

## CRISPRi Screening in Cancer Spheroids - BME 402

### *Progress Report 11*

**Reporting Period:** April 11, 2025 - April 17, 2025

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<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:**

- 2D cell passaging
  - Bleached both flasks
- RT-qPCR step 3 and data analysis
  - Ana
  - Althys
  - Julia
- $\gamma$ H2AX staining: Trial 2
  - Emily
  - Jayson

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

**Current design:** Cells seeded in 6 well plate at 75k cells/cm<sup>2</sup> with 0.75% methylcellulose in full DMEM (10% FBS, 1% p/s).

### **Materials and expenses:**

1. D-MEM (1x) Delbecco's Modified Eagle Medium:
  1. Brand: gibco
  2. Volume: 500 mL
  3. Content added (by us): 10% FBS (fetal bovine serum), P/S
2. Trypsin 0.05% (1x):
  1. Brand: cytiva
  2. Volume: 125 mL
3. Fetal Bovine Serum, Value FBS:
  1. Brand: gibco
  2. Volume: 500 mL
4. PBS pH 7.4 (1x):
  1. Brand: gibco
  2. Volume: 500 mL
5. A549 Cell Line
6. Poly-HEMA and Methylcellulose Sigma Aldrich Total: \$289.40

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

1. Analyze RT-qPCR results and  $\gamma$ H2AX stain results
2. Present poster
3. Incorporate preliminary report feedback when drafting the final report

### **Next week's individual goals:**

- Althys Cao
  - qPCR data analysis and graphing
  - Work on section of final poster and final report
- Ana Martinez
  - qPCR data analysis and graphing
  - Finish final poster section, work on final report section/editing
- Emily Rhine
  -
- Julia Salita
  - qPCR data analysis and graphing
  - Finish final poster section, work on final report section/editing
  - Update website and lab archives
- Jayson O'Halloran
  - Analyze results from second  $\gamma$ H2AX stain
  - Finish poster and begin working on final report
  - Help with anything else related to the project that needs wrapping up

**Table 1.** Project Timeline.

<b>Week #</b>	<b>Task</b>
1	<b>Choose project Assign roles</b>
2	<b>Finish first progress report BSAC meeting First client meeting</b>
3	<b>PDS, Brainstorm, Research</b>
4	<b>Brainstorm, Literature Search, Design matrix criteria and design ideas (at least three) due</b>
5	<b>Preliminary Oral Presentation</b>
6	<b>Preliminary Report, Electronic Notebook, Peer/Self Evaluation, Decide on final design</b>
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
  - Step 3 of RT-qPCR
  - Trial 2 of gamma-H2AX staining
  - Work on poster and final report
- Althys Cao
  - Step 3 of RT-qPCR
  - Worked on section of final report and poster
- Ana Martinez
  - Prepared for and performed Step 3 of RT-qPCR

- Engineering Expo and reflection
- Met with team
- Emily Rhine
  - Seeded spheroids for  $\gamma$ H2AX stain trial two
  - Worked on final report and poster draft
  - Updated LabArchives and benchling
- Julia Salita
  - Practice origami for Engineering Expo
  - Prepared for Step 3 of RT-qPCR, however it had to be moved due to illness and I was unable to help perform it.
  - Cut out all pages for DNA origami
  - Finish outreach activity guide
- Jayson O'Halloran
  - Seed spheroids for second  $\gamma$ H2AX stain
  - Engineering expo and outreach
  - Team and client meeting

**Table 2. Activities**

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	4/11	Engineering EXPO	1.5	6	77
	4/15	Team meeting	1		
	4/16	qPCR protocol prep	1		
	4/17	Step 3 qPCR	2.5		
Ana Martinez	4/11	Engineering Expo	2	6.75	69.25
	4/15	Team meeting	1.25		
	4/16	qPCR (step 3) protocol prep	1		
	4/17	Step 3 qPCR	2.5		
	4/17				
Emily Rhine	4/11	Engineering Expo	2.5	8.5	85
	4/11	Seed spheroids	2		
	4/11	Update LabArchives/ benchling	1		
	4/15	Team meeting	1		
	4/15-4/16	Outreach report & summary guide	2		
Julia Salita	4/11	- Practice origami for Engineering Expo	1	3.5	57
	4/15	- Prepared for Step 3 of RT-qPCR	0.5		

	4/10	- Cut out all pages for DNA origami	1		
	4/16	- Finished outreach activity guide	1		
Jayson O'Halloran	4/11	Engineering expo	2.5	7	72
	4/11	Seeding spheroids and	2		
	4/15	passaging	1		
	4/15	Team meeting	1.5		
		Outreach final deliverables			