

Materials you will need:

Human ES medium

DMEM/F12	Invitrogen	10565-042		
Pen/strep	Invitrogen	15140-122	1X	1 mL
L-Glutamine	Invitrogen	25030-081	2 mM	2 mL
nonessential amino acids	Invitrogen	11140-050	0.1 mM	2 mL
2-mercaptoethanol	Sigma	M7522	0.1 mM (1000x)	0.2 mL
knockout serum replacement	Invitrogen	10828-028	20%	40 mL
bFGF	Invitrogen	13256-029	5 ng/mL	0.2 mL
Total				200 mL

hFibroblast medium

DMEM	Invitrogen	11965-092		
Pen/strep	Invitrogen	15140-122	1X	2.5 mL
L-Glutamine	Invitrogen	25030-081	2 mM	5 mL
FBS	Invitrogen	16000-044	10%	50mL
Total				500 mL

High titer retrovirus or lentivirus expressing hOct4, hSox2, hKLF4 and hc-Myc. The minimum titers you will require are approximately $>10^6$ IFU/ml.

Procedure

Lentiviral transduction of human dermal fibroblasts

1. When hFib cells reaches 80% confluence, aspirate medium, wash twice with PBS, cover cells with 0.05% trypsin, and incubate for 5 min at 37°C.
2. Inactivate trypsin with hFib medium, collect cells in a 15 ml conical tube.
3. Centrifuge cells at 100xg at room temperature for 3 min and discard the supernatant.
4. Resuspend the cells in 1 ml hFib medium and count the cell number using a hemacytometer.
5. Plate 1×10^5 cells in each well of 6-well plate, and incubate cells at 37°C, 5% CO₂, for 6 hours.
6. Aspirate medium to remove dead cells, and add 2 ml of fresh hFib medium.
7. Add lentiviruses carrying hOCT4, hSOX2, hKLF4 and hc-MYC, respectively, with an MOI of 5. Infect one well with lentiviruses at MOI 10 and one well with empty vector as control.
8. Or infect the cells with virus supernatant mixture (0.5 ml each and 2 ml in total).
9. Add 10 µl of 200x TransDux™ solution into each well, and mix gently by swirling the plate.
10. Repeat steps 7 and 8 next two to three days (decrease the c-MYC MOI in these steps to an MOI of 1 to 2).
11. One day after final infection, remove the viral supernatant, wash three times with PBS, and add 3 ml of hFib medium.
12. Four days after infection, plate 1×10^6 mitomycin C treated MEF cells in a 100-mm dish or two 60-mm dishes (precoated with 0.1% gelatin). Incubate until the next day.
13. On day 5 after third infection, trypsinize the infected cells and plate them in a 100-mm dish at different cell densities between 5×10^4 to 2×10^5 cells or in a 60-mm dish at densities between 2×10^4 to 1×10^5 cells.
14. Two days later, aspirate medium and replace with hES medium.
15. Change medium everyday with hES medium.
16. After about 3-4 weeks, check the colony formation and pick the colonies with ES-like morphology manually for expansion in hES media.

Example of pre-iPSC colony (after 10-15 days), note the loosely organized colony layered on top of the feeder cells.



Example of a more mature iPSC colony (after 3-4 weeks), note the large and flat appearance.

