CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 10

Reporting Period: April 3, 2025 - April 10, 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:

- 2D cell passaging
- RT-qPCR step 1 (RNA extraction) and step 2 (cDNA synthesis) protocols
    - Ana
    - Althys
    - Julia
- γH2AX staining protocol
    - Emily
    - Jayson

Difficulties / advice requests: We struggled with not having enough cells for gamma-H2AX staining due to the cell pellets not being secure enough such that frequent aspiration led to us losing a significant amount of cells.

Current design: Cells seeded in 6 well plate at 75k cells/cm^2 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. Finish RT-qPCR
2. Redo γH2AX stain
3. Incorporate preliminary report feedback when drafting the final report

Next week's individual goals:

- Althys Cao
    - Complete Step 3 of RT-qPCR
    - Continue passaging cells
    - Work on section of final report and poster
- Ana Martinez
    - Complete Step 3 (qPCR) of the RT-qPCR protocol
    - Continue passaging cells
    - Work on section of final report and poster
- Emily Rhine
    - Seed new 3D - 6 well plate
    - Seed new 2D - 6 well plate
    - Redo γH2AX stain
    - Complete section of final report draft
    - Begin assigned poster section
- Julia Salita
    - Complete Step 3 of RT-qPCR
    - Continue passaging cells
    - Work on section of final report and poster

    - Update website
- Jayson O'Halloran
    - Seed new 3D - 6 well plate
    - Seed new 2D - 6 well plate
    - Redo γH2AX stain
    - Begin working on final report and poster

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

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- Althys Cao
    - Helped with RNA extraction and cDNA synthesis
    - Prepared for Engineering Expo
- Ana Martinez
    - Helped with RNA extraction and cDNA synthesis steps
    - Continued passaging 2D A549 WT flasks
    - Continued preparing for outreach event at Engineering Expo
    - Met with team, client, and advisor
- Emily Rhine
    - Continue 2D passaging. Make more polyHEMA plates. Prepare for γH2AX stain. Met with team, client, and advisor.
- Julia Salita
    - Helped with RNA extraction steps
    - Continued preparing Engineering Expo
    - Met with team, client, and advisor
- Jayson O'Halloran
    - Passaged 2D cells
    - Prepared for yH2AX stain
    - Met with client and advisor

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	4/4 4/6 4/7 4/7 4/9	Advisor, client meetings RT-qPCR protocol review , team meeting Dissociate 3D spheroids RNA extraction (step 1 of RT-qPCR) cDNA synthesis (step 2 of RT-qPCR)	2 2 2 3 1.5	10.5	71
Ana Martinez	4/4 4/6 4/6 4/7, 4/9 4/7 4/7 4/9	Advisor, client meetings RT-qPCR protocol review Team meeting 2D passaging Advisor meeting RNA extraction (step 1 of RT-qPCR) cDNA synthesis (step 2 of RT-qPCR)	2 1.25 0.5 2 0.5 3 1	10.25	62.5
Emily Rhine	4/4	BSAC	1	13.5	76.5

	4/4 4/4 4/4 4/6 4/8	Meeting Advisor + client Spheroid seeding -2D passaging -Add etoposide to cells -γH2AX stain	2 1 0.5 3 6		
Julia Salita	4/7 4/4-10 4/4-8	- Helped with RNA extraction steps - Continued preparing Engineering Expo - Met with team, client, and advisor	3 0.5 1	4.5	53.5
Jayson O'Halloran	4/4 4/6 4/8 4/9	-Advisor and Client meeting, passaging, spheroid seeding -Add Etoposide to cultures & team meeting -yH2AX stain -Upload images to lab archives	4 3 5 1	13	65