Manipulating Cellular Senescence with RRM2

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Introduction

Dr. Nicholas Graham's Lab is focused on using systems biology approaches to understand cancer and other human diseases. To acquire a holistic understanding of cellular processes, the Graham Lab collects large data-sets including information from proteins, metabolites and genes. By integrating this data, the Graham Lab creates data-driven computational models of cell biology and diseases.

During the SHINE program, I had the opportunity to work with in the Graham Lab and be involved in one of their projects on cellular senescence.

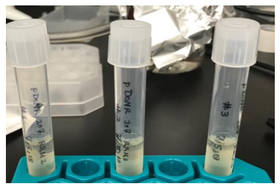
Objective & Impact of Professor's Research

In cells, glucose is one of the most important nutrients for cell metabolism. In addition to energy generation, glucose can be used to produce molecules needed for DNA synthesis. Normal, non-cancerous cells can divide up to 40 times before they reach a state called senescence when they stop growing and no longer divide. Cancer cells, however escape this mechanism and acquire uncontrolled growth. During senescence, the pathway that is responsible for DNA synthesis is shutdown. What Dr. Graham's lab is interested in is the understanding of this pathway and its role in senescence. This summer, I worked with the RRM2 gene that encodes the production of the RRM2 protein, which is a key enzyme in controlling the DNA synthesis pathway.

If the RRM2 enzyme can be controlled by doctors, this enzyme could have big implications in the world of cancer treatment. Possibly applications include the suppression of rapidly dividing cancer cells by shutting down their growth pathway.

Skills Learned

- Gateway Cloning (Method of DNA cloning)
- Polymerase Chain Reaction
- Miniprep and Miraprep (for DNA extraction)
- BCA Assay (to measure protein concentration)
- Spectrophotometry
- Gel Electrophoresis (To verify expected DNA fragment by size. Based on how fast or slow different molecules travel in a solution with an electrical field)



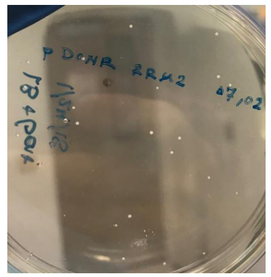


Figure 1 & 2.
PDONR-207 RRM2 plate (right) culture and liquid (left) culture. Liquid cultures are used in Miniprep and liquid cultures are made from colonies that originate from plate cultures. (PC: Alireza Delfarah)

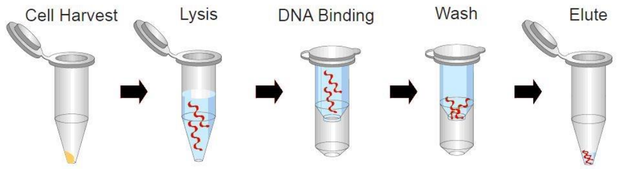


Figure 3. Illustration of Miniprep protocol (PC: fairbiotech.com)

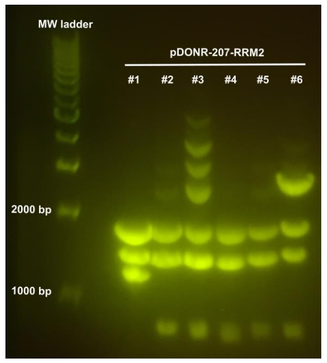


Figure 4. Six individual pDONR-207 RRM2 clones under UV light after gel electrophoresis. Only clone #1 shows the expected DNA fragments.

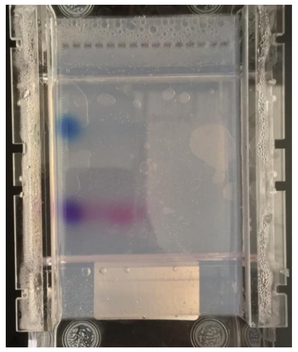


Figure 5. Molecular Ruler and multiple pDONR-207 samples in an agar gel block after electrophoresis (PC: Marcus Gutierrez)

How This Relates to Your STEM Coursework

Before I entered the SHINE program, I had very little grasp on chemistry and why it is so important in the world we live in today. I did not think you would use chemistry knowledge in cancer research. I also did not fathom how frequently multiple STEM fields overlap such as chemistry and biology or chemistry and mathematics.

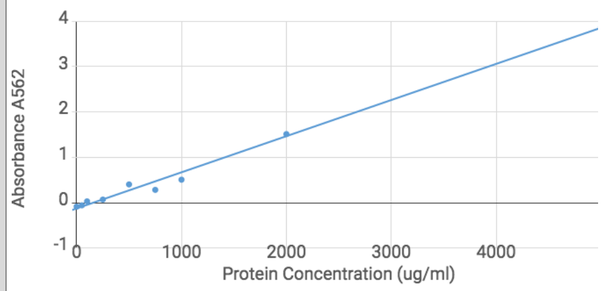
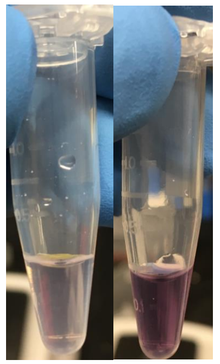


Figure 6
BCA assay standard curve to measure protein concentration with spectrophotometry. (PC: Marcus Gutierrez)

Figure 7
Protein samples from BCA Assay. The intensity of the purple is directly related to how much protein is in the solution. So in one sample, there is a lot more protein present which is why it is darker than the other. (PC: Marcus Gutierrez)



When I return to school, I will retain this knowledge by being cognizant of how important it is to not prioritize one STEM course. Instead, I will focus on making sure I understand each and every course as a whole. I can share this with my colleagues and classmates to prevent them from prioritizing one course at the expense of another for the sake of percentages.

Project Goal Until End of Program

Create pLenti-PGK-neo-RRM2 lentiviral plasmid to be used to increase expression of RRM2 protein through viral infection in cells. Neomycin would be used to select cells that successfully received the plasmid.

Advice for Future SHINE Students

For future SHINE students, you have made a great decision in attending SHINE and your future will greatly benefit from it. Make sure to use your time at USC doing the most as it will go by in an instant. Talk to not only your mentor but the other university students in your lab as well since you can learn a lot from them. In addition to your mentors, become acquainted with your fellow SHINE students, a lot of them have great stories and experiences to share. Most importantly, don't stress yourself out! You have many resources at your disposal to use if you find yourself overwhelmed and you should enjoy yourself while also working hard.

Acknowledgements

I would like to thank Brian Boyle, Mr. Aburto, and Ms. Ross for giving me the opportunity to attend SHINE. I would also like to thank my mentor, Alireza Delfarah, along with Professor Nicholas Graham for guiding my lab research throughout the summer. Thanks to Dr. Katie Mills and Dr. Megan Herrold of SHINE. Finally, I would like to thank the following for motivating me to attend SHINE and do great things; Aurora Velasco, Elizabeth Garica, Melissa Valencia, Claudia Galicia, Elvia Altimirano, Ms.Gutierrez, Mr. C, Ms. Martinez, Mr. Agarwal, Ms. Erhard, Abner Espinoza, Isis Mares, Michelle Miranda, Liliana Reyes