CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 2

Reporting Period: January 31st, 2025 - February 6, 2025

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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 γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- Team met with clients to lay out expectations and next steps
- Team continued passaging and made new polyHEMA stock
- Team determined timeline of experiments for the next 3 weeks

Difficulties / advice requests: The team had some difficulty filtering our new polyHEMA stock solution (via a manual syringe filter and a vacuum filter). We will consider revisiting our protocol for making the solution and tweaking it as needed for future experiments.

Current design: N/A

Materials and expenses: N/A

Major team goals for the next week:

1. Repeat spheroid experiment
    a. Seed spheroids into premade PolyHema plates
    b. Image spheroids
2. Meet with client to review Gamma-H2AX stain and qPCR protocols

Next week's individual goals:

- Althys Cao
    - Continue gamma-H2AX and qPCR research
    - Write protocol for next round of spheroid formation
    - Help supervise the spheroid formation process
- Ana Martinez
    - Continue gamma-H2AX and qPCR research
    - Help team seed spheroids for cell viability assay
    - Meet with team/client to discuss cell viability assay results
    - Continue passaging A549 WT vial 3 cells
- Emily Rhine
    - Continue research into Gamma-H2AX stain and qPCR. Continue passing A549 WT vial 3. Seed spheroids.
- Julia Salita
    - Gamma-H2AX and qPCR research
    - Perform cell viability assay, image, and analyze the data
    - Continue to passage vial 3
    - Update website
    - Update predicted timeline
- Jayson O'Halloran
    - Continue to passage A549 cells
    - Research yH2AX staining protocol efficiency and compare to ours
    - Continue to make spheroids

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
    - Finished PDS
    - Continued work on Preliminary presentation
    - Established client and advisor expectations
    - Passage 3-5
    - Made PolyHema
- Althys Cao
    - Worked on preliminary presentation section
    - Continued research
    - Continued passaging cells
    - Met with advisor to finalize expectations for this semester
- Ana Martinez
    - Worked on preliminary presentation slides/timeline
    - Met with advisor and client
    - Continued passaging A549 WT vial 3 cells
- Emily Rhine
    - Worked on meeting notes, background research, preliminary presentation, and passaging.
- Julia Salita
    - Continued work on Preliminary presentation
    - Established client and advisor expectations
    - Made more PolyHema stock solution

- Jayson O'Halloran
    - Passaged A549 Cells
    - Met with advisor and client
    - Had team meeting regarding cell staining and qPCR

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	2/4 2/5	- Team meeting (timeline & presentation) - Coat plate with PolyHEMA - Help with PolyHEMA filtering	1 1	2	6.5
Ana Martinez	2/4 2/5	- PolyHEMA filtering - Passage 5, finish PolyHEMA filtering - Team meeting -qPCR primers search	0.5 1.5 1 0.5	3.5	8
Emily Rhine	1/31, 2/3 2/4 2/4 2/4 1/31-2/6	-Passage 3 & 4 -Filter PolyHema -Team Meeting (Timeline) -Preliminary Presentation -Background research	-1 -0.5 -0.5 -1 -0.5	3.5	9
Julia Salita	2/4 1/31 2/3 2/4	- Preliminary presentation - Established client and advisor expectations - Made more PolyHema stock solution - team meeting	0.5 1 1 1	3.5	7.5
Jayson O'Halloran	1/31 2/4 2/5	-Passage 3 and client meeting -Team meeting - Staining methods research -Primer research -Preliminary Presentation	3 1 1 .5	5.5	9