

CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 5

Reporting Period: February 21, 2025 - February 27, 2025

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:

- Finish Preliminary report
- Plan spheroid experiment 3 & qPCR
- Passage 12 - 15

Difficulties / advice requests: Data analysis is taking longer than expected.

Current design: N/A

Materials and expenses: N/A

Major team goals for the next week:

1. Finalize timeline for RT-qPCR workflow
2. Continue passaging spheroids for determining spheroid doubling time

3. Based on doubling time, determine amount of time to get 2 million cells for RNA extraction

Next week's individual goals:

- Althys Cao
 - Finished with preliminary report and LabArchives
 - Continue passaging
 - Help with RT-qPCR timeline, look more in depth into methods and result analysis
- Ana Martinez
 - Submit preliminary report
 - Update LabArchives
 - Continue passaging cells/spheroids
 - Meet with client, advisor, and team
- Emily Rhine
 - Submit preliminary report
 - Continue research, note taking, and passaging cells
 - Meet with client and advisor
 - Help develop RT-qPCR timeline and ideal spheroid formation protocol
- Julia Salita
 - Submit preliminary report
 - Continue research, note taking, and passaging cells
 - Meet with client and advisor
 - Update lab archives
 - Help develop RT-qPCR timeline and ideal spheroid formation protocol
- Jayson O'Halloran
 - Submit preliminary report
 - Passage cells
 - Work on spheroid cell measurement quantification

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
 - Spheroid formation for determining spheroid doubling time
 - Met with client to go over RT-qPCR workflow, timeline, etc
 - Ordered qPCR SOX2 primer and probe set
- Althys Cao
 - Continued editing timeline
 - Caught up on work, continued research on qPCR and gammaH2AX staining
 - Continue passaging, made polyHEMA plates and edited spheroid formation protocol
- Ana Martinez
 - Worked on my section of preliminary report/editing
 - Continue passaging cells
 - Meet with client, advisor, and team
- Emily Rhine
 - Continued work on preliminary report
 - Meet with client, advisor, and team
 - Passaged vial 3 of A549s
- Julia Salita
 - Met with client and advisor
 - Completed Results section of preliminary report
 - Attended team meeting to discuss future
 - Analyzed spheroid imaging and Cell titer-glo assay data
- Jayson O'Halloran

- Met with client and advisor
- Continued passaging cells
- Ordered qPCR SOX2 primer and probe set

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	2/25	- Team meeting	1	3	17.75
	2/6	- Recalculate spheroid seeding protocol, seed spheroids, making polyHEMA plates	2		
Ana Martinez	2/21	-Passage 12 & client meeting	1.5	7	25.5
	2/24	-Passage 13 & polyHEMA plate	1.5		
	2/25	-Team meeting	1		
	2/22-2/26	-Preliminary report	3		
Emily Rhine	2/21	-Passage 12 & client meeting	1.5	5.5	30
	2/22	-Preliminary Report	2		
	2/25	-Team Meeting	1		
	2/26	-Passage 14 & spheroid seeding	1		
Julia Salita	2/21	-Passage 12 & client meeting	1.5	15	34
	2/22-26	-Preliminary Report	1.5		
	2/25	-Team Meeting	1		
	2/21-26	-Data Analysis	11		
Jayson O'Halloran	2/21	-Passage 12 and client meeting	1.5	5	27
	2/25		1		
	2/22-2/27	-Team meeting	2		
	2/27	-Preliminary report -Report meeting	0.5		