

# CRISPRi Screening in Cancer Spheroids - BME 402

## *Progress Report 1*

**Reporting Period:** January 21st, 2025 - January 30th, 2025

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<b>Advisor:</b>	Paul Campagnola	pcampagnola@wisc.edu
<b>Team:</b>	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  $\gamma$ H2AX: a histone variant that is a sensitive marker for DNA damage.

### **Brief status update:**

- Team met to determine roles and begin passaging A549 vial 3.

**Difficulties / advice requests:** N/A

**Current design:** N/A

**Materials and expenses:** N/A

### **Major team goals for the next week:**

1. Finish PDS
2. Finalize expectations for this next semester with client
3. Finish preliminary presentation and present to advisor
4. Continue background research

5. Continue passaging

**Next week's individual goals:**

- Althys Cao
  - Finish preliminary presentation section & present
  - Continue research
  - Continue passaging cells
  - Meet with advisor to finalize expectations for this semester
- Ana Martinez
  - Finish my preliminary presentation section
  - Present as a group to advisor
  - Continue research
  - Meet with advisor and client
  - Continue passaging
- Emily Rhine
  - Finish my section of the preliminary presentation section and present to advisor.  
Continue background research and passaging.
- Julia Salita
  - Finish the preliminary presentation and present
  - Continue to research
  - Maintain cell line
- Jayson O'Halloran
  - Finish the preliminary presentation and present it to advisor
  - Have first client meeting of the semester
  - Continue passaging cells

**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	<b>Final Design</b>
8	<b>Order materials, consider submitting invention disclosure</b>
9	<b>Fabrication, show and tell</b>
10	<b>Fabrication</b>
11	<b>Fabrication</b>
12	<b>Design Testing and Modification, Poster Draft Review</b>
13	<b>Design Testing and Modification, Final Report</b>
14	<b>Poster Presentation, Final Report, Final Electronic Notebook, Team Evaluation, Peer/Self Evaluation</b>

**Previous week's goals and accomplishments:**

- Team
  - Thawed new batch of A549 for next round of passages
  - Freeze down 4 new vial
  - Began work on preliminary presentation
- Althys Cao
  - Thawed new batch of A549 with team
  - Started research on gamma-H2AX research
  - Did other first-day activities
- Ana Martinez
  - Thawed new batch of A549 cells with team
  - Reviewed PDS and previous objectives for semester
  - Helped team with other first-day activities
- Emily Rhine
  - Outlined potential experiments and project goals for the entire semester. Helped complete Vial 3 Passage 1. Observed and took notes for freezing down cells. Created and edited preliminary presentation. Edited and left comments on PDS.
- Julia Salita
  - Thawed a new batch of A549 with team
  - Assigned roles and updated website
  - Completed passage 1 and froze down 4 new vials of A549 cells
- Jayson O'Halloran
  - Thawed new batch of A549 cells with team
  - Went back and looked at previous objectives for this semester
  - Had first team meeting

**Table 2. Activities**

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	1/23	- Research	1	4.5	4.5
	1/24	- Met with team & thawed new batch of A549	2.5		
	1/29	- Passage 1	1		
Ana Martinez	1/24	- First team meeting	1.5	4.5	4.5
	1/24	- Thawed new batch of A549 cells	1		
	1/24-1/25	- Review of PDS, previous objectives	1		
	1/29	- Passage 2	1		
Emily Rhine	1/21	-Experiment Timeline	1.5	5.5	5.5
	1/22	-Background research	1		
	1/24	-Met with team to thaw vial	1		
	1/24	-Worked on PDS and preliminary presentation	1		
	1/27	-Passage 1 vial 3 & freeze down 4 vials	1		
Julia Salita	1/24	- Met with team & thawed new batch of A549	1	4	4
	1/27	- Passage 1	1		
	1/29	- review PDS and past semester research	2		
Jayson O'Halloran	1/23	-Previous semester research review	1	3.5	3.5
	1/24	-Thawed A549 cells and met with team	2.5		