GENETIC MANIPULATION OF AcMNPV BACMID BY HOMOLOGOUS RECOMBINATION

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Genetic manipulation of Autographa californica multinucleocapsid nucleopolyhedrovirus (AcMNPV bacmid) is generally performed by using transposition process at lacZ gene between transposon attachment sites. This attachment sites had been genetically engineered into AcMNPV genome for heterologous gene expression under polyhedrin baculovirus strong promoter. For genetic engineering of other genes, homologous recombination is proposed in this study. AcMNPV chitinase gene was selected as a model and green fluorescent protein (GFP) gene as a marker. GFP gene from a transfer vector was homologous recombined with AcMNPV bacmid DNA at specific chitinase gene. Chitinase gene was expected to be knocked out whereas the GFP gene was expressed when the recombinant AcMNPV bacmid transfected into insect cells. Since the bacmid is the shuttle vector between baculovirus and bacteria, it replicates in both insect cells and E. coli cells. Isolation of the recombinant bacmid can therefore be performed by colony selection method instead of a traditional method, plaque purification, which usually takes 2-4 weeks for selection. This study offers a method for genetic engineering of any genes of the bacmid rather than limiting at the transposition attachment site that is generally used for bacmid. In addition, colony selection of recombinant bacmid after homologous recombination can be used for separation of recombinant and parental bacmid in shorter time compare to plaque purification.

Keywords: Autographa californica multinucleocapsid nucleopolyhedrovirus bacmid, chitinase, green fluorescent protein gene, homologous recombination

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Introduction : Generally Autographa californica multinucleocapsid nucleopolyhedrovirus bacmid (AcMNPV bacmid) was used for expression of heterologous gene by transposition process which limit at one position i.e. the Tn attachment site in the lacZ gene of the bacmid (Luckow et al, 1993). For genetic manipulation of any other genes, which there is no Tn attachment site available, other methods must be carried out. In this study, homologous recombination was proposed to disrupt expression of chitinase gene (Gritsun et al, 1997).

Methods : AcMNPV chitinase gene was cloned into pGEM-T Easy vector after that polGFP gene was inserted in the middle of chitinase gene. Recombinant plasmid was designated pGEM Chi-GFP. The pGEM Chi-GFP and AcMNPV bacmid was cotransfected into insect cells (SF-9). Extracellular virus was purified by plaque purification. The recombinant baculovirus (Acchi’ gfp’) which had green fluorescent plaque was isolated and amplified in SF-9 cells. Viral DNA was extracted from infected insect cells and electro-transformed into competent E. coli DH10B cells. Colony selection for isolation of recombinant bacmid (Acchi’ gfp’) was carried out. Recombinant bacmid (Acchi’ gfp’) which had GFP gene was confirmed by PCR analysis. Recombinant bacmid (Acchi’ gfp’) extracted from E. coli was transfected into SF-9 cells to produce recombinant baculovirus (Acchi’ gfp’). AcMNPV chitinase of infected insect cells which had been interrupted by the present of GFP was assayed by
use fluorescent chitin oligomer analogues substrate.

**Results and Discussion : Construction of pGEM Chi-GFP transfer vector.**

A transfer vector, pGEM Chi-GFP, which contains chitinase gene with GFP gene in the middle was constructed as shown in Fig 1.

![Map of pGEM Chi-GFP transfer vector.](image)

**Isolation of recombinant baculovirus (Acchi-gfp⁺) by plaque purification.**

After cotransfection between pGEM Chi-GFP and bacmid, isolation of green fluorescent baculovirus was performed by plaque purification (Fig 2).

![Green fluorescent plaque of recombinant baculovirus (Acchi-gfp⁺).](image)

**Colony selection of recombinant bacmid (Acchi-gfp⁺).**

Extracted DNA from GFP expressing infected insect cells was performed and electrotansformed into competent *E. coli* DH10B cells. Individual colony containing recombinant bacmid was isolated. The genomic DNA from these colonies was extracted and analyzed by PCR (Fig 3 and 4).

![Diagrammatic presentation showing the position of primers on recombinant bacmid (Acchi-gfp⁺).](image)

**Chitinase assay of recombinant baculovirus (Acchi-gfp⁺).**

Recombinant bacmid (Acchi-gfp⁺) that had been confirmed by PCR for GFP insertion was assayed for its chitinase activity (Fig 5).

![Chitinase activity assay of recombinant baculovirus (Acchi-gfp⁺) with fluorescent chitin oligomer analogous.](image)

**References :**
