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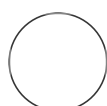
## Lentivirus production

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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

### SAFETY WARNINGS



Has to be performed in a biosafety level 2 laboratory.

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We use this protocol and it's working

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## Lentivirus production

1d 1h 10m

- 1 Plate  $\sim 3.6 \times 10^6$  Lenti-X HEK293T cells (Takara) in 10 cm dish in 10 mL standard DMEM. Cells should be  $\sim 80\%$  confluent at the time of transfection.

### Note

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  $\mu\text{L}$  Lipofectamine 3000 to 600  $\mu\text{L}$  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.

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  $\mu$ L PBS, aliquot and store at -80 °C until use.