Konstruksi plasmid rekombinan pengekspresi antigen E7 HPV-16 pada sistem ekspresi mamalia untuk pengembangan vaksin dna terapeutik HPV-16 = The construction of recombinant plasmid expressing HPV-16 E7 antigen on mammalian expression system to develop therapeutic HPV-16 dna vaccine

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Abstrak

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Human papillomavirus HPV adalah virus DNA yang dapat menginfeksi bagian basal sel epitel leher rahim wanita melalui luka sehingga meningkatkan kasus kanker serviks di Indonesia. Penelitian mengenai pengembangan obat terhadap penyakit kanker serviks berbasis vaksin DNA terapeutik telah dilakukan melalui konstruksi plasmid rekombinan antigen E7 HPV-16 pada sistem ekspresi mamalia. Vektor plasmid pcDNA 3.1 5.428 pb yang digunakan pada penelitian berhasil dikonstruksi melalui proses digesti pada situs NheI dan ligasi dengan fragmen acak gen E7 294 pb sehingga membentuk plasmid rekombinan pcDNA-E7 CADB. Plasmid rekombinan hasil ligasi diklona ke dalam sel E. coli TOP 10 melalui proses transformasi heat shock. Analisis hasil penelitian menunjukkan bahwa 5 koloni mengandung plasmid rekombinan pcDNA-E7 CADB. Analisis orientasi arah gen melalui PCR dan digesti pada 5 koloni menghasilkan 2 koloni plasmid positif dengan arah orientasi 5 rsquo; ke 3 rsquo; pada koloni nomor 5 dan 7. Kedua koloni menunjukkan bahwa fragmen gen E7 CADB berhasil disisipkan pada vektor pcDNA 3.1 dan berhasil diklona ke dalam E. coli TOP 10.
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ABSTRACT
Human papillomavirus HPV is a DNA virus that can infects the basal cells of the female cervix through wounds in which it may increase the risk of cervical cancer in Indonesia. There has been a drug development research to treat cervical cancer based on therapeutic DNA vaccine via constructing recombinant plasmid of HPV 16 E7 in mammalian expression system. pcDNA 3.1 plasmid vectors 5.428 bp which were used in this research are successfully construced through the digestion process at NheI site and the ligation process with shuffling fragments of E7 gene 294 bp which created pcDNA E7 CADB recombinant plasmid. Recombinant plasmid which is the result of the ligation process is cloned into TOP 10 Escherichia coli cell through a transformation process called heat shock. The result of this research displays 5 colonies containing pcDNA E7 CADB recombinant plasmid. Analysis of gene direction orientation through PCR and digestion of 5 colonies displays positive plasmid on 2 colonies with 5 rsquo ndash 3 rsquo direction on colony unit 5 and colony unit 7. Result of the 2 colony shows that E7 CADB gene fragment successfully inserted into the NheI site of pcDNA 3.1. It also resulted in cloning completion of E7 CADB gene fragments into TOP 10 E. coli.