Expression of gut fungal cellulase in a bacterial host

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Current practice allows the creation of biofuels from agriculture (corn, etc.) but it is not economical.

The use of any agriculture waste would present higher yields without the cost, acreage, and societal impacts associated with large grain production.

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The Big Picture

- The purpose of this research is to use the plasmids to create chemicals on a large scale

- Uses and applications include:
  - Pharmaceuticals
  - Bio-fuels
The Research

- Our goal is to clone cellulase, an enzyme from fungus

- From there we will put it into a more industrial organism, *E. coli* bacteria

- The cellulase will be tagged with GFP which will fluoresce when correctly ligated

- Ultimately it will be able to break down biomass in order to make chemical products
Lab Methods: Molecular Cloning

1. Cutting out the insert
2. Cutting the vector
3. Ligation
4. Transformation

Molecular Cloning of GFP
Project Workflow

Generate source materials
Project Workflow

Generate source materials

Run materials on a gel to confirm success
Generate source materials → Run materials on a gel to confirm success → Overdigest insert and vector
Project Workflow

1. Generate source materials
2. Run materials on a gel to confirm success
3. Overdigest insert and vector
4. Cleanup reaction and nanodrop to confirm concentration
Generate source materials

Run materials on a gel to confirm success

Overdigest insert and vector

Ligate insert and vector and plate for overnight growth

Cleanup reaction and nanodrop to confirm concentration
Project Workflow

1. Generate source materials
2. Run materials on a gel to confirm success
3. Check and screen colonies in selective medium overnight
Project Workflow

1. Generate source materials
2. Run materials on a gel to confirm success
3. Overdigest insert and vector
4. Check and screen colonies in selective medium overnight
5. Make index plate of cultures
6. Cleanup reaction and nanodrop to confirm concentration

Make index plate of cultures
Generate source materials

Run materials on a gel to confirm success

Overdigest insert and vector

Check and screen colonies in selective medium overnight

Ligate insert and vector and plate for overnight growth

Cleanup reaction and nanodrop to confirm

Make index plate of cultures

Miniprep cultured plasmids
Project Workflow

1. Generate source materials
2. Run materials on a gel to confirm success
3. Overdigest insert and vector
4. Check and screen colonies in selective medium overnight
5. Ligate insert and vector and plate for overnight growth
6. Cleanup reaction and nanodrop to confirm concentration
7. Make index plate of cultures
8. Miniprep cultured plasmids
9. Screen cultures by restrictive analysis
Lab Methods

- Using enzymes to cut DNA plasmids
  - Restriction enzymes are found naturally in bacteria
  - Used to cut DNA plasmid at specific sequences to create "sticky ends"

- DNA ligase, can attach or rejoin DNA fragments with complementary ends.

- Assays are used to quantify the protein content of your sample
Data Sources

- The data we obtain in the lab is used to confirm the success of our ligations (genetic engineering)
- We cut the DNA into bands with restriction enzymes chosen for their specific restriction sites
- If our insert correctly linked to our vector, we should see a specific number of bands with an accurate number of kilobases
Agarose gel electrophoresis is used in molecular biology to separate a DNA.

- The DNA is separated by fragment length.
- As the current runs through the agarose gel, DNA fragments are pulled through based on size.
- The gel is then imaged and analyzed.
- DNA can also be extracted from the gel with UV illumination.
Results

- My first ligation consisted of piecing in a gene sequence (GFP) into a vector (pET-28a)
- To confirm that this was successful I ran a gel electrophoresis
- Using the enzymes Sac1 and Xho1 I inserted the GFP sequence
- Because the ligation was successful, the image to the right shows 3 bands at 5.2kb/0.55kb/0.28kb
- The 1kb ladder is our measuring guide
Challenges

- With most of the problems we are able to problem solve a solution
  - Increase the concentration of our samples
  - Change the thermocycler protocol
Moving Forward

- Despite minor setbacks, I did successfully create a platform to be used in *E. coli* bacteria.

- We created it using a bacterial plasmid (pET 28a) because of its ability to be reproduced in large quantities.
Future Work

- When the sequence is transformed into the *E. coli* bacteria it will inform us of two things by fluorescing:
  - If our cellulase is active
  - If antisense is controlling it
I successfully PCR amplified and cloned a cellulase which was fused to GFP and expressed it in *E. coli*. This caused the lysate to glow green.

This green construct will enable further studies such as the research into whether antisense is controlling the cellulase.
Classroom Application

- Industrial uses of microbes
- E. coli bacteria as a machine for replication
- Plasmids and restriction enzymes
- Genetic engineering
- Molecular cloning
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Abstract

In this project I worked with Dr. Kevin Solomon in the department of Chemical Engineering. The goal of my project was to molecularly clone a cellulase from the fungus *P. finn* and insert in the *bacteria E. coli*. Cellulases are enzymes that break down complex plant biomass into fermentable sugars. These sugars serve as feed stocks for microbial processes that generate a wide array of compounds including fuels, medicines, and bulk chemicals that form the majority of the items that we use as a society.

In completing this project we used the tools of molecular cloning, including gel electrophoresis, restriction digests, polymerase chain reactions (PCR), colorimetric assays, and microbiological techniques.