KONSTRUKSI PLASMID UNTUK EKSPRESI GEN PET (*POLYETHYLENE TEREPHTHALATE*) HIDROLASE DAN PENGARUH PROMOTER ANDERSON J23102 TERHADAP PERTUMBUHAN *Escherichia coli*

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INTISARI

PET (*Polyethylene Terephthalate)* merupakan polimer penyusun botol plastik kemasan. Polimer ini dapat didegradasi oleh enzim PETase yang diisolasi dari bakteri *Ideonella sakaiensis*. Optimasi produksi enzim PETase dapat dilakukan melalui *heterologous gene expression* pada bakteri yang telah terstandarisasi dalam rekayasa genetik, salah satunya yaitu *Escherichia coli*. Aktivitas promoter dan konstruksi vektor merupakan dua hal yang penting dalam *heterologous gene expression.* Selain itu, adanya teknik perakitan cepat terhadap komponen genetik juga dapat mempermudah *heterologous gene expression,* salah satunya melalui metode *MoClo Assembly* yang berdasar pada prinsip kerja enzim endonuklease restriksi tipe IIS. Penelitan ini dilakukan untuk mengetahui strategi konstruksi *expression cassette* dalam mengekspresikan PETase dengan MoClo *Assembly* melalui perakitan level 1 PETase-RFP dan level 0 PETase, melakukan perakitan promoter Anderson J23102 untuk ekspresi PETase dan GFP (pJ02B2PETase:Gm), serta mengetahui pengaruhnya terhadap pertumbuhan sel *Escherichia coli.* Pada perakitan level 1 PETase-RFP dengan MoClo *Assembly*, penurunan aktivitas enzimatis pada BsaI dan Eco31I sangat berpengaruh terhadap keberhasilan perakitan plasmid rekombinan. GFP yang diekspresikan mengindikasikan secara tidak langsung bahwa gen PETase pada pJ02B2PETase:Gm mampu diekspresikan dengan menggunakan promoter Anderson J23102 yang diperoleh dari CIDAR MoClo *Parts Library* (Densmore). Perbandingan kurva pertumbuhan *E. coli* transforman pJ02B2PETase:Gm dan non-transforman (*wildtype*) menunjukkan bahwa pertumbuhan *E. coli* transforman lebih lambat dibandingkan non transforman.

Kata kunci: *Escherichia coli*, MoClo *Assembly*, Promoter Anderson, PET, PETase

PLASMID CONSTRUCTION FOR PET (*POLYETHYLENE TEREPHTHALATE*) HYDROLASE GENE EXPRESSION AND THE EFFECT OF ANDERSON PROMOTER J23102 ON THE GROWTH OF *Escherichia coli*

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ABSTRACT

PET (Polyethylene Terephthalate) is a type of plastic polymer which is commonly used for food-beverage packaging. PET can be broken down into its building blocks using PETase which was first isolated from bacteria *Ideonella sakaiensis*. A heterologous gene expression in a standard engineered organism, such as *Escherichia coli* will be important for future implementation of PETase. The promoter activity and expression cassette construction are two concerns of heterologous gene expression. This study aims to construct the PETase expression cassette using MoClo assembly, a type IIS restriction endonuclease-based assembly technique for rapid construction of genetic parts. In addition, level 0 of PETase for MoClo assembly will be assembled. Furthermore, to learn about the effect of Anderson promoter J23102 on the growth of bacteria, Anderson promoter J23102 will be used for PETase and GFP expression by constructing pJ02B2PETase:Gm plasmid. The assembly of PETase and RFP transcriptional units using MoClo assembly was highly affected by the performance of the enzyme, since there is no recombinant plasmid were formed with low activity of BsaI. The expression of GFP indicated that the PETase gene in pJ02B2PETase:Gm was able to be expressed under the J23102 promoter regulation. The comparison of the growth curves of the transformant *E.coli* pJ02B2PETase:Gm and the non-transformant (wildtype) showed that the growth of the transformant *E.coli* was slower than the non-transformant.

Keywords: *Escherichia coli*, MoClo Assembly, Anderson Promoter, PET, PETase