CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 7

Reporting Period: October 18, 2024 - October 23, 2024

Client:	Carley Schwartz	cischwartz@wisc.edu
	Dr. Gaelen Hess	ghess3@wisc.edu
Advisor:	Paul Campagnola	pcampagnola@wisc.edu
Team:	Althys Cao (Leader)	nvcao@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.

Current design:

Cell Line

A549 was the cell line selected based on the team's decision matrix. Reasons for this selection were as follows:

- A549 is an adherent cell line which exhibits particularly useful mutations like EGFR, TP53, PIK3CA, ALK, and PTEN.
- A549 has a doubling time of approximately 22 hours. This means that the cell line will grow rapidly enough for the team to work with, and thus the team will need to passage the cells 2-3 times a week. Moreover, because the Hess lab recommends a maximum of 20 passages to be done on each cell line, cells will be able to be in culture for approximately 7 weeks before they are bleached and re-thawed.
- A549 is very sensitive to bleomycin (Z-score average of -1.04), somewhat sensitive to cisplatin (-0.12), and very sensitive to oxaliplatin (-1.45).

Spheroid Formation

The treated tissue culture plates method was selected based on the team's decision matrix. Reasons for this selection as follows:

- The treated tissue culture plates method involves using hydrophilic polymer-coated plates, which prevent cell adhesion to wells and cause cells to self-assemble into spheroids. This method of plate-coating is not particularly labor-intensive and the method in general is not time-consuming because spheroids tend to form after up to 4 days.
- The treated tissue culture plates method is a scaffold-free method, meaning it will not require animal-derived materials that would otherwise induce batch-to-batch variability in spheroid shape and size.

Materials and expenses: N/A for week 8

Major team goals for the next week:

1. Continue passaging cells
    a. Find average doubling time
2. Establish a assaging plan for the rest of the semester
3. Meet to determine a good spheroid formation protocol and ask Carley to order materials
4. Take photos of cells and tissue culture work for show and tell.

Next week's individual goals:

- Althys Cao
    - Additional spheroid formation protocol research (within the scheme of using low attachment plates) and finalize the protocol so Carley can order needed materials. Also look into how to make PolyHEMA plates
- Ana Martinez
    - Share spheroid formation protocol research with the team. Begin putting together cell images for show and tell. Continue passaging cells. Look into ordering additional materials for spheroid formation.
- Emily Rhine
    - Finish additional spheroid formation protocol research and share with the team. Take more photos of A549s for show and tell. Create progress report template. Continue passaging cells. Catch advisor up on 2 weeks of progress.
- Julia Salita
    - Continue cell passaging
    - Finalize a spheroid formation protocol
    - Meet with the team to proceed with the spheroid protocol
- Jayson O'Halloran
    - Finalize a spheroid formation protocol and review options from literature with team and client
    - Put together a portfolio of images from our design project
    - Material ordering as needed

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
    - Reviewed preliminary report with client and advisor
    - Continued to establish A549 cell doubling time
    - Established project timeline
- Althys Cao
    - Established passaging timeline, continued passaging cells and compare doubling time with literature values, asked client if imaging confluent A549 cells is possible for pictures for final poster presentation.
- Ana Martinez
    - Continued passaging cells to characterize cell line's approximate doubling time. Updated clients and advisor on progress and received feedback on preliminary report. Took additional images of cell culture for show and tell.
- Emily Rhine
    - Updated client and advisor on progress and received feedback. Took notes at team, advisor, and client meetings. Created progress report template. Brainstormed ideas for show & tell. Took photos of A549s for show and tell.
- Julia Salita
    - I had hoped to accomplish a few tasks this week however, due to a family emergency I was unable to complete work on this project this past week.
- Jayson O'Halloran
    - Established a passaging timeline

- Final poster presentation considerations
- Helped establish a reasonable A549 cell line doubling time

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	10/18 10/23 10/23-24	- Advisor/client meeting - Passage 5 - Spheroid formation protocol research	2 1.5 1.5	5	46.5
Ana Martinez	10/18 10/23 10/23-10/24	- Advisor/client meetings - Passage 5 - Spheroid formation protocol research	2 1.5 1.5	5	43.5
Emily Rhine	10/18 10/21 10/23	-Passage 3 -Passage 4 -Spheroid protocol research	1.5 1.5 2	5	39
Julia Salita	N/A	N/A	0	0	31.75
Jayson O'Halloran	10/18 10/21 10/25	-Advisor/Client meeting -Spheroid protocol research - Passage 6 & Client Questions	2 2 2	6	40