# Construction of Plasmid DNA for Heterologous Expression of Ester Forming Enzymes

Melody Cymbor, Chemical Engineering
Mentor: Dr. Arul Varman, Assistant Professor
School for Engineering of Matter, Transport and Energy

## Introduction

**Abstract**

Developing a microbial system increasing production of sustainable solvents like ethyl lactate have environmental and economic impacts. Prior bioinformatics analysis showed ATF1, ATF2 and LgATF1 to be potential genes. In silico and in vitro laboratory studies were performed to confirm their functionality in ester biosynthesis.

PCR was used to amplify both a small section of DNA (*cgATF1 gene) and the plasmid (pCRB204)* determined by primers. *Then, CPEC cloning was performed where insert and vector* get compatible ends to connect to each other. To finish, heat shock bacterial transformation in E.Coli of these genes was performed followed by Colony PCR and gel electrophoresis in order to verify that the desired gene is present in the plasmid.

**Motivation**

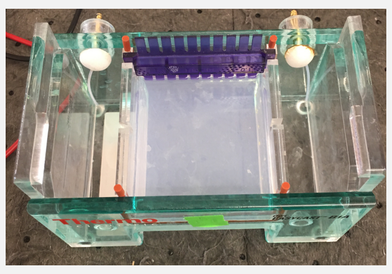
- Current chemical solvents' industries are petroleum-based, nonbiodegradable, toxic and non-renewable
- Ethyl lactate could be used as an biodegradable alternative, renewable
- Increasing the production of sustainable solvents such as ethyl lactate is urging
- Using agricultural products as raw materials
- Exploring more such genes that can improve its yield
- Exploring new esterase or acyl alcohol transferase genes
- The economic possibilities of its production as a green solvent is very consequent
- However the production means remains costly

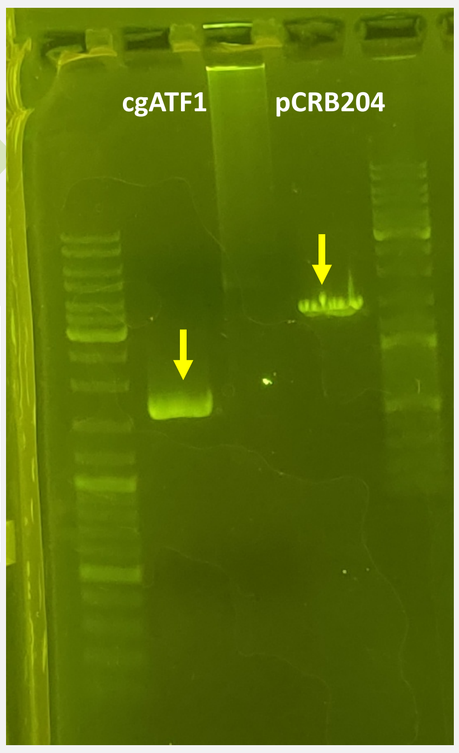
Goal: Will ethyl lactate be able to be produced in a more efficient way?

## Approach/Results/Discussion

***PCR Amplification of***
*Insert: cgATF1 gene*
*Vector: pCRB204*

Agarose gel electrophoresis






Gel purify the fragments

***CPEC Cloning***

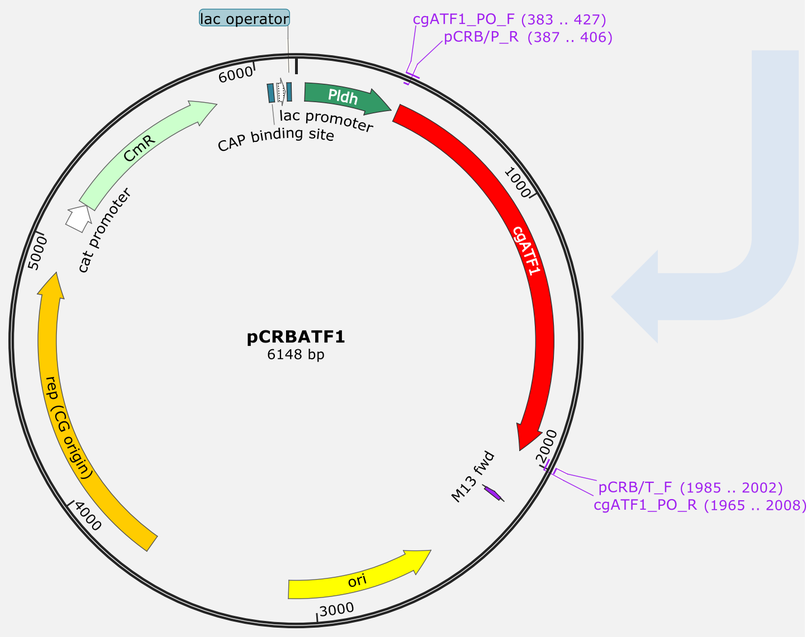
***CPEC REACTION MIX***

| Initial concentration | Volume per 20 µl reaction | Final amount per 20µl reaction |
|---|---|---|
| Phusion HF buffer (5x) | 4 µl | 1x |
| dNTP mix (40 nM) | 0.4 µl | 0.8nM |
| Phusion high-fidelity DNA polymerase (2 U $\mu l^{-1}$) | 0.2 µl | 1 u |
| Vector DNA | Variable | 100-200 ng |
| Insert gene library (-ies) | Variable | -- |
| $ddH_20$ | Up to 20 µl | |

*Plasmid synthesized due to overlaps in CPEC*

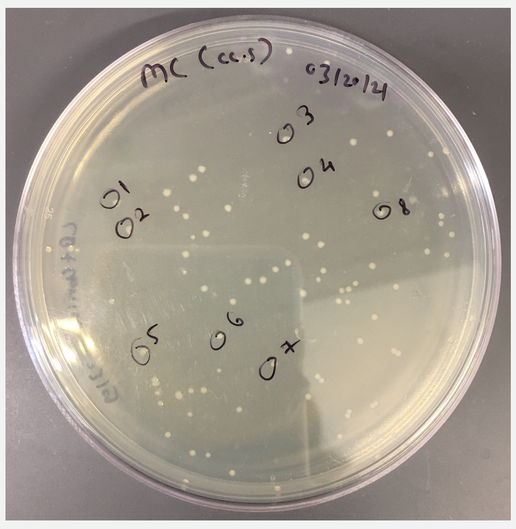
***CPEC REACTION PROTOCOL ON PCR MACHINE***

| Cycle number | Denature | Slow ramp anneal | Anneal | Extend |
|---|---|---|---|---|
| 1 | 98°C, 30s | --- | --- | --- |
| 2-26 | 98°C, 10s | 70 to 55°C, 3min | 55°C, 30s | 72°C, 10-20s per kb |
| 27 | --- | --- | --- | 72°C, 5 min |

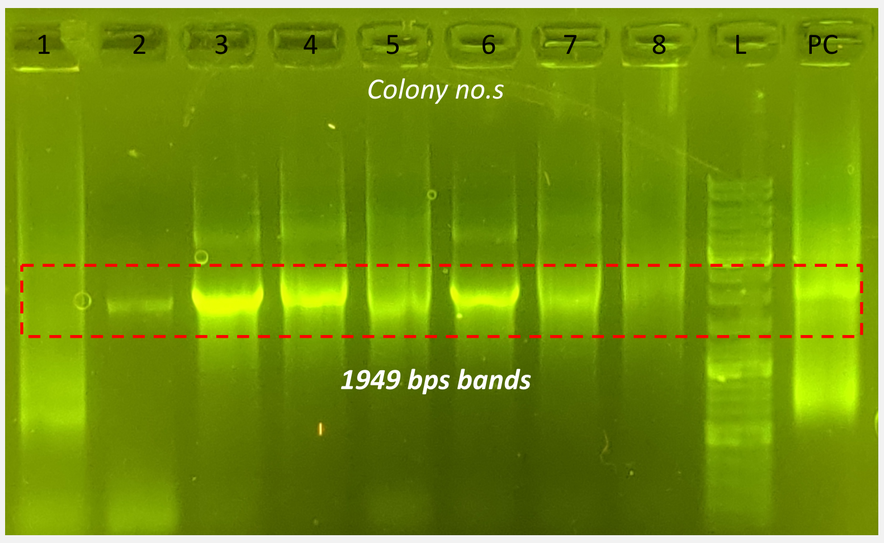



***Transformation In E.Coli***

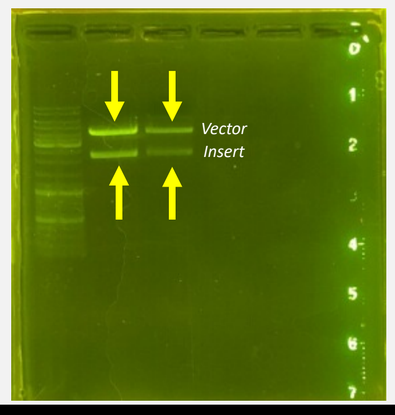
*Transformant colonies*




***Colony PCR***




***Successful cloning***




***Inoculate the positive colonies***

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***Future prospect:***
*Perform plasmid-prep and purify the plasmid for transforming into Corynebacterium glutamicum*

FURI

Ira A. Fulton Schools of Engineering
Arizona State University