Feb 06, 2025 Version 2

Lentivirus preparation for neuronal transdifferentiation V.2

Nature Cell Biology

In 2 collections

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx317wg8j/v2

Ching-Chieh Chou[1], Judith Frydman[1]

[1]Department of Biology, Stanford University

Ching-Chieh Chou
Stanford University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx317wg8j/v2

External link: https://doi.org/10.1038/s41556-025-01623-y

Protocol Citation: Ching-Chieh Chou, Judith Frydman 2025. Lentivirus preparation for neuronal transdifferentiation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx317wg8j/v2Version created by Ching-Chieh Chou

Manuscript citation:
Chou, CC., Vest, R., Prado, M.A. *et al.* Proteostasis and lysosomal repair deficits in transdifferentiated neurons of Alzheimer's disease. *Nat Cell Biol* **27**, 619–632 (2025). https://doi.org/10.1038/s41556-025-01623-y

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: February 06, 2025

Last Modified: February 06, 2025

Protocol Integer ID: 119733

Keywords: ASAPCRN, Lentiviruse, lentivirus preparation for neuronal transdifferentiation, lentivirus preparation, lentivirus, neuronal transdifferentiation, protocol for the preparation, preparation

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Abstract

This is a protocol for the preparation of Lentiviruses.

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

Culturing HEK293T cells

2 Culture HEK293T cells in a 10-cm dish or T-75 flask and place in a 37°C, 5% CO_2 incubator

3 Monitor cell growth daily and passage when the cells reach 80–90% confluence (~every 2–3 days).

4 Before passaging, pre-warm the complete DMEM and 0.05% Trypsin-EDTA at 37°C.

5 Aspirate the old medium and rinse the cells once with 5 mL PBS.

6 Add 3-5 mL 0.05% Trypsin-EDTA and incubate at 37°C for 3 min until cells detach (tap the flask gently if needed). Add 5–10 mL complete DMEM to neutralize trypsin.

7 Transfer the cell-containing solution into a conical tube and centrifuge at 300 × g for 5 min.

8 Aspirate the supernatant and resuspend the pellet in fresh DMEM. Plate cells at the desired density.

Generation of Lentiviruses

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

10 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

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

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

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

14 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.

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

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

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

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

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

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

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

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