

Lentivirus Production

Nabila Aboulaich

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 (*pCMV-dR8.91* or *pCMV-dR8.74 psPAX2*) and envelop plasmid (*pMD2.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. Seeding media (see Recipes)
10. Harvest media (see Recipes)

Equipment

1. Tissue culture incubator (37 °C, 5% CO₂)
2. Centrifuges
3. 6 cm tissue culture plates (Thermo Fisher Scientific)
4. 45 um filter (Thermo Fisher Scientific)
5. Storage tubes

Procedure

1. Seed 293T packaging cells at $1.3-1.5 \times 10^5$ cells/ml (6 ml per plate) in seeding media in 6 cm tissue culture plates.
2. Incubate cells for 24 h (37 °C, 5% CO₂), 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 envelop 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% CO₂), 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% CO₂).
 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