Rabies pseudotyped EIAV packaging system –PEI transfection protocol

Seed 2.5 x 10^6 293T cells in one 15cm dish in 15 ml DMEM with 10% serum and 1% pen strep. For a standard prep from 12 dishes you will need to start with 3 dishes. Grow until 80% confluent (~ 3 days) and then split 1:4 to give twelve 15cm dishes. Allow cells to grown until 60% confluent (~11 x10^6, approx 24 hours)

PEI transfection

2 hours prior to transfection, remove medium and replace with 15ml fresh pre-warmed growth medium.

Prepare your DNA mix as follows. Amounts are given for a 1x15 cm dish. Scale up as appropriate for the number of dishes you have.

Vector	**amount(ug)**	**x12**	**Size (kb)**
EIAV vector	5	24	8-9
pCEV53b	7.5	90	8.8
phCMV.Rb.G	5	60	5.8

Per plate - add DNA to 500uL of pre-warmed Optimem medium. Add 70uL PEI (1ug/ul in 1xPBS pH4.5) to the mixture (4:1 ratio of PEI:DNA). Vortex briefly (10sec) and leave for 5 – 10 min at room temp.

Add the transfection complex drop-wise to a 15cm plate, swirl briefly to mix and incubate for 6-8 hrs in 3.5% CO2, 35 degrees C. Replace medium with 10 ml of fresh growth medium +25mM HEPES and incubate until 48 hours post-transfection. For viral preps where in vivo toxicity might be a problem, lower the serum concentration to 3% in the collection medium.

Note: 16 hours after transfection add Sodium Butyrate (10mM final concentration, make a 1M stock solution of TC grade Sodium butyrate in water and filter sterilize) to each dish. This is known to increase the titer of lentivirus pseudotyped with non-VSV glycoproteins and may also increase the titer of VSV.G pseudotyped vector.

Virus collection.

1. Remove medium from each dish and pool. Store @ 4 degrees. Add 5 ml fresh GM + 25mM HEPES and incubate overnight (60-72 hours post transfection).
2. Collect 2nd lot of medium from each dish and pool with previous harvest.
3. Remove plasmid carry-over by digestion with DNaseI (1mg/ml stock). Use 1 ug per mL of viral supernatant. Also add 1 ul of 1M MgCl2 per ml of supernatant and incubate at RT for 30 min followed by 4 degrees for 2-4 hours
4. Filter supernatants through a 0.22um filter unit and proceed to purification steps.

Virus purification.

Follow purification steps as described in Tiscornia, Singer & Verma (2006) with the following modifications.

ALL STEPS SHOULD BE CONDUCTED AT 4 DEGREES. DO NOT USE THE SECOND SUCROSE PURIFICATION SPIN AS SIGINIFICANTLY LOWERS THE INFECTIOUS TITER OF THE EIAV VECTOR.

THE FIRST CONCENTRATION SPIN CAN ALSO BE PERFORMED AT 7,000 X G FOR 16-20 HOURS TO PREVENT DAMAGE TO THE VIRAL ENVELOPE, ESPECIALLY IF PACKAGING LARGE GENOMES.

To Make the PEI solution – pH 1xPBS to 4.5 using H.Cl. Add 50 mg linear PEI (Polysciences #23966-2) to 50ml 1xPBS pH4.5. Place in a 75 degree waterbath and vortex every 10 min until completely dissolved. Cool to room temp and filter sterilize through a 0.22um syringe filter. Aliquot and store at -20.