

Transfection of FlashBac DNA and transfect vector into SF9 Cells using
ESCORT reagent

*This procedure was specially adapted for transfection of FlashBac viral DNA and appropriate transfect vector.

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 9×10^5 cells per well.
2. Incubate plates for 30-60m in a 27C incubator
3. In a cryo tube mix: 6uL ESCORT / 75uL ESF921 (No antibiotics) / 5uL FlashBacBacmid DNA. (100ng), and 1ul of 0.5ug/ul transfer vector
4. (Optional control reaction: take 5ul of FlashBac control vector [100ng DNA/ul] + 5ul Bacmid DNA, mix with 6ul of Escort and 71ul of ESF921)
5. Slow vortex several times and incubate for 15min/RT (up to 45min)
6. Add 610ul of ES921 (-Antibiotics) to the Escort-DNA tube
7. Aspirate 1 well.
8. Add 700uL of the transfection cocktail into a the well
9. Incubate for 5-6h in a humidity chamber in an incubator
10. Aspirate medium and re-feed with 2ml ESF921 (+antibiotics) per transfected well. Place in a humidity chamber for the duration of the incubation.
11. Check cells each day, Harvest virus 4-5 days post transfection.