Gateway Cloning Enzymes of Glycolysis

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Introduction

The Graham lab focuses on using system biology approaches to comprehend and develop new therapeutic approaches for cancer and other human diseases.

During the SHINE program, I had the opportunity to work in the Graham lab and be involved in my PhD mentor, Belinda Garana's project in enzymatic regulation of glycolysis in breast cancer cells.

Objective

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Use gateway cloning to put genes for enzymes in a lentiviral destination vector.

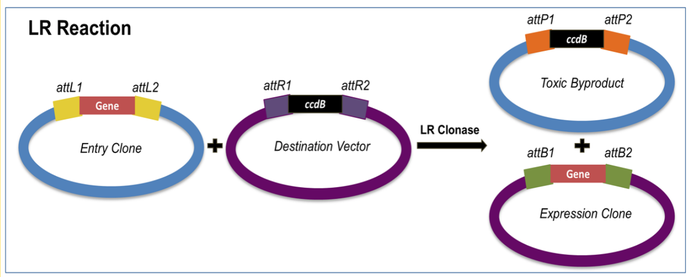
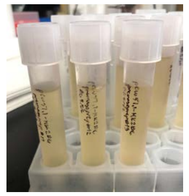


Figure 1
Moves the gene of interest from the entry vector to the destination vector. (Retrieved from: https://blog.addgene.org/plasmids-101-gateway-cloning)

Skills Learned

- **Gateway cloning**
  - Tool to move the gene of interest from the entry vector to the destination vector.
- **Bacteria culture**

Plate



Liquid Culture

Figures 2 and 3 Allows us to amplify the DNA and use antibiotics to select for colonies with the desired reaction product.

- **Plasmid DNA Mini-Prep**

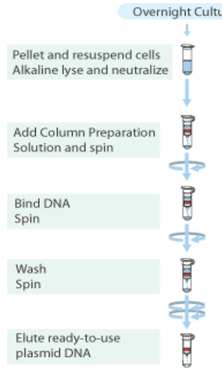
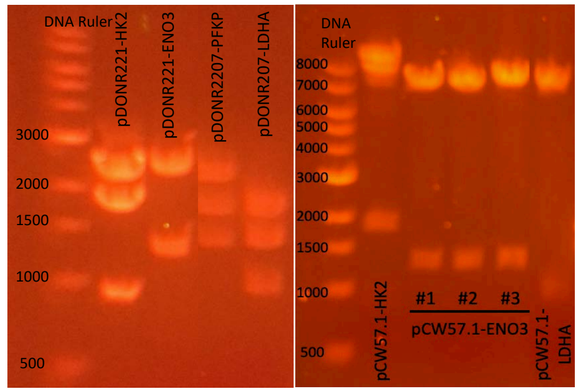


Figure 4
Extracts DNA from bacteria. (Retrieved from: https://www.sigmaaldrich.com/life-science/molecular-biology/dna-and-rna-purification/plasmid-miniprep-kit.html)

- **Nanodrop**

Figure 5
Uses ultraviolet light to see the DNA concentration after doing the DNA Mini-Prep.

- **Gel Electrophoresis**



Entry Vectors **Destination Vectors**

Figures 6 and 7
A method used to separate DNA fragments by size using an electric current through agarose gel.

Impact of Graham Lab's Research

Gateway cloning is a powerful tool because the lentiviral destination vector allows us to:

- Create virus to infect breast cancer cells
  - Adds copy of gene to DNA
  - Increases the amount of enzymes
- Measure how much the enzyme affects the metabolism of glucose
  - Choose which enzymes to target for possible therapy for breast cancer

How This Relates to Your STEM Coursework

When starting SHINE, at first I was not comfortable pipetting because I had never used those kind of pipettes and now I feel very comfortable pipetting liquids into other liquids and transferring small volumes of it gave me a more steady hand as well. Processes like making bacteria liquid culture plates and tubes, using a centrifuge, making agarose gel for processes like gel electrophoresis has become more comfortable to me. With this I also learned more about polymerase chain reaction, the central dogma of biology and better understand how gateway cloning works. All of these things could be really helpful when taking my AP Biology class next year in high school.

Next Steps for Me/Advice for Future SHINE Students

To future SHINE students, you will not regret attending the SHINE program because SHINE:

- Gives you an actual hands on experience in the type of field you are interested in.
- Is an advantageous learning experience; don't be afraid to ask you mentors questions, you don't need to stress yourself out.

I will continue to use this experience to give me an idea of what I would like to do in life. I will also do some research on chemical engineering because I found it a field I am interested in.

Acknowledgements

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