

Lentiviral Transfection/Transduction

I. Materials needed prior to initiating transfection:

- 1. DNA Preps:** The cleanliness of DNA to be transfected is critical. Plasmids should be prepped previously from Qiagen or Marligen Maxi Prep kits (Marligen kit is recommended). Phenol/Chloroform cleaning is usually detrimental. Three plasmids are used: GAG-pol, Envelope (usually VSVG), and the transfer plasmid of interest.
- 2. HEK 293T cells:** 293T cells are best for viral production. The cells should have a "rounded" morphology and not as adherent as NIH3T3 cells. A good culture of 293T cells disattach from a plate by simple pipetting media on the monolayer (no scraping or Trypsing necessary; **Avoid trypsin**). **Important note:** Because HEK 293T cells are of human origin, BSL-2 level precautions need to be taken in their maintenance and handling. You must receive Jackson Laboratory BSL-2 training before working with these cells, and before planning a transfection. 293T cells require DMEM with 10% Heat-Inactivated FBS, .02% 1M HEPES, .01% 1M Sodium Pyruvate, 0.01% of 1M Non-Essential Amino Acids, and streptomycin/penicillin.
- 3. Transfection method:** The standard Calcium Phosphate method is utilized without DMSO or glycerol shock. Reagents needed: (1) 2X HBS (50mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄. Final pH must be 7.1 (critical)) (2) dH₂O (DNase, RNase free, not DEPC treated). (3) 2M CaCl₂. Avoid using 70% ETOH while performing the transfection. Spray all instruments before beginning the transfection, and ensure all ETOH has evaporated before use.

II. Transfection Protocol

Day 1:

1. Ensure that you have 293T cells at a density of 80-90% confluency. Utilize one 10-cm plate of 293T cells per transfection, and 10 mLs of 293T media per dish.

Day 2:

1. Change media on the 293T plates to fresh 293T media.

For each transfection:

2. Mix 5ug of Gag-pol plasmid, 5ug of Env plasmid, and 10ug of transfer plasmid in sterile microfuge tube. Bring the volume in the tube to 438 uL with dH₂O. Add 62 uL of 2M CaCl₂ per tube. Mix gently. Incubate for 5 minutes at room temperature, underneath the (BSL-2) hood.
3. In a fresh microfuge tube, add 500 uL of 2XHBS for each transfection, close and flick the tube.
4. Open the tube and use the empty pipette tip to "blow" 100uL of air into the 2XHBS tube that has received the DNA/CaCl₂ mixture. Repeat the "blowing" one more time.
5. Repeat step 4 until the contents of the 500 uL tube of DNA/CaCl₂ mix are finished, and mixed with the contents of the 2XHBS tube. Close the tube.
6. Incubate the mixture at room temperature in the hood for 20 minutes. The mixture, after 20 minutes, should appear "cloudy".
7. Mix the transfection mixture well and add dropwise to a plate of 293T cells. Swirl the plate gently as the addition occurs. Some pieces of the 293T monolayer may dislodge. Slow down the swirling if dislodging occurs.
8. Incubate the transfected 293T plates for at least 18 hours in the BSL-2 tissue culture incubator. Do not exceed 30 hours.

Day 3:

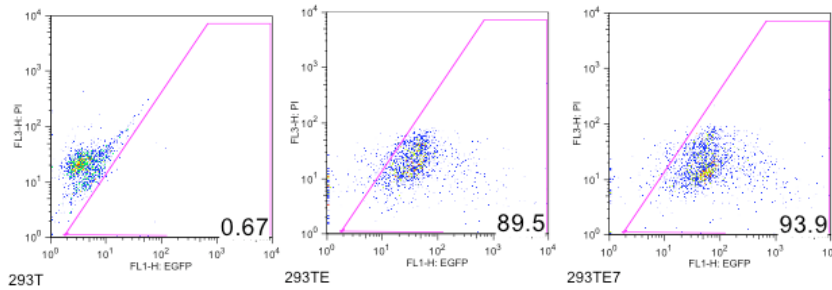
9. Remove the media from the transfected 293T plates, add 10 mLs fresh 293T media.

Day 4:

10. Check the 293T cells. Some cells may be dislodging from the plate or dying. This is normal. Let the media turn yellow.

Day 5:

11. Harvest the supernatant about 60-72 hours post transfection. If fluorescent markers are used for quality control, check the fluorescence of the 293T cells that produced the virus. All the 293T cells should fluoresce as compared to control cells by flow cytometry. See flow figure below:
From left to right: untransduced control, two positive samples



12. Harvesting and storing the supernatant: Filter the supernatant through 100uM sterile mesh into a 50mL conical tube. For best results, use the virus immediately. However, you can store the virus at -80°C for months.

II. Transduction protocol for suspension cells:

13. Suspend the target cells (usually primary splenocytes) in fresh medium at a concentration of 10^5 - 10^7 cells/mL.
14. In a 12-well tissue culture plate, add 1 mL of the cells suspension, 1 mL of filtered viral supernatant, 2 uL of 10mg/mL Hexadimethrine Bromide (a.k.a. polybrene, Sigma).
15. Mix gently and incubated cells for 20 minutes in the hood at room temperature.
16. Centrifuge the plate(s) for 90 minutes at 1500xg at 32°C.
17. Carefully remove the plates from the centrifuge so as not to disturb the cells. Incubate cells overnight (18 hours) with the virus in the BSL-2 TC incubator.

Day 6:

18. Transfer the transduced cells to a 50 mL conical centrifuge tube (pooling cells that have been transduced with the same virus).
19. Centrifuge the cells at 400xg for 5 minutes. Discard supernatant. Add 50 mL of fresh media or PBS and centrifuge again at 400xg for 5 minutes.
20. Repeat the previous step twice, and the cells are ready for use in downstream growth or assays. You should be able to transduce primary splenocytes between 90-95%, see flow figure below:

