Passaging hiPS Cells on Matrigel using ReLeSR

In general, human induced pluripotent stem cells (hiPSCs) should be passaged when the colonies become large and before they start touching each other (60-80% confluency). Human iPS cells that are maintained on Matrigel-coated surfaces in defined, feeder-independent media such as mTeSR are typically passaged approximately every 5 - 7 days at a 1:10 to 1:40 split ratio. For hiPS cells growing on Matrigel-coated dishes, cells may be passaged as clumps or aggregates using a number of different non-enzymatic dissociation reagents such as ReLeSR or Gentle Cell Dissociation Reagent.

Materials and reagents:

- ReLeSR (StemCell Tech. Cat# 05782)
- mTeSR (StemCell Tech. Cat# 05850) supplemented with primocin (InvivoGen ant-pm-2)
- DMEM/F12 (Life Tech. Cat# 11330-057)
- DPBS (Life Tech. Cat# 14190-144)
- Matrigel (Corning Cat# 354277)-coated plates (6-well, Fisher Cat# 07-200-83)
- Cell scrapers (Corning Cat# 3010)

1. Prepare freshly coated Matrigel plates at least 1 hr prior to use according to manufacturer's instructions. Aspirate excess Matrigel and rinse each well (6-well plate) with 1 mL DMEM/F12. Add 2 mL of mTeSR media (at room temperature) to each well and store plate in a 37°C, 5% CO₂ incubator until needed.

2. To prepare hiPS cells for passaging using ReLeSR, first remove any regions of cellular differentiation, if necessary, by scraping with a pipette tip.

3. Aspirate media and wash cells with 1 mL DPBS.

4. Add 1 mL of ReLeSR to each well and aspirate within 1 minute.

5. Transfer the plate to the 37°C incubator for 5-7 min (~ 5.5 min). *(Optimal dissociation times may have to be determined for each cell line)*

6. Remove plate from incubator and add 1 mL mTeSR down side of wall of each well. The hiPSC colonies will lift from the plate and areas of differentiated colonies will remain adherent to plate.

7. To break up large detached colonies into small clumps, firmly tap plate for 30 seconds or gently pipet up and down, slowly once, with a 5 mL serological pipet. Alternatively, if detached colonies remain large, transfer large clumps to a 50 mL conical tube using a 5 mL serological pipet and pipet up and down once slowly with a P1000 pipet tip.

8. Transfer cell aggregates to Matrigel-coated wells with 2 mLs mTeSR at desired dilution (usually around 1:10 to 1:40) depending on cell density and growth rate of cell line.