

CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 12

Reporting Period: April 18, 2025 - April 24, 2025

Client:	Carley Schwartz Dr. Gaelen Hess	cischwartz@wisc.edu ghess3@wisc.edu
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Team:	Althys Cao (Leader) Ana Martinez (Communicator) Emily Rhine (BSAC) Julia Salita (BWIG) Jayson O'Halloran (BPAG)	nvcao@wisc.edu almartinez4@wisc.edu erhine@wisc.edu jsalita@wisc.edu 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 γ H2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- 2D cell passaging
 - Bleached both flasks
- RT-qPCR step 3 and data analysis
 - Ana
 - Althys
 - Julia
- γ H2AX staining: Trial 2 and data analysis
 - Emily
 - Jayson

Difficulties / advice requests: N/A

Current design: Cells seeded in 6 well plate at 75k cells/cm² with 0.75% methylcellulose in full DMEM (10% FBS, 1% p/s).

Materials and expenses:

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

Major team goals for the next week:

1. Present final presentation
2. Continue working on final report

Next week's individual goals:

- Althys Cao
 - Present final poster
 - Finish final report
 - Update LabArchives
 - Evaluations and reflections
- Ana Martinez
 - Present final presentation with team
 - Continue working on final report sections
 - Update LabArchives as needed
 - Team, peer, and self evaluations/reflection
- Emily Rhine
 - Present final poster
 - Finish final report
 - Update LabArchives
 - Complete evaluations and reflections
- Julia Salita
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- Jayson O'Halloran
 - Finish final deliverables

- Wrap up project
- Final meetings with team, client, and advisor

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
 - Step 3 of RT-qPCR redo
 - Trial 2 of gamma-H2AX staining
 - RT-qPCR, gamma-H2AX results graphing, data analysis
 - Finish poster, work on final report

- Althys Cao
 - qPCR data analysis and graphing
 - Work on section of final poster and final report
- Ana Martinez
 - Prepared for and performed Step 3 of RT-qPCR redo
 - Graphed/analyzed data for RT-qPCR
 - Finished my sections of poster
 - Met with team, client, and advisor
- Emily Rhine
 - Attend client, advisor, and team meetings
 - Work on final report and final poster presentation
 - Completed yH2AX stain trial 2
- Julia Salita
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- Jayson O'Halloran
 - Client, Advisor, and Team meetings
 - Work on final deliverables
 - yH2AX stain 2

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	4/18 4/21 4/22-23 4/22	Client meeting RT-qPCR Step 3 (redo) & analysis Poster Advisor meeting	0.5 4 5 0.5	10	87
Ana Martinez	4/18 4/21 4/22 4/19-4/23 4/22	Client meeting RT-qPCR Step 3 (redo) & analysis, getting data Advisor, team meeting Poster	0.5 3.5 1 4	9	78.25
Emily Rhine	4/18 4/18 4/18 4/19 4/21 4/22 4/21-4/23	Client meeting Work on final report Add Etoposide and seed 2D yH2AX stain trial 2 yH2AX Data Analysis Advisor & team meeting Poster	0.5 1 1.5 7 2 1 4	17	102
Julia Salita					57

Jayson O'Halloran	4/18	Client meeting	0.5	14	86
	4/18	Etoposide adding	1.5		
	4/19	yH2AX stain	7		
	4/20	Lab archives uploads	1		
	4/22	Advisor & Team meeting	1		
	4/20-4/23	Poster	3		