Thawing, Propagation, and Freezing Protocol for JAX human iPSC lines

(KOLF2.1J and gene edited derivatives)

Each cryovial purchase from JAX contains ~0.5 x 10^6 cells in 500µl.

Cryovials should be stored in liquid N2 upon receipt. The cryovials are shipped on dry ice or in a dry shipper (outside of US, Canada, and Puerto Rico). Prior to storage, confirm the cryovial 10-digit barcodes match the values adjacent to the Product# in the packing slip (Figure 1).

Figure 1: Cryovial tube format and barcode ID

JAX human iPSC lines are delivered in screwcap cryotubes with unique 10-digit numbers on the label. The packing slip will have this 10-digit number adjacent to the Product Number, enabling the user to identify which tube contains which genetically engineered cell line.

Reagent list

Thawing & Propagation

- StemFlex™ Medium (Cat# A3349401; Gibco Life Technologies): Carefully review the vendor-provided "User Guide" before use as the working media is the composition of two components.
- RevitaCell™ Supplement (Cat# A26445-01, Gibco Life Technologies) referred to as RevitaCell in protocol
- Synthemax II-SC Substrate (Cat# 3535, Corning) referred to as Synthemax in protocol
- Sterile Distilled Water (Cat# 15230-204, Gibco Life Technologies)
- ReLeSR™ (Cat# 05872, STEMCELL Technologies)
- DPBS (Cat# 14190144, Gibco Life Technologies)
- Cell Lifter (Cat# CLS3008, Corning)
- 6-well Tissue Culture-treated Dish
- 10cm Tissue Culture-treated Dish
- 5ml Self-Standing Sterile Transport Tube (Cat# 22-010-1223, Globe Scientific)

Freezing

- ACCUTASE™ (Cat# 07920, STEMCELL Technologies)
- DMSO Hybri-Max™ (Cat# D2650, Sigma) referred to as DMSO in protocol
- KnockOut™ Serum Replacement (Cat# 10828028, Gibco) referred to as KOSR in protocol
- Vital Dye (i.e. Trypan Blue)
- Cryovials

Protocol

Cryo-Recovery (Single Frozen Cryovial into a single well of 6-well TC dish)

Before Starting:
Prepare 1mg/ml Synthemax stock solution (40X) by dissolving 10mg lyophilized Synthemax II-SC in 10ml sterile water (can be stored at 4°C)

1. ***For every clone to be thawed,*** treat a single well of a 6-well plate with 2ml sterile water + 50µl of 1mg/ml Synthemax stock (40X stock) and let stand at room temperature (RT) in a tissue culture (TC) hood for 2 hours. Aspirate the Synthemax solution from wells after 2 hours of incubation.
2. ***For every clone to be thawed***, prepare 4 ml of StemFlex media containing RevitaCell (1:100 dilution of 100X RevitaCell stock) and let warm to RT.
3. Remove the cryovial from the liquid nitrogen tank.
4. Hold the tube in the hood in your hand to thaw.
5. Once thawed, use a 200 µl pipet tip to transfer the entire 0.5 ml cell thawed suspension to a 5ml tube (Cat# 22-010-1223, Globe Scientific) 1 ml StemFlex + RevitalCell media.
6. Centrifuge for 5 min at 300 x g to pellet the cells.
7. Aspirate the media, taking care not to disturb the cell pellet and resuspend the cell pellet in 0.5 ml StemFlex + RevitaCell by gentle trituration.
8. Transfer the cell suspension to one well of a Synthemax-treated 6-well plate containing 2.5 ml StemFlex + RevitaCell and swirl gently.
9. Place 6-well plate in 37°C/5% CO_2 incubator.
10. Next day, change the media to StemFlex (without RevitaCell) and then every 2 days thereafter. (Note: See Figure 2 to appreciate the cell morphology change)
11. When the plate is 80% confluent (3-4 days), freeze into 5 x 0.5 vials OR pass 1:15 to expand the cell line stock (See Passage and Expansion). (Note: We recommend passaging 1:15 to 3 x 10cm plates to allow the subsequent freezing of many cryovials to ensure future experiments can access an inventory of low passage stocks.)

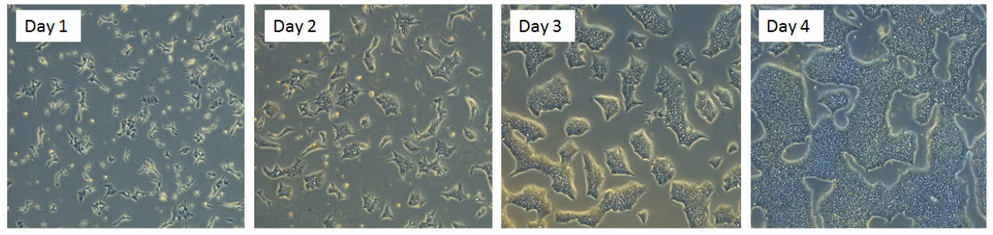

Figure 2: iPSC Morphology in Culture

Growth and morphology of KOLF2-C1 human iPS cells after thawing single cells.
Following exposure to a ROCK inhibitor (Revitacell) for 1 day, individual iPS cells will appear elongated. Once ROCK inhibitor is removed from the media on Day 1 and by Day 4, the iPS cells revert to a normal morphology and are ready for freezing or passaging. Non-enzymatic dissociation to small clumps, e.g., with ReLeSR, is used for subsequent passaging of cells. At a dilution of 1:15, the cells will reach confluency in 3-4 days.

Passage & Expansion (6-well to 3 x 10cm dishes)

1. Prepare 10cm TC plates: ***For each clone in a single well of 6-well dish***, coat 3 x 10cm TC dishes with 8 ml of Synthemax per dish. Let stand in a TC hood for 2 hours. (Note: Less than 6 ml will bead up leaving the plate uncoated in places.)
2. Prewarm 36 ml StemFlex media to room temperature in TC hood.
3. Wash the 6-well dish well with 1X PBS. Add 2 ml ReLeSR to each well and incubate in the TC hood for 5 min.
4. Aspirate ReLeSR and add 3 ml StemFlex per well. Scrape well and triturate cells several times to break cells into small clumps. (Note: The number of times depends on speed of pipetting and bore of pipette. Check cells under microscope. Pipette additional times if required.)
5. Transfer 1ml of cell suspension to Synthemax-coated dishes containing 9 ml StemFlex media (3 x 10cm TC plate). (Note: To get even plating of clumps, swirl dishes in one direction then the other. Finish with up and down and side-to-side motion.)
6. Change media after 2 days with new StemFlex media. When cells reach 80% confluency, plan to freeze the cells (~3-4 days post-plating).

Freezing

1. Pre-warm ACCUTASE at room temperature.
2. Pipette old media from 10cm plate into sterile conical tube.
3. Wash 10cm plate with 8 ml of PBS and then aspirate PBS.
4. Add 6ml of ACCUTASE to each 10cm plate.
5. Incubate plate(s) for 7 minutes in 37°C incubator.
6. Aspirate ACCUTASE (Note: Cells on Synthemax will still adhere to dish during aspiration).
7. Add 5ml of collected media from sterile tube to 10cm plate.
8. Using a cell lifter, thoroughly scrape plate to dislodge all cells.
9. Gently pipette media up and down 3 times while washing plate surface with the media/cell mixture, and transfer to sterile conical tube.
10. Count cells using a haemocytometer (include a vital dye such as Trypan blue)
11. Spin down cells, and aspirate media.
12. Resuspend cells in appropriate volume of freezing media (90% KOSR + 10% DMSO) to a concentration of 1 x 10^6 live cells/ml of freezing media. Aliquot 500µl (0.5ml) freezing media/cell mixture per tube.
13. After capping tubes, transfer to -80°C for 24 hours.
14. Transfer cryovials to liquid N2 for long term storage. (Note: One cryovial should be thawed to test the viability of the frozen Lot. Expand it for 2-3 days and then subject it to pathogen testing. This will validate the quality of your frozen Lot for future experiments.)