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TRANSDUCTION OF i³NEURONS (Support Protocol 4)

In 1 collection

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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-1 α , 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.

ATTACHMENTS

[fernandopulle2018.pdf](#)

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

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mediated differentiation

MATERIALS

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

☒ Lenti-X™ 293T Cell Line **Takarabio Catalog #632180**

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

☒ Opti-MEM™ Reduced Serum Medium **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 **Gibco, ThermoFisher Catalog #11320033**

☒ Fetal Bovine Serum, qualified, heat inactivated, United States **Thermo Fisher Scientific Catalog #16140071**

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

☒ psPAX2 **addgene Catalog #12260**

☒ pMD2.G **addgene Catalog #12259**

☒ pAdVantage(TM) Vector, 20ug **Promega Catalog #E1711**

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

☒ Lipofectamine 3000 **Thermo Fisher Scientific Catalog #L3000015**

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

☒ ViralBoost Reagent **Contributed by users Catalog #VB100**

- Inverted microscope
- i³Neurons (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 START INSTRUCTIONS

For each new viral preparation introduced to iPSCs, i³Neurons, or i³LMNs, 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

Note

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

- 4 Warm two tubes with 150 μ L 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.

Note

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 .

21 *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](https://www.clontech.com/products/29110)).

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