iPSC Culture Protocol

Reagents required:

Dispase - Stemcell Technologies #07923 100ml (£42)	**Store at -20 °C, (aliquot into 4ml volumes)**
PBS - Gibco #2014-05 500ml (£8.65)	Store at RT
MTeSR1 medium - Stemcell Technologies #05850 500ml (£213.00)	Store 400ml media at 4 °C and 100ml 5x supplement at -20 °C (aliquot into 8 x 12.5ml volumes)
DMSO - TC own brand	Store at RT
Matrigel - #354277 5ml (£235.72)	Store at -20 °C (aliquot according to dilution factor which is Lot dependent.
DMEM medium – Gibco #11330-057 500ml (£9.85)	Store at 4 °C
ROCK inhibitor (Y-27632) - Millipore #SCM075 (£440)	Make up 1mM stock and store at -20 °C in 200µl aliquots

Day 1: Preparation of 3cm Matrigel plates

- Thaw 1x aliquot of Matrigel O/N at 4°C.
- On ice, add aliquot to 25ml of cold DMEM.
- Immediately use 1ml/3cm dish ensuring complete coverage.
- Either seal with Parafilm and store at 4 °C or leave at RT for 1h before use.

Preparation of Media

- Thaw 1x 12.5 ml aliquot of 5x supplement O/N at 4°C.
- Add to 50ml of mTeSR1 media.
- Use at **RT** or store at 4°C for up to 2 weeks.

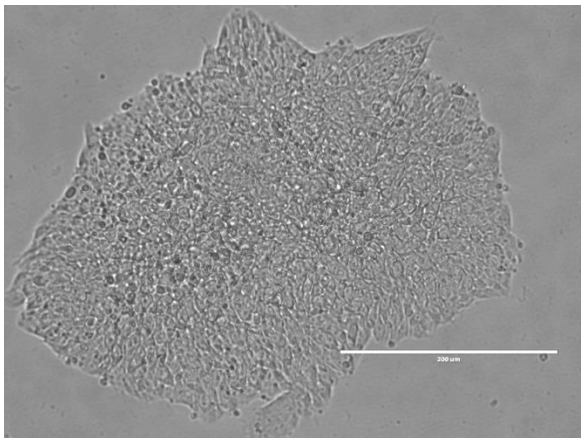
Day 2: Thawing of cells

- Remove vial from LN_2 and place into 37 °C water bath.
- Swirl gently and spray with IMS.
- Transfer cells to universal and slowly add 5ml of complete mTeSR media.
- Centrifuge for 5’ at 300xg (1500rpm).
- Aspirate supernatant and resuspend cells in 4ml mTeSR +10µm ROCK inhibitor (10µl of 1mM stock/ml).
- Aspirate excess Matrigel from dishes and add 2ml of cell suspension/dish.
- Place plates into 37°C incubator and move side to side to evenly distribute cells.
- Media change daily with the addition of fresh ROCKi (once established ROCKi only needs to added every other day).

<u>Days 7-9:</u> Passaging of cells

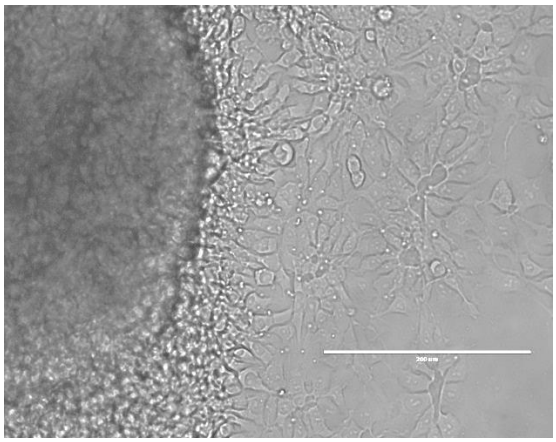
- Prepare Matrigel dishes or take out stores dishes from 4°C and leave at RT for 1h.
- Warm media and Dispase to RT.
- Generally split 1:3-1:9.
- Aspirate media from cells and rinse with 1ml RT PBS.
- Aspirate PBS and add with 1ml of RT Dispase and place plate into 37°C incubator for 5min.
- Ensure the layer of cells remains attached if they do peel off collect, spin down and resuspend in 3-9 ml of media (depending on split ratio required).
- If attached add 1ml of media and gently use a cell scrapper to dislodge colonies. Collect cells trying not to create a single cell suspension and centrifuge for 5min at 300xg (1500rpm).
- Resuspend in 3-9ml of media (depending on split ratio) and add 1ml of suspension to each new well in a total of 2ml/dish + ROCKi.

A



Undifferentiated human IPSCs cells on Matrigel at the optimal time of passaging

B



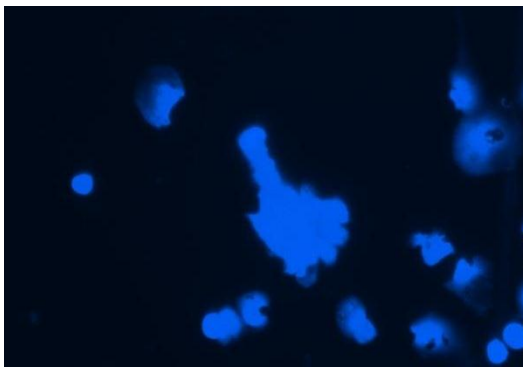
Cell differentiation observed on the periphery

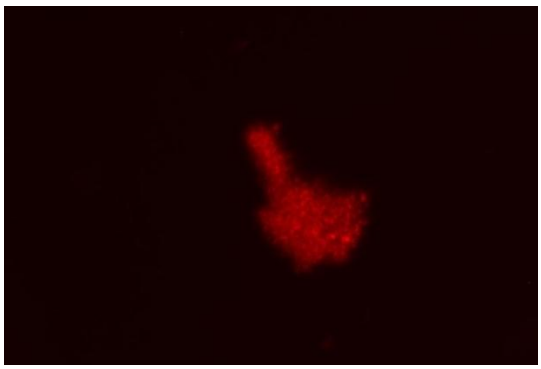
Cryopreservation

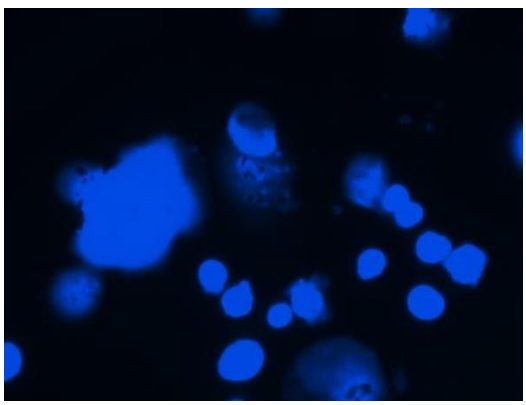
- Freeze approx. 6x3cm^2 dishes at ~60-75% confluency /per vial.
- Remove media and rinse each dish with 1ml PBS.
- Add 1ml RT Dispase to each dish for 5min at 37°C.
- Inactivate by adding 1ml of cold media per dish.
- Pool dishes together and transfer to a universal and centrifuge for 5min at 300xg (1500rpm).
- Aspirate media and resuspend in 10ml cold media, spin down and resuspend in 1ml of cold freezing media (mTeSR1 +15% DMSO +ROCKi).
- Transfer to cryovessel and place in -80°C before transferring to LN_2 storage.

<u>Day 1:</u> TRA-1-60 Characterisation via Immunofluorescence

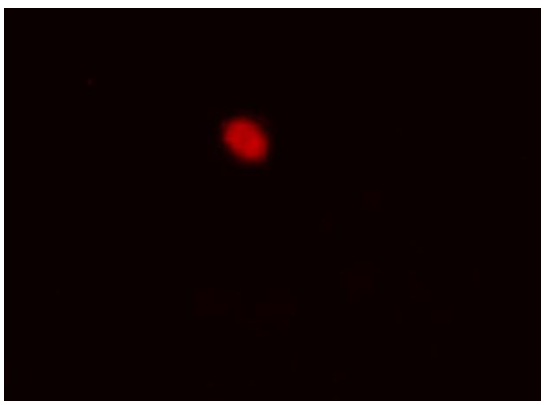
- Use 1xconfluent 3cm dish.
- Aspirate media and gently wash with PBS x1.
- Add 1ml RT Dispase to each dish for 5min at 37^{o}C.
- Inactivate by adding 1ml of cold media per dish.
- Centrifuge for 5min at 300xg (1500rpm).
- Wash with 5ml of RT PBS and Centrifuge for 5min at 300xg (1500rpm).
- Resuspend in 200µl of PBS.
- Cytospin onto 2 slides (100µl/slide) 5min at 5000RCF.
- Fix cells with 2% PFA for 10min at RT.
- Wash x3 with PBS.
- Block with 2% BSA in PBS for 30min at RT.
- Stain with anti-human TRA-1-60-R Alexa Flour 594 for 1h at RT (1:100 in 2% BSA).
- Wash x3 with PBS.
- Counterstain with DAPI, wash and mount for microscope analysis.







DAPI



TRA-1-60

From advice please see Lisa