CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 5

Reporting Period: October 4, 2024 - October 10, 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 presented preliminary presentation
- Team started establishing times to go into labs to start working on cell cultures

Difficulties / advice requests: The team does not have difficulties for week 6. However, we would appreciate feedback from our client advisor on our preliminary report (will be sent by end of week) so we can implement appropriate changes that will inform our work with cell culture, spheroid formation, etc. in the coming weeks. Additionally, the team would appreciate feedback regarding what type of treated tissue culture plates (ultra-low attachment vs. polyHEMA-coated) are preferred or more readily available at the Hess Lab.

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 6

Major team goals for the next week:

1. Continue passaging cells
2. Discuss preliminary report with client and advisor
3. Establish a timeline for the rest of the semester
4. Begin planning for show and tell Friday, November 1st

Next week's individual goals:

- Althys Cao
    - Meet with team and clients at Hess Lab to start cell culture

    - Meet with team to divide lab work
    - Discuss preliminary report with client
    - Finalize protocols if possible - determine what variables to change to optimize spheroid protocol
- Ana Martinez
    - Meet with the team and clients at Hess Lab to begin practicing cell culture.
    - Discuss preliminary report with client.
    - Continue updating research on design notebook.
- Emily Rhine
    - Come into Hess lab to help feed and passage cells. Begin paper layout and experiment brainstorm for paper to parallel research conducted. Brainstorm what to bring in to show & tell.
- Julia Salita
    - Meet with the team and clients at the Hess Lab
    - Divide lab work
    - Begin working in the Hess lab on cell culturing
- Jayson O'Halloran
    - Begin to work in the Hess lab on cell culturing
    - Go over preliminary report with client Q/A
    - Update design notebook and begin to put together a materials/BPAG 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
    - Received feedback on preliminary presentation from client and advisor.
- Althys Cao
    - Worked on preliminary presentation
    - Updated LabArchives
- Ana Martinez
    - Finished preliminary presentation.
    - Worked on preliminary report sections.
    - Began planning roles/responsibilities for cell culture, spheroid formation, etc.
- Emily Rhine
    - Finish preliminary presentation and begin research and work on preliminary report.
- Julia Salita
    - Finish preliminary presentation
    - Work on preliminary report
    - Begin preparing to work in the lab
- Jayson O'Halloran
    - Finished preliminary presentation and began to work on the preliminary report. Decided roles and responsibilities for lab work/prepare to culture cell lines.

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	10/3-4 10/4	- Preliminary Presentation - Preliminary Report	4 1	5	34.5
Ana Martinez	10/3-10/4 10/4-10/10	- Finalized/Presented Preliminary Presentation -Preliminary Report	2 3	6	32
Emily Rhine	10/4-10/10	-Preliminary Report -Human Cancer Safety Research	3 0.5	3.5	32
Julia Salita	10/4 10/4-10/10	- Preliminary presentation - Preliminary report	3 1	4	25.5
Jayson O'Halloran	10/4-10/10	-Preliminary Report - Cancer DNA damage research -Biosafety 2 Preparation	2.5 1 0.5	4	29