CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 9

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

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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:

- 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:

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 (poly-HEMA)-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 9

Major team goals for the next week:

1. Receive materials
    a. Prepare PolyHEMA plates
2. Draft spheroid formation protocols
    a. Vary [A549] across 3 spheroid formation attempts
3. Thaw new vial of A549s
4. Begin final deliverables
    a. Final report
    b. Poster

Next week's individual goals:

- Althys Cao
    - Continue cell maintenance. Prepare PolyHEMA plates. Check on different techniques and how to analyze results for qPCR.
- Ana Martinez
    - Thaw new cell vial for spheroid size experiment. Finalize accutase amount requirement for spheroid dissociation for viability study. Prepare Poly-HEMA plates for spheroids.
- Emily Rhine
    - Prepare PolyHEMA TC plates. Continue cell maintenance. Thaw new cells for spheroid experiments. Begin work on final report. Create progress report. Take notes on client, team, and advisor meetings
- Julia Salita
    - Prepare the TC plates as per the chosen protocol.
    - Continue cell maintenance.
    - Thaw new vial of cells for spheroid experiments.
    - Begin work on final report
- Jayson O'Halloran
    - Finish ordering new materials
    - Continue to passage cell line
    - Begin to create spheroids once all materials are in

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
    - Continued to establish A549 cell doubling time via passaging MWF
    - Established project timeline and client-specific goals for current semester
    - Narrowed down spheroid formation protocols to help order materials
- Althys Cao
    - Presented at show and tell and received/gave feedback. Continued maintenance of cells. Met with client to clarify team expectations and next-semester goals.
- Ana Martinez
    - Presented at show and tell and received/gave feedback. Passaged cells to establish doubling time. Met with client to clarify team expectations and next-semester goals regarding gamma-H2AX staining.
- Emily Rhine
    - Presented at show and tell and received feedback. Continued passaging cell line to help establish a consistent doubling time. Met with client to better understand team goal expectations and project motivation.
- Julia Salita
    - Passaged cells
    - Met with team about materials
    - Met with advisor to discuss project status

- Jayson O'Halloran

- Passaged cells
- Met with client to go over semester goals and answer any lingering questions

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	11/1 11/4 11/5 11/6	Show and tell presentation Team meeting to finalize materials list and email client Advisor meeting Passage 11	2 3 0.5 1	6.5	56.5
Ana Martinez	11/1 11/4 11/5 11/6	Show and tell presentation Team meeting, finalize materials list/email client Advisor meeting Passage 11	2 2.5 0.5 1.5	6.5	55.5
Emily Rhine	11/1 11/1 11/1 11/2 11/2 11/4 11/5	-Show and tell -Client Meeting -Passage 9 -Materials list -Spheroid Formation matrix -Team meeting -Advisor Meeting	-2 -0.5 -1 -0.5 -2 -1 -0.5	7.5	53
Julia Salita	11/1 11/4 11/4 11/5	-Show and tell -Passage 10 -Team meeting -Advisor Meeting	2 1 2 0.5	5.5	42.75
Jayson O'Halloran	11/4 11/5 11/5 11/6 11/7	-Team meeting -Advisor meeting -Materials sheet - Spheroid optimization and media research -Notebook Update	1 0.5 0.5 3 2	7	50