Lentivirus Production

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Materials

Packaging cell line: 293FT better to use early passaged cells
Medium: DMEM with 10% FBS with or without P/S for culturing 293FT cells
DMEM with 20% FBS without P/S for making virus
Lipofectamine2000 30 μl/10 cm dish, 5 μl/well of 6-well plate
Opti-MEM
Transfection plasmids stored at -20°C, common plasmid box:
psPAX2, 2nd generation lentiviral packaging plasmid. (Addgene Catalog # 12260)
pMD2.G, VSV-G envelope expressing plasmid. (Addgene Catalog # 12259)
Lentiviral plasmid: e.g. pLent-EGFP-puro 11 to 12 μg/10 cm dish
0.45 μm PES filter for filtering viral supernatant
PEG8000 for concentration of virus (stored at cell culture room 4°C)

Prepare 293FT cells, split cells 2 to 3 times a week, cells should remain healthy.

Transfection Steps:

Prepare plasmids mixture 0.5 - 1 ml/10 dish
0.25 or 0.5 ml Opti-MEM
30 μl Lipofectamine2000
Mix and leave at RT for 5 min

0.25 ml or 0.5 ml Opti-MEM
psPAX2  9.2 μg
pMD2.G  2.8 μg
pLent-EGFP  11 to 12 μg
Mix DNA with Lipofactamine2000 at RT for 30 min
Passage 293FT cells.
Count cell number and suspend cells in DMEM with 20% FBS.
Cell number ~ 7.5 x 10^5/ml, 12 ml /10 cm dish, ~ 8.5 to 9 x 10^6/10 cm dish.
Add plasmid mixture into 12 ml cell suspension and place into a 10 cm dish evenly.
Also can place cells in the dish first then add the plasmid mixture dropwise evenly. Incubate cells no medium change.

**Harvest virus at 48 or 72 hours post transfection.**
Centrifuge the medium with virus at ~500 g for 3 minutes and filter the viral supernatant through a 0.45 μm PES filter. Viral supernatant can be stored at 4°C for less than 1 week. It is better to concentrate and aliquot the viral supernatant to store at -80°C.

**Concentrate viral supernatant**

Mix PEG8000 and viral supernatant at 1:4 ratio (3 ml PEG in 12 ml viral sup.).
Mix and shaking gently on ice or at 4°C for 5 to 6 h.
Spin down at 1600 g for 60 min at 4°C.
Remove the supernatant and re-suspend the viral pellet in PBS, 1/20 original volume. e.g. 2ml in a 2 ml tube, the pellet can be suspended in 100 μl PBS.
Aliquot the concentrated virus if need and store at -80°C.