

# CRISPRi Screening in Cancer Spheroids - BME 400

## *Progress Report 1*

**Reporting Period:** September 6th, 2024 - September 12th, 2024

<b>Client:</b>	Carley Schwartz Dr. Gaelen Hess	cischwartz@wisc.edu ghess3@wisc.edu
<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:**

- First meeting with client scheduled in-person at 9/13 at WIMR, which will also include a tour of the Hess Lab.
- Group started training required to join the Hess Lab.

**Difficulties / advice requests:** N/A for week 2

**Current design:** N/A for week 2

**Materials and expenses:** N/A for week 2

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

1. Finish PDS
2. Finalize expectations for project with client

3. Start research
4. Finish training

**Next week's individual goals:**

- 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. Finish PDS and review document with the team before submission. Individual brainstorming session.
- 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 1.** Project Timeline.

<b>Week #</b>	<b>Task</b>
<b>1</b>	<b>Choose project Assign roles</b>
<b>2</b>	<b>Finish first progress report BSAC meeting First client meeting</b>
<b>3</b>	<b>PDS, Brainstorm, Research</b>
<b>4</b>	<b>Brainstorm, Literature Search, Design matrix criteria and design ideas (at least three) due</b>
<b>5</b>	<b>Preliminary Oral Presentation</b>
<b>6</b>	<b>Preliminary Report, Electronic Notebook,</b>

	<b>Peer/Self Evaluation, Decide on final design</b>
<b>7</b>	<b>Final Design</b>
<b>8</b>	<b>Order materials, consider submitting invention disclosure</b>
<b>9</b>	<b>Fabrication, show and tell</b>
<b>10</b>	<b>Fabrication</b>
<b>11</b>	<b>Fabrication</b>
<b>12</b>	<b>Design Testing and Modification, Poster Draft Review</b>
<b>13</b>	<b>Design Testing and Modification, Final Report</b>
<b>14</b>	<b>Poster Presentation, Final Report, Final Electronic Notebook, Team Evaluation, Peer/Self Evaluation</b>

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

- Team
  - Submitted project proposal. Met with our assigned advisor.
- Althys Cao
  - Completed project proposal. Met with team and advisor. Team meeting afterwards to finalize meeting time, brainstorm questions for client and assign PDS roles. Started research.
- Ana Martinez
  - Completed project proposal, as well as met with my team to assign responsibilities and complete first-week activities for BME design.
- Emily Rhine
  - Completed project proposal. Met with the team and advisor to discuss roles and responsibilities. Set up PDS document and drafted client questions.
- Julia Salita
  - Completed project proposal and first day activities. Met with advisor and team to discuss course logistics and expectations. Started a preliminary search on CRISPR and cancer spheroids.
- Jayson O'Halloran
  - Finished project proposal. Both the first team and advisor meeting have been completed.

**Table 2. Activities**

<b>Name</b>	<b>Date</b>	<b>Activity</b>	<b>Time (h)</b>	<b>Week Total (h)</b>	<b>Sem. Total (h)</b>
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Althys Cao	9/10	- CRISPR and spheroid research	1	3.5	3.5
	9/10	- Team meeting - Started training to join Hess Lab	1.5 1		
	9/11				
Ana Martinez	9/10	- Spheroids and research	1.5	5	3.5
	9/10	- CRISPR research	1		
	9/10	- Hess Lab Canvas trainings - Assigned PDS roles amongst teammates	2 0.5		
Emily Rhine	9/8	- Client questions	0.5	5	5
	9/9	- Cancer spheroids research	1.5		
	9/10	- CRISPR research	1.5		
	9/10-9/12	- PDS	1.5		
Julia Salita	9/11	- Cancer Spheroid research - CRISPR screening research	1 0.75	3.25	3.25
	9/12	-Continued both research	1.5		
Jayson O'Halloran	9/9/24	- CRISPR research	1	4	4
	9/10/24	- Cellular engineering and Spheroid research	2		
	9/11/24	- $\gamma$ H2AX research	1		