Comparing Two Different Heat Shock Promoters for Gene Expression

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Abstract

- Thermal control of gene expression is an important yet largely unexplored area of research in synthetic biology. One potential approach to achieve this is through the use of heat shock promoters (HSPs) in DNA transcription.
- DNA transcription, as the first step of gene expression, forms mRNA from DNA by involving various transcription factors, enzymes, and promoters that are responsive to heat.
- Heat shock promoters are segments of DNA located before structural genes. Once activated by heat, HSPs act as "landing pads" for RNA polymerase to bind onto and begin transcription.

By integrating HSPs into the genome of particular cells, we can spatiotemporally control gene transcription.

- To achieve greatest efficiency in HSP-related therapies, our research compares the gene expression of two HSPs in the presence of focused ultrasound, to understand their respective advantages and disadvantages.
- This project is an extension of our lab's HSP comparison between HSP1 and HSP2, made possible with the usage of cloning techniques, such as PCR and bacterial transformation, as well as cell culture for in-vitro studies.

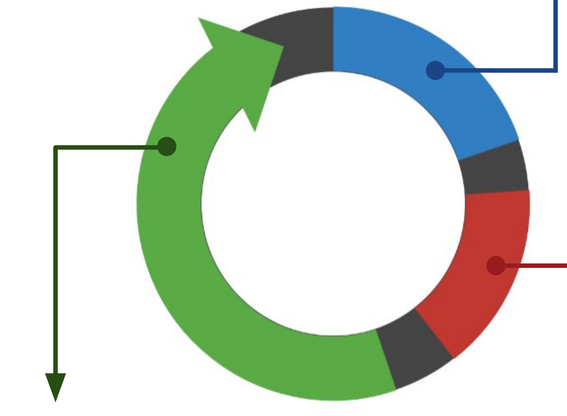
Objective

To identify which heat shock promoter is most inducible to heat shock

By observing the amount of gene product for each HSP via intensity of red glow from translation of genetic marker **RFP**

Plasmid Diagram

HSP1/HSP2
- Heat shock promoter
- Independent variable
- 2 options



pHR
- Backbone for GOI
- Antibiotic resistant
- Originates from viral DNA

GOI
- Gene of interest (GOI)
- Includes various genetic markers for activity detection

All diagrams not to scale.

Cloning Methodology

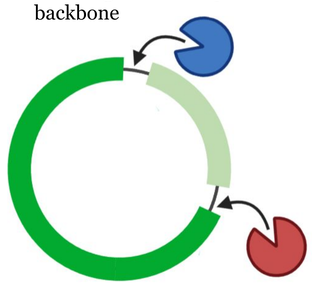
PCR

- To amplify each fragment of DNA (3 total) using primers, DNA polymerase, nucleotides, and the template DNA

- Gives us more DNA to work with

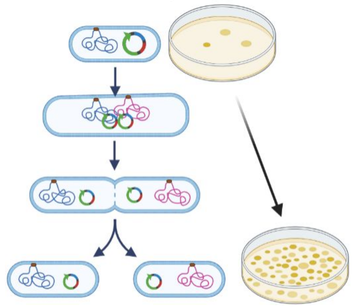
Enzymatic Digestion

- 2 different restriction enzymes cut out the backbone
- Then, we ligase (glue) the two PCR fragments into pHR backbone



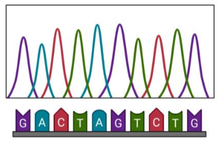
Bacterial Amplification

- Integrate plasmid into competent cells (*E. coli*), select and culture a transformed colony



- *E. coli* acts like a cloning machine for our engineered plasmid; then we extract the plasmids

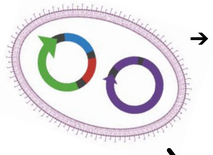
Sanger Sequencing



- An external company Sanger sequences the plasmid to identify the nucleotide sequence
- Verifies plasmids have proper ligation/ "glued together"

Lentivirus Production

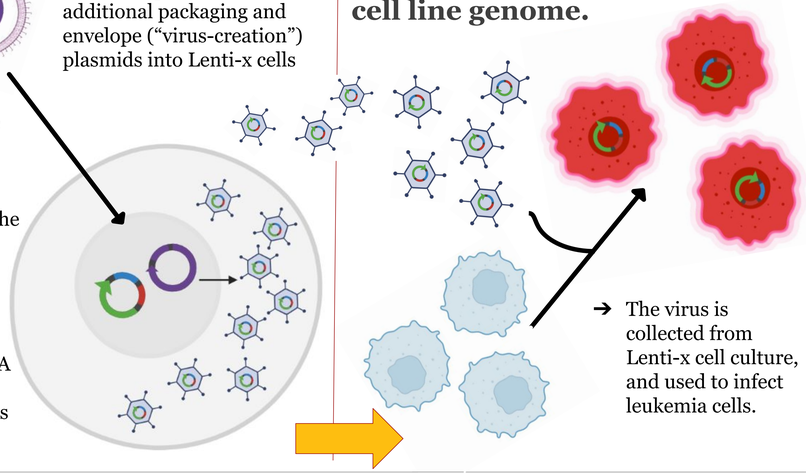
Transfection



- Transfection kit used to insert our plasmid and additional packaging and envelope ("virus-creation") plasmids into Lenti-x cells
- Essentially, the plasmid is wrapped in a membrane and "hugged" into the Lenti-x cells
- The cells will translate the introduced DNA and begin to generate viruses

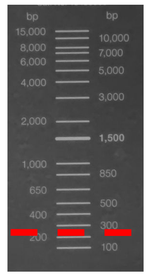
Transduction

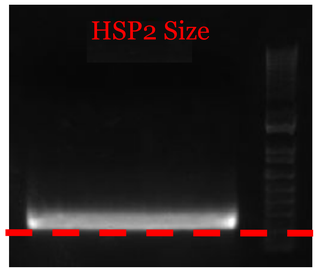
Virus infection integrates our engineered plasmid into leukemia cell line genome.



- The virus is collected from Lenti-x cell culture, and used to infect leukemia cells.

Results





Gel UV Imaging

Is UV detection of DNA fragment size following gel electrophoresis.

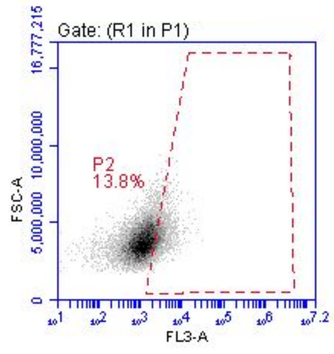
- HSP2 size ≈ 200 bp
  - Target size.

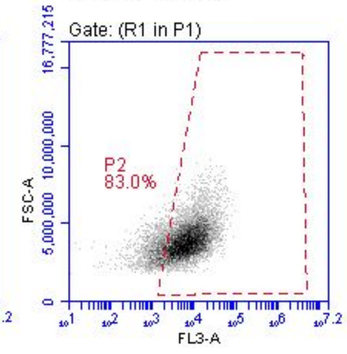
Flow Cytometry

Utilizes lasers to detect the fluorescence of cells.

With some additional calculations, we used it to detect the concentration of viruses produced.

- HSP1:
  - 13.8% infection rate
    - Successful virus transduction!
- HSP2:
  - 83.0% infection rate
    - Successful virus transduction!





Conclusions and Future Work

- In this summer's research, we have:
  - Engineered plasmids to compare differences in gene expression between HSP1 and HSP2 using cloning techniques.
  - Successfully made lentivirus to infect cancer cells with our engineered plasmid.
  - Effectively visualized results with gel UV imaging and flow cytometry.

In the future, we hope to compare the efficiency of the two heat shock promoters in the presence of heat.

Citations

(1)
DNA Learning Center. *Sanger Sequencing*. www.youtube.com. https://www.youtube.com/watch?v=6ldtdWjDwes (accessed 2023-07-28).

Contact and Acknowledgements

bridge.usc.edu/bugs

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