Culturing iPSCs in Mouse embryonic fibroblasts (MEFs)

Mouse embryonic fibroblasts are the feeder cells for the iPSCs cells.

Passage 0 cells (p0) are expanded to p4. At passage p4 the cells are irradiated to go into senescence. They still produce all the factors that are needed for the hES cells to stay alive.

MEFs are stored and frozen in FBS 10% DMSO. This needs to be diluted when the cells are thawed

MEFs expansion

Freeze MEFs in 1mL aliquots and calculate how many dishes can be prepared from 1 vial. *Concentration of MEFs needed for hiPS cells- confluency 90% / 300 000 – 500 000 cells.*

Seeding MEFs for iPSCs culture (for 6mm dishes)

Prepare in advance:
Pork skin gelatin (Add 0.5g of gelatin powder in 500mL MQ and autoclave)
Gelatin coated dishes (Add 3mL per dish and incubate at 37C for at least 30 minutes)
MEF's medium (450mL DMEM-Glutamax, 50mL FBS, filter sterilize with 0.22um steric cup)

1. Prepare a 15mL Falcon with 7mL MEFs medium
2. remove gelatine from dishes and add 3ml of MEF medium prewarmed.
3. Take a MEFs vial from -80C and thaw it at 37C water bath
4. Add MEFs to Falcon
5. Centrifuge 4minutes at 200rcf
6. Ressuspend in 1mL medium, pipette up and down 2x (so that no clumps are visible)
7. Add 29 mL MEF medium to falcon
8. Add 1mL of cell suspension/dish
9. place the dish(es) with MEFs into incubator. Important : Spread cells in the dish to create an homogeneous distribution

Passaging iPSCs grown on MEFs

Prepare in advance:
HuES medium(100mL KOSR,5mL L-Glu, 5mL NEAA (add 3.6uL of β-Mercaptanol to it), 2.5mL P/S, up to 500mL with DMEM-F12, filter sterilize with 0.22um steric cup, add 20ng of bFGF(2 vials) after filtering)

1. Prepare HuES:Y27 1:1000 (1uL Y-27 per mL of HuES) 4mL per new dish
2. Change medium to iPSCs, add 2mL of HuES:Y-27 and place at 37C for at least 30 minutes
3. Wash MEF dishes 2x with PBS (2mL) and add 2mL of HuES:Y-27
4. Spray microscope with ethanol before putting inside the hood
5. Pick 8-10 colonies into new dish
6. Add 3mL HuES medium to back up dish and save until confirmation that new dish is ok.

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Freezing iPSCs grown on MEFs

1. Have a box with ice ready outside the hood
2. Prepare a 15mL falcon with 1mL HuES medium per each dish
3. Pick all good colonies in the dish
4. Collect to falcon
5. Centrifuge 4 minutes 160rcf
6. Ressuspend in 1mL mFeSR
7. Place in ice
8. Place in -80C inside purple box (this box allows for gradual cool down)
9. Take to Liq nitrogen the day after