CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 3

Reporting Period: September 20th, 2024 - September 26th, 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 came up with 3 cell lines to use (all already available in Hess Lab) and 3 spheroid formation protocols.
- Team generated two design matrices and rated the designs based on different criteria and narrowed down to the most viable cell line and protocol.

Difficulties / advice requests: The team does not have difficulties for week 4. However, we would appreciate feedback from our client and advisor for our design matrix so we can make appropriate changes in time for our preliminary presentation next Friday.

Current design: As will be outlined in the design matrices (will be sent out shortly), the team has agreed on a winning cell line and a spheroid formation protocol. The cell line selected was A549 and the spheroid formation protocol will involve the use of treated tissue culture plates for low attachment.

Materials and expenses: N/A for week 4

Major team goals for the next week:

1. Meet with client to review design matrix
2. Create preliminary design presentation
3. Meet as a team to practice for presentation

Next week's individual goals:

- Althys Cao
    - Prepare preliminary presentation slides with team to send by Wednesday and practice for presentation on Friday. If time allows, do deeper research into formation protocol and determine variables to change. Finish training
- Ana Martinez
    - Compile my research into relevant points for my presentation slides (to be assigned as a team). Edit presentation slides as a team and then send to our advisor for feedback. Practice my presentation slides to be ready for next Friday.
- Emily Rhine
    - Create and edit slides for presentation. Give presentation to advisor and classmates and receive feedback. Meet with the team to decide when and what cell line to order.
- Julia Salita
    - Prepare the preliminary presentation slides and present to our advisor and other peers to receive feedback. Prepare to start working with the chosen cell line
- Jayson O'Halloran
    - Begin to work on cell line creation after selecting a cell line from the preliminary design matrix. Work on/give preliminary presentation to advisor and classmates with the intent on getting feedback for our initial cell line and spheroid formation method. Finish most of the required training for the Hess Lab.

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
    - Finish PDS
    - Finalize expectations for project with client
    - Start research
    - Finish training
- Althys Cao
    - Began literature search to understand more about forming spheroid and about CRISPR screening protocol, as well as finding out if there have been any instances of CRISPR applications in 3D cell layers.
    - Go through as many trainings as possible to prepare for Hess Lab tour.
    - Work on and finalize PDS with the team
- Ana Martinez
    - Began my literature search to gain further familiarity with spheroid formation protocols and CRISPR screening for cancer. Completed trainings required by Hess Lab.
    - Plan to meet with our client and tour the Hess Lab, as well as work with my team and complete the PDS.
- Emily Rhine
    - Continued cell line and spheroid formation research. Completed individual PDS section and edited entire document.
- Julia Salita
    - Continue research to better understand the background behind the project. Meet with the clients and tour the Hess lab. Finish PDS outline, rough draft, and final

draft. Take a group picture and upload it to the website.

- Jayson O'Halloran
    - Continue to do research into how lung cancer can be stained for γH2AX. Look into how spheroid formation in 3D can be created using the cell lines we are proposing for the design matrix. Work on the design matrix with team members. PDS was completed.

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	9/19-9/25 9/23 9/24 9/25 9/24-25	-Cell line/drug sensitivity/spheroid research -Client Meeting -Team Meeting -Advisor Meeting -Design Matrix	4 0.5 1 0.5 1.5	7.5	22
Ana Martinez	9/22-9/23 9/24-9/26 9/24	-Cell line/spheroid research -Design Matrix sections -Team meeting	3.5 1.5 1.5	6.5	20.5
Emily Rhine	9/19-9/25 9/23 9/24-9/26 9/24	-Cell line research -Client Meeting -Design Matrix -Team meeting	4 0.5 1.5 1	7	21
Julia Salita	9/23-25 9/24 9/25-26 9/25	-Cell line research -Team Meeting -Design Matrix -Advisor Meeting	2 1.5 2 0.5	6	15.5
Jayson O'Halloran	9/22 9/23 9/24-9/26 9/24	-Cell line research -Spheroid method research -Design Matrix -Team meeting	3 1 1 1	6	18