Enabling study of Human Immune Dysfunction using yeast gene OST3/UNGI

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Introduction

OST3 is a yeast gene for Obligosaccharyl transferase, a homolog of the Magnesium Transport 1 gene in humans. This human gene produce XMEN disease. XMEN disease is an X-Linked immune system deficiency where the amount of CD4+ T cells present in the body are reduced in function. XMEN is caused by a mutation in the MAGT1 gene. The MAGT1 gene produces a protein called magnesium transport. As shown in Magnesium transport is important because it activates CD4+ T cells, allowing for infections to be effectively detected. A mutation in the MAGT1 gene, reduces the function and effectivity of CD4+ T cells. For the individual, this mutation would result in a higher risk of developing infection, pneumonia or cancer. The only known effective treatment for XMEN is stem cell therapy.

Methods

- **Primer Design**
  - Defines which part of the sequence will be amplified
- **PCR**
  - Amplifies gene
- **Gel Electrophoresis**
  - Separates macromolecule
- **Gateway reaction**
  - DNA fragments cloned and properly placed in Vectors
- **Transformation**
  - Transfer DNA into bacteria
- **Mini Prep**
  - Purifies DNA from bacteria
- **Restriction Mapping**
  - Map DNA. Restriction enzymes digest specific DNA fragments

PCR/Gel electrophoresis

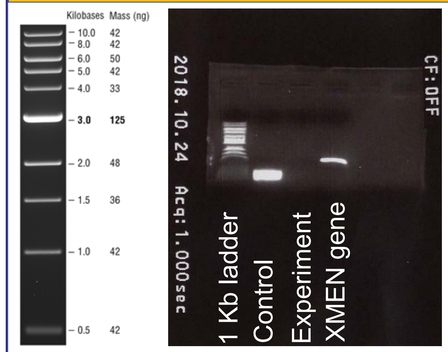

Figure 2. XMEN, control and 1 kb ladder bands appeared on the Gel electrophoresis. The experiment gene, UNG1, was not amplified by PCR while the MAG1 gene was amplified by PCR.

("Biomaker Insight", Kjell Kirschbaum, July 13,2016, http://biomarkerinsights.qiagen.com/2016/07/13/new-tool-for-successful-end-point-multiplex-pcr)

Transformation Bacterial Colonies

Figure 3. The colonies, located on the positive control showing that the Topoisomerase worked. Their were approximately 20-30 isolated colonies present in the plate. The colonies located on the XMEN plate indicates that the OST3 gene was cloned into the vector, and transformed into bacteria.

Restriction enzyme Mapping

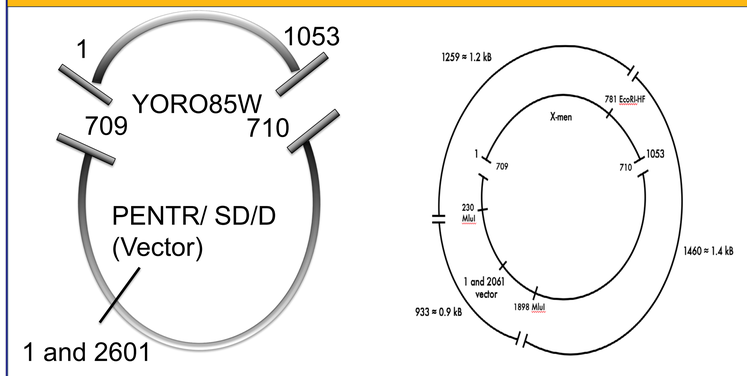

Figure 4. The restrictive enzymes (MluI-HF and XhoI-HF) were used for OST3 gene. The gene length was 1,053 base pairs.

Digestion/ Gel electrophoresis

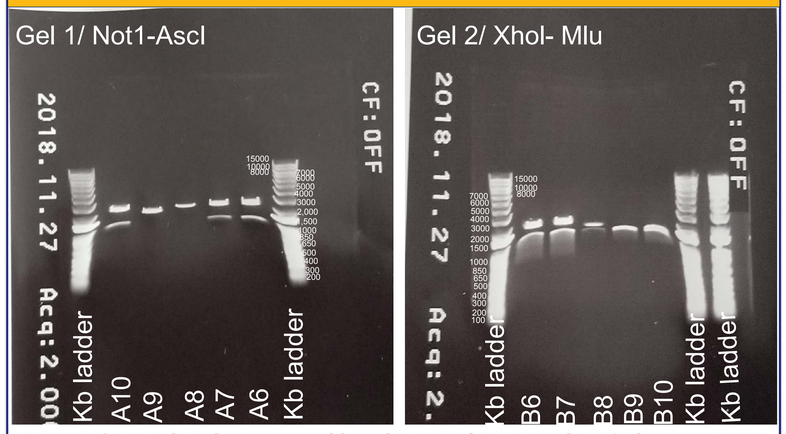

Figure 5. In Gel 1, the expected band sizes of 2601 and 1053 base pairs were presented in A10, A7 and A6, receiving bands between 2000 and 1500 base pairs. In Gel 2, the expected band sizes was 2012, 933 and 707, presented in B6 and B7. In both Gel 1 and Gel 2, the restriction digest worked and the plasmid was digested.

Conclusion/Discussion

The original gene of interest was UNG1. UNG1 is a X linked immune system deficiency where B cells are not able to go through antibody switching. UNG1 gene was unable to be replicated because the PCR did not work causing the switch to OST3 gene.

- OST3 PCR was successful shown and described on Figure 2
- Transformation for OST3 was successful which was shown through colonies presented on the Vector shown and described on Figure 3
- Digestion was successful for OST3, and this was shown through the bands located on Figure 5
- The proper restive enzymes were selected and cut the DNA fragments for OST3.
- OST3 was successfully cloned

In future experiments, I hope to Conduct more experimental research on OST3 to see…

- How the human gene may reacts to different genes work using yeast
- Effectively clone UNG1 gene using yeast

Literature Cited

Burgers PM and Klein MB (1986) Selection by genetic transformation of a Saccharomyces cerevisiae mutant defective for the nuclear uracil-DNA-glycosylase. J Bacteriol 166(3):905-13 PMID: 3519585

Reference, Genetics. "XMEN". *Genetics Home Reference*, 2018, https://ghr.nlm.nih.gov/condition/x-linked-immunodeficiency-with-magnesium-defect-epstein-barr-virus-infection-and-neoplasia#statistics. Accessed 25 Nov 2018.

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