Generation of Lentivirus

Reagents

- 293T: ATCC
- Fugene 6 transfection reagent: Roche cat# 11814443001
- FBS: Invitrogen cat# 16000-044
- DMEM: Invitrogen cat# 11965-118
- Pen/strep: Invitrogen cat# 15140-155
- L-glutamine: Invitrogen cat# 25030-156
- Non essential amino acid (NEAA): Invitrogen cat# 11140-050
- Amicon Ultra Ultracel 100K: Millipore: cat# UFC910024
- Beckman centrifuge tubes: Beckman: cat# 344058

293T/fibroblast media (500 mls):

DMEM (450 ml)
10% FBS (50 ml)
Pen/strep (5ml)
L-glutamine (5ml)
NEAA (5 ml)

Reagent setup

Lentiviral vectors: Prepare all vector plasmids with Qiagen Maxiprep kits, including lentiviral packaging vectors and lentiviral transfer vectors

A. Production of Virus

1. Seed $2x10^6$ 293T cells on a 100-cm tissue culture dish and incubate overnight until cells reach ~70% confluence (~1-2 days). Replace with 10 ml of fresh media 2 hours before transfection.

2. Mix lentiviral transfer vector and packaging vectors in 600 ul of DMEM in an eppendorf tube. Add 50 ul of Fugene6 directly to the DNA mixture, making sure Fugene6 does not come in contact with the plastic. Gently vortex tube containing transfection mixture and incubate at RT for 20 min.

Second generation packaging system

Transfer vector	10 ug
pMD2.G	5 ug
psPAX2	5 ug

Third generation packaging system

Transfer vector	10 ug
pMDL g/pRRE	5 ug
pRSV-Rev	2.5 ug
pMD2.G	2.5 ug

3. Add transfection mixture dropwise to cells; incubate 4 hrs to overnight (16 hours) and replace with fresh medium.

4. Collect virus-containing medium 48 hours after transfection and replace with fresh medium. Collect virus every 12 hours for up to 3 times total. Keep all viral media at 4C until all collections are done. Pass viral media through a 0.45uM low protein-binding filter. At this point, the viral supernatant can be used to infect cells, frozen at -80C or concentrated.

Concentration using Amicon Ultra Centrifugal Filters:

Transfer viral supernatnat to Amicon Filter and spin filter in tabletop centrifuge at 3000 rpm for 10-20 min at 4C. Concentrated virus can be aliquoted and stored at -80C. Thaw an aliquot on ice before use; do not refreeze.

Concentration by ultracentrifugation:

1. Transfer viral supernatant to 33ml Beckman ultracentrifugation tubes and spin for 2 hrs at 24,000 rpm using SW32Ti or SW28Ti.

2. Pour off supernatant carefully and resuspend viral pellet using 100-ul pipette tip with appropriate amount of DMEM to concentrate virus 100x. It may take 30 min to overnight at 4C for the pellet to completely dissolve.

3. Store virus in aliquots at -80C. Thaw an aliquot on ice before each use; do not refreeze.