



VSV.G pseudotyped retroviral packaging system –PEI transfection protocol

Seed 2.5×10^6 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 90% confluent (~ 3 days) and then split 1:4 to give twelve 15cm dishes. Allow cells to grow until 60% confluent.

PEI transfection

2 hours prior to transfection, remove medium and replace with 15ml fresh pre-warmed growth medium containing 25mM HEPES.

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)	Size (kb)
Transfer plasmid	5	~8-9
pCL plasmid	7.5	8.8
pMDL.G (VSV.G)	5	5.8

Per plate - add DNA to a tube containing 0.5ml of pre-warmed Optimem medium. Add PEI from stock solution (1mg/ml in 1xPBS) at a ratio of 4:1 v/w (PEI:DNA). Vortex at max speed for 10-15 seconds and incubate for 5 min at 37 degrees.

Add the transfection complex drop-wise to a 15cm plate, swirl briefly to mix and incubate for 8 hours at 3.5% CO₂, 35 degrees C. Replace medium with 10 ml of fresh growth medium + 25mM HEPES and incubate as above until 48 hours post-transfection.

Note: 16 hours after transfection you can 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 increase the titer of VSV.G pseudotyped retrovirus.

Virus collection.

1. Remove medium from each dish and pool. Store @ 4 degrees. Add 5 ml fresh GM+25mM HEPES and incubate overnight as above (60-72 hours post transfection).
2. Collect 2nd lot of medium from each dish and pool with previous harvest.
3. Spin medium at 3,000xg for 15 min @ 4 degrees to pellet cell debris.
4. Filter supernatants through a 0.22um filter unit (Millipore Durapore or equivalent low protein binding, fast flow membrane) and proceed to purification steps.

Virus purification.

Follow purification steps as described for lentivirus in Tiscornia, Singer & Verma (2006).

To Make the PEI solution – pH 1xPBS to 4.5 using HCl. Add 50 mg of 25KD linear PEI (Polysciences #23966-2) to 50ml 1xPBS pH4.5. Place in a 75 degree waterbath and



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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.