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Expansion and maintenance of human induced pluripotent stem cells (iPSCs) V.1

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ABSTRACT

This protocol describes the maintenance and expansion of iPSCs in the adherent culture via single-cell passaging.

MATERIALS

Reagents:

- KnockOut™ DMEM (KO DMEM basal medium) (ThermoFisher Scientific, CAT# 10829018)
- Matrigel (CAT# 354277)
- mTeSR™1 complete kit (mTeSR basal medium + 5x Supplement) (Stem Cell Technologies, CAT# 85850_C)
- Phosphate-buffered saline, pH 7.4 (PBS) (Life Technologies, CAT# 10010056)
- Penicillin-Streptomycin (10,000 U/mL) (ThermoFisher Scientific, CAT# 15140122)
- ROCK inhibitor Y-27632 (ROCKi) (Bio-Techne, CAT# 1254)
- TrypLE (Life Technologies, CAT#12604013)

Preparing iPSC Maintenance (iMM) Media:

- 100 mL of mTesR 5X Supplement
- 400 mL of mTesR basal medium
- 5 mL of 100x Penicillin-Streptomycin

Keywords: iPSCs, Single-Cell Passaging

BEFORE START INSTRUCTIONS

Sterile working techniques are an absolute must to ensure cell viability and vitality. This includes, but not limited to, filtering of all media to be used with 0.22 µm filter, sterilisation of gloves, stripettes, falcons, or any materials to be in contact with cells or cell media.

All growth factors should be added fresh on the day of intended use, or within 48 hours. Prior to use media must be warmed preferentially to 37ºC, or room temperature at the very least, as these cells are temperature-sensitive.

Cells should be regularly checked under brightfield microscope for monitoring of normal growth and identification of potential contamination.

Expansion of iPSCs by thawing onto Matrigel

1 Day -1: Preparing plates for replating

Note

Geltrex can be substituted for Matrigel at any stage of the protocol.

Matrigel should be stored at -80ºC and prepared in KO DMEM basal medium on manufacturer's dilution instructions. Once prepared it should be kept cold at all times.

1.1 Add 1 mL/well of Matrigel to each well of a 6-well plate one day prior to thawing the iPSCs.

1.2 Place at 37ºC overnight (for at least 1 hour before using or up to 48 hours).

2 Day -1: Preparing iPSC Maintenance Medium (iMM) for thawing

2.1 Thaw mTesR 5X Supplement (sold in a single package with mTesR basal medium) at 4^{o}C

overnight.

2.2 Prepare iPSC Maintenance Medium (iMM; see **Materials**) and store at 4ºC.

3 Day of thawing: Preparing spinning tubes for thawing

3.1 Add ROCKi (1:1000) to iMM media and pre-warm in 37^{O}C water bath (2.5 mL of media per well of a 6-well plate).

3.2 Calculate the desired volume of Phosphate-buffered Saline (PBS) media and pre-warm in 37^{O}C water bath (1 mL of media per well of a 6-well plate).

3.3 Add 9 mL of KO DMEM basal medium to a 15 mL falcon (spinning tube) for each vial to be thawed and prewarm.

4 Thawing of iPSCs

4.1 Thaw cryovial containing iPSCs in water bath until only a small component remains frozen.

Note

Swirl the vial to thaw the iPSCs and check frequently to ensure the vial do not get completely thawed while in the water bath.

Be very careful to wipe down the vial with ethanol after removing from the water bath, and try to not place lid underwater to remove the risk of contamination.

If inexperienced, do 1 or maximum 2 vials at a time as a time delay will affect the viability of your cells.

4.2 Carefully transfer contents of cryovial to pre-warmed spinning tubes.

4.3 Centrifuge at 350g for 5 min.

4.4 While spinning, aspirate Matrigel from each well and replace with 1mL iMM media + 10 µM ROCKi (1:1000).

4.5 Aspirate media from cell pellet in spinning falcon and replace with 1 mL iMM media + 10 µM ROCKi (1:1000), slowly and gently resuspending the pellet.

4.6 Transfer whole 1 mL to each well with 1 mL iMM media + 10 µM ROCKi (1:1000) and swirl plate gently in a figure 8 motion.

Maintenance of iPSCs

5 **Feeding iPSCs**

Replace iMM media daily and split (by colony passage or single cell passage) when cells have

reached 100% confluency and continue until enough have been obtained to start your experiment.

Note

Check cells daily under a light microscope to track confluency and health.

These cells are particularly temperature sensitive and mTesR basal medium should be warmed briefly to at least room temperature before use.

Single cell passaging of iPSCs is not recommended for more than 2 weeks unless regularly karyotyped and SNPs.

Cells at 100% confluency should be split 1:2 or 1:3. If cells don't look particularly healthy or are growing slower then split 1:2.

6 Day before split: Preparing for split

Matrigel wells of a 6-well plate as previously described in **step 1.1.**

7 Splitting iPSCs by single-cell passaging

7.1 Aspirate media from wells and rinse with 1mL PBS.

7.2 Aspirate PBS and add 1 mL of room-temperature TrypLE per well of 6-well plate.

7.3 Incubate at 37°C for ~3 minutes, until cells come off by pipetting.

Note

Check if cells have dissociated by tapping gently on side of plate.

7.4 Neutralize TrypLE by collecting well contents and adding to pre-warmed spinning falcon.

7.5 Spin at 350g for 5 minutes and replate cells as previously described, following **steps 4.3. to 4.6.**