CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 6

Reporting Period: October 11, 2024 - October 17, 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 started passaging A549 cell line to determine our cell line's doubling time.

Difficulties / advice requests:

- Review the possibility of running another cell line**.**

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 7

Major team goals for the next week:

1. Continue passaging cells
    a. Find average doubling time
2. Meet with client and advisor Oct 18,2024
3. Establish a timeline for the rest of the semester
    a. Passaging plan

Next week's individual goals:

- Althys Cao
    - Establish passaging timeline, continue passaging cells and compare doubling time with literature values, ask client if imaging confluent A549 cells is possible for pictures for final poster presentation.
- Ana Martinez
    - Establish a passaging timeline based our cell line's determined doubling time. Brainstorm model/prop/imaging options we could present at our show and tell and final poster presentation.
- Emily Rhine
    - Establish a team passaging timeline. Establish a general project timeline. Continue passaging cells. Brainstorm model/prop to bring in to the final poster presentation.
- Julia Salita
    - Continue to passage cells and establish a passaging schedule. Brainstorm and prototype a prop/model for show and tell/ the final poster presentation.
- Jayson O'Halloran
    - Establish a passaging timeline, talk about the possibility of starting another cell line.
    - Look into final poster presentation.
    - Continue to pass cells and develop a plan for CRISPRi screening.

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
  - Finished preliminary report
  - Began passaging A549 cells
- Althys Cao
  - Finished preliminary report and updated LabArchives notebook
  - Passaged A549 cells
- Ana Martinez
  - Finished preliminary report.
  - Met with client and thawed A549 cells.
  - Conducted 2nd passage for A549 cells.
- Emily Rhine
  - Finished preliminary report. Met with Carley to begin tissue culture: passaging A549 cells. Updated LabArchives.
- Julia Salita
  - Finished preliminary report
  - Met with the client to thaw A549 cells.
  - Conducted 1st passage of A549 cells.
- Jayson O'Halloran
  - Started cell culturing A549
  - Went over preliminary report with client Q/A
  - Updated design notebook and began to put together a materials/BPAG sheet

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	10/11 10/11 10/10-12 10/16	- Cell Passaging - Client Meeting - Preliminary Report, Notebook - Cell Passaging	1 1 4 1	7	41.5
Ana Martinez	10/10-10/12 10/11 10/11, 10/16	- Preliminary Report, LabArchives Notebook - Client Meeting - Cell Passaging	3.5 1 2	5.5	38.5
Emily Rhine	10/11 10/11	-Cell Passaging -Client Meeting	1 1	2	32
Julia Salita	10/11-14 10/11 10/14 10/14	- Preliminary Report - Client meeting - Cell Passaging - Lab Archives	2 2 1.5 0.75	6.25	31.75
Jayson O'Halloran	10/11 10/14 10/16	-Preliminary Report -Cell Passaging -Mutations in Lung Cancer	2 2 1	5	34