TRANSFORMASI PLASMID REKOMBINAN PEMBAWA GEN *PhaA* PADA *Escherichia coli* BL21

Azizah Tyas Nugrahanty
20/454736/BI/10431

Dosen Pembimbing: Ganies Riza Aristya, S.Si., M.Sc., Ph.D.

INTISARI

Polyhydroxybutyrate (PHB) merupakan biopolimer yang ramah lingkungan dan dapat disintesis dari bakteri. Salah satu gen yang berperan penting dalam sintesis PHB pada bakteri adalah *PhaA.* Gen *PhaA* akan mengkode *acetoacetyl-CoA* pada *Escherichia coli* sebagai substrat utama biosintesis PHB. Penelitian ini bertujuan untuk melakukan transformasi gen *PhaA* pengkode *acetoacetyl-CoA* pada *Escherichia coli* BL21, serta melakukan evaluasi hasil transformasi melalui pendekatan PCR, Sanger *sequencing*, dan pemotongan dengan enzim restriksi. Insersi plasmid rekombinan yang membawa gen *PhaA* pada sel *E. coli* BL21 dilakukan dengan metode elektroporasi dan diseleksi dengan menumbuhkan bakteri pada medium LB yang mengandung ampisilin. Berdasarkan hasil PCR koloni dan Sanger *sequencing*, diperoleh bahwa gen *PhaA* telah berhasil ditransformasikan pada *E. coli* BL21 yang ditunjukkan dengan kesesuaian terhadap gen target *PhaA* (KP681582.1). Hal ini didukung dengan data *digest test* menggunakan enzim restriksi *Nco*I dan *Sbf*I yang menunjukkan adanya pita gen *PhaA* pada 1195 bp.

Kata Kunci: *E. coli* BL21, gen *PhaA*, plasmid rekombinan, *polyhydroxybutyrate*, transformasi genetik

TRANSFORMATION OF RECOMBINANT PLASMID CARRYING THE *PhaA* GENE IN *Escherichia coli* BL21

Azizah Tyas Nugrahanty
20/454736/BI/10431

Supervisor: Ganies Riza Aristya, S.Si., M.Sc., Ph.D.

ABSTRACT

Polyhydroxybutyrate (PHB) is an environmentally friendly biopolymer and can be synthesized from bacteria. One of the genes that plays an important role in PHB synthesis in bacteria is *PhaA*. The *PhaA* gene is encoding acetoacetyl-CoA in *Escherichia coli* as the main substrate for PHB biosynthesis. This research aims to carry out transformation of the *PhaA* gene encoding acetoacetyl-CoA in *Escherichia coli* BL21, as well as evaluating the transformation results using PCR approaches, Sanger sequencing, and digest with restriction enzymes. Insertion of the recombinant plasmid carrying the *PhaA* gene into *E. coli* BL21 cells was carried out using the electroporation method and selected in LB medium containing ampicillin. Based on the results of colony PCR and Sanger sequencing, it was found that the *PhaA* gene had been successfully transformed in *E. coli* BL21 as indicated by suitability for the *PhaA* target gene (KP681582.1). This is supported by digest test data using the restriction enzymes *Nco*I and *Sbf*I which shows the presence of the *PhaA* gene band at 1195 bp.

Keyword: *E. coli* BL21, genetic transformation, *PhaA* gene, polyhydroxybutyrate, recombinant plasmid