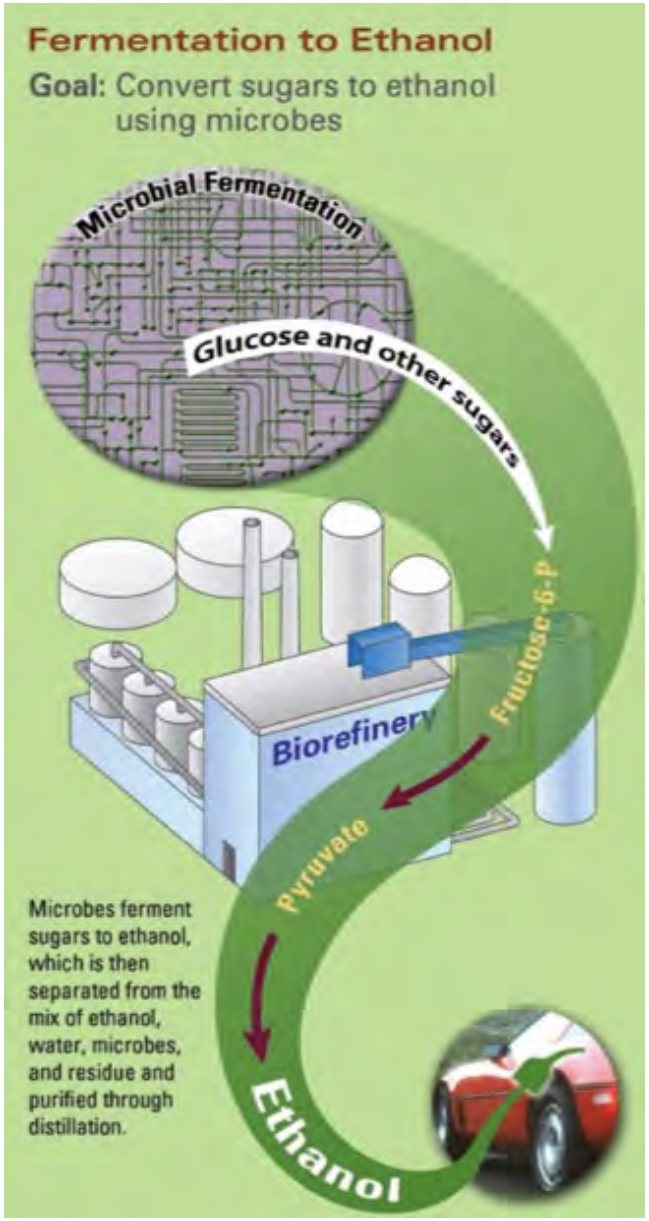
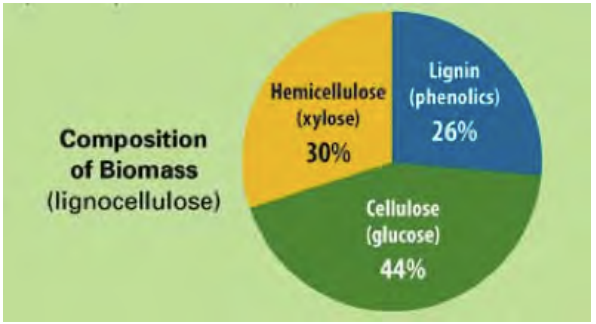
Expression of gut fungal cellulase in a bacterial host

Megan Cotich
Mentor: Kevin Solomon



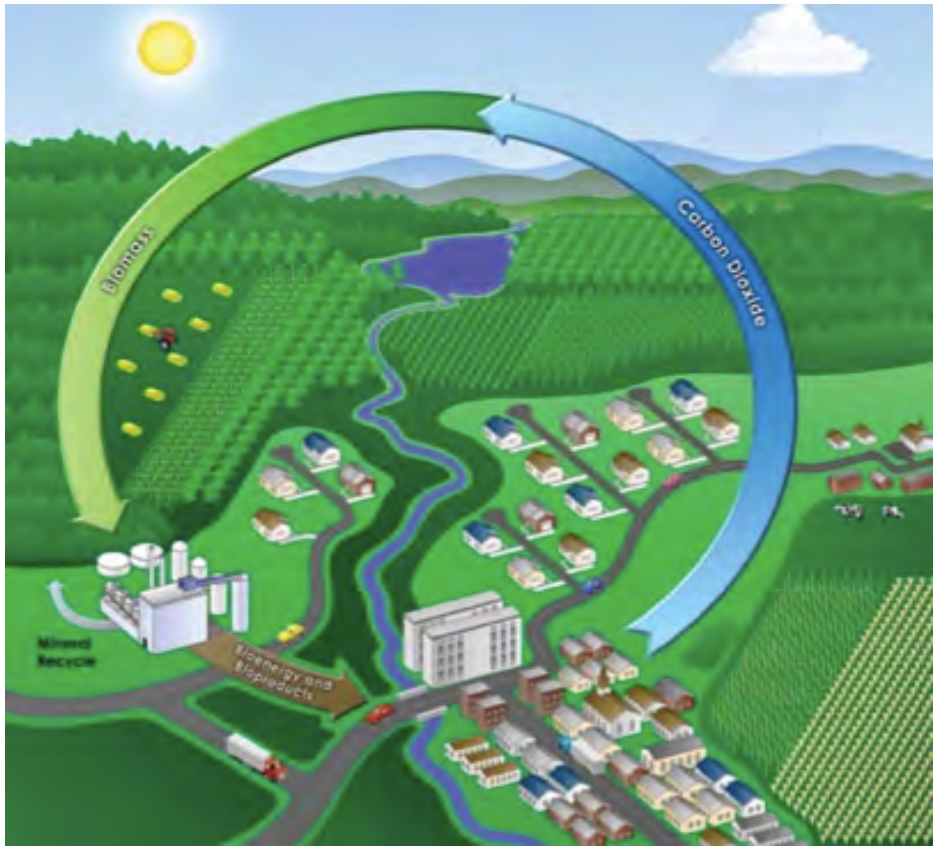
The Big Picture

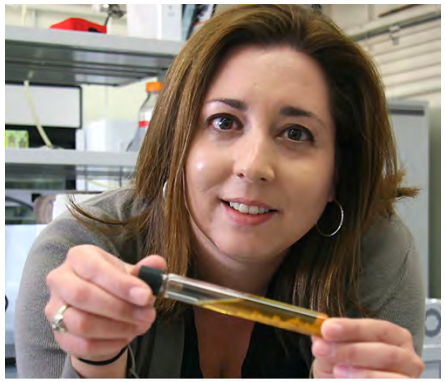
- Current practice allows the creation of bio-fuels from agriculture (corn, etc.) but it is not economical
- The use of any agriculture waste would present higher yields without the cost, acreage, and societal impacts associated with large grain production
- This research is funded by the U.S. Dept. of Agriculture (USDA) and the Dept. of Energy (DOE)



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





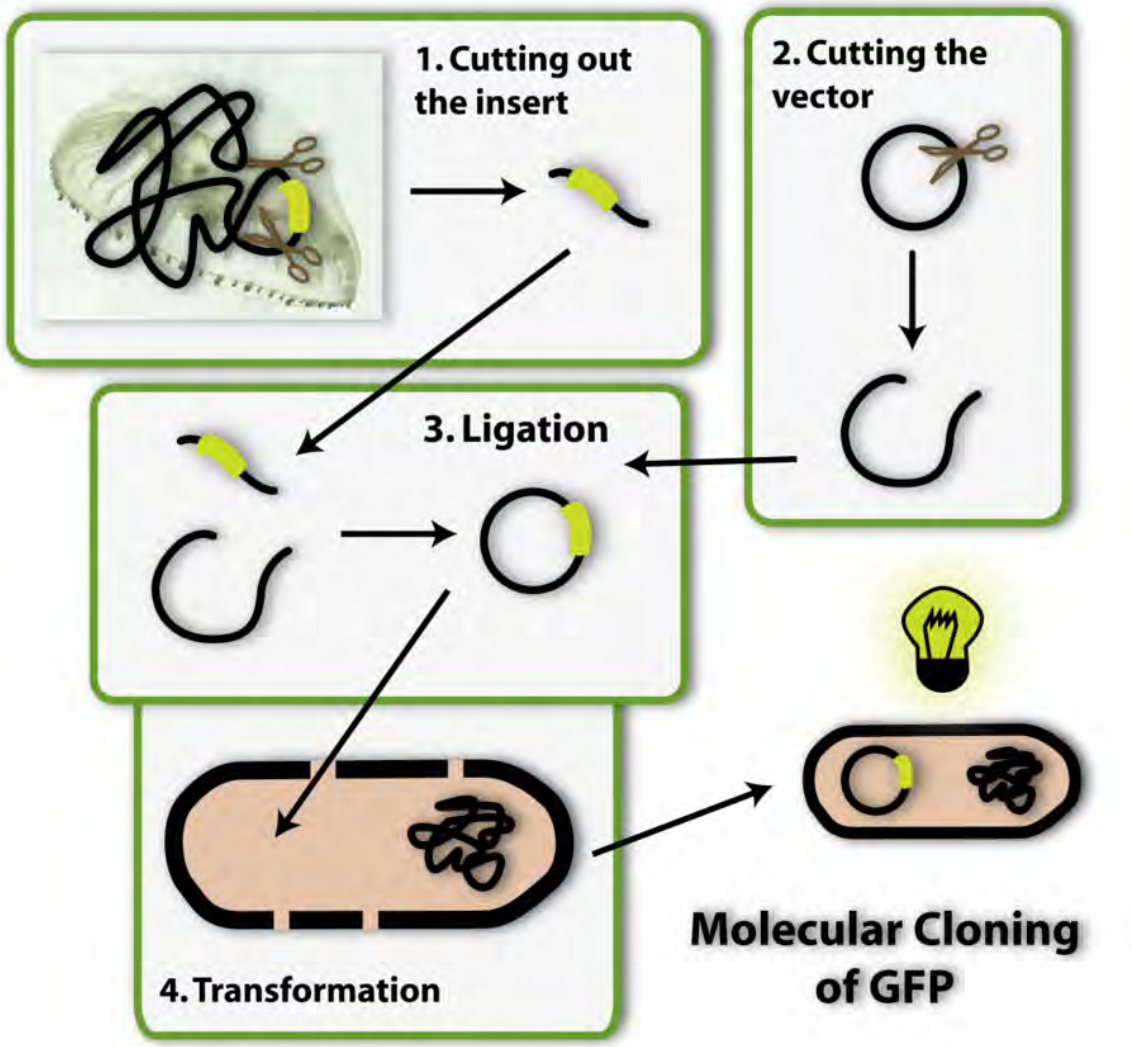
U.S. DEPARTMENT OF ENERGY

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

Anaerobic gut fungi colonizing wheat straw

Lab Methods: Molecular Cloning



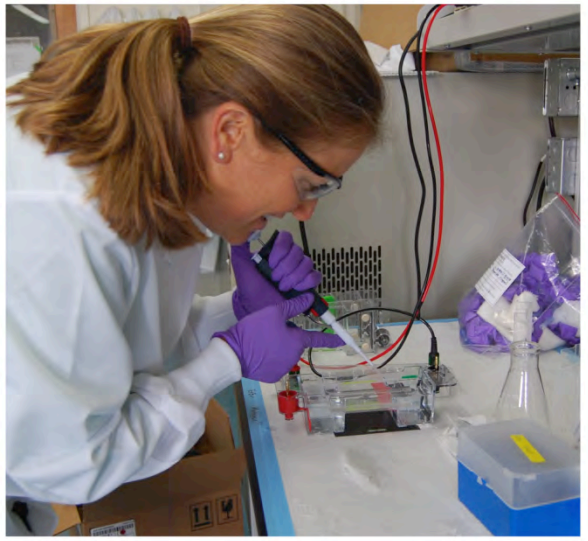
Project Workflow

Generate source materials

Project Workflow

Generate source materials

Run materials on a gel to confirm success



Project Workflow

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

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Overdigest insert and vector

Cleanup reaction and nanodrop to confirm concentration

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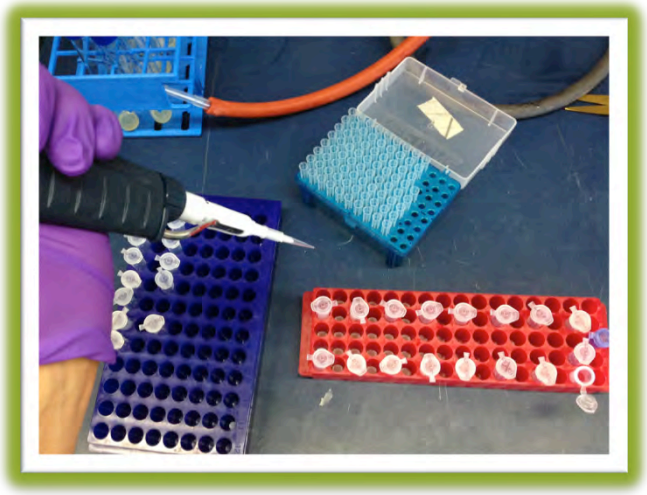
Ligate insert and vector and plate for overnight growth



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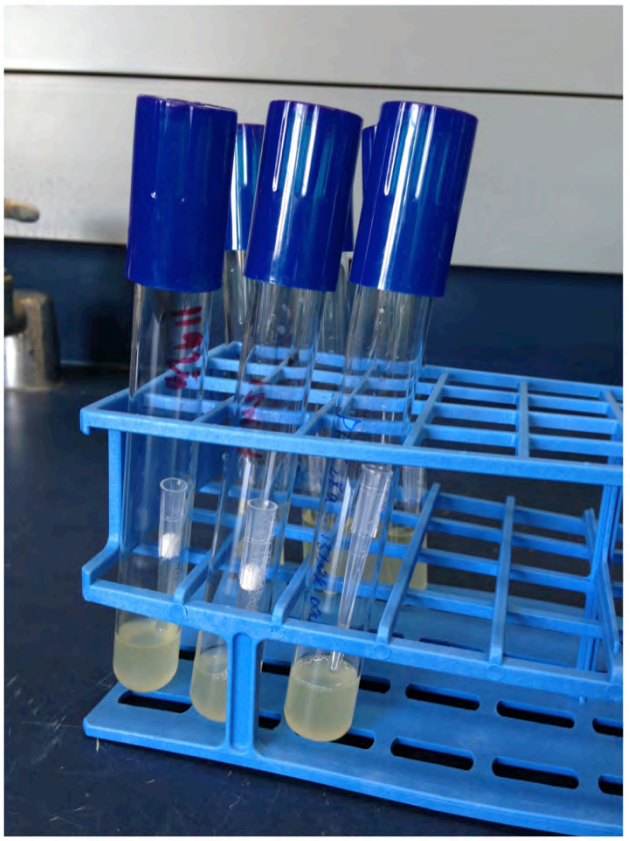
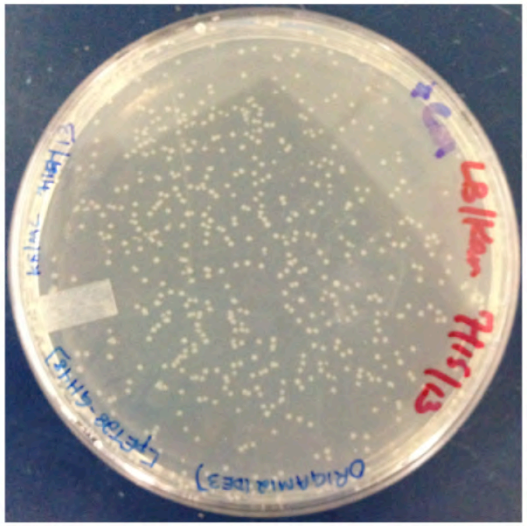


Check and screen colonies in selective medium overnight



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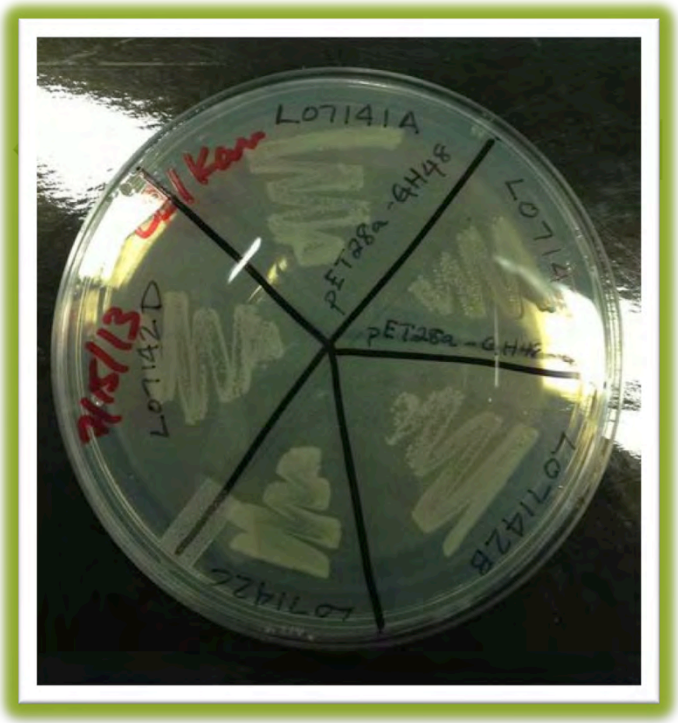

Project Workflow

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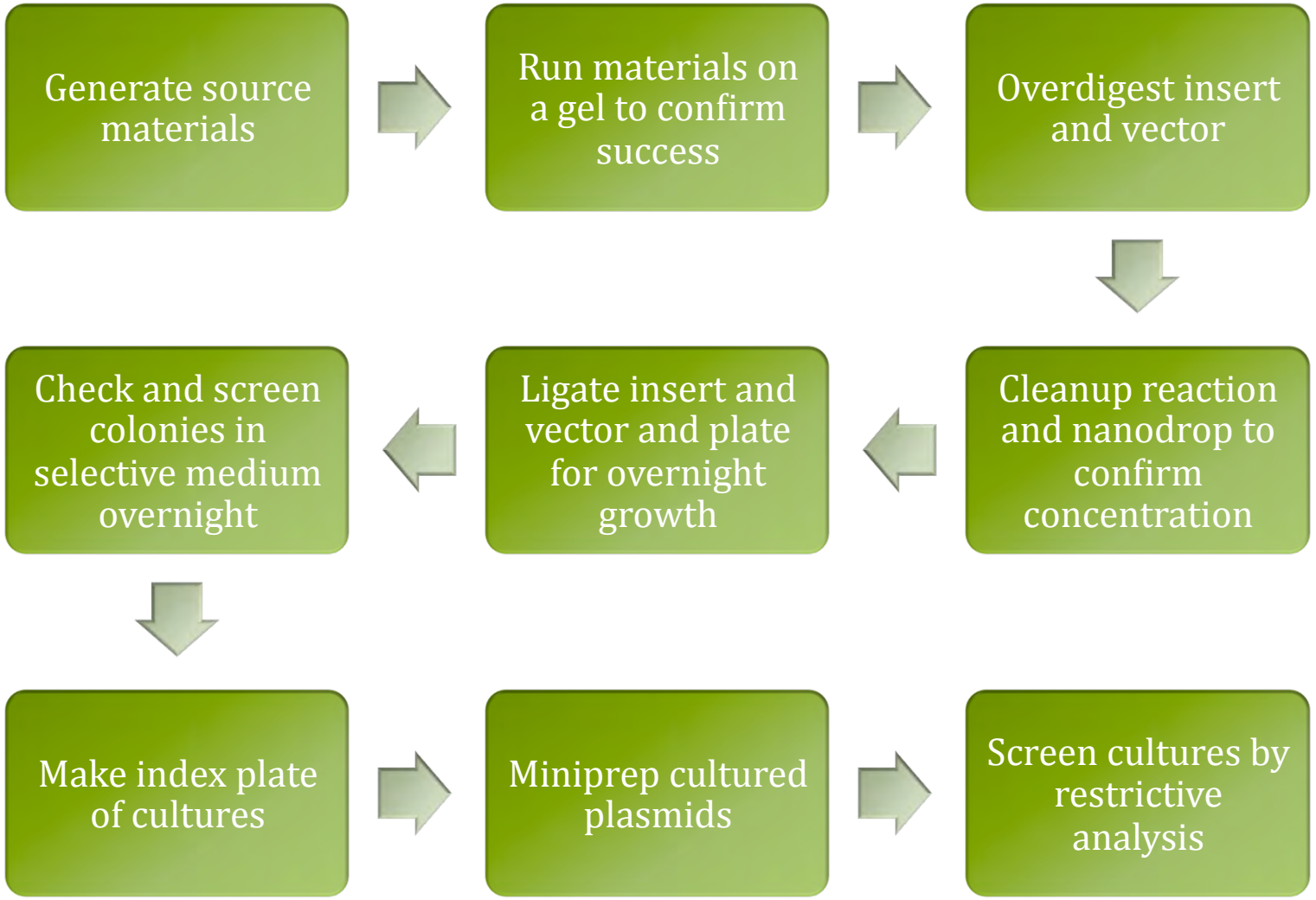
Make index plate of cultures

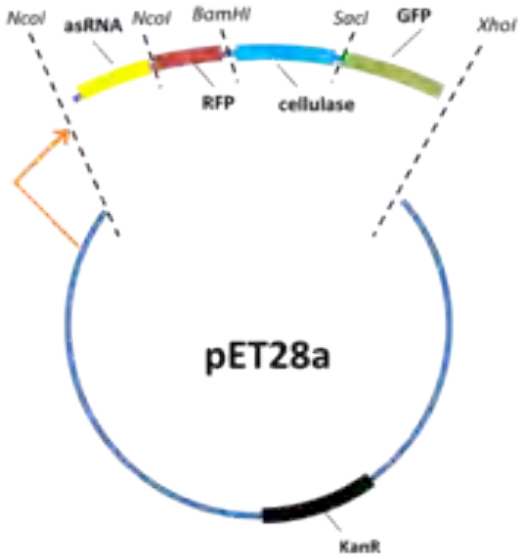
Miniprep cultured plasmids

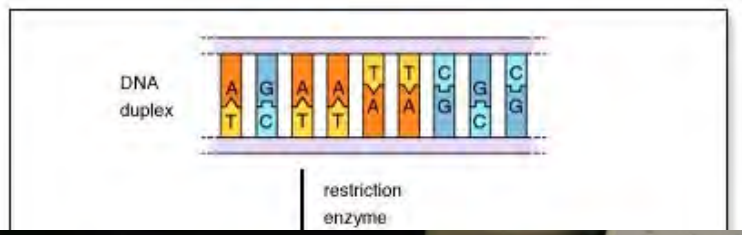
Project Workflow

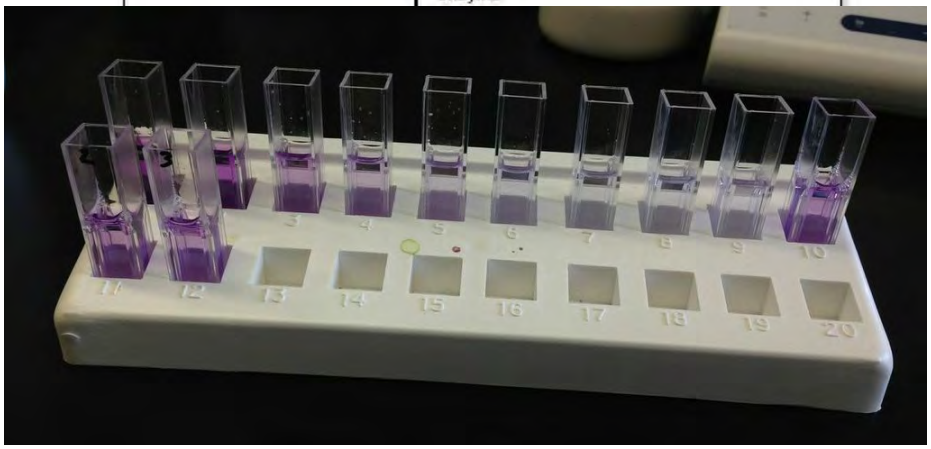


Lab Methods

- 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

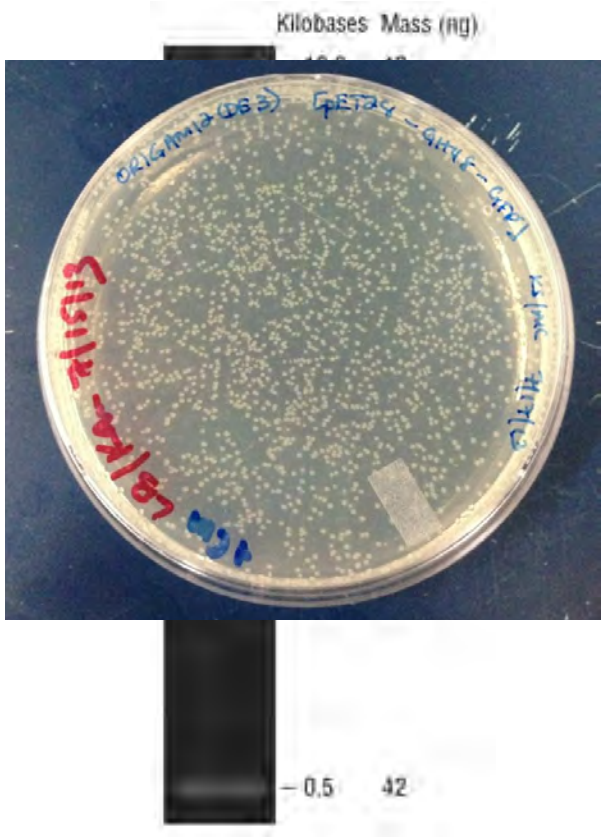






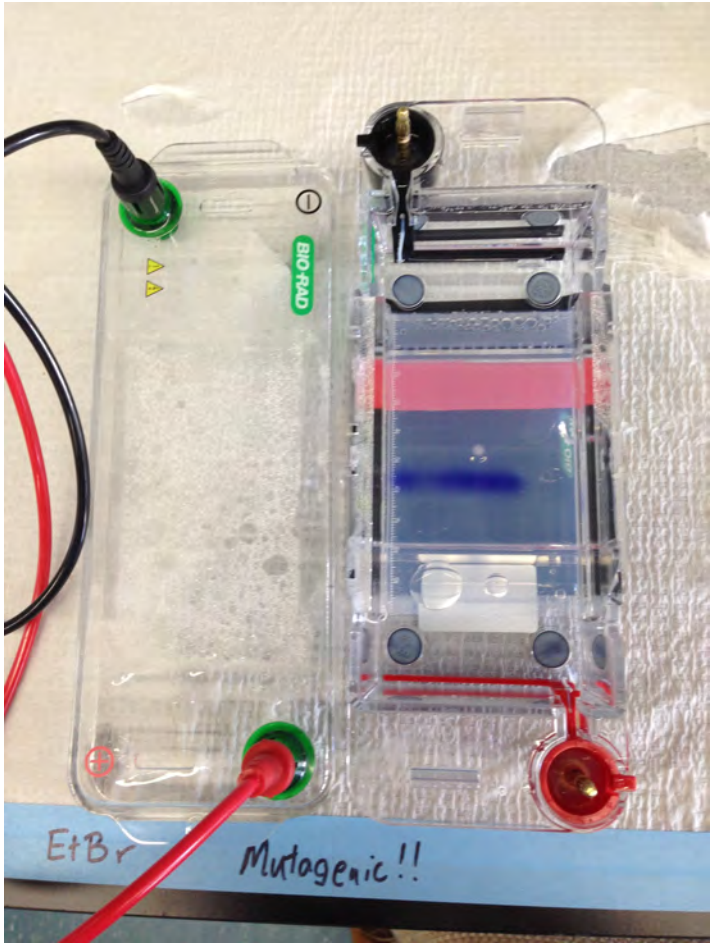
Data Sources

- 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



Data Retrieval Method

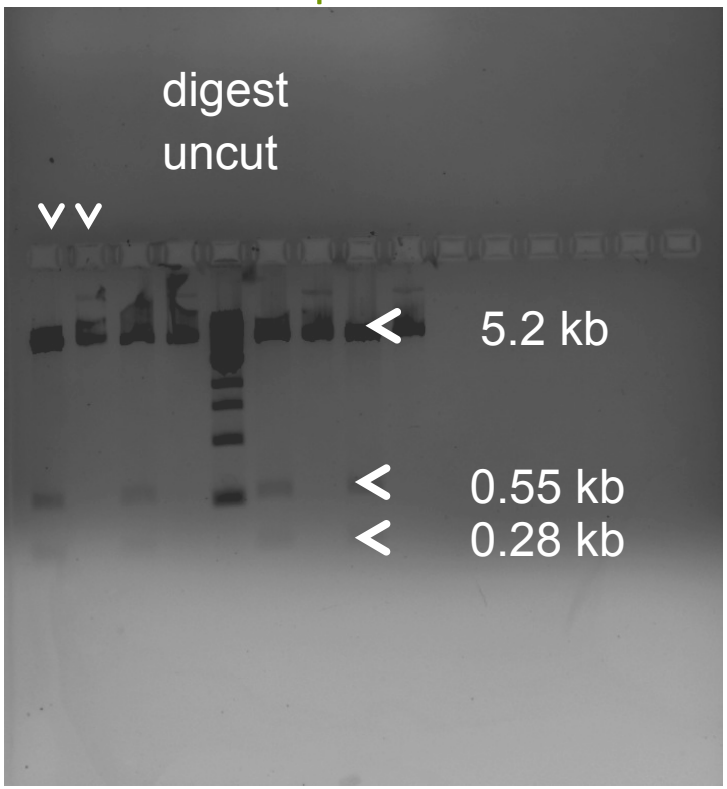
- Agarose gel electrophoresis is used in molecular biology to separate a DNA
- The DNA is separated by fragment length
- As the current runs through the agarose gel, DNA fragments are pulled through based on size
- The gel is then imaged and analyzed
- DNA can also be extracted from the gel with UV illumination



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

pET-28A GFP Ligation Samples A-D

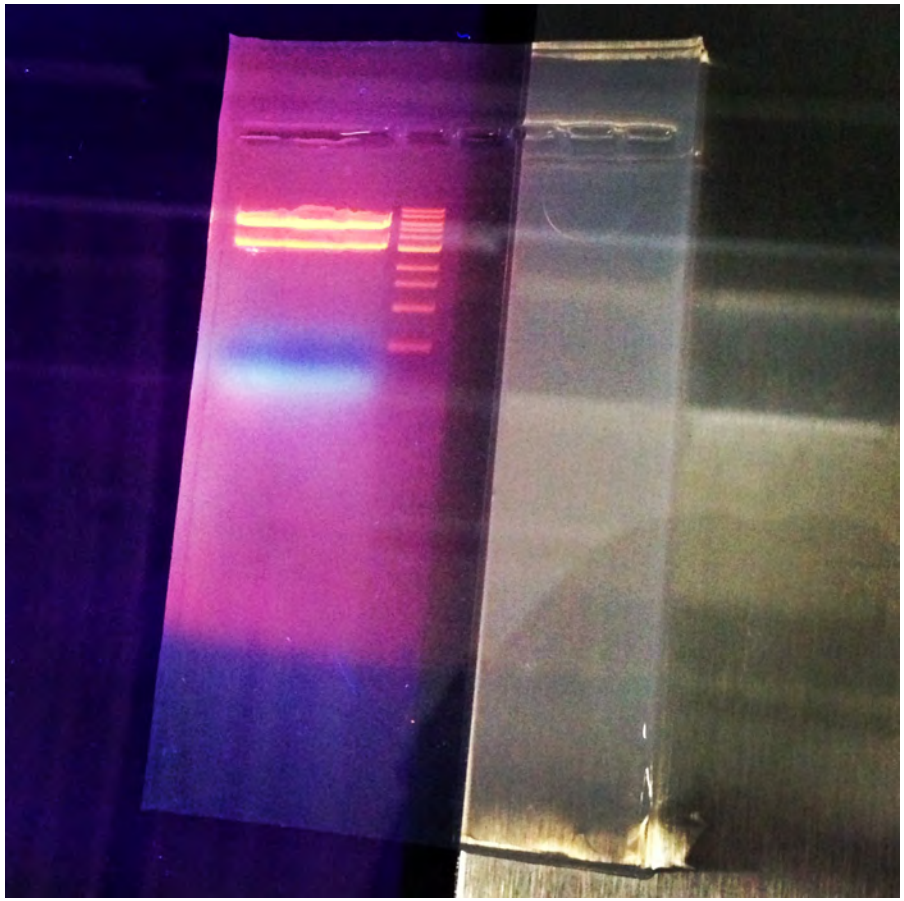


Challenges

Troubleshooting

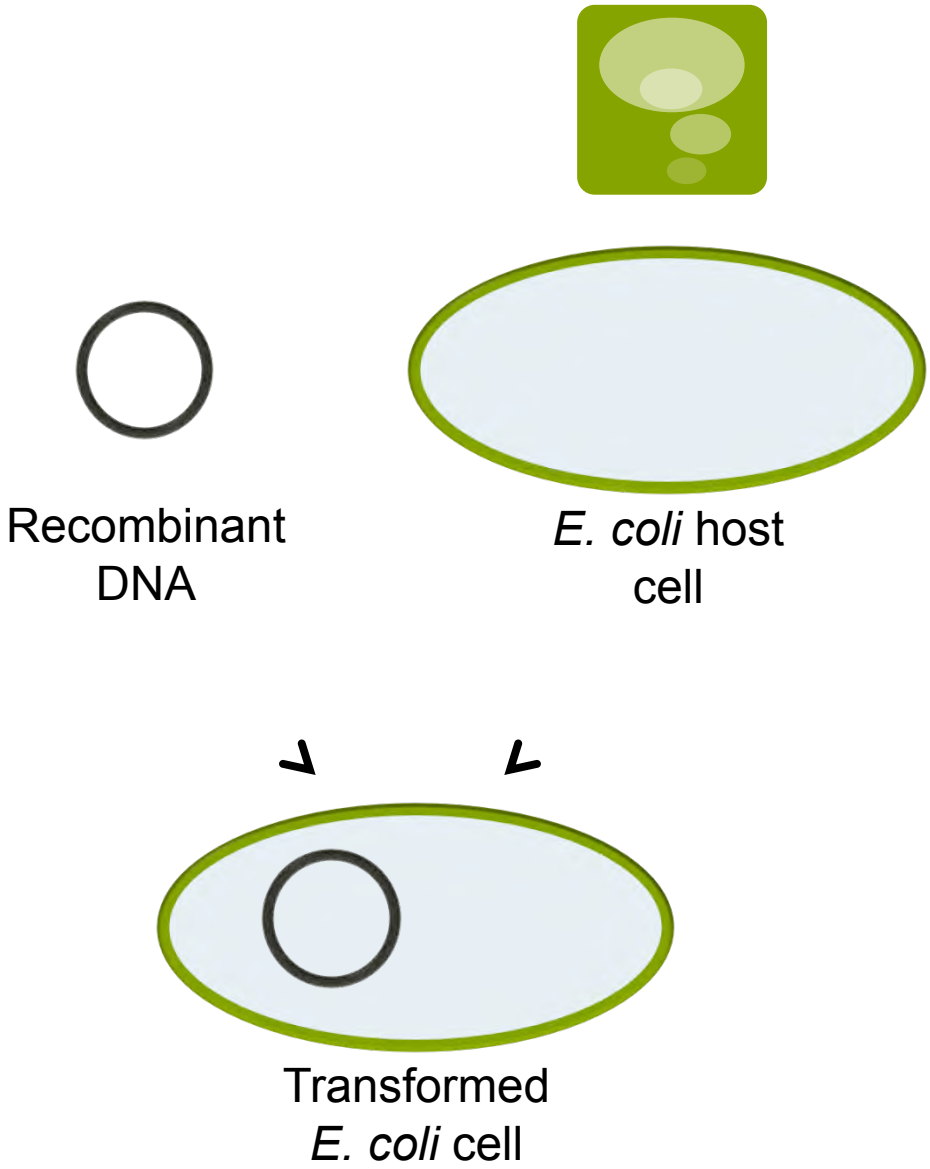
- With most of the problems we are able to problem solve a solution
  - Increase the concentration of our samples
  - Change the thermocycler protocol

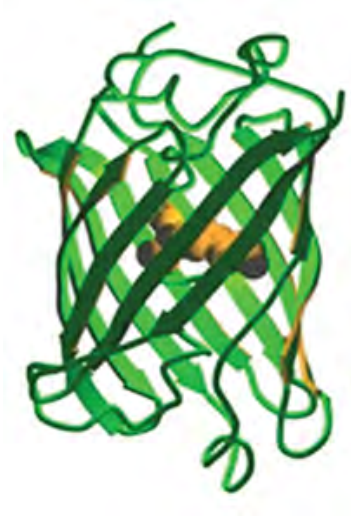
Successful PCR



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





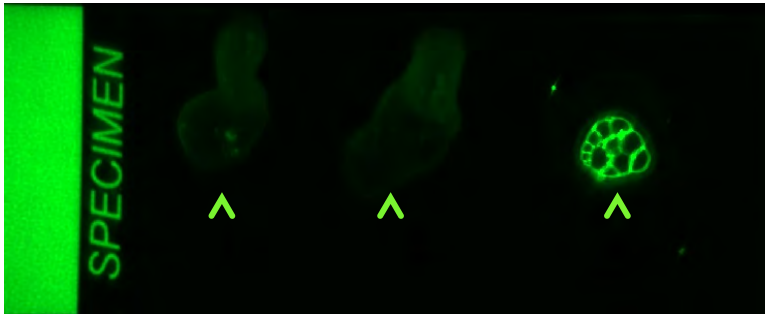
Future Work

- 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

Conclusion

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.



GH48 GFP

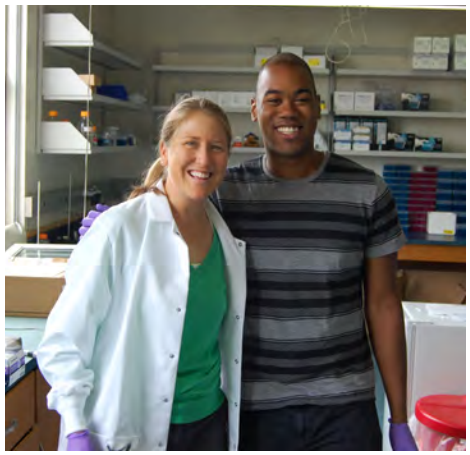
GH48

No expression

Classroom Application

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

Acknowledgments



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O'Malley Lab Group

Frank Kinnaman

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UCSB Materials Research Lab

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.