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Lentivirus production for primary neuron transduction

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ABSTRACT

This protocol describes the production of lentiviruses to transduce mouse primary neurons and has to be performed in a biosafety level 2 laboratory

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SAFETY WARNINGS

This protocol describes the production of lentiviruses to transduce mouse primary neurons and has to be performed in a biosafety level 2 laboratory

1 Expand HEK293T cells (LentiX 293T cell line, Takara) for lentiviral packaging to 70-85% confluency in DMEM Glutamax (+4.5 g/L D-glucose, - pyruvate) supplemented with 10% Fetal Bovine Serum (FBS)(Sigma), 1% G418 (Gibco), 1% Non-Essential Amino Acids (Thermo Fisher) and 1% HEPES (Biomol).

NOTE: Only low passage cells should be used.

2 For lentiviral production, plate cells (~ 4.8 x 106) in a three-layered 525 cm2 flask (Falcon).

3 On the following day, transfect cells with 59.52 µg expression plasmid, 35.2 µg packaging plasmid psPAX2 (RRID:Addgene_12260) and 20.48 µg envelope plasmid pVsVg (gift from Dieter Edbauer) using 345.6 µl TransIT-Lenti transfection reagent (Mirus) in 9.6 mL DMEM without FBS. 1d

4 Incubate transfection mix for 20 min at room temperature and exchange the cell medium in the meantime. 20m

5 Add 10 mL of transfection mix to the flask, followed by incubation overnight.

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6 Exchange the medium on the following day. 1d

7 After 48-52 h, collect culture medium containing the viral particles and centrifuge for 10 min at 1,200 x g. 2d

8 Filter the supernatant through 0.45 µm pore size filters using 50 mL syringes and add 20 mL Lenti-X concentrator (Takara) to filtered supernatant.

9 Incubate overnight at 4 °C and centrifuge samples at 1,500 x g for 45 min at 4 °C. 1d

10 Remove the supernatant and resuspend the lentivirus pellet in 150 µL TBS-5 buffer (50 mM Tris-HCl pH 7.8, 130 mM NaCl, 10 mM KCl, 5 mM MgCl2).

11 After aliquoting, store lentivirus at -80 °C.

12 Thaw virus preparation immediately before adding to freshly prepared neuronal culture medium.

13 Remove a fifth of the medium from cultured neurons and add the equivalent volume of virus-containing medium.

NOTE: Volume of concentrated virus to be added to the neurons and the length of transduction should be determined empirically.

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