

# Defective *Bombyx mori* Nuclear Polyhedrosis Virus Genomes Maintained in *Escherichia coli* for the Generation of Occ<sup>-</sup> and Occ<sup>+</sup> Baculovirus Expression Vectors

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We have generated a novel *Bombyx mori* Nuclear Polyhedrosis Virus genome which can be maintained in *Escherichia coli* that facilitates rapid and efficient generation of recombinant baculovirus expression vectors. This genome, designated bBmGOZA, lacks a portion of the essential ORF1629 gene, but includes a mini-F replicon and selectable kanamycin-resistance marker. The bBmGOZA can replicate only in *E. coli* and can be rescued by recombination with a transfer vector containing an intact ORF1629 gene. There is no background of non-recombinant virus. To construct occlusion-positive recombinants for oral infection to the silkworm, bBpGOZA is generated by transferring the polyhedrin gene under p10 promoter to bBmGOZA and can be used conveniently in mass production. The structure of both bacmids were analyzed and confirmed by southern blot and restriction enzyme digestion profile. In the comparison of the recombination efficiency, mean titers of the progeny of cotransfection with pBmKSK3-LacZ and bBmGOZA or bBpGOZA gave rise to over 10<sup>3</sup> times than that of wild-type BmNPV-K1 and the progeny viruses from both bacmids were determined almost as recombinant through  $\beta$ -galactosidase assay. The plaques made by bBmGOZA or bBpGOZA with pBmKSK3-LacZ were blue color in X-gal stained plate, but only bBpGOZA produced occlusion-positive plaques. In protein examination by SDS-PAGE, the  $\beta$ -galactosidase band was shown in both cells infected by bBmGOZA or bBpGOZA with pBmKSK3-LacZ, but polyhedrin constituting the occlusion body was only expressed in cells infected by bBpGOZA and pBmKSK3-LacZ.