

Development of Biotechniques for Genome Analysis of *Cotesia plutellae* Polydnavirus

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A braconid wasp, *Cotesia plutellae*, has been recommended as useful component to apply integrated pest management of the diamondback moth, *Plutella xylostella*, in southeastern Asia. The wasp had polydnavirus (CpPDV), which causes immune-depression of hosts, *P. xylostella* larvae. In this study, we newly developed plasmid capture system (PCS system) in order to clone CpPDV genome, of which comprised a number of circular DNA segments, in *Escherichia coli* cell. An *E. coli* origin of replication for amplification and a drug-resistant gene for selection were simultaneously inserted between Tn7 left (L) and right (R) end, and the final donor plasmids, pPCS-S and pPCS-L, were constructed. The pPCS-S may transfer a pUC19 origin of replication and an ampicillin resistance marker, and the pPCS-L transfer a mini-F replicon and a kanamycin resistance marker. These PCS donors were applied to clone segments of CpPDV genome by *in vitro* transposition using TnsABC* transposase. In result, 21 genome segments were cloned and their sequences were partially analyzed. To express interesting genes derived from genome clones, we constructed two defective viral genomes (ApGOZA and AcGOZA) maintained in *E. coli* for the rapid generation of baculovirus expression vectors, and transfer vectors (pOBI and pOBII) for the production of a recombinant baculovirus in which a foreign protein is actually incorporated into viral polyhedra. In conclusion, these techniques can be successfully applied from cloning to expression for genome analysis of CpPDV.