Subkloning gen EGFRvIII di pPICZalpha dan transformasi plasmid rekombinan pPICZalpha-EGFRvIII-BFP ke pichia pastor = Subcloning EGFRvIII gene in pPICZalpha and transformation recombinant plasmid pPICZalpha-EGFRvIII-BFP into pichia pastoris

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Abstrak

ABSTRAK
Epidermal growth factor receptor variant III (EGFRvIII) adalah salah satu varian mutan dari protein human EGFR. Mutasi yang terjadi pada EGFR menyebabkan terjadinya kanker. Berbagai mutan EGFR, termasuk EGFRvIII, telah banyak dipelajari karena potensinya sebagai molekul target dalam terapi kanker. Gen penyandi domain ekstraselular EGFRvIII telah berhasil dikonstruksi pada penelitian sebelumnya untuk studi ekspresi protein sebagai molekul target dalam terapi kanker. Penelitian bertujuan untuk subkloning gen EGFRvIII ke dalam plasmid ekspresi pPICZ? dan transformasi plasmid rekombinan ke dalam sel Pichia patoris SMD1168H. Fragmen gen EGFRvIII diperoleh melalui amplifikasi secara in vitro dengan teknik PCR plasmid pJ404-EGFFRvIII-bfp. Gen EGFRvIII tersebut telah terfusi dengan gen bfp penyandi blue fluorescent protein BFP pada ujung-C. Fusi gen tersebut disubklon ke dalam plasmid pPICZ? pada situs XhoI untuk memperoleh plasmid pPICZa-EGFRvIII-bfp . Plasmid rekombinan ditransformasikan ke dalam sel E. coli TOP10 F rsquo; dengan metode kejut panas. Plasmid rekombinan diseleksi dan dikarakterisasi dengan analisis PCR dan sequencing. Plasmid yang telah dikonfirmasi susunan basa dan ukurannya ditransformasikan ke dalam sel P. pastoris SMD1168H dengan metode elektroporasi untuk ekspresi protein rekombinan. Hasil penelitian menunjukkan bahwa fusi gen EGFRvIII-bfp 1317 bp telah berhasil disubklon ke dalam plasmid pPICZ? dan plasmid rekombinan pPICZ?-EGFRvIII-bfp berhasil ditransformasikan ke dalam P. patoris SMD1168H dengan efisiensi transformasi sebesar 90 CFU/ g DNA plasmid.
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ABSTRACT
Epidermal growth factor receptor variant III EGFRvIII is one of the mutant variant of the EGFR protein. Mutations that occur in EGFR cause cancer. Various mutants of EGFR, including the mutant variant III have been widely studied because of their potential as target molecules in cancer therapy. The extracellular domain coding EGFRvIII gene has been successfully constructed in previous studies for the study of protein expression as a molecule target in cancer therapy. The objective of research are to subclone the EGFRvIII gene into the pPICZ expression plasmid then recombinant plasmid transformation into Pichia patoris SMD1168H cells. Epidermal growth factor receptor variant III EGFRvIII gene fragment was obtained with in vitro amplification by PCR of pJ404 EGFFRvIII bfp plasmid. Epidermal growth factor receptor variant III EGFRvIII gene has been fused with the bfp gene encoding blue fluorescent protein BFP at the C end. The gene fusion was subcloned into the pPICZ at the XhoI site to obtain the pPICZa EGFRvIII bfp plasmid. Recombinant plasmid was transformed into E. coli TOP10 F 39 cells by heat shock method. Recombinant plasmids were selected and characterized by PCR analysis and sequencing. The confirmed plasmid of the base structure and size is transformed into the P. pastoris SMD1168H cell by an electroporation method for the expression of the recombinant protein. Results showed that the fusion of the EGFRvIII bfp 1317 bp gene

was successfully subcloned into the pPICZ and the recombinant plasmid pPICZ EGFRvIII bfp was successfully transformed into P. patoris SMD1168H with a transformation efficiency of 90 CFU g DNA plasmid.