Transformation Associated Recombination of an AgseNPV-B bacmid

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Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *A. segetum* (Agse) NPV-B, *A.ipsilon* (Agip) NPV and *A. segetum* granulovirus (AgseGV) from the genera *Alpha-* and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon*. All four baculoviruses have a potential for being used as biological control agent against *Agrotis* pests. Despite genomic sequence information, studies about the infection cycle on a molecular and cellular level are still in favor. These studies require bacmids, recombinant baculoviruses, for their genomic manipulation and successive functional studies of genes in larvae and cell culture assays. In the past Bacmid construction was performed either in insect cell culture or in recombination's with *E. coli*. These methods were either time-consuming or lacked in the recombination quality of large and highly repetitive genomes like for *E. coli*.

This study presents a novel method for the construction of bacmids; a yeast transformation associated recombination (TAR) cloning. TAR cloning in yeast simplifies the recombination of large (semi-) synthetic-DNA constructs, e.g. as experiments of C. Venter have shown for *Mycoplasma genitalae*, or modification of entire pathways. Furthermore it allows the recombination of numerous different DNA fragments, also from different species background, in one single step. The AgseNPV-B genome was therefore decomposed in thirty fragments with overlapping ends. In addition to that the shuttle vector pYES-1L with homologues overlaps was amplified. This shuttle vector allows a replication of the Bacmid in *S. cerevisae* and *E. coli*. This study investigated the possibilities of facilitating the fragment construction via long range PCRs and a comparison of the transformation efficacy between chemically– and electro-competent yeast cultures