

# Large-scale lentivirus production for barcode library

## Materials

- HEK293T cells
- 4 x T175 flasks
- VSV-G and PSPAX2 envelope and packaging plasmids
- Opti-mem
- PEI transfection reagent
- 0.45 um filters
- 50 mL syringes
- Amicon Ultra 15 mL filters (100 KDa cut-off)

**Day 0:** Plate 15 million HEK cells into a T175 (x 4 flasks) at around 2pm

**Day 1:** Transfection. HEK cells should be ~80% confluent.

Transfection (amount per T75)	Concentration needed (ug)	Conc of plasmid	Amount needed (ul)
Barcode Plasmid	15		
VSV-g	5		
PSPAX2	10		

- 1) Add the DNA to 750 uL Opti-mem in an Eppendorf, vortex and spin down. Incubate at RT for 5 mins. (Have a separate Eppendorf for each plate of HEK cells).
- 2) Add 180ul of PEI (1mg/ml PEI (6ul per 1ug DNA)), vortex and spin down. Incubate for 10 mins at RT.
- 3) Remove DMEM media from HEK cells
- 4) Add DNA-PEI-Opti-mem mix to 20mL of pre-warmed Opti-MEM and add to HEK cells.

**Day 3:** Harvest 48hr virus, filter through a 0.45 micron filter and store in 50ml falcon tubes at 4 degrees. Add more Opti-mem to the HEK cells for a second harvest.

**Day 4:** Harvest remaining virus and filter again through a 0.45 micron filter and pool both days of virus. Concentrate both pools 100X using 2-4 x Amicon 15ml ultra filters (should end up with 1600ul of virus from 160mls). Centrifuge at 3000G for 5 minutes. Check filters and keep adding more virus until all is concentrated. Each filter should have around 400-500uL left after centrifugation. Make sure all the remaining virus is removed from the columns by washing the sides with the remaining Opti-mem and virus. Aliquot virus into 50, 20 and 10uL aliquots and snap freeze on dry ice. Store at -80 degrees.

## Transduction

The transduction is really cell type dependent and you will need to conduct titration tests. Our leukaemia mouse cells are hard to transduce so we conduct our transductions in 48-well plates to maximise cell contact with the viral particles. We plate 500,000 cells per well in a total of

500 uL of media + around 50 uL of concentrated virus + Polybrene (8.5ug/mL final conc). We spinfect for 1.5 hours at 1250 G and then leave in the 37-degree incubator for a further 5 hours. We then wash off the polybrene, add fresh media and check the transduction efficiency based on the fluorescent protein percentage 48 hours later. We get around 3-5% with this protocol. As a comparison, 10 uL of the same virus will transduce K562 cells at 50-70% without any spinfection or polybrene. You want to aim between 1-10% for your cells and you may have a protocol that works already in your hands.