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Subkloning Gen Sintetik anti-TfR-scFv dan Fusi Gen scFv-egfp ke dalam Vektor Ekspresi pPICZ A pada Escherichia coli TOP10F = Subcloning of anti-TfR-scFv Synthetic Gene and scFv-egfp Fusion Gene into pPICZ A Expression Vector on Escherichia coli TOP10F

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Abstrak

Gen sintetik anti-TfR-scFv telah dikonstruksi untuk mengkode protein rekombinan anti-TfR-scFv. Protein rekombinan tersebut dirancang untuk menghambat ikatan antara molekul transferin dengan reseptor transferin (TfR). Gen enhanced green fluorescent protein (egfp) digunakan dalam penelitian sebagai reporter gene untuk mengetahui ekspresi gen anti-TfR-scFv. Penelitian bertujuan untuk mensubkloning gen anti-TfR-scFv dan fusi gen scFv-egfp ke dalam vektor ekspresi pPICZ A pada Escherichia coli (E. coli) TOP10F'. Gen anti-TfR-scFv yang berada di dalam pJ-TfR-scFv diamplifikasi menggunakan teknik PCR. Fragmen gen anti-TfR-scFv yang berukuran 747 bp kemudian diligasi pada situs restriksi EcoRI pada vektor ekspresi pPICZ A dan ditransformasi ke dalam E. coli TOP10F' untuk memperoleh vektor rekombinan konstruksi pertama (pPICZ_TfR). Gen egfp yang berukuran 753 bp diligasi dengan vektor rekombinan pPICZ_TfR dan ditranformasi ke dalam E. coli TOP10F' untuk memperoleh vektor rekombinan konstruksi kedua (pPICZ_TfR_EGFP). Hasil penelitian menunjukkan bahwa kedua vektor rekombinan baik pPICZ_TfR maupun pPICZ_TfR_EGFP telah berhasil ditransformasikan ke dalam E. coli TOP10F' dengan efisiensi transformasi 4,94 x 103 cfu/g dan 6,74 x 103 cfu/g plasmid DNA pada medium LSLB yang mengandung 25 g/ml antibiotik zeocin. Hasil verifikasi menggunakan PCR, digesti, dan sekuensing menunjukkan bahwa gen anti-TfR-scFv dan fusi gen scFv- egfp berhasil disubkloning ke dalam vektor ekspresi pPICZ A.

.....The anti-TfR-scFv synthetic gene is a gene encoding single chain variable fragment that prevents the bond between transferrin receptor (TfR) and transferrin molecule. The enhanced green fluorescent protein (egfp) gene was used in this study as reporter gene for monitoring expression of anti-TfR-scFv gene. The study was aimed to subclone anti-TfR-scFv synthetic gene and scFv-egfp fusion gene into pPICZ A expression vector on E. coli TOP10F'. The anti-TfR-scFv synthetic gene had been cloned previously in the cloning vector pJ-TfR-scFv and was amplified by PCR technique. Furthermore, the 747 bp fragment of anti-TfR- scFv synthetic gene was ligated into EcoRI restriction site in pPICZ A expression vector and transformed into E. coli TOP10F' in order to obtain type I recombinant vector named pPICZ_TfR. The 753 bp fragment of egfp gene was ligated to recombinant vector pPICZ_TfR in order to obtain type II recombinant vector named pPICZ_TfR_EGFP. The results showed that both of recombinant vectors pPICZ_TfR and pPICZ_TfR_EGFP were successfully transformed into E. coli TOP10F' with efficiency of transformation 4,94 x 103 cfu/g dan 6,74 x 103 cfu/g DNA plasmid in LSLB medium containing 25 g/ml zeocin. The results of verification by PCR method, digestion, and sequencing showed that anti-TfR-scFv synthetic gene and scFv-egfp fusion gene were successfully subcloned into pPICZ A expression vector.