

Subklona Gen Sintetik Lipase *Thermomyces Lanuginosus* Tsikl. 1899 menggunakan peptida sinyal asli pada *Pichia Pastoris* Guillerm. Phaff 1956 dan karakterisasi parsial produk gennya = Subclone Synthetic Lipase Gene *Thermomyces Lanuginosus* Tsikl 1899 with original signal peptide into *Pichia Pastoris* Guillerm Phaff 1956 and partial characterization of the gene product

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Abstrak

Lipase merupakan salah satu enzim yang penting di industri enzim, dan banyak digunakan dalam pembuatan zat aditif makanan, kosmetik, dan industri farmasi. Penelitian sebelumnya yang dilakukan di PTB-Laptiab BPPT, pengklonaan gen sintetik *T. lanuginosus* lipase pada *B. subtilis* memiliki aktivitas lipase yang rendah yaitu sebesar 1,488 U/mg.

Penelitian ini bertujuan untuk mengklona gen sintetik *T. lanuginosus* lipase TLL ke dalam vektor ekspresi *Pichia pastoris* menggunakan sinyal peptida asli TLL. Gen TLL yang mengandung sinyal peptida asli diamplifikasi dengan PCR, dan disisipkan ke dalam pPICZ? A di antara situs XhoI dan XbaI, kemudian ditransformasikan ke dalam sel kompeten *E. coli* DH5?.

Hasil transformasi dipilih dua rekombinan positif untuk dilakukan analisis sekuensing. Hasil sekuensing, kedua rekombinan mengandung gen target lipase. Plasmid yang telah dikonfirmasi kemudian dilinearisasi dan ditransformasikan ke dalam *P. pastoris* X-33 dengan menggunakan metoda elektroporasi. Gen *T. lanuginosus* lipase berhasil diintegrasikan ke dalam kromosom *P. pastoris* X-33, yang ditunjukkan dengan terbentuknya zona bening pada media Yeast extract Peptone Dextrose Tributyrin YPD.TB agar yang mengandung zeocin.

Thermomyces lanuginosus lipase memiliki daerah open reading frame ORF 916 bp yang mengkode 291 asam amino dengan massa molekul teoritis 35 kDa. Enzim rekombinan *T. lanuginosus* memiliki suhu optimum 80 C dan pH optimum 8.

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Lipase is one of the most important industrial enzymes, which is widely used in the preparation of food additives, cosmetics, and pharmaceutical industries. In the previous study, we have cloned synthetic *Thermomyces lanuginosus* lipase gene into *Bacillus subtilis* and *Escherichia coli* and resulting low expression of enzyme activity.

The aim of this research was to construct the *T. lanuginosus* lipase TLL gene into *P. pastoris* vector expression with TLL original signal peptide. *Thermomyces lanuginosus* lipase gene was amplified by PCR and contained original signal peptide and then inserted into pPICZ A between XhoI and XbaI site, and transformed into competent cell *E. coli* DH5. From the transformant, two of positive recombinants were analyzed by sequencing analysis.

As the result, both of two recombinant have a positive target gene which has lipase gene. The correct plasmid was linearized and then was transformed into *P. pastoris* X 33 by electroporation method.

Thermomyces lanuginosus synthetic gene lipase has been successfully integrated into chromosome of *P. pastoris* X 33, which revealed by clear zones around the colony on Yeast extract Peptone Dextrose

Tributylin YPD.TB plate with zeocin.

The *Thermomyces lanuginosus* lipase had an open reading frame of 916 bp encoding TLL of 291 amino acids with theoretical molecular mass of 35 kDa. The recombinant enzyme, *T. lanuginosus* lipase had optimal temperature at 80 C and optimal pH at pH 8.0.