



# Investigation of Esterases' Activity on Fluorescein Diacetate (FDA)

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## Abstract

Peroxisomes are the organelles that are found in all eukaryotic cells. They are mostly related to lipid metabolism and oxidation of substances. In the eukaryotic cells, proteins can also be targeted towards the peroxisomes, known as the matrix protein import [3]. The membrane of these peroxisomes is essential as they allow passage for certain important substances. In this project, we are going to find out the most active esterase with the most effective digestion on fluorescein diacetate that will then be inserted in the peroxisome to test its membrane permeability. Five candidates of esterases will be cloned, expressed using golden gate cloning method and purified from the *E. coli*. *In vitro* activity assay of FDA and five different esterases will determine the best candidate that can be used in our *in vivo* assay in the peroxisome. In future work, we will be localizing the best esterase with the FDA to find out the permeability of the peroxisomal membrane. Being able to bioengineer the peroxisomal membrane will be advantageous in isolating proteins and other biochemical substances.

## Introduction

### Esterases<sup>3</sup>

- Five genes encoding the esterases/hydrolases with potential activity on FDA identified from the yeast, *S. cerevisiae*.
- YJL068C, IAH1, PPE1, EEB1, EHT1
- Functions: Hydrolyze fatty acid esters, isoamyl acetate; Demethylate phosphoprotein in mitochondria; Synthesize fatty acid ethyl esters.

### Fluorescein Diacetate (FDA)<sup>2</sup>

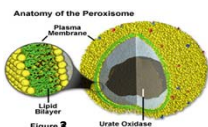
- Colorless in solution.
- When hydrolyzed, fluorescein is produced; fluorescence produced.



- Used in measuring the activity of the enzyme and staining for cells visibility.

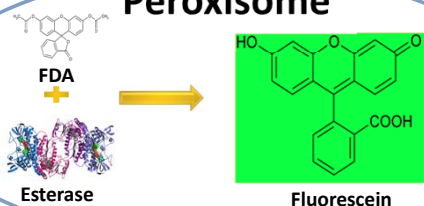
### Peroxisomes<sup>1</sup>

- Found in all eukaryotic cells.
- Worked together with other organelles such as chloroplast.
- Functions: Oxidation of substances.
  - Hydrogen Peroxide breakdowns.
  - Post translational proteins import.
  - Lipid metabolism.
- Impermeable to large substances.
- Small metabolites are able to enter.
- The extent of permeability is still unknown.



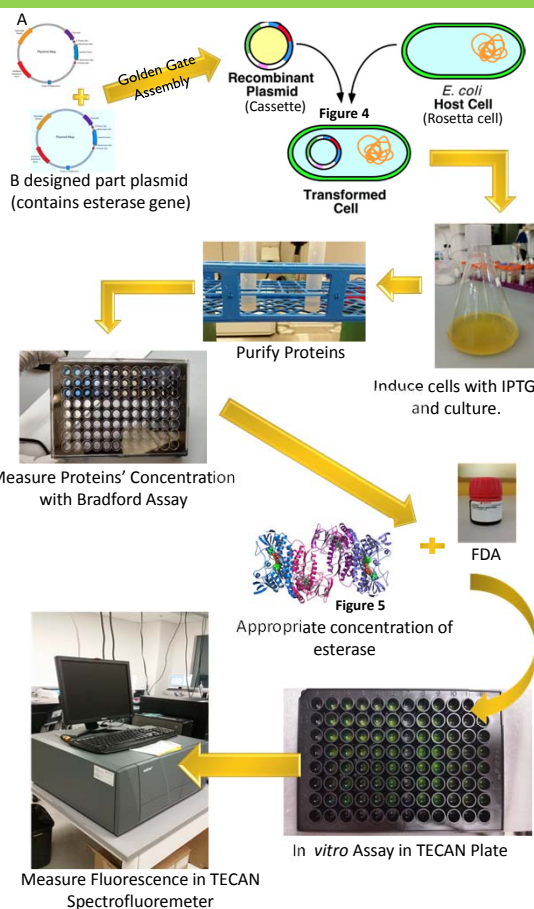
## Motivation

### Peroxisome



- To test for membrane permeability of peroxisomes.
- FDA and the best esterase will be targeted towards the peroxisome with a tag in the yeast, *S. cerevisiae*.
- The entire reaction of fluorescein diacetate and the best esterase will again take place inside a peroxisome.
- The movement and position of the fluorescein will be observed under the fluorescent microscope.
- Depending on the movement of the fluorescein, the permeability of the peroxisome can be tested.

## Method

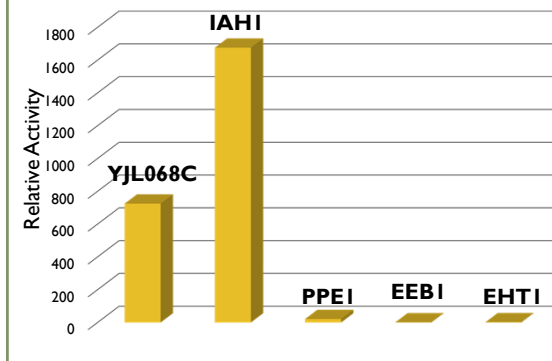


## Results

- All columns with FDA and esterases produced fluorescence.
- Fluorescein produced in green column as shown in the figure.
- All esterases showed activities on FDA.



### Enzymatic Activity On FDA



- YJL068C protein had the enzymatic activity of 724 relative to EEB1.
- IAH1 HAD the relative activity of 1676 to EEB1.
- PPE1 only had 21 relative activity.
- EEB1 and EHT1 only had low activity of 1.
- IAH 1 esterase had the highest relative activity on FDA.

## Conclusion and Future Work

We found out that IAH1 coding protein, Isoamyl acetate-hydrolyzing esterase, had the most efficient activity on fluorescein diacetate. This esterase will be used in testing the permeability of the peroxisomal membrane by localizing it together with the FDA in yeast, *S. cerevisiae*. The previous experiments have indicated that peroxisomes may be permeable to substances, but the extent of the permeability remains a mystery. Being able to bioengineer peroxisomal membrane will be advantageous in isolating the proteins, allowing us to mass produce the drugs and other substances easily.

## Citations

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