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NU iPSC general culture

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We use this protocol and it's working.

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Abstract

KOLF2.2J iPS cells are grown on substrate in StemFlex media

Coating Plates with Substrate

1 **Option A**: Matrigel coating

1.1 Reconstitute aliquot of Matrigel (200ul) with cold medium (20mL, 1:100) and mix well. (Cold
tips)

1.2 Immediately use Matrigel solution to coat 6-well plates (1mL for each well, 19 wells) & gently rock to spread evenly.

1.3 Place in 37 °C incubator for 30 minutes or room temperature 1 hour.

2 **Option B:** Synthemax coating

2.1 Prepare the required volume of a 1:40 dilution of Synthemax to coat the plates (2 ml water+50 µl 1 mg/ml Synthemax for each well of a 6-well plate; 8 ml water+200 µl 1 mg/ml Synthemax for each 10 cm dish).

2.2 Leave plates in hood for at least 2 h (or at 37 °C for at least 1 h).

2.3 Plates can be prepared ahead of time and stored dry at 4 °C for up to 3 months.

Thawing Cells

3 We generally thaw a vial of one million cells KOLF2.2J cells into one well of a 6-well plate.

3.1 Treat one well of a 6-well plate with Matrigel and stand at room temperature for 1 h.

3.2 Prepare 6 ml of StemFlex media containing ClonR2 (10x, 1:10 dillution) or rock inhibitor y-27632 (10 nM, 1:1000 dillution) in Falcon tube. Aspirate Matrigel and add 2 mL StemFlex media containing ClonR2 or rock inhibitor.

3.3 Remove 1 vial of cells in the 96-well Matrix plate or cryovial from the liquid nitrogen tank.

3.4 Hold the tube between your index finger and thumb to thaw quickly. The layer of mineral oil will thaw first. For cryovial, hold the top, put the bottom into 37 °C waterbath to thaw until a tiny ice left.

3.5 Once thawed, with a pipettor, transfer the cell suspension under the oil layer or the cell suspension from cryovial to a 15 mL Faclon tube containing 3 ml StemFlex+ ClonR2 or rock inhibitor media.

3.6 Centrifuge for 5 min at 300 x g to pellet the cells

3.7 Aspirate the media, taking care not to disturb the cell pellet and resuspend the cell pellet in 0.5 ml StemFlex + ClonR2 or rock inhibitor by gentle trituration.

3.8 Transfer the cell suspension to one well of a Matrigel-treated 6-well plate containing 2 ml StemFlex + ClonR2 or rock inhibitor and distribute evenly

3.9 Place 6-well plate in 37°C/5% CO2 incubator and rock plate gently to make sure cells are well distributed.

3.10 Next day, change the media to StemFlex (without ClonR2 or rock inhibitor) and then every 2 days thereafter.

3.11 When the plate is 80% confluent (3-4 days), freeze into 3 × 1 cryovials OR pass 1:15 to expand the cell line stock (See Passage and Expansion). (Note: We recommend passaging 1:15 to 3 × 10cm plates to allow the subsequent freezing of many cryovials to ensure future experiments can access an inventory of low passage stocks.)

3.12 The next day, replace the media in the well with StemFlex without ClonR2 or rock inhibitor and change the media every two days thereafter.

*Note: To ensure the cells are growing optimally, we recommend passing the cells at a
dilution of 1:12 with ReLeSR at least once before initiating genome editing experiments.

Passaging Cells with ReLeSR

4 On reaching 80% confluence, passage the iPS cells at a dilution of 1:12 using ReLeSR as follows:

4.1 Aspirate the culture media and wash the cells with 3 mL room temperature D-PBS (Life Technologies, 14190–094).

4.2 Add 1 ml room temperature ReLeSR (Stem Cell Technologies, 05872) and incubate at room temperature in the hood for 5 min then aspirate ReLeSR. On Metrigel substrate, the iPS cells should not detach from the plate.

4.3 Add 2 ml room temperature StemFlex media (3 ml per 6-well). Use a cell lifter (Corning, 3008) to gently scrape the cells from the surface of the plate. Break up the cells to small clumps by pipetting up and down 2–3 times using a 5ml pipette.

4.4 Transfer 0.2 ml to one well of a new Metrigel -coated well of a 6-well plate containing 2 ml StemFlex media. Check under the microscope to make sure the cell clumps evenly distributed across the well. Place cells in a 37 °C/5% CO2 incubator. Change the media every 2 days until the cells reach confluence, usually in 3 to 4 days.

*Note: When using ReLeSR, ROCK inhibitor is not required for passaging of small clumps
of cells.

Freezing Cells

5 Generally, one well of 6-well plate will yield 3 vials of KOLF2.2J cells.

5.1 Pre-warm ACCUTASE at room temperature.

5.2 Pipette old media from the 6-well plate into sterile conical tube.

5.3 Wash each well of a 6-well with 1 ml of PBS and then aspirate PBS.

5.4 Add 1ml of ACCUTASE to each well of 6-well plate.

5.5 Incubate plate(s) for 7 minutes in 37ºC incubator.

5.6 Cells on Metrigel-coated plate will detach, add 2 mL medium. Disperse the medium by pipetting over the cell layer surface several times. and transfer to sterile conical tube.

Cells on Synthemax-coated plate will still adhere to plate. Aspirate ACCUTASE, add 2 mL medium and Using a cell lifter, thoroughly scrape plate to dislodge all cells.Gently pipette media up and down 3 times while washing plate surface with the media/cell mixture, and transfer to sterile conical tube.

5.7 Count cells using a haemocytometer (include a vital dye such as Trypan blue)

5.8 Centrifuge for 5 min at 300 x g to pellet the cells, and aspirate media.

5.9 Resuspend cells in appropriate volume of freezing media (90% KOSR + 10% DMSO) to a concentration of 1×10^6 live cells/ml of freezing media. Aliquot 1 mL freezing media/cell mixture per cryovial.

5.10 After capping tubes, put the cryovials into Cell Freezing Container (Corning, 432001 or Thermo Fisher Scientific, 15-350-50), and transfer to -80^{o}C for overnight.

5.11 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.