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Construction of Transfer Vector for Production of Baculovirus Occlusion Bodies that Contain Novel Recombinant Crystal Protein

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Objectives

Baculovirus occlusion bodies have been recently engineered to incorporate foreign protein such as the *Bacillus thuringiensis* (Bt) Cry1Ac protein for improvement of insecticidal activity. In this study, *polyhedrin*, *cry1Ac*, *egfp* and *cry1Ca* genes were fused to produce occlusion bodies that contain novel recombinant crystal protein by homologous recombination between *cry1Ac* and *cry1Ca* genes in insect cells.

Materials and Methods

Materials - Bt δ-endotoxin *cry1Ac* and *cry1Ca* genes, EGFP gene, bApGOZA vector : pGEM-T Easy, pGEM-5Zf(-), pOB I

Methods - PCR, vector construction, transformation, sequencing, transfection

Results and Discussion

The *cry1Ac, cry1Ca* and EGFP genes were amplified by PCR from pProAc, pBacPHcry1C and pEGFP as the templates, respectively. The amplified genes were fused in pGEM5Zf(-) vector in two orders, *cry1Ac*·EGFP·*cry1Ca* and *cry1Ca*·EGFP·*cry1Ac*. These full fusion genes were transferred into restriction sites, *Xho* I and *Not* I , at the back of *polyhedrin* gene of pOB I (named as transfer vectors, pBacPAC-F and pBacPCA-F, respectively). The fusion constructs were confirmed by restriction endonuclease analysis, DNA sequencing and PCR. pBacPAC-F and pBacPCA-F were cotransfected with bApGOZA in Sf9 cells and the recombinant viruses were confirmed by PCR.

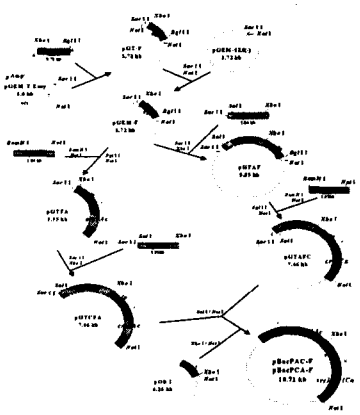

Fig. 1. Flow chart for the construction of vectors, pBacPAC-F and pBacPCA-F.

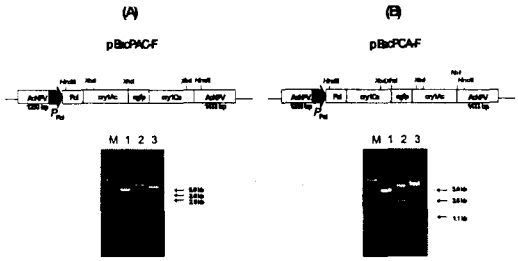

Fig. 2. Restriction analysis of the transfer vectors, pBacPAC-F(A) and pBacPCA-F(B).

References

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