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Lentivirus production

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Akio Mori[1], Robert Edwards[1]

[1]University of California San Francisco

Akio Mori

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the production of lentivirus in HEK293T cells for the transduction of primary neuronal cultures.

Materials

Cells

- HEK293T cells (early passage) (CRL-3216, ATCC)

Plasmids

- Gene of interest in lentiviral backbone
- Accessory plasmids: pMDLG/pRRE, pIVS-VSVG, pRSV-Rev

Reagents

- FuGENE transfection reagent (E2311, Promega)
- Neurobasal medium (21103-049, Gibco)
- Poly-L-lysine for coating 6-well plates (P2636, Sigma-Aldrich)

Consumables

- 6-well tissue culture plates
- 0.45 µm filter units

Cell seeding

1 Coat 6-well plates with poly-L-lysine for 1 h, and wash twice with PBS.

2 Seed early-passage (< P10) HEK293T cells onto coated 6-well plates to reach ~70–80% confluency the next day.

Transfection

3 Prepare a DNA mixture per well:
a. In 200 µL Opti-MEM, combine:
- 1.5 µg plasmid encoding the gene of interest (pFUGW, pJHUG, or pJHUIC)
- 0.5 µg each of accessory plasmids (pMDLG/pRRE, pIVS-VSVG, pRSV-Rev)
b. Add 12 µL FuGENE HD transfection reagent to the DNA solution.
c. Mix gently by vortexing.
d. Incubate at room temperature for 10–15 min to allow complex formation.

4 Remove HEK cells' medium and replace it with 1.8 mL of fresh medium per well.

5 Add the transfection mixture to each well and incubate overnight at 37°C, 5% CO_2.

Virus production

6 The next morning, remove the transfection medium and replace it with 2 mL neurobasal medium or HEK cells' medium per well.

7 Incubate cells for 36–48 h to allow virus production.

8 Collect the culture medium containing lentivirus. Filter the collected medium through a 0.45 µm filter to remove cellular debris. Use the viral supernatant immediately for neuronal transduction, or aliquot and store at -80°C.

Infection of primary neurons

9 Add 20–40 µL viral supernatant per cloning ring of primary neuronal culture to achieve efficient transduction.