Konstruksi vektor rekombinan gen VDAC3 pada plasmid pET100/D-TOPO = Recombinant vector construction of VDAC3 gene on pET100/D-TOTP plasmid

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Abstrak

ABSTRAK
Protein Voltage dependent anion channel 3 (VDAC3) merupakan salah satu kandidat antigen untuk pengembangan metode imunokontrasepsi pada pria. Penelitian bertujuan membuat konstruksi vektor rekombinan gen VDAC3 pada vektor pET100/D-TOPO melalui metode directional TOPO® cloning. Fragmen gen VDAC3 target berukuran 600 bp diamplifikasi dari cDNA yang berasal dari mRNA sel sperma manusia dengan primer spesifik gen VDAC3 ekson 6--10. Gen VDAC3 target disisipi sekuens CACC pada ujung 5? untuk ligasi pada vector menggunakan topoisomerase I. Vektor rekombinan hasil transformasi dengan metode kejutan panas pada sel E. coli TOP10 diseleksi menggunakan medium ampisilin. Analisis transforman dengan PCR colony menggunakan primer gen VDAC3 rekombinan menghasilkan pita DNA berukuran 607 bp. Hasil penelitian menunjukkan gen VDAC3 telah berhasil dikonstruksi ke dalam plasmid pET100/D-TOPO.
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ABSTRACT
Voltage-dependent anion channel 3 (VDAC3) protein is one of antigen candidate for the development of methods immunecontraception in males. The research aims to create a recombinant gene vector construction of VDAC3 gene on pET100/D-TOPO vector via directional TOPO® cloning method. VDAC3 target gene fragment size 600 bp was amplified from cDNA derived from mRNA of human sperm cells with the gene specific primers VDAC3 exons 6--10. VDAC3 target gene inserted at the end of the 5? CACC sequence for ligation to the vector using topoisomerase I. Recombinant vector which is transformed by heat shock on the cell E. coli TOP10 selected using ampicillin medium. Analysis of transformants by colony PCR using the primers VDAC3 recombinant gene produced 607 bp DNA band size. The results of the study showed VDAC3 gene has been successfully constructed into the plasmid pET100/D-TOPO.