1. PURPOSE

To describe the procedure for passaging of iPSCs maintained on Matrigel using Pick-to-Keep (PTK)

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)

BD Lo-Dose™ U-100 Insulin Syringes (FisherSci, Cat #329461)

5ml and 10ml sterile serological pipettes

Filtered 1000uL Pipette tips

Unfiltered 200uL Pipette tips

Biohazardous Sharps Waste Container (inside BSC)

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. Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 1 ml of complete mTeSR media to each well

3.2. You should have a total volume of at least 2.5mls per well after cells have been added

3.3. Aspirate spent media.

3.4. Add 1ml of mTeSR per wells needed for PTK.

3.5. Working under microscope inside the BSC at 4X objective, use an insulin syringe needle to cut iPSC colonies into small clumps (see Figure A)

NOTE: Uncap the syringe one time and discard the cap right away. Avoid injury by never re-capping a needle

3.5.1. Take great care to use the lightest pressure possible in order to avoid scratching through the coating substrate and plasticware, as the insulin syringe is incredibly sharp

3.5.2. It is recommended to select at least 10 colonies per well of a 6-well plate to obtain sufficient adhesion and proliferation the following week

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3.6. Carefully dispose the used syringe into a biohazardous sharp waste inside the BSC
3.7. Place a P1000 micropipette tip inside a P200 tip.
3.8. Use the P1000+P200 tip to gently nudge or lift the cut colony pieces off the plate similar to a fine cleaning motion (see Figure B)
3.9. Add 1 ml of mTesR to the well with picked clumps and gently rinse the well to collect all the pieces
3.10. Tilt the PTK plate and gently resuspend to rinse the well and pool the clumps at the bottom of the well
3.11. Transfer floating clumps to new plate.
3.12. 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.
3.13. Do not move the plate for 24 hours
3.14. The next day, check for colony attachment and proliferation

Figure A

Figure B

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