Digesti gen EGFsyn dan plasmid PJ404 sebagai persiapan subkloning dan ekspresi protein hEGF ke periplasma escherichia coli = Digestion of EGFsyn gene and plasmid PJ404 as preparation for subcloning and expression hEGF protein to escherichia coli periplasm

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Abstrak

Human Epidermal Growth Factor (hEGF) merupakan polipeptida yang terdiri atas 53 asam amino. Protein hEGF berfungsi untuk proliferasi sel epitel dan epidermis secara in vitro dan in vivo. Protein hEGF dikode oleh gen EGF. Gen EGFsyn telah dikonstruksi secara sintetik untuk optimasi kodon pada Escherichia coli. Optimasi kodon berfungsi untuk meningkatkan ekspresi protein rekombinan pada E. coli. Subkloning gen EGFsyn ke plasmid pJ404 yang mengandung gen peptida sinyal endoxylanase sangat diperlukan dalam ekspresi protein hEGF rekombinan pada E. coli. Kendala ekspresi protein rekombinan pada E. coli yaitu terbentuknya badan inklusi di sitoplasma. Peptida sinyal endoxylanase digunakan untuk mentranslokasi protein ke periplasma. Digesti gen EGFsyn dan vektor pJ404 dengan enzim NheI dan BamHI diperlukan untuk persiapan subkloning dan ekspresi protein hEGF ke periplasma. Hasil penelitian menunjukkan bahwa gen EGFsyn dan plasmid pJ404 berhasil dipotong dan siap digunakan untuk subkloning.
<hr>Human Epidermal Growth Factor (hEGF) is a polypeptide consisted of 53 amino acids. Its function is to promote epithel and epidermis cells proliferation in vitro and in vivo. It is encoded by EGF gene. An EGFsyn has been constructed to optimize codon usage in Escherichia coli. Codon optimization enhances recombinant protein expression in E. coli. Subcloning EGFsyn gene to a plasmid carrying endoxylanase signal peptide gene is important for recombinant hEGF expression in E. coli. One of the problem expressing recombinant protein in E. coli is the accumulation of inclusion bodies in cytoplasm. Endoxylanase signal peptide is used to translocate protein to periplasm. Digestion of EGFsyn gene and plasmid pJ404 by enzyme NheI and BamHI is needed for preparation of subcloning and hEGF expression to periplasm. The results showed that EGFsyn gene and plasmid pJ404 was digested and can be used for subcloning.