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Lentivirus preparation for neuronal transdifferentiation V.1

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We use this protocol and it's working

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Abstract

This is a protocol for the preparation of Lentiviruses.

Troubleshooting

Preparation of culture medium and plasmids

1 1. HEK293T medium @4C lasts for 1 month. A total of 500 mL:
- 435 ml DMEM (High glucose, GlutaMAX) (Thermo Fisher Scientific)
- 50 ml Fetal bovine serum (FBS) (Thermo Fisher Scientific)
- 5 ml Penicillin-Streptomycin (Thermo Fisher Scientific)
- 5 ml HEPES (1M; Thermo Fisher Scientific)
- 5 ml Sodium pyruvate (100 mM; Thermo Fisher Scientific)
- Sterilized by filtration through a 0.22 µm filter

2. Virus medium @4C lasts for 1 month. A total of 500 mL:
- 470 ml DMEM (High glucose, GlutaMAX) (ThermoFisher Scientific)
- 10 ml Fetal bovine serum (FBS) (Thermo Fisher Scientific)
- 5 ml Penicillin-Streptomycin (Thermo Fisher Scientific)
- 5 ml MEM NEAA (100X; Thermo Fisher Scientific)
- 5 ml HEPES (1M; Thermo Fisher Scientific)
- 5 ml Sodium pyruvate (100 mM; Thermo Fisher Scientific)
- 0.5 ml beta-mercaptoethanol (Thermo Fisher Scientific)
- Sterilized by filtration through a 0.22 µm filter

3. OptiMEM+GlutaMAX (Thermo Fisher Scientific)

4. Lipofectamine 2000 (Thermo Fisher Scientific)

5. Plasmids:
- Transfer: FUW-M2rtTA (Addgene plasmid# 20342), pTet-O-Ngn2-puro (Addgene plasmid# 52047), Tet-O-FUW-Ascl1 (Addgene plasmid# 27150), Tet-O-FUW-Brn2 (Addgene plasmid# 27151), Tet-O-FUW-Myt1l (Addgene plasmid# 27152)
- Packaging: psPAX2 (Addgene plasmid# 12260)
- Envelope: pMD2.G (Addgene plasmid# 12259)

6. Millex syringe 0.22 & 0.45 µm filter

Generation of Lentiviruses

2 Seed 6×10^6 HEK293T cells in a 10-cm dish for each transfer plasmid and culture to reach 80% confluence next day.

3 Prepare DNA and Lipofectamine 2000 dilutions.
- 5 µg Transfer plasmid
- 4 µg Packaging plasmid

- 2.5 µg Envelope plasmid
- DNA diluted into 300 µL OptiMEM
- 23 µL Lipofectamine 2000, diluted into 300 µL OptiMEM

4 Vortex DNA and Lipofectamine dilutions, centrifuge for 5 sec and incubate at room temperature for 5 mins.

5 Combine DNA and Lipofectamine dilutions, vortex, centrifuge and incubate the mixture for additional 15 mins.

6 Remove old HEK293T medium, rinse the cells with OptiMEM once and then add 5 mL OptiMEM to each dish.

7 Add a total of 600 µL of Lipofectamine/DNA mixture to the cells dropwise across the whole dish, gently shake in vertical × horizontal direction a few times and return the dishes to the incubator.

8 After 6-hr incubation, remove the transfection medium and add 9 mL of fresh Virus medium to each dish.

9 For additional 24-hr incubation, collect the viral particle-containing medium from each dish and store at 4°C.

10 After medium collection, add fresh 8 mL of pre-warm Virus medium to each dish.

11 After 24-hr incubation, collect the medium and pool it with the first collection.

12 The cells can now be bleached and disposed of accordingly.

13 Seal the pooled medium with parafilm and centrifuge in the swinging bucket at 400xG for 5 mins at 4°C to pellet cell debris.

14 Carefully take the cleared supernatant and pass through a 0.45 µm filter.

15 Viral supernatant is stored at 4°C until use and good for 2 weeks.