# GENETIC ENGINEERING OF YARROWIA LIPOLYTICA Po1g FOR THE CONSOLIDATED BIO-PROCESSING OF BIO-DIESEL FROM LIGNOCELLULOSIC BIOMASS

## INTRODUCTION

A Crabtree-negative ascomycete yeast called Y. lipolytica first caught attention for its exceptional proteolytic and lipolytic abilities. Wild-type isolates of this yeast often come from lipid- and/or protein- rich environments, particularly from meat and dairy products, in agreement with the high levels of secreted enzymatic activity.

By expressing glycoside hydrolase, the cel-48 gene introduced by genetic engineering of Y. lipolytica will considerably aid in the breakdown of lignocellulosic biomaterial.

1. Purification of Recombinant Plasmid PYLEX1-Cel48
2. Linearisation of the PYLEX1-Cel48
3. *Y. lipolytica* Po1g transformation
4. Screening for Recombinant Clones by colony PCR
5. Agarose Gel Electrophoresis

## RESULTS

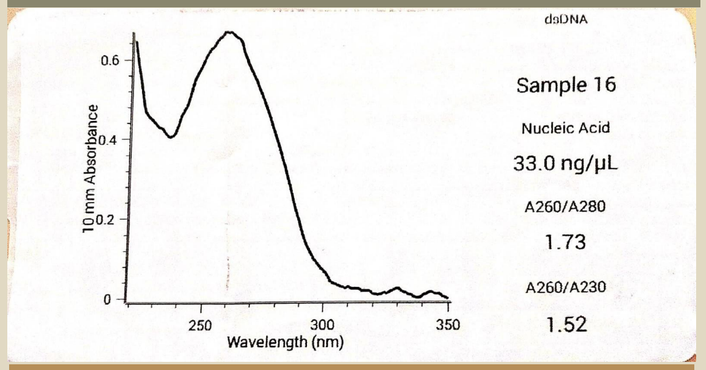
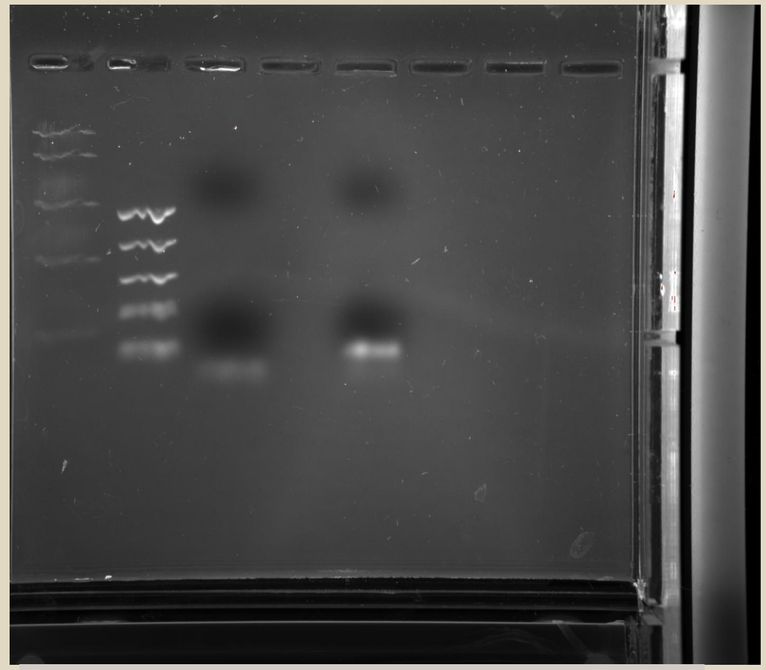



Figure1. The DNA purity of pYLEX1-Cel 48 at absorbance ratio 260/280 AND 260/230, quantification results shows that nucleic acid content is 33.0ng/µl.

Figure2.Plate containing transformed Y. lipolytica cells



Reconstituted cells of Y. lipolytica/Pylex1-cel48, Pylex1-cel48 (positive control), and negative control, respectively, are used as the PCR products in wells (2, 3, and 4 in Figure 3). Primer dimers were visible, and no bands were generated.

## CONCLUSION

The colonies for transformed Y. lipolytica cells were observed on the agar plate and the pYLEX1-Cel 48 cloned DNA sample in TOP10 E. coli was pure, but the gel electrophoresis problems, such as inadequate DNA quantity and DNA degradation by nuclease, prevented the insert from being located at the desired base pair. Thus, the experiment needs revision for the verification of plasmid insert through PCR by overcoming the problems in previous experiment.