An efficient method of constructing homologous recombinant baculovirus with PCR-amplified fragments

HOU Songwang (侯松旺)1, 2, CHEN Xinwen (陈新文)1, WANG Hanzhong (王汉中)1 & HU Zhihong (胡志红)1

1. Joint-lab of Invertebrate Virology and Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China;
2. Key Laboratory of Agricultural Microbiology, Ministry of Agriculture, Huazhong Agriculture University, Wuhan 430072, China

Correspondence should be addressed to Hu Zhihong (email: huzh@pentium.whiov.ac.cn)

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Abstract This paper describes a rapid method of constructing homologous recombinant baculovirus in E. coli with PCR-amplified fragments. By using this method, the traditional steps of constructing transfer vector are omitted. The method is based on phage λ red system which can promote the recombination between the homologous fragments with the length above 36 bp. Taking HaSNPV as an example, this paper describes the rapid recombination process by using chloramphenicol resistance gene (Cmr) to replace orf135 in HaSNPV genome. A pair of primers with length of 60 bp was synthesized, in which 40 bp was homologous to the each end sequence of orf135, and the rest 20 bp was homologous to the each end sequence of Cmr. By using these primers, a linear fragment containing the complete Cmr gene between 40 bp of homologous arms of orf135 was generated by PCR with the plasmid pKD3 which contains Cmr as the template. By transforming the linear fragment into the E. coli containing the bacterial artificial chromosome of HaSNPV and with the help of a plasmid expressing λ recombinase, the recombinants on which the homologue replacement had taken place were selected by chloramphenicol resistance. This method greatly shortens the process of constructing recombinant baculovirus since the process was performed in E. coli and does not need to construct transfer vectors. It can be further used for gene replacement and gene deletion of other large viral genomes.

Keywords: phage λ red system, baculovirus Bacmid, gene replacement, gene deletion, HaSNPV, recombinant virus.

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Baculovirus is a rod-shaped virus containing a large circular dsDNA genome with the size of 80—180 kb[1]. Baculoviruses have been used as insecticides for biological control of forest and agricultural pests[2]. In addition, baculovirus is of great interest as it can be used as efficient eukaryotic expression vector[3], surface display vector[4], and gene therapy vector[5]. Till April 2002, the complete genome sequences of 13 baculoviruses have been reported. The functional genomics has now become the most interesting field in the baculovirus research. Generating a target gene-deletion recombinant virus plays an important role in analyzing the functions of the genes. The traditional method of constructing recombinant baculovirus is by cotransfecting insect cells with the DNA of transfer vector and wild type virus, and afterwards the recombinant virus is se-
lected by plaque purification. The method takes a long time and the recombinant frequency is low\cite{3}. Recently, we developed a new strategy to construct the recombinant baculovirus in *E. coli* based on phage \(\lambda\) red system and bacterial artificial chromosome (Bacmid)\cite{6}. However, it still needed the construction of a transfer vector and the homologous recombination took place between 1 kb of homologous arms. Phage \(\lambda\) red system has the potential to promote homologous recombination between homologous arms with a length above 36 bp\cite{7,8}. In this paper, we describe a method of rapidly constructing recombinant baculovirus using the PCR products with homologous arms of 40 bp based on phage \(\lambda\) red system. The whole process did not need the construction of any transfer vector.

*Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV) is a specific pathogen of the cotton pest *H. armigera*. It has been developed as an efficient viral insecticide and has been commercially produced\cite{9,10}. Recently, the complete sequence of HaSNPV has been finished, which contains 135 ORFs with 20 unique genes\cite{11}. *Orf135* is one of the unique genes. In this work, *orf135* was taken as an example for generating homologous recombinant baculovirus by using the new method.

## 1 Materials and methods

### 1.1 Bacterial strains and plasmids

BW25113 (pKD46 & HaBacHZ8) was constructed and preserved in our lab\cite{6}, which contained HaSNPV bacterial artificial chromosome (HaBacmid) and could express phage \(\lambda\) red recombinase. Plasmid pKD3 which contained the chloramphenicol resistant gene (\(C_{mR}\)) was kindly provided by Dr. Wanner\cite{8}.

### 1.2 Preparation of linear fragments containing 40 bp homologous arms of *orf135*

The linear fragment for generating *orf135*-replaced recombinant HaSNPV was obtained by PCR method. Primers used for PCR were: HP1: 5′ GTA ACG TGT CGC CAG TGG TTA ACC GTG TGC TTT GCA AAC TA TAA GTA GGC TGG AGC TGC TTC G; HP2: 5′ GAA ATG GCA CTC CTT CAA CGC ATA TTT TGT TCA TTA CCA TAT GAA TAT CCT CCT TAG. Both primers were made up of 40 bp of H fragment (italic) and 20 bp of P fragment. Of which, H part was homologous to *orf135*, and P part was homologous to \(C_{mR}\). The amplified \(C_{mR}\) contained its original promoter, and its direction was opposite to the transcriptional direction of *orf135*. In order to avoid the expression of *orf135* promoter after the gene deletion, a TAA terminal codon (bold) that lied in the same open reading frame of *orf135* was introduced in HP1 between H and P fragments.

The annealing temperature of the first 10 rounds of PCR was calculated with Oligo software taking the sequence of P1 and P2 parts as the primers, while the annealing temperature of the last 20 rounds of PCR was done with the software in the internet (http://www.biophys.usi-duesseldorf.de/local/POLAND) and taking the whole sequence of HP1 and HP2 as the primers.