Packaging of shRNA constructs into Viral Particles and Infection of Target Cells

Day 1

1. Plate 293FT cells in 4mL DMEM/10%FBS on a 60mm plate (1*10^6 cells). Prepare one 60mm plate for each construct that must be packaged into viral particles. Aliquots of 293FT cells are stored in liquid nitrogen Rack 3 Box 11

Day 2

2. Begin transfection ~24h later. For each transfection, place 200µl sterile 150mM NaCl into a 1.5mL eppendorf tube.
3. Add 0.5µg of each packaging vector (PI, PII, PIII or pCMV-VSV-G, pΔ8.9). Do not premix packaging vectors.
4. Add 1µg lentiviral plasmid and gently mix
5. Add 16µl TurboFect. Gently mix and incubate at RT for 15 min.
6. Add to 293FT cells by gently dripping into the media. Incubate overnight at 37°C.

Day 3

7. In the morning, aspirate media from the 293 FT cells and replace with 4 mL of appropriate target media. Be careful not to disturb the 293FT cells as they are loosely adherent.
8. Plate target cells for infection tomorrow.

Day 4

9. Prior to infection; pretreat target cells with polybrene (Sigma H9268, stored in Media Fridge). 1000x stock prepared in sterile water is stored at 4°C. Add 1µl of stock per mL of target cell media (10µl for 10 mL media). Return to incubator for 30-60 minutes.
10. Pipet viral media from the 293FT cells into 15mL conical tubes. Add back 4mL media to 293FT packaging cells.
11. Add 1µl polybrene stock per mL viral media (total 4µl for 4mL viral media). Filter through 0.45um syringe filter.
12. Aspirate media from target cells
13. Split viral media equally among target cells and return to incubator for 1 hr. For cells in suspension, resuspend 1x10^7 cells in 200ul viral media and spin at 2000rpm for 1hr at RT.
14. Aspirate viral media (off of target cells) and replace with normal target cell media (10mL). Incubate at 37°C overnight. For cells that are difficult to infect, add an additional volume of polybrene-containing media and incubate for an additional 1hr or up to overnight

Day 5

15. Repeat day 4. (This greatly increases transfection efficiency)

Day 6

16. Aspirate target cell media and replace with fresh media containing 2µg/mL puromycin to begin positive selection of infected cells. Puromycin stock is 1000x and stored at -20°C.

Days 9-13

17. Observe puromycin resistant colonies. Feed and expand as necessary.
18. Screen for knockdown by western blot and PCR. Begin cloning of successful knockdowns
19. Freeze aliquots for storage