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Reprogramming of mouse fibroblasts into iPSCs

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Abstract

This protocol is derived from the original method to generate induced pluripotent stem cells (iPSCs)[1, 2], and describes the reprogramming of mouse embryonic fibroblasts (MEFs) and mouse tail tip fibroblasts (TTFs), using pMXs-based retrovirus encoding Oct4, Sox2, Klf4, and c-Myc.

Subject terms: **Cell biology**

Keywords: **Reprogramming** **induced pluripotent stem cell** **fibroblast.**

Reagents

MEF/TTF medium
DMEM (Gibco; 10569-010)
10% FBS (Gibco; 16000-044)
2 mM Glutamax (Gibco; 35050-061)
0.1 mM 2-mercaptoethanol (Gibco; 21985-023)
100 units/ml penicillin and 100 µg/ml streptomycin (Gibco; 15140-122)

Plat-E medium
DMEM (Gibco; 10569-010)
10% FBS (Gibco; 16000-044)
2 mM Glutamax (Gibco; 35050-061)
0.1 mM 2-mercaptoethanol (Gibco; 21985-023)

ESC medium
Knockout DMEM (Gibco; 10829-018)
10% FBS (Gibco; 16141-079)
10% KSR (Gibco; 10828-028)
2 mM Glutamax (Gibco; 35050-061)

0.1 mM nonessential amino acids (Gibco; 11140-050)
0.1 mM 2-mercaptoethanol (Gibco; 21985-023)
10^3 units/ml leukemia inhibitory factor (Stemgent; 03-0011-100)
100 units/ml penicillin and 100 µg/ml streptomycin (Gibco; 15140-122)

DPBS (Gibco; 14190-144)

0.25% Trypsin-EDTA (Gibco; 25200-056)

FuGENE HD Transfection Reagent (Promega; E2311)

Opti-MEM® I Reduced Serum Medium (Gibco; 31985-047)

Procedure

Preparation of MEFs
1. Isolate mouse embryos at E13.5 (embryonic day 13.5) and wash them with DPBS.
2. Remove heads, tails and visceral tissues from the embryos.
3. Mince the remaining bodies and then incubate them in 0.25% trypsin-EDTA solution (1 ml per embryo) at 37°C for 10–15 min
4. After incubation, add an equal volume of MEF medium.
5. Pipet up and down thoroughly to dissociate the cells.
6. Transfer cellular suspension into 150 mm dishes (three embryos per dish) and culture at 37°C with 5% CO_2.
7. Change fresh MEF medium every day from the next day.

Preparation of TTFs
1. Cut the tail tips from adult mice.
2. Peel the tail tips and mince them into 1–2 mm pieces.
3. Place the pieces on 100 mm dishes (two tail tips per dish) and culture in TTF medium.
4. 2 days later, change fresh TTF medium every other day.
5. After large amounts of TTFs migrate out, remove the pieces by washing with DPBS.
6. Change fresh TTF medium every day.

Reprogramming of fibroblasts
1. Maintain Plat-E packaging cells in Plat-E medium.
2. Split cells into 6-well plates at a density of 8×10^6 cells per plate.
3. On the next day, transfect Plat-E cells with pMXs plasmids encoding Oct4, Sox2, Klf4 and cMyc individually, using FuGENE HD, according to manufacturer's instruction.
For each well, dilute 3 µg plasmid and 9 µl FuGENE HD transfection reagent in 200 µl Opti-MEM I

Reduced Serum Medium. The DNA/FuGENE HD mixtures are incubated for 30 min at room temperature, and added onto Plat-E cells.

4. Change fresh Plat-E medium 1 day after transfection.

5. After an incubation for 24 h, collect virus-containing supernatants, supplement with polybrene (1:2000 dilution), and filter the mixtures through 0.45 μM cellulose acetate filters.

6. Combine and mix Oct4, Sox2, Klf4 and c-Myc viruses with equal volume.

7. Incubate MEFs (at passage 2 or 3) or TTFs (at passage 1 or 2) that are cultured on 0.1% gelatin-coated plates with virus/polybrene-containing supernatants for 24 h.

8. Change ESC medium after transduction.

9. Continuously culture reprogramming cells with ESC medium for at least 2 weeks.

References

1. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676 (2006).

2. Shi, Y. et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 3, 568-574 (2008).

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Competing financial interests

The authors declare no conflicting financial interests.

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