Culture and Maintenance of induced pluripotent stem cells (iPSCs)

(Note: The following protocol is specific for 6 well plate).

Coating: Use Geltrex® LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Catalog# A1413202, ThermoFisher Scientific) to coat the plate before seeding the cells.

Procedure:

Thaw pre-aliquoted Geltrex® on ice. Dilute it 1:100 with pre-chilled DMEM/F12 (Catalog# 11320033, ThermoFisher Scientific)

Pipette sufficient amount of diluted Geltrex® to cover the surface area of cell culture vessel (1ml/well for 6 well plate) and move the plate back and forth to spread it evenly on the surface.

Incubate the plate at 37°C for at-least 1 hour.

Equilibrate the plate at room temperature (RT) for 1 hour before seeding the cells.

Passaging iPSCs

Procedure:

Wash cells once with 1 mL of 0.5µM EDTA solution in PBS (without Calcium and Magnesium)

Pipette 1 ml/well of 0.5µM EDTA solution and incubate for 4mins at RT. The incubation time varies with temperature and cell lines.

Inspect cells under the microscope. Slowly aspirate EDTA solution just when the edges of the colonies start to detach.

Add 1 mL of room temperature equilibrated iPSC culture media (TeSR™-E8™, Catalog# 05990 or mTeSR™,Catalog# 85850, STEMCELL™ Technologies) and re-suspend the cells gently (Note: Avoid creating single cell suspension).

Seed cells into Geltrex® coated plate at 1:4 dilution from a (70-80%) confluent well.

Move the plate back and forth to ensure even distribution of the cells in the well.

Incubate the plate at 37° C at 5% CO_2 and avoid moving the plate for at-least 12 hours.

Change media every day. iPSC culture media should be brought at room temperature before feeding the cells.

(Note: Passage cells every 4-5 day)

Freezing iPSCs

Procedure:

Once cells reach 80-90% confluency, wash the wells once with 1 mL of 0.5µM EDTA solution.

Pipette 1 mL of 0.5µM EDTA solution and incubate for 4mins at RT.

Aspirate EDTA solution once the edges of the colonies start to detach.

Add 2 mL of DMEM/F12 equilibrated at RT to collect the cells and transfer it to 15 mL falcon tube.

Let the cells to settle down for 15mins.

Centrifuge the cells at 1200 rpm for 5mins.

Aspirate DMEM/F12 completely.

Use 1 ml of iPSC freezing medium pre-chilled at 4°C to gently re-suspend the cells. (Freezing media composition: 10% DMSO {Catalog# D2650-5X5ML, Sigma Aldich} in knockOut Serum Replacement {Catalog# 10828028, ThermoFisher Scientific})

Transfer cells into a cryo-vial (freeze ~ 2.0×10^6 cells in one cryo-vial)

Transfer cryo-vial to Mr.Frosty (pre-chilled at 4°C) and then store it at -80°.

Transfer the cells to liquid nitrogen for long term storage.

Defreezing iPSCs

Coat with Geltrex® and equilibrate cell culture plate as instructed above.

Thaw iPSCs containing cryo-vial in a water bath at 37° C until small ice crystal is visible.

Using, 2mL serological pipette or 1mL micropipette with larger opening (VWR® Wide Orifice Pipet Tips), gently transfer the content into a 15mL falcon tube.

Add 4 mL of DMEM/F12 equilibrated at RT drop by drop to the falcon tube and gently shake the tube now and then during the process.

Centrifuge cells at 1200 rpm for 5 minutes

Carefully aspirate the medium and re-suspend the cells with iPSC culture media supplemented with 1µM ROCK inhibitor.

Cells from 1 cryo-vial can be seeded into a maximum of 2 wells of a 6-well plate. Move the plate back and forth to spread it evenly on the well.

Incubate the cells at 37° C at 5% CO2 and avoid moving the plate for at least 12-16 hours.