CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 6

Reporting Period: February 28, 2025 - March 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:

- Passage 0-2
- Spheroid passaging
- First trial of spheroid dissociation & determined ways to improve current protocol
- Seeded spheroids for next trial of determining doubling time and to prep for qPCR
- Determined timeline for upcoming 10 days

Difficulties / advice requests: Optimization of accutase spheroid dissociation in order to establish spheroid doubling time.

Current design: N/A

Materials and expenses: N/A

Major team goals for the next week:

1. Finalize timeline for RT-qPCR workflow
2. Continue passaging spheroids for determining spheroid doubling time
3. Based on doubling time, determine amount of time to get 2 million cells for RNA extraction

Next week's individual goals:

- Althys Cao
    - Continue optimizing spheroid dissociation and passaging protocol
    - Determine doubling time to then establish cell seeding timeline for qPCR
    - Continue passaging 2D A549 cells
- 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 optimization of accutase spheroid dissociation protocol. Continue passaging 2D WT A549s. Plan timeline for RT-qPCR based on spheroid doubling time.
- Julia Salita
    - Continue 2D passaging
    - Dissociate spheroids for doubling time experiment
    - Work with team to optimize protocols and plan a RT-qPCR timeline
- 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 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
  - Finish Preliminary report
  - Plan spheroid experiment 3 & qPCR
  - Passage 12 - 15
- Althys Cao
  - Worked on method and materials part of preliminary report; helped with graphing and edit of result and analysis part
  - Did calculations and seeded spheroids for 24-well plate
  - Did some additional qPCR research to fully catch up
  - Worked on team to update timeline for qPCR
- Ana Martinez
  - Finished editing preliminary report
  - Continued passaging 2D WT A549s
  - Continued spheroid dissociation/passaging protocol to determine doubling time
- Emily Rhine
  - Finish editing and submitted preliminary report.
  - Establish timeline of experiments and deliverables at client, advisor, and team meetings
  - Optimize accutase dissociation.
- Julia Salita
  - Continued passaging 2D A549 cells
  - Completed data analysis and preliminary report

    - Met with Advisor and client
    - Met with team to discuss specifics surrounding RT-qPCR timeline and spheroid experiment

- Jayson O'Halloran
    - Submitted preliminary report
    - Passage cells
    - Work on spheroid cell measurement quantification

Table 2. Activities

Name	Date	Activity	Time (h)	Week Total (h)	Sem. Total (h)
Althys Cao	2/27-3/3 2/28 2/28 3/2 3/4 3/5	- Preliminary Report - Client meeting - Support spheroid passaging and dissociation - Additional qPCR research - Team meeting - Calculations + seed spheroid for 24-well plate	8 1 2 1 0.5 2.5	15	42.75
Ana Martinez	2/28-3/3 2/28 3/4 3/5	- Preliminary Report - Spheroid passaging - Team meeting, emails - Passaging, prepping polyHEMA plate	3.5 1.5 1 1.5	7.5	33
Emily Rhine	2/28-3/3 2/28 2/28 2/28 3/3 3/4 3/5	-Preliminary Report -BSAC meeting -Client meeting -Spheroid passaging and dissociation -Accutase dissociation -Team meeting -Spheroid dissociation research	1 1 1 2 2 0.5 1	8.5	38.5
Julia Salita	3/3 2/28-3/3 2/28 3/4	-Passaging -Completed data analysis and preliminary report -Met with Advisor and client -Met with team	0.75 1.25 1 1	4	38
Jayson O'Halloran	2/28-3/3 2/28	-Preliminary report -Spheroid passaging and client	2 3	6	33

	3/4 3/6	meeting - Team meeting - Update notebook	 0.5 0.5		