1. PURPOSE

To describe the procedure for thawing iPSC colonies for maintenance and expansion.

2. SUPPLIES

Complete mTeSR Plus Medium (StemCell Technologies, Cat #05825)

Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)

5ml and 10ml sterile serological pipettes

Sterile 15ml conical tube

3. PROCEDURE

NOTE: You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, **the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting**.

NOTE: 1 cryovial should be thawed into 1 well of a 6 well plate

3.1 Add 9mls of cold mTeSR medium to a sterile 15ml conical.

NOTE: A 1:10 ratio is recommended to effectively dilute the Cryostor CS10 (1ml of cells and 9mls of mTeSR).

3.2 Remove cells from the LN_2 tank.

3.3 Thaw cells quickly in a 37°C water bath using a "figure 8" motion until you see a pea sized ball of ice.

NOTE: You must observe the cryovial at all times to ensure it does not thaw entirely

3.4 Cap the conical and gently invert the tube 4-5 times to mix the CryoStor CS10 and mTeSR.

3.5 Centrifuge the conical/cell mixture for 1 minute at 1000rpm.

3.6 While cells are spinning, aspirate Matrigel from dish and add an 1ml of mTeSR to the well (for a final volume of 2.5mls per well after cells have been added).

3.7 Aspirate the medium from cells and re-suspend cells in 1.5mls of fresh mTeSR.

3.8 Plate the cells into the new well.

NOTE: 1 cryovial will typically thaw into one well of a 6-well plate.

3.9 Place the plate in the 37°C incubator with 5% CO_2 and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.10 Do not move the plate for 24 hours.

3.11 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate.

3.11.1 If there is very little attachment the day after thawing, don't change the media until the next day to give the cells more time to settle and attach firmly.

3.12 Change the media every day until ready to be used or passaged.

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