F306 Expression of Human Hepatitis B virus Polymerase and Its Domains in Insect Cells with Baculovirus Expression Systems

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DNA polymerase (P protein) activity is necessary for HBV replication. Researches have revealed that expressions of P protein and its functional domains in heterologous systems are significantly important for development of new diagnosis reagents and design of new therapeutical drugs. So we constructed different transfer vectors to express this enzyme and its domains in insect cells. Full length P protein gene with the template sequence for reverse transcription,ε , and the domain of reverse transcriptase were cloned into transfer vector pBlueBacHis 2, which would result in histidine-tagged fusion proteins. These constructs were designated as pBBH-Pε and pBBH-RT, respectively. Another full length P gene without ε and terminal protein domain were cloned into pBacPAK 8 (pBP-PΔε) and pBacPAK 9 (pBP-TP). These two recombinants will produce non-fusion proteins. After transfections of SF9 cells with transfer vectors and Bac-N-Blue DNA or BacPAC 6 DNA recombinant viruses were isolated using plaque assay and identified by PCR using insert-specific or baculovirus-specific primers. The recombinant polymerase was expressed.

F307 Molecular cloning and characterization of Mn-superoxide dismutase gene from *Candida* sp.

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The manganese-containing superoxide dismutase (MnSOD) is a major component of the cellular defence mechanisms against the toxic effects of the superoxide radical. Within the framework of studies on oxidative stress-responsible enzymes in the *Candida* sp., sequence encoding the MnSOD was isolated and examined in this study. Specific primer was designed based upon conserved regions of MnSOD sequences from other organisms, and was used in PCR on reverse-transcribed *Candida* poly(A^+) RNA. The PCR product was used to screen an *Candida* genomic lambda library and the nucleotide sequence of positive clone was determined. The deduced primary sequence encodes a 25 kDa protein, which has the conserved residues for enzyme activity and metal binding. The 28 N-terminal amino acids encoded by the *Candida* cDNA comprise a putative mitochondrial transit peptide. Potential regulatory elements were identified in the 5′ flanking sequences. Northern blot analysis showed that the transcription of MnSOD gene was induced 5- to 10-fold in response to mercury, cadmium ions and hydrogen peroxide.