Production of lentiviruses in HEK293T cells
2nd Generation lentiviral system

1. Background and purpose of the procedure

Lentiviral transduction is an effective method for creating a stable cell line with a DNA cassette of interest integrated into its genomic DNA. The second-generation system has one packaging plasmid which includes all the important packaging components: Gag, Pol, Rev, and Tat. To produce virus, it requires a single packaging plasmid, an envelope plasmid, and a transfer vector (i.e., with the gene of interest to be expressed).

2. Materials and equipment

- HEK293T cells (ATCC, CRL-3216)
- Complete cell culture medium (DMEM, Gibco, 11995073) with FBS (FB Essence, VWR, 10803-034) and antibiotics (Penicillin/Streptomycin, Corning, 30-002-CI)
- D-PBS, without Ca^{++} and Mg^{++} (Gibco, 14190144)
- Opti-MEM Serum-free media (Gibco, 11995)

- Lentiviral packaging vector psPAX2 (Addgene, 12260)
- VSV-G envelope vector pMD2.G (Addgene, 12259)
- pLV transfer vector containing the gene of interest

- T75 TC-treated Flask
- 15 mL and 50 mL polypropylene tubes
- Cell counter/Hemacytometer

3. Method

1. Day 1

a. For each plasmid to be transfected, plate 3 x 10^6 HEK293T cells in 10-15 mL of media in a T75 TC-treated flask

b. Incubate the cells at 37˚C, 5% CO_2 overnight

2. Day 2

a. Perform the transfection in the late afternoon, transfection mix should only be incubated with the cells for 15-18 h maximum

b. In a 1.5 mL microcentrifuge tube, mix:

   i. 1 mL of Opti-MEM
   ii. 10 µg of transfer vector (pLV)
   iii. 10 µg of packaging vector (psPAX2)
   iv. 5 µg of envelope vector (pMD2.G)
   v. 75 µL of Lipofectamine reagent (3 µL:1 µg of DNA) *[yellow cap tube]*
   vi. 50 µL of Lipofectamine 3000 (2 µL:1 µg of DNA) *[red cap tube]*

c. Vortex for 10 sec

d. Incubate the mix for 15-30 min at room temperature

e. Gently add the DNA:Lipofectamine 3000 mix to the cells being careful to not detach the cells from the flask
f. Swirl the flask gently to disperse the mixture evenly
g. Incubate the cells for 15-18 h at 37˚C, 5% CO_2

3. Day 3

a. In the morning, change the media to remove the transfection reagent. Replace with 20 mL of fresh complete DMEM. Be careful to not detach the cells from the flask
b. Incubate the cells for 48 h at 37˚C, 5% CO_2

4. Day 5

a. Harvest media containing the lentiviral particles and transfer to a 50 mL polypropylene tube
b. Spin media at 1,500 rpm for 5 min any cells or cells debris that were collected during harvesting
c. Viruses may be stored at 4˚C for short-term storage (1 day to 1 week) or frozen at -80˚C for long term-storage (up to 1 year, aliquots into smaller tubes to minimize freeze/thaw cycles)
d. Add 10 mL of fresh complete DMEM. Be careful to not detach the cells from the flask [optional]
e. Incubate the cells for 48 h at 37˚C, 5% CO_2 [optional]

5. Day 6 [optional]

a. Harvest media containing the lentiviral particles and transfer to a 15 mL polypropylene tube
b. Spin media at 1,500 rpm for 5 min any cells or cells debris that were collected during harvesting
c. Viruses may be combined with the viruses harvested on Day 5 and stored at 4˚C for short-term storage (1 day to 1 week) or frozen at -80˚C for long term-storage (up to 1 year, aliquots into smaller tubes to minimize freeze/thaw cycles)

4. References

Chu C, Xin A, Zhou Y, Zhang Y. A simple protocol for producing high-titer lentivirus. Acta Biochim Biophys Sin (Shanghai). 2013 Dec;45(12):1079-82. doi: 10.1093/abbs/gmt112. Epub 2013 Oct 10. PMID: 24113089.

Producing lentivirus in HEK293T cells using a 2nd Generation lentiviral system, from David P. Turner, Medical University of South Carolina, Hollings Cancer Center

5. Revision history

Revision #	Date	Prepared by
1.0	**2021-10-07**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		