1. PURPOSE

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

2. SUPPLIES

Complete mTeSR Plus Medium (Basal medium + 5x Supplement) (StemCell Technologies, Cat # 05825)

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

ReLeSR (StemCell Technologies, Cat # 05872)

DPBS without Ca and Mg

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 PBS -/- 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 ReLeSR to each well and incubate at room temp for 30secs.

3.6 Aspirate ReLeSR.

3.7 Incubate at 37°C for 6 minutes.

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

3.8 Add 1ml of mTeSR to the wall of the well and tap plate gently for 30 secs.

3.9 Add the 1ml cell suspension to a 15ml conical and add an appropriate volume of mTeSR and pipette up and down to break up the clumps.

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

3.11 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.

3.12 Do not move the plate for 24 hours.

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

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

4 Troubleshooting

PROBLEM	POSSIBLE CAUSE	SOLUTION
Colonies are still whole after 5-minute incubation	Cells may need a longer incubation period	Place the plate back into the incubator and check for colony break up every 3 minutes
Colonies will not dislodge after 5-minute incubation	Cells may need a longer incubation period	Place the plate back into the incubator and check for colony break up every 3 minutes
Colonies have all attached to the center of the plate	Colonies did not evenly spread across the plate	Make sure to perform step 3.10. Perform this step in the incubator so after the plate is rocked back and forth it won't be disturbed Place plates in the back of the incubator to ensure they are not bumped after passing Open and close the incubator door gently to prevent any dislodging
Colonies did not attach to the plate after passaging	Matrigel plates were old or died out Cell line may have difficulties attaching	Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated Cell line may require a double concentration of Matrigel. Coat a new plate.
Differentiation is still abundant in the newly passaged well	6 minute incubation period may be too long	Decrease incubation times by 2-3 minutes for lines that display high rates of differentiation

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