Lentivirus Production

Abstract

[Abstract] Lentivirus is a common tool used to introduce a gene into mammalian or other animal cells. This protocol is to produce lentivirus stocks from hairpin-pLKO.1 plasmid.

Materials and Reagents

1. 293T packaging cells (ATCC)
2. Hairpin-pLKO.1 vector (GE Healthcare Dharmacon)
3. Packaging vectors: 2nd generation packaging plasmids containing *gag*, *pol*, and *rev* genes (*p*CMV-dR8.91 or *p*CMV-dR8.74 psPAX2) and envelop plasmid (*p*MD2.G) (Addgene)
4. Lipofectamine 2,000 (Life Technologies, Invitrogen™)
5. OptiMEM serum free media (Life Technologies, Invitrogen™)
6. DMEM (Life Technologies, Invitrogen™)
7. Fetal Bovine Serum (FBS) (Life Technologies, Invitrogen™)
8. Pen/Strep (Life Technologies, Invitrogen™)
9. 6 cm tissue culture plates (Thermo Fisher Scientific)
10. 45 um filter (Thermo Fisher Scientific)
11. Storage tubes
12. Seeding media (see Recipes)
13. Harvest media (see Recipes)

Equipment

1. Tissue culture incubator (37 °C, 5% CO_2)
2. Centrifuges

Procedure

1. Seed 293T packaging cells at 1.3-1.5 x 10^5 cells/ml (6 ml per plate) in seeding media in 6 cm tissue culture plates.
2. Incubate cells for 24 h (37 °C, 5% CO_2), or until the following afternoon. After ~24 h, the cells should be ~70% confluent.
3. Transfect packaging cells:

a. Prepare a mixture of the 3 transfection plasmids:
   900 ng packaging plasmid
   100 ng envolop plasmid
   1 µg pLKO.1 plasmid
   10-30 µl OptiMEM media
b. Dilute Lipofectamine 2,000 with OptiMEM: 10 µl Lipofectamine + 90 µl OptiMEM. Add the Lipofectamine reagent dropwise and mix by swirling the tip or gently flicking the tube (do not mix by pipetting or vortexing). Incubate 5 min at room temperature.
c. Add the 3 plasmid mix dropwise to the diluted Lipofectamine reagent and mix by swirling the tip or gently flicking the tube.
d. Incubate the transfection mix for 20-30 min at RT.
e. Carefully transfer the transfection mix to the packaging cells in Seeding media. The packaging cells can be sensitive to perturbation - take care not to dislodge the cells from the plate. The total volume of transfection mix should be 100 to 125 µl per plate.

4. Incubate cells for 18 h (37 °C, 5% CO_2), or until the following morning.
5. Change media to remove the transfection reagent and replace with 6 ml harvest media for viral harvests.
6. Incubate cells for 24 h (37 °C, 5% CO_2).
7. Harvest media containing lentivirus at ~40 h post-transfection. Transfer media to a storage tube. Replace with 6 ml harvest media.
8. Repeat viral harvesting every 12-24 h and replace with 6 ml harvest media. Viral titer tends to decrease in later harvests; typically collect a total of 2-3 time points. After the final harvest, discard the packaging cells. The viral harvests may be pooled as desired.
9. Spin the media containing virus at 1,250 rpm for 5 min to pellet any packaging cells that were collected during harvesting. Pass the supernatant through 45 µm filter and transfer to a sterile storage tube.
10. Virus may be stored at 4 °C for short periods (hours to days), but should be frozen at -20 °C or -80 °C for long-term storage. To reduce the number of freeze/thaw cycles, aliquot large-scale virus preps to smaller storage tubes prior to long-term storage.

Recipes

1. Seeding media
   DMEM + 10% FBS without Pen/Strep
2. Harvest media
   DMEMD + 30% FBS + 1x Pen/Strep