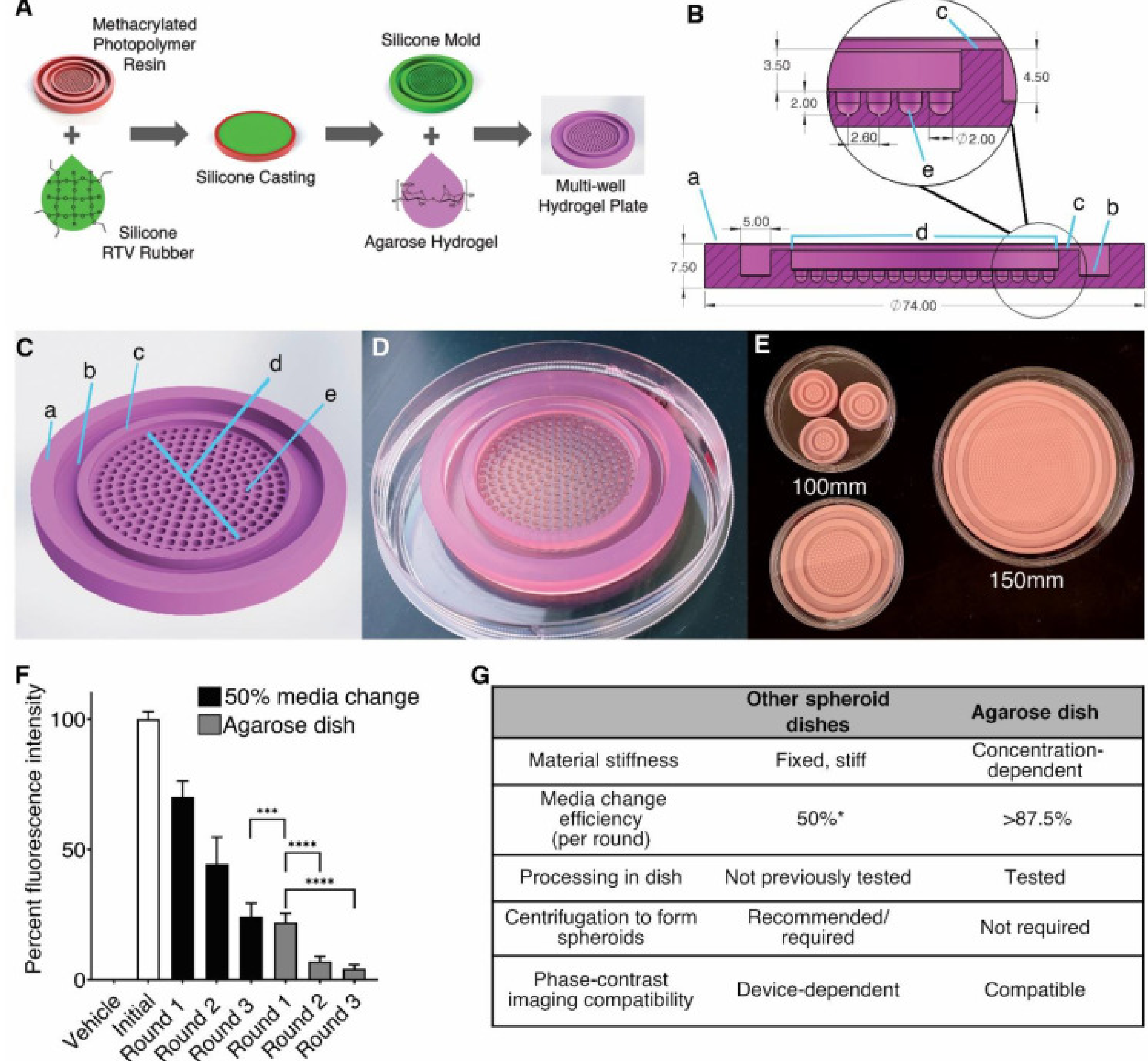
Summary:

Hydrogel Casting [1]

Molecular biology grade agarose powder (Fisher Scientific, Hampton, NH, USA) was dissolved in the corresponding volume of DMEM/F12 50:50 mix (Difco, Detroit, MI, USA) depending on the desired concentration and then boiled using a standard microwave oven until dissolved. Inside a biosafety cabinet, boiled agarose solution was poured over the silicone mold and left to solidify for 10 to 15 min at room temperature. Once solidified, hydrogel plates were transferred into appropriate TC dishes. The hydrogel dishes were then UV irradiated for at least 30 min before use. For storage, approximately 3 mL of basic culture media (i.e., serum-free DMEM/F12) was added to each TC dish, and each dish was sealed with Parafilm to maintain hydrogel hydration. The hydrogel plates were stored in a 4 °C fridge for up to a maximum of 2 weeks.

Cell and Spheroid Culture [1]

Three cell lines were used in this study: wild-type human lung epithelial carcinoma, A549 (ATCC® CCL-185™), endothelial EAhy (EA.hy.926, ATCC® CRL-2922™) lentivirally transduced with Azurite gene, and lung fibroblast HFL1 (ATCC® CCL-153™) transduced with mCherry gene. These cells were maintained in complete growth medium. Cells were dissociated using 0.25% Trypsin-EDTA (Gibco, Waltham, MA, USA) and counted using a Bio-Rad automated cell counter. Three milliliters of 3D growth media containing the appropriate number of cells was seeded into the culture chamber (Figure 1(Bd,Cd)). The specific cell seeding densities used are found in the specific sections below. The agarose dish cultures were not spun down by centrifugation to form spheroids. The cultures were incubated at 37 °C with 5% CO_2. The medium was changed every two to three days. Images were taken using a phase-contrast microscope (Amscope, Irvine, CA, USA).



	Other spheroid dishes	Agarose dish
Material stiffness	Fixed, stiff	Concentration-dependent
Media change efficiency (per round)	50%*	>87.5%
Processing in dish	Not previously tested	Tested
Centrifugation to form spheroids	Recommended/ required	Not required
Phase-contrast imaging compatibility	Device-dependent	Compatible

[1]

Creation of a scalable, micropatterned hydrogel mold. (**A**) Workflow for creating a multi-well casted hydrogel plate for 3D culture. Methacrylated photopolymer resin is 3D printed to create a cast for the room-temperature vulcanizing (RTV) silicone negative mold. The silicone negative mold is used to create the final multi-well hydrogel plate for 3D culture. (**B**) Agarose hydrogel dish cross-section (a = outer wall, b = channel, c = inner wall, d = culture chamber, and e = culture pocket or recess). Dimensions expressed in mm. (**C**) Agarose hydrogel dish CAD render. (**D**) Photo of a molded 217-well micropatterned agarose hydrogel dish in a standard 100 mm tissue culture dish. The dish was cast using 2% agarose in DMEM/F12 media. (**E**) Silicone rubber molds in 3 scaled sizes: (top left) 19-well, (bottom left) 217-well, and (right) 919-well. Left molds are in a 100 mm tissue culture dish while the right mold is in a 150 mm dish for reference. (**F**) Media change simulation using fluorescein isothiocyanate (FITC) in PBS. Fifty percent media change was used to simulate media change in commercially available dishes. Data normalized to vehicle control (0%) and initial solution (100%). Bars indicate the means and error bars indicate the SD of 36 technical replicates from 3 independently run experiments by independent researchers. *** $p < 0.001$; **** $p < 0.0001$, one-way ANOVA with Welch's correction. (**G**) Table comparing the major advantages of the agarose dish over commercially available micropatterned plates. * suggested media change in most commercially available spheroid plates. [1]

- "The initial mold was 3D printed using the methacrylated photopolymer. This mold was then used to create a reusable silicone mold. Agarose hydrogel solution was poured over the silicone mold and allowed to harden, creating a readily

available micropatterned 3D culture dish. An outline and video of the method for creating a silicone molded multi-well hydrogel plate is provided (Figure 1A and Video S1)." [1]

- During 3D culture, dissociated cells were seeded directly into the culture chamber in 3 mL of 3D growth media (Figure 1(Bd,Cd)). The maximum volume of the culture chamber is 5 milliliters and each microwell has an interior volume of approximately 8 microliters abased on our design. Due to gravity and optimal ultra-low adhesive hydrogel concentration, our design allows cells to drop into the microwells forming aggregates within 24 h. The inner wall of our design was deliberately made lower than the outer wall to aid in media changing. To change media, the user simply tilts the whole device, allowing the old media to flow over the inner wall to the channel (Figure 1(Bb)), where media is quickly aspirated without risk of losing the spheroids. The depth of the microwells prevents the spheroids from drifting out of their wells in the culture chamber when the device is tilted. Fresh media is then added dropwise onto the inner wall (Figure 1(Bc)) where it flows safely into the culture chamber with minimal disturbance to the growing spheroids (Video S2). Downstream processes like enzymatic dissociation and fixation can also be carried out in the agarose dish after draining the culture chamber of media as previously described. [1]

References

[1] J. C. Valdoz et al., “An Improved Scalable Hydrogel Dish for Spheroid Culture,” Life, vol. 11, no. 6, p. 517, Jun. 2021, doi: 10.3390/life11060517.

Conclusions/Action items:

- Summary: A methacrylated photopolymer 919 well was 3D printed. Each culture chamber is 5 milliliters and each microwell has an interior volume of approximately 8 microliters abased on their design. [1]
- Propose this plate preparation protocol to the team and client at Friday (10/25) meeting
- Continue research on spheroid formation protocols

Protocols Matrix_Spheroids_11/2/2024

Emily Rhine - Nov 02, 2024, 5:57 PM CDT

Title: Protocols Matrix Spheroids

Date:11/2/2024

Content by: Emily

Content:

Emily Rhine - Nov 02, 2024, 5:58 PM CDT

Design matrix for Spheroid Protocols.

Design Criteria (weight)	1. K. Han et al.		2. F. R. Fernandes et al.		3. [illegible] et al.		4. J. C. [illegible] et al.	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
Cost (25)	medium	20/25	low	25/25	high	N/A	medium	N/A
Time* (25)	Overnight*	25/25	24 hours	20/25	2–10 days	N/A	Mold—1 day Hydrogel—2 hours	N/A
Scalability (20) [illegible]	[illegible]	20/20	[illegible]	20/20	[illegible]	N/A	[illegible]	N/A
Complexity (15) steps	3 Main steps	15/15	3 Main steps	15/15	high	N/A	high	N/A
Required machinery & materials (15)	[illegible]	15/15	[illegible]	15/15	[illegible]	N/A	[illegible]	N/A
Total Score (100)	85		85					

*[illegible]

Download

Protocols_Matrix.pdf (72.8 kB)

Emily Rhine - Nov 02, 2024, 6:04 PM CDT

Conclusions/Action Items:

- Meet with team to discuss this first materials draft
- Use materials list to draft a order sheet to send the client
- Order materials by 11/5 at the latest!!
- Consider other protocols if needed
  - Poly-HEMA plate coating:
    - C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.
    - “In vitro differentiation of human embryonic stem cells to hemogenic endothelium and blood progenitors via embryoid body formation.” Accessed: Oct. 29, 2024. [Online]. Available: https://star-protocols.cell.com/protocols/499
  - Other spheroid protocols:
    - T. Stiff, S. Bayraktar, P. Dama, J. Stebbing, and L. Castellano, “CRISPR screens in 3D tumourspheres identified miR-4787-3p as a transcriptional start site miRNA essential for breast tumour-initiating cell growth,” Commun. Biol., vol. 7, no. 1, pp. 1–11, Jul. 2024, doi: 10.1038/s42003-024-06555-1.
    - L. Carroll, B. Tiwari, J. Curtin, and J. Wanigasekara, “U-251MG Spheroid generation using low attachment plate method protocol,” May 2021, Accessed: Oct. 29, 2024. [Online].

Loading [MathJax]/extensions/Safe.js

Available: https://www.protocols.io/view/u-251mg-spheroid-generation-using-low-attachment-p-bszmnf46

- "Corning® Spheroid Microplates - Spheroid Formation Protocol." Accessed: Oct. 29, 2024. [Online]. Available: https://www.corning.com/catalog/cls/documents/protocols/CLS-AN-308.pdf
- Y. T. Phung, D. Barbone, V. C. Broaddus, and M. Ho, "Rapid Generation of In Vitro Multicellular Spheroids for the Study of Monoclonal Antibody Therapy," Journal of Cancer, vol. 2, p. 507, Oct. 2011, doi: 10.7150/jca.2.507.

Loading [MathJax]/extensions/Safe.js

Design Matrix Research

Emily Rhine - Sep 27, 2024, 11:18 PM CDT

Title: Design Matrix Research

Date: 9/27/2024

Goal: Complete necessary research to add scientific data to values assigned in design matrix.

Content:

Emily Rhine - Sep 27, 2024, 11:05 PM CDT

Viability (20):

Cell viability is a measure of the proportion of live, healthy cells within a population [1]. For this category, viability is a measure of how robust the cell is and how well it can withstand various stresses. The highest ranked cell line is predicted to be the healthiest after fluid shear stress, transfection, and cisplatin treatment.

NCI-H23 cells were assigned a score of 4/5 for its and >20% cell death after 72 hours when subjected to [3 μM] of cisplatin and its sensitivity to cisplatin treatment [2]. The NCI H23 cell line showed a higher expression level of DNA repair proteins after cisplatin treatment compared to A549 cells. In previous experiments for both NCI-H23 and A549, a negative correlation between cell viability and DNA damage induction upon cisplatin treatment has been noted [2]. A549 cells were assigned a score of 5/5 for its >20% cell death after 72 hours when subjected to [3 μM] of cisplatin and its greater resistance to cisplatin treatment compared to NCI-H23 cells [2]. OVCAR-5 scored a 3/5 for its severe morphological changes—decreased spheroid-forming capacity, reduced cytoskeleton organization, and chromosomal instability— after exposure to <1 dyne/cm^2 fluid shear stress and >50% cell death after 72 hours when subjected to [2.5 μM] cisplatin [3], [4].

The type of transfection done to these three cell types may either help or hinder the cell health depending on the transfection virus used (lentivirus or siRNA)and what it is used in conjunction with (Nrf2, cisplatin, or both). The trend in scientific literature indicates that these three cell types have the same viability, within 7% ±3%, when transfected under the same conditions [2], [3], [4]. Since the vectors encoding the components necessary for CRISPR/Cas are large, they result in low transfection efficiency and cell viability [5]. To overcome those obstacles, the team can add exogenous small plasmids that increase transfection efficiency up to 40-fold and cell viability up to 6-fold [5].

Emily Rhine - Sep 27, 2024, 11:06 PM CDT

Genetic Mutation (15):

Analysis of genetic mutations inherent to various cell lines is crucial in determining the ideal cell line for our project. A high score in this category means genetic mutations that are helpful must work with for any step in the adhesion process, Cas9 attachment, CRISPR edit, or screen. Using Han's ten selected lung cancer cell lines, a set of ideal cell line mutations were created including: TP53 (tumor protein p53), a gene that encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains [23]; EGFR, a cell surface protein that binds to epidermal growth factor leading to cell proliferation [24]; and KRAS, Kirsten rat sarcoma viral oncogene homolog causes malignancies based on an error encoding a protein that is a member of the small GTPase superfamily [25]. These mutations may either cause or enhance the malignancy of existing cancer cells. Since TP53 is the most frequently mutated gene in human cancer, the presence of this mutation is crucial for biological relevance to half of all cancer cases [26].

NCI-H23 has all of the key mutations including TP53, KRAS, EGFR, a high degree of c-myc DNA amplification, and no counterproductive mutations, so it was given the highest score of 5/5 [6]. A549 scored second highest with 3/5 because it contains EGFR and TP53 which are key, but also PIK3CA, ALK, and PTEN which aren't key but won't impede the success of the project [15]. The protein encoded by PIK3CA, phosphatidylinositol 3-kinase, is oncogenic and represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2 [27]. ALK, anaplastic lymphoma receptor tyrosine kinase, a

chromosomal rearrangement which results in creation of multiple fusion genes in tumorigenesis. PTEN guards the genome by controlling multiple processes of chromosome inheritance [28]. Although OVCAR-5 has KRAS, a key mutation, it was ranked the lowest at 2/5 due to the lack of essential protein TP53 and the presence of additional mutation CLDN4, which is a mutation of integral membrane proteins [29], [30], [31].

Emily Rhine - Sep 27, 2024, 11:06 PM CDT

Uniform Properties (20):

This criteria outlines the reliability of the spheroid formation to produce uniform properties. The ideal formation protocol has no batch-to-batch variability. Minimal variation in size, shape, and porosity is expected so the experiment will have a higher chance of reproducibility and fewer outliers. It is important to note that research has shown that differences in spheroid formation arise from the differences in cell-biomaterial interactions rather than due to differences in cell viability or proliferation [42].

The treated tissue culture plates scored the highest in this category, 4/5, due to their minimal variation between batches. Additionally, treated tissue culture plates have a larger amount of spheroids per batch— see *Scalability* section— making it easier to harvest a uniform size or shape [32]. Due to the low-throughput nature of Hanging Drop, making it harder to harvest uniform spheroids, and due to its 10% to 15% size variation between drops, this method scored a 3/5 [43]. Matrigel, animal-based basement membrane extract, has a batch-to-batch variation that can hinder reproducibility, so a score of 2/5 was assigned to it [42]. Cells seeded in matrigel also tend to form large cell aggregates with looser morphology rather than spheroids with round, regular shape [42].

Emily Rhine - Sep 27, 2024, 11:17 PM CDT

References

[1] "Overview of Cell Viability and Survival," Cell Signaling Technology. Accessed: Sep. 27, 2024. [Online]. Available: https://www.cellsignal.com/science-resources/cell-viability-and-survival

[2] M. M. Silva, C. R. R. Rocha, G. S. Kinker, A. L. Pelegrini, and C. F. M. Menck, "The balance between NRF2/GSH antioxidant mediated pathway and DNA repair modulates cisplatin resistance in lung cancer cells," *Sci. Rep.*, vol. 9, p. 17639, Nov. 2019, doi: 10.1038/s41598-019-54065-6.

[3] A. R. Hyler *et al.*, "Fluid shear stress impacts ovarian cancer cell viability, subcellular organization, and promotes genomic instability," *PLoS ONE*, vol. 13, no. 3, p. e0194170, Mar. 2018, doi: 10.1371/journal.pone.0194170.

[4] R. Zhang, X. Chen, S. Fu, L. Xu, and J. Lin, "A small molecule STAT3 inhibitor, LLL12, enhances cisplatin- and paclitaxel-mediated inhibition of cell growth and migration in human ovarian cancer cells," *Oncol. Rep.*, vol. 44, no. 3, pp. 1224–1232, Sep. 2020, doi: 10.3892/or.2020.7667.

[5] J. N. Søndergaard, K. Geng, C. Sommerauer, I. Atanasoai, X. Yin, and C. Kutter, "Successful delivery of large-size CRISPR/Cas9 vectors in hard-to-transfect human cells using small plasmids," *Commun. Biol.*, vol. 3, p. 319, Jun. 2020, doi: 10.1038/s42003-020-1045-7.

[6] "NCI-H23 [H23] - CRL-5800 | ATCC." Accessed: Sep. 25, 2024. [Online]. Available: https://www.atcc.org/products/crl-5800

---

[15] B. C. Baguley *et al.*, "Resistance mechanisms determining the in vitro sensitivity to paclitaxel of tumour cells cultured from patients with ovarian cancer," *Eur. J. Cancer Oxf. Engl. 1990*, vol. 31A, no. 2, pp. 230–237, 1995, doi: 10.1016/0959-8049(94)00472-h.

---

[23] "TP53 tumor protein p53 BCC7 LFS1 P53 TRP53 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/tp53

[24] "EGFR epidermal growth factor receptor ERBB ERBB1 HER1 PIG61 mENA | Sigma-Aldrich." Accessed: Sep. 20, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/egfr

[25] "KRAS Kirsten rat sarcoma viral oncogene homolog C-K-RAS CFC2 K-RAS2A K-RAS2B K-RAS4A K-RAS4B KI-RAS KRAS1 KRAS2 NS NS3 RASK2 | Sigma-Aldrich." Accessed: Sep. 19, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/kras

[26] R. Mirgayazova *et al.*, "Therapeutic Editing of the TP53 Gene: Is CRISPR/Cas9 an Option?," *Genes*, vol. 11, no. 6, p. 704, Jun. 2020, doi: 10.3390/genes11060704.
[27] "PIK3CA phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha CLOVE CWS5 MCAP MCM MCMTC PI3K p110-alpha | Sigma-Aldrich." Accessed: Sep. 27, 2024. [Online]. Available: https://www.sigmaaldrich.com/US/en/genes/pik3ca
[28] S.-Q. Hou, M. Ouyang, A. Brandmaier, H. Hao, and W. H. Shen, "PTEN in the maintenance of genome integrity: From DNA replication to chromosome segregation," *BioEssays News Rev. Mol. Cell. Dev. Biol.*, vol. 39, no. 10, p. 10.1002/bies.201700082, Oct. 2017, doi: 10.1002/bies.201700082.
[29] "OVCAR-5 Human Cancer Cell Line | SCC259." Accessed: Sep. 27, 2024. [Online]. Available: https://www.emdmillipore.com/US/en/product/OVCAR-5-Human-Cancer-Cell-Line,MM_NF-SCC259?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
[30] E. Ó hAinmhire, S. M. Quartuccio, W. Cheng, R. A. Ahmed, S. M. King, and J. E. Burdette, "Mutation or Loss of p53 Differentially Modifies TGFβ Action in Ovarian Cancer," *PLoS ONE*, vol. 9, no. 2, p. e89553, Feb. 2014, doi: 10.1371/journal.pone.0089553.
[31] "CLDN4 claudin 4 [Homo sapiens (human)] - Gene - NCBI." Accessed: Sep. 27, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/gene/1364
[32] T. Riss and O. J. Trask, "Factors to consider when interrogating 3D culture models with plate readers or automated microscopes," *In Vitro Cell. Dev. Biol. Anim.*, vol. 57, no. 2, pp. 238–256, 2021, doi: 10.1007/s11626-020-00537-3.

---

[42] R. Teixeira Polez, N. Huynh, C. S. Pridgeon, J. J. Valle-Delgado, R. Harjumäki, and M. Österberg, "Insights into spheroids formation in cellulose nanofibrils and Matrigel hydrogels using AFM-based techniques," *Mater. Today Bio*, vol. 26, p. 101065, Jun. 2024, doi: 10.1016/j.mtbio.2024.101065.
[43] N.-E. Ryu, S.-H. Lee, and H. Park, "Spheroid Culture System Methods and Applications for Mesenchymal Stem Cells," *Cells*, vol. 8, no. 12, p. 1620, Dec. 2019, doi: 10.3390/cells8121620.

Emily Rhine - Sep 27, 2024, 11:25 PM CDT

CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

BME 400

September 27, 2023

Section [illegible]

Design Matrix

Client: Carley Schwartz cischwartz@wisc.edu
Dr. Gaelen Hess [illegible]

Advisor: Paul Campagnola pcampagnola@wisc.edu

Team: Althys Cao (Leader) [illegible]
Ana Martinez (Communicator) [illegible]
Emily Rhine (BSAC) erhine@wisc.edu
Julia Salita ([illegible]) jsalita@wisc.edu
Jayson O'Halloran (BPAG) [illegible]

Table 1. Design matrix for Cell Line.

Design Criteria (weight)	NCI-H23		A549		OVCAR-5	
	Score X/5	Weighted score	Score X/5	Weighted score	Score X/5	Weighted score
Viability (20)	4	16	5	20	3	12
Adhesion (20)	3	12	4	16	4	16
Reproduction Speed (20)	3	12	5	20	4	16
Drug Sensitivity (15)	4	12	5	15	3	9
Genetic Mutation (15)	5	15	3	9	2	6
Ease of Procurement (10)	5	10	5	10	5	10
Total Score (100)	77		90		69	

Download

Unlinked_Zotero_CRISPRi_Screening_in_Cancer_Spheroids_Design_Matrix_.pdf (156 kB)

Condensed Passaging Protocol_10/21/2024

Emily Rhine - Oct 21, 2024, 11:30 AM CDT

Notes

Condensed A549 Passaging Protocol

1) Gather supplies:
   - Trypsin (1x)
   - Media (DMEM)
   - sterological pipettes
   - bleach bucket (10% concentrate)
   - PBS
   - Cells (T-75 flask)

2) Aspirate Media (writting side)

3) Wash gently with 2mL PBS
   ↳ gently slush back & forth

4) Aspirate PBS (tipped toward writting side)

5) Add 3mL Trypsin to back of flask & incubate 5-10 min
   ↳ until all cells floating/detached

6) Add 7mL media & gently swish to mix

7) Open lid to eppendorf 1.5mL pipette

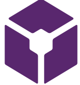
8) Remove 0.5mL of mixed cell media to eppendorf

9) After following cytoFLEX protocol split accordingly.

10) Cleanup as needed.

Download

15F1A8E6-0BB4-485E-9015-82DAC5446B54.jpg (626 kB)



Hess Lab Training Requirements_9/10/2024

Emily Rhine - Oct 12, 2024, 4:41 PM CDT

Lab Safety

Required Training

Before starting any work in the lab, you must complete the following sets of training. These can be found on Canvas https://it.wisc.edu/services/canvas/ or https://ehs.wisc.edu/training/

General:

2020-21 HIPAA Privacy & Security Training

SMPH: SMPH: Creating a Respectful and Welcoming Learning Environment (2020)

Preventing Sexual Harassment and Sexual Violence at UW-Madison

Cybersecurity Training (https://it.wisc.edu/it-projects/cybersecurity-awareness-training/#started)

Research-Based:

-Biosafety Required Training

-Chemical Safety: The OSHA Lab Standard

-Chemical Safety: Cryogen Safety Training

-Chemical Safety: Fume Hood Safety Training

-Chemical Safety: Hazard Communication

-Disposing of Hazardous Chemicals

-Biosafety 102: Bloodborne Pathogens for Laboratory and Research

-Biosafety 205: Bio HazMat Shipping Training (Both Campus Transport of Biologicals & Dry Ice Training Path and Dangerous Goods Training Paths)

-Biosafety 105: Biosafety Cabinet Use

-Biosafety 106: Autoclave Use

-Biosafety 107: Centrifuge Safety

Emily Rhine - Sep 27, 2024, 3:56 PM CDT

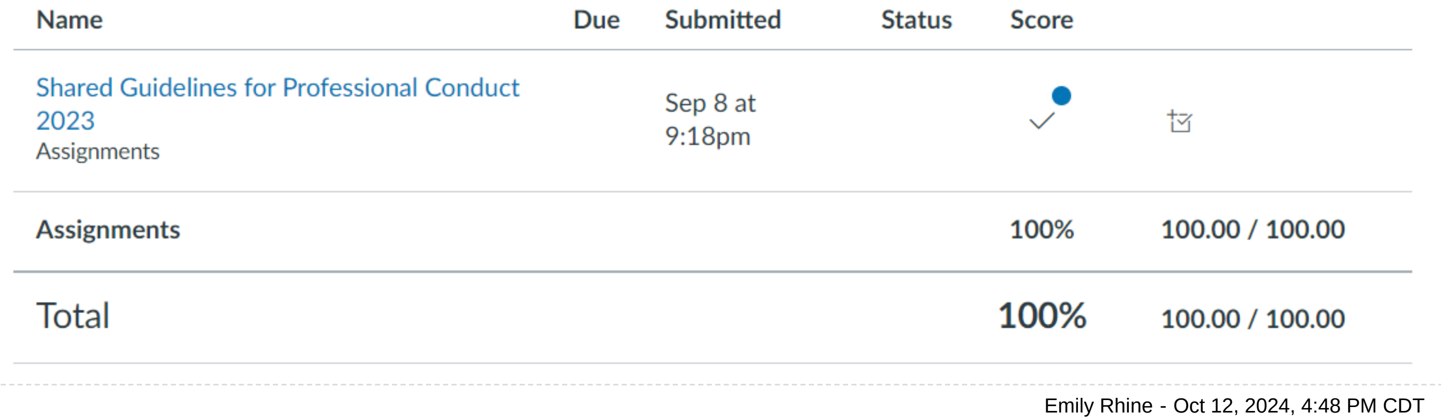
https://compliance.research.wisc.edu/TILT/Details/8785095

Emily Rhine - Sep 10, 2024, 2:40 PM CDT

Course	Assignment	Completion	Expiration
2022-23 HIPAA Privacy & Security Training	HIPAA Training Quiz	1/25/2023	
2023-24 HIPAA Privacy & Security Training	HIPAA Attestation	10/28/2023	
Biosafety 102: Bloodborne Pathogens for Laboratory and Research	Biosafety 102: Bloodborne Pathogens Safety in Research Quiz 2024	8/15/2024	8/15/2025
Biosafety 105: Biosafety Cabinet Use	Biosafety 105: Biosafety Cabinet Use Quiz	8/31/2023	No Expiration
Biosafety 106: Autoclave Use	Biosafety 106: Autoclave Use: Safety and Efficacy - Verification Quiz	8/31/2023	No Expiration
Biosafety 107: Centrifuge Safety	Biosafety 107: Centrifuge Safety Verification Quiz	8/31/2023	No Expiration
Biosafety Required Training	Biosafety Required Training Quiz 2023	3/12/2023	3/12/2028
Chemical Safety: Hazard Communication - Identifying Chemical Hazards	Final Quiz	8/31/2023	8/31/2028
Chemical Safety: The OSHA Lab Standard	Final Quiz	3/12/2023	

Emily Rhine - Oct 12, 2024, 4:44 PM CDT

Loading [MathJax]/extensions/Safe.js



Name	Due	Submitted	Status	Score	
Shared Guidelines for Professional Conduct 2023 Assignments		Sep 8 at 9:18pm		✓	
Assignments				**100%**	**100.00 / 100.00**
Total				**100%**	**100.00 / 100.00**

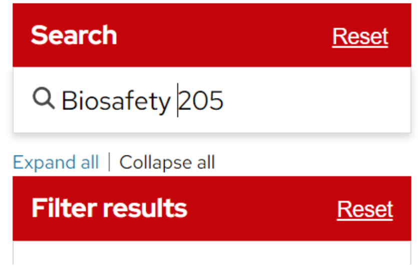
Emily Rhine - Oct 12, 2024, 4:48 PM CDT

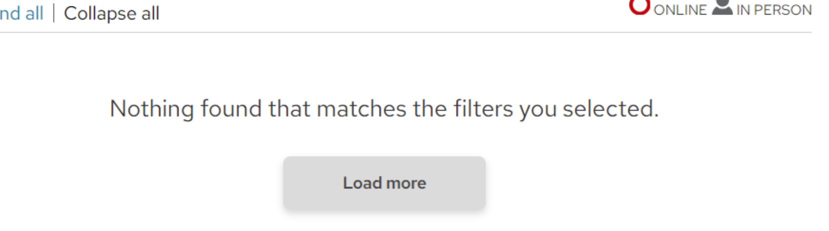
Substituted "ListenWIse" program for "Preventing Sexual Harassment and Sexual Violence at UW-Madison"

4. Certify Completion of GetWIse@Home (Required for completion of the GetWIse@Home Annual Refresher course) GetWIse Requirement Completion	Sep 3 at 9:27pm	1 / 1

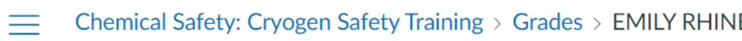
Emily Rhine - Oct 12, 2024, 4:48 PM CDT

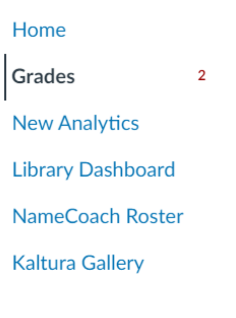
Biosafety 205 no longer offered



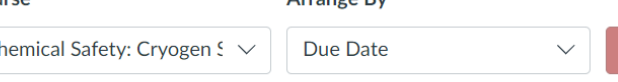


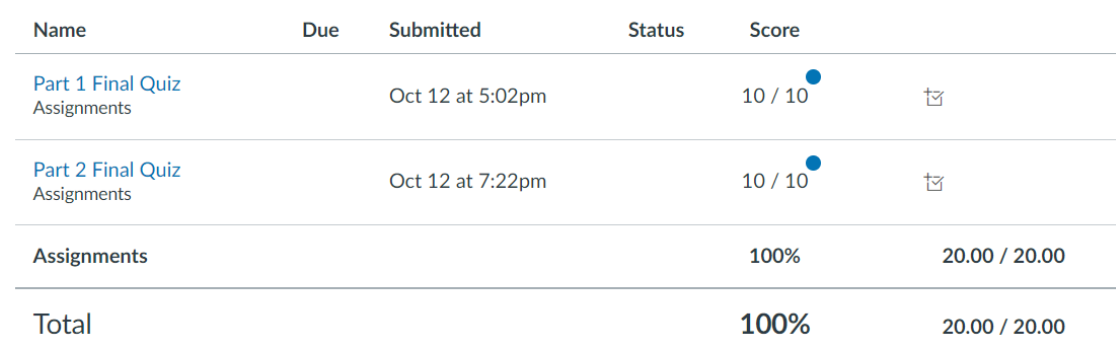
Emily Rhine - Oct 12, 2024, 7:23 PM CDT





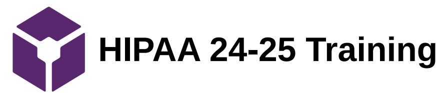
Print Grades





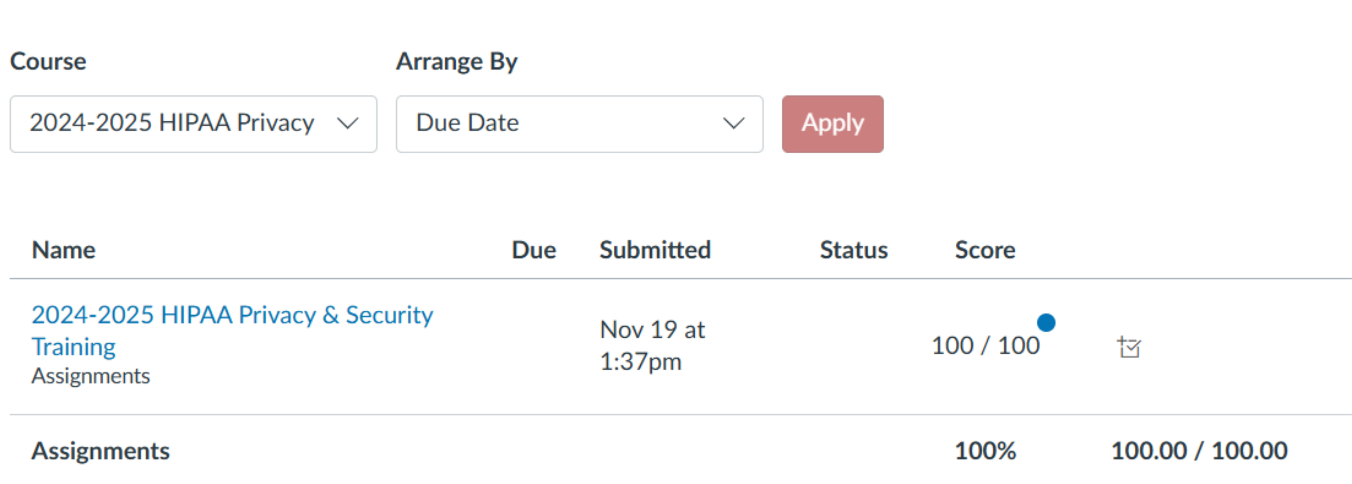
Name	Due	Submitted	Status	Score	
Part 1 Final Quiz Assignments		Oct 12 at 5:02pm		10 / 10	
Part 2 Final Quiz Assignments		Oct 12 at 7:22pm		10 / 10	
Assignments				100%	20.00 / 20.00
Total				**100%**	20.00 / 20.00

Loading [MathJax]/extensions/Safe.js



HIPAA 24-25 Training

Emily Rhine - Nov 19, 2024, 1:39 PM CST



Grades for EMILY RHINE

Print Grades

Course: 2024-2025 HIPAA Privacy

Arrange By: Due Date

Apply

Name	Due	Submitted	Status	Score	
2024-2025 HIPAA Privacy & Security Training Assignments		Nov 19 at 1:37pm		100 / 100	
Assignments				**100%**	**100.00 / 100.00**
Total				**100%**	**100.00 / 100.00**

Show and Tell Presentation_10/30/2024

Emily Rhine - Oct 30, 2024, 5:02 PM CDT

Title: Show and Tell Presentation

Date: 10/30/2024

Content By: Emily

Content:

Emily Rhine - Oct 30, 2024, 5:01 PM CDT

Download

Show_and_Tell_11_1_2024.pdf (1.37 MB)

Emily Rhine - Oct 30, 2024, 5:03 PM CDT

Conclusions & Action Items:

- Show presentation to other BME 400 students at Show and Tell
- Prepare 1 minute elevator pitch
- Ask for specific advice about spheroid formation or stemness markers

Loading [MathJax]/extensions/Safe.js

Preliminary Materials Sheet_11/2/2024

Emily Rhine - Nov 02, 2024, 5:55 PM CDT

Title: Preliminary Materials Sheet

Date:11/2/2024

Content by: Emily

Content:

Emily Rhine - Nov 02, 2024, 5:55 PM CDT

Item	Amount	Vendor	Cost	Associated Protocol	Link
[illegible]	[illegible]	[illegible]	[illegible]	1,2,3,4	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	1,2,3,4	[illegible]
RPMI 1640 growth medium		[illegible]		1,2	[illegible]

Download

BPAG_Expense_Sheet_Materials_-_Sheet1.pdf (51.7 kB)

Emily Rhine - Nov 02, 2024, 5:57 PM CDT

Conclusions/Action Items:

- Meet with team to discuss this first materials draft
- Get materials approved and ordered by the client
- Add materials as needed
- Materials based on those listed in "Protocols Matrix" entry

Loading [MathJax]/extensions/Safe.js

Final Poster Section_11/22/2024

Emily Rhine - Nov 22, 2024, 9:45 AM CST

Title: Final Poster Section

Date:11/22/2024

Content By: Emily

Goals: Create final report document from template and complete my section.

Content:

FINAL DESIGN

Cell Line: A549

- Non-small cell lung cancer (NSCLC)
  - Adenocarcinoma
  - Adherent
  - 50 µm cell diameter
- Maintenance
  - Doubling time: 22 hours
  - Confluency 5,000,000 cells/10 mL
  - 20 passages maximum
- Cell Procurement
  - ATCC: $555
  - Provided by Hess Labs

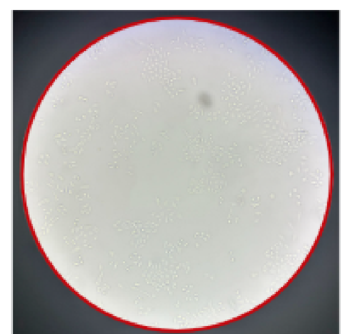


Figure #: 10x Brightfield image of A549 Passage 3 cells taken at 48% confluency.

DESIGN CRITERIA

✓ Select suitable cancer cell line
✓ Select and optimize spheroid formation protocol
✓ Scale 3D culture to prepare for genome-wide screen
✓ Adhere to all Biosafety Level 2 (BSL-2) standards
✓ Budget of $1000

Future Work:

- Run 3 trials of best seeding density to confirm results
- Optimize Methylcellulose concentration for spheroid formation protocol
- Optimize PolyHEMA plate preparation
- Begin γH2AX staining

https://docs.google.com/presentation/d/1e2kUBM1243vdwKiIt_wWo1ssHczuEipB/edit#slide=id.p1

Conclusions/Action Items:

- Update poster as needed before the presentation 12/6/2024
- Decide between team who will print poster
  - Everyone pitches in to help pay for the poster (equal)
- Divide up final report
- Send final poster draft to Dr. C to get it reviewed before we print it

SOX2_Stemness Gene_12/17/2024

Emily Rhine - Dec 17, 2024, 7:26 PM CST

Title: SOX2_Stemness Gene

Date: 12/17/2024

Content By: Emily

Content:

Link: SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine

Summary:

SOX-2

- "**In poly-HEMA culture, A549 cells** formed substratum-detached **spheroids** with characteristics of intermediate epithelial to mesenchymal transition (EMT) and **exhibited greater expression of SOX2 than did control 2D cells**." [1]
- "**Knockdown of SOX2 markedly suppressed the growth of A549 cell aggregates in poly-HEMA culture conditions** and furthermore increased their sensitivity to the anticancer drug vinblastine with concomitant downregulation of the activity of the anti-apoptotic AKT kinase." [1]
- "Interestingly, a small molecule, RepSox, which replaces SOX2, stimulated A549 cell growth in poly-HEMA 3D culture condition." [1]
- "SOX2 may therefore be an invaluable therapeutic target of NSCLC." [1]

Application:

- Since the expression of SOX2 is shown to increase in 3D versus 2D environment, we can use this expected outcome to monitor the health and functionality of our spheroids.
- A qPCR test for SOX2 gene expression would be a great way for us to confirm these results.
    - For exact qPCR/real-time qPCR protocol see original article
- SOX2 is deemed important for A549 tumor microenvironment recapitulation due to its ability to be targeted by cancer drugs such as vinblastine. [1]

References

[1] C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.

Conclusions/Action items:

- In the final report Jayson stated:
    - "For instance, SOX2 is a transcription factor associated with stemness and tumor progression in non-small cell lung cancer (NSCLC). It regulates self-renewal, differentiation, and pluripotency, making it a key

driver of cancer stem cell (CSC) properties. SOX2 is frequently co-expressed with surface markers such as CD133 and CD44, which are commonly used to identify CSCs. CD133, a marker of tumorigenicity, is often upregulated in SOX2-expressing cells and is linked to enhanced self-renewal and tumor initiation [1]. Similarly, CD44, which plays a role in cell adhesion and migration, is associated with SOX2 in promoting metastasis and resistance to therapies [1]. These surface markers, in conjunction with SOX2, delineate a subpopulation of CSCs that contribute to lung cancer progression, recurrence, and therapeutic resistance, making them a target for gene therapies in NSCLC.

- Review this paper as necessary for qPCR testing and biological relevance/ motivation for selecting SOX2.
- Finish and submit final report

Goals and Experiments for Spring 2025_12/17/2024

Emily Rhine - Dec 17, 2024, 7:51 PM CST

Title: Goals and Experiments for Spring 2025

Date: 12/17/2024

Content:

1. Redo spheroid experiment with 50k and 75k seeding densities to confirm results
    a. Alter methylcellulose levels to confirm optimization
    b. Test spheroid dissociation with Accutace protocol
    c. Select ideal seeding density based on data gathered
2. Calculate whether, using the ideal seeding density, it is possible/realistic to scale up spheroid formation to the 50 million cells necessary for the genome wide screen
    a. calculate time. materials necessary, and complexity
3. Use qPCR to determine whether or not expression of SOX2 increases in our spheroids to confirm ideal cellular conditions and similarity to tumor environment
    a. "A549 with CRISRPi to add gRNA and look for specific mutation --> See loss in staining if specific proteins are selected to be removed with gRNA" -Gaelen/Carley
4. Lentiviral transduce cells with γH2AX, a biomarker for DNA double strand breaks (dsbs), so the team can understand the effects of gene knockdown via lentivirus.
    a. See "Spheroid Formation Protocols_10/25/2024" entry excerpt below
    b. See of methylcellulose levels alter γH2AX presentation - Carley S.
5. ~~CRISPRi genome-wide screen~~ (Technically not a goal for us to complete according to the client(s))

Conclusions/Action Items:

- Finish and submit final report
- Send proposed timeline to team over winter break to better prepare for the the start of spring semester
- Touch base with the client Week 1 to establish a plan for experiments and their goals for us

Emily Rhine - Dec 17, 2024, 7:51 PM CST

Excerpt taken from "Spheroid Formation Protocols_10/25/2024" client meeting notes

- γ-H2AX
    - Marker on histone due to phosphorylation (happens when changes in DNA damage occur)
    - The inverse could also be true: if genome stability increases → decrease in γ-H2AX
    - Therefore, can notice both high and low γ-H2AX populations
        - Take top 20% (γ-H2AX enriched for DNA repair) and bottom 20% of γ-H2AX population (endogenous expression meaning: within the cell and not from external DNA expression such as plasmids)
            - Why 20%: there are both targeting and non-targeting control guide RNAs in genome-wide screening → 20% will capture negative controls

Loading [MathJax]/extensions/Safe.js

- **γ-H2AX staining process** (basic procedure - as if don't have spheroids) ***Takes 4-4.5 hours

1. Infect cells with lentivirus
    a. Will introduce plasmid with guideRNA, which will target some gene that is involved in gamma-H2AX/DNA damage
        i. The plasmid also has fluorescence: mCherry (positive)
            1. Therefore, associate mCherry+ cells with guideRNA infection into cell
    b. Will not get 100% infection → ideal so we can have controls (with no plasmid aka no vector and no mCherry - negative)
    c. **Wait 3-5 days so that DNA damage caused by lentivirus infection itself will be "healed" → only downstream data and can be ignored**
2. Gather population of cells (3-5 million)
3. Fix cells
    a. Immobilize and kill cells
    b. This allows us to stain, image, sort, and other downstream steps
    c. Dissociate cells from spheroid here?
4. Permeabilize cells
    a. Makes holes in cells so "stuff" can get in them (because we are doing an intracellular staining)
    b. Dissociate cells from spheroid here?
5. Antibody 1
    a. In our case, this will be gamma-H2AX
    b. We actually have a primary-conjugated antibody (already has fluorescence so do not need a secondary antibody)
        i. Our fluorophore will be APC - Alexa fluorophore 647
    c. Dissociate cells from spheroid here?
6. Flow Cytometry
    a. Will have graph: mCherry vs. FSC
    b. In each population, will have some cells positive for mCherry and some negative for mCherry (control, not lentivirally infected)
    c. Will "gate" mCherry+ cells and average their gamma-H2AX expression
        i. Two more subplots:
            1. mCherry+ only with APC gamma-H2AX
                a. Expected to see increase in APC gamma-H2AX

Loading [MathJax]/extensions/Safe.js

   b. On plot, “peak” will be shifted higher/to the right on x-axis (APC gamma-H2AX)

2. mCherry- only with APC gamma-H2AX

   a. Expect to see decrease in APC-gamma-H2AX (because no lentiviral infection = gene was not knocked down = no increase in DNA damage)

   b. On plot, “peak” will be shifted lower/to the left on x-axis (APC gamma-H2AX)

NOTE: will need to refer to current spheroid CRISPR screening gamma-H2AX staining protocols to see the process (do we dissociate spheroids before fixing, after fixing, or after permeabilizing?)

- **Possible: if earlier dissociation, may be losing some of DNA markers)**
- **Our job: find a “starting point” for when to dissociate based on literature, then test staining at alternative disassociation points and compare whether there are differences in results**

Loading [MathJax]/extensions/Safe.js

γ-H2AX_12/17/2024

Emily Rhine - Dec 17, 2024, 8:18 PM CST

Title: γ-H2AX

Date: 12/17/2024

Content:

Google query: "gamma h2ax staining protocol"

Link: Quantitative γ-H2AX immunofluorescence method for DNA double-strand break analysis in testis and liver after intravenous administration of $InCl_3$

Summary:

- "It is well known that a severe cell injury after exposure to ionizing radiation is the induction of DNA double-strand breaks (DSBs). After exposure, an early response to DSBs is the phosphorylation of the histone H2AX molecule regions adjacent to the DSBs, referred to as γ-H2AX foci. The γ-H2AX assay after external exposure is a good tool for investigating the link between the absorbed dose and biological effect. However, less is known about DNA DSBs and γ-H2AX foci within the tissue microarchitecture after internal irradiation from radiopharmaceuticals." [1]
- "H2AX phosphorylation and the formation of γ-H2AX foci in the testis are not restricted to radiation effects. The formation of haploid germ cells requires the formation and repair of meiosis-specific DNA DSBs through programmed changes in the chromatin structure [35, 36]. The phosphorylation and dephosphorylation of H2AX throughout the different stages of the spermatogenic cycle creates specific staining patterns correlated to germ cell development" [1]

γ-H2AX immunofluorescence labeling

The specimens were rinsed three times for 5 s each in phosphate buffer saline (PBS 0.1 M, pH 7.4) and dehydrated in a graded alcohol (EtOH) series (70–100%). They were then further immersed in equal volumes of ethanol (100%) and xylene (100%), followed by xylene (100%). The testes and the liver lobes were then infiltrated with 100% paraffin for 1 h at 58 °C, followed by immersion in fresh (100%) paraffin at 58 °C overnight. From the paraffin blocks, consecutive sections were made on a rotation microtome (Rotary Microtome, HM 360, Microm International GmbH, Waldorf, Germany). Five micrometer-thick sections of the testis and liver tissues were collected on SuperFrost Plus microscope slides (G Menzel, Braunschweig, Germany) and dried for 16–18 h at 37 °C. The sections were then deparaffinized, starting with immersion in 100% xylene, followed by immersion in a graded alcohol series down to 70% EtOH. Antigen retrieval was performed by immersing the slides in citrate buffer (pH 6.0) containing a detergent (0.5% Tween 20) that was heated to 90 °C for 10 min. The slides were allowed to cool to room temperature (RT, around 20 °C), incubated in acetone (100%, about 5 s), and then rinsed in PBS three times for 5 min each.

For γ-H2AX immunofluorescence labeling, two different primary antibodies against γ-H2AX were used: one mouse monoclonal anti-phospho-histone H2A.X, (clone JBW301, Merck Millipore, Darmstadt, Germany) and one rabbit polyclonal anti-γ-H2AX (Thermo Scientific Art no. PA1-25001). First, the sections were encircled with a silicon pen and immersed three times for 5 min each in a washing solution of PBSTX (0.1%) (phosphate buffer saline 0.1 M, pH 7.5, 0.1% Triton X-100) at RT. The slides were incubated with a blocking solution of PBSTX (0.1%) containing 1% bovine serum albumin (BSA) for 60 min at RT. The primary anti-γ-H2AX antibodies were diluted in 1% BSA/PBSTX (0.05%) to a concentration of 1 μg/mL for the monoclonal and 1.25 μg/ml for the polyclonal. Sections were incubated with the γ-H2AX antibody solution in a moisture chamber for 16–18 h at 4 °C. Randomly selected sections from non-exposed and exposed animals were incubated without the primary antibodies, i.e., they were used as specificity controls of the γ-H2AX labeling and secondary antibody binding. Sections were then rinsed in PBS three times for 5 min each under gentle shaking.

To visualize the primary antibody binding sites, sections were incubated with secondary goat antibodies against mouse IgG conjugated with Alexa Fluor 488 (Jackson Immunoresearch, Baltimore, MD, USA) or against rabbit IgG conjugated with Alexa Fluor 568 (Invitrogen. Art no. A11036). Secondary antibodies were diluted 1:150 (13.3 μg/mL) in 1% BSA/PBS, and sections were incubated for 60 min at RT. Sections were then rinsed in PBS three times for 5 min each and were incubated with 4′,6-diamidino-2-phenylindole (DAPI, nuclear labeling, Invitrogen, USA) at a concentration of 0.1 μM for 20 min at RT. Sections were then rinsed with PBS for 5 min before being mounted and coverslipped using DAKO fluorescent mounting medium (Carpenteria, USA). [1]

References

[1] A. Stenvall, E. Larsson, B. Holmqvist, S.-E. Strand, and B.-A. Jönsson, “Quantitative γ-H2AX immunofluorescence method for DNA double-strand break analysis in testis and liver after intravenous administration of 111InCl3,” EJNMMI Res, vol. 10, p. 22, Mar. 2020, doi: 10.1186/s13550-020-0604-8.

Conclusions/Action Items:

- Review protocol next semester
- Find alternate protocol to compare to
- Condense protocol and create materials list from it

2024/09/09 - Cancer Cell Line

Althys Cao - Oct 11, 2024, 9:47 AM CDT

Title: Cancer Cell Line Research

Date: 2024/09/09

Content by: Althys

Present: self

Goals: introductory research about cancer cell line - what is it? what is the difference between cancer cell line and cancer? what are the different genetic mutations and what they mean?

Content:

- Cancer cell lines are *in vitro* models used in research, derived from a tumor
- Primary cancer cell lines are taken directly from cancerous tissues
    - primary tumors contain high heterogeneity due to various subclones with different genetic mutations
- "Immortal" cancer cell lines can continually grow and divide over time under lab conditions
    - modified chemically for improved amneability to gene editing
    - Typically dominated by a single clone, lack heterogeneity, not respresentative of the true responses of cancers to treatments and therapies
    - Pros and cons:

Advantages	Disadvantages
Relatively inexpensive alternative to animal models. They are also easy to maintain and store.	Susceptible to genetic and phenotypic drift as mutations arise.
Most cell lines can grow indefinitely when cultured correctly.	Identifying the cancer subtype the cell line is derived from isn't always possible.
They require much less maintenance as compared to animal models.	Cell lines are highly susceptible to contamination by microorganisms, and some (like HeLa) can contaminate other cell lines.
Cell lines are all genetically similar which makes them easy and inexpensive to sequence in contrast to tissue samples from patients.	There are inherent variabilities in each tumor sample because some cell lines were developed from metastasis instead of primary tumors.
Results are highly reproducible and they are amenable to CRISPR editing.	Cell-cell and cell-matrix interactions are lost in vitro making these cells less useful in translational research.

- Mutations:
    - TP53 gene, "tumor-suppressor gene", which repairs DNA damage (so cells don't become cancer cells) or results in cell death (via apoptosis). This gene is mutated in around 50% of cancer cells.
    - Oncogene KRAS, which encodes for proteins that control cell division.

References: https://www.verywellhealth.com/the-p53-gene-its-role-in-cancer-2249349

Conclusions/action items:

- Cancer cell lines used for this project will be obtained from either Hess Lab or other labs in WIMR, likely to be "immortal" cell lines.
- Note for mutations when research different cell lines to be used.

2024/09/17 - ECM-Free and ECM-based Cancer Spheroid Formation and CRISPR-Cas9 Screening Protocol

Althys Cao - Oct 11, 2024, 9:05 AM CDT

Title: Cancer Spheroid Formation and CRISPR-Cas9 Screening Protocols

Date: 2024/09/17

Content by: Althys

Present: self

Goals: look into some previous studies that use CRISPRi screening for 3D cancer spheroids and look at their protocols for 3D cancer spheroid formation and CRISPR-Cas9 screening and compare the differences as well as similarities among them.

Content:

1. **Paper 1:**"CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities" [1] (note: this paper was referred to a lot in the past 2 client meetings and was recommended to read for further understanding of project)
    - 10 cell lines were used: NCI-H1437, NCI-H1568, NCI-1650, NCI-1975, NCI-H322, NCI-H1792, NCI-H2009, NCI-H23, NCI-H358 and A549 from American Type Culture Collection (ATCC).
    - ECM-free scalable method propagate 3D spheroids.
    - 3D models more accurately showed growth phenotypes that resembled the observed ones in tumors. Some genes that were enriched in human lung cancers also had more significant effects in 3D models.
    - Seeding densities and methylcellulose concentrations (to change viscosity of growth medium for cancer spheroids) optimized to propagate 200 million cells (more than the needed amount of 100-150 million cells []).
    - Protocol:
        - 3D cultures:
            - Cells were seeded at a density of 50,000 cells/cm^2 on pre-treated ultra-low attachment or polyhema-covered plates in 500uL RPMI 1640 medium with 0.75% methylcellulose. Spheroids were then split every 3-4 days.
            - Spheroids were collected by centrifugation and dissociated into single cells using accutase.
        - Genome-wide CRISPR screens:
            - CRISPR library was synthesized by Aligent. There were total of around 210,000 single guide RNAs (sgRNAs) with around 10 sgRNAs/gene, 13,500 of which are negative control sgRNA.
            - Plasmid libraries were created to drive expression of sgRNA and mCherry (fluorescence protein used for labelling purposes), then transfected to produce lentiviral pools and transduced into cell lines.
            - Samples were used to calculate growth and/or tested for drug resistance.
2. **Paper 2:**"3D Culture Models with CRISPR Screens Reveal Hyperactive NRF2 as a Prerequisite for Spheroid Formation via Regulation of Proliferation and Ferroptosis" [2]
    - A549 and H1437 cell lines were used, from ATCC.
    - Protocol:
        - 3D cultures:
            - Cells were cultured in RPMI 1640 + 10% FBS + 1% Pen/Strep

        - For growth of spheroids, cells were also supplemented with 4% Matrigel
    - Genome-wide CRISPR screens:
        - Pooled lentiCRISPR library, cloning of sgRNAs into lentiGuide-puro backbone, verification, and titering of virus was created and performed
        - Cells were transduced with lentiCas9-blast and then with the lentiGuide-puro library at an expected MOI of 0.25 and coverage of 500 cells per sgRNA.
  - The study's CRISPRi library gene list as well as its phenotypes have a strong overlap with those from paper 1 described above, suggesting that inner cells of ECM-based 3D spheroids may be similar to ECM-free 3D spheroids. Also, A549 and H1437 cells can produce ECM in ECM-free environments.

References:

[1] K. Han *et al.*, "CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities," *Nature*, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

[2] N. Takahashi *et al.*, "3D Culture Models with CRISPR Screens Reveal Hyperactive NRF2 as a Prerequisite for Spheroid Formation via Regulation of Proliferation and Ferroptosis," *Mol. Cell*, vol. 80, no. 5, pp. 828-844.e6, Dec. 2020, doi: 10.1016/j.molcel.2020.10.010.

Conclusions:

- 3D cancer spheroid models that can be utilized for CRISPR-Cas9 screens have been implemented in previous research, with and without using an exogenous extracellular matrix (ECM)
- Depending on the cell line, some cell lines can produce their own ECM, meaning an ECM-free method is still sufficient

Action items:

- Discuss with client how important it is for the team to replicate cell-to-ECM interactions, discuss with client about Matrigel-based protocol
- Look more into low attachment plates, define variables to tweak in low attachment plates method

2024/09/18 - Standards Research

Althys Cao - Oct 11, 2024, 10:10 AM CDT

Title: Standards Research

Date: 2024/09/18

Content by: Althys

Present: self

Goals: research standards for PDS

Content:

- As cells used in the project will be derived from human tissues, they will be classified as BSL-2 and thus require proper PPE handling
- The use of human cell lines and other organisms in research for this project is outlined in various standards and regulations including Section 29 CFR 1910.1030 *(OSHA Bloodborne Pathogens),* Section 21 CFR 876.5885 *(Tissue culture media for human ex vivo tissue and cell culture processing applications),* and a comprehensive cell line guideline by Geraughty, *et al* (2014):
    - Bloodborne pathogens are microorganisms in the blood that can infect and cause diseases in humans [1].
    - Human cell lines are designated by the FDA as Class II (Special Controls) [2].
    - If applicable, genetic modification processes on the cell line must be detailedly-noted and kept track of, including but not limited to, sequence details, insertion vectors and modes, and antibiotic resistance markers [3]. Following modification using lentivirus, cell lines may be additionally tested to confirm non-infectivity [3].
- Human cell lines that may be needed for this project are already available in the client's lab, or can be obtained from another lab, meaning they already comply with the requirements listed above. In the case that biological research materials are obtained from another lab or institute, an MTA (material-transfer agreements) must be signed by both the provider and recipient to define their rights regarding the materials [3].
- In the unlikely case that new human cell lines must be acquired, additional ethical and authentication considerations must be taken into account:
    - Ethical considerations: the Patient Consent Form and associated Patient Information Sheet will be needed to explain the need for the specimen and the purpose and importance of the research [3]. Patient/ donor and original tissue information such as age, sex, clinical history, site of origin, nature of tissue specimen, stage and grade of cancer or other disease/ pathology, and other important information must also be recorded.
    - For cell line authentication, short tandem repeat (STR) is the recommended profiling method, outlined in ASN-0002 *(Authentication of Human Cell Lines: Standardization of STR Profiling)* provided by the American National Standards Institute (ANSI) and the American Type Culture Collection (ATCC). STR is a DNA-based sequence method that allows for detection of mutations and adventitious agents, as well as confirmation of the origin of the interested cell line [4].

References:

[1] "1910.1030 - Bloodborne pathogens. | Occupational Safety and Health Administration." Accessed: Sep. 17, 2024. [Online]. Available: https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030

[2] “CFR - Code of Federal Regulations Title 21.” Accessed: Sep. 17, 2024. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=876.5885

[3] R. J. Geraghty *et al.*, “Guidelines for the use of cell lines in biomedical research,” *Br. J. Cancer*, vol. 111, no. 6, pp. 1021–1046, Sep. 2014, doi: 10.1038/bjc.2014.166.

[4] J. L. Almeida, K. D. Cole, and A. L. Plant, “Standards for Cell Line Authentication and Beyond,” *PLoS Biol.*, vol. 14, no. 6, p. e1002476, Jun. 2016, doi: 10.1371/journal.pbio.1002476.

Conclusions/action items: Cells used for this project should already be available in the lab, therefore the ethical and authentication considerations for creation of new cell lines should not be a concern. The main concern should be about safety - have to follow proper safety considerations and wear appropriate PPE

2024/09/23 - Drug Sensitivity and DNA Damage Inducing Drugs

Title: Drug Sensitivity and DNA Damage-Inducing Drugs

Date: 2024/09/23

Content by: Althys

Present: self

Goals: Drug sensitivity comes up quite a lot during cancer cell line research. I want to understand more about the parameter regarding drug sensitivity as w
DNA damage in various cancer cell lines.

Content:

Z-scores are drug-gene correlation scores, used to describe whether a cell line is sensitive or resistant to a certain drug in comparison to other cell lines [1]
negative Z-score means that a cell line is *sensitive* to a drug [2]. A difference of **±1.7** is significant [2].

Anticancer drugs used to induce DNA damage in cancer cell lines to induce cell death [3], [4], [5]:

- Bleomycin: induces dsDNA break, extent of damage can be studied using gamma-H2AX foci staining [5]
- Cisplatin: platinum core with two chloride leaving groups and two amine nonleaving groups, form intrastrand DNA crosslinks. Platinum-based analogs
  - Carboplatin
  - Oxaliplatin
- Methotrexate: prevents DNA synthesis by inhibiting DHFR
- Topoisomerase poison:
  - Camptothecin: topoisomerase I poison, binds to DNA-topoisomerase complex to inhibit strand ligation.
  - Doxorubicin, daunoribicin: topoisomerase II poison.
- Drugs that alkylate DNA bases:
  - cyclophosphamide
  - chlorambucil
  - melphalan
  - nitrosoureas (carmustine, lomustine, and semustine)
  - triazenes (e.g., dacarbazine and temozolomide)
  - mitomycin C
  - streptozotocin
- Blocking nucleotide metabolism pathway:
  - pyrimidine analogs:
    - 5-fluorouracil (5-FU)
    - capecitabine
    - floxuridine
    - gemcitabine
  - purine analogs:
    - 6-mercaptopurine
    - 8-azaguanine
    - fludarabine
    - cladribine
- Inhibit enzymes important for DNA synthesis:
  - methotrexate
  - aminopterin
  - pemetrexed

References:

1. Genomics of Drug Sensitivity in Cancer Database: https://www.cancerrxgene.org/news#:~:text=The%20z%2Dscore%20describes%20whether,screened%

2. Z-score paper: https://ncbi.nlm.nih.gov/pmc/articles/PMC10376793/

3. Chemically-Induced DNA Damage in Cancer: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6032311/

4. Another comprehensive drug paper: https://www.sciencedirect.com/science/article/pii/S1074552113001312

5. Bleomycin:
https://www.sciencedirect.com/science/article/pii/S1383574217301205#:~:text=This%20antibiotic%20is%20an%20S%2Dindependent%20clastogen%20an

Conclusions/action items:

- Look for Z-scores for different cancer cell lines regarding the drugs mentioned above.
  - Look for a z-score of -1.7 or below (important to note that some other sources mention -1 or below as significant, meaning a z-score of -1.7 to -1
- Ask the client which kind of drugs is appropriate to look at for the project, as in, which of the drugs create the kind of DNA damage that the client is loo

2024/09/23,24 - Cancer Cell Line Research

1. NCI-H23 Cell Line

Title: NCI-H23 Cell Line Research

Date: 2024/09/23

Content by: Althys

Present: self

Goals: In Han *et al*, 2020 Carley's 3D Spheroid paper], NCI-H23 cancer cell line is the most referred to, even in meetings with both clients. I want to do moı handle? 3. Any study with DNA damage as treatment to kill H23 cell line? 4. Ability to form spheroids?

Content:

- Non-small lung cancer cell line, originally from 51yo male with lung adenocarcinoma [1]
- High proliferation rate: double every 38 hours [1]
- Resistance to apoptosis/programmed cell death [1]
- Mutations [1]:
  - K-ras 12
  - p53: in the 246 codon
  - TP53, which encodes for p53 tumor suppressor protein (common in lung cancer)
  - deletion in CDKN2A gene, which encodes for p16 tumor suppressor protein (common in lung cancer)
- Colony forming efficiency of 9.7% in soft agarose [2]
- Studies with DNA damage-inducing drugs [3]:
  - bleomycin: z-score = -2.57 for 10uM of drug, z-score = -1.28 for 50uM
  - cisplatin: z-score between -1.49 to -0.73
  - oxaliplatin: 0.22 - 0.6
- Ability to form spheroid: interestingly, some paper can form H23 spheroids while some cannot [4], [5], [6]

References:

1. NCI-H23 Transfection Reagent: https://altogen.com/product/nci-h23-transfection-reagent-lung-adenocarcinoma/

2. ATCC NCI-H23: https://www.atcc.org/products/crl-5800#:~:text=Characteristics&text=This%20line%20was%20derived%20from,a%20patient%20prior%20to%20therapy.&text=Male%20Clinical%20data-,Thi

3. Drug sensitivity: https://pubchem.ncbi.nlm.nih.gov/cell/4007#section=Small-Molecule-BioAssays

4. Cannot form H23 spheroids: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7718371/

5. Can form H23 spheroids: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7368463/

6. Can form H23 spheroids (2): https://www.researchgate.net/figure/The-AMG510-sensitivity-of-3D-NCI-H23-spheroids-under-normoxia-conditions-a-The-bı

Conclusions/action items:

Althys Cao - Oct 11, 2024, 8:08 AM CDT

2. A549 Cell Line

Title: A549 Cell Line Research

Date: 2024/09/24

Content by: Althys

Present: self

Loading [MathJax]/extensions/Safe.js

Goals: A549 is used in both Han *et al*, 2020 ECM-free model and in Takahashi *et al*, 2020 ECM-based model. There are noticeable overlap in the CRISPR gene library between Han *et al* and Takahashi *et al,* attributed to the fact that ECM-free 3D colony cells may partly resemble the inner cells of ECM-based 3D spheroids, and that A549 cells can produce ECM by themselves in ECM-free 3D conditions. It is unknown what the effects of cell-to-ECM interactions to CRISPRi screening and gamma-H2AX foci staining (and that the client does not place high importance in good replication of cell-to-ECM interactions); however, it is still interesting to see if we can incorporate A549. Guiding questions are the same as above (1. What disease are they associated with? 2. How robust are they/what is their growth rate/how much stress can they handle? 3. Any study with DNA damage as treatment to kill H23 cell line? 4. Ability to form spheroids?) but with the addition of 5. Mechanisms of ECM development by A549?

Content:

- Non-small lung carcinoma epithelial cell [1]
- Isolated from 58yo Caucasian male [1]
- Adherent growth properties [1]
- High proliferation rate: double every 22 hours, can sometimes take up to 40 hours [1]
- Mutations: KRAS mutation and EGFR wild type [2]
- Z-scores with DNA damage-inducing drugs [3]:
    - bleomycin: -0.9 -> -1.14
    - oxaliplatin: -1.1 -> 1.6
    - cisplatin: -0.12

References:

1. https://www.atcc.org/products/ccl-185#detailed-product-information

2. https://oncology.labcorp.com/a549-model-non-small-cell-lung-cancer

3. https://www.cancerrxgene.org/cellline/A549/905949

Conclusions/action items:

Loading [MathJax]/extensions/Safe.js

2024/09/26 - Low Attachment Plates Method / Treated Tissue Culture Plates Method

Althys Cao - Oct 11, 2024, 10:34 AM CDT

Title: Low Attachment Plates Method

Date: 2024/09/26

Content by: Althys

Present: self

Goals: first design idea for spheroid formation protocol is using low attachment plates method, as seen in Han *et al,* 2020. Determine what are low attachment plates and how to actually form spheroids with these plates.

Content:

Treated tissue cultures method is a scaffold-free method [1]. It uses low attachment plates, which are coated with a hydrophilic polymer (such as hydrogel or poly-HEMA) to prevent specific and nonspecific cell adhesion to the culture vessel [1]. Thus, high cell-to-cell interactions allow cells to cluster together, form cell aggregates and eventually into 3D spheroids (Figure ___) [2]. The whole process usually takes up to 4 days [2].

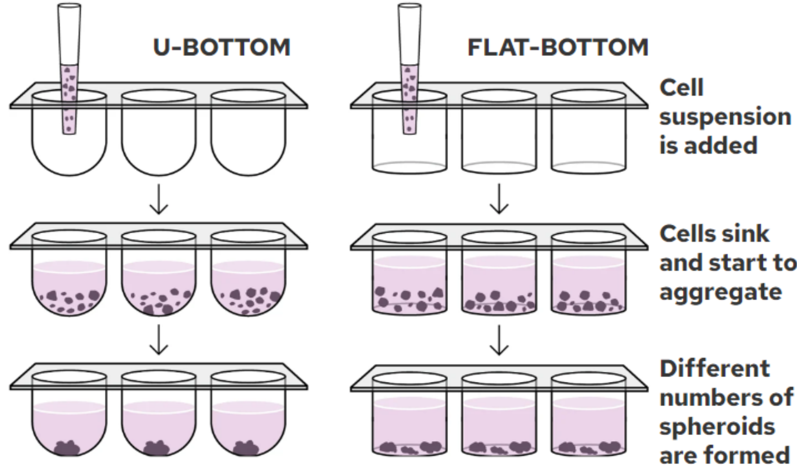


Figure __. Spheroid formation by treated tissue culture plates/low attachment plates with U-bottom wells (left) and flat-bottom (right) [1].

References:

[1] W. Asghar *et al.*, “In Vitro Three-Dimensional Cancer Culture Models,” *Cancer Target. Drug Deliv. Elus. Dream*, pp. 635–665, Jul. 2013, doi: 10.1007/978-1-4614-7876-8-24.

[2] L. Carroll, B. Tiwari, J. Curtin, and J. Wanigasekara, “U-251MG Spheroid Generation Using Hanging Drop Method Protocol,” May 2021, Accessed: Sep. 27, 2024. [Online]. Available: https://www.protocols.io/view/u-251mg-spheroid-generation-using-hanging-drop-met-btstnnen

Conclusions/action items: I really like this method, mainly because it is not labor intensive or time consuming, which is very important as the team will need high amounts of spheroids due to the large scale of genome-wide CRISPR screening. The only thing that can contribute to its batch-to-batch variability is if cells are cultured in FBS solution (prior to seeding to form spheroids), but that is a former step so it should not be a big concern.

2024/09/26 - Hanging Drop Method

Althys Cao - Oct 11, 2024, 11:09 AM CDT

Title: Hanging Drop Method

Date: 2024/09/26

Content by: Althys

Present: self

Goals: Hanging drop is another scaffold-free method to form spheroids - look into it

Content:

Currently, two methods to form hanging drops exist. The first method is the traditional method, involving dispensing small amounts of cell suspension on a surface, usually the underside of a petri dish, then flipping the dish upside down to form hanging drops (Figure 1) [2]. However, this method has a high risk of droplet fusion (if space is limited), loss of droplet shape (because of frequent media change), and droplet evaporation [3], [4]. The second method using hanging drop plates negates these risks [4]. Each plate consists of a lid to maintain sterility and a main component, which has a water reservoir to prevent evaporation and access holes on top where cells can be added to form hanging drops on the bottom (Figure 2a) [4]. Hanging drops create a flexible spheroid cell-to-liquid interface, allowing cells to gravitationally aggregate and form because of high cell-to-cell interactions (Figure 2b) [1]. The whole process usually takes up to 4 days [2].

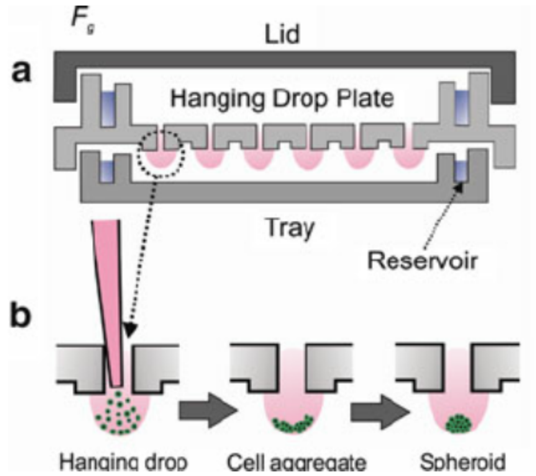


Figure 2. Spheroid formation by hanging drop plate [1].
a. Composition of a hanging drop plate, b. Spheroid formation.

References:

[1] W. Asghar *et al.*, “In Vitro Three-Dimensional Cancer Culture Models,” *Cancer Target. Drug Deliv. Elus. Dream*, pp. 635–665, Jul. 2013, doi: 10.1007/978-1-4614-7876-8-24.

[20] L. Carroll, B. Tiwari, J. Curtin, and J. Wanigasekara, “U-251MG Spheroid Generation Using Hanging Drop Method Protocol,” May 2021, Accessed: Sep. 27, 2024. [Online]. Available: https://www.protocols.io/view/u-251mg-spheroid-generation-using-hanging-drop-met-btstnnen .

[21] “Generation and Cultivation of 3D Cell Models,” ibidi. Accessed: Oct. 10, 2024. [Online]. Available: https://ibidi.com/content/1037-generation-and-cultivation-of-3d-cell-models.

[22] A. P. P. Guimaraes, I. R. Calori, H. Bi, and A. C. Tedesco, “SpheroMold: modernizing the hanging drop method for spheroid culture,” *Front. Drug Deliv.*, vol. 4, Jun. 2024, doi: 10.3389/fddev.2024.1397153.

[23] “Perfecta3D Hanging Drop Plates - 96-Well.” Accessed: Oct. 10, 2024. [Online]. Available: https://www.selectscience.net/product/perfecta3d-hanging-drop-plates-96-well

Conclusions/action items: If we were to use hanging drop method, it will have to use hanging drop plates because of the sheer amount of spheroids needed. However, the client is against this protocol and this protocol does not produce as good results as low attachment plates.

2024/09/26 - Matrigel Method

Althys Cao - Oct 11, 2024, 11:24 AM CDT

Title: Matrigel Method

Date: 2024/09/26

Content by: Althys

Present: self

Goals: look into Matrigel method, a scaffold-based method

Content:

The Matrigel method is a scaffold-dependent method [1]. Cells are cultured in low attachment plates for 4 days to form aggregates, then Matrigel is added to encourage cell-to-ECM interactions which will form spheroids after another 2 days (Figure 1) [2].

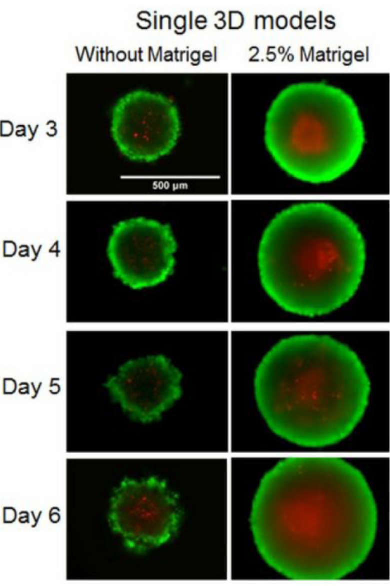


Figure 1. Comparison of single 3D spheroids grown without and with Matrigel [3]. Green indicates live cells and red indicates dead cells.

References:

[1] W. Asghar *et al.*, “In Vitro Three-Dimensional Cancer Culture Models,” *Cancer Target. Drug Deliv. Elus. Dream*, pp. 635–665, Jul. 2013, doi: 10.1007/978-1-4614-7876-8-24.
[2] S. S. Nazari, “Generation of 3D Tumor Spheroids with Encapsulating Basement Membranes for Invasion Studies,” *Curr. Protoc. Cell Biol.*, vol. 87, no. 1, p. e105, 2020, doi: 10.1002/cpcb.105.
[3] M. A. Badea *et al.*, “Influence of Matrigel on Single- and Multiple-Spheroid Cultures in Breast Cancer Research,” *SLAS Discov.*, vol. 24, no. 5, pp. 563–578, Jun. 2019, doi: 10.1177/2472555219834698.

Conclusions/Action Items: While the client does not know the importance or effects of cell-to-ECM interactions on gamma-H2AX staining after knocking down genes and think it will be very interesting to study, he still recommends against the Matrigel method due to the high batch-to-batch variability from using animal-derived matrices.

2024/10/28 - PolyHEMA Plate Formation Protocol

Althys Cao - Nov 15, 2024, 10:38 PM CST

Title: PolyHEMA Plate Making Protocol

Date: 2024/10/28

Content by: Althys

Present: self

Goals: look up protocols to make polyHEMA-coated plates ourselves as those are the team's chosen plates for spheroid formation protocol moving forward.

Content:

From: “In vitro differentiation of human embryonic stem cells to hemogenic endothelium and blood progenitors via embryoid body formation.” Accessed: Oct. 29, 2024. [Online]. Available: https://star-protocols.cell.com/protocols/499

i. Poly-HEMA solution is made by dissolving 6 g of Poly-HEMA in 500 mL of 95% ethanol (25 mL of tissue culture grade H2O in 475 mL of biological grade ethanol) for 10–12 h on a heated stirrer at 30°C.
ii. The solution is sterilized using a 0.2 μm filter (Nalgene) and kept at 20°C until needed.
iii. 10 cm tissue culture dishes are coated with 4 mL of Poly-HEMA solution and the dishes are allowed to dry completely for 24 h at 40°C in a ventilated oven. Similarly, 6-well plates are coated with 1 mL of Poly-HEMA solution per well. Plates and dishes are kept with their lids on during the drying step in the oven to maintain sterility.
iv. Coated dishes and plates can be sealed with parafilm and stored at 20°C for 3 months.

Conclusions/action items: Applicable protocol (materials and protocol do not seem complicated). Will discuss with team and compare this with other plate making protocols

2024/10/28 - Additional Spheroid Formation Protocols using Low Attachment Plates

Althys Cao - Nov 15, 2024, 10:46 PM CST

Title: Additional Spheroid Formation Protocols using Low Attachment Plates

Date: 2024/10/28

Content by: Althys

Present: self

Goals: look up some extra protocols (apart from Han *et al* protocol) to form spheroids (even better is A549 is used) and define some parameters that are in play (such as centrifugation, any additional content that is added to the spheroid formation media)

Content:

1. From: J. R. Ferrarone *et al.*, "Genome-wide CRISPR screens in spheroid culture reveal that the tumor suppressor LKB1 inhibits growth via the PIKFYVE lipid kinase," *Proc. Natl. Acad. Sci.*, vol. 121, no. 21, p. e2403685121, May 2024, doi: 10.1073/pnas.2403685121.
    a. Plate: low-attachment, V-bottom 96-well plates (S-bio MS-9096VZ)
    b. Seeding density: 1000 cells/well
    c. 100 uL media/well
    d. Spin plates at at 300g, 5 min, then incubated overnight
    e. 80uL of media is aspirated from the plate the following day then 80uL of methylcellulose-containing medium is added
        i. 1.2g of methylcellulose per 100 mL for methylcellulose-containing medium (1.2e-5g/uL)
        ii. Final methylcellulose concentration: 9.6e-6g/uL
    f. Full protocol: Single spheroid growth assays were performed by seeding 100 cells per well (for H2030), 500 cells per well (for Calu-1, H1792, SW1573, and 634T cell lines), or 1,000 cells per well (for A549 and MOR cell lines) into low-attachment, V-bottom 96-well plates (S-bio MS-9096VZ) containing 100 µL of regular media per well. The plate was spun at 300 × g for 5 min and then placed in an incubator overnight. The following day, 80 µL of medium was slowly aspirated from each well, and then, 80 µL of methylcellulose-containing medium was layered on top of each spheroid. For all 96-well assays, a total of 100 µL of medium (with or without added methylcellulose) was used per well. Images of live cells were taken every 4 h using an Incucyte Zoom or Incucyte S3 live cell imaging system. The volume of an individual spheroid was derived from the total cross-sectional area of the spheroid. The start of each spheroid growth assay (T = 0 h) was approximately 8 h after the addition of methylcellulose. For all live cell imaging experiments, a minimum of three replicates were performed for each condition. Wells were excluded from analysis if the Incucyte failed to image a spheroid at any time point.

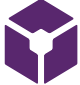
2. From: T. Stiff, S. Bayraktar, P. Dama, J. Stebbing, and L. Castellano, "CRISPR screens in 3D tumourspheres identified miR-4787-3p as a transcriptional start site miRNA essential for breast tumour-initiating cell growth," *Commun. Biol.*, vol. 7, no. 1, pp. 1–11, Jul. 2024, doi: 10.1038/s42003-024-06555-1.
    a. NOTE: this study did not use A549
    b. ultralow attachment plates (Corning, # CLS3471)
    c. Seeding density: 1.5e3 for HCC1395 and 1e3 for MCF7 (cells/well)
    d. Cells were grown in serum-free DMEM/F12 medium (Gibco) supplemented with B27 (1:50, Gibco), 20 ng/mL basic fibroblast growth factor (bFGF, Biolegend), and 20 ng/mL EGF (Sigma).
    e. The percentage of sphere formation efficiency was calculated as a ratio between the number of formed spheres divided by the number of cells seeded, multiplied by 100.
    f. Full protocol: For sphere formation assay, BC cells were plated in ultralow attachment plates (24 well) at a density of 1.5 × 103 for HCC1395 and 1 × 103 for MCF7 cells/well, and formed spheres with a size larger than 50 µm were counted under the microscope. The percentage of sphere formation efficiency

was calculated as a ratio between the number of formed spheres divided by the number of cells seeded, multiplied by 100. For each condition at least 6 wells of a 24 well plate were counted and 3 independent biological replicates performed.

3. From: L. Carroll, B. Tiwari, J. Curtin, and J. Wanigasekara, “U-251MG Spheroid generation using low attachment plate method protocol,” May 2021, Accessed: Oct. 29, 2024. [Online]. Available: https://www.protocols.io/view/u-251mg-spheroid-generation-using-low-attachment-p-bszmnf46
    a. **Dispense 200 µl/well into ultra-low attachment 96-well plates** using a multichannel pipette ensuring pipette tips do not touch the surface of the wells to protect surface coating
    b. **Centrifuge** the Nunclon™ Sphera™ 96-Well plates **at 1250rpm for 5 minutes** and transfer the plates to an incubator (37 °C, 5% CO2, 95% humidity).
    c. **After 24h incubation, the media must be replenished. Remove 100µl media without disrupting tumorspheres and add 100µl of fresh media (DMEM + 10%FBS + 1%Ab)** into each well and incubate at 37°C (5% CO2, 95% humidity). The sides of wells should be used to remove or add media and pipetting should be carried out at average or below average speeds to avoid disruption to spheroids
    d. U-251 MG Human glioblastoma astrocytoma **spheroids will usually form in 3-7** days from the starting date depending on seeding density and visually confirm tumor spheroid formation daily using Bright-field microscope.

4. N. Abe-Fukasawa, K. Otsuka, A. Aihara, N. Itasaki, and T. Nishino, “Novel 3D Liquid Cell Culture Method for Anchorage-independent Cell Growth, Cell Imaging and Automated Drug Screening,” *Sci Rep*, vol. 8, no. 1, p. 3627, Feb. 2018, doi: 10.1038/s41598-018-21950-5.
    a. The main difference with this protocol is that it also implements LA717 (low-molecular weight agar). While LA717 does not serve as a scaffold, it allows for more uniform cell dispersion in the medium (cell do not gather to form clumps) → spheroids are relatively homogenous in size. A concentration of 0.03% (w/v) of LA717 was used
    b. Full protocol: Each human cancer cell line was seeded at a density of 5,000-20,000 cells/mL into the appropriate medium containing 0.03% (w/v) of LA717, and was dispensed into 96-well flat-bottom low-attachment plates using 100–200 µL/well.

Conclusions/action items: Some parameters include: whether centrifugation is involved in the process to speed up the cell aggregate process (and subsequently spheroid formation) and addition of extra chemicals (for example, in Han *et al*, methylcellulose is added for viscosity and in Abe-Fukasawa *et al*, LA717 is added to ensure uniformity in spheroid size), and cell density. Furthermore, I think that U-bottom wells would be a better option than flat-bottom wells as they would produce one spheroid per well. Based on client meetings, I think that the Han *et al* protocol is most likely going to be the one that the team will stick with at the end, but I will present these options to the team and clients for their opinions.



2024/09/09 - Spheroids as a Type of 3D Cell Culture

ANA MARTINEZ - Sep 22, 2024, 3:24 PM CDT

Title: Article: Spheroids as a Type of Three-Dimensional Cell Cultures—Examples of Methods of Preparation and the Most Important Application

Date: 09/09/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To gain a more thorough understanding of spheroids and their use in 3D cell culture, as well as the most common methods of forming spheroids.

Content:

Search Engine: Google

Query: "spheroid formation types"

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503223/

- Cell-cell and cell-ECM interactions differ from 2D to 3D cultures and between cell layers in spheroids structures --> this can affect cytotoxicity results
- Spheroids are one type of 3D cell culture that more precisely mimic the natural cell microenvironment
- Spheroids are cell aggregates that self-assemble in an environment that prevents attachment to a flat surface, through the help of integrins (membrane proteins) and ECM proteins
  - Integrins - involved in activation of focal adhesion kinase (FAK); involved in cell adhesion, migration, and growth
- 3 basic steps to spheroid formation:
  - 1) Dispersed/single cells aggregate due to long-chain ECM fibers consisting of RGD motifs that allow to bind cell-surface integrin --> leads to upregulated cadherin expression
  - 2) Cadherin accumulates on cell membrane surface
  - 3) Hemophilic cadherin-cadherin binding between neighboring cells allows for "tighter" connections between cells --> forms spheroids
- Spheroid Formation Method # 1: Hanging Drop
  - Limitations:
    - Low-throughput
    - Spherical geometry
    - High shear force environment
    - Complicated/time-consuming manipulations (changing medium, adding compounds)
    - Some cell lines do NOT form compact spheroids
  - Strengths:
    - Relatively simple equipment is needed (not specialized, but practical) --> cell suspension placed on the well/lid of a plate
    - Cells remain in direct contact with each other and the ECM throughout
    - Can be used to co-culture several cell lines
  - Basic Steps:
    - Add drops of cell suspension on well/lid of a culture plate
    - Turn this surface upside down
    - Cell suspension will become a hanging drop held by surface tension --> can add methyl cellulose to increase this
    - Microgravity will concentrate cells at the bottom of the drop
- Spheroid Formation Method # 2: Hydrogels
  - Typically use non-adhesive agarose hydrogels --> no influence of an ECM
  - Strengths:
    - Ease of maintenance
    - Can control microtissue size
    - Large amount of microtissues per plate
    - Some micromolds for hydrogel preparation are commercially available (ex: see Vantangoli et al. (2015))
    - Allows for versatility for controlled microtissue production
  - Basic steps:
    - Cells seeded on hydrogel with recesses where cells will sink

    - These cells will self-assemble into 3D spheroid microtissues
    - Cells in homogeneous suspension self-assemble into spheroids, while cells in heterogeneous suspension self-segregate to form multilayered structures
- Spheroid Formation Method #3: Rotary Cell Cultures
  - Limitations:
    - Longevity of cultures (tends to be low)
    - Variation in spheroid size is higher
    - Mechanical damages of cells
  - Strengths:
    - One of the simplest methods
    - Produces spheroids on a large-scale (high-throughput)
  - Basic steps:
    - Cell culture is in a bottle with an agitator
    - Cells cannot attach to the substrate --> start aggregating and self-assembling
    - Variation: rotate flask around a horizontal axis --> simulation of microgravity with minimal hydrodynamic forces --> does NOT destroy cells, forms bigger and morphologically similar spheroids
- Spheroid Formation Method #4: Cell Suspension with Addition of Nanofibers
  - Involves adding polymer nanofibers to a suspension of adherent cells
  - Cells bind to nanofibers via vitronectin and fibronectin from the media's serum --> these proteins adsorb on nanofibers, then cells attach to them and form spheroids
  - Strengths:
    - Nanofibers allow for increased spheroid production
    - Reduced cell death (due to cell non-adherence)
- Spheroid Formation Method #5: Magnetic Levitation Method
  - Basic Steps:
    - Magnetic force overcomes gravitational force
    - Cells treated with paramagnetic iron oxide nanoparticles overnight --> cells uptake
    - After washing, cells are lifted with trypsin and seeded into low-adhesive plates
    - Magnet is placed on top of plate lid --> labeled cells are pulled up under magnetic forces
    - Spheroids created within few hours
      - Like the "opposite" of hanging drop method!
  - Strengths:
    - Cells are able to associate into 3D cell culture and produce ECM, retaining cellular activity
    - Potentially a cheaper substitute for expensive and labor-intensive method based on human tumor xenografts from immunodeficient mice
- Spheroid Formation Method #6: Microfluidic Systems
  - Aimed to address non-microfluidic methods' limitations, such as:
    - Differences in spheroid diameters
    - Low-throughput
    - Labor intensity
    - Reduction of oxygen and nutrients (at cells in middle)
    - Increase in osmolality
    - Increase in metabolite level
  - Strengths (more specific):
    - Controlled mixing
    - Chemical concentration gradients are formed
    - Lower reagent consumption
    - Control of shear stress and pressure on cells
    - Constant perfusion
    - Dynamic environment = better reflection of in vivo environment
  - Basic components:
    - Microfluidic device is made of microwells connected by microfluidic channels
    - Microchannels are prepared by etching or forming on the surface of neutral materials (ex: silicon, glass, PDMS)
    - Cells are cultured above layers made of matrix coated porous membrane/in direct contact with endothelial cells
    - Immune and tumor cells flow through channels
- Spheroid Formation Protocol #7: Bioprinting
  - Basic steps:
    - Cell layers and supporting biological materials are positioned precisely to mimic functions of tissue/organ
    - Computer program controls the deposition of droplets --> creates 3D structures
  - Limitations:
    - Expensive equipment (bioprinters)

    - Poor choice of bioinks (particularly in laser-assisted bioprinting)
  - Strengths:
    - Relatively fast to generate
    - Inexpensive to generate
    - High resolution
    - Provides high viability of cells
    - Better vascularization compared to other 3D culture models
    - Better reflection of tumor microenvironment and heterogeneity (particularly in later stages of tumors) compared to other models

Conclusions/action items:

From this article, I have learned that there are a lot of different methods of generating spheroids. I have experience with the hanging drop technique and using different amounts of methyl cellulose to modulate spheroid size/compactness. However, based on what our client tells us at our next meeting, we may find that the Hess Lab may be leaning towards another specific method/has had a bad experience with certain methods. Also, we will have to wait until we learn more about our team's budget before we pick a method, as these vary greatly in expense.

2024/09/10 - What is CRISPR Screening?

ANA MARTINEZ - Sep 10, 2024, 10:39 PM CDT

Title: Article: What is CRISPR screening? The role of oligo libraries in identifying genetic sequences of importance

Date: 09/10/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To gain a better understanding of what CRISPR screening and its applications.

Content:

Link: https://www.idtdna.com/pages/education/decoded/article/overview-what-is-crispr-screening

---

What is CRISPR screening?

- "Large-scale genetic loss-of-function experimental approach designed to find the equivalent of a few needles in a haystack"
- Facilitates the discovery of key genes/genetic sequences that "code" for a specific function/phenotype for a cell type
- Result: short list of candidate genes/genetic sequences that appear to participate in producing the physiological effect being studied
- CRISPR = method of making double-strand cuts at specifically targeted DNA sites; cell DNA repair systems are then "used" to mend these cuts
  - Problems:
    - Repair process is often imprecise
    - Can result in mutations that knock out targeted gene --> often the reason why scientists call for CRISPR screening

More on Uses of CRISPR Screening

- Identify genes/DNA sequences that cause cells to be resistant or sensitive to a drug
- Identify genes/DNA sequences affecting susceptibility to toxins
- Identify components of a cellular pathway (i.e. cancer --> if pathway has gone awry)
- Identify genes/DNA sequences leading to a particular disease state (i.e. cancer)

How does CRISPR screening work?

- Idea: have a mixed population of cells with a different gene knocked out in each cell --> to eliminate options
- Some cells will die, others will survive/excel in growth and become the predominant cell type
- After growing for a few days, next-generation sequencing (NGS) is performed on cell population to determine which sequences are present and which are depleted/absent
- Result: identify sequences necessary for survival under normal conditions
  - Can also do in the context of drug treatment/cancerous conditions, etc
- Generally, 6-8 target sites per gene are recommended (for pooled, vector-based screens) --> allows for increased probability of cutting
- Control sequences
  - Negative controls - designed NOT to have genetically-mediated physiological effects; can help identify any indirect confounding effects from experimental materials and procedures. Include:
    - Non-targeting DNA sequences - can either be designed without any homology to cell genome OR have homology to a sequence that does not have an identifiable protospacer-adjacent motif (PAM) nearby
    - Controls that target regions of the genome not known to contain any genes - these are "safe-targeting" control sequences, but may still cause unexpected biological effects
    - Sequences targeting genes already known NOT to have any effect on the physiological response under study - another type of "safe-targeting" control
  - Positive controls - necessary to know something about the biological effect being studied (ex: if a specific gene is known to be involved in effect, the pool should include several sequences targeting that gene)
- After target and control sequences are identified, one then designs a pool of oligos which will be used to make lentiviruses

The Cas enzyme in CRISPR screening

- Necessary for cells to express a CRISPR-associated (Cas) enzyme for CRISPR screening to work!
- CRISPR guide RNA targets the Cas protein to intended site

- Cas enzyme cuts the DNA
- Methods to deliver Cas enzyme into cells:
  - Using a cell line that stably expresses a Cas enzyme
  - Producing a pool of lentiviruses that contain both the gene for the desired Cas enzyme and the DNA coding for the guide DNA

Conclusions/action items: Discuss what I learned about CRISPR screening with my team and client, and how it may be different from the CRISPRi screening we will use for cancer spheroids. Clarify with the client what screening would entail when it comes to our application of finding tumor-specific factors affecting genome stability. Add relevant information into product design specifications and future deliverables.

ANA MARTINEZ - Oct 11, 2024, 4:06 PM CDT

Search Engine: Google

Query: "CRISPR screening background"

2024/10/11 - Stemness Markers in A549

ANA MARTINEZ - Oct 11, 2024, 5:31 PM CDT

Title: Stemness Markers in A549

Date: 10/11/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To get a better understanding of common stemness markers indicative of spheroid formation in non-small cell lung cancer lines, particularly A549.

Content:

Search Engine: Google

Query: "stemness markers used to verify A549 spheroid formation"

Link:

Background on SOX2

- "The stem cell transcription factors SOX2, OCT4, KLF4, C-MYC, and NANOG, which were originally identified in induced pluripotent stem cells, have been detected in a variety of tumors"
- "In particular, a number of associations were recently found between SOX2 and lung cancer.[21–23] "
- What is SOX2?
  - SOX2 is a transcription factor that, together with OCT3/4 and NANOG, plays a pivotal role in the developmental stage when the repetitive branching pf the airways allows for the primary bronchial tree to form, a process that efficiently increases the surface area-to-volume ratio[25] .
- SOX2 in Lung Cancer
  - Lung Squamous Cell Carcinoma (LUSC): Genomic amplification is seen in 20%, increased expression in 90%[26]
  - Small Cell Lung Cancer (SCLC): seen in 27%[27]
  - Lung Adenocarcinoma (LUAD): increased expression in nearly 20%[28]
  - These numbers suggest that SOX2 mediates a major tumorigenic effect in lung cancers (particularly SCLC and LUSC) regardless of genetic alteration
  - Gap in knowledge: little was previously known about stemness markers (including SOX2) in the tumor progression of NSLC
- Previous Work with SOX2 Knockdown
  - Bass et al[26] reported that knocking down SOX2 in RNA interference (RNAi) experiments reduced proliferation and anchorage-independent growth of LUSC in soft agar
  - Thus, SOX2 is believed to be important in promoting tumor growth in LUSC

Chungyoul et al., 2018 Work

Purpose: This study sought to determine the role of SOX2 in the growth of extracellular matrix-detached cells (in spheroids) during cancer progression.

Methods Overview:

- Generated spheroids for A549 cells using poly-HEMA plates (using the "treated tissue culture plates" method we selected). This served as a 3D, ECM-detached cell growth model of tumor progression.

Loading [MathJax]/extensions/Safe.js

- Examined the role of stemness markers using small interfering RNA (siRNA) and small molecule inhibitor in comparison to standard 2D culture.

Results Overview:

- A549 cells cultured in poly-HEMA formed substratum-detached spheroids with intermediate epithelial-to-mesenchymal transition (EMT).
- These cells exhibited increased gene expression of SOX2 compared to 2D cells.
  - Note that other stemness markers, as well as EMT markers and cell cycle markers, were also upregulated in expression for these cells.
    - For stemness markers, the highest upregulation from 2D to 3D was seen in SOX2 and KLF4.
  - See figure 1 below for a graphical depiction of these gene expression observations:

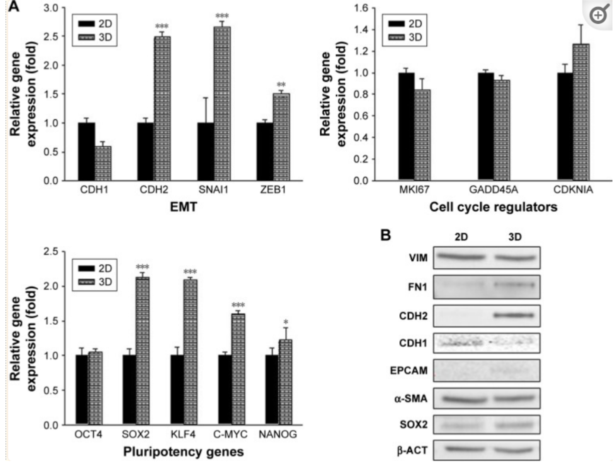


Figure 1: "Gene expression patterns for A549 cell spheroids. (**A**) Quantitative real-time RT-PCR of transcript levels for EMT markers (CDH1, CDH2, SNAI1, and ZEB1), cell cycle markers (MKI67, GADD45A, and CDKN1A), and pluripotent markers (OCT4, SOX2, KLF4, C-MYC, and NANOG). Data shown are representative of two independent experiments, and the values represent the mean ± SD of triplicate samples. The expression of each mRNA was normalized to that of GAPDH mRNA in the same sample and is presented as the fold-change over that of 2D culture control cells. Differences in expression levels were evaluated for significance using one-sided *t*-tests with unequal variance (*$P < 0.01$, **$P < 0.05$, ***$P < 0.001$). (**B**) The protein expression changes were estimated by Western blot. β-Actin was used as an internal control."

- When SOX2 was knocked down, there was decreased cell aggregate (spheroid) growth under poly-HEMA culture conditions.
- SOX KO cells were also more sensitive to the anticancer drug vinblastine with associaed down regulation of AKT kinase (an anti-apoptotic protein).
- RepSox, a small molecule that can replace SOX2's function in reprogramming cells, was found to stimulate spheroid growth under poly-HEMA culture conditions.

Conclusions/action items: This study's findings provide a strong indication that SOX2 contributes to anchorage-independent growth in A549 cells (as shown in poly-HEMA cultured spheroids). This makes SOX2 a strong candidate for stemness markers indicative of spheroid formation for our qPCR test (see "qPCR Testing Protocol" entry in Protocols section). Also found in this study was that KLF4 expression was upregulated in A549 spheroids. I will be sure to discuss these findings with client and continue searching for other potential markers.

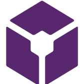
ANA MARTINEZ - Nov 15, 2024, 6:43 PM CST

References:

Loading [MathJax]/extensions/Safe.js

[1] C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” *OncoTargets Ther.*, vol. 11, pp. 6197–6207, Sep. 2018, doi: 10.2147/OTT.S175810.

Loading [MathJax]/extensions/Safe.js



2024/09/22 - Common Mutations in Lung Cancer Cell Lines

ANA MARTINEZ - Sep 22, 2024, 1:06 PM CDT

Title: Article: Authenticated Lung Cancer Cell Lines

Date: 9/22/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To gain a better understanding of the common somatic mutations in lung cancer cell lines, and common cell lines with these mutations.

Content:

Search engine: Google

Query: "mutations in lung cancer cell lines"

Link: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

2) https://www.ncbi.nlm.nih.gov/gtr/genes/79633/

Types of Lung Cancer (Background)

- Non-small cell lung cancer (NSCLC) --> ~85% of diagnoses
  - Squamous cell carcinoma
    - Arises from the proximal airway
    - More closely correlated with smoking than other NSCLC's
    - Originates in bronchial epithelium cells (lining the airways)
  - Adenocarcinoma
    - Arises from the distal airway
    - Dominant subtype in non-smoking patients
    - Originates in mucus-producing glands and cells
- Small cell lung cancer (SCLC) --> ~ 15% of diagnoses
  - Arises from neuroendocrine cells
  - Closely correlated to heavy carcinogen exposure
  - Originates in tracheal basal cell progenitors and pulmonary neuroendocrine cells

Common Mutations

- "The most frequently reported somatic mutations in lung cancer are the genes for TP53, LRP1B, KRAS, KEAP1, KMT2C, FAT4, CDKN2A, EGFR, and FAT2"
- More on each mutation:
  - TP53:
    - "Encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains"
    - "Encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism"
    - "Mutations in this gene are associated with a variety of human cancers, including hereditary cancers"
  - LRP1B:
    - "Belongs to the low density lipoprotein (LDL) receptor gene family"
    - "These receptors play a wide variety of roles in normal cell function and development due to their interactions with multiple ligands"
  - KRAS:
    - "A Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily"
    - "A single amino acid substitution is responsible for an activating mutation"

    - "The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma"
  - KEAP1:
    - "Encodes a protein containing KELCH-1 like domains, as well as a BTB/POZ domain. Kelch-like ECH-associated protein 1 interacts with NF-E2-related factor 2 in a redox-sensitive manner and the dissociation of the proteins in the cytoplasm is followed by transportation of NF-E2-related factor 2 to the nucleus. This interaction results in the expression of the catalytic subunit of gamma-glutamylcysteine synthetase"
    - In other words, acts as a suppressor of tumor metastasis
  - KMT2C:
    - "Member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family"
    - "Encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger"
    - "Protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation"
  - FAT4 (source: 2)):
    - "Protein encoded by this gene is a member of the protocadherin family"
    - "Gene may play a role in regulating planar cell polarity (PCP)"
    - "Studies in mice suggest that loss of PCP signaling may cause cystic kidney disease, and mutations in this gene have been associated with Van Maldergem Syndrome 2"
  - CDKN2A:
    - "Gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control"
    - "This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene"
  - EGFR:
    - "Protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family."
    - "Cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation"
    - "Mutations in this gene are associated with lung cancer"
  - FAT2:
    - "Gene is the second identified human homolog of the Drosophila fat gene, which encodes a tumor suppressor essential for controlling cell proliferation during Drosophila development"
    - "Gene product is a member of the cadherin superfamily, a group of integral membrane proteins characterized by the presence of cadherin-type repeats. In addition to containing 34 tandem cadherin-type repeats, the gene product has two epidermal growth factor (EGF)-like repeats and one laminin G domain"
    - "Protein most likely functions as a cell adhesion molecule, controlling cell proliferation and playing an important role in cerebellum development"
- Cell Lines Associated with Mutations

Mutated gene	Cell lines
TP53	PC9, NCIH-322, LC-2/AD, DMS 273, NCI-H69/CPR, COR-L279, NCI-H727, SHP-77, DMS153, LUDLU-1
LRP1B	NCIH-322, DMS 273, CALU 1, COR-L279, NCI-H727, SHP-77, DMS153, CORL-47, DMS 79, DMS 454
KRAS	A549, CALU 1, CORL23, NCI-H727, SHP-77, SKLU1, MOR/CPR, NCIH358
KEAP1	A549, SHP-77, LUDLU-1, MOR/CPR, DMS 454
KMT2C	A549, CORL23, COR-L47, SKLU1, COR-L105, DMS 454, SKMES1, NCIH358
FAT4	A549, NCI-H727, COR-L47, ChaGo-K-1
CDKN2A	PC9, CORL23
EGFR	PC9, SK MES 1
FAT2	NCI-H358

Table 1. Lung cancer cell lines with specific mutations

-
- Cell lines with most mutations:
  - PC9 (3): TP53, CDKN2A, EGFR
  - A549 (4): KRAS, KEAP1, KMT2C, FAT4
  - SHP-77 (3): TP53, LRP1B, KRAS
  - NCI-H727 (3): TP53, LRP1B, FAT4
  - CORL23 (3): KRAS, KMT2C, CDKN2A
  - COR-L47 (3): LRP1B, KMT2C, FAT4
  - *Note: yellow include 2+ relevant mutations, orange include 3+ relevant mutations

Conclusions/action items:

Research further these 6 cell lines and compare them with cell lines/mutations from Han et. al paper (2020) to further narrow down my list for the design matrix. Investigate these cell lines for whether or not they meet the following "ideal" criteria:

2024/09/22 - Relevant Lung Cancer Cell Lines

Title: Relevant Lung Cancer Cell Lines Research

Date: 9/22/24

Content by: Ana Martinez

Present: Ana Martinez

Goals: To further investigate and narrow down relevant lung cancer cell lines previously identified such that they meet the "ideal cell line criteria" (see below).

Content:

Search engine: Google

Query: "lung cancer cell lines", "adherent", "doubling time", "susceptible to DNA damage"

Links:

1) https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC6

2) https://www.nature.com/articles/s41586-020-2099-x#MOESM5

3) https://www.sigmaaldrich.com/US/en/product/sigma/cb_90071810

4) https://www.researchgate.net/publication/347158320_CloneSeq_A_Highly_Sensitive_Single-cell_Analysis_Platform_for_Comprehensive_Characterization_of_Cells_from_3D_C

5) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5650413/#:~:text=Platinum%2Dbased%20chemotherapeutic%20drugs%20are,%2C%20etoposide%20and%205%2Dfluorouracil

6) https://www.sigmaaldrich.com/US/en/product/sigma/cb_86012804

7) https://www.cellosaurus.org/index.html

8) https://www.synthego.com/a549-cells#:~:text=A549%20cells%20doubling%20time%20is,in%20liquid%20nitrogen%20vapor%20phase

9) https://cancerci.biomedcentral.com/articles/10.1186/s12935-019-1037-1#:~:text=This%20study%20revealed%20that%20cisplatin,therapy%20in%20the%20clinical%20setting

10) https://www.atcc.org/products/crl-2195

11) https://www.sigmaaldrich.com/US/en/product/sigma/cb_98110201

12) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4304157/#:~:text=Because%20CDDP%20is%20frequently%20used,proliferation%20observed%20from%20combination%20trea

13) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5783418/#:~:text=These%20data%20suggest%20that%20COTI,with%20these%20first%2Dline%20agents.&text=COTI%2D2%2

14) https://www.sigmaaldrich.com/US/en/product/sigma/cb_92031919

15) https://www.researchgate.net/publication/21827223_Sensitivity_to_novel_platinum_compounds_of_panels_of_human_lung_cancer_cell_lines_with_acquired_and_inherent_re

-----

Cell lines with 2+ relevant mutations (source: 1))

- PC-9 (3): TP53, CDKN2A, EGFR
- A-549 (4): KRAS, KEAP1, KMT2C, FAT4
- SHP-77 (3): TP53, LRP1B, KRAS
- CORL23 (3): KRAS, KMT2C, CDKN2A
- *Note: yellow include 2+ relevant mutations, orange include 3+ relevant mutations --> KMT2C and FAT4 were not analyzed in Han et al. (2020) paper (source: 2)), so NOT c

For reference: Ideal Cell Line Characteristics (per Dr. Hess, Carley)

- Good viability
- Adherent
- Grow/expand fast
- Susceptible to DNA Damage (ex: platinum-based chemotherapy)
- Relevant genetic mutations (see above)

Further Research on Cell Line Characteristics

- PC-9
  - Origin: human adenocarcinoma (remains undifferentiated), non-small cell lung cancer (source: 3))
  - Morphology: heterogeneous mixture of round cells and spindle-shaped cells (source: 3))
  - Growth mode: adherent/suspension (source: 3))
  - Doubling time: 1.5 days (2D), 2.5 days (3D) (source: 4))
  - Can become resistant to carboplatin (platinum-based chemotherapy) (source: 5))
- A-549
  - Origin: human lung carcinoma, non-small cell lung cancer (source: 6))
  - Morphology: epithelial (source: 6))
  - Growth mode: adherent (source: 6))
  - Doubling time: 18-27 hours (source: 7))
  - Sensitivity to Platinum-Based Chemotherapies:
    - Can become resistant to carboplatin (source: 5))

    - Transfection with Cas9 targeting BRCA2 gene leads to increased sensitivity to Cisplatin (source: 8))
      - But also shown to become resistant to cisplatin (source: 9))
- SHP-77
  - Origin: human lung carcinoma, small cell lung cancer (source: 10))
  - Morphology: epithelial (source: 10))
  - Growth mode: adherent/aggregates in suspension (source: 11))
  - Doubling time: 85-96 hours (source: 7))
  - Sensitivity to Platinum-Based Chemotherapies:
    - Shown greatest sensitivity when treated with a combination of carfilzomib (CFZ) and cisplatin (CDDP) (source: 12))
    - COTI-2 is shown to enhance the cytotoxic activity of cisplatin, with no induced resistance to therapy (source: 13))
- CORL23
  - Origin: human lung carcinoma, non-small cell lung cancer (source: 14))
  - Morphology: epithelial (source: 14))
  - Growth mode: adherent (source: 14))
  - Doubling time: 30-35 hours (source: 7))
  - Sensitivity to Platinum-Based Chemotherapies:
    - Shown to exhibit cross-resistance to cisplatin, carboplatin, iproplatin, tetraplatin, and a series of 10 novel ammine/amine dicarboxylate platinum(IV) compounds

Conclusions/action items:

From this list, it looks like A-549 seems to be the cell line that overall addresses all the "ideal" criteria. Because SHP-77 is small cell lung cancer (most rare form), it is likely we will disregard this

2024/09/22 - Relevant Ovarian Cancer Cell Lines

ANA MARTINEZ - Sep 23, 2024, 1:13 PM CDT

Title: Relevant Lung Cancer Cell Lines Research

Date: 9/22/24

Content by: Ana Martinez

Present: Ana Martinez

Goals: To further investigate and narrow down ovarian cancer cell lines such that they meet the "ideal cell line criteria" (see below).

Content:

Search engine: Google

Query: "ovarian cancer cell lines", "adherent", "doubling time", "susceptible to DNA damage"

Links:

1) https://depmap.org/portal/data_page/?tab=allData

2) https://www.atcc.org/products/htb-161

3) https://www.cellosaurus.org/index.html

4) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8566917/

5) https://www.atcc.org/products/crl-3585

6) https://bmccancer.biomedcentral.com/articles/10.1186/s12885-020-06752-1#:~:text=We%20used%20two%20platinum%2Dsensitive,between%2015%20and%2025%20passages

7) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7589258/

____

General Note: I selected this list of ovarian cancer cell lines based off of previous spheroid work with them in the Kreeger Lab and Campagnola Lab.

Cell lines with 2+ relevant mutations (source: 1))

- OVCAR3: TP53
- OVCAR8: TP53, KRAS
- OV90: TP53, CDKN2A, SMAD4
- OVCA433: none
- *Note: yellow include 2+ relevant mutations, orange include 3+ relevant mutations (relevant: TP53, BRCA1/2, PIK3CA, KRAS, CDK2A, SMAD4 --> source: 1))

For reference: Ideal Cell Line Characteristics (per Dr. Hess, Carley)

- Good viability
- Adherent
- Grow/expand fast
- Susceptible to DNA Damage (ex: platinum-based chemotherapy)
- Relevant genetic mutations (see above)

Further Research on Cell Line Characteristics

- OVCAR8
  - Origin: adenocarcinoma (ascites) (source: 2))
  - Morphology: epithelial (source: 2))

  - Growth mode: adherent (source: 2))
  - Doubling time: 24-31.93 hours (source: 3))
  - Sensitivity to Platinum-Based Chemotherapies:
    - Observed to develop inherent resistance to carboplatin and induced resistance to paclaxitel (source: 4))
- OV90
  - Origin: adenocarcinoma (papillary serous) (source: 5))
  - Morphology: epithelial (source: 5))
  - Growth mode: adherent (source: 5))
  - Doubling time: 36-46.34 hours (source: 3))
  - Sensitivity to Platinum-Based Chemotherapies:
    - Shown to have synergistic sensitivity to combination therapy with verteporfin and platinum cisplatin (CDDP), carboplatin (CP), and paclaxitel (source: 6))
      - Can develop resistance to cisplatin (source: 7))

Conclusions/action items:

Of these two cell lines, OV90 seems to address the "ideal" criteria more fully and will therefore be selected as my proposed ovarian cancer cell line for the design matrix. This will be along with A-549 in lung cancer (see previous entry). I will compare my findings with my teammates so we can select the most appropriate cell lines for the design matrix.

2014/09/23 Relevant Lung Cancer Cell Lines (cont.)

ANA MARTINEZ - Sep 23, 2024, 1:06 PM CDT

Title:

Date: 09/23/24

Content by: Ana Martinez

Present: Ana Martinez

Goals: To further investigate the NCI-H23 cell line used in the Han et al. (2020) paper and determine whether it meets the "ideal cell line criteria" (see below).

Content:

Search engine: Google

Query: "NCI-H23 cell line", "adherent", "doubling time", "susceptible to DNA damage"

Links:

1) https://www.nature.com/articles/s41586-020-2099-x#MOESM5

2) https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cancer-research/lung-cancer-cell-lines?srsltid=AfmBOooLdVOVqlo2ZC62O7v9UqlJM91bFqPHxgjsZ5BWjRzeVd21CYke

3) https://www.atcc.org/products/crl-5800

4) https://www.cellosaurus.org/index.html

5) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2957658/#:~:text=If%20oxaliplatin%20is%20compared%20to,H226%20appears%20sensitive%20to%20oxaliplatin.

General Note: I selected this lung cancer cell line based on the Han et al. (2020) paper (source: 1)), in whih they used this cell line to make 3D spheroids for screening.

Cell line and relevant mutations

NCI-H23: TP53, LRP1B, KEAP1, KRAS, FAT2

- Recall that frequently reported somatic mutations in lung cancer are TP53, LRP1B, KRAS, KEAP1, KMT2C, FAT4, CDKN2A, EGFR, and FAT2 (source: 2))
- Note: yellow include 2+ relevant mutations, orange include 3+ relevant mutations --> KMT2C and FAT4 were not analyzed in Han et al. (2020) paper (source: 1)), so NOT considered "relevant"

For reference: Ideal Cell Line Characteristics (per Dr. Hess, Carley)

- Good viability
- Adherent
- Grow/expand fast
- Susceptible to DNA Damage (ex: platinum-based chemotherapy)
- Relevant genetic mutations (see above)

Further Research on NCI-H23 Cell Line Characteristics

- Origin: adenocarcinoma, non-small cell lung cancer (source: 3))
- Morphology: epithelial (source: 3))
- Growth mode: adherent (source: 3))
- Doubling time: 25-39.1 hours in RPMI 1640 medium + 10% FBS, up to 71 hours in ACL-3 medium (+BSA for ~57 hours) (source: 4))
- Sensitivity to Platinum-Based Chemotherapies:
    - Observed to be the most sensitive (least resistant) to cisplatin as well as oxaliplatin out of the following cell lines: A549, H520, H460, H23 (source: 5))

Conclusions/action items: The NCI-H23 appears to be another great option for a cell line, in that it meets all of the "ideal" criteria. Based off its sensitivity to platinum-based therapies and its doubling time range, this is evidence that NCI-H23 might be a better option than A549 for the design matrix. I will propose this cell line as my top contender for the design matrix, followed by A549 and OV90.

2024/09/09 - Spheroids as a Type of 3D Cell Culture - Copy

ANA MARTINEZ - Sep 22, 2024, 3:26 PM CDT

Title: Article: Spheroids as a Type of Three-Dimensional Cell Cultures—Examples of Methods of Preparation and the Most Important Application

Date: 09/09/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To gain a more thorough understanding of spheroids and their use in 3D cell culture, as well as the most common methods of forming spheroids.

Content:

Search Engine: Google

Query: "spheroid formation types"

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503223/

- Cell-cell and cell-ECM interactions differ from 2D to 3D cultures and between cell layers in spheroids structures --> this can affect cytotoxicity results
- Spheroids are one type of 3D cell culture that more precisely mimic the natural cell microenvironment
- Spheroids are cell aggregates that self-assemble in an environment that prevents attachment to a flat surface, through the help of integrins (membrane proteins) and ECM proteins
  - Integrins - involved in activation of focal adhesion kinase (FAK); involved in cell adhesion, migration, and growth
- 3 basic steps to spheroid formation:
  - 1) Dispersed/single cells aggregate due to long-chain ECM fibers consisting of RGD motifs that allow to bind cell-surface integrin --> leads to upregulated cadherin expression
  - 2) Cadherin accumulates on cell membrane surface
  - 3) Hemophilic cadherin-cadherin binding between neighboring cells allows for "tighter" connections between cells --> forms spheroids
- Spheroid Formation Method # 1: Hanging Drop
  - Limitations:
    - Low-throughput
    - Spherical geometry
    - High shear force environment
    - Complicated/time-consuming manipulations (changing medium, adding compounds)
    - Some cell lines do NOT form compact spheroids
  - Strengths:
    - Relatively simple equipment is needed (not specialized, but practical) --> cell suspension placed on the well/lid of a plate
    - Cells remain in direct contact with each other and the ECM throughout
    - Can be used to co-culture several cell lines
  - Basic Steps:
    - Add drops of cell suspension on well/lid of a culture plate
    - Turn this surface upside down
    - Cell suspension will become a hanging drop held by surface tension --> can add methyl cellulose to increase this
    - Microgravity will concentrate cells at the bottom of the drop
- Spheroid Formation Method # 2: Hydrogels
  - Typically use non-adhesive agarose hydrogels --> no influence of an ECM
  - Strengths:
    - Ease of maintenance
    - Can control microtissue size
    - Large amount of microtissues per plate
    - Some micromolds for hydrogel preparation are commercially available (ex: see Vantangoli et al. (2015))
    - Allows for versatility for controlled microtissue production
  - Basic steps:
    - Cells seeded on hydrogel with recesses where cells will sink

        - These cells will self-assemble into 3D spheroid microtissues
        - Cells in homogeneous suspension self-assemble into spheroids, while cells in heterogeneous suspension self-segregate to form multilayered structures
- Spheroid Formation Method #3: Rotary Cell Cultures
    - Limitations:
        - Longevity of cultures (tends to be low)
        - Variation in spheroid size is higher
        - Mechanical damages of cells
    - Strengths:
        - One of the simplest methods
        - Produces spheroids on a large-scale (high-throughput)
    - Basic steps:
        - Cell culture is in a bottle with an agitator
        - Cells cannot attach to the substrate --> start aggregating and self-assembling
        - Variation: rotate flask around a horizontal axis --> simulation of microgravity with minimal hydrodynamic forces --> does NOT destroy cells, forms bigger and morphologically similar spheroids
- Spheroid Formation Method #4: Cell Suspension with Addition of Nanofibers
    - Involves adding polymer nanofibers to a suspension of adherent cells
    - Cells bind to nanofibers via vitronectin and fibronectin from the media's serum --> these proteins adsorb on nanofibers, then cells attach to them and form spheroids
    - Strengths:
        - Nanofibers allow for increased spheroid production
        - Reduced cell death (due to cell non-adherence)
- Spheroid Formation Method #5: Magnetic Levitation Method
    - Basic Steps:
        - Magnetic force overcomes gravitational force
        - Cells treated with paramagnetic iron oxide nanoparticles overnight --> cells uptake
        - After washing, cells are lifted with trypsin and seeded into low-adhesive plates
        - Magnet is placed on top of plate lid --> labeled cells are pulled up under magnetic forces
        - Spheroids created within few hours
            - Like the "opposite" of hanging drop method!
    - Strengths:
        - Cells are able to associate into 3D cell culture and produce ECM, retaining cellular activity
        - Potentially a cheaper substitute for expensive and labor-intensive method based on human tumor xenografts from immunodeficient mice
- Spheroid Formation Method #6: Microfluidic Systems
    - Aimed to address non-microfluidic methods' limitations, such as:
        - Differences in spheroid diameters
        - Low-throughput
        - Labor intensity
        - Reduction of oxygen and nutrients (at cells in middle)
        - Increase in osmolality
        - Increase in metabolite level
    - Strengths (more specific):
        - Controlled mixing
        - Chemical concentration gradients are formed
        - Lower reagent consumption
        - Control of shear stress and pressure on cells
        - Constant perfusion
        - Dynamic environment = better reflection of in vivo environment
    - Basic components:
        - Microfluidic device is made of microwells connected by microfluidic channels
        - Microchannels are prepared by etching or forming on the surface of neutral materials (ex: silicon, glass, PDMS)
        - Cells are cultured above layers made of matrix coated porous membrane/in direct contact with endothelial cells
        - Immune and tumor cells flow through channels
- Spheroid Formation Protocol #7: Bioprinting
    - Basic steps:
        - Cell layers and supporting biological materials are positioned precisely to mimic functions of tissue/organ
        - Computer program controls the deposition of droplets --> creates 3D structures
    - Limitations:
        - Expensive equipment (bioprinters)

        - Poor choice of bioinks (particularly in laser-assisted bioprinting)
    - Strengths:
        - Relatively fast to generate
        - Inexpensive to generate
        - High resolution
        - Provides high viability of cells
        - Better vascularization compared to other 3D culture models
        - Better reflection of tumor microenvironment and heterogeneity (particularly in later stages of tumors) compared to other models

Conclusions/action items:

From this article, I have learned that there are a lot of different methods of generating spheroids. I have experience with the hanging drop technique and using different amounts of methyl cellulose to modulate spheroid size/compactness. However, based on what our client tells us at our next meeting, we may find that the Hess Lab may be leaning towards another specific method/has had a bad experience with certain methods. Also, we will have to wait until we learn more about our team's budget before we pick a method, as these vary greatly in expense.

2024/12/17 - Spheroid Sizes

ANA MARTINEZ - Dec 17, 2024, 9:54 PM CST

Title: Article: "Static systems to obtain 3D spheroid cell models: a cost analysis comparing the implementation of four types of microwell array insterts"

Date: 12/17/2024

Content by: Ana Martinez

Present: Ana Martinez

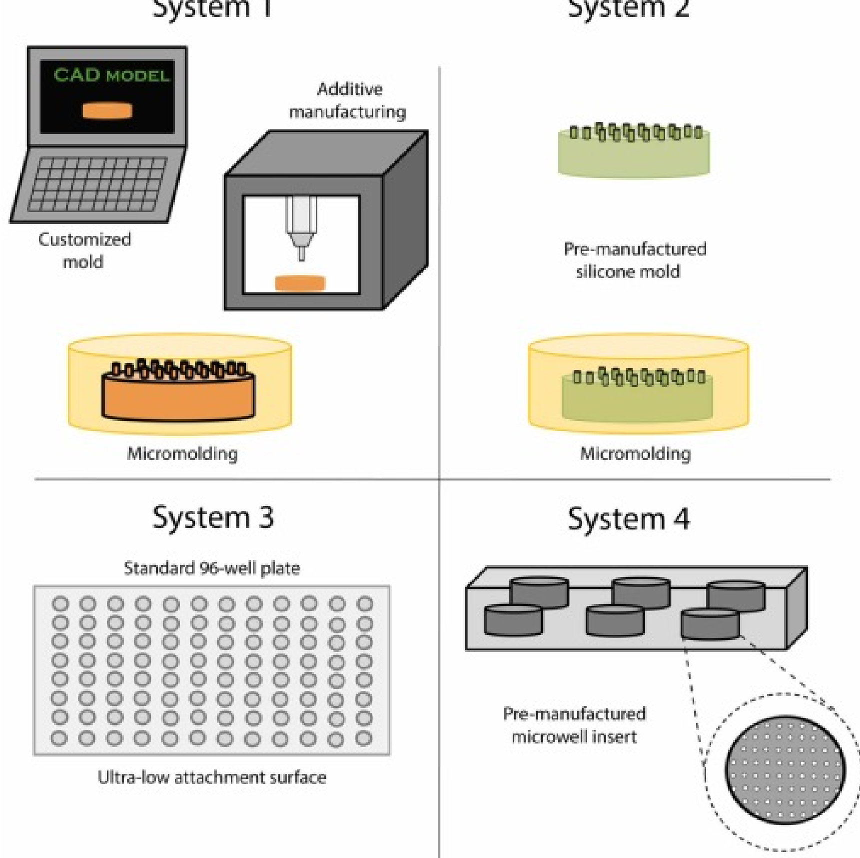
Goals: To learn more about different spheroid characteristics for 3D culture, with an emphasis in learning more about commonly-observed spheroid sizes to validate our findings for our spheroid density variation assay.

Content:

- 3D cell culture systems have proven to provide more accurate cell models than 2D to mimic the natural microenvironments and organs, as reviewed extensively by Achilli et al. [2], Sart et al. [3] and Decarli et al. [4]
- "Enabling precise geometric control of the 3D cell culture models is one of the most relevant technical challenges of this area [9]"
    - Possible to obtain cell aggregates of any diameter, but cell viability is limited in structures of very large sizes due to impaired nutrient, metabolite, and oxygen diffusion [9].
    - Spheroids specifically have a typical diameter range from 50-800 µm, depending mostly on the intended application and the method used to produce it [10].
    - Heterogeneity in diameter and volume across spheroid "batches" is yet another problem because large vs. small spheroids respond differently to treatments/applications [11]
        - Large spheroids (starting at 500 µm diameter): useful to recreate the pathophysiological environment of solid tumors with a hypoxic core and severe oxygen/nutrient concentration gradients [11], [12] --> useful for testing antitumor compound testing alternative to chemotherapy/radiotherapy cytotoxicity trials [13]
        - Small spheroids (up to 200 µm diameter): efficiently supplied with oxygen/nutrients through diffusion and do not experience a hypoxic core [14] --> useful for tissue engineering and regenerative medicine applications [15]
        - Differences in other things with diameter variations:
            - Cell proliferation and differentiation [20], [21]
            - Performance on cytotoxicity tests [22], [23]
            - Data reproducibility with drug screening, efficacy, and cytotoxicity trials [11]
    - Another problem is scale of production, depending on the application
        - Few amounts (in the order of hundreds of spheroids) are needed for developmental biology and congenital disease studies

- Larger amounts are needed for drug screening, cytotoxicity and efficacy trials --> especially when multiple parameters are simultaneously analyzed in high-throughput systems [27], [28]
- Much, much larger amounts (ex: up to 5 × 10^7 cells for 1 mL of a lung-mimicking cellularized structure [29]) are needed when using spheroids as building blocks to construct larger 3D-bioprinted tissues --> call for using higher-throughput platforms or easier scale-up methods, such as spheroid formation in suspension (ex: spinner flask bioreactor)

- Analysis of 4 main spheroid formation systems:



- "Fig. 1. Graphic view of the four selected microwell array systems with similar diameter values to obtain 3D cell culture models. Our in-house developed system (1), and the three commercially available systems (2, 3 and 4). The four systems were developed using different technological concepts."
- --------
- "Table 1. Main characteristics of the four selected microwell array systems to obtain 3D cell models, developed by using different technological concepts and based on the criterion of resulting in spheroids with similar diameter range."

Product	Type of product	Technological concept	Cavities size (µm)	Number of spheroids per product
System 1:Developed by Decarli and co-authors, 2021[33]	Micromolded hydrogel array prepared and transferred to a 6-well plate	Customized resin mold obtained by additive manufacturing	600	164
System 2: MicroTissues® 3D Petri Dish, micro-mold spheroids, ref Z764019–6EA, by MicroTissues Inc. and available from Sigma-Aldrich	Micromolded hydrogel array prepared and transferred to a 12-well plate	Pre-manufactured silicone mold	800	81
System 3:96-well Spheroid Microplates, Ultra Low Attachment surface, ref 4515, by Corning	96-well plate, ready to use	Ultra-low attachment surface to reduce cellular adhesion to the well plate	635	96
System 4: AggreWell™800 6-well plate starter kit, ref 34860, by Stemcell Technologies	Microwell system 6-well plate, ready to use	Pre-manufactured microwell system approach	800	1500 per well 9000 per product

-

Conclusions/action items: From this article I was able to learn a bit more about additional applications for spheroids, including ones that include large-scale production methods. I also was able to confirm that our spheroid formation method using hydrophilic plates (poly-HEMA coated, serve the same purpose as ultra-low attachment) tends to yield consistently-sized spheroids of about 635 um in diameter depending on the specifics of the spheroid formation setup. Additionally, through this article I was able to confirm that the largest average spheroid size we observed in our first spheroid seeding density variation experiment (~ 800 um^2 area = ~ 100 um diameter) was within the range of 50-800 um diameter range typically observed in literature. However, because our observed number was on the lower end of this range, our team will plan on tweaking spheroid densities and methylcellulose concentrations in hopes of increasing this number to more well within the range as well as decreasing overall spheroid size variability.

2024/10/11 - Spheroid Formation Protocol

ANA MARTINEZ - Oct 11, 2024, 8:40 AM CDT

Title: Spheroid Formation Protocol

Date: 10/11/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To outline an initial protocol for spheroid formation, based on Han et al, 2020 [1].

Content:

Materials:

- Pre-treated ultra-low attachment plates (Corning, no. 3261) or polyhema (Sigma, no. P3932) coated tissue culture plates.
- Chosen cell line
- Full cell culture growth medium for chosen cell line
- Methylcellulose (0.75%; Fisher, no. M-352)
- Accutase (Innovative Cell Technologies, no. AT104)

Procedure:

1. Seed cells at multiple densities ranging from 200,000-150,000 cells/cm² (with 500 µL growth medium/cm²).
2. Monitor cell growth and death rates using an automated fluorescent microscope optimized for live-cell imaging (i.e. IncuCyte S3 or IncuCyte ZOOM, Essen Bioscience).
    a. Monitor cell growth rates by mCherry expressed in the cell line.
        i. Estimate the number of live cells in spheroids by dividing total integrated mCherry intensities of spheroids by the average integrated mCherry intensity of single live cells measured during initial seeding.
    b. Monitor death rates by Sytox Green signal. Sytox Green should be added at 100 nM final concentration at the beginning of the experiment.
        i. Estimate the number of dead cells by dividing the total integrated Sytox Green intensities of spheroids by the average integrated Sytox Green intensity of a single dead cell.
3. From the above results, choose a cell density which shows ~30% peak cell death rate within 24 hours after initial seeding. For all subsequent experiments, initially seed cells with this cell density in 500 µL medium containing 0.75% methylcellulose. The methylcellulose will prevent excessive aggregation of cells in spheroid culture and maintain even spheroid size.
4. Split spheroids every 3-4 days.
    a. To passage cells, collect spheroids in methylcellulose media and dilute in PBS (~3 medium volumes) to reduce the medium's viscosity prior to centrifugation.
5. Centrifuge spheroids at 800g for 15 minutes. Remove methylcellulose media and PBS from the spheroid pellets.
6. Add Accutase to the pellets to dissociate the spheroids into single cells.
    a. In this protocol, 10 mL of Accutase per 100 million cells in spheroids were used, which were incubated for ~30 minutes until spheroids were fully dissociated into single cells. Single cells were then re-seeded at the starting density in step 3).

References

[1] K. Han *et al.*, "CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities," *Nature*, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s41586-020-2099-x.

Conclusions/action items: Determine what media A549 cells are cultured in at Hess Lab and add this to the above protocol. Begin practicing cell culture passaging and spheroid formation with a non-essential cell line for practice, then move on to using 549 cells to determine if the cell density and % methylcellulose (viscosity) parameters need to be adjusted for our cell line.

2024/10/11 - High-Throughput CRISPR Screen Protocol

ANA MARTINEZ - Oct 11, 2024, 8:49 AM CDT

Title: High-Throughput CRISPR Screen Protocol

Date: 10/11/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To outline an initial protocol for high-throughput, pooled CRISPR-Cas9 loss-of-function screening, based on Mathiowetz et al, 2023 [1].

Content:

This protocol was originally written for use with HuH7 and U-2 OS cells. However, the protocol steps to test dose-response cytotoxicity can be applied to any cell type, including A549 cells which will be used for our project.

Materials: See “key resources” table in protocol [1] for a more detailed description of necessary reagents for the CRISPR screening process.

Procedure:

<u>Generate Cas9-Expressing Cells</u>

The Cas9 endonuclease and targeting sgRNAs can be introduced together or separately through lentiviral transduction. This integrates Cas9 and sgRNAs non-specifically into the genome, which is appropriate for almost all CRISPR screens in immortalized cells. Through this protocol, constitutive Cas9 expression is induced through lentiviral transduction. Cas9 is then stably expressed and remains active throughout the screening process.

1. Plate 300,000 HEK293T cells into one well of a 6-well culture plate in 1 mL DMEM + 10% FBS such that they are at ∼50% confluence 24 h later.
2. After 24 h, transfect HEK293T cells with Mirus LT1 transfection reagent, 500 ng 3rd generation lentiviral packaging vector mix (equal parts pMDLg/pRRE [Addgene # 12251], pRSV-Rev [Addgene # 12253], and pMV2.g [Addgene # 12259]), and 500 ng pLenti-Cas9-blast (Addgene # 52962) at a 3:1 Mirus:DNA ratio. Follow Mirus Bio’s “TransIT-LT1 Full Transfection Protocol” [2].

    Critical: To ensure sufficient viral titers are reached, all library and packaging plasmids should be endotoxin-free and the packaging HEK293T cells need to be healthy prior to transfection.

3. Incubate cells for 72 h.
4. After 72 h, collect viral supernatant through a 0.45 μm filter. Use immediately or store at 4°C for up to one week or −80°C for up to six months.

    Critical: When working with lentivirus, bleach all media and supplies and turn on ultraviolet light in the biosafety cabinet for thirty minutes to inactivate viral particles.

5. Plate 100,000 HuH7 cells into two wells of a 6-well tissue culture plate in DMEM + 10% FBS such that cells are at ∼80% confluence 24 h later.
6. After 24 h, introduce 0.5 mL fresh DMEM + 10% FBS and 0.5 mL pLenti-Cas9-blast lentivirus-containing medium to the cells in one well with 8 μg/mL polybrene. Incubate for 24 h.

    Note: Keep one well uninfected as a control for the antibiotic selection.

7. After 24 h, remove viral medium and replace with DMEM + 10% FBS.

7. After 24 h, remove viral media and replace with DMEM + 10% FBS.
    a. Expand cells for 24 h and then begin antibiotic selection with 4 μg/mL blasticidin.

        Note: With the amount of lentivirus added in step 6, ∼30%–50% of cells should be infected. Cells can therefore start selection at high confluency and not overgrow the plate.

    b. Replace the selection media every 3–4 days and split cells as necessary until all control cells have died.

        Note: The concentration of selection antibiotic is dependent on the cell line. This concentration should be determined in advance with antibiotic kill curves.

8. Once all control cells have died, replace media for Cas9 cells with fresh DMEM + 10% FBS without antibiotic to allow cells to recover. These are now your “Cas9” cells.

    Note: Cas9 pools or clonal cells can be used for CRISPR screening. To avoid clonal bias from the genetic background, this protocol calls for screening pools of Cas9 cells and does not select monoclonal cells.

9. Validate Cas9 expression by Western blot.
10. Freeze cells at −80°C and store in liquid nitrogen.

    Note: It is useful to expand these cells and store in excess since they can be used for subsequent screens or individual gene knockouts.

    Note: After introduction into cells, it is important to make sure that Cas9 is active (steps 11-16). To test Cas9 activity, independently infect cells with a lentiviral plasmid encoding: 1. mCherry plus a non-targeting sgRNA (control) and 2. mCherry plus an mCherry-targeting sgRNA. Employ flow cytometry to measure mCherry expression. Cells expressing active Cas9 will cleave the mCherry DNA and appear as an mCherry negative population. Conversely, cells lacking active Cas9 will fail to cleave the mCherry DNA and appear as an mCherry positive population. Due to the long half-life of mCherry, it may take up to 1–2 weeks to distinguish the active Cas9 (e.g., mCherry-negative) cells.

11. Repeat steps 1–4 to make lentiviral media containing a control sgRNA or mCherry-targeting sgRNA (see “key resources” table [1] for sequences) cloned into pMCB320 lentiviral vector (Addgene # 89359).
12. Plate 100,000 HuH7 Cas9 cells into three wells of a 6-well plate in DMEM + 10% FBS so that cells reach ∼80% confluence 24 h later.
13. After 24 h, introduce viral media containing the control sgRNA or the mCherry-targeting sgRNA cloned into the pMCB320 lentiviral vector with 8 μg/mL polybrene to two of the wells. Incubate for 24 h.
14. After 24 h, remove viral media and replace with DMEM + 10% FBS. Expand cells for 48 h and then begin antibiotic selection with 2 μg/mL puromycin (see note above on antibiotic concentrations). Replace the selection media every 3–4 days until all control cells have died.
15. Once all control cells have died, replace media with fresh DMEM + 10% FBS without antibiotics to allow cells to recover.
16. Measure mCherry fluorescence by flow cytometry to validate Cas9 activity.

Dose Response Analysis to Determine Concentration of Cytotoxic Compounds

An optimal concentration of your choice compound to induce cell death is crucial to achieving the maximum dynamic range of the screen readout. For a drug-resistance screen, a sub-lethal concentration of drug that causes very modest cell death (∼5%) in 24-48 h should be determined. Presumably, the depletion of a drug resistance factor will lead to a substantial increase in the sensitivity to the drug, leading to a depletion of the sgRNA over time. For a drug-sensitivity screen, an initial drug concentration that causes ∼50% cell death should be determined. However, as pools of surviving cells from the initial selection will become resistant to cell death induced by the drug, a slightly higher concentration may be required for each subsequent treatment cycle to achieve ∼50% death.

Note: This is a specific example for identifying ferroptosis resistance factors using known ferroptosis inducing compounds. However, this protocol can be extrapolated to any treatment or condition that provides a selective pressure on cell viability.

17. On day 0, seed ∼5,000 U-2 OS cells in each well of a 96-well plate such that the final volume per well is 200 μL.

18. On day 1, aspirate the media from the 96 well-plate and replace it with 100 µL fresh media.
19. Prepare a 2× final solution of the compound at varying concentrations by serial dilution in media containing 60 nM SYTOX Green Dead Cell Stain.

    Note: 8–12 different concentrations are recommended to ensure that the optimum concentration is within the standard curve. It is recommended to begin with a 10-point, 5-fold dilution series.

20. Slowly add 100 µL compound containing media back to each well so that the final volume of media in each well is 200 µL with 30 nM SYTOX Green Dead Cell Stain.
21. Monitor cell death using an Incucyte Live-Cell Analysis System (Essen Biosciences), taking images every 2 h for 24–48 h total. Dead cells will be SYTOX green-positive.
22. On day 2 or 3, determine the percentage of cell death by dividing the number of dead cells (SYTOX green-positive) by the total number of cells (visualized by phase imaging).

    Note: Due to some limitations of the Incucyte system and the dramatic difference in cell morphology, thresholding and automatically counting total cell number using phase images can sometimes be difficult and inaccurate. Generating a cell line that stably expresses mCherry or using a genetically encoded live-cell nuclear marker (e.g., Incucyte Nuclight reagents) greatly improves the accuracy of the counting for live cells.

    Note: If an Incucyte Live-Cell Analysis System is not available, a CellTiter-Glo 2.0 Cell Viability Assay can be used to determine the sub-lethal dose of the drug.

23. Choose a concentration of drug that results in ∼5% cell death. Use this concentration for the CRISPR screen.

<u>Determine the Dynamic Range for Fluorescence-Based Assays</u>

The confidence of screen results depends on the dynamic range of the fluorescence reporter. A greater distance between the high and low fluorescence intensity bins will result in less biological noise and will increase the confidence of positive results and reduce the occurrence of false positives and negatives [3]. When possible, it is useful to determine the dynamic range of a cell population using a positive control prior to screening to ensure that cells with altered phenotypes can be accurately sorted by FACS.

Note: Fluorescence can arise from a fluorescent reporter protein or a fluorescent dye. To obtain the highest dynamic range from a reporter protein, it may be useful to sort cells to obtain a population with uniform fluorescence levels. For fluorescent dyes, test multiple concentrations and incubation times.

Note: It is important to establish the timeframe and treatment conditions before performing the screen itself. For example, it may take several days for a genetic perturbation or drug to produce a measurable effect on a fluorescent reporter. Cells may also need to be differentiated or pretreated with drugs or nutrients. Therefore, optimize conditions and establish a timeline for seeding cells, inducing genetic perturbations, differentiating (if applicable), and treating cells, and carry it over to the screen to yield the most robust results.

24. Choose a positive control gene (if possible) that is known to influence levels of the fluorescent reporter. Generate a knockout cell line or treat cells with a drug targeting the positive control protein. Confirm that the expected increase or decrease in fluorescence is detectable by flow cytometry.
25. Measure fluorescence by flow cytometry to validate that a change in fluorescence is detected and to determine the dynamic range of your assay.
    a. In this example, HuH7 cells were treated with 1 µg/mL triacsin C or 100 µM oleic acid to deplete or increase neutral lipid storage, respectively. Cells were treated with 1 µg/mL BODIPY 493/503 to label neutral lipids and fluorescence was measured by flow cytometry. The 10× decrease and 5× increase in fluorescence intensity will be the target dynamic range of fluorescence for this CRISPR screen.

        Note: Fluorescence intensity can diminish over time. Incubate cells on ice for multiple hours (as long as the FACS sort will be) and check that fluorescence does not change during sorting. We have not found this to be an issue with GFP-based reporters. If necessary, cells can be fixed prior to FACS to ensure fluorescent marker stability over time.

Note: In some cases, there are no drugs or known regulators to manipulate or validate the system. In the absence of a positive control to validate the fluorescence reporter, it is possible to move directly to the screen.

Prepare sgRNA Library

Many genome-scale and small-scale libraries are deposited on Addgene. This protocol used the Bassik Human CRISPR Knockout Library (Addgene # 101926-101934), which is composed of 9 sublibraries, or the custom Human Lipid Droplet and Metabolism Library (Addgene # 191535). Each sgRNA library will need to be amplified and packaged into lentivirus. Alternatively, pre-packaged lentivirus can be purchased directly from Addgene.

26) Follow the Bassik Lab's "Liquid Culture Library Plasmid Re-amp Protocol" [4].
27) Measure DNA concentration using the Qubit dsDNA HS Assay.

Preparing for Deep Sequencing

To identify the sgRNAs present in each cell population, genomic DNA (gDNA) is extracted from frozen cell pellets and guide sequences are amplified by PCR.

Note: This protocol modifies the above protocol for increased yield. For the DNA precipitation step, increase the spin time if centrifuging at a slower speed to fully precipitate DNA. Elute with Qiagen Buffer EB (10 mM Tris-Cl, pH 8.5; Cat # 19086) instead of Buffer AE. Spin at 4,500 × g for 5 min. Elute 2–3 times with new Buffer EB each time (do not reload eluate).

28. Extract gDNA from cell pellets using Qiagen QIAamp DNA Blood Midi Kit (Cat # 51183) according to the manufacturer's instructions [5].
29. Measure the gDNA concentration by nanodrop. 100 µg gDNA per 20 × 10^6 cells are typically obtained.
30. Amplify the integrated sgRNA (PCR1) with the following reagents and reaction program:

Critical: To make sure the diversity and coverage of sgRNAs is not lost during PCR1, multiple 100 µL PCR reactions are required for screen preparation. Empirically, we recommend 1 PCR reaction for every 2,500 sgRNA in the library (e.g., For a customized library that has 25,000 sgRNAs, at least 10 PCR reactions are needed, and therefore a minimum of 100 µg gDNA is required).

Note: Although this protocol calls for 10 µg of genomic DNA per 100 µL PCR1 reaction, DNA input can be decreased to 5 µg or less if necessary.

Table 1. PCR1 reaction master mix reagents and volumes.

Reagent	Volume (µL)
gDNA template (10 µg)	x
Herculase II polymerase	2
oMCB_1562 (100 µM)	1
oMCB_1563 (100 µM)	1
5× Herculase buffer	20
dNTPs (100 nM)∗	1
ddH2O	75-x

∗25 nM per dNTP.

Table 2: PCR1 cycling conditions.

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	2 min	1

Denaturation	98°C	30 s	18
Annealing	59.1°C	30 s	
Extension	72°C	45 s	
Final Extension	72°C	3 min	1
Hold	4°C	∞	

Note: As the amount of genomic DNA collected from cell samples can be limited, especially for FACS-based screens, it is highly recommended to run a single (or "pilot") PCR1 to ensure all conditions are correct and yield an amplified fragment.

31) Pool and mix all amplicons of the PCRs from the same gDNA sample. Add Illumina sequencing indexes with the following reagents and reaction program:

Table 3: PCR2 reaction master mix reagents and volumes.

Reagent	Volume (μL)
PCR1 Product	5
Herculase II polymerase	2
oMCB_1440 (100 μM)	0.8
oMCB_1439 (100 μM)	0.8
5× Herculase buffer	20
dNTPs (100 nM)∗	2
ddH2O	69.4

Table 4: PCR2 cycling conditions.

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	2 min	1
Denaturation	98°C	30 s	20
Annealing	59.1°C	30 s	
Extension	72°C	45 s	
Final Extension	72°C	3 min	1
Hold	4°C	∞	

Note: Though PCR1 uses 18 cycles, it was empirically determined that 20 cycles for PCR2 resulted in the best signal-to-noise ratio.

Note: Selecting index adapters with diverse sequences for pooled libraries is CRITICAL: for successful sequencing and data analysis. For information on how to optimize the color balance of the index adapters see the "Index Adapters Pooling Guide" published by Illumina.

Note: Only one 100 μL PCR2 reaction is sufficient to achieve sequencing depth.

32) Load PCR products onto 2% TBE-agarose gel.
33) Run the sample at 120 V for 50 min. Excise the brightest band.

Note: The size of the band is expected to be 280 bp but may run higher due to overloading of the gel.

34) Purify DNA products using QiaQuick Gel Extraction Kit (Cat # 28706) according to the manufacturer's instructions [6].

Note: We slightly modified this protocol for increased yield. When dissolving the gel, add 4 volumes Buffer QG instead of 3. For the DNA precipitation step, add 3 M sodium acetate pH 5.2 at a 1:100 ratio. For the wash step, wash with Buffer PE two times instead of once. Elute DNA in Buffer EB, not water.

35) Check DNA concentration by Qubit dsDNA HS Assay. We typically obtain 30 ng/mL DNA.
36) Verify DNA quality using a fragment analyzer. DNA fragments should run as a single band at ∼300 bp.
37) If the DNA runs as a single band at ∼300 bp without contamination at other sizes, pool equal amounts of DNA from each screen sample so that the final concentration is 3 nM. Send the pooled library for deep sequencing.

Note: The molecular weight of the DNA can be calculated based on the nucleotide sequence.

a. 50 µL of the pooled library at 3 nM and 30 µL of the Bassik custom sequencing Illumina sequencing primer (oMCB1672_new10gCRKO) at 100 µM are typically sent. Both are sent in Qiagen EB (10 mM Tris, pH 8.5).

38) Analyze deep sequencing data using casTLE.

References

[1] A. J. Mathiowetz, M. A. Roberts, D. W. Morgens, J. A. Olzmann, and Z. Li, "Protocol for performing pooled CRISPR-Cas9 loss-of-function screens," *STAR Protoc.*, vol. 4, no. 2, p. 102201, Mar. 2023, doi: 10.1016/j.xpro.2023.102201.
[2] "Transit-LT1 transfection reagent – 0.4 mL," Mirus Bio, Accessed: Oct. 10, 2024. [Online]. Available: https://www.mirusbio.com/product/transit-lt1-transfection-reagent-0-4-ml/
[3] T. Nagy and M. Kampmann, "Crispulator: A discrete simulation tool for pooled genetic screens," *BMC Bioinformatics*, vol. 18, no. 1, Jul. 2017. doi:10.1186/s12859-017-1759-9.
[4] A. Truong, Liquid culture library plasmid re-amp Protocol, Accessed: Oct. 10, 2024 [Online]. Available: https://media.addgene.org/cms/filer_public/05/f3/05f37d31-3984-4531-9a27-6d591a4b5c5e/liquid_culture_library_plasmid_reamp_bassik.pdf.
[5] QIAamp® DNA BLOOD MIDI/maxi handbook, Accessed: Oct. 10, 2024 [Online]. Available: https://www.qiagen.com/us/resources/download.aspx?id=bf32146a-77fd-40c2-8743-c28974f7935b&lang=en.
[6] QIAquick® Spin Handbook, Accessed: Oct. 10, 2024. [Online]. Available: https://www.qiagen.com/ye/resources/download.aspx?id=a9de0fd4-e405-4bb7-b3a0-a74b336d613e&lang=en.

Conclusions/action items: Determine more details about what Hess Lab does for CRISPR screening, particularly whether they introduce the Cas-9 protein and the sgRNAs into cells separately or together, and where they send the sgRNA library for sequencing. Confirm with client additional details regarding this protocol from Mathiowetz et al., 2023 [1] and whether they apply to Hess Lab.

2024/10/11 - qPCR Testing Protocol

ANA MARTINEZ - Oct 11, 2024, 5:07 PM CDT

Title: qPCR Testing Protocol

Date: 10/11/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To outline an initial protocol by Hess Lab [1] for qPCR. This test will be to determine the expression level of stemness markers indicative of spheroid formation in spheroids formed via our treated tissue culture plates spheroid formation method.

Content:

Materials: RNA Quick Extract (https://biosearchtech.a.bigcontent.io/v1/static/manual_QER090150_QuickExtract-RNA)

Procedure:

RNA Extraction

1. Harvest 10^3-10^6 cells (at least 10^5 recommended). Pellet cells at 1000g for 5 minutes at 4°C. Remove supernatant.

   Note: You can use half the cells if you half the amount of RNA quick extract you use in Step 3.

2. Optional: Wash cells in PBS, by adding 500 µL of PBS and centrifuge for 5 minutes at 1000g and 4 minutes. Aspirate supernatant.
3. Add 100 uL of ice-cold RNA quick extract. Vortex mix for 1 minute.
4. This mixture can be stored at -70°C.

cDNA Synthesis

5. This protocol is for 20µL/2µg of RNA protocol. Prepare in PCR tubes:

RNA (2µg)	x
Oligo dT (0.5µg/µL)	2
H_2O	8-x

   Note: If using a transcript specific primer, Oligo concentration should be 0.5-1 µM in final RT reaction. I usually use 2µL of 10µM stock.

6. Heat sample at 70°C for 5 minutes. Place heated sample on ice for 5 minutes.
7. Add 10µL of following mix:

5X AMV RT Buffer	4
10mM dNTPs	1
RNAsin	0.25
AMV RT	0.75
H_2O	4

8. Incubate at 42°C for 2 hours.
9. If going into a PCR, add 50 uL of Milli-Q-H2O and proceed to set up qPCR reaction.

Setting up qPCR: SYBR Green Method

10. **Designing the primers***:* If you do not have preset primer pairs for qPCR, you can get them from literature or you can also gather them from (https://www.idtdna.com/site/order/qpcr/predesignedassay).
11. **Designing the experiment***:* For these experiments, you require a housekeeping gene as a loading control in addition to your genes of interest. We have designed primers for GAPDH and RPL19 (see below).

Table 1: qPCR Primers designed by Hess Lab.

Name	Sequence
oGH1850 hGAPDH qPCR For	agccacatcgctcagacac
oGH1851 hGAPDH qPCR Rev	gcccaatacgaccaaatcc
hRPL19 For	ATGTATCACAGCCTGTACCTG
hRPL19 Rev	TTCTTGGTCTCTTCCTCCTTG

12. You will need the following samples:

Experimental sample is measured at 1:10 dilution in triplicate – with designed primers.
Experimental sample is measured at 1:10 dilution in triplicate – housekeeping primers.
Primer curve of control template to be done (1 well each dilution) done in duplicate for each primer set consisting of:

No dilution
1:5 dilution
1:25 dilution
1:125 dilution
1:625 dilution
1:3125 dilution
Empty sample

13. Set up the Following Master Mix:

Table 2: Master Mix Reagents.

Reagent	1X	25X
H_2O	14.32	358
5x AMV Buffer	4.4	110
50 mM $MgCl_2$	0.28	7
10mM dNTP	0.4	10
100 µM 5' Primer	0.15	3.75
100 µM 5' Primer	0.15	3.75
10X Sybr Green (in DMSO)	0.2	5
Promega GoTaq Polymerase	0.1	2.5
Total:	20	500

14. Prepare enough of this mix for all experimental samples, all primer curve samples, plus extra.
15. Prepare 1:10 dilution of each sample from cDNA for use as template.
16. Prepare dilution series of control sample for primer curves.
17. Add 20 uL to each well, then add 2 µL sample template (Final reaction volume is 22 µL).
18. Set up the following:

95ºC 5 min
95ºC 30 s
55ºC 30 s
72ºC 1 min
75ºC 10 s
Plate read
Go to 2 39 times
72ºC 5 min
Melting curve 70-94°C read every 0.2°C, hold for 10 s between reads

19. **Analysis:**
    a. Get the value with CT or Cq values.
    b. Using these values for the different primer pairs, generate a standard curve (the curve should be linear with the CT values on the axis and logarithmic y-axis for the relative amount of cDNA added.
    c. Using the equation for this curve, you can use the CT values obtained for your experimental samples to interpolate the relative amount of your specific gene and the housekeeping gene.
    d. Using these values, you can determine the change in this ratio for your sample to determine if the expression of your gene has been perturbed.

References:

[1] qPCR Protocol · Benchling, Accessed: Oct. 10, 2024. [Online]. Available: https://benchling.com/uw_hesslab/f/lib_xtPaCTIU-extraction-and-staining-protocols/prt_Va3aG4Cc-qpcr-protocol/edit.

Conclusions/action items: Confirm with client on whether the RNA QuickExtract Kit or the Qiagen RNAeasy Kit is best/accessible to us for RNA extraction and cDNA synthesis, as well as the SYBR Green method vs. TaqMan method for setting up the qPCR. Confirm with client whether he deems SOX2 and OCT4 as appropriate stemness markers to test for with qPCR (see "Stemness Markers for A549" entry in Research Notes section).

2024/11/15 - polyHEMA stock protocol

ANA MARTINEZ - Nov 15, 2024, 6:14 PM CST

Title: polyHEMA stock solution protocol

Date: 11/15/2024

Content by: Ana Martinez

Present: Ana Martinez, Emily Rhine

Goals: To outline a step-by-step protocol of making poly-HEMA stock which we will use for coating our tissue culture plates when making spheroids.

Content:

Materials:

- 1.3 g poly-HEMA
- 33 mL 99% ethanol
- 1x beaker that can hold 33 mL
- 1x heated stirrer plate
- 1x magnetic stir bar
- 1x 0.2 µm filter (Nalgene) or something similar
- 1x 250 mL glass bottle with screw cap

  Steps:
  - Add poly-HEMA and 99% ethanol into beaker.
  - Mix solution overnight at 37°C on heated stirrer plate.
  - Filter solution into glass bottle.
  - Keep solution at 20°C (room temperature) until needed.

References:

[1] C. Choe, H. Kim, S. Min, S. Park, J. Seo, and S. Roh, “SOX2, a stemness gene, induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance to vinblastine,” OncoTargets and therapy, vol. 11, p. 6197, Sep. 2018, doi: 10.2147/OTT.S175810.

Conclusions/action items: Optimize this protocol as needed if poly-HEMA solution does not form as desired. Scale up material amounts to reflect our large-scale need for our genome-wide CRISPR screening project.

2024/11/15 - Methylcellulose stock protocol

ANA MARTINEZ - Nov 15, 2024, 6:13 PM CST

Title: Methylcellulose stock solution protocol

Date: 11/15/2024

Content by: Ana Martinez

Present: Ana Martinez

Goals: To outline a step-by-step protocol for making 2% (w/v) methylcellulose stock solution for spheroid formation.

Content:

Materials:

- 2 g Methylcellulose
- 100 mL deionized water (diH_2O)
- 1x beaker that can hold 100 mL
- 1x heated stirrer plate
- 1x magnetic stir bar
- 1x 500 mL glass bottle with screw cap

Steps:

- Add methylcellulose into beaker
- Autoclave solution using Gravity 2 (G2) setting with tin foil cover on beaker opening
- Add diH_2O and stir bar into beaker
- Mix solution at 80°C until homogeneous
- Move solution into fridge and mix overnight at 4°C
- Transfer solution into glass bottle for storage
- Keep solution at 4°C until needed

References:

[1] Abbaspour, Ali. "OVCA Spheroid Development Protocol," Google Drive, Feb. 2022. Accessed: Nov. 15, 2024. [Online]. Available: https://drive.google.com/file/d/1h7T-q4y4xLdRilO4qIk9YCFNvc1m7H8a/view?usp=sharing

Conclusions/action items: Optimize this protocol as needed if Methylcellulose solution does not form as desired. Scale up material amounts to reflect our large-scale need for our genome-wide CRISPR screening project.

2024/11/15 - Live/Dead Flow Cytometry Assay Protocol

Title: Live/Dead Flow Cytometry Assay Protocol

Date: 11/15/2024

Content by: Ana Martinez

Present: Ana Martinez, Althys Cao

Goals: To outline Han et al (2020)'s protocol for determining % cell death for dissociated spheroids. This metric will help us optimize the seeding density ne cell death within 24 hours after initial seeding.

Content:

- Spheroids were centrifuged at 800g for 15 min and media/PBS was removed from spheroid pellets.
- Accutase (Innovative Cell Technologies, #AT104) was added to spheroid pellets to dissociate the spheroids into single cells. These were incubated for about 30 min single cells.
  - Han et al used 10 ml of Accutase per 100 million cells in spheroids
  - We will need to translate this ratio into our system of using welled (24 or 96) plates to form spheroids using a range of cell seeding densities (25,000, 50,000,
- Perform flow cytometry on cell sample from each cell density group (3 trials each?)
- Divide number of live and dead cells (separately) counted by the predetermined average number of spheroids formed for each cell density group to get an estimate

References:

[1] K. Han *et al.*, “CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities,” *Nature*, vol. 580, no. 7801, pp. 136–141, Apr. 2020, doi: 10.1038/s415

Conclusions/action items: Modify protocol once team decides whether we will use 24 or 96-well plates for this assay. Confirm with team whether 3 trials p flow cytometry.

2024/12/12 - BME Design CytoFLEX Operation Protocol

ANA MARTINEZ - Dec 12, 2024, 9:49 PM CST

Title: CytoFLEX Operation Protocol

Date: 12/12/2024

Content by: Ana Martinez, Emily Rhine

Present: All

Goals: To outline the protocol followed by our team for operating the Hess Lab's CytoFLEX machine.

Content:

Once the CytoFLEX and its corresponding computer have been turned on, the following steps must be followed:

1. In the top left click Cytometer > Daily clean
    a. Load Blue tube and press run (3 minutes)
    b. Load DI water and press run (1 minute)
2. To create a new experiment click file> new> from template>Browse
    a. Click into BME Team cell counting file on computer drive Documents>CytoFLEX>BME Team
3. Name file after current date and passage number
4. Vortex sample
5. Uncap sample in 1 mL Eppendorf container and place in CytoFLEX
6. Select fast in settings on left side
7. Click record
8. Once a dot plot of the events has been created right click on the plot>properties
    a. Auto set x-axis
    b. Auto set y-axis
9. Right click on graph and select polygon tool
10. Gate the cells in a oblong hexagon fashion trying to bin all relevant events (general note: live cells are larger than dead cells)
11. Save file
12. Rerun daily clean (step 1)

Conclusions/action items: Update this protocol as needed for the percent viability assay next semester. Revisit Hess Lab's general CytoFLEX protocol when making these edits.

2024/12/12 - Hess Lab General CytoFLEX Operation Protocol

ANA MARTINEZ - Dec 12, 2024, 9:59 PM CST

Title: Hess Lab General CytoFLEX Operation Protocol

Date: 12/12/2024

Content by: Ana Martinez, Emily Rhine

Present: All

Goals: To outline the general protocol for operating the Hess Lab's CytoFLEX machine.

Content:

Cytoflex Equipment Overview:

- On the back of the machine is on the switch
- To the left is two containers
- Sheath: this is the fluid that is fed through the machine when running cell samples, there is a larger container labeled sheath fluid to refill it when the alarm goes off
- Waste: This is where your ran samples end up, these will include cells so when you need to empty it you put in some bleach and water

Software Use:

- CytExpert is the application that allows you to analyze your samples
- From the Start page, select New Experiment or New Experiment from Template to start a new experiment, or Open Experiment to continue an existing experiment.
- NOTE Templates contain information on hardware and software settings including channels used, gain settings, flow rate, and stop criteria.
- If a tube has not been created, use the Tube toolbar buttons to create a new tube. To modify a tube's property, highlight the tube name then select located on the bottom, left section of the Acquisition screen.
- Familiarize yourself with the toolbar.
- Create the desired histograms or dot plots using the Plots tool
- Fluorescence key
- Set the desired sample flow rate, then select on the Acquisition panel to start the lasers and fluidics.
- Load a sample tube into the sample port, then select to start acquisition and display data.
- Forward scatter (FSC) that detects scatter along the path of the laser, and side scatter (SSC) which measures scatter at a ninety-degree angle relative to the laser
- This really just means the size of the cells moving through
- Dead cells are smaller than alive ones
- Use the Threshold tool or select to set discrimination to eliminate undesired populations on the plot.
- Use the Scale and Gain tools or select to move the population displayed on the plot to the desired location.
- Once done - Follow the Daily Clean procedures, which consist of: Run FlowClean cleaning fluid for 3 minutes. Run DI water for 3 minutes.

QC:

- Select Start QC in the QC menu. The Acquisition screen is now replaced by the QC screen.
- Ensure that the QC fluorosphere lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, import the lot-specific target value file. Refer to Importing Lot-Specific Target Values in Chapter 4, Instrument Quality Control of the CytoFLEX Flow Cytometer Instructions for Use.

- QC beads are found in the hess lab fridge outside
- QC must be passed daily before use

More detailed instructions: https://med.nyu.edu/research/scientific-cores-shared-resources/sites/default/files/cytoflex-quick-start-guide.pdfCytoflex Equipment Overview:

- On the back of the machine is on the switch
- To the left is two containers
  - Sheath: this is the fluid that is fed through the machine when running cell samples, there is a larger container labeled sheath fluid to refill it when the alarm goes off
  - Waste: This is where your ran samples end up, these will include cells so when you need to empty it you put in some bleach and water

Software Use:

- CytExpert is the application that allows you to analyze your samples
- From the Start page, select New Experiment or New Experiment from Template to start a new experiment, or Open Experiment to continue an existing experiment.
  - NOTE Templates contain information on hardware and software settings including channels used, gain settings, flow rate, and stop criteria.
- If a tube has not been created, use the Tube toolbar buttons to create a new tube. To modify a tube's property, highlight the tube name then select located on the bottom, left section of the Acquisition screen.
- Familiarize yourself with the toolbar.
- Create the desired histograms or dot plots using the Plots tool
  - Fluorescence key
- Set the desired sample flow rate, then select on the Acquisition panel to start the lasers and fluidics.
- Load a sample tube into the sample port, then select to start acquisition and display data.
  - Forward scatter (FSC) that detects scatter along the path of the laser, and side scatter (SSC) which measures scatter at a ninety-degree angle relative to the laser
    - This really just means the size of the cells moving through
      - Dead cells are smaller than alive ones
- Use the Threshold tool or select to set discrimination to eliminate undesired populations on the plot.
- Use the Scale and Gain tools or select to move the population displayed on the plot to the desired location.
- Once done - Follow the Daily Clean procedures, which consist of: Run FlowClean cleaning fluid for 3 minutes. Run DI water for 3 minutes.

QC:

- Select Start QC in the QC menu. The Acquisition screen is now replaced by the QC screen.
- Ensure that the QC fluorosphere lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, import the lot-specific target value file. Refer to Importing Lot-Specific Target Values in Chapter 4, Instrument Quality Control of the CytoFLEX Flow Cytometer Instructions for Use.
- QC beads are found in the hess lab fridge outside
- QC must be passed daily before use

More detailed instructions: https://med.nyu.edu/research/scientific-cores-shared-resources/sites/default/files/cytoflex-quick-start-guide.pdf

References:

[1] "Cytoflex Basics · Benchling." Accessed: Dec. 12, 2024. [Online]. Available: https://benchling.com/uw_hesslab/f/lib_tD5E8G7L-cell-line-maintenance/etr_syJbLT7n-cytoflex-basics/edit

Conclusions/action items: Revisit this protocol as needed for the percent viability assay next semester. Compare with the BME Design CytoFLEX protocol when making these revisions.

2024/11/15 - A549 Cell Line Maintenance Data

ANA MARTINEZ - Nov 15, 2024, 7:09 PM CST

Title: A549 Cell Line Maintenance Data

Date: 11/15/2024

Content by: All

Present: All

Goals: To outline cell line maintenance - related data when passaging our A549 cells.

Content:

Notes

- The A549 cell line is a human alveolar basal epithelial cell line
  - They are adherent, squamous, and have an epithelial like apearance and are a larger cell line so they take up more volume
  - They are a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells
- Media: DMEM + 10% FBS + P/S
- The doubling time ranges between 20-40 hours (we will need to keep track of this)
- They are good for 20 passages

Passaging Schedule

For adherent cell lines while media is important to ensure your cells get proper nutrients confluency is really based on the surface area of the flask or dish they are forming their monolayer on. A T75 flask is 75cm^2 and a T50 is 150cm^2 and based on the area, cell size, and how fast your cells double you can determine how often you need to passage to ensure they don't get overgrown. You typically want to keep them around/under 80% confluent.

Flask	Surface Area	Seeding Density (million)	Cells at Confluency (million)	PBS Volume	Trypsin Volume	Resuspension Volume	Final Volume
T-75	75	1	5	2	3mL	7 mL	10
		On the cytoflex 100% confluency with this cell line would be .5 million cells per mL			You may need 3 since these cells can be a bit more adherent than others	8 (needs to be x3 the amount of trypisin to neutralize it)	

If they double between 20-28 hours:

- You will do a Monday, Wednesday, Friday passaging schedule
  - How this works: on Monday your cells are at confluency in 10 mL of media, the next time you will be in lab is Wednesday so you need to cut your cells back. Between Monday and Wednesday your cells will double twice (x2 x2). Thus, you need to cut them back to a point where by Wednesday (48 hours later) they won't be overgrown but won't be too low either. After going through the process of PBS, trypisin, and resuspending the cells in media to get a volume of 10 mL you will keep 2 mL of this volume and bleach the remaining 8 mL. The way this works is if

Loading [MathJax]/extensions/Safe.js

you have roughly 1 million cells in 1 mL of media and you keep 2 mL you're saving 1 million cells, this will double to 2, and then double again to 4 million which roughly puts you at 80% confluency. On Friday though you won't be in till Monday so you will need to save only 1 mL of cell solution volume.

  - This math is not perfect and passaging does not need to be perfect, but for the health of your cells it is important to be roughly on track. This is will also allow you to begin to recognize how well or poorly your cells are growing.
  - To start, you will use the cytoflex to count the cells everytime to determine how many you have and how much you need to cut them back by based on the average growing rate

If they double between 30-48 hours:

- You will do a Monday, Wednesday, Friday passage schedule but will be cutting them back less
  - How this works: On Monday your cells are at confluency in 10 mL of media, the next time you will be in lab is Wednesday so you need to cut your cells back. Between Monday and Wednesday your cells will double once. Thus, you need to cut them back to a point where by Wednesday (48 hours later) they won't be overgrown but won't be too low either. After going through the process of PBS, trypisin, and resuspending the cells in media to get a volume of 10 mL you will keep 4 mL of this volume and bleach the remaining 6 mL. The way this works is if you have roughly .5 million cells in 1 mL of media and you keep 4 mL you're saving 2 million cells, this will double to 4 which roughly puts you at 80% confluency. On Friday though you won't be in till Monday so you will need to save only 2 mL of cell solution volume.

https://www.cellseeker.org/cellcalc/growth-rate-and-doubling-time/

Date	Passage Number	Cytoflex Confluency	Cells per mL	Total cells in solution	Doubling time	Cut Back To
10/11/24	0 (thaw)	N/A	N/A	1000000	Thaw	
10/14/24	1	5309	530900	5309000	28.2	1000000
10/16/24	2	5094	509400	5094000	20.4	750000
10/18/24	3	1956	195600	1956000	36.2	1200000
10/21/2024	4	2393	239300	2393000	72.3	2000000
10/23/2024	5	9156	915600	9156000	21.9	1500000
10/25/2024	6	3147	314700	3147000	44.9	1750000
10/28/2024	7	3708	370800	3708000	54.0	1875000
10/30/2024	8	8804	880400	8804000	21.1	1750000
11/1/2024	9	4187	418700	4187000	42.9	1650000
11/4/2024	10	4506	450600	4506000	46.9	1750000
11/6/2024	11	8117	811700	8117000	21.7	1700000
11/8/2024	12	4208	420800	4208000	35.7	1650000
11/11/2024	13	3305	330500	3305000	68.4	1725000
11/13/2024	14	8124	812400	8124000	21.5	1710000
11/15/2024	15	4973	497300	4973000	33.1	1650000
New flask at p20						

Loading [MathJax]/extensions/Safe.js

Rack Number	Box Number	Cell Type	Position	Box Name

Rack 15	4	A549 WT	52	Hess Stocks 4
Rack 15	4	A549 WT	53	Hess Stocks 4
Rack 15	4	A549 WT	54	Hess Stocks 4
Rack 15	4	A549 WT	55	Hess Stocks 4
Rack 15	4	A549 WT	56	Hess Stocks 4
Rack 15	4	A549 WT	57	Hess Stocks 4
Rack 15	4	A549 WT	58	Hess Stocks 4
Rack 15	4	A549 WT	59	Hess Stocks 4

References:

[1] “A549 Cell Line Maintenance · Benchling.” Accessed: Nov. 15, 2024. [Online]. Available: https://benchling.com/uw_hesslab/f/lib_tD5E8G7L-cell-line-maintenance/etr_5Skd74TL-a549-cell-line-maintenance/edit

Conclusions/action items: Continue updating data for each passage until we reach passage 20. After this, we will keep track of the same data using a new vial of A549 cells.

ANA MARTINEZ - Dec 12, 2024, 9:36 PM CST

For vial 2:

11/15/2024	0 (thaw)	N/A	N/A	1000000	Thaw	1000000	
11/18/2024	1	9296	929600	9296000	22.4	1750000	1.8825301205
11/19/2024	2	3535	353500	3535000	23.7	1750000	4.9504950495
11/20/2024	3	3426	342600	3426000	22.7	1500000	4.3782837128
11/22/2024	4	2985	298500	2985000	50.4	1600000	5.3601340034
11/25/2024	5	3934	393400	3934000	55.5	1700000	4.3213014743

Loading [MathJax]/extensions/Safe.js

2024/11/15 - Tong Lecture Notes

ANA MARTINEZ - Nov 15, 2024, 7:15 PM CST

Title: Tong Lecture Notes

Date: 11/15/24

Content by: Ana Martinez

Present: Ana Martinez

Goals: To outline what was talked about at the Tong Entrepreneurship Lecture, given by the two starters of Tasso, inc.

Content:

- "The future of healthcare is in the home" - via delivery (Amazon, blood draw, etc)
- Needed a consumer-grade, consumer friendly, transportable blood draw device
- Scraped together whatever they could to start prototyping
- Started asking around — got referred to the Law and Entrepreneurship Clinic - helped set up law documents, gave them legal advice, helped them write patent
- Worked with David Beebe, ended up helping him as grad students editing, writing grants, eventually got a paper published
- Got an award tiered for projects that benefit minorities/disadvantaged
- Open microfluidics: combined with blood draw device to make their device more efficient, connect a tube to it, and do this at very low costs
- Eventually figured out that the vacuum used to draw blood "cinched" off skin capillaries and limited blood being drawn — opportunity to improve this portion of the device and eventually tripled the amount of blood they could draw after addressing this!
- Tasso inc. now does most of anti-doping blood tests in US sports, Olympics, etc
- Tasso has been successful because of its grassroots nature - working with customers and the problem they face, focus in, one step at a time
- COVID helped Tasso a lot — at-home antibody testing was needed!
- When scaling up: quality is key, otherwise if one customer has a problem then word-of-mouth referrals will go down
- FDA: split up lancet and tube into two parts — tube was already cleared, and lancet became a class I device (very easy clearance!) - by being creative, were able to beat a lot of competitors to FDA clearance
- Tasso means badger in Italian

Conclusions/action items: This was a really interesting talk! I enjoyed learning about the whole process from the first idea, to prototyping, to scaling up, and eventually to more complex financial strategies that Tasso and its founders went through. This will definitely be a lecture I will look back at for inspiration and ideas in my current design project as well as future projects.

CRISPR Screening in Cancer Spheroids (article 1)

JULIA SALITA - Sep 11, 2024, 11:54 AM CDT

Title: CRISPR Screening in Cancer Spheroids (article 1)

Date: 9/11/2024

Content by: Julia Salita

Present: Self

Goals: To understand CRISPR use with cancer spheroids

Content:

1. unclear which gene mutations are functional cancer drivers
    1. "have to scalably investigate these genes in relevant cancer models to assign causality and identify cancer specific vulnerabilities"
    2. have to look into the genes in cancer models to see what causes it and point out its weaknesses
2. In vivo and in vitro models are used to look at cancer but both have their limitations
    1. In Vivo (genetically engineered mouse models)
        1. summarizes' tumor growth and microenvironment
        2. limited by scale, time and cost
    2. In Vitro (Xenograft based models)
        1. limited in scale and manipulability
3. 2D cell culture has limitations in many disease features; ex: hypoxia, altered cell-cell contacts and rewired metabolism.
    1. in vitro organoid models lesson some of those missing pieces but can't be scaled like that
4. DepMap: use CRISPR screens to characterize cancer dependencies on a genome scale in hundreds of cell lines to reveal many (previously) undiscovered cancer drivers.
5. in article made scalable way to (re)produce lung cancer spheroids and use CRISR screens genome-wide in both 2D monolayers and 3D spheroids.

Conclusions/action items:

What is CRISPR screening

JULIA SALITA - Sep 11, 2024, 12:40 PM CDT

Title: What is CRISPR screening

Date: 9/11/2024

Content by: Julia Salita

Present: Self

Goals: To understand what CRISPR screening is an its subsequent parts.

Content:

1. What is CRISPR screening
    1. "CRISPR screening is a large-scale genetic loss-of-function Experimental approach designed to find the equivalent of a few needles in a haystack."
    2. helps the discovery of key genes or sequences that cause a certain function or phenotype for a cell type
    3. Hypothesis of CRISPR screening: "that there are a few genetic sequences or genes in the genome that have a certain physiological effect, ant that these few genetic sequences can be identified"
    4. Success in CRISPR screening is a list of genes or genetic sequence that potentially partake in producing the specific physiological effect that is being investigated
    5. starts with a broad hypothesis but ends with creating narrower hypotheses that need to be further investigated individually using other biological methods
    6. CRISPR is a method to cut double-strand DNA at targeted sites.
    7. When used in in genomic DNA in cells, the cells DNA repair system mends the cut, however the repair process is imprecise and results in mutations that knock out the targeted gene.
    8. The knockout event is what most scientists want to happen when they use CRISPR screening
2. CRISPR screening examples:
    1. identify genes or DNA sequences that cause cells to be resistant or sensitive to a drug
    2. identify genes of DNA sequences that affect susceptibility to environmental toxins
    3. identify components of cellular pathway
    4. identify genes of DNA sequences that lead to a particular disease state
3. How does CRISPR screening work?
    1. mostly done in cell culture
    2. basic idea: knock out every gene that could be important, but only one gene per cell
    3. the mixed cell population with different knocked out genes in each cell sit and some will die, some will live, and some may even excel in growth, becoming the predominant cell type.
    4. then Next generation sequencing (parallel sequencing) is performed to see which sequences are there and which are depleted (absent)
        1. high-throughput, rapid, and scalable sequencing alternative to first generation sanger sequencing. allows for sequence of millions of DNA fragments from hundreds of samples on a single sequencing run.
    5. identifies genetic sequence that are necessary for survival in normal conditions
    6. used to identify specific genes or sequenced that survive under special conditions (drug treatment of other physiological conditions of interest)
    7.

Loading [MathJax]/extensions/Safe.js

Conclusions/action items:

JULIA SALITA - Sep 12, 2024, 12:45 PM CDT

Title: What is CRISPR screening (continued)

Date: 9/12/2024

Content by: Julia Salita

Present: Self

Goals: To understand what CRISPR screening is an its subsequent parts.

Content:

1. What is CRISPR screening
    1. "CRISPR screening is a large-scale genetic loss-of-function Experimental approach designed to find the equivalent of a few needles in a haystack."
    2. helps the discovery of key genes or sequences that cause a certain function or phenotype for a cell type
    3. Hypothesis of CRISPR screening: "that there are a few genetic sequences or genes in the genome that have a certain physiological effect, ant that these few genetic sequences can be identified"
    4. Success in CRISPR screening is a list of genes or genetic sequence that potentially partake in producing the specific physiological effect that is being investigated
    5. starts with a broad hypothesis but ends with creating narrower hypotheses that need to be further investigated individually using other biological methods
    6. CRISPR is a method to cut double-strand DNA at targeted sites.
    7. When used in in genomic DNA in cells, the cells DNA repair system mends the cut, however the repair process is imprecise and results in mutations that knock out the targeted gene.
    8. The knockout event is what most scientists want to happen when they use CRISPR screening
2. CRISPR screening examples:
    1. identify genes or DNA sequences that cause cells to be resistant or sensitive to a drug
    2. identify genes of DNA sequences that affect susceptibility to environmental toxins
    3. identify components of cellular pathway
    4. identify genes of DNA sequences that lead to a particular disease state
3. How does CRISPR screening work?
    1. mostly done in cell culture
    2. basic idea: knock out every gene that could be important, but only one gene per cell
    3. the mixed cell population with different knocked out genes in each cell sit and some will die, some will live, and some may even excel in growth, becoming the predominant cell type.
    4. then Next generation sequencing (parallel sequencing) is performed to see which sequences are there and which are depleted (absent)
        1. high-throughput, rapid, and scalable sequencing alternative to first generation sanger sequencing. allows for sequence of millions of DNA fragments from hundreds of samples on a single sequencing run.

Loading [MathJax]/extensions/Safe.js

5. identifies genetic sequence that are necessary for survival in normal conditions
6. used to identify specific genes or sequenced that survive under special conditions (drug treatment of other physiological conditions of interest)
7.

Conclusions/action items:

Loading [MathJax]/extensions/Safe.js

Spheroids as a Type of Three-Dimensional Cell Cultures—Examples of Methods of Preparation and the Most Important Application

JULIA SALITA - Sep 24, 2024, 1:21 PM CDT

Title: Spheroids as a Type of Three-Dimensional Cell Cultures—Examples of Methods of Preparation and the Most Important Application

Date: 9/24/2024

Content by: Julia Salita

Present: Self

Goals: to find a cell line that we can use to make into spheroids

Content:

1. 96-well NanoCulture plate
2. colon cancer cell lines HCT-116wt, HCT-116 wt/GFP and HCT-116 HRP EGFP

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503223/#:~:text=3%2D%20and%206%2Dday%20spheroids,by%20spheroid%20formation%20%5B42%5D

Conclusions/action items:

1. Look more into these cell lines and their formation

colorectal carcinoma spheroids

JULIA SALITA - Sep 24, 2024, 1:56 PM CDT

Title: colorectal carcinoma spheroids

Date: 9/24/2024

Content by: Julia Salita

Present: self

Goals: to understand more about colorectal carcinoma spheroids, and see if they are a good option to pursue.

Content:

1. first link:
   1. HCT116, DLD-1 and SW620 were used in this article.
   2. spheroid formation capacity in ultra low attachment round bottom 96-well plates
2. Second Link:
   1. "Enrichment of CSC-related features in HT-29 and Caco-2 (for the first time without applying special scaffold/biochemical) spheroids, suggests spheroid culture as robust, reproducible, simple and cost-effective model to imitate the complexity of in vivo tumors including self-renewal, drug resistance and invasion for in vitro research of CRC-CSCs."- second link
      1. I think this would be a good thing to investigate more. It seems Colorectal carcinoma makes good spheroids.

https://www.nature.com/articles/s41598-018-19384-0#:~:text=Formation%20of%20colon%20cancer%20spheroids,round%20bottom%2096%2Dwell%20plates.

https://cancerci.biomedcentral.com/articles/10.1186/s12935-021-01898-9#:~:text=Relapse%20and%20metastasis%20in%20colorectal,detailed%20morphological%20and%20molecular%20characteristics.

https://www.eurekaselect.com/article/33210

https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/biot.200700228

https://onlinelibrary.wiley.com/doi/10.1111/cas.13155

https://link.springer.com/article/10.1186/s12943-017-0691-y

Conclusions/action items:

1. once again look into the specific properties of it
2. talk with group about helping find it/ understanding the wording of the articles

Mutation Research

JULIA SALITA - Nov 13, 2024, 10:34 AM CST

Title: Julia Salita

Date: 03- Oct- 2024 (added 13- Nov-2024)

Content by: Julia Salita

Present: Julia Salita

Goals: To understand common genetic mutations that may contribute to causing cancer

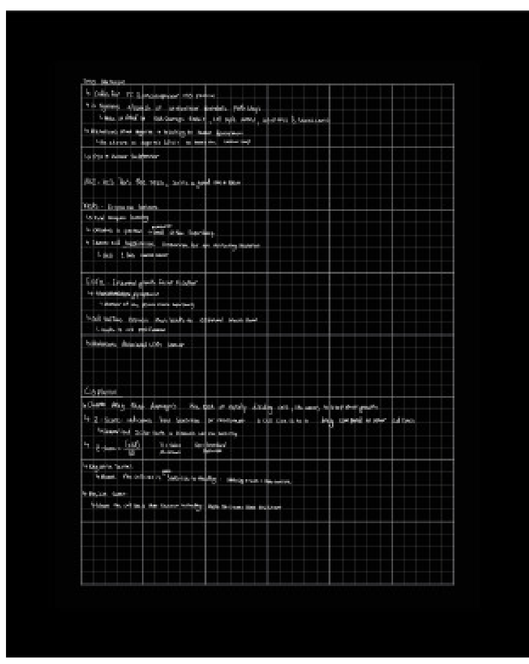
Content:

See notes attached below

Conclusions/action items:

1. add to preliminary presentation

JULIA SALITA - Nov 13, 2024, 10:42 AM CST



Download

Note_Oct_3_2024_1_.pdf (708 kB)

Understanding qPCR

JULIA SALITA - Nov 13, 2024, 10:39 AM CST

Title: Understanding qPCR as explained by our client

Date: 08-Nov-2024

Content by: Julia Salita

Present: Whole team

Goals: to understand how a qPCR works and what results/data would be produced

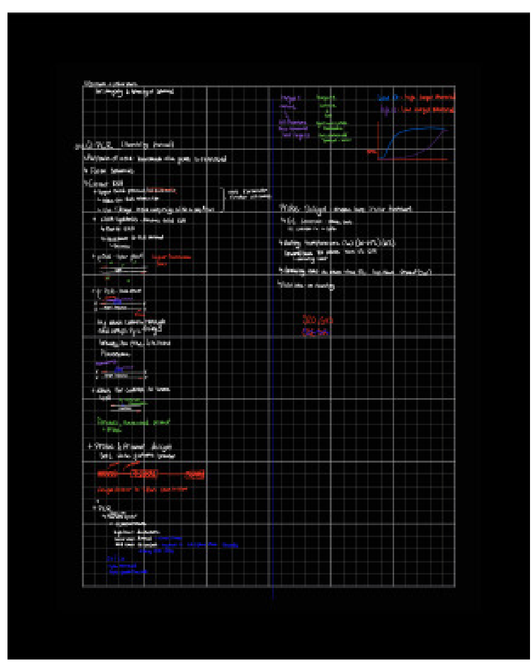
Content:

See notes below

Conclusions/action items:

1. Discuss with team on whether to proceed or not

JULIA SALITA - Nov 13, 2024, 10:43 AM CST



Download

Note_Nov_8_2024.pdf (1.08 MB)

Spheroid Formation Protocols

JULIA SALITA - Nov 16, 2024, 3:32 PM CST

Title: Spheroid formation using Ultra-Low Attachment Surface in MCF-7 Human Breast cancer cell line

Date: 11/16/2024 (inputted - no Idea when initially found)

Content by: Julia Salita

Present: Myself

Goals: To find a way to form spheroids using Ultra-Low Attachment plates

Content:

1. They didn't make the plates they used pre-made Ultra-Low Attachment Surface (Catalog number 3815_ but I couldn't find it) and TCT (430639) made by Corning.
2. Did a few other things with the cells as well but for spheroid:
    1. seeded onto an Ultra-Low Attachment surface with 384 wells, black with clear bottom microplate. with a density of 80,000 cells/cm^2 (0.49 mL/cm^2) and incubated for 96 hours (4 days).
    2. they did immunostaining with spheroids so they did that here, along with a few other things
        1. fixed and permeabilized for a few hours as well as exposed to other chemicals that are not as relevant for our project

Conclusions/action items:

1. could be good start for a seeding density and timing of incubation, but we would most likely need to make our own plates, because of money, and control over variables of concentrations in the coating.

JULIA SALITA - Nov 16, 2024, 3:32 PM CST

Loading [MathJax]/extensions/Safe.js

Download

Corning_ULA_Surface_Promotes_Spheroid_Formatn_in_MCF-7.pdf (244 kB) Original article

JULIA SALITA - Nov 16, 2024, 3:51 PM CST

Title: Spheroid formation from Corning using multiple types of cell line including A549

Date: 16-Nov-2024 (input- originally found Oct 24th)

Content by: Julia Salita

Present: Whole team

Goals: to find a spheroid formation protocol to have a base for forming necessary spheroids

Content:

1. Culture and maintain as normal, using standard methods
    1. they used F-12K (Kaighn's Mod.) medium
2. Seeding densities used: 40, 200, 1,000, 5,000, and 10,000 cells/ 100uL of media per well (96-well spheroid microplates from Corning, Catalog number: 4520)
3. shperoids analyzed at 0, 24, 48, and 72 hours using a CEll Titer-Glo 3D cell viability assay
4. using same seeding method for all cell lines used:
    1. harvest cells, single cell suspension
    2. perform 5mL dilutions for each seeding density to seed eight wells per seeding density for each time point (96 well plate per cell line)
    3. add 100 uL of cell suspension to 8 wells per each concentration (add control by adding media without cells) avoiding scratching the well to not scrape the plate coating off
    4. incubate plate and evaluate ate specified hours

Conclusions/action items:

1. This seems like a good thing to maybe do but with just our cell line to see what is the best seeding density and time.

JULIA SALITA - Nov 16, 2024, 3:51 PM CST

Loading [MathJax]/extensions/Safe.js

Corning® Spheroid Microplates

Spheroid Formation Protocol

Methods and Materials

Table 1. Seeding Density Preparation

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
40	400	2,000
200	2,000	10,000
1,000	10,000	50,000
5,000	50,000	250,000
10,000	100,000	500,000

Download

CLS-AN-308.pdf (497 kB) Full article

JULIA SALITA - Nov 16, 2024, 3:52 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items:

Loading [MathJax]/extensions/Safe.js

10/14/2024 WIMR Lab Visit- Cell Passage 1 - Copy

JULIA SALITA - Oct 14, 2024, 12:06 PM CDT

Title: Cell Passage 1

Date: 10/14/2024

Content by: Julia Salita

Present: Julia Salita and Jayson O'Halloran

Goals: To correctly passage our cells, get a better understanding of how to passage cells, learn to use the required equipment, and understand why each step of passaging cells is important.

Content:

1. See file below for in lab notes
2. Start as normal: look at your cells get, everything ready, then aspirate the current media, use 2mL of PBS to wash the cells GENTLY, aspirate the PBS out, **you're supposed to add 2mL of trypsin but we needed 3mL**, then let sit in the incubator for about 3 minutes, then add 7 mL of D-MEM, and mix up and down with the serological pipet making sure to wash the back of the flask, take about 0.5 mL and put it in a small centrifuge tube and close it, then go to they cytoflex
3. Fill in table: Run the cytoflex and get the # of alive cells (# of events in the shape you make) and multiply by 100. This will give you cells per mL. We got 5309 which means there is 530,900 cells in 1mL which in 10 mL is 5,309,000 cells total in flask.
    1. We were at about 110% confluency (100% = 5,000,000 cells) with 5,309,000 cells.
4. Then we used a doubling time calculator (https://www.cellseeker.org/cellcalc/growth-rate-and-doubling-time/) to calculate that they had a doubling time of 28.2 hr.
    1. We cut back the cells to 1,000,000 cells, so we left a volume of 1.9 mL of the ~10mL solution of the current flask and added back in 8.1 mL of D-MEM for anew total of 10mL.
5. Then record the passage and put the cells back in the incubator.

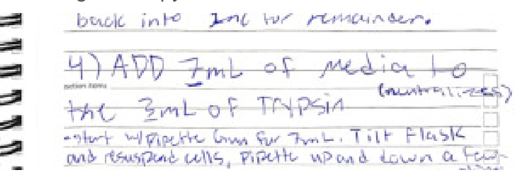
Conclusions/action items:

1. Passage cells again on Wednesday October 16th.

JULIA SALITA - Oct 14, 2024, 12:06 PM CDT

10/14/24
Cell Passage 1
Passage

1) Remove Previous media using aspirator. ~~dispose~~ of
2) Add 2mL of PBS (front side) gently using pipette gun, back side. After, remove using aspirator (Put tips in bleach)
3) Add 2mL of Trypsin to flask, back side to dissociate. Incubate for 5 min, after 2-3 lightly shake and check to see dissociation. Add 1mL of Trypsin If not fully diss. Put


back into 1mL for remainder.

4) ADD 7mL of media to the 3mL of TRYPSIN (neutralizes)

- start w/ pipette gun for 7mL. Tilt Flask and resuspend cells, pipette up and down a few

Download

CamScanner_10-14-2024_.pdf (1.19 MB)

Passage Records

JULIA SALITA - Nov 13, 2024, 12:11 PM CST

Title: Passage Records

Date: Added 13-Nov-2024

Content by: Julia Salita

Present: Julia for all, Jayson for some

Goals: to passage and take care of our cell line

Content:

Performed 5 passages as of when inserted

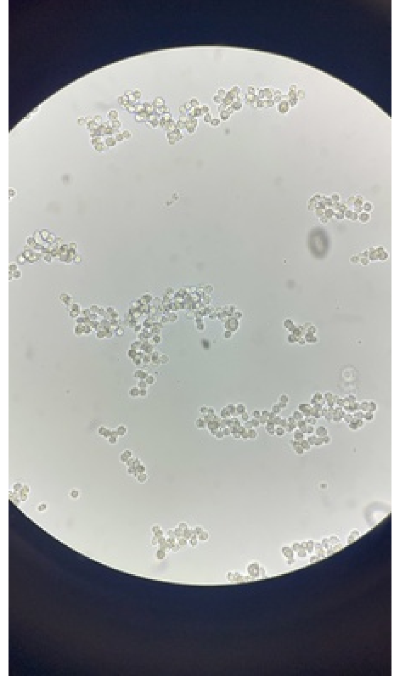
1. Passage 1: 14-Oct-2024 , No image
2. Passage 6: 25-Oct-2024 , No image
3. Passage 7: 28-Oct-2024
4. Passage 10: 04-Nov-2024
5. Passage 13: 11-Nov-2024

See some images below

Conclusions/action items:

Continue to passage.

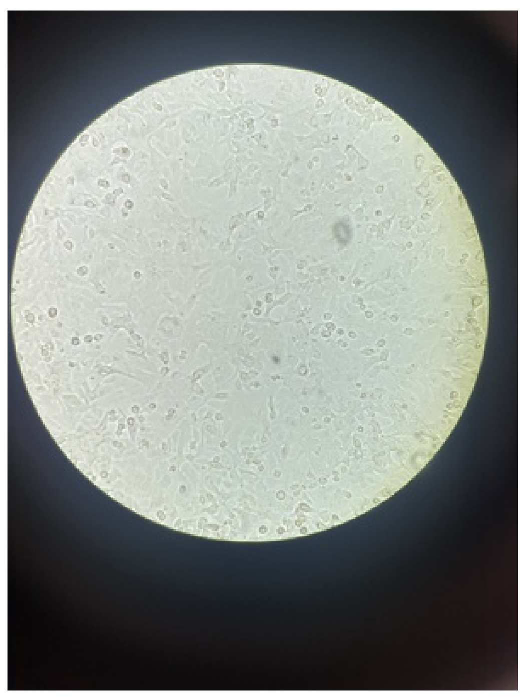
JULIA SALITA - Nov 13, 2024, 12:09 PM CST



Download

IMG_2702_1_.JPG (2.22 MB) Cells detached with trypsin during passage 7

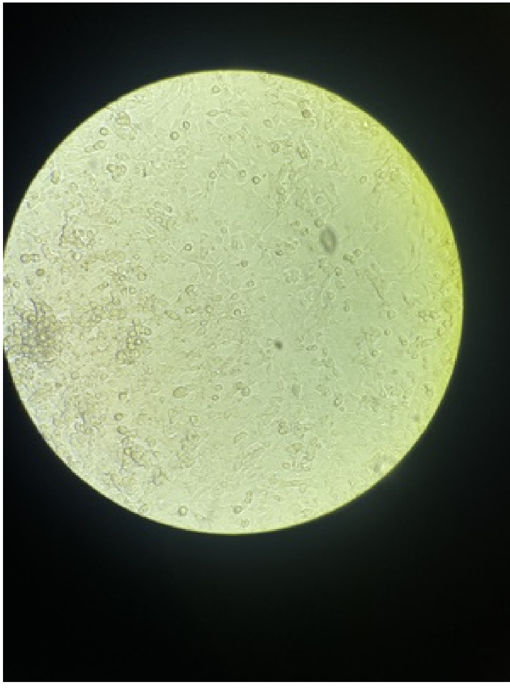
JULIA SALITA - Nov 13, 2024, 12:11 PM CST



Download

IMG_2788_1_.jpg (2.82 MB) Adhered cells before passage 10

JULIA SALITA - Nov 13, 2024, 12:13 PM CST



Download

IMG_2814_1_.jpg (3.01 MB) Adhered cells before passage 13

2024/09/09 Introductory Research I

JAYSON O'HALLORAN - Sep 11, 2024, 7:21 PM CDT

Title: Introductory Research I

Date: 9/09/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the field of CRISPR engineering

Resource: [1] D. Shriner , “What are genome editing and CRISPR-Cas9?: Medlineplus Genetics,” MedlinePlus, https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/ (accessed Sep. 9, 2024).

Content:

Genome Editing:

- Technologies that enable scientists to modify an organism's DNA.

- Allows addition, removal, or alteration of genetic material at specific genome locations.

- Includes various methods, with CRISPR-Cas9 being a prominent example.

CRISPR-Cas9:

- Stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9.

- Adapted from a bacterial immune defense mechanism.

- Bacteria use CRISPR arrays to remember and cut viral DNA using Cas9 enzyme.

- Researchers use guide RNA and Cas9 to target and cut specific DNA sequences in cells.

- Cas9 is commonly used, but other enzymes like Cpf1 can also be employed.

Applications and Potential:

- Used in research to understand diseases and for potential treatment.

- Explored for treating single-gene disorders (e.g., cystic fibrosis, hemophilia, sickle cell disease) and complex diseases (e.g., cancer, heart disease, HIV).

- Promises significant advancements in medicine.

Ethical Considerations:

- Most editing is done on somatic cells, which are not inherited by future generations.

- Editing germline cells or embryos can affect future generations and raises ethical issues.

- Concerns include potential use for enhancing traits and long-term impacts.

- Germline and embryo editing are illegal in the U.S. and many other countries due to ethical and safety concerns.

Conclusions/action items:

- In conclusion, CRISPR-Cas9 has emerged as a new tool in genetic engineering, providing scientists/researchers with precision and efficiency in editing DNA. Its ability to target specific genetic sequences and make precise modifications holds immense promise for advancing medical research, developing new treatments, and improving agricultural practices. However, the technology also brings significant ethical and safety considerations, particularly concerning off-target effects and germline editing. As more progress is gained in CRISPR, it is crucial to navigate potential ethical concerns carefully, ensuring that its applications are regulated and used responsibly to maximize benefits while minimizing risks.

- Continue to do research, specifically on CRISPR mechanisms, spheroid formation, and cell line creation

- Finish Hess Lab paperwork

- Meet with design team this week

- Finish progress report 1

2024/09/10 Spheroid Research II

JAYSON O'HALLORAN - Sep 12, 2024, 9:55 PM CDT

Title: Spheroid Research II

Date: 9/10/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of spheroids and their uses

Resource: [2] K. Białkowska, P. Komorowski, M. Bryszewska, and K. Miłowska, “Spheroids as a type of three-dimensional cell cultures-examples of methods of preparation and the most important application,” International journal of molecular sciences, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503223/ (accessed Sep. 10, 2024).

Content:

Abstract

- Importance of Cell Cultures: Cell cultures are essential for testing materials, drugs, and understanding cell biology and mechanisms.

- Limitations of 2D Cultures: Traditional two-dimensional (2D) cultures, typically monolayers, do not accurately replicate the natural cell environment, lacking essential cell–cell and cell–extracellular matrix (ECM) interactions.

- Advantage of 3D Cultures: Three-dimensional (3D) cultures, particularly spheroids, provide a more realistic in vitro model that better mimics the complexity of living tissues.

- Spheroid Applications: Spheroids are utilized in drug testing, nanoparticle evaluation, and disease modeling, highlighting their versatility and relevance in R&D.

Introduction

- Cell Sourcing: Cells can be obtained directly from tissues or from established cell lines, which are adapted for laboratory use, allowing for controlled experimental conditions.

Spheroids as a Type of 3D Cell Culture

- Spheroids: Spheroids are defined as aggregates of cells that self-assemble in environments that prevent attachment to flat surfaces, allowing for more natural cell behavior.

Formation Process:

  - Initial Aggregation: Dispersed cells aggregate due to long-chain ECM fibers that contain RGD (arginine-glycine-aspartic acid) motifs, facilitating binding to cell-surface integrins.

  - Cadherin Expression: This binding leads to the upregulation of cadherin, a protein crucial for cell adhesion.

  - Tightening Connections: Hemophilic cadherin–cadherin interactions between neighboring cells strengthen the connections, resulting in the formation of stable spheroids.

- Role of Integrins: Integrins are involved in activating focal adhesion kinase (FAK), which is linked to tumor invasiveness and poor patient prognosis when overexpressed.

Methods of Spheroid Preparation

Hydrogels:

- Provide a versatile platform for controlled microtissue production, allowing for the creation of spheroids with specific shapes and sizes.

- Some cell lines require ECM proteins in the culture medium to form spheroids effectively.

Rotary Cell Cultures:

- Cells are cultured in bottles with agitators, preventing attachment and promoting aggregation.

- This method is simple and allows for large-scale spheroid production but has drawbacks, such as variability in spheroid size and potential mechanical damage to cells.

Microgravity Simulation

- A variation involves rotating flasks around a horizontal axis, simulating microgravity and minimizing hydrodynamic forces, leading to larger and more uniform spheroids.

- Hanging Drop Method: Cells are cultured in droplets suspended by surface tension, allowing them to aggregate without a solid substrate.

- Microfluidic Systems: These systems provide precise control over the microenvironment, enhancing spheroid viability and homogeneity.

Applications of Spheroids

- Phenotypic Stability: Spheroids maintain their phenotypic characteristics over time, making them reliable for long-term studies.

- Co-culture Capabilities: Primary human hepatocytes (PHH) can be co-cultured with non-parenchymal cells (e.g., Kupffer cells), enhancing their viability and mimicking liver tissue interactions.

- Proteome Analysis: Studies show that spheroids exhibit fewer changes in protein expression compared to 2D cultures, indicating a more stable cellular environment. After 24 hours, 457 proteins showed altered expression in 2D cultures, while spheroids demonstrated significantly fewer changes.

- Inter-individual Differences: Spheroids retain the unique characteristics of the donor liver tissue, which is a driver for personalized medicine and understanding individual responses to drugs.

- Functional Studies: Spheroids maintain hepatocyte-specific functions, such as stable albumin secretion, over prolonged culture periods.

- They are effective models for studying liver pathologies, such as cholestasis (indicated by bile acid accumulation) and steatosis (indicated by increased neutral lipids), facilitating drug screening and understanding disease mechanisms.

Conclusions/action items:

- In conclusion, I found this paper incredibly helpful for developing a background in spheroid creation. It clearly outlines various 3D cell culture techniques and explains why they are better than traditional 2D methods, which often don't mimic the natural environment of cells. The insights on different spheroid formation methods, like using hydrogels and microfluidic systems, are particularly useful for our project. I also appreciate the focus on extracellular matrix components and cell interactions, as these factors are necessary for achieving consistent and viable spheroids.

- Continue to do research, specifically on CRISPR mechanisms, spheroid formation, and cell line creation

- Finish Hess Lab paperwork

- Finish progress report 1

2024/09/11 Initial γH2AX Research III

JAYSON O'HALLORAN - Sep 12, 2024, 10:27 PM CDT

Title: Initial γH2AX Research III

Date: 9/11/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of γH2AX and its properties as a biomarker

Resource: [3] A. Ivashkevich, C. E. Redon, A. J. Nakamura, R. F. Martin, and O. A. Martin, "Use of the γ-H2AX assay to monitor DNA damage and Repair in translational cancer research," Cancer letters, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3329565/ (accessed Sep. 11, 2024).

Content: Information regarding γH2AX from the article above

Biomarker for DNA Damage: The formation of γH2AX in response to DNA double-strand breaks (DSBs) provides the basis for a sensitive assay of DNA damage in human biopsies. This assay is particularly useful for monitoring the clinical response to DNA-targeted therapies such as chemotherapy and radiation therapy [T4].

Clinical Trials and Monitoring: Since its discovery, γH2AX has been extensively used in basic research, but its application as a pharmacodynamic marker in clinical studies is a relatively recent development. In the last four years, there has been a sharp increase in its use to monitor chemotherapy-induced DNA damage in cancer patients, with more than 35 clinical trials utilizing γH2AX for evaluation of drug response [T1].

Sensitivity and Methodology: The assay can be performed using various techniques, including immunoblotting and enzyme-linked immunosorbent assay (ELISA), but microscopy is preferred for clinical samples due to its ability to detect large numbers of γH2AX molecules at DNA break sites, creating bright foci that allow for sensitive detection of individual DSBs [T2]. Microscopy also provides valuable information about the extent of DNA damage and the differential response in tumor tissues [T2].

Assessment of Treatment Efficacy: The γH2AX assay has been applied to monitor the formation and persistence of DNA damage in human cancer cells. For instance, studies have shown that γH2AX levels increase in cultured tumor cells, confirming the genotoxic effect of therapies such as radioimmunotherapy [T3]. This monitoring can help assess the anti-tumor efficacy of treatments and guide clinical decisions.

Radiosensitivity and Individualized Treatment: Another important application of the γH2AX assay is the assessment of radiosensitivity in prospective patients. Monitoring normal tissue response in parallel with clinical tumor outcomes could form a basis for routine monitoring of individual radiosensitivity to therapy [T3].

High Throughput and Automation: Recent developments in automation have led to the creation of high throughput analysis platforms, such as the Rapid Automated Bio dosimetry Tool (RABiT), which is designed for high throughput bio dosimetry and can handle large numbers of samples for dosimetry screening in response to radiation incidents. Such systems are adaptable to routine clinical investigations [T1].

Conclusions/action items:

- In conclusion, the γH2AX assay is a powerful and sensitive tool for detecting DNA damage in cells. When it comes to screening for gamma-H2AX (γH2AX), cells or tissue samples are first fixed to preserve their structure. They are then

permeabilized to allow antibodies to enter. After blocking non-specific binding, the samples are incubated with an anti-γH2AX primary antibody, followed by a fluorescently labeled secondary antibody. The samples are then mounted and examined under a fluorescence or confocal microscope to detect and quantify γH2AX foci, which indicate DNA double-strand breaks. (Screening for our project)

- Continue to do research into week 3

- Finish Hess Lab paperwork

- Finished progress report 1

- Meet with advisor and client on 9/13/24

2024/09/15 Cell Line Research IV

JAYSON O'HALLORAN - Oct 11, 2024, 1:22 AM CDT

Title: Cell Line Research IV

Date: 9/15/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of cell lines used in modeling lung cancer.

Resource: [4] A. F. Gazdar, L. Girard, W. W. Lockwood, W. L. Lam, and J. D. Minna, “Lung cancer cell lines as tools for Biomedical Discovery and Research,” Journal of the National Cancer Institute, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2935474/ (accessed Sep. 15, 2024).

Content:

- The role of tumor cell lines in understanding the molecular biology of lung cancer and the ability to translate these findings to clinical applications would have been severely hampered and delayed without the availability of cell lines.

- Ongoing debate exists regarding the relevance of cell lines and in vitro models for cancer research.

- Cell lines typically maintain the expression of most "hallmarks of cancer," except for angiogenesis, which requires stromal tissues.

- Genomic instability in cancer leads to various genetic and epigenetic changes, including driver and passenger mutations.

- Lung cancer cell lines help distinguish driver mutations from passenger mutations through functionality tests, which are challenging in tumor tissues.

- Key contributions include the identification of TP53 mutations and insights into copy number variations and allele-specific imbalances.

- Initial cytogenetic observations in small cell lung cancer (SCLC) lines revealed frequent deletions on chromosome 3, leading to the discovery of tumor suppressor genes like RASSF1A and FUS1.

- MYC amplification was observed in SCLC lines, influencing the understanding of NMYC and the role of MYC family members in lung cancer.

- High-density genomic techniques have identified recurrent gains and losses in the lung cancer genome, with significant overlap between cell lines and tumors.

- Lung cancer cell lines revealed the role of RB in SCLC pathogenesis and established a model for tumor suppressor pathways.

- The LKB1 gene is frequently mutated in non-small cell lung cancer (NSCLC) and located at a common loss site in cell lines.

- The TITF1 gene, a master transcription factor, was identified as an oncogene frequently amplified in lung cancer.

- Activating mutations in the EGFR kinase domain were discovered through cell line studies, establishing a link to sensitivity to tyrosine kinase inhibitors.

- Cell lines have contributed to understanding both intrinsic and acquired resistance to EGFR-targeted therapies.

<u>γH2AX</u>

- DNA Damage Response: γH2AX acts as an early marker for double-strand breaks (DSBs), indicating activation of DNA repair mechanisms in lung cancer cells.

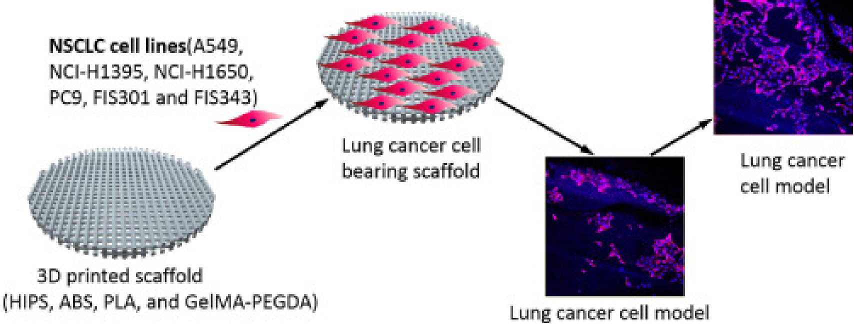
- Genomic Instability: Elevated γH2AX levels in lung cancer cell lines suggest genomic instability, a key hallmark of cancer.

- Evaluating Treatment Efficacy: Measuring γH2AX can help assess the effectiveness of radiotherapy and chemotherapy in inducing DNA damage in lung cancer cells.

- Understanding Resistance Mechanisms: Analyzing γH2AX dynamics provides insights into how lung cancer cells may develop resistance to therapies through unresolved DNA damage.

- Pathway Insights: Correlating γH2AX accumulation with mutations in key genes (e.g., TP53, LKB1) offers insights into the molecular pathways involved in lung cancer progression and treatment response.

Image of possible route for future model of spheroids being held together on a scaffold.



Conclusions/action items:

- Finalize PDS

- Client meeting

- Advisor Meeting

- More research

- Design matrix

2024/09/18 Types of Cancer Cell Lines Research V

JAYSON O'HALLORAN - Sep 30, 2024, 7:09 PM CDT

Title: Types of Cancer Cell Lines Research V

Date: 9/18/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the different cell lines in cancer research

Resource: [5] R. Sinha, A. Luna, N. Schultz, and C. Sander, “A pan-cancer survey of cell line tumor similarity by feature-weighted molecular profiles,” Cell reports methods, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9017219/ (accessed Sep. 18, 2024).

Content:

1. Immortalized Cancer Cell Lines

Description: These are cell lines derived from patient tumors that have been modified to proliferate indefinitely in vitro.

Pros:

Availability: "Immortalized cancer cell lines, derived from patient tumors and grown and maintained in vitro, are the most commonly used experimental model in cancer research" .

Consistency: They provide a stable environment for experiments, allowing for reproducibility.

Historical Data: Extensive background data available for many lines facilitates comparative studies.

Cons:

Genetic Drift: "Cell lines generally have more genomic alterations than primary tumors" .

Limited Heterogeneity: "Cell lines typically do not represent all subtypes of cancers nor do they reflect tumor heterogeneity" .

2. Patient-Derived Xenografts (PDXs)

Description: Tumor tissues from patients are implanted into immunocompromised mice, allowing for the growth of human tumors in a living organism.

Pros:

Tumor Microenvironment: "Retain the original tumor microenvironment, providing a more accurate model of human cancer."

Genetic Fidelity: "Better preserve the genetic and phenotypic characteristics of the original tumor."

Cons:

Time-Consuming: Establishing PDX models can take a long time and requires significant resources.

Variability: Differences in host responses can lead to variability in tumor behavior.

3. Organotypic Cultures

Description: These cultures maintain the architecture and cellular composition of the original tissue.

Pros:

Physiological Relevance: "More closely mimic the in vivo environment compared to traditional cell lines."

Cell-Cell Interactions: "Preserve important interactions between different cell types within the tumor."

Cons:

Complexity: More complex to establish and maintain than standard cell lines.

Limited Lifespan: Often have a shorter lifespan and may not be as amenable to long-term studies.

4. Stem Cell-Derived Lines

Description: These lines are derived from cancer stem cells, which are thought to drive tumor growth and metastasis.

Pros:

Heterogeneity: "Capture the diversity of cancer cell populations, including those that are resistant to treatment."

Targeting Cancer Stem Cells: Useful for studying therapies aimed at eradicating cancer stem cells.

Cons:

Isolation Challenges: Difficult to isolate and maintain in culture.

Variability: May exhibit significant variability in behavior and response to treatments.

5. Established Cell Lines (e.g., HeLa, A375)

Description: Well-characterized cell lines that have been used extensively in research.

Pros:

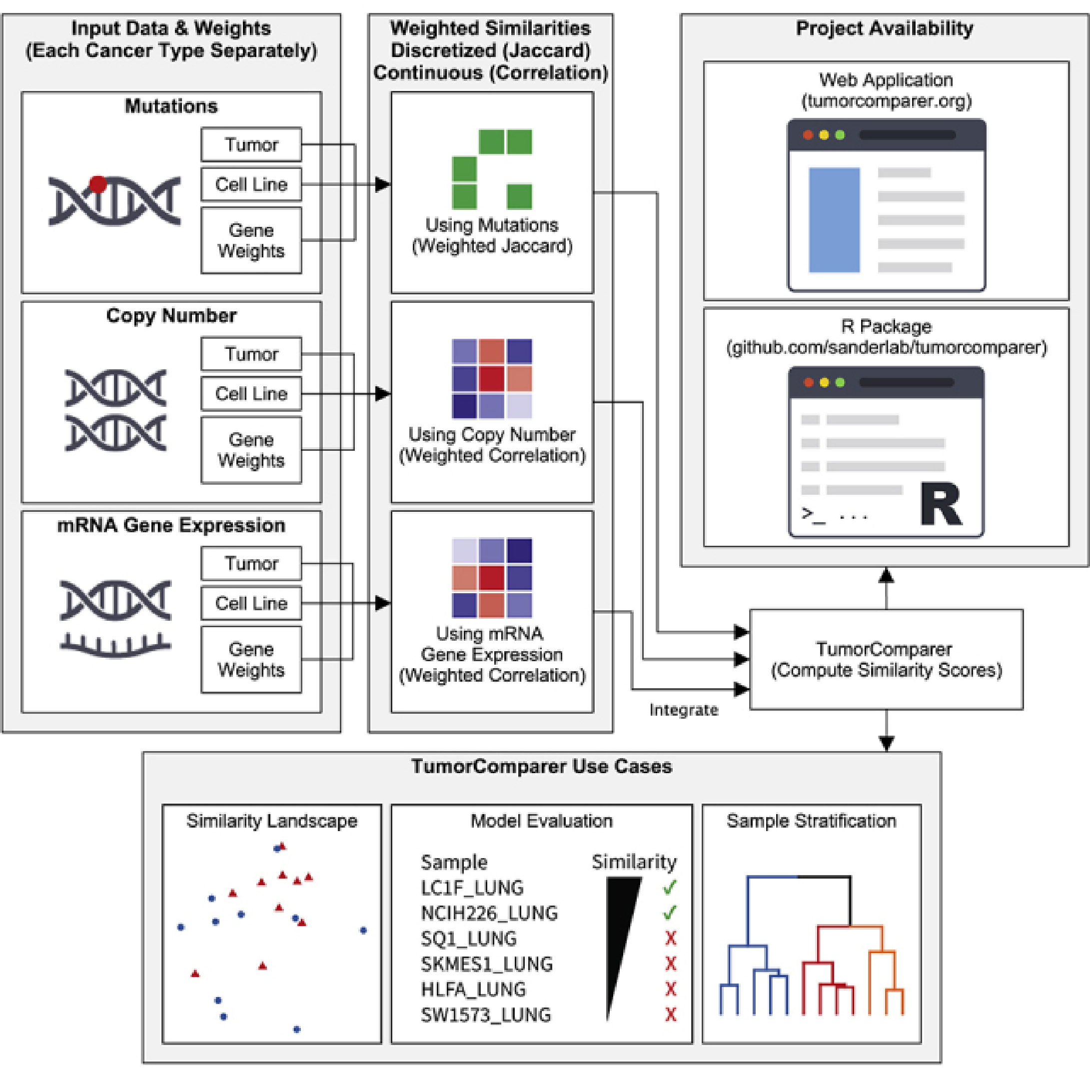
Extensive Literature: "A wealth of information and protocols available for these lines" .

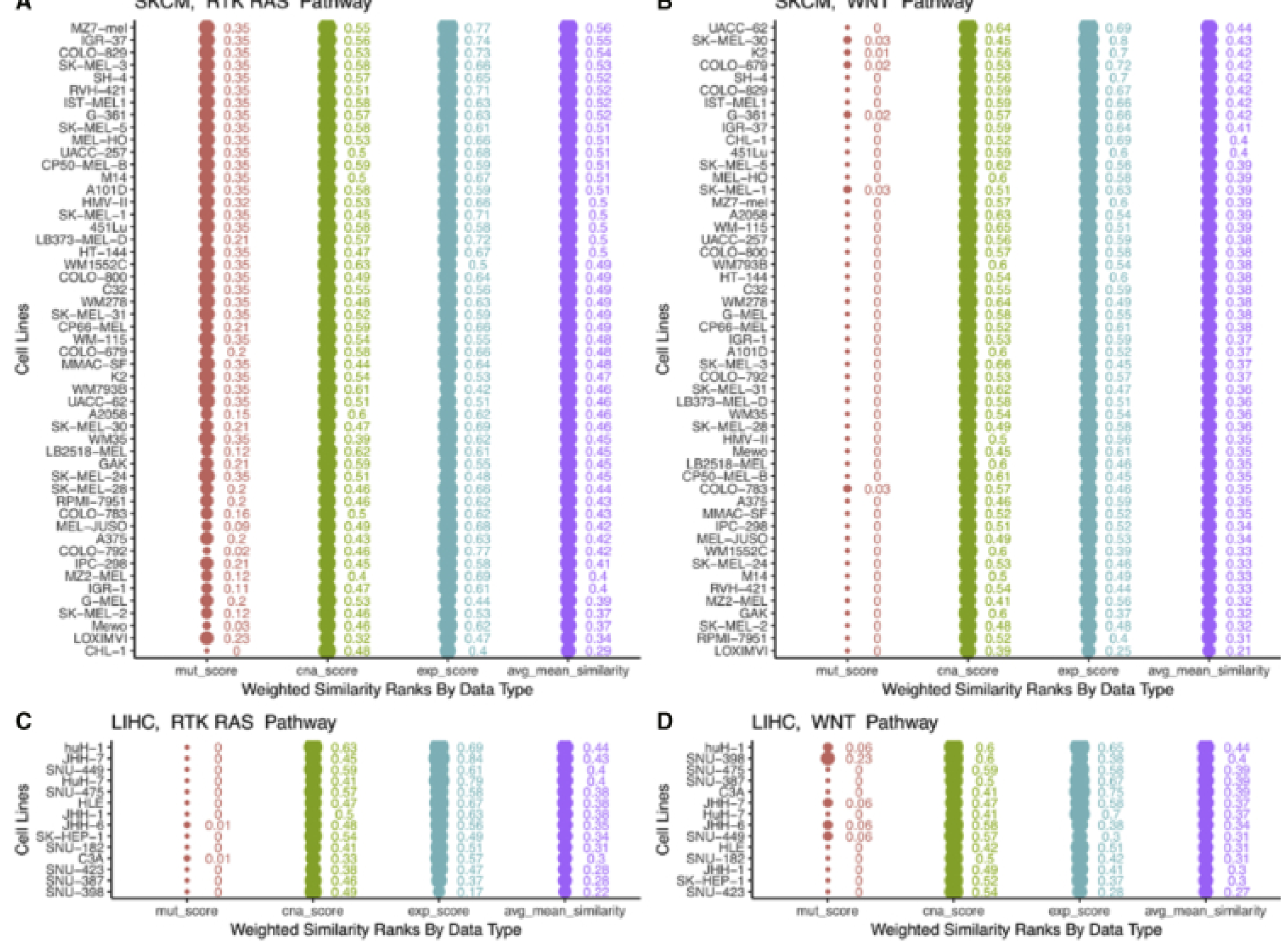
Ease of Use: Generally easy to culture and manipulate genetically.

Cons:

Misidentification: "The issue of misidentification of cell lines is an important and well-known challenge in the field" .

Limited Relevance: "The widespread use of cell lines with a low genomic resemblance to tumors is concerning"


Input Data & Weights
(Each Cancer Type Separately)
Mutations
Tumor
Cell Line
Gene Weights
Copy Number
Tumor
Cell Line
Gene Weights
mRNA Gene Expression
Tumor
Cell Line
Gene Weights
Weighted Similarities
Discretized (Jaccard)
Continuous (Correlation)
Using Mutations (Weighted Jaccard)
Using Copy Number (Weighted Correlation)
Using mRNA Gene Expression (Weighted Correlation)
Project Availability
Web Application (tumorcomparer.org)
R Package (github.com/sanderlab/tumorcomparer)
R
Integrate
TumorComparer (Compute Similarity Scores)
TumorComparer Use Cases
Similarity Landscape
Model Evaluation
Sample
Similarity
LC1F_LUNG
NCIH226_LUNG
SQ1_LUNG
SKMES1_LUNG
HLFA_LUNG
SW1573_LUNG
Sample Stratification



The similarity of cell lines and tumors varies by gene set—the best matches might be quite different for different gene sets/pathways

The top two panels show the similarity scores of SKCM tumors and melanoma cell lines when using uniform weights on all features, and genes from (A) RTK-RAS pathway and (B) WNT pathway. Similarly, the bottom two panels (C and D) show corresponding scores for liver cancer cell lines, compared with TCGA LIHC tumors. SKCM cell lines show similar/better similarity scores when using the RTK-RAS pathway than the WNT pathway, whereas LIHC cell lines show lower scores with the RTK-RAS pathway than with the WNT pathway—this is consistent with the frequency of alterations in the member genes of the RTK-RAS and WNT pathways in these cancer types.

Conclusions/action items:

- Client meeting

- Advisor Meeting

- More research

- Design matrix finished

2024/09/21 A549 Cell Line Research VI

JAYSON O'HALLORAN - Nov 15, 2024, 6:41 PM CST

Title: A549 Cell Line Research VI

Date: 9/21/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the A549 cell line and how it can be used in our project.

Resource: [6] “Everything You Need to Know About A549 Cells ,” Synthego A549 Cells , https://www.synthego.com/a549-cells (accessed Sep. 21, 2024).

Content:

A549 Cells: Origin And General Information

- A549 is the most commonly used human non-small cell lung cancer cell line for both basic research and drug discovery.

- A549 Cell Line Origin: The A549 cell line consists of hypotriploid alveolar basal epithelial cells. This cell line was first developed by D. J. Giard et al. in 1972 by removing and culturing pulmonary carcinoma tissue from the explanted tumor of a 58-year-old Caucasian male.

A549 Cells: Morphology and Characteristics

- A549 cells are squamous in nature and are responsible for the diffusion of water and electrolytes across the alveoli. When grown in vitro, these cells grow as a monolayer, adhering to the culture flask.

- These cells have the ability to synthesize lecithin and contain a high percentage of unsaturated fatty acids that are responsible for the maintenance of membrane phospholipids.

A549 Cell Line Information

Information on the cell line such as karyotype, cell size, doubling time, etc.

- Karyotype: A549 cell line is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. It is common to have modal numbers of 64 and 67 with higher ploidies occurring at an infrequent rate (0.4%).

- Cell Size: In a morphological analysis of A549 cells, the mean cell diameter of the cells from inverted microscopy and TEM images was estimated to be 14.93 µm and 10.59 µm respectively.

- Doubling time: These cells are easy to grow. A549 cells doubling time is typically 22 hours, although it can sometimes take up to 40 hours.

- Storage: To ensure that the A549 cells remain viable, the culture should be stored in liquid nitrogen vapor phase pre thaw. Post thaw they can be stored in an incubator with the right media and growth factors.

- Growth Medium: The A549 cells are typically cultured using a base medium F12/K (Gibco/Invitrogen). 10% fetal bovine serum (FBS) is added to the base medium to make the complete growth medium. The cells can also be cultured in complete media consisting of Dulbecco’s MEM (DMEM) modified with 10% FBS.

Research and Applications of A549 Cells

- The A549 cell line has been tested and approved by the FDA and finds use in a variety of applications, including in manufacturing constructs for use in clinical trials.

Adenovirus Production

- The A549 cell line has been used for adenovirus production. Most significantly, it has been utilized for replicating adenovirus constructs that do not need complementation by the viral oncogene, early region 1A (EA1). It has also been used as a negative control in assays to measure the replication of adenoviruses that lack E1A as well as a target cell line for the detection of replication competent adenoviruses.

Disease Modeling

- The A549 cells have been used to model the alveolar Type II pulmonary epithelium. Studies have shown that this can be particularly useful in research for studying the metabolic processing of lung tissue and for identifying mechanisms of drug delivery to the tissue. This cell line has been utilized not only for studying lung cancer but also for other infections related to the lungs like allergies, asthma, and respiratory infections.

A few examples of research studies where A549 cells were used for disease modeling.

- P53, also known as tumor protein is a gene that codes for a protein that regulates the cell cycle and acts as a tumor suppressant. M3814 is a selective pharmacologic inhibitor of the serine/threonine kinase DNA-dependent protein kinase (DNA-PK), and plays a vital role in non homologous end-joining. In a recent study (the one linked), the researchers used isogenic p53-null/wild-type A549 and HT-1080 cell lines to show that M3814 blocks the repair of radiation-induced double-stranded breaks and enhances p53 phosphorylation and activation.

- In another study, the A549 cell line was used to investigate the role of chemokines during the initial local response to infection with Mycobacterium tuberculosis in the human lung. The researchers found that the human alveolar epithelial cell line infected with M. tuberculosis produced the chemokines MCP-1 and IL-8 through upregulation of their respective mRNAs. The production of these cytokines only depended on the intracellular mycobacterial growth and was not related to virulence.

Drug Development

- These cells have proved to be great for in vitro as well as in vivo testing of novel drugs like docetaxel, paclitaxel and, bevacizumab. The in vivo tests are conducted through xenografting, while in vitro tests are conducted in cell culture. The A549 CDX mouse model (cell line derived xenograft) is the most commonly used xenograft lung cancer model.

Enzymatic Studies

- The human A549 cell line has been used to test the ability of 2-(2,4-dihydroxyphenyl)thieno-1,3-thiazin-4-one (BChTT) in the inhibition of cancer cell proliferation and to understand its mechanism of action on a molecular level. It has also been used to study the effects of insulin and insulin-like growth factor 1 on the apoptosis and proliferation of the A549 cells.

CRISPR Delivery

- CRISPR Cas9, as we already know, is a great tool for precisely editing the DNA. However, delivery of the Cas protein into the cells is still an under researched area with a lot of potential for innovation (hence the need for our design project). In a recent study, researchers constructed Cas9 ribonucleoprotein complexes conjugated to the 7D12 nanobody and demonstrated the transfection of Cas9 mediated by receptors into A549 cells via binding to the epithelial

growth factor receptor. They also exhibited that transfection with a Cas9 ribonucleoprotein targeting the BRCA2 gene resulted in an increased sensitivity to Cisplatin, a chemotherapeutic drug and led to a synthetic dose lethality in the cells.

Conclusions/action items:

- The A549 cell line possesses all of the characteristics of a cell line that our team would chose, currently it is the top choice for this semester.

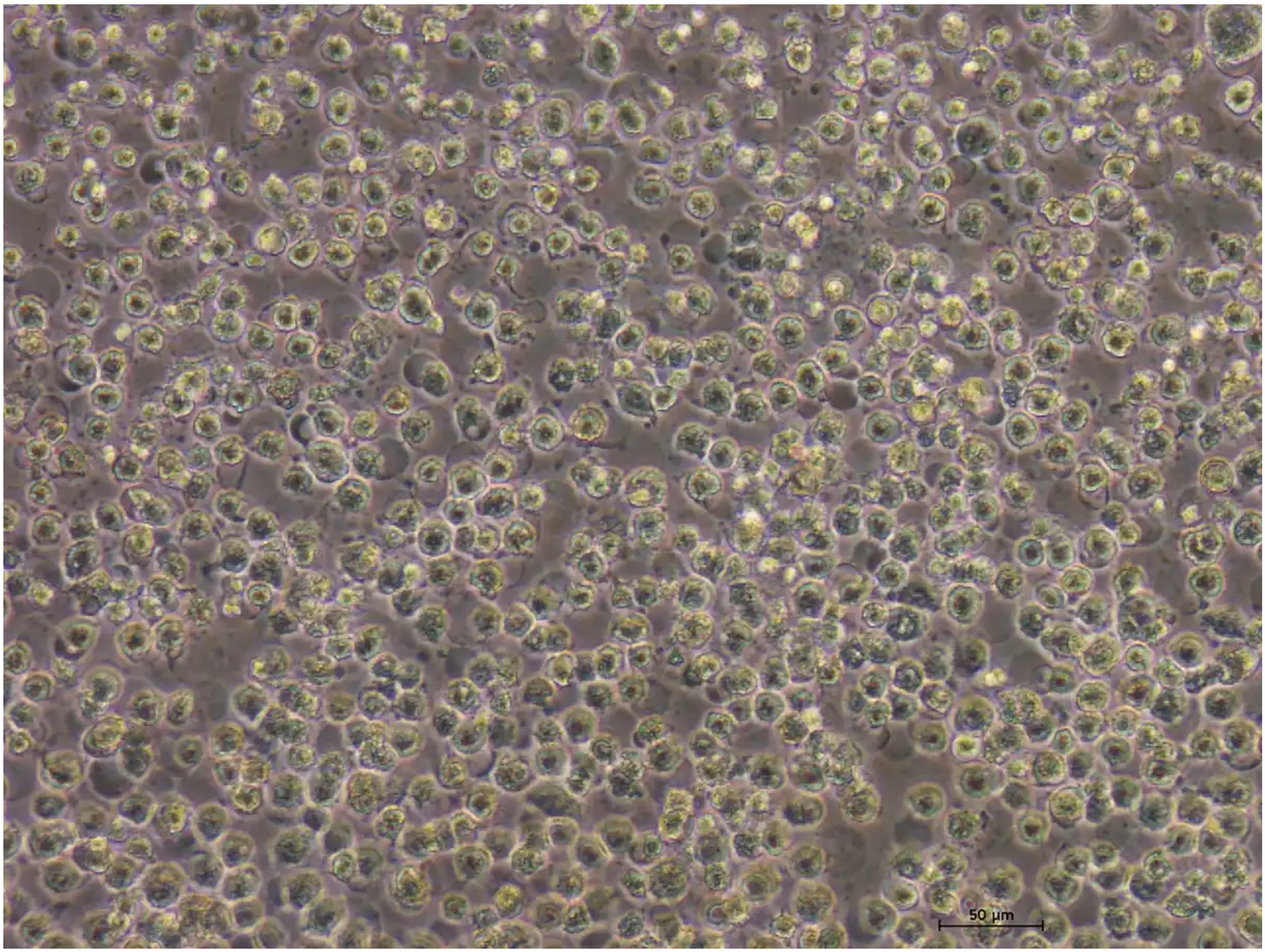


Image of the A549 cell line

2024/09/23 OVCAR-5 Cell Line Research VII

JAYSON O'HALLORAN - Oct 06, 202

Title: OVCAR-5 Cell Line Research VII

Date: 9/23/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the OVCAR-5 cell line and how it can be used in our project.

Resource: [7] M. Acland et al., “Chemoresistant cancer cell lines are characterized by migratory, amino acid metabolism, protein catabolism and IFN1 signa perturbations,” Cancers, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9179525/#:~:text=We%20developed%20carboplatin%2Dresistant%20cell,compared%20to%20parental%20 (accessed Sep. 23, 2024).

Content: OVCAR-5 cancer cell line is a good model for metastatic gastrointestinal carcinoma.

Pros:

Relevance to Human Ovarian Cancer: As a model derived from a human tumor, OVCAR-5 maintains some of the genetic and phenotypic characteristics of cancer, making it relevant for research.

Established Protocols: Extensive protocols and methodologies have been developed for this cell line, including growth conditions, transfection techniques, a sensitivity assays.

Spheroid Formation: OVCAR-5 can form multicellular spheroids, allowing us to study the tumor microenvironment and various drug responses in a 3D conte

CRISPR Compatibility: OVCAR-5 is amenable to CRISPR/Cas9 and CRISPRi (CRISPR interference) technologies for gene editing and regulation studies.

Cons:

Availability: Not widely available anymore, a discontinued cell line.

Heterogeneity: OVCAR-5 may not fully represent the heterogeneity of ovarian tumors, limiting its use for DNA damage assessment and screening.

Drug Resistance: This cell line often displays intrinsic resistance to certain chemotherapeutic agents, which can skew our drug response data.

Lack of Microenvironment: Being a cell line, OVCAR-5 does not fully recapitulate the complex tumor microenvironment found in vivo.

Limited Life Span: Like many cell lines, OVCAR-5 can undergo genetic drift over passages, which may affect experimental outcomes.

Spheroid Formation

Spheroid Formation:

Description: Spheroids are 3D aggregates of cells that better mimic in vivo tumors compared to traditional 2D cultures.

Pros:

Increased Relevance: Mimics the structure and behavior of solid tumors, offering insights into cellular behavior in a more physiologically relevant context.

Drug Testing: Allows for better assessment of therapeutic efficacy and mechanisms of resistance.

Cons:

Standardization: Variability in spheroid size and structure can complicate reproducibility.

Growth Conditions: Requires specific conditions for optimal spheroid formation, which may not be easily standardized in our case.

CRISPRi Screening

CRISPRi:

Description: CRISPR interference is a technique that uses a catalytically inactive Cas9 (dCas9) to inhibit gene expression.

Pros:

Targeted Gene Regulation: Allows for precise downregulation of genes without permanent modifications, useful for functional genomics studies.

High Throughput: Can be adapted for high-throughput screening to identify genes involved in drug resistance or other cancer-related pathways.

Cons:

Off-target Effects: Potential for unintended interactions with non-target genes, which can complicate interpretation.

Dependence on Transcriptional Activity: Effectiveness can vary based on the target gene's promoter activity, leading to inconsistent results.

Gamma-H2AX Staining

Gamma-H2AX Staining:

Description: Gamma-H2AX is a marker for DNA double-strand breaks. Staining for gamma-H2AX is commonly used to assess DNA damage response.

Pros:

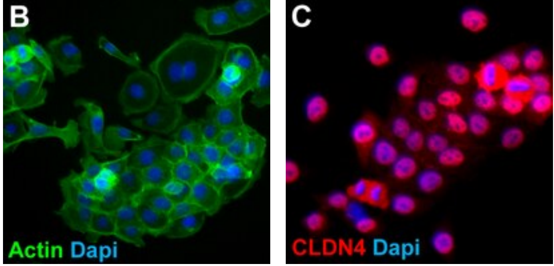
Sensitive Marker: Effective in detecting DNA damage, providing insights into the effects of therapies and cellular stress responses.

Quantitative: Can be quantified using flow cytometry or microscopy, allowing for detailed analysis of DNA damage across different conditions.

Cons:

Interpretation Challenges: Elevated gamma-H2AX levels can be influenced by various factors (e.g., cellular stress), making it essential to correlate with othe confirm DNA damage specifically.

Cell Cycle Dependency: DNA damage response can vary with the cell cycle phase, complicating the interpretation of results without proper controls.



Images of OVCAR-5

Conclusions/action items:

OVCAR-5 is a widely used cell line for studying ovarian cancer due to its relevance, established protocols, and compatibility with CRISPR and spheroid for ability. While it has significant advantages, we have to consider its limitations, especially in terms of tumor heterogeneity, drug resistance, and the complexi interpreting experimental data.

2024/09/26 NCI-H23 Cell Line Research VIII

JAYSON O'HALLORAN - Oct 11, 2024, 1:21 AM CDT

Title: NCI-H23 Cell Line Research VIII

Date: 9/26/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the NCI-H23 cell line and how it can be used in our project.

Resource: [8] Kaczmarczyk et. al, Comparative microsomal proteomics of a model lung cancer cell line NCI-H23 reveals distinct differences between molecular profiles of 3D and 2D cultured cells https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8487723/pdf/oncotarget-12-2022.pdf (accessed Sep. 26, 2024).

Content:

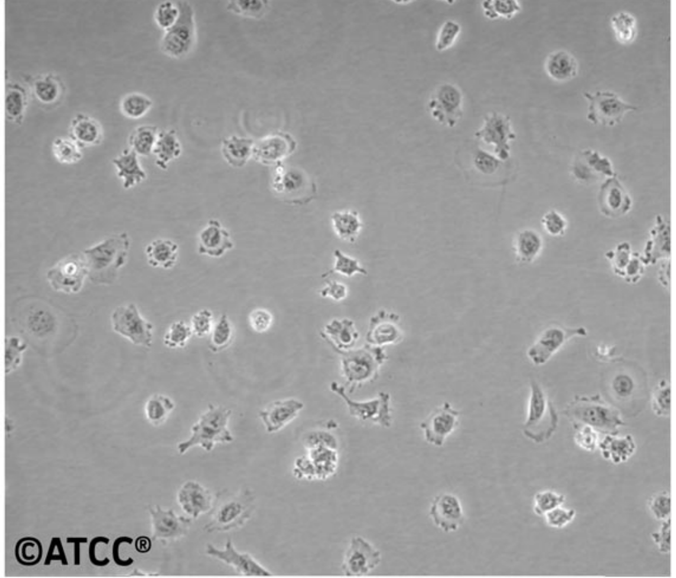
This study generated a cell surface resource and atlas for the NCI-H23 cell line, revealing phenotypical changes at the proteome level specific to different culture types. A novel antibody-free and gel-free proteomic method was established to quantify proteoform- and allele-specific changes in KRasWT and KRasG12C mutant expression within complex protein mixtures using comparative bottom-up proteomics. The resource identifies significantly dysregulated protein species in a culture-dependent manner and highlights proteins unique to 3D- and 2D-cultures, which conventional 2D-culture alone would not reveal. Capturing these culture-dependent changes is essential for establishing a baseline for accurate comparisons, especially when testing drug candidates.

Significant metabolic changes were observed that correlate with culture conditions, and the proteomic resource provides insights into the differences in enrichment of biological functions and protein networks. These findings help elucidate the transformation of NCI-H23 cells in 3D-culture, particularly regarding the activation of malignant tumor stroma. Notably, interactions among CD molecules play a critical role in regulating phenotypical changes under 3D culture. Unique expression of proteins such as CD99, CD146, and CD239 in 3D-cultured cells indicates the development of a tumor microenvironment conducive to angiogenesis and metastasis, likely influenced by increased hypoxia in 3D conditions.

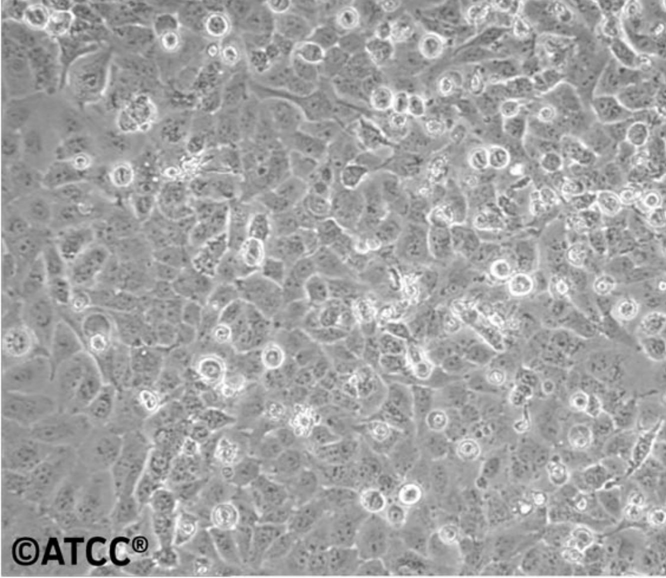
This proteomic resource offers a valuable set of protein targets for future research and emphasizes the advantages of 3D-culture in bridging conventional 2D in vitro cultures and in vivo animal testing models. The proteomic strategy demonstrated here is broadly applicable to other patient-derived cancer cell lines. Given that each patient-derived cell line represents an individual tumor phenotype, comparative proteomic profiling across a wide range of cell lines, such as those in the NCI-60 panel, is essential to address heterogeneity and create a comprehensive molecular atlas for preclinical testing. Furthermore, the identified phenotypical differences between 3D- and 2D-cultured NCI-H23 cells underscore the necessity of characterizing 3D cell models in preclinical studies to mitigate high drug attrition rates. Ultimately, utilizing 3D-cultured cells may serve as a crucial precursor or alternative to traditional animal models.

Images Via ATCC

ATCC Number: **CRL-5800**
Designation: **NCI-H23 [H23]**



Low Density



High Density

Conclusions:

The NCI-H23 cell line is a well-characterized human non-small cell lung cancer (NSCLC) model that originates from a lung adenocarcinoma. This cell line is particularly significant due to its homozygous KRas4BG12C mutation, which is commonly associated with aggressive tumor behavior and poor prognosis in lung cancer patients. The presence of this mutation makes NCI-H23 an ideal candidate for studying the molecular mechanisms underlying KRas-driven oncogenesis and for testing targeted therapies aimed at this pathway. NCI-H23 cells can be cultured in both two-dimensional (2D) and three-dimensional (3D) environments, with the latter providing a more physiologically relevant model that better mimics the in vivo tumor microenvironment.

In 3D culture systems, NCI-H23 cells can form spheroids. Spheroid formation is facilitated by various techniques, including the use of ultra-low attachment plates or specialized scaffolds that promote cell aggregation. The resulting spheroids not only exhibit a more differentiated phenotype but also show altered gene expression profiles compared to their 2D counterparts. This makes them particularly useful for evaluating the efficacy of chemotherapeutic agents and targeted therapies, as well as for studying the tumor microenvironment's influence on cancer progression and treatment resistance. (we are using tissue treated plates)

In addition to spheroid formation, NCI-H23 cells are also employed in studies assessing DNA damage response, particularly through the use of yH2AX staining, done in this article.

2024/10/01 Matrigel Research IX

JAYSON O'HALLORAN - Oct 13, 2024, 1:27 AM CDT

Title: Matrigel Research IX

Date: 10/01/24

Content by: Jayson O'Halloran

Goals: To gain an understanding the process of matrigel in spheroid formation.

Resource: [9] M. Balas, R. Badea, and A. Olaru, "Advances in Multicellular Tumor Spheroids: A Review," , vol. 3, no. 1, pp. 83-92, 2019. https://www.sciencedirect.com/science/article/pii/S247255522206782X (accessed Oct 1, 2024).

Content:

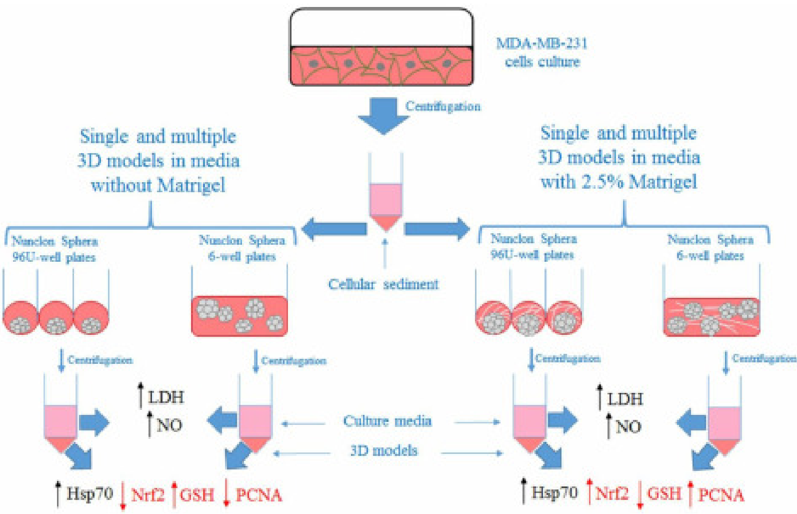
Matrigel is derived from Engelbreth-Holm-Swarm (EHS) mouse sarcoma and contains a complex mixture of extracellular matrix proteins, including laminin, collagen IV, and growth factors, which are helpful for cell adhesion, proliferation, and differentiation. In the context of the article, Matrigel enhances the formation of multicellular tumor spheroids (MCTSs) by providing a supportive environment that mimics in vivo conditions, leading to improved morphology, increased proliferation (as indicated by PCNA expression), and reduced hypoxia.

Pros of Matrigel: 1. Enhanced Cell Growth: Promotes rapid spheroid formation (2-3 days) and supports high viability and proliferation rates. 2. Improved Morphology: Spheroids formed in Matrigel exhibit better circularity and compaction (cell line dependent) 3. Reduced Hypoxia: Matrigel's biological activity helps maintain oxygen levels within spheroids, which is critical for studying tumor biology.

Cons of Matrigel: 1. Variability: The composition can vary between batches, leading to inconsistent experimental results. 2. Animal-Derived: Being derived from mouse tumors raises ethical concerns and may introduce variability in human applications. 3. Influence on Metabolism: Additional components in Matrigel can affect cancer cell metabolism, potentially confounding experimental outcomes. (Cons seem to outweigh the benefits here)

In the study, the authors demonstrated that Matrigel not only facilitates the formation of well-defined 3D models but also highlights the importance of extracellular matrix components in cancer research, suggesting that Matrigel-enhanced spheroids can be representative of actual tumors, but it is often dependent on the cell line chosen and what the ultimate goal of the research is.

Use of Matrigel in the Article:



Matrigel Drop 3D invasion assay

3D Tumorsphere

Leading edge of migrating cancer cells

3D Matrigel drop

Invading cancer cell

24 well plate

Conclusions:

Matrigel is a gelatinous protein mixture used in cell culture to create 3D structures like spheroids. It contains extracellular matrix (ECM) components such as laminin and collagen, which provide a supportive environment that mimics in vivo conditions. When cells are mixed with liquid Matrigel and allowed to solidify, they interact with each other and the ECM, leading to cellular aggregation and proliferation. Over time, this process results in the formation of compact multicellular structures called spheroids. For the purposes of our design project Matrigel doesn't quite display the properties that we want in our cell lines, it also lacks in uniformity for a screen and for staining (DNA damage).

2024/10/03 Hanging Drop Method Research X

JAYSON O'HALLORAN - Oct 13, 2024, 1:38 AM CDT

Title: Hanging Drop Method Research X

Date: 10/03/24

Content by: Jayson O'Halloran

Goals: To gain an understanding of the the Hanging drop spheroid formation.

Resource: [10] R. Foty, “A simple hanging drop cell culture protocol for generation of 3D spheroids,” Journal of visualized experiments : JoVE, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3197119/ (accessed Oct. 3, 2024).

Content:

The hanging drop spheroid formation method is a straightforward technique for generating 3D cellular aggregates that better mimic in vivo conditions compared to traditional monolayer cultures. In this protocol, adherent cells are detached, counted, and resuspended before being placed as drops on an inverted culture dish lid. The lid is then positioned over a hydration chamber to maintain moisture, allowing cells to aggregate and form spheroids through direct cell-cell adhesion. This method requires no specialized equipment, making it accessible and cost-effective, and can accommodate the inclusion of different cell types or biological agents. The resulting spheroids can be used for various applications, including studying cell behavior, interactions, and tissue engineering, with notable findings related to cell sorting based on adhesion properties. Overall, this method provides a physiologically relevant model for analyzing cellular dynamics in a 3D context.

Example Protocol:

Protocol for Hanging Drop Spheroid Formation

Main 1 Preparation of a Single Cell Suspension

1. Grow adherent cell cultures to 90% confluence.

2. Rinse the monolayers twice with PBS.

3. Drain excess PBS and add 2 mL of 0.05% trypsin-1 mM EDTA (for 100 mm plates).

4. Incubate at 37°C until cells detach.

5. Stop trypsinization by adding 2 mL of complete medium and gently triturate with a 5 mL pipette until cells are in suspension.

6. Transfer the cell suspension to a 15 mL conical tube.

7. Add 40 µL of a 10 mg/mL DNAse stock and incubate for 5 minutes at room temperature (RT).

8. Vortex briefly and centrifuge at 200 × g for 5 minutes.

9. Discard the supernatant and wash the pellet with 1 mL of complete tissue culture medium. Repeat this step.

10. Resuspend the cells in 2 mL of complete tissue culture medium.

11. Count the cells using a hemacytometer or an automated cell counter, and adjust the concentration to 2.5×10^6 cells/mL.

Main step 2 Formation of Hanging Drops

1. Remove the lid from a 60 mm tissue culture dish and add 5 mL of PBS to the bottom. This acts as a hydration chamber.

2. Invert the lid and use a 20 μL pipettor to deposit 10 μL drops onto the bottom of the lid, ensuring sufficient spacing between drops (at least 20 drops per dish).

3. Invert the lid onto the PBS-filled bottom chamber and incubate at 37°C with 5% CO_2 and 95% humidity.

4. Monitor the drops daily until cell sheets or aggregates have formed, using a stereo microscope for assessment.

5. Once sheets form, transfer them to round-bottom glass shaker flasks containing 3 mL of complete medium.

6. Incubate in a shaking water bath at 37°C and 5% CO_2 until spheroids form.

Side Notes

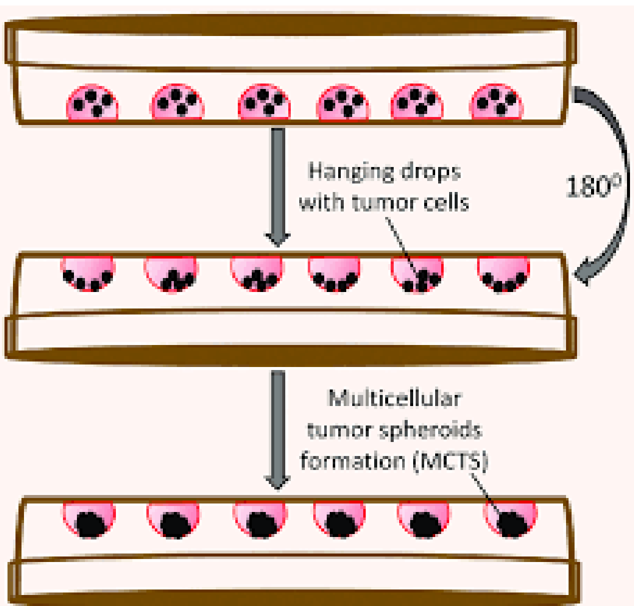
- Adjust the cell concentration based on cell size if necessary.

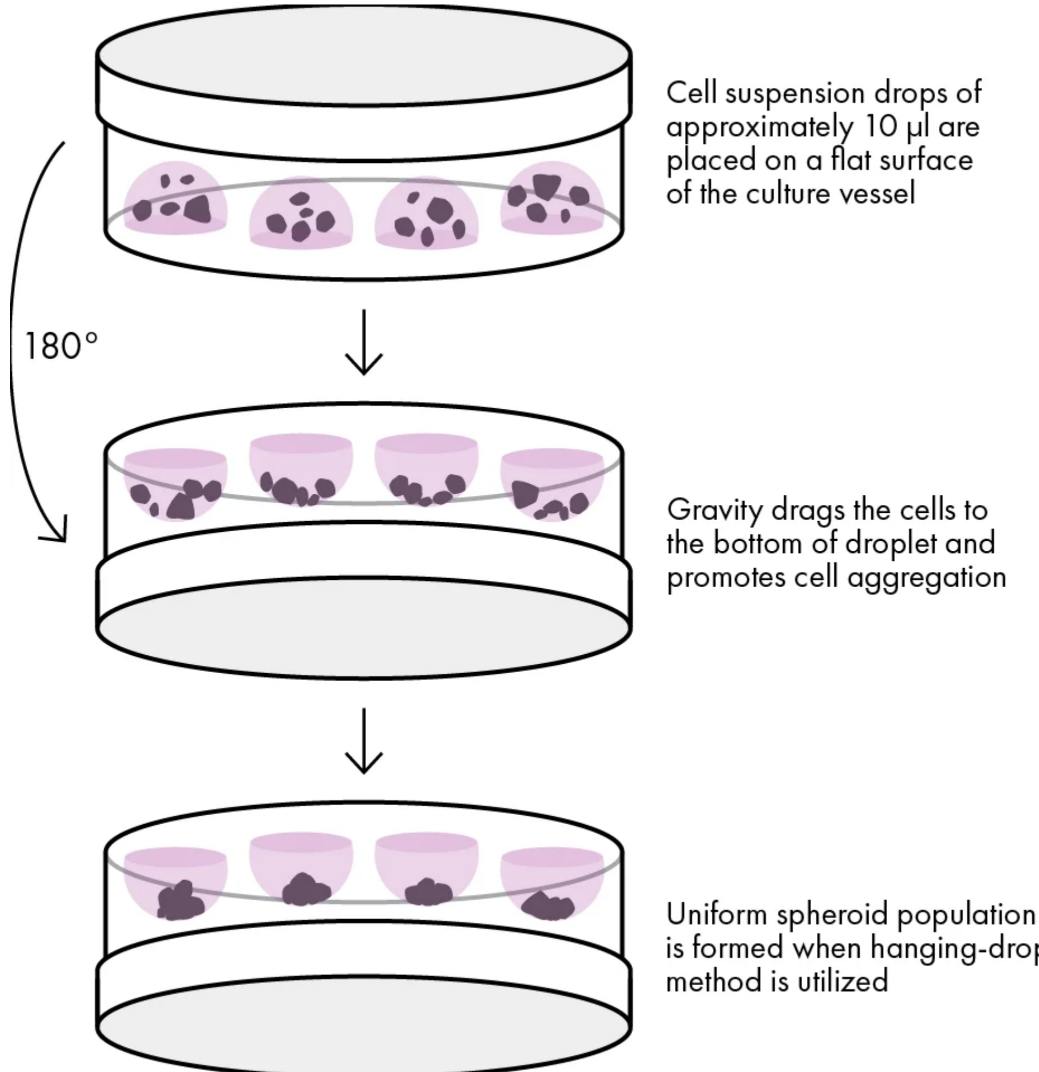
- Cells can be stained with membrane-intercalating fluorescent dyes prior to hanging drop formation.

- Two different cell types can be differentially stained and mixed in a 1:1 ratio before forming hanging drops (e.g., cancer and stromal cells).

- Cells can be detached using 0.05% trypsin/2 mM calcium to preserve cadherin function.

- Depending on the experiment, sheet sizes can be measured, or aggregates can be used for mechanical testing or biochemical/molecular analysis.





Conclusions:

In conclusion, the hanging drop method for spheroid formation is a valuable technique that provides a simple, cost-effective way to generate 3D cellular aggregates, closely mimicking in vivo conditions. Its advantages include ease of use, the ability to co-culture different cell types, and minimal equipment requirements. However, it is important to note that this method may present challenges for certain cell lines that do not aggregate well or require specific conditions for optimal growth. Variability in cell behavior can lead to inconsistent spheroid formation and may affect experimental outcomes. In our case the Hanging Drop Method could be used but it is important to also consider other options. It is definitely better than Matrigel at forming the spheroids we want.

2024/10/07 Treated Tissue Culture Plates Research XI

JAYSON O'HALLORAN - Oct 13, 2024, 1:48 AM CDT

Title: Treated Tissue Cultured Plates Research XI

Date: 10/07/24

Content by: Jayson O'Halloran

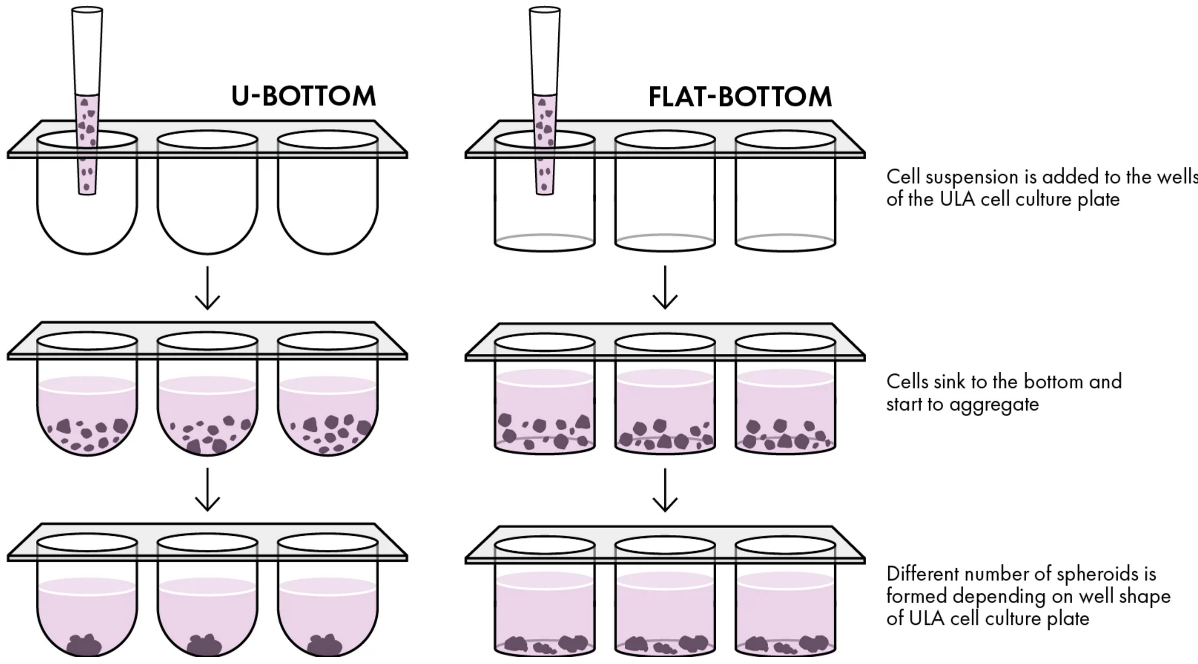
Goals: To gain an understanding of how Treated Tissue Cultured Plates can form spheroids.

Resource: [11] “3D cell culture models: Learning center,” 3D Cell Culture Models | Learning Center | UPM Biomedicals, https://www.upmbiomedicals.com/resource-center/learning-center/what-is-3d-cell-culture/3d-cell-culture-models/?gad_source=1&gclid=CjwKCAjwvKi4BhABEiwAH2gcw50g29l2ky8QDWPZUOwSUjHtM5q3kLRI9cX_0t_MsCUBc5R_Y73ksBoCZz4QAvD_BwE (accessed Oct. 7, 2024).

Content:

Ultra-low attachment (ULA) plates were created to produce large scale scaffold-free 3D-cell cultures. These plates are made using liquid overlay techniques where the bottom of a cell culture dish is coated with a non-adhesive material, which prevents cell adhesion and protein absorption. Commonly, ULA-plates are produced by covalently binding a hydrophilic and biologically inert material on the surface of a plate.

When the cell suspension is added to a well of an ULA cell culture plate, cells sink to the bottom of the well, but do not attach to the culture surface which facilitates their aggregation and spheroid formation. There are various well bottom shapes of ULA cell culture plates available, such as U bottom, Flatbottom, Spindle bottom and V-bottom.



U-bottom ULA plates have been created to form and analyse homogenous spheroid populations. Like the hanging drop technique, the U-shape uses gravity to pull down the cells to the bottom of the well. The round geometry will force the cells to aggregate and form a 3D cell complex. The size and shape of cell complex can be easily adjusted by calculating the wanted cell density in the well.

Compared to U-bottom plates, Flat-bottom ULA plates form heterogenous spheroid populations. In each well, cells can freely move on the flat surface and randomly form a cell aggregate. Between plate wells, there may occur varying number of spheroids with different sizes.

ULA Plates are compatible with liquid robotic systems which increases their usage in HTS applications. Downstream analyses and visualization can be done in the same plate as cell culturing. However, in ULA-plate the spheroids float in suspension and are not physically in a fixed position. In live cell imaging, this can cause difficulties to obtain and maintain optical focus when small movements can get spheroids on the

move. Together with this, biological relevance of ULA cell culture plates should be considered because this method lacks tissue-like stiffness and moreover cell-matrix interaction.

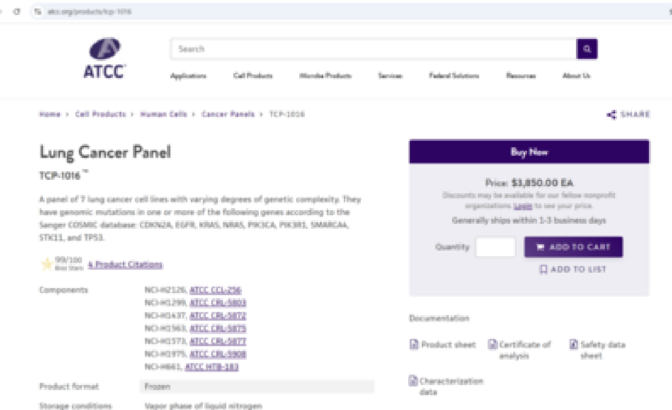
Conclusions:

Ultra-Low Attachment (ULA) plates are particularly beneficial for spheroid formation of lung cancer cell lines like A549 and NCI-H23 due to their unique design that facilitates 3D aggregation. The non-adhesive surface prevents cells from attaching, allowing them to sink and aggregate at the bottom of the wells, which promotes the formation of homogenous spheroid populations. The U-bottom configuration effectively uses gravity to encourage cell clustering, creating a consistent environment for spheroid development, which is crucial for studying tumor behavior and drug responses.

Moreover, ULA plates offer compatibility with high-throughput screening (HTS) applications and enable downstream analyses directly in the same culture environment. However, it is essential to recognize potential challenges, such as maintaining optical focus during live cell imaging, as spheroids float and may shift positions. Out of all the options considered for spheroid formations, the ULA plates method is slightly better than the hanging drop method, and much better than matrigel for our specific cell lines.

ATCC Lung Cancer Cell Line

JAYSON O'HALLORAN - Sep 20, 2024, 11:41 AM CDT

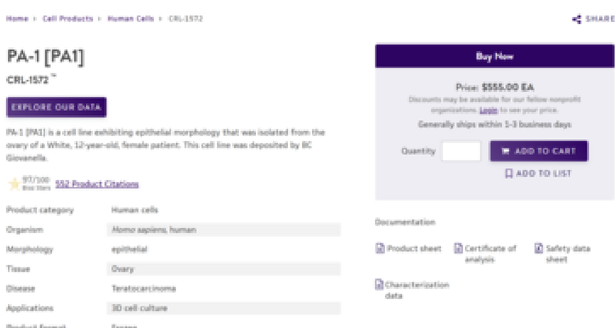


Download

Screenshot_2_.png (256 kB)

Ovarian cancer cell line I PA-1

JAYSON O'HALLORAN - Nov 15, 2024, 5:42 PM CST

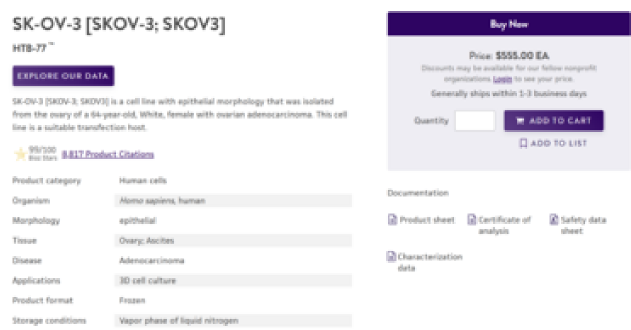


Download

Screenshot_9_.png (180 kB)

Ovarian cancer cell line II SK-OV-3

JAYSON O'HALLORAN - Nov 15, 2024, 5:45 PM CST

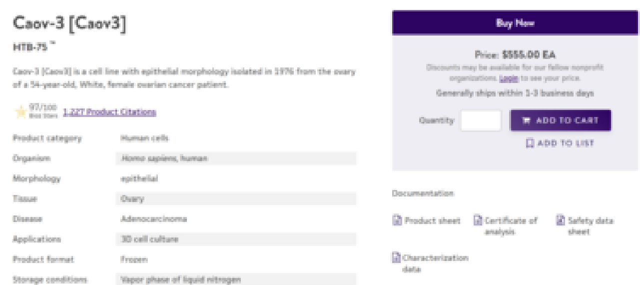


Download

Screenshot_136_.png (198 kB)

Ovarian cancer cell line III Caov-3

JAYSON O'HALLORAN - Nov 15, 2024, 5:48 PM CST



Download

Screenshot_139_.png (162 kB)

Colon cancer cell line I CACO-2

JAYSON O'HALLORAN - Nov 15, 2024, 5:50 PM CST

PROPERTIES

product name	CACO-2 Cell Line human, from human colon(Caucasian colon adenocarcinoma), 86010202
biological source	human colon (Caucasian colon adenocarcinoma)
growth mode	Adherent
karyotype	Hypertetraploid, modal no. 96
morphology	Epithelial
products	Not specified
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	cancer
shipped in	dry ice

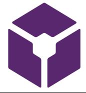
Show More

Related Categories

Mammalian Cell Lines

Download

Screenshot_140_.png (74.5 kB)



Colon cancer cell line II COLO 205

JAYSON O'HALLORAN - Nov 15, 2024, 5:54 PM CST

PROPERTIES

product name	COLO 205 Cell Line human, 87061208
biological source	human colon
description	Human Caucasian colon adenocarcinoma
growth mode	Suspension
karyotype	Hypertriploid modal no 71-78
morphology	Round and refractile cells in suspension. Some cuboidal cells in the monolayer
products	Carcinoembryonic antigen (CEA)
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	cancer

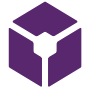
Show More

Related Categories

Mammalian Cell Lines

Download

Screenshot_141_.png (78 kB)



Colon cancer cell line III COLO 741

JAYSON O'HALLORAN - Nov 15, 2024, 6:05 PM CST

PROPERTIES

product name	COLO 741, 93052621
biological source	human colon (pelvic wall metastasis)
growth mode	Adherent
karyotype	Not specified
morphology	Fibroblast-like
products	Not specified
receptors	Not specified
technique(s)	cell culture | mammalian: suitable
relevant disease(s)	metastasis
shipped in	dry ice

Show More

Related Categories

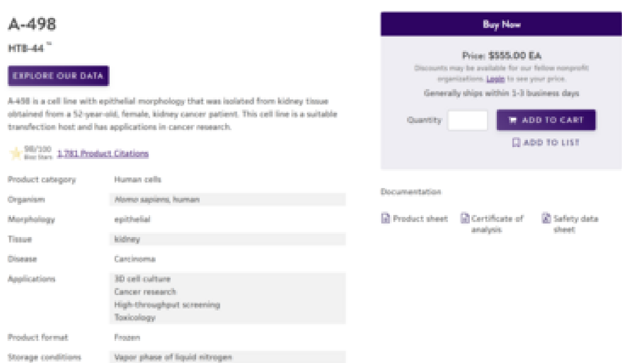
Mammalian Cell Lines

Download

Screenshot_142_.png (66.2 kB)

Kidney cancer cell line A-498

JAYSON O'HALLORAN - Nov 15, 2024, 6:07 PM CST

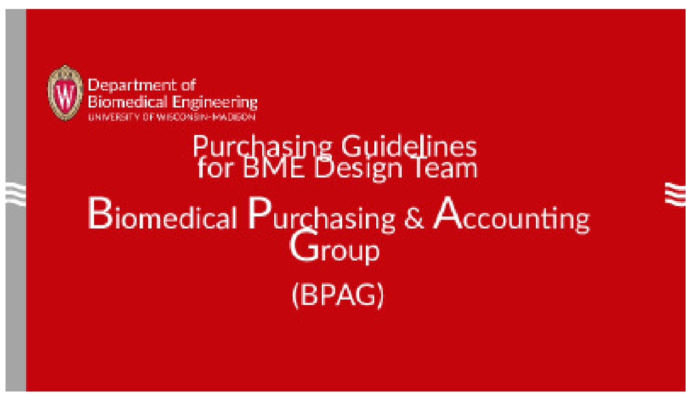


Download

Screenshot_143_.png (192 kB)

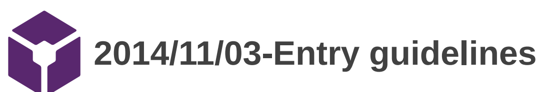
9/27/2024 BPAG Slides

JAYSON O'HALLORAN - Sep 27, 2024, 12:11 PM CDT



Download

BPAG__Purchasing_Guidelines_for_BME_Design_Teams.pptx (569 kB)



2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

Althys Cao - Sep 23, 2024, 10:26 AM CDT

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: