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

Materials

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.

Nutristem, XF/FF Culture Medium (Stemgent, 01-0005)
DMEM (Invitrogen, 11965-092)
Matrigel hESC-qualified Matrix (BD, 354277)

Thaw Nutristem overnight at room temperature or 4°C, and warm to 37°C before cell culture use.

Procedures

Preparation of Matrigel-coated plates

1. Thaw Matrigel at 4°C on ice overnight. This product gels rapidly at room temperature, so take care to keep all components and tubes cold while working.
2. Aliquot the thawed Matrigel according to the dilution factor provided with the product. Aliquots should be kept at -20°C.
3. For use, thaw one aliquot (1 ml of 1:1 dilution) at 4°C on ice overnight. Add it to 25 ml of cold DMEM to coat four 6-well plates. Store plates at 4°C; move to 37°C 1 hour prior to use.

Passaging iPSCs from MEF to Matrigel

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 Nutristem 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 Nutristem medium.
6. Wash the well once with 1 ml of Nutristem 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 Nutristem 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 Matrigel by removing the medium and adding 1 ml of Nutristem medium per well.
10. Add 1 ml of resuspended cells per well.
11. Change the medium daily, adding 2 ml of Nutristem per well of a 6-well plate until colonies are large enough to passage (usually around 5 days).

Contact: Sarah Dowey: sdowey1@jhmi.edu, (410) 614-1244