FACLR
FRIEDREICH'S ATAXIA CELL LINE REPOSITORY

Napierala Lab protocol for culturing human iPSCs (May 2022)

For details, please refer to "Maintenance of Human Pluripotent Stem Cells in mTeSR™1" Technical Manual (STEMCELL Technologies).

Necessary Reagents

Reagent	Company	Catalog Number
BD MATRIGEL HESC-QUALIFIED	Fisher Scientific	08-774-552
mTeSR™1 Complete Kit	STEMCELL Technologies	85850
Cryostor CS10	STEMCELL Technologies	07930
Dispase 1U/mL, 100mL	STEMCELL Technologies	07923
Stemolecule Y27632	Stemgent	04-0012
DMEM/F12	Life Technologies	11320-082

Passaging of iPSCs

Different passaging conditions are necessary depending on purpose: A. splitting or B. splitting and freezing. The splitting ratio depends on the number of colonies before splitting/freezing, and on the nature of the colony (e.g. growth rate, amount of differentiation, etc.). If the colonies are at an optimal density, the cells can be passaged every 4 - 7 days using 1:3 to 1:6 splits (i.e. the aggregates from 1 well of a 6-well plate can be plated into 3 wells of a 6-well plate). If the colonies are too dense or too sparse, adjust the split ratio accordingly. If there are some differentiating cells, mark them and remove regions of differentiation by aspiration before splitting.

1.) Prepare new plate(s) by coating wells with Matrigel (0.3 ml/well for 24-well plate, 0.5 ml/well for 12-well plate, 1 ml/well for 6-well plate), and incubate the plate for 1 h at room temperature. Aspirate Matrigel, but do not aspirate all so that the plate does not dry out. Add mTeSR™1 media supplemented with 10 μM Y-27632 (ROCK inhibitor) to the wells (0.5 ml/well for 24-well plate, 1 ml/well for 12- well plate, 2 ml/well for 6-well plate).

2.) Aspirate medium from the iPSC culture and rinse with DMEM/F12 (1.5 - 2 ml/well).

3.) Add 1 ml/well (6-well plate) of Dispase at a concentration of 1 mg/ml. Place at 37°C for 3-4 min.

4.) After incubation, the colony edges will appear slightly folded back but the colonies should remain attached to the plate.

5.) Remove Dispase and gently rinse each well 2 times with 1.5 - 2 ml of DMEM/F12 per well to dilute any remaining Dispase.

There are different ways of splitting colonies. **A** is preferred when the ratio of splitting is small (e.g. 1:1 or 1:2); **B** is preferred if the ratio needs to be larger.

A. Splitting

Add 1 ml/well of mTeSR™1 and carefully cut colonies to a proper size using a 2 ml glass pipette, then gently scrape colonies off with a cell scraper and transfer cell aggregates to the new plate paying attention to equally

divide the amount of cells between the new wells. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the wells. Place the plate in a 37°C incubator (5% CO_2). Ensure that the newly seeded colonies are evenly dispersed across the entire surface of a Matrigel-coated plate. Uneven distribution of cell clumps may result in differentiation of iPSCs.

B. Splitting and freezing

Add 1 ml/well of mTeSR™1 (or DMEM/F12) and carefully cut colonies using a 2 ml glass pipette, gently scrape off colonies using a cell scraper, and then transfer aggregates to a 15 ml conical tube. Add an additional 1 ml of mTeSR™1 (or DMEM/F12) to rinse remaining cells from the plate, and transfer the suspension to the same tube. Centrifuge at 200-250 x g for 5 min at room temperature.

If passaging the cells, remove the supernatant and resuspend the pellet with fresh mTeSR™1+10 μM Y27632 media by pipetting up and down 3 times to get the proper cell aggregate size, then seed the cell aggregates into a new Matrigel-coated plate.

If freezing the cells, carefully remove the supernatant. For each well (cryovial) of iPSC aggregates collected in the 15 ml tube, add cold (4°C) 0.7-1 ml of CryoStor CS10 freezing media using a 2 or 5 ml glass pipette. Pipette the suspension **gently** up and down several times (sometimes it can be 2, but also can be 5-6 times). Perform the minimum amount of pipetting necessary to achieve resuspension, trying to avoid excess pipetting. Perform this step very cautiously to not disperse cell clumps too much, but to make an evenly scattered suspension of cells. Transfer the cell suspension to cryovials. Freeze the vials gradually (-1°C/minute) using a freezing container placed at -80°C overnight, then transfer the vials to liquid nitrogen for long-term storage.

General information and technical considerations

Cells are cultured according to the "Maintenance of Human Pluripotent Stem Cells in mTeSR™1" Technical Manual by STEMCELL Technologies with some modifications depending on cell type and specific characteristics.

1.) Change the media every day; 6-well plate: 1.5 - 2 ml per well (depends on amount/size of colonies on wells); 12-well plate 0.7 - 1 ml; 24-well plate 0.5 - 0.7 ml.

2.) It is good practice to check every well/plate every day to seek out unsuitable cells and/or colonies (e.g. differentiating iPSC colonies or clumps and somatic cells that did not undergo efficient reprogramming). Mark these places using an objective marker and aspirate them while changing the medium.

3.) Sometimes you may have several marked places in one well. If so, do not aspirate all the media at first. Instead, aspirate some colonies/cells from one well, wash the well with media, aspirate the next area of colonies/cells, wash, and aspirate the media, etc. Be attentive and work **precisely** but also **quickly** so as to not leave the colonies without media for more than **30 sec**.

4.) Make sure that the end of the glass Pasteur pipette is not broken before beginning aspiration steps. A pipette with a broken end does not pick up the cells, it will only aspirate medium.

MATRIGEL (BD Biosciences):

Matrigel aliquots are prepared according to the manufacturer's recommended procedure and the unique product information sheet that accompanies each vial of purchased Matrigel (BD Biosciences). Thaw the vial on ice prior to making the aliquots, then store the aliquots at -80°C.

Prepare a small container (e.g. plastic beaker) with ice and place in it an aliquot of Matrigel taken from -80°C. Place the container into a refrigerator (+4°C) and allow the aliquot to thaw (usually 1.5 - 2 hours). When working with Matrigel, it is best to have **cold** plates and pipettes/tips/tubes that have been kept at -20°C (at least 4°C). Transfer the thawed portion of Matrigel (kept on ice) to 25 ml of cold DMEM/F12 media using aseptic technique. **Keep it cold**. Coat a sufficient number of wells for your immediate use. Coated plates

should be left at room temperature at least 1 hour prior to use. **Do not allow the Matrigel to evaporate!** Use plastic wrap or Parafilm to seal the plates if the plates need to be left for a longer period of time. You can prepare additional plates and keep them for up to 2 weeks at +4°C, but it is difficult to predict how many wells you will need within that time. Rather, it is better to store the unused diluted Matrigel at +4°C for up to 2 weeks. Be sure to wrap the screw-cap of the tube with Parafilm and label the tube with the date of dilution.

mTeSR™1:
Prepare complete media according to the procedure recommended by STEMCELL Technologies.

Additional comments

1.) Always warm mTeSR™1 at room temperature, **do not warm at 37°C**.

2.) Aliquot mTeSR™1 if necessary, as it will lose activity if kept at +4°C for more than one week. The portioned medium can be frozen at -20°C for one month and go through one freeze-thaw cycle. Note: aliquot only 40 ml into a 50 ml conical tube to allow for expansion of the media upon freezing.

3.) Passage/split the iPSCs using mTeSR™1 complete medium without antibiotics but with ROCK inhibitor (Y- 27632) [10 µM].

ROCK inhibitor solution (Stemgent):
Prepare a 10 mM solution of Stemolecule Y27632 in DMSO according to manufacturer's recommendation (Stemgent, catalog number 04-0012). Aliquot ~50 µl per cryovial and store at -20°C. Protect from light and avoid multiple freeze/thaw cycles.

Dispase solution (1 U/ml) (STEMCELL Technologies):
Portion into 4-, 6-, and 10 ml aliquots and store at -20°C. Thaw slowly at +4°C or room temperature, and warm it shortly before using it. Quick thawing at 37°C, multiple freeze/thaw cycles, or long warming of the enzyme all make it less active.

DMEM/F12 medium (Life Technologies):
Pure medium is used as a rinsing solution before and after the use of Dispase and during cell freezing. It is also used to prepare the diluted Matrigel solution.