Lenti-Virus Protocol

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A. For packing virus in 6-well plate: (if in 12-well plate, reduce all to 1/2)

1. Mix the following plasmids in a 1.5 ml eppendorf tube,
   1.5 μg Lenti-vector + 1.5 μg packing plasmids = 3 μg total
   0.5:0.3:0.2 (pMDL =0.75 μg, VSV-G=0.45 μg, REV=0.3 μg)
   add 200 μl OPTI-MEM, mix well.

2. Mix 7.5 μl LF2K in 200 μl OPTI-MEM, incubate for 5 min.

3. Mix the LF2K mixture with plasmids mixture, incubate for 15-20 min.

4. During the incubation time, trypsinize 293T:

   Add 1 ml 0.05% Trypsin & EDTA to a full 100 mm plate (~1.2-1.4 x 10^7 cells),
   Add 5 ml fresh medium to terminate reaction,
   Count cells and adjust to a concentration of 2x 10^6 cells / ml.

5. Aliquot 1 ml cells to a well of 6-well plate (2x 10^6 cells / well), then add another 1 ml medium.

6. Add transfection mixture to the well, mix well, and move back to 37C incubator quickly.

7. 8-12 h later, change medium (2 or 2.5 ml), and incubate for another 24-36 h.

8. Harvest virus. (virus can be stored at 4C for one week, otherwise, aliquot virus and store at -80C for further use)

B. Infection:

9. Seed cells that need to be infected in a 12-well or 6-well plate. The best confluence is 40-70%, depending on the different applications.

10. For 12-well plate: (if in 6-well plate, duplicate all)
    Infect mouse cells: 400-800 μl virus + 800-400 μl fresh medium =1.2 ml total
    Infect human cells: 100-500 μl virus + 1100 μl-700 μl

11. Add 8-10 μg/ml polybrene, spin for 30 min at 2500 rpm at 37C.

12. 12-24 h later, change medium.