Standard human iPSC/ESC Culture for long-term growth on MEFs
(Version: March 2011)

If you receive frozen vials of our iPSCs (normally cultured on MEFs), please read before proceeding. Each vial shipped from our laboratory should be thawed into one well of a 6-well plate, unless otherwise noted.

Materials

Mouse Embryonic Fibroblast (MEF) Medium

- 450 ml DMEM (Invitrogen, 11965-092)
- 50 ml defined FBS (Hyclone, SH3007003)
- 5 ml MEM non-essential amino acids (Invitrogen, 11140050)
- 5 ml Glutamax (Invitrogen, 35050061)
- 5 ml antibiotic-antimycotic (Invitrogen, 15240062)

Combine all components and sterile filter. Store at 4°C. Use within 3 weeks. The medium should be warmed to 37°C before use.

Embryonic Stem Cell (ESC) Medium

- 470 ml Knockout DMEM (Invitrogen, 10829018)
- 120 ml Knockout serum replacement (Invitrogen, 10828028)
- 6 ml Glutamax (Invitrogen, 35050061)
- 6 ml antibiotic-antimycotic (Invitrogen, 15240062)
- 6 ml MEM non-essential amino acids (Invitrogen, 11140050)
- 0.6 ml 2-mercaptoethanol (Invitrogen, 21985023)
- 10 ng/ml bFGF (PeproTech, 100-18B or other validated sources)

Combine all components and sterile filter. Store at 4°C. Use within 3 weeks. The medium should be warmed to 37°C before use.

Freezing Medium

- 8 ml FBS (Hyclone, SH3007003)
- 2 ml DMSO (Mediatech, 25-950-CQC)

Combine all components and sterile filter. Store at 4°C. Use within 3 weeks.

We also use CryoStem ESC freezing medium (Stemgent, 01-0013-51), which appears to lead to better recovery.

Procedures

Plating MEFs

1. Coat an appropriately sized plate with 0.1% gelatin. Use 1 ml of gelatin per well of a 6-well plate; the amount can be decreased for smaller wells.
2. Let the plate rest for 20 minutes; then aspirate the gelatin solution.
3. Thaw and resuspend irradiated MEFs from CF-1 mice (GlobalStem, GSC-6001G, 4-5 million per vial) in MEF medium. Centrifuge at 300 *g* for 5 minutes.
4. Plate the cells in 2 ml of MEF medium at a density of 200,000 cells per well of a 6-well plate. Incubate overnight for use the following day.

For standard culture, iPSCs plated on MEF should have ESC medium changed daily, with 2 ml of medium used per well of a 6-well plate. After 4-5 days, when colonies are of a sufficient size, they can be passaged. At near confluency, the split ratio is 1:3.

Passaging iPSCs

Collagenase (Type IV, Sigma, C5138) should be dissolved in ESC medium at a concentration of 1 mg/ml.

1. Wash wells to be split with 1 ml of PBS.
2. Add 1 ml of collagenase solution per well of a 6-well plate and incubate at 37°C for 10 minutes, or until curling of the colony edges is observed.
3. Carefully remove the collagenase solution and add 1 ml ESC medium to the well.
4. Scrape the well using a cell scraper (Sarstedt, 80-130) to lift the colonies.
5. Pipette the cells into a 15 ml conical tube containing 10 ml of ESC medium.
6. Wash the well once with 1 ml of ESC medium and add it to the tube containing the cells.
7. Centrifuge the tube at 300 *g* for 5 minutes.
8. Aspirate the supernatant and gently resuspend the pellet in 1 ml of ESC medium for each well of a 6-well plate you would like to use. Be careful not to break up the pellet too much, as single cells are unlikely to attach and form colonies.
9. Prepare a 6-well plate of MEF by removing the MEF medium and adding 1 ml of ESC medium per well.
10. Add 1 ml of resuspended cells per well.

Freezing iPSCs

1. After lifting colonies with collagenase and centrifuging as described above, remove supernatant.
2. Cells should be resuspended in 0.5 ml ESC medium for each vial to be frozen. We recommend freezing 2-3 wells of a 6-well plate per vial.
3. Slowly add 0.5 ml freezing medium for each vial to be frozen and carefully resuspend the cell pellet.
4. Transfer 1 ml of the cell solution per cryovial and place in a controlled-rate freezing container (Nalgene, 5100-0001). Store at -80°C for 24 hours and then transfer to a liquid nitrogen storage freezer.
5. Optional: Adding ROCK inhibitor (Stemgent, Y27632) during freezing and/or after thaw to the plating medium can help with recovery.

Thawing iPSCs

1. Remove the vial from the freezer and thaw in a 37°C waterbath. Slowly swirl the vial and do not submerge it completely. When only a small ice crystal is left, remove the vial from the waterbath and clean the outside with 70% ethanol.
2. In a biosafety cabinet, transfer the cell suspension to an empty 50 ml conical tube.
3. Rinse the vial with 1 ml of pre-warmed ESC medium.
4. Slowly add the rinse dropwise to the cell suspension while gently shaking the tube.
5. Add 10-15 ml of pre-warmed ESC medium dropwise to the cell suspension.
6. Centrifuge at 300 *g* for 5 minutes.
7. Aspirate the supernatant and resuspend the pellet in an appropriate amount of medium for plating.

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