CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 8

Reporting Period: October 23, 2024 - October 31, 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:

- Team finished passaging 7 and 8.
- Team compiled a list of protocols to make low-attachment plates and form spheroids to send to the clients.

Difficulties / advice requests:

- A549 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. We are currently fine-tuning the volume of cells we passage to find a more consistent doubling time.

- The team would appreciate feedback from client on our suggestions for poly-HEMA plate preparation and spheroid formation protocols (from the literature) to start working with.

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 8

Major team goals for the next week:

1. Continue passaging cells

    a. Find average doubling time
2. Establish a timeline and list of expectations for the rest of the semester
3. Determine a good preliminary spheroid formation protocol
4. Ask Carley to order materials

Next week's individual goals:

- Althys Cao
    - Present show and tell slides and gather advices given by other groups. Hopefully hear back from the clients about their opinions on the list of protocols and finalize protocols and materials. Continue passaging cells to finalize doubling time and amount of remaining cells for next passaging to have a consistent supply of cells.
- Ana Martinez
    - Present show and tell slides and gather/discuss advice given to us by other groups with the team, client, and advisor. Finalize determination of our starting spheroid formation protocol and order necessary materials. Continue passaging cells to establish our cell line's doubling time.
- Emily Rhine
    - Present show and tell slides. Create a progress report template. Continue passaging cells.
- Julia Salita
    - Continue to passage cells
    - Present show and tell slides and gather feedback
    - Determine which spheroid formation protocol to use and order necessary materials.
- Jayson O'Halloran
    - Present show and tell slides and gather feedback from peers, client, and advisor.
    - Continue to passage cells.
    - Work on materials sheet.

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
- 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
    - Shared spheroid formation protocol research with the team. Captured additional cell culture images for show and tell presentation. Continued passaging cells to establish a doubling time. Met with client to clarify specific goals (primarily the γ-H2AX staining process).
- Emily Rhine

    - 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 twice
    - Met with team about spheroid formation protocols
    - Met with client to review goals and clarify γ-H2AX staining process
- 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	10/25 10/28-29 10/30	- Client meeting - Plate & Spheroid Formation Protocols List - Passage 8	1 1 1.5	3.5	50
Ana Martinez	10/25 10/30	- Client meeting - Passage 8 - Spheroid Formation Protocol Document - Show and Tell Presentation	1 1.5 2 1	5.5	49
Emily Rhine	10/25 10/25 10/28-10/31 10/28	-Client Meeting -BSAC Meeting -Show and Tell Slides and Pitch -Spheroid Formation Protocol Comparison	-1 -1 -3.5 -1	6.5	45.5
Julia Salita	10/25, 28 10/24 10/25 10/31	Cell Passaging Team meeting Client meeting Updated website	3 1 1 0.5	5.5	37.25
Jayson O'Halloran	10/25 10/25	Cell passaging Client meeting	2 1	3	43