Maintenance of Primary Human Dermal Fibroblast

Purpose: Human dermal fibroblasts are a commonly used somatic cell for iPSC and direct conversion disease modeling applications. Isolated from a dermal punch biopsy, these cells are easily cultured and actively proliferate for approximately 15-20 passages. A basic maintenance approach for human dermal fibroblasts is described below. Primary dermal fibroblasts will reach senescence through prolonged maintenance in culture. Care and intentionality should be given to ensure prolonged access to proliferative and robust cells. Designing and maintaining banking strategies that preserve earlier passage material is critical.

Reviving human dermal fibroblasts from cryopreservation

Materials:

- Fibroblast media:
  DMEM
  15% FBS
  1% NEAA
  1% Glutamine/Glutamax
  10ng/mL FGF2 (optional – can be added to support slow growing cell lines)
- Gelatin coated 6-well plate (0.1% gelatin for at least min at 37°C) (optional – not needed for most lines. Uncoated tissue culture dishes, flasks, and plates work well in most cases)

Procedure:

1. Aliquot 3mL of fibroblast media to a 15mL conical tube.
2. Collect banked fibroblast vial from cryo-storage on dry ice.
3. Transfer vial to 37°C water bath to thaw. Remove vial from the water bath when a small piece of ice remains (roughly the size of a grain of rice).
4. Transfer thawed cell solution to the prepared 15mL tube (step 1). Rinse the cryo-vial with an additional 1mL of fibroblast media and transfer to the 15mL tube.
5. Pellet by centrifugation at 1000RPM (~200G) for 5 minutes.
6. Aspirate supernatant and resuspend pelleted cells in 2mL of fibroblast media.
7. Plate cells to 1 well of 6-well plate (gelatin coating optional) and transfer to 37°C/5% CO^2 incubator.
8. Allow fibroblast to recover overnight. Replenish media completely every 2-3 days. Passage fibroblast 1:6 before confluency is reached.

Passaging human dermal fibroblasts

Materials:

- Fibroblast media
- PBS-/-
- TrypLE or Trypsin
- Gelatin coated plate(s) (optional – see note above)

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Procedure:
Indicated volumes are for a 6-well plate. Scale up or down as needed

1. Aspirate culture media from wells being passaged and rinse once with 0.5-1mL of PBS-/-.
2. Add 0.5-1mL of enzyme and incubate at 37°C for 5 minutes.
3. While enzyme is incubating, prepare a 15mL conical tube with 3mL of fibroblast media.
4. After enzyme incubation is complete, transfer cell solution to the prepared 15mL conical tube.
5. Wash the well with an additional 1mL of fibroblast media to recover residual cells and transfer them to the 15mL conical tube. If performing a cell count, collect a small sample for counting.
6. Pellet by centrifugation at 1000RPM (~200G) for 5 minutes. If needed, count cells during the centrifugation step.
7. Aspirate supernatant and resuspend cell pellet to achieve desired cell seeding density. For routine passaging, a 1:6 ratio is recommended.
8. Seed fibroblasts to gelatin-coated plates (optional) and allow to cells recover overnight. Replenish media completely every 2-3 days. Passage cells before confluency is reached.

Preparing human dermal fibroblasts for cryopreservation

Materials:

- Fibroblast media
- Cryopreservation media (fibroblast media with 10% DMSO)
- PBS-/-
- TrypLE or Trypsin
- Labeled cryo-vials
- Foam freezing rack or Mr. Frosty

Procedure:

Follow steps 1-6 of passaging protocol

7. Aspirate supernatant and resuspend cells in cryopreservation media to ~500K cells/mL. Higher densities are ok if needed for downstream applications.
8. Aliquot 1mL of cell solution to pre-labeled cryo-vials. Transfer vials to a freezing rack and store at -80°C overnight. Transfer cells to permanent vapor phase cryo-storage the following day. Prolonged storage at -80°C will result in reduced viability upon thaw and should be avoided. When transferring cells to cryo-storage we strongly encourage mapping boxes for documentation needs and easy retrieval of vials.

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