

CRISPRi Screening in Cancer Spheroids - BME 400

Progress Report 4

Reporting Period: September 27th, 2024 - October 3, 2024

Client:	Carley Schwartz Dr. Gaelen Hess	cischwartz@wisc.edu ghess3@wisc.edu
Advisor:	Paul Campagnola	pcampagnola@wisc.edu
Team:	Althys Cao (Leader) Ana Martinez (Communicator) Emily Rhine (BSAC) Julia Salita (BWIG) Jayson O'Halloran (BPAG)	nvcao@wisc.edu almartinez4@wisc.edu erhine@wisc.edu jsalita@wisc.edu 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 γ H2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- The team is prepared to propose our preliminary final design to the client and advisor in the preliminary design presentation
- Team worked on preliminary presentation.

Difficulties / advice requests: The team does not have difficulties for week 5. However, we would appreciate feedback from our client advisor on our preliminary presentation so we can make appropriate changes in time for Friday's presentation and next week's preliminary report.

Current design: As will be outlined in the design matrices and the preliminary presentation (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 5

Major team goals for the next week:

1. Make sure all Biosafety 2 training is complete
2. Meet with client to discuss preliminary report and proposed final design
3. Complete Hess lab-specific tissue culture training
4. Thaw and begin passaging chosen cell line

Next week's individual goals:

- Althys Cao
 - Work on preliminary report
 - Update LabArchives
 - Meet with Ms. Schwartz to begin training in lab, work with team to divide lab work
- Ana Martinez
 - Work on and edit preliminary report
 - Meet with team to begin assigning roles/times for cell culture and spheroid formation
 - Meet with client and advisor
- Emily Rhine
 - Meet with the team to set a timeline and schedule cell thawing, passaging, and general care (feeding) of our chosen cell line.
 - Meet with client and advisor
 - Update LabArchives
- Julia Salita
 - Update LabArchives
 - Write, edit, and complete the preliminary report
 - Meet with team to decide on a schedule to start working with the chosen cell line
 - Start working with the cell line
 - Meet with client and advisor
- Jayson O'Halloran
 - Begin cell line creation in the lab
 - Meet with client and advisor
 - Work on preliminary report with the team

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
 - Created preliminary design presentation
 - Met as a team to practice for presentation
- Althys Cao

- Finished design matrix
- Prepared preliminary presentation slides with team to send by Wednesday and practice for presentation on Friday.
- Did deeper research into formation protocols.
- Ana Martinez
 - Finished and submitted design matrix
 - Compiled my research into relevant points for preliminary presentation
 - Worked on preliminary presentation and received corresponding feedback from advisor
- Emily Rhine
 - Finished, proofread, and uploaded design matrix to LabArchives.
 - Complete more research on various cell lines, spheroid formation protocols, and the article the client provided.
- Julia Salita
 - Completed the design matrix and uploaded it to the website
 - Worked on the preliminary presentation
 - Continued to read and research about the designs present in the design matrix
- Jayson O'Halloran
 - Finished the design matrix and various trainings for the Hess Lab. Worked on preliminary presentation and gained feedback on cell lines and spheroid formation options from advisor/clients.

Table 2. Itemized list of individual activities.

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	9/25-27	Design Matrix	3	7.5	29.5
	9/27-9/30	Research	1.5		
	9/30-10/3	Preliminary Presentation	3		
Ana Martinez	9/27	Finish Design Matrix	2	6.5	27
	9/29-9/30	Additional Research	1.5		
	9/30-10/3	Preliminary Presentation	3		
Emily Rhine	9/27,10/1	Update Notebook	1	8	28.5
	9/27	Design Matrix	3		
	9/27-9/28	Research	1		
	9/27-10/3	Preliminary Presentation	3		

Julia Salita	9/27 10/2 10/3	Design Matrix Preliminary Presentation Research	2 2 2	6	21.5
Jayson O'Halloran	9/27 9/29 10/1	-Design Matrix -Cell line & CRISPRi research -Preliminary Presentation	2 3 2	7	25