SBI System Biosciences
Accelerating Discoveries in Functional Genomics

System Biosciences
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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 lentivirus expressing mouse/human mir-302bcad/367. The minimum titers you will require are approximately >10^6 IFU/ml.

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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 x 10^5 cells in each well of 6-well plate, and incubate cells at 37°C, 5% CO_2, for 6 hours.
6. Aspirate medium to remove dead cells, and add 2 ml of fresh hFib medium.
7. Add lentiviruses carrying mouse/human mir-302bcad/367 clusters, with an MOI of 5-10. Infect one well with lentiviruses at MOI 10 and one well with empty vector as control.
8. Add 10 μl of 200x TransDux™ solution into each well, and mix gently by swirling the plate.
9. Repeat steps 7 and 8 next two to three days.
10. One day after final infection, remove the viral supernatant, wash three times with PBS, and add 3 ml of hFib medium.
11. Four days after infection, plate 1 x 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.
12. On day 5 after thirst infection, trypsinize the infected cells and plate them in a 100-mm dish at different cell densities between 5 x 10^4 to 2 x 10^5 cells or in a 60-mm dish at densities between 2 x 10^4 to 1 x 10^5 cells.
13. Two days later, aspirate medium and replace with hES medium.
14. Change medium everyday with hES medium.
15. After about 3-4 weeks, check the colony formation and pick the colonies with ES-like morphology manually for expansion in hES media.

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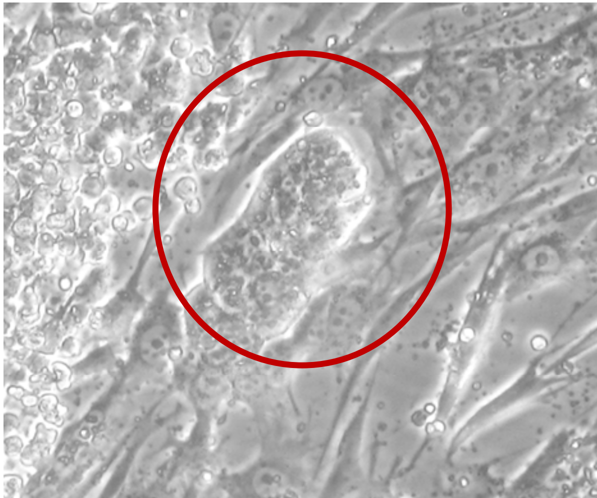
Example of pre-iPSC colony (after 10-15 days), note the loosely organized colony layered on top of the feeder cells.



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