Genetic Engineering of Cell Cycle Genes in Pancreatic Beta Cells

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Abstract

Diabetes is a chronic disease affecting millions of Americans every day. This illness stems from the body's inability to use insulin effectively and produce sufficient insulin. Our bodies contain specialized cells called pancreatic beta cells, which are responsible for fabricating insulin and play a crucial role in storing and transporting glucose in our bodies for cells to use in later processes. Through a series of meticulous procedures and testing, this research explores the idea of developing an effective system that will allow us to manufacture an adenovirus which later will be used to infect these pancreatic beta cells to promote cell proliferation. Using different procedures such as mini prep, enzyme digestions, DNA extractions, gel electrophoresis, and gateway cloning systems, we have been able to first develop a plasmid which we can introduce into a viral vector to create the adenovirus. Through our use of CRISPR and CRISPRa technology, we will be able to screen the adenovirus and target different genes, as well as locate specific cell cycle inhibitors and activators that could be responsible for regulation of cell proliferation in pancreatic beta cells. This experiment is significant in the advancement of medicine, treatments, and technology for diabetes. It will provide a new alternative and treatment to regulate and treat diabetes. Our research is ongoing, and new results are continuously collected.

Introduction

Diabetes is a chronic illness that results from the pancreas not making enough insulin or the body not being able to use insulin effectively (World Health Organization, 2023). Insulin is a crucial hormone that utilizes the sugar we ingest from food to be used in our bodies for energy. When insulin is used efficiently, we are able to have a controlled blood sugar level and have extra glucose stored for energy (Mayo Clinic, 2023). There are two types of diabetes Type I and Type II. Type I diabetes is usually recognized in childhood or adolescence. There is no cure for this type of diabetes and can only be controlled through diet and lifestyle. Type II diabetes can also begin in childhood and adulthood but is more common in older adults. For this type of diabetes, weight loss, healthy diet, and exercise can help manage it but not cure it.

A newly developed therapeutic approach to genetic disorders is the editing of specific mutated genes. CRISPR/Cas is one of the engineered nuclease used to assemble a double-strand break for a targeted sequence. Cas9 is needed to read, recognize, and create a double-strand break in the targeted sequence. CRISPR uses the Cas9 to distinguish the DNA sequence and split each strand using a different nuclease domain (Lotfi et al., 2023). The use of the CRISPR/Cas9 complex can lead to gene editing to improve production of pancreatic beta cells, the cells that are responsible for insulin production.

Results

Results of skills gained in the lab

1. Miniprep: We have mastered the use of the mini prep protocol, which has enabled us to learn how to purify DNA and find the concentration of the DNA in our given samples.
2. Enzyme Digestion: We have executed enzyme digestions to determine different sites in which the DNA will be cut by the enzymes. Allowing us to create active sites for the LR Clonase process, as well as determine the samples that have resulted in clones.
3. Cell Culturing: We have become proficient in cell culturing, allowing us to determine the health, confluency (concentration) and proper growth of our cells. With the expectation of preparing our cells to become infected by our adenovirus in future.
4. Gel electrophoresis: Gel electrophoresis is a skill we have acquired to separate our DNA into critical parts which we will use to manufacture our recombinant DNA. Through this we will be able to determine which samples are our clones by determining their band sizes.
5. Imaging: We have gained this skill in the lab by imaging the gels we have prepared along with our growing colonies in order to track the critical DNA fragments needed.
6. Picking colonies: Picking colonies is a skill we have attained, that allows us to grow our colonies which will be used later during the mini prep protocol. As a result, we will be able to effectively determine the amount of DNA contained in different colonies and samples.

Results of Imaging and DNA Concentrations

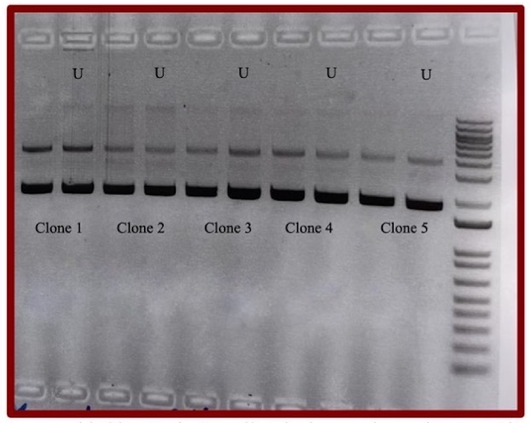
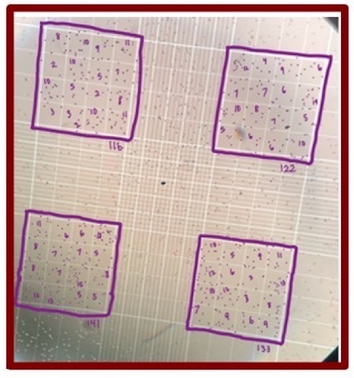


Image provided by Hyeje Sumajit. The image above shows a gel with potential LMNA candidates digested with Bsa1 and undigested to determine creation of successful clones through confirmation with expected band sizes.

Sample	Yield (µg)
LR1-CA-1	5.505
LR1-CA-2	12.756
LR1-CA-3	10.623
LR1-CA-4	6.954
LR1-CA-5	12.636
LR1-CA-11	14.016
LR1-CA-12	12.624
LR1-CA-13	3.336
LR1-CA-14	25.869
LR1-CA-15	13.275

The above table shows the yield of DNA collected from each sample after undergoing mini prep, which is a procedure that helps to purify DNA from our samples.



133.5/0.1*2*2000 µL = 5.34 million cells in tube. 5340000/2*6 = 16.02 million cells in plate. Cells counted using hemacytometer, a device used to section off 30µL of media solution containing cells

Methods

Routine Lab Procedures for Cloning:

- Cell Culturing
- Mini Prep
- Gel Electrophoresis
- Enzyme Digestion
- PCR Testing
- Imaging
- Picking/Growing Colonies

Overall Project Pathway:

1. We will introduce a pEntry clone + ccdb cassette destination vector by using LR Clonase to produce a pAdExpression Clone + toxic byproduct.
2. Next, we aim to manufacture an Adenovirus in which we will be able to introduce into a HEK293T cell line.
3. We will validate our system to ensure appropriate CRISPR activity
4. After CRISPR validation, we will introduce the adenovirus transduce to our islets of pancreatic beta cells to confirm increased proliferation

Conclusion/Discussion

This project serves several purposes. Overall, this project aims to evaluate genes regulating beta-cell proliferation through whole genome CRISPR screening in human beta cells. With the CRISPR/Cas9 complex and LR Clonase, the goal is to develop an adenovirus vector that can be used to transfect pancreatic beta cell islets. Future goals of this research include successful transfection of pancreatic beta cell islets and ultimately developing a novel treatment method for increased beta cell proliferation. The successful creation of such a treatment would be revolutionary for diabetic patients. This project is currently in the process of developing the adenovirus vector that would be used for transfection and testing this vector on HEK293T cells, a human cell line originating from the kidney. This project is ongoing and consequently, definitive results have not been concluded. However, the research lab anticipates successful completion of creating an adenovirus vector that increases beta cell proliferation.

References

1. Lotfi, M., Butler, A. E., Sukhorukov, V. N., & Sahebkar, A. (2024). Application of CRISPR-Cas9 technology in diabetes research. Diabetic Medicine, 41(1), e15240. https://doi.org/10.1111/dme.15240
2. Mayo Foundation for Medical Education and Research. (2023, September 15). Diabetes. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444
3. World Health Organization. (2023, April 5). Diabetes. World Health Organization. https://www.who.int/health-topics/diabetes#tab=tab_1.