

## Transfection of Bacmid DNA into SF9 Cells using ESCORT reagent

\*This procedure was specially adapted for transfection of bacmid DNA derived from the Bac-to-Bac system (Invitrogen)

The Bacmid DNA we use is generally produced using standard mini-prep procedure, as described in the Bac-to-Bac user manual. We found that it is not beneficial to use a commercial mini-prep kit or columns.

### Reagents:

Escort transfection reagent (Sigma Aldrich; cat. # E9770)

ESF921 serum-free media (Expression Systems; cat. # 96-001)

### Procedure:

1. From a log-phase growing SF9 cells, prepare 6well plates with  $9 \times 10^5$  cells per well.
2. Incubate plates for 30-60m in a 27C incubator

Each reaction is for 3 wells.

3. In a 15ml polypropylene tube mix: 15uL ESCORT reagent; 230uL serum-free media (Antibiotics Free) and 8uL Bacmid DNA.
4. Pipette-mix several times and incubate for 15min/RT (& up to 45min)
5. Add 2ml of serum-free media (no antibiotics) to the Escort-DNA tube
6. In a sterile hood, aspirate 3 wells out of the 6 well plate
7. Add 700uL of the transfection cocktail into each well
8. Incubate for 5-6h in a humidity chamber, at a 27C incubator
9. Aspirate medium and re-feed with 2ml serum free media
10. Check cells for expression after 48-72h, Harvest 72-96h post transfection.