SOP for Thawing, Culturing, and Freezing Human iPSCs
Stanford CVI Biobank

The purpose of this document is to outline the standard operating procedures (SOP) for thawing and culturing human iPSCs.

From Life Technologies:
Essential 8™ (E8) Medium: A1517001

From Stem Cell Tech:
Gentle Cell Dissociation Reagent (EDTA): 07174

From Fisher Scientific:
mTeSR: NC0722499
DMEM/F12 Medium: 10-092-CM
Y27632 2HCl (ROCK Inhibitor) 50mg: 50-863-7
BD Matrigel, hESC Qualified: 354277
Bambanker: NC9582225

Note: This is for thawing one vial of iPSC

Thaw Protocol:

1. Dilute Matrigel 1:200 in DMEM/F12 and coat wells of a 6-well plate. Incubate the matrigel coated plates for 30 minutes at 37°C.
2. Prepare 2 ml of mTeSR medium in a 15 ml tube for washing.
3. Prepare another 2 ml of mTeSR medium + 10uM ROCK Inhibitor in a 15 ml tube for resuspension.
4. Remove frozen vial of iPSCs from liquid nitrogen storage and place in 37°C water bath until most, but not all, cells are thawed.
5. Remove cells from the freezing vial and add to the prepared tube of washing mTeSR medium.
6. Centrifuge at 200 g for 5 minutes and discard the washing supernatant.
7. Resuspend the cells in 2 ml of mTeSR medium plus ROCK inhibitor.
8. Aspirate excess Matrigel from well surfaces and plate cells into 1 well of a six well plate. Incubate cells in a 37°C, 5% CO2 incubator.
9. The next day, replace the spent mTeSR + 10uM ROCK Inhibitor medium with 1 ml each of fresh mTeSR and E8 medium (no ROCK Inhibitor in either).
10. The next day, replace with only E8 media. Replace the medium daily until the cells reach 85% confluence.

Passage of hiPSC with EDTA

1. Ideally cells should have reached 85% confluence in 4 days. Use a split ratio of 1:6.
2. Aspirate culture medium

3. Add 1 mL per well of Gentle Cell Dissociation Reagent (EDTA), incubate for 7 min at RT (in hood)
4. During this time set up a 15 mL Falcon tube with fresh E8-RI medium (i.e. 12 mL for 1:6 split)
5. Aspirate EDTA from well
6. With a P1000 tip, add 1 mL of the E8-RI medium and blast against cell surface to dissociate cells. Cells should come off easily. Transfer to the Falcon tube.
7. Matrigel-coated plate: aspirate excess Matrigel
8. Plate out cells at 2 mL per well**.**
9. The next day, replace the spent E8 + ROCK Inhibitor medium with fresh E8 medium (no ROCK Inhibitor). Replace the medium daily until the cells reach 85% confluence.

iPSC Freezing Protocol

1. Aspirate culture medium
2. Add 1 mL of EDTA per well, incubate for 7 min at RT (in hood)
3. Aspirate EDTA from well
4. With a P1000 tip, add 1 mL of freezing medium (Bambanker) and blast against cell surface to dissociate cells. Cells should come off easily. Transfer cell suspension into freezing tube (1 well per tube).
5. Place freezing tubes in a freezing container and place the container in -80°C freezer overnight.
6. After 24 hours move the freezing vials to liquid nitrogen storage.

For more information please contact:

Joseph C. Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Professor, Dept of Medicine & Radiology
Email: joewu@stanford.edu

Justin Vincent
Manager, SCVI Biobank
Email: justin81@stanford.edu

Ioannis Karakikes, PhD
Co-Director, SCVI Biobank
Email: ioannis1@stanford.edu

Rinkal Chaudhary
Co-manager, SCVI Biobank
Email: rinkalc@stanford.edu