CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 3

Reporting Period: February 7, 2025 - February 13, 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:

- Completed spheroid experiment 1 and planned spheroid experiment 2
- Passage 6-8
- Meeting with client to potentially order Cell-Titer Glo 3D and either SOX2 primer (qPCR) or antibody (immunostaining)

Difficulties / advice requests: The team had some difficulties with some steps of the Cell-Titer Glo 2D for the spheroid cell viability assay. Hoping to get clearer results, we will take another look at our protocol and re-run the assay next week with either the Cell-Titer Glo 2D kit the Hess Lab currently has or, if approved, with the 3D version.

Current design: N/A

Materials and expenses: N/A

Major team goals for the next week:

1. Seed spheroids into premade PolyHEMA plates
2. Image spheroids, replace media and prepare positive/negative control wells for Cell Titer Glo
3. Perform Cell Titer Glo
4. Continue passaging A549 WT vial 3 cells

Next week's individual goals:

- Althys Cao
    - Learnt about Cell Titer Glo assay protocol, did first trial
    - Updated new timeline with team
    - Coat plates with polyHEMA
- Ana Martinez
    - Continue qPCR, immunostaining, and gamma-H2AX research
    - Help team seed spheroids for cell viability assay/Cell Titer Glo
    - Meet with advisor/client to discuss next steps
    - Continue passaging A549 WT vial 3 cells
- Emily Rhine
    - Repeat spheroid experiment and continue passaging. Continue research into ICC, antibody stain, and live/dead stain. Attend and take notes for client and advisor meeting.
- Julia Salita
    - Repeat spheroid experiment with updated method of using Cell Titer Glo
    - Continue passaging cells
    - Meet with the advisor/ client about next steps.
- Jayson O'Halloran
    - Repeat spheroid experiment and passage friday.
    - Finalize whether to do antibody stain or qPCR, ask client
    - Look ahead into yH2AX staining/refresh on protocol

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
    - Repeated spheroid experiment
        - Seeded spheroids into premade PolyHema plates
        - Imaged spheroids
    - Met with client to review Gamma-H2AX stain and qPCR protocols
- Althys Cao
    - Finished and presented preliminary presentation
    - Review Cell Titer Glo assay
    - qPCR research
- Ana Martinez
    - Finished and presented preliminary presentation
    - Helped team update project timeline
    - Reviewed Cell Titer Glo assay protocol
    - Researched qPCR SOX2 primers and antibodies with team
    - Continue passaging A549 WT vial 3 cells
- Emily Rhine
    - Finished and presented preliminary presentation slides. Attended and took notes for client meeting and team meeting. Completed necessary research and added it to Benchling & LabArchives. Kept up with timeline for spheroid formation, cell seeding, passaging, and CellTiter-Glo in 2D.
- Julia Salita
    - Preliminary presentation

    - Team meeting about timeline and BME outreach
    - Complete cell titer glo experiment
    - Meet with client on next steps
- Jayson O'Halloran
    - Continue to passage A549 cells
    - Research yH2AX staining protocol efficiency and compare to ours
    - Continue to make spheroids

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	2/7 2/10 2/11 2/12	Preliminary presentation qPCR primer research Client meeting Cell Titer Glo assay Updated new timeline w team PolyHEMA plate	0.5 0.5 0.5 2.5 1 0.75	5.75	12.25
Ana Martinez	2/7 2/7 2/7 2/7 2/11 2/12	qPCR primer research Preliminary Presentation Client Meeting Spheroid formation, passaging Team Meeting Passage 8, PolyHEMA plate	1.5 0.5 0.5 1 1 1	5.5	13.5
Emily Rhine	2/7 2/7 2/7 2/7-2/9 2/10 2/11	Preliminary presentation Client meeting Spheroid Formation & Passaging Update LabArchives & Benchling with research CellTiter-Glo & Imaging Team Meeting	-0.5 -0.5 -2 -4 -2 -1	10	19
Julia Salita	2/7 2/7 2/7 and 10 2/10 2/11	Preliminary presentation Client meeting Spheroid Formation & Passaging CellTiter-Glo & Imaging Team Meeting	0.5 0.5 2 2.5 1	6.5	14
Jayson O'Halloran	2/7 2/7 2/7 2/11	-Preliminary presentation -Client meeting -Spheroid formation -Team meeting	-0.5 -0.5 -2 -1	6	15

	2/12	-SOX2 antibody research and cancer drug research	-2		