1. PURPOSE

To describe the procedure for chemical passaging of iPSCs maintained on Matrigel using Versene

2. SUPPLIES

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

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

Versene (Life Technologies, Cat # 15040-066)

5ml and 10ml sterile serological pipettes

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**.

3.1 Prior to passaging your cells, check colonies in a microscope and using a cleaning tool remove any areas of differentiation from the culture.

3.2 Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 2mls of complete mTeSR media to each well (for a total of 2.5mls per well after cells have been added).

3.3 Aspirate spent media.

3.4 Rinse the wells with one volume of Versene and aspirate

3.4.1 Use 1ml for a single well of 6-well plate

3.4.2 Use 1.5ml for a single 60mm dish

3.4.3 Use 0.5ml for a single well of a 12-well dish

3.5 Add a volume of Versene to each well.

3.6 Incubate at 37°C for 4-5 minutes.

Optional: Check cells under microscope after 4-5 minutes to check for the breaking up of colonies.


Before


After

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3.7 Aspirate Versene and gently rinse the wells with a single volume of mTeSR.

NOTE: Typically, the cells should **NOT** lift from the plate at this point. You will lose a minimal number of cells**. If too many cells have lifted off the plate**, add additional media to the well and collect the cells into a sterile 15ml conical. Centrifuge the conical for 1 minute at 1000rpm. Proceed to step 3.8.

3.8 Add an appropriate volume of mTeSR and pipette up and down to dislodge the cells.

NOTE: If you have collected and centrifuged your cells, you will use this step to break up your cell pellet.

3.9 Pass cells at desired density into a new Matrigel coated TC dish.

EXAMPLE: Add 3mls of mTeSR at step 3.8, then distribute 0.5ml of cell suspension to each well for a 1:6 split.

3.10 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.

3.11 Do not move the plate for 24 hours.

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

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

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