293 Transfection for Lentiviral Preparation.

1. Plate 10 x 10^6 293.T in 20 ml on a 15 cm^2 plate 24 hours before transfection. In general, two 15cm plates per virus. It is essential that the cells be well-maintained and of relatively low passage number. On day of transfection you do not want the cells to be overgrown, but at a confluence of 70-80%.

2. We use the MIRUS- Transit293 reagent for transfection according to the manufacturer’s instructions. Basically mix 80-120ul of Transit in 2ml of serum free DMEM media. Let sit at room temp. In another tube mix the following DNAs:
   For 4 plasmid, system (recommended),
   20 μg vector,
   5 μg VSVG
   5 μg RSV-REV
   10 μg pMDL g/p RRE

3. Add the DNA mixture to the media with Transit.

4. Let incubate at room temp. for 20-30 min.

5. Take plate of 293T out of the incubator (plate remains in incubator for long as possible), and add transfection mixture drop wise all over the plate. Gently swirl plate from front to back, and return to incubator.

6. 36-48 hours after transfection, harvest viral supernatant and spin @ 2000 rpm, 7min at 4°C in a 50ml tube.

7. Filter viral SN through .45 um filter. Add 35ml of filtered supernatant to an ultracentrifuge tube. Balance tubes with additional media.

8. Spin tubes using a SW-28 rotor @ 25,000 rpm, 90 min, 4°C. Decant liquid and leave tube upside down on kimwipe for 10 min. Aspirate remaining media being careful not to touch bottom of tube.

9. Add 15-50μl cold PBS (for embryo infections, or any volume you wish) and leave tube at 4°C O/N with no shaking.

10. To resuspend, hold tube at angle and pipet fluid over pellet 20 times, being careful not to touch pellet with tip.

11. Aliquot or use virus. Virus should be aliquotted, flash-frozen in liquid nitrogen and stored at -80. Avoid multiple freeze-thaws.

*Adapted protocol provided by Sokol Haxhinasto, Ph.D.*
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