iPSCs

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TRANSDUCTION OF i^{3}NEURONS (Support Protocol 4)

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.5wvg7e6

Neurodegeneration Method Development Community

ABSTRACT

Lentiviral infection of neurons can be used for a variety of applications in microscopy and biochemistry, provided that the transgene is driven by promoters not silenced in iPSCs or neurons (e.g., CAG, PGK In EF-1α, not CMV, see Critical Parameters and Troubleshooting). The precise parameters for optimal viral production and supernatant collection/concentration will depend on the type of virus used, viral packaging mechanism, viral packaging cell line, and specific transgene introduced. Highly efficient results have been observed with lentivirus produced by Lenti-X HEK cells, with transgenes carried in the pLEX backbone.

EXTERNAL LINK

https://doi.org/10.1002/cpcb.51

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Fernandopulle, M. S., Prestil, R., Grunseich, C., Wang, C., Gan, L., & Ward, M. E. (2018). Transcription-factor mediated differentiation of human iPSCs into neurons. Current Protocols in Cell Biology, e51. doi:https://doi.org/10.1002/cpcb.51

fernandopulle2018.pdf

MATERIALS TEXT

- Poly-L-ornithine (PLO) coating solution (see Table 3)
- Lenti-X HEK cells (Clontech, cat. no. 632180)

Lenti-X™ 293T Cell Line
by Takarabio
Catalog #: 632180

- Opti-MEM (ThermoFisher, cat. no. 31985062)

Opti-MEM™ Reduced Serum Medium
by Thermo Fisher Scientific
Catalog #: 31985062

- DMEM/F12 medium (Gibco, cat. no. 11320033) containing 10% (v/v) FBS, heat inactivated (Gibco, cat. no. 16140071)

DMEM/F-12
by Gibco, ThermoFisher
Catalog #: 11320033

Fetal Bovine Serum, qualified, heat inactivated, United States
by Thermo Fisher Scientific
Catalog #: 16140071

- psPAX (Addgene, cat. no. 12260), pMD302 (Addgene, cat. no. 12259), pAdvantage (Promega cat. no. E1711) viral packaging plasmids

psPAX2
by addgene
Catalog #: 12260

pMD2.G
by addgene
Catalog #: 12259

pAdVAntage(TM) Vector, 20ug
by Promega
Catalog #: E1711

- pLEX or equivalent lentiviral vector with desired transgene
- Lipofectamine 3000 with P3000 reagent (ThermoFisher, cat. no. L3000015)

Lipofectamine 3000
by Thermo Fisher Scientific
Catalog #: L3000015

- 500× ViralBoost reagent (ALSTEM, cat. no. VB100)

ViralBoost Reagent
Catalog #: VB100

- Inverted microscope
- i^3Neurons (See Basic Protocols 5 and 6)
- Cortical Neuron Culture Medium (CM, see Table 4)

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

For each new viral preparation introduced to iPSCs, i^3Neurons, or i^3LMNs, titer should be assessed with a serial dilution of viral supernatant relative to the culture medium (E8, CM, or MM, respectively). This dilution typically begins with a 1:1 mixture of culture medium and viral supernatant, and reduces by 50 % in each subsequent well. Viral supernatant-containing medium should be removed from cells approximately 24 hr after addition, and replaced with fresh culture medium. With lentivirus, maximal transgene expression typically occurs 3 to 5 days after infection. Depending on the particular transgene, vector, and viral packaging system, initial expression can often be assayed 2 days after infection.

Day 0

1 Coat a 6-well dish with PLO (see Basic Protocol 6).

2 Plate 2.5 × 10^6 Lenti-X HEK cells in 6-well dish well in **1.5 ml** DMEM/F12 containing 10 % FBS (HEK medium).

Day 1

3 Check cells for confluency under an inverted microscope.
Cells should be > 95 % confluent for maximal viral production efficiency

If necessary, wait a day to transfect so that the cells are nearly confluent.

4 Warm two tubes with **150 µl** Opti-MEM each to **Room temperature** .

5 Add **1.6 µg** psPAX, **0.6 µg** pMD302, **0.2 µg** pAdvantage, **2.4 µg** pLEX viral vector, and **10 µl** P3000 reagent to tube A.

6 Gently flick to mix.

7 Add **1.87 µl** Lipofectamine 3000 to tube B.

8 Flick to mix.

9 Incubate tubes at **Room temperature** for **00:05:00** .

10 Add the contents of tube A into tube B with a P200.

11 Immediately flick the tube several times to mix.

12 Incubate tube at **Room temperature** for **00:20:00** to **00:40:00** .

13 Add contents of tube dropwise to to the medium of Lenti-X cells with a P1000.

14 Agitate plate to evenly distribute transfection solution.

Day 2

15 Replace medium on Lenti-X HEK cells with **3 ml** of warm, fresh HEK medium supplemented with **6 µl** of 500× ViralBoost reagent.

16 Check cells with a fluorescent microscope to ensure expression of any fluorescent proteins in the viral vector.

Day 4

17 Check cells under inverted microscope for multinucleated morphology (indicating viral production) and fluorescent protein production.

18 Collect medium from well.

19 Filter to purify virus from floating HEK cells.

Instead of filtering, medium may be centrifuged at > **10000 x g** for **00:10:00** at **4 °C** to remove cells/debris, followed by transferring the supernatant to a new microcentrifuge tube.

20 Either use immediately or freeze aliquots at **-80 °C** .

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Optional: Replace medium on HEK cells with **1 ml** to **2 ml** fresh HEK medium, to be collected the following day. This process can be repeated until Day 6 to collect more virus. Be aware that viral titer decreases with each collection. Lentiviral preps can also be concentrated using various commercially available reagents (e.g., Clontech, cat. no. 631231).