General Guidelines for Handling Human iPSC cells

- iPSC are cryopreserved in plastic cryovials and shipped on dry ice. If storing the iPSC before thawing, store in liquid nitrogen vapor. Storage directly in liquid nitrogen may result in cracking of o-rings.
- It is highly recommended that a small number of vials are cryopreserved as a master stock before beginning any experimentation.

Media and Reagents

Matrigel

mTeSR or E8 medium

ROCK Inhibitor (Y-27632)

Versene

Cryopreservation Medium: 90% Knock-out Serum Replacement, 10% DMSO

Coating Plates with Matrigel

1. Reconstitute aliquot of Matrigel with cold medium and mix well. (This should be performed according to manufacturer's instructions)
2. Immediately use Matrigel solution to coat 6-well plates.
3. Gently swirl plate to spread the Matrigel solution evenly across the plate.
4. Place in 37°C incubator for 30 minutes to use plates immediately or seal with parafilm and store at 2-8°C for up to 7 days. To use plates after storage at 2-8°C, remove parafilm and incubate for 30 minutes at 37°C.
5. Aspirate Matrigel and add warm mTeSRl or E8 (culture medium).

Thawing Human iPS cells

1. Remove iPSC from liquid nitrogen vapor or dry ice and thaw quickly in 37°C water bath.
2. Transfer cell suspension into sterile 15ml conical containing warm culture medium and gently mix cells.
3. Centrifuge conical tube containing cells at 228 ± 24 g for 2 min at room temperature.
4. Aspirate supernatant and gently resuspend cells into 2 ml of warm culture medium supplemented with 10M Y-27632.
5. Plate cells in 1 well of 6-well plate coated with Matrigel.
6. Replace with fresh medium without Y compound, it is important to remove the Y compound from

the medium within 20 hours.

7. Maintain cells by daily medium exchange. **WARNING**: Failure to replace medium daily can result in spontaneous differentiation.

8. Colonies should be observed within 2-5 days.

Passaging of Human iPSC

Passage iPSCs when colonies approach borders of an adjacent colony. Ideally, iPSCs should be passaged before individual colonies begin differentiating in the center of colony (approximately 700 microns in diameter). To avoid spontaneous differentiation, do not allow colonies to overgrow.

Split ratios range from 1:3 to 1:6 and are indicated on the Certificate of Analysis for each iPSC line. We recommend that you record the passage information for each cell line to determine the exact growth kinetics of a particular line in your laboratory.

1. Remove spent medium from culture and rinse with PBS.
2. Add 1 ml/well Versene at room temperature until edges of colonies begin to roll up.
3. Aspirate enzyme solution.
4. Wash each well with 1ml of culture medium.
5. Add 1 ml of warm culture medium to cells and, using cell scraper, gently dislodge cells from plate.
6. Transfer the detached cell aggregates to a 15 ml conical tube. Rinse each well with 1 ml of culture medium to collect any remaining aggregates and add to conical tube containing cells.
7. Resuspend cells in appropriate volume of growth medium for the appropriate cell density for cell culture vessel
8. Seed cells onto prepared Matrigel coated plates.

Cyropreservation of Human iPSC

1. Remove spent medium from culture and rinse with PBS.
2. Add 1 ml/well Versene at room temperature until edges of colonies begin to roll up.
3. Aspirate enzyme solution.

4. Wash each well with 1ml of culture medium.
5. Add 1 ml of warm culture medium to cells and, using cell scraper, gently dislodge cells from plate.
6. Transfer the detached cell aggregates to a conical tube. Rinse each well with 1 ml of culture medium to collect any remaining aggregates and add to conical tube containing cells.
7. Centrifuge conical tube containing cells at 228 ± 24 g for 2 min at room temperature.
8. Remove supernatant and resuspend cells in pre-cooled cryopreservation medium (1 ml for each well of a 6 well plate to be harvested).
9. Transfer 1 ml of cell suspension to cryovials on ice.
10. Place cryovials in isopropanol freezing container and store at -80°C overnight.
11. Transfer vials to liquid nitrogen vapor.

Troubleshooting Tips

Problem	Observation of problem	Possible causes
Spontaneous differentiation	Morphology of differentiated cells can vary but is commonly characterized by 1. hypertrophic colonies 2. colonies without distinct borders 3. flattened cells	1. Low confluency or suboptimal passaging of cells (see "low viability after passage") 2. Poor or inappropriate matrigel quality-this can be prevented by testing matrigel prior to using in an experiment
Non-uniform distribution of colonies within culture vessel	Areas within culture vessel with highly confluent iPSC colonies AND areas with few or no iPSC colonies	Rock plates back and forth gently immediately following plating - usually rocking plates back and forth and then side to side and diagonally produces a fairly uniform distribution
Low Viability after Passage	Little to no cell colonies are visible 24 hours after passage	During passaging, clusters may have been disrupted into a single cell suspension-decrease pipetting of cells during passaging
Low Viability after Recovery from Cryopreservation	Little to no cell colonies are visible within 4 days after recovery	Lack of or insufficient ROCK inhibitor in thawing medium.

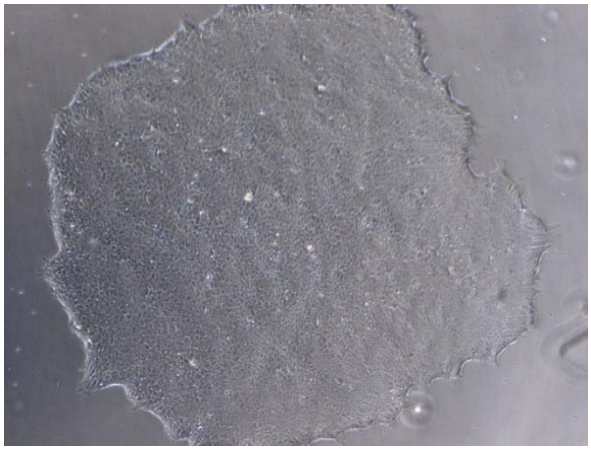

Image 1: Typical image of a human induced pluripotent stem cell (iPSC) colony. Note the distinct borders of the colony and morphology of the iPSCs.

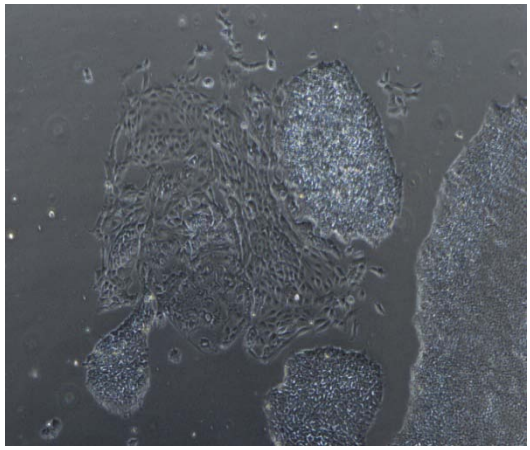

Image 2: Image of a differentiated colony. Note the flattened morphology of the cells and lack of distinct borders.