CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 8

Reporting Period: March 14, 2025 - March 20, 2025

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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:

- 2D cell passaging
- Spheroid passaging
- Third and fourth trial of spheroid dissociation & determined ways to improve current protocol
    - Evaluated spheroid doubling time/ confluency
- We will seeded spheroids for next trial of determining doubling time and to prep for qPCR after spring break

Difficulties / advice requests: Optimization of accutase spheroid dissociation in order to establish spheroid doubling time.

Current design: Cells seeded in 24 well plate at 75k cells/cm^2 with 0.75% methylcellulose in full DMEM (10% FBS, 1% p/s).

Identity	Events	Cells/mL	Cells/ well	Starting amount of cells	Confluency (Cell#final/Cell #initial)
Full	253	28111.1111111111	67466.6666666666	142500	0.4734502924
Full-1 -tube 8	671	74555.5555555555	178933.3333333333	142500	1.2556725146
Full-2 -tube 9	1189	132111.1111111111	317066.6666666666	142500	2.2250292398
Full-3 -tube 10	826	91777.7777777778	220266.6666666667	142500	1.5457309942
Full-4 -tube 11	822	91333.3333333333	219200	142500	1.538245614
Full-5 -tube 12	854	94888.8888888889	227733.3333333333	142500	1.598128655
Full-6 -tube 13	899	99888.8888888889	239733.3333333333	142500	1.6823391813
Serum free	253	28111.1111111111	67466.6666666666	142500	0.4734502924
Serum free 1	127	14111.1111111111	33866.6666666666	142500	0.2376608187
Serum free 2	163	18111.1111111111	43466.6666666666	142500	0.3050292398
Serum free 3-tube 5	204	22666.6666666667	54400.0000000001	142500	0.381754386
Serum free 4-tube 6	280	31111.1111111111	74666.6666666666	142500	0.5239766082
Serum free 5-tube 7	315	35000	84000	142500	0.5894736842

Figure 1. Day 5 Spheroid Dissociation_Confluency Tracker_3/17/25

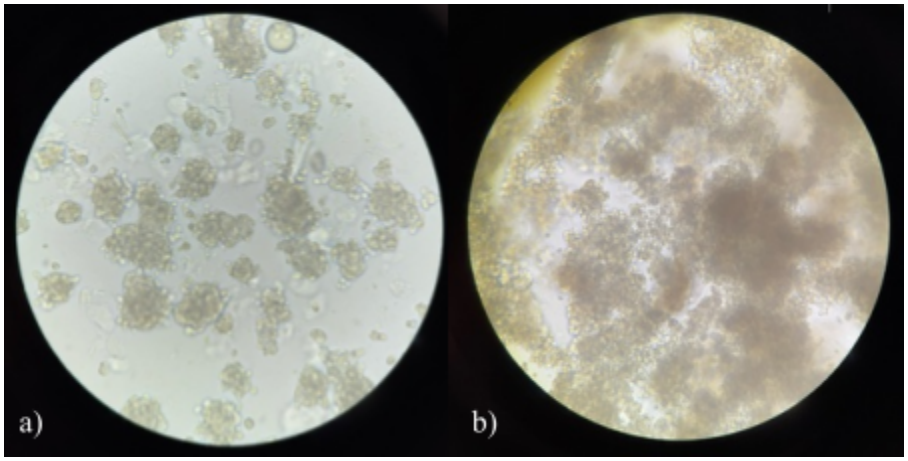

Figure 2: Side-by-side comparison of media conditions a) Full DMEM 20x brightfield b) SFM 20x brightfield.

Materials and expenses:

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. A549 Cell Line
6. Poly-HEMA and Methylcellulose Sigma Aldrich Total: $289.40

Major team goals for the next week:

1. Spring break: 3/22 - 3/29
2. Prepare spheroids for qPCR
3. Meet with client and advisor to plan out the next month of experiments

Next week's individual goals:

- Althys Cao
    - Prepare for qPCR: seed more spheroids and establish timeline
    - Start on planning gammaH2AX staining
- Ana Martinez
    - Establish schedule and prepare for qPCR after spring break
    - Client, advisor, team meeting
        - Discuss spheroid dissociation updates
    - LabArchives update
- Emily Rhine
    - Establish plan for qPCR spheroids using 24 well plate
    - Client, advisor and team meeting
    - Finalize spheroid dissociation protocol
- Julia Salita
    - Update lab archives
    - Prepare for qPCR
    - Discuss results from spheroid dissociation with clients
    - Attend show and tell, as well as team, client, and advisor meetings.
- Jayson O'Halloran

- Prepare for qPCR after spring break
- Show client results from spheroid dissociation
- Work on yH2AX staining timeline
- Keep updating lab archives

Table 1. Project Timeline.

Week #	Task
1	Choose project Assign roles
2	Finish first progress report BSAC meeting First client meeting
3	PDS, Brainstorm, Research
4	Brainstorm, Literature Search, Design matrix criteria and design ideas (at least three) due
5	Preliminary Oral Presentation
6	Preliminary Report, Electronic Notebook, Peer/Self Evaluation, Decide on final design
7	Final Design
8	Order materials, consider submitting invention disclosure
9	Fabrication, show and tell
10	Fabrication
11	Fabrication
12	Design Testing and Modification, Poster Draft Review
13	Design Testing and Modification, Final Report
14	Poster Presentation, Final Report, Final Electronic Notebook, Team Evaluation, Peer/Self Evaluation

Previous week's goals and accomplishments:

- Team
  -
- Althys Cao

    - Support with 3rd trial of spheroid dissociation/passaging protocol to determine doubling time
    - Keep updating timeline for qPCR
- Ana Martinez
    - Helped team with 3rd trial of spheroid dissociation/passaging protocol to determine spheroid doubling time
    - Continued passaging 2D WT A549s
    - Met with team, advisor, and client to discuss updates
- Emily Rhine
    - Optimized spheroid dissociation protocol to limit cell loss and cell death
    - Met with team, advisor, and client to discuss past experiments and plan future experiments
- Julia Salita
    - Completed passage 7 for both flasks
    - Assisted in running spheroid dissociation through the Cytoflex
    - Met with team
- Jayson O'Halloran
    - Spheroid dissociation
    - Continued passaging A549 2D culture
    - Updated Lab Archives

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	3/13 3/14 3/14 3/18 3/19	Coat 4 96-well plates with polyHEMA Client meeting Assist with spheroid seeding Team meeting & Modify accutase dissociation protocol for 96-well plate Spheroid dissociation from 96-well plate Reseed spheroids into 24-well plate 2D Passaging	1 0.5 0.5 1 4	7	53.75
Ana Martinez	3/14 3/14 3/14 3/14 3/18 3/19	Advisor meeting Client meeting Flask passaging Spheroid seeding Team meeting Spheroid dissociation, passaging	0.5 0.5 0.5 1 1 2.5	6	45

Emily Rhine	3/14 3/14 3/14 3/14 3/17 3/18 3/14-3/20	BSAC Client meeting Flask passaging Spheroid seeding Spheroid dissociation Team meeting LabArchives/Benchling entries	1 0.5 0.5 0.5 3 1 1.5	8	53.5
Julia Salita	3/17 3/18	Passaging and spheroid dissociation help Team meeting	1.5 1	2.5	45
Jayson O'Halloran	3/14 3/16 3/17 3/18	Spheroid passaging, 2D passaging, client meeting Lab archives qPCR protocol review Team meeting	3 1 1 1	6	43