

Viral Particles Production

Use filter tips, double gloves, bleach waste, GMO disinfectant, disinfect all plastic material before disposal.

Day 1 - HEK replate

1. Re-seed HEK cells to obtain 10-20% confluence (ideally early in the morning)
2. After HEK adhesion (late afternoon) proceed with transfection using either Fugene or Lipofectamine 3000

Day 1A - Transfection by Fugene

4. Confirm plasmids concentration and prepare the mix of packaging plasmids
 - plasmid #9: 1750ng
 - plasmid #10: 3240ng
 - plasmid of interest: 5000ng.
6. Equilibrate Optimem to RT
7. Vortex and spin down Fugene
8. Dilute plasmid mix in 1mL Optimem, vortex, spin down
9. Add 30uL of Fugene, mix by inversion incubate 5-15minutes at RT
10. Apply dropwise to 10cm dish
11. Incubate overnight

Day 1B - Transfection by Lipofectamine

4. Confirm plasmids concentration and prepare the mix of packaging plasmids
 - plasmid #9: 1750ng
 - plasmid #10: 3240ng
 - plasmid of interest: 5000ng.
5. Equilibrate Optimem and Lipofectamine components to RT
6. Vortex and spin down components
7. **Solution A:** Dilute 35uL of Lipofectamine in 875uL of Optimem, vortex, spin
8. **Solution B:** Dilute 20uL of P3000 from Lipofect kit in 875uL of Optimem, add your plasmids, mix by pipetting
9. Mix A and B, incubate 10-15 min at RT
10. Apply dropwise to 10cm dish
11. Incubate overnight

Day 2 - Exchange media

Change media to 10 %KOSR in DMEM high Glucose (15mL)

Day 3-5 collect media with viral particles

1. Collect media
2. Put fresh 10 %KOSR in DMEM high Glucose (15mL)
3. SPin down debris in collected media using 3000g for 10min
4. Filter using 0.45um filter
5. Store at 4°C

Day 5 Concentrate particles

NOTE: may not be needed for well transducible cells such as HEK, Cal51

1. Use Vivaspin Concentrator spin columns
2. Centrifuge particles at 3000g at 4°C for 15 min
3. Check if they are concentrated enough if not repeat (I concentrate 20-40x for CMs)
4. Store concentrated at 4°C for up to 1 month

Transduction test

1. Seed your cells in 24 well
2. Test ratio of viral particles / media in range 4to1 to 0.25to1
3. Check after 24hours what concentration works best

Transduction

1. Prepare culture media with Polybrene 5-10ug/mL stock 10mg/mL 7.5ug/mL works good(VV)
2. Add particles dropwise to wells
3. Check after 24 hours

Plasmids

- Packaging #9: Addgene #12259; Stored in Sofia's freezer top right drawer
- Packaging #10: Addgene#12260; Stored in Sofia's freezer top right drawer
- YAP1-OE #124: Addgene#74942; in Vladimir's green box in IPSc

Material

Transfection reagents (IPSCs fridge)

- Lipofectamine 3000 Thermofisher Web Link; CatNo: L3000-15; Current Lot:2153940
- Eugene HD transfection reagent; Promega Web Link ; CatNo: E2311; Lot:000064305J
- Optimem Thermofisher web link

Collection and concentration

- 50mL syringe
- 0.45um filters
- Concentration columns Vivaspin 20PES, 100kDa; in western-blot cabinet bottom shelf, the one with blue tops

Transduction

- Polybrene Sigma Aldrich: 10mg/mL; in cell culture freezer, upper right mbd drawer in 50mL falcon (20F1 B2)