Preparation of Lentiviral Vectors

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HIV-1-based lentiviral vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G) are generated by transient transfection of three plasmids, the packaging construct (pCAG-HIVgp), in which all accessory genes (*vif*, *vpr*, *vpu*, and *nef*) and regulatory genes (*tat* and *rev*) have been deleted, the VSV-G- and Rev-expressing construct (pCMV-VSV-G-RSV-Rev), and the self-inactivating (SIN) lentiviral vector construct into 293T cells (1-3).

Methods

1. Harvest exponentially growing 293T cells by trypsinization (0.25% trypsin-0.02% EDTA) and seed 5×10^6 cells in poly-lysine coated 10-cm dish in 10 ml of complete DMEM medium (*see* Note 1).

2. Incubate the cells for 24 h at 37˚C in a humidified incubator with an atmosphere of 10% CO_2. The cells should be about 75% confluent at the time of transfection.

3. Transfection: Prepare a total volume of 450 μl of plasmid DNA solution containing 17 μg of the SIN vector plasmid (*see* Note 2), 10 μg of the packaging plasmid (pCAG-HIVgp), and 10 μg of the VSV-G- and Rev-expressing plasmid (pCMV-VSV-G-RSV-Rev) in a 5-ml polystyrene tube (Falcon 2058) or a 1.5-ml microfuge tube. Add 50 μl of 2.5 *M* $CaCl_2$ and then 500 μl of 2× BBS with gentle mixing. Incubate the mixture for 10-20 min at room temperature. Mix gently by pipetting and transfer the calcium phosphate-DNA solution dropwise to the dish. Rock the dish gently to mix the medium.

4. Incubate the cells for 12-16 h at 37˚C in a humidified incubator with an atmosphere of 3% CO_2.

5. Remove the medium by aspiration and add 7.5 ml of prewarmed (37˚C) complete DMEM medium containing 10 μ*M* Forskolin.

6. Incubate for 48 h at 37˚C in a humidified incubator with an atmosphere of 10% CO_2.

7. Remove the vector-containing medium and filter through a 0.45-μm filter (*see* Note 3).

8. Transfer the filtered medium to a conical ultracentrifuge tube (Beckman 358126) that fits a Beckman SW28 rotor including conical inserts (Beckman 358156) and centrifuge at 50,000g (19,400 rpm in a Beckman SW28 rotor) for 2 h at 20˚C.

9. Discard the supernatant and resuspend the pellet in an appropriate volume (up to 50 μl per dish) of HBSS by pipetting. Try to avoid introducing bubbles. When dealing with small-scale preparations of vector, the following step can be omitted; store the vector suspension at –80˚C.

10. Transfer the vector suspension to an ultracentrifuge tube (Beckman 344057) that fits a Beckman SW55TI rotor and fill the tube with HBSS. Centrifuge at 50,000g (24,000 rpm in a Beckman SW55TI rotor) for 2 h at 20˚C.

11. Discard the supernatant and resuspend the pellet in an appropriate volume (up to 10 μl per dish) of HBSS by pipetting. Try to avoid introducing bubbles.

12. Transfer the vector suspension to a tightly capped tube. Vortex the tube until no solid material is visible. If any debris is still visible, remove it by brief centrifugation and discard. Store the vector suspension in aliquots at –80˚C (*see* Note 4).

Notes

1. For large-scale preparation, 15-cm dishes can be used. Scale up all materials in proportion to the area of the dishes.

2. The latest SIN lentiviral vectors contain the central polypurine tract (cPPT) sequence, which improves the transduction efficiency (4-6), and the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), which enhances the expression of the transgene (7).

3. It is possible to add fresh medium and to collect vector-containing medium a second time from the cells. Add 7.5 ml of prewarmed (37˚C) complete DMEM medium containing 10 μ*M* Forskolin to the dish immediately after withdrawing the medium and continue incubation for another 48 h. Vector titers will be about 75% lower.

4. The titer of vectors is determined by measuring the amount of HIV-1 p24 gag antigen using an ELISA kit. (Commercial kits are available from PerkinElmer, Inc. (Boston, MA) and Advanced Biotechnologies, Inc. (Columbia, MD)). 1 ng of p24 would represent 5,000-10,000 infectious units (IU) on 293T or HeLa $CD4^+$ cells. In the case of vectors containing marker genes, the titer of vectors can be determined by infection of 293T or HeLa $CD4^+$ cells with serial dilutions of the vector stocks. Most vector stocks after two rounds of ultracentrifugation have titers ranging between 5×10^8 and 2×10^9 IU/ml. The titer of the vector stocks remains stable for at least 6 months when they are stored in closed tubes at –80˚C. In general, the vector stocks can be frozen and thawed several times with gradually loss of infectivity. If the yields of the vector are low, the number of infectious viral particles should be determined in samples taken at various stages during the purification to determine where losses are occurring.

Materials

Complete DMEM medium:
Dulbecco's Modified Eagle's Medium (SIGMA D5796) supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate.

5 mM Forskolin (500×):
Forskolin (SIGMA F3917) 25 mg/12.18 ml DMSO, store at –20˚C

Poly-lysine coated dish:
Add 3 ml poly-L-lysine (dilute 0.01% solution (SIGMA P4832) 1:5 with PBS) to 10-cm dish for a minimum of 10 min at room temperature, then aspirate off.

Calcium phosphate transfection buffer:
150 mM Na_2HPO_4 1.065 g/50 ml

2× BBS
50 mM BES 2.665 g
280 mM NaCl 4.091 g
1.5 mM Na_2HPO_4 2.5 ml of 150 mM Na_2HPO_4
↓
Autoclaved Milli-Q H_2O to 245 ml
Adjust pH 6.95 with 2N NaOH (about 2.5 ml)
Autoclaved Milli-Q H_2O to 250 ml
↓
0.22-μm filter
↓
aliquot (e.g. 12.5 ml) and store at –20˚C

2.5 M $CaCl_2$ $CaCl_2 2H_2O$ 18.38 g/50 ml
↓
0.22-μm filter (pre-wetted with Milli-Q H_2O)
↓
aliquot (e.g. 10 ml) and store at –20˚C

Ref.: Mol Cell Biol. vol. 7, 2745-2752 (1987)

HBSS:
Hank's Balanced Salt Solution (GIBCO 14025-092)

References

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