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Producing and Concentrating Lenti-Cre for Mouse Infections

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Abstract

Lentiviral vectors offer versatility as vehicles for gene delivery. They can transduce a wide range of cell types and integrate into the host genome, which results in long-term expression of the transgene (Cre) both in vitro and in vivo. This protocol describes how lentiviral particles are produced, purified, and concentrated.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Dulbecco's modified Eagle's medium (DMEM)

Fetal bovine serum (FBS)

FuGENE 6 (Promega)

Hank's balanced salt solution (HBSS)

HEK293 T cells (Invitrogen R700-07)

Lentivirus-Cre (transfer vector expressing Cre; Addgene plasmid 17408)

Minimal essential medium (MEM)

Packaging plasmids (CMV-VSV-G [Addgene plasmid 8454] and Δ 8.2 [Addgene plasmid 8455])

Paraffin

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Penicillin/streptomycin

Ethanol (100%)

Equipment

Biosafety hood (BL2)

Incubator (37°C, 5% CO_2)

Microcentrifuge tubes, polypropylene

DO NOT use polystyrene tubes.

Refrigerated centrifuge (Sorvall Legend X1R)

Syringe filter (0.45 μm; Whatman 6780-2504)

Tissue culture dishes (6 cm)

Viral centrifuge tubes (Beckman 344058)

METHOD

While high titer suspensions can be produced easily, it is important to use caution when handling lentiviruses. Preparation of viruses should always occur in a biosafety hood according to biosafety level 2 research guidelines. Research with lentiviruses pseudotyped with VSV-G to enable infection of human cells, should be performed in accordance with your environmental health and safety office and Institutional Animal Care and Use Committee (IACUC).

Day 1

1. For each plasmid to be transfected, plate 7×10^5 HEK293T cells in 5 mL of DMEM in a 6-cm tissue culture dish. Incubate cells overnight in an incubator at 37°C with 5% CO_2.

Although cells should be passaged regularly in DMEM containing 10% FBS with penicillin/streptomycin, at this step, cells should be plated without penicillin/streptomycin.

Day 2

2. In polypropylene microcentrifuge tubes, make a cocktail for each transfection. Combine the following:

1 μg lentivirus-Cre expression vector,

250 ng VSV-G plasmid, and

750 ng Δ8.2 plasmid.

Then bring the volume up to 20 μL with serum-free MEM.

The ratio of expression plasmid to packaging plasmid to envelope plasmid can be varied to obtain optimal viral production.

3. In a separate polypropylene tube, create a master mix of FuGENE 6 transfection reagent in serum-free MEM. Each reaction will require 6 µL of FuGENE 6 and 74 µL of MEM. Add the MEM into the tube first and then directly add the FuGENE into the MEM. Do not allow FuGENE to come in contact with the wall of the tube before it has been diluted. Mix by flicking the tube and incubate 5 min at room temperature.

4. Add 80 µL of FuGENE master mix to each tube from Step 2 for a total volume of 100 µL. Mix by flicking the tube and incubate for 20 min at room temperature.

5. Without touching the sides of the dish, gently add the FuGENE:DNA mix dropwise to the HEK293 cells. Swirl to disperse the mixture evenly. Incubate the cells overnight (i.e., 12–15 h) in a 37°C, 5% CO_2 incubator.

Day 3

6. In the morning, aspirate the medium to remove the transfection reagent. Replace with 5 mL of fresh DMEM containing 10% FBS and penicillin/streptomycin. Incubate the cells for 24 h at 37°C, 5% CO_2.

Day 4

7. Collect medium from cells and transfer to polypropylene storage tubes. The medium contains the lentiviral particles. Store at 4°C. Add 5 mL of fresh medium containing antibiotics to the cells and incubate for 24 h at 37°C, 5% CO_2.

Day 5

8. Harvest medium from cells and pool with medium from Day 4. Centrifuge the medium at 1,250 rpm for 5 min to pellet any HEK293 cells that were inadvertently collected during harvesting.

9. Filter the medium through a 0.45-µmfilter. Virus may be stored at 4°C for a few days, but should be frozen at −20°C or −80°C for long-term storage.

Do not use a 0.22-µmfilter, as it may shear the viral envelope. Freeze/thaw cycles decrease the efficiency of the virus, so either use the virus without freezing or divide into aliquots before freezing.

Day 6

10. Rinse viral centrifuge tubes with 100% ethanol. Air dry the centrifuge tubes in the biosafety hood, with the UV light on for 10 min.

11. Add lentivirus to the UV-sterilized centrifuge tubes and bring the volume to 30 mL with DMEM medium.

12. Centrifuge the virus at 25,000 rpm for 2 h at 4°C in a refrigerated centrifuge.

13. Decant the supernatant without disrupting the pellet. Place the centrifuge tubes upside down on a tissue to remove any access medium for 10 min at room temperature.

14. Depending on the size of the pellet, resuspend in 100–400 μL of HBSS. Cover the centrifuge tubes with paraffin and store overnight at 4°C.