

## CRISPRi Screening in Cancer Spheroids - BME 402

### *Progress Report 4*

**Reporting Period:** February 14, 2025 - February 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  $\gamma$ H2AX: a histone variant that is a sensitive marker for DNA damage.

#### **Brief status update:**

- Completed spheroid experiment 2
- Analyzed luminescence data from experiment 1
- Passage 9-11

**Difficulties / advice requests:** N/A

**Current design:** N/A

**Materials and expenses:** N/A

#### **Major team goals for the next week:**

1. Continue passaging A549 WT vial 3 cells
2. Analyze experiment 2 data and compare to experiment 1 data

3. Meeting with client to discuss qPCR workflow and order SOX2 primer

**Next week's individual goals:**

- Althys Cao
  - Edit timeline for rest of semester
  - Complete preliminary report
  - Catch up on work, continue research on qPCR and gammaH2AX staining
  - Continue passaging, make polyHEMA plates and edit spheroid formation protocol as needed
- Ana Martinez
  - Complete my section of preliminary report and help team with edits
  - Continue passaging
  - Meet with client, advisor, and team
- Emily Rhine
  - Complete preliminary report
  - Continue research, note taking, and passaging cells
  - Meet with client and advisor
- Julia Salita
  - Complete preliminary report
  - Complete data analysis on collected data
  - Continue passaging
  - Meet with client, advisor, and team
- Jayson O'Halloran
  - Complete preliminary report
  - Order SOX2 primer
  - Edit timeline for rest of semester and talk about future goals with client

**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	<b>Final Design</b>
8	<b>Order materials, consider submitting invention disclosure</b>
9	<b>Fabrication, show and tell</b>
10	<b>Fabrication</b>
11	<b>Fabrication</b>
12	<b>Design Testing and Modification, Poster Draft Review</b>
13	<b>Design Testing and Modification, Final Report</b>
14	<b>Poster Presentation, Final Report, Final Electronic Notebook, Team Evaluation, Peer/Self Evaluation</b>

**Previous week's goals and accomplishments:**

- Team
  - Completed spheroid experiment 1 and cell titer glo assay. Met with client and advisor for project update.
- Althys Cao
  - Did calculations and seeded cells for positive control for CellTiter Glo assay
  - Imaged spheroids (tested a few plate options but none produced good pictures)
- Ana Martinez
  - Continued qPCR primer search
  - Seeded cells for spheroid formation for viability assay repeat
  - Imaged spheroids for viability assay repeat
  - Met with team to assign preliminary report sections
- Emily Rhine
  - Continued research, LabArchives updates, Benchling Updates, Passaging A549s, and Imaged spheroids from exp 2. Met with client and advisor for project check ins. Helped perform cell titer glo assay.
- Julia Salita
  - Met with client
  - Passaged
  - Imaged spheroids
  - Performed cell titer glo assay
- Jayson O'Halloran
  - Continue to passage A549 cells
  - Research qPCR primers for SOX2
  - Start preliminary report
  - Continue to make spheroids

**Table 2. Activities**

<b>Name</b>	<b>Date</b>	<b>Activity</b>	<b>Time (h)</b>	<b>Week Total (h)</b>	<b>Sem. Total (h)</b>
Althys Cao	2/16	- Calculations for cell seeding for CellTiter Glo	0.5	2.5	14.75
	2/17	- Imaging trial 2 spheroids - Seed cells for CellTiter Glo	2		
Ana Martinez	2/14	- Spheroid formation for experiment trial 2	1.5	5	18.5
	2/14	- Client/advisor meeting	1		
	2/17	- Imaging trial 2 spheroids	1.5		
	2/18	- qPCR primer search/team meeting	1		
Emily Rhine	2/14	-Passage 9	-0.5	5.5	24.5
	2/14	-Exp 2	-1		
	2/14	-Client and Advisor meeting	-1		
	2/16	-Lab Archives Update	-1.5		
	2/17	-Passage 10 & Imaging	-2		
	2/18	-Team Meeting	-0.5		
Julia Salita	2/14	-Advisor meeting	0.5	5	19
	2/14	-Client meeting	0.5		
	2/17	-Spheroid imaging	2		
	2/19	-Passage 11 & cell titer glo	2		
Jayson O'Halloran	2/14	-BPAG meeting	0.5	6	22
	2/14	-Client meeting, passaging	2		
	2/16	-mCherry and CRISPRi	1		
	2/18	research	0.5		
	2/19	- Team meeting - Cell titer glo	2		