Pattra Moonjit 2006: Cloning and Expression of Canine Parvovirus VP2 Capsid Protein Gene. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Theerapol Sirinarumitr, Ph.D. 65 pages.
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Canine Parvovirus (CPV) is the single stranded DNA virus. It causes serious contagious disease which is important for population of dogs worldwide. The purposes of this study were to develop the polymerase chain reaction (PCR) technique for the detection of CPV, to clone VP2 gene of CPV and to express recombinant VP2 protein using baculovirus expression system. Hence VP2 protein can induce the protective antibody against CPV. Fecal samples were used for the extraction of CPV DNA. For CPV detection, a set of specific primer to VP2 gene, named VP2 400 bp, was designed to amplify the partial fragment of VP2 gene. For VP2 gene cloning , two sets of gene specific primer pairs were used for the amplification of two-third of 5′end and of VP2 gene by PCR. The first set of primer pair, containing *BamH*I and *Sal*I restriction sites, gave amplicon of two-third of 5′end of VP2 gene approximately 1 kb and the second set primer pair, containing *Xba*I and *Sal*I restriction sites, gave one-third of 3′ end of VP2 geneapproximately 700 bp. The PCR products were purified and then cloned into the cloning vector, and were digested with restriction enzymes. Subsequently the restriction products were cloned into the plasmid pFastBac™ Htb. Two VP2 gene fragments were ligated at *Sal*I restriction site to get full-length of plasmid pFastBac- VP2 gene which had the insert approximately 1,755 bp. Clones were proved by restriction enzyme digestion, PCR and DNA sequencing. The recombinant plasmids pFastBac-VP2 were used to transform DH-10Bac to generate recombinant baculovirus DNA. Subsequently, recombinant baculovirus DNA was used to transfect insect cell for the production of the recombinant baculovirus. Recombinant protein was checked by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE), dot blotting and western blotting using rabbit anti CPV antibody and mouse-antihistidine monoclonal antibody. Dot blot analysis using rabbit anti-CPV and mouse-antihistidine monoclonal antibody showed the positive result of the recombinant VP2 protein. Western blot analysis using mouse-antihistidine monoclonal antibody showed a distinct band approximately 68 kDa which was the same size of as that of SDS-PAGE. However, western blot analysis using rabbit anti-CPV antibody did not give the obvious result.

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