The RNAi Core

Version 5 (11/01/2012)

Lentiviral production

Introduction:

This contains the protocol for lentiviral production in 6 cm plate.

Lentiviral production consists of the following steps:

Day 1 (pm)	Seed 293T cells
Day 2 (pm)	Transfect cells with 3 plasmids
Day 3 (am)	16 hours post-transfection – change media; replace with BSA-containing media
Day 4 (am)	24 hours after media change – harvest virus (1) ; replace with BSA-containing media
Day 5 (am)	24 hours after harvest (1) – harvest virus (2); combine harvest (1) and (2); discard cells

I. Materials

1. Cell line: 293T packaging cells <recommended: healthy; avoid confluent culture, and no continuous subculture more than 10 passages>
2. Seeding media: DMEM+10% FBS (Biological Industries, Cat. 04-001-1A) + 0.1x Pen/Strep (Gibco, Cat. 15140122)
3. BSA-containing media: DMEM+10% FBS + 1% (W/V) BSA < Bovine Serum Albumin (MP Biomedicals, Cat. 02199896)> + 1x Pen/Strep
4. Plates: 6 cm tissue culture plate
5. Transfection-quality plasmid DNA:
   TRC library plasmid: pLKO.1-shRNA vector
   Packaging plasmid: pCMV-ΔR8.91 (containing gag, pol and rev genes)
   Envelope plasmid: pMD.G (VSV-G expressing plasmid)
6. OPTI-MEM serum-free media (Gibco, Cat. 31985-070)
7. Transfection reagent: TransIT-LT1 (Mirus Bio.) < alternative: X-tremeGENE HP DNA Transfection Reagent (Roche) >
8. Sterile Polypropylene storage tube

II. Instructions

1. Day 1: Seed 293T cells in 6 cm tissue culture plate. (seeding density: 3*10^5

cells/ml; seeding volume: 5 mL) and incubate cells in the incubator (37 °C, 5% CO2) until the following afternoon.

Note: [1] The cells are seeded in low-antibiotic growth media (DMEM + 10% FBS + 0.1x Pen/Strep).

[2] Before transferring to a culture incubator, keep cells settled for 30 min at room temperature to reduce uneven distribution.

2. Day 2: The cell density should be among 70% to 90% confluence for transfection with TransIT-LT1 or X-tremeGENE HP DNA transfection reagent. Allow transfection reagent, DNA and OPTI-MEM to equilibrate to room temperature.

a. Prepare a mixture of three plasmids:

Reagent	per 6 cm plate
pCMV-ΔR8.91	2.25 µg
pMD.G	0.25 µg
hairpin-PLKO.1	2.5 µg
OPTI-MEM to total volume	250 µl

Note: The cell density at transfection, DNA concentration for transfection and reagent to DNA ratio should be optimized according to the different transfection reagent kit.

b. Dilute transfection reagent, briefly vortex the reagent vial before using:

Reagent	per 6 cm plate
TransIT-LT1	15 µl
OPTI-MEM to total volume	250 µl

Add the TransIT-LT1 reagent into OPTI-MEM, pipette gently or flicking the tube gently to mix completely and incubate at RT for 5 min.

c. Add the plasmid mixture into the diluted transfection reagent and mix by swirling the tip or gently flicking the tube to mix completely

d. Incubate the transfection mix for 20-30 minutes at room temperature.

e. Add the transfection complex to the cells in a dropwise manner. 293T cells would be sensitive to perturbation, therefore take care not to dislodge the cells from the plate.

f. Incubate cells in the incubator (37 °C, 5% CO2) for 18 hours.

3. Day 3: Change media to remove the transfection reagent and replace with 5 mL BSA- containing media per plate. Incubate cells for 24 hr (37 °C, 5% CO2).

4. Day 4: Harvest media containing lentivirus at 40 hours post-transfection. Transfer media to a polypropylene storage tube and store at 4 °C. Replace with 5 mL BSA-

containing media for further harvest.

5. Day 5: Harvest media containing lentivirus at 64 hr post-transfection and then discard the cells. Pool the viral harvests and spin the media at 1250 rpm for 5 minutes to pellet the cells that were collected during harvest. Transfer the supernatant to a polypropylene storage tube and then aliquot to smaller storage tubes to reduce the numbers of freeze/thaw cycles.
   Note: Virus may be stored at 4 °C for a few days but should be stored at -80 °C for long-term storage.

Version Notes:
BSA-containing media for viral harvests: By comparison with growth media, viral harvest media containing 1 g/100 mL supplemental BSA can improve virus yield by ~2 fold.