CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 7

Reporting Period: March 7, 2025 - March 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:

- Passage 3-5
- Spheroid passaging
- Second trial of spheroid dissociation & determined ways to improve current protocol
    - Evaluate spheroid doubling time
- Seeded spheroids for next trial of determining doubling time and to prep for qPCR

Difficulties / advice requests: Optimization of accutase spheroid dissociation in order to establish spheroid doubling time. We find that we keep losing a significant amount of cells during the process such that our doubling time is inaccurately low. New PolyHema stock solution may be affecting experimental results (improper filtering), so we also plan to make a new batch with a 30-min UV sterilization of plates instead of vacuum filtration [1].

[1] "Do polyHEMA coated plates need to be sterilised for use in embryoid body formation?," ResearchGate. Accessed: Mar. 13, 2025. [Online]. Available:

https://www.researchgate.net/post/Do_polyHEMA_coated_plates_need_to_be_sterilised_for_use_in_embryoid_body_formation

Current design:

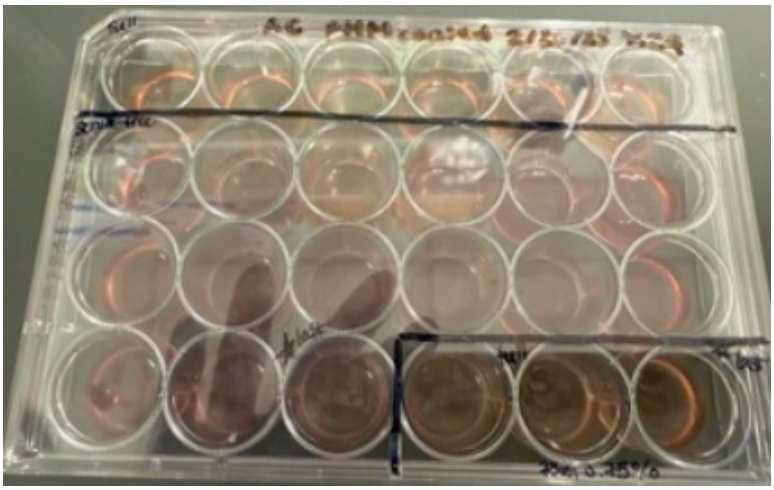

Figure 1. Cells seeded 3/5/25 at 75k cells/cm^2 with 0.75% methylcellulose. Full DMEM wells are A1-A6; D4-D6, and SFM wells are B1-B6; C1-C6; and D1-D3.

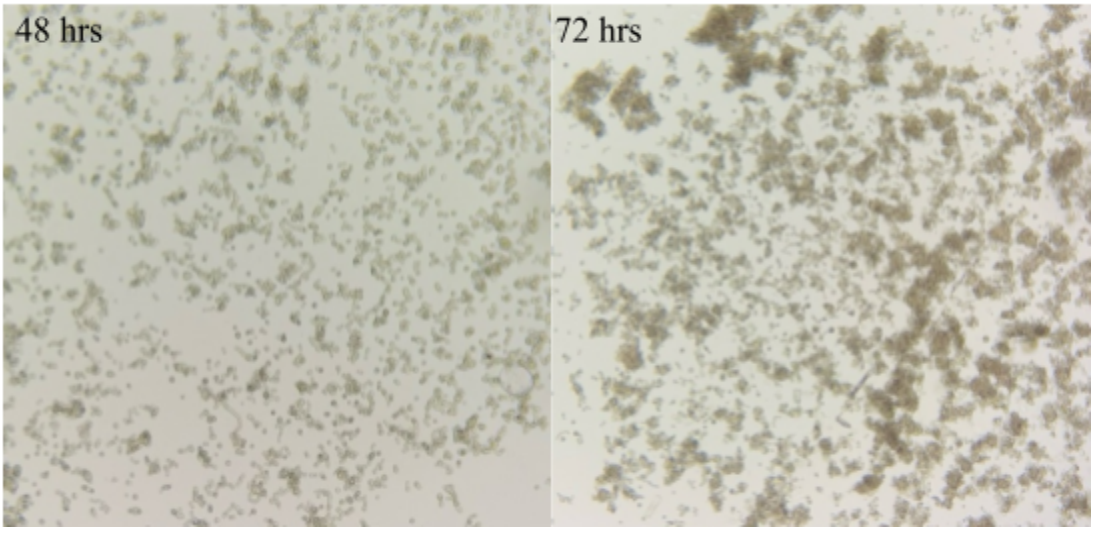

Figure 2. Well A1 (Full DMEM) at various time points (4x brightfield).

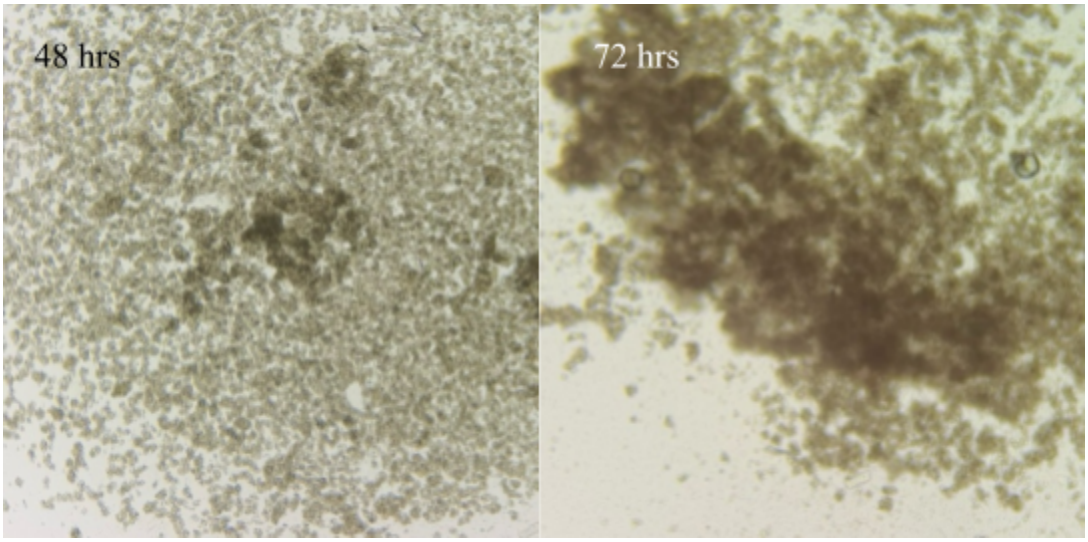

Figure 3. Well B1 (SFM) at various time points (4x brightfield).

Materials and expenses:

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. Continue to optimize spheroid passaging and dissociation protocol
2. Establish spheroid doubling time
3. Make new PolyHEMA stock (with UV sterilization instead of vacuum filtration)
4. Plan qPCR and gamma-H2AX stain

Next week's individual goals:

- Althys Cao
    - Support with 3rd trial of spheroid dissociation/passaging protocol to determine doubling time
    - Keep updating timeline for qPCR
- Ana Martinez
    - Help team with 3rd trial of spheroid dissociation/passaging protocol to determine spheroid doubling time
    - Work with team/client to establish a more specific timeline for RT-qPCR based on spheroid doubling time
    - Continue passaging 2D WT A549s
    - Update LabArchives
- Emily Rhine
    -
- Julia Salita
    - Assist with third spheroid dissociation/passaging protocol
    - Establish a more specific RT-qPCR timeline
    - Continue passaging update Lab archives
- Jayson O'Halloran
    - Spheroid dissociation
    - Continue passaging A549 2D culture
    - Begin qPCR
    - Update Lab Archives

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
    - Attempt spheroid passaging and dissociation protocols in order to gain information on the doubling time and ideal media conditions
- Althys Cao
    - Seeded spheroids in 24-well plate to determine doubling time
      Help plan next experiments
- Ana Martinez
    - Continue optimization of spheroid dissociation/passaging protocol to determine spheroid doubling time
    - Work with team to establish a more specific timeline for RT-qPCR based on spheroid doubling time
    - Continue passaging 2D WT A549s
- Emily Rhine
    - Continue A549 WT and spheroid research in LabArchives. Take notes on all team, client, and advisor meetings. Establish and aid in optimizing spheroid passaging and dissociation protocols.
- Julia Salita
    - Assessed spheroids from experiment
    - Passage 2D cells
    - Attend team meeting
- Jayson O'Halloran
    - Continue passaging spheroids and 2D cells
    - Met with advisor and client
    - Finalize timeline for qPCR based on spheroid doubling time analysis

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)

Althys Cao	3/10 3/11 3/12	Spheroid dissociation Team meeting Seed spheroids	2 1 1	4	46.75
Ana Martinez	3/8 3/11 3/10-3/11 3/12	Spheroid feeding Team meeting Spheroid dissociation research Passaging 2 flasks	2 1 2 1	6	39
Emily Rhine	3/7 3/8 3/10 3/11 3/11	Passage 2 flasks Spheroid feeding Spheroid dissociation Team meeting LabArchives Research	1 1 2.5 1 1.5	7	45.5
Julia Salita	3/10 3/10 3/11 3/12	Passage 2 flasks Spheroid dissociation Team meeting Make Methylcellulose	1 2 1 0.5	4.5	42.5
Jayson O'Halloran	3/7 3/10 3/11	Passaging Spheroid formation research Team meeting	2 1 1	4	37