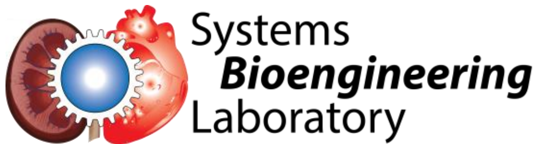

iPSC culture and maintenance

Prepared by: Nanditha Anandakrishnan
Version: 1.0
Date: 30-Sep-2020

- Preparing Matrigel coated plates
  1. Reconstitute Matrigel (Corning Cat No. 354277) with cold DMEM/F12 (Thermofisher Cat No. 11330-032) according to manufacturer's instructions and mix well.
  2. Use 1 mL Matrigel solution to coat each well of a 6-well plate. Gently swirl plate to coat the plate evenly.
  3. Place in 37°C incubator for 60 minutes to use plates immediately or seal with parafilm and store at 4°C for up to 7 days.
  4. To use plates after storage at 4°C, remove parafilm and incubate for 30 minutes at 37°C.
  5. Aspirate Matrigel and add 2 mL of warm mTeSR1 (Stemcell Technologies Cat No. 85850) or Stem Flex (Thermofisher Cat No. A3349401) culture medium to each well of a 6 well plate.

- Thawing Cryopreserved iPS cells
  1. Thaw cryopreserved iPSC by immersing the cryovial in a 37°C water bath for not more than 2 minutes.
  2. Add 1mL of prewarmed media to the cryovial and gently pipette to completely thaw the contents of the cryovial.
  3. Add the thawed cell suspension dropwise to warm culture medium in a sterile 15 ml conical tube, bring the volume up to 10 ml and gently mix cells.
  4. Centrifuge cells at 200 xg for 3 min at room temperature.
  5. Aspirate supernatant and gently resuspend cells into 6 ml of warm culture medium supplemented with 10µM Y-27632 or 1 µM Thiazovivin Rho kinase (ROCK) inhibitor (Stemcell Technologies # 72302 or Selleckchem # S1459).
  6. Plate cells in 3 well of 6-well plate coated with Matrigel and gently swirl plate to evenly distribute cells.
  7. Replace with fresh medium without ROCK inhibitor within 20 hours after thawing.
  8. Change media daily.

- Passaging of iPSCs
  1. iPSCs are ready to be passaged when adjacent colonies start to merge. Colonies must be cleaned under a microscope if there are a significant number of spontaneously differentiating cells.
  2. iPSCs are passaged with no-spin method using Versene (Thermofisher Cat No. 15040066) or ReLeSR (Stem cell Technologies Cat No. 05872) as dissociation reagents.
  3. Versene/0.5mM EDTA: Aspirate medium from the wells. Add 0.5 mL per well of Versene for 5minutes or until edges of colonies begin to roll up. Aspirate Versene, add 1 mL of culture medium and use a cell scraper to gently dislodge cells from the plate. Pipette the suspension carefully to not break the colonies down to single cells and transfer to a 15 mL conical tube. Rinse the plate with culture medium to collect any remaining clumps. Proceed to step 5.
  4. ReLeSR: Add 0.5 mL of ReLeSR per well and incubate at room temperature for 1 minute. Aspirate ReLeSR and incubate plate with cells at 37 C for 3-4 minutes. Add 1 mL of culture media and gently pipette the cells carefully to not break the colonies down to single cells. Transfer the suspension to a 15 mL conical tube. Proceed to step 5.
  5. Re-suspend cells in appropriate volume of growth medium for the appropriate cell density for cell culture plate. One confluent well of a 6-well plate can be passaged into 3-6 wells of a 6 well plate freshly coated with Matrigel.
  6. Rock plate gently to achieve a uniform cell distribution. Change media every day.

- Cryopreservation of iPSCs
  1. Each well of a 6-well plate can be frozen in separate cryovials with a final volume of 1 mL. Cryopreservation medium: 50% Growth medium, 40% KO serum (Thermofisher Cat No. 10828028) and 10% DMSO. 90% Growth medium and 10% DMSO has also been validated to work well with our iPSC lines.
  2. Follow passaging protocol until step 3/4.
  3. After aspirating ReLeSR/Versene, add 0.5 mL culture media per well and pipette gently to resuspend cells.
  4. Transfer the detached cell clumps to a 15 mL conical tube and add 0.4 mL KO serum and 100 µL of DMSO for every 0.5 mL of cell suspension in growth medium.
  5. Transfer 1 ml of cell suspension to each cryovial and place them isopropanol freezing container and store at -80°Covernight.
  6. Transfer vials to liquid nitrogen vapor for long term storage.