CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 2

Reporting Period: September 13th, 2024 - September 19th, 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:

- Currently working on required trainings
- Started more in-depth research to understand PDS requirements
- Finished first draft of PDS

Difficulties / advice requests: N/A for week 3

Current design: N/A for week 3

Materials and expenses: N/A for week 3

Major team goals for the next week:

1. Assign the proposed spheroid protocols a rank to determine which method will be used.
2. Assign proposed cell lines a rank to determine which line will be used.

Next week's individual goals:

- Althys Cao
    - Go more in depth about different cell lines (if applicable, see more into cell lines that have been used in 3D spheroids projects and understand what works for them). Look into different variables for spheroid formation protocols. Help with design matrix.
- Ana Martinez
    - Continue my research of cell line options, with particular emphasis on lung cancer cell lines. Continue research on spheroid formation methods. Come up with a few cell lines to propose for the design matrix. Help to establish design matrix criteria to evaluate cell lines proposed by the team.
- Emily Rhine
    - Continue researching potential cell lines. Propose a specific cell line for the design matrix. Help the team evaluate proposed designs. Continue updating LabArchives as needed.
- Julia Salita
    - Continue research for potential cell line options. Draw up a preliminary design matrix for cell lines. Keep researching spheroid formation methods.
- 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.

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
    - Read literature provided by clients including cancer spheroids, CRISPR-Cas9, and CRISPRi. Began relevant PDS research. Meet with team and advisor to set expectations for the semester.
- 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
    - Meet clients and tour the Hess Lab. Continue to do research in cancer spheroid protocol and cell line creation. Finish PDS by the end of next week.

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	9/13,16 9/13 9/15 9/16-9/17 9/17	-Client meeting/Hess Lab Tour -Advisor meeting -Training -Research and work on PDS -Edit PDS	3 0.5 0.5 5 2	11	14.5
Ana Martinez	9/13, 9/16 9/13 9/14-9/17 9/16-9/18	-Hess Lab tour/client meeting -Advisor meeting -Research and work on my PDS section - Edit PDS	2 0.5 3.5 3	9	12.5
Emily Rhine	9/13 9/13-9/17 9/13-9/17 9/13, 9/16 9/18, 9/19	-Advisor Meeting -Research for PDS -Edit PDS -Client Meetings -Team Meetings	0.5 2 2 3 1.5	9	14
Julia Salita	9/13 9/13-9/17 9/16	- Client meeting/lab tour - Advisor Meeting - Work on PDS - Client meeting	2 3.5 0.5	5.5	9.5
Jayson O'Halloran	9/14-9/17 9/16 9/18-9/19	-Research and PDS - Client Meetings -Trainings	4 2 2	8	12