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DIRECT CLONING OF MELANOMA ANTIGEN 1 (MAGE-1) AND THE E2 GENE OF HOG CHOLERA VIRUS (HCV) FROM THE BLUNT END PCR PRODUCT USING pET 101/D-TOPO

Abstract

The objective of this study was to clone the full length of coding region of Mage-1 and E2 gene of Hog Cholera Virus (HCV) using the plasmid pET101/D-TOPO to get the corresponding recombinant plasmid. Samples were obtained from the liver tissue with hepatocellular carcinoma and a pig spleen infected by HCV from Sukoharjo Middle Java. cDNA were amplified by PCR using GMTOPOF-GMTOPOR for Mage-1 and RS-1-RS-2 for E2 HCV. The purified PCR products were cloned into pET101/D-TOPO as direct cloning expression vector. The gene target was transformed into E. coli Top10. Analysis of the recombinant plasmid was undertaken by sequencing and restriction test. The PCR of Mage-1 gene resulted fragments at +1105bp for first round PCR and at +931bp for the second round. The PCR of E2 gene HCV produced fragment at +1200 bp. The DNA targets were cloned into pET101/D-TOPO from the blunt end PCR product directly. The sequence of full length of coding region of Mage-1 contained 927 nucleotides that encoded 309 amino acids residues. The sequence of the full length coding region of E2 gene HCV was encoded by 1218 nucleotide. EcoRV enzyme cuts vector pET101/D-TOPO at nucleotide positions at 545 and 4775bp. The result of EcoRV restriction had band at 4230 and 2450 for pETGM/MAGE1-HCC and 4200 and 2700bp for pETRS/E2-SH. The recombinant plasmid pETGM/MAGE1-HCC and pETRS/E2-SH were obtained from the blunt end PCR product for direct cloning using pET101/D-TOPO. (FMI 2013;49:101-108)

Keyword : direct, cloning, Mage-1, E2, Hog, Cholera, Virus, pET101/D-TOPO, ,

Daftar Pustaka :