Lentivirus production

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ABSTRACT

This protocol describes the production of lentiviruses to transduce HEK293T cells and has to be performed in a biosafety level 2 laboratory

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SAFETY WARNINGS

Has to be performed in a biosafety level 2 laboratory.

Lentivirus production 1d 1h 10m

1 Plate ~3.6x106 Lenti-X HEK293T cells (Takara) in 10 cm dish in 10 mL standard DMEM. Cells should be ~80% confluent at the time of transfection.

NOTE: Only low passage cells should be used.

2 Next day, remove 5 mL medium and replenish with fresh medium.

3 Warm up reduced serum medium e.g. Opti-MEM (Gibco) and transfection reagent to room temperature (RT). This protocol was performed with Lipofectamine 3000 transfection reagent (Thermo).

4 Add 24 µL Lipofectamine 3000 to 600 µL Opti-MEM, mix by vortexing and incubate 5 min at RT. 5m

5 In another tube, mix 6 µg plasmid containing gene of interest, 5 µg packaging plasmid psPAX2 (RRID:Addgene_12260), 1 µg envelope plasmid pMD2.G (RRID:Addgene_12259) and 24 µL P3000 reagent (provided by manufacturer along with Lipofectamine 3000 reagent) in 600 µL Opti-MEM, mix by vortexing and incubate 5 min at RT.

6 Mix contents of both tubes and incubate for 15 min at RT. 15m

7 Add DNA-lipid complex to cells dropwise.

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8 2 days later, collect virus-containing medium and centrifuge for 5 min at 1,000 x g. 2d

9 Collect supernatant in a fresh tube and proceed with concentration.

Concentration 1d 1h 10m

10 Add Lenti-X concentrator (Takara) to clarified virus-containing medium at 1:4 dilution and mix well by gently inverting tube.

11 Incubate overnight or 2 h at 4 °C. 1d

12 Next day, centrifuge for 45 min at 1,500 x g at 4 °C followed by gently aspirating supernatant. 45m

13 Resuspend viral pellet in 100 - 1000 µL PBS, aliquot and store at -80 °C until use.

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