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Lentivirus Production Protocol

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Abstract

This protocol describes producing lentiviral particles by transiently transfecting HEK-293 based packaging cells with a transfer vector plus packaging and envelope plasmids, harvesting and clarifying viral supernatant, and then transducing target cells in a 6-well format. Transduced cells are recovered in fresh media and subsequently selected to generate a stable, enriched population before expansion outside the lentivirus workspace.

Expected results include robust viral production from high-transfection-efficiency HEK-293T or HEK-293FT cells and efficient gene delivery to target cells, enhanced by polybrene during transduction. After antibiotic selection, surviving cells should show stable integration and consistent expression of the lentiviral construct (e.g., fluorescent reporters or other transgenes) with minimal residual non-transduced cells.

Materials

- Transfer vector: pARR3tk-eGFP/SV40-mCherry
- Lentiviral packaging vector: psPAX2
- Lentiviral envelop vector: pMD2.G
- HEK293T cells (ATCC, CRL-3216)
- DMEM (Hyclone, # SH30022.01)
- FBS (Thermo Fisher Scientific, 10438026)
- Pen/Strep (Hyclone, SV30010)
- PBS (VPC Core)
- Trypsin (Hyclone, SH30042.01)
- ProFection Mammalian Transfection System (Promega, E1200)
- Sterile double distilled water (ddH_2O)
- Lipfectamine 3000 Reagent (Thermo Fisher, L3000015)
- 70% Ethanol (VPC Core)
- Long sleeved 26 regular nitrile gloves (VPC Core)
- Isolation gowns (VPC Core)
- 10 cm tissue culture plate (Fisher, 08-772-4A)
- 15 and 50 mL Falcon tubes (Corning, 430828)
- Filter-tips (P2/P10, P20, P200, P1000)
- Premade Lentivirus
- LNCaP cells (ATCC, CRL-1740)
- LAPC4 cells (a kind gift from Dr. Charles Sawyers lab)
- VCaP cells (ATCC, CRL-2876)
- RPMI 1640 (Hyclone, SH30027.01)
- Polybrene (Santa Cruz, sc-134220)
- Puromycin dihydrochloride (Invitrogen, A1113803)
- 6-well tissue culture plate (Corning, 3516)
- 0.45 Steriflip™ Sterile Disposable Vacuum Filter Units (Fisher, SE1M003M00)
- CO_2 incubator
- 4°C fridge
- Benchtop Vortex
- Microscope
- Single-channel Pipettes (P2, P10, P20, P200, P1000)
- Centrifuge (4°C, Beckman Coulter)

Safety warnings

Biohazard Concerns
- Lentivirus is a modified HIV virus that is mostly unable to replicate in a host, and must be handled with caution at all times.
- When working with these viruses, work only in BL2+ designated hoods and viral vector rooms.
- All handling, storage, and disposal of biohazardous waste must be in accordance with Institute rules and regulations, Occupational Safety and Health Administration (OSHA), Environment Protection Agency (EPA) and Municipal Waste Association (MWA).

Before start

Before production of lentivirus, seed target cells at 1-4×10^5/well cells in a 6-well plate in 2 mL of media supplemented with 5-10% FBS (Day 0).

- Note: Target cells should be at least 20-30% confluent at the time of transduction (Day 1).

Lentivirus Preparation in HEK-293 cells

1 Plate 4 × 10^6 HEK-293 cells of choice in a 10 cm dish in 7 mL of DMEM supplemented with 5-10% FBS and 1% Pen/Strep.
- NOTE: The difference in transduction protocols for HEK-293, HEK293T, and HEK293FT cells lies in their inherent transfection efficiency and growth characteristics, driven by the presence of the SV40 large T-antigen (T-Ag), making 293T/FT cells ideal for high-yield viral packaging (lentivirus, AAV) and protein expression, while standard HEK-293s are simpler for general use or stable cell lines.

2 Mix the following in a 15 mL Falcon tube containing 750µL Opti-MEM.
1. Lenti plasmid (eg: pARR3tk-eGFP/SV40-mCherry): 15 µg
2. Packaging plasmid (eg: psPAX2): 10 µg
3. Envelope plasmid (eg: pMD2.G): 5 µg
4. Add 60µL P3000 Reagent.

3 In a separate tube combine 45µL of Lipofectamine 3000 with 750µL Opti-MEM. Mix well.

4 Combine DNA and Lipofectamine tubes.

5 Incubate at room temperature for 15 minutes.

6 Add the precipitate drop-wise for even distribution onto HEK-293 cells.

7 Swirl plates gently to mix.

8 Incubate overnight (~16 hours) at 37°C.

9 Aspirate medium from each plate.

10 Add 5 mL DMEM supplemented with 5-10% FBS and 1% Pen/Strep containing 10mM HEPES.

11 Incubate for 48 hours.

12 Collect the supernatant in a 50 mL Falcon tube.

13 Centrifuge at 2,000 x rpm 4°C for 5 min.

14 Filter the supernatant using a 0.45 µm Steriflip tube to remove cellular debris. The virus containing supernatant can be used immediately to transduce cells in Lentiviral Room, or stored for up to 24 hours at 4°C in the designated lentiviral refrigerator.

Transduction of Target Cells with Lentivirus

15 **Day 0 (24 hrs before the transduction; *can be performed in any Bio-safety Cabinet in the centre)***
Seed target cells at 1-4×10^5/well in a 6-well plate in 2 mL of media supplemented with 5-10% FBS.
- Note: Cells should be at least 20-30% confluent at the time of transduction.

16 **Day 1 (the day of transduction)**
Transfer plates/dishes of target cell to be transduced to the lentivirus room.

17 Remove culture medium from the plates and add 2-3 mL/well of virus containing medium along with 8 µg/mL Polybrene.

18 **Day 2 (24 hrs post transduction)**
Replace Lentivirus/Polybrene mixture with fresh media (supplemented with 10% FBS and 1% Pen/Strep).
Incubate cells for 48 hrs.

19 **Day 4 (72 hrs post transduction)**
Replace the media and add 1 µg/mL puromycin for selection for additional 72 hours.

20 **Day 7 (144 hrs post transduction)**
Replace media with fresh media supplemented with 10% FBS and 1% Pen/Strep containing 1 µg/mL puromycin.

21 Subculture the cells at least twice before removing from Lentivirus room.

22 Take the cells out of the lentivirus room and continue culturing/selection in the regular culture room.