Cultivation protocols for PSCs

I. Manual passaging

1. Check colonies under the microscope.
2. Prepare new Matrigel dish with fresh media (CM/E8/mTeSR1).
3. Remove differentiated or bad-looking colonies by scraping.
4. Select best colonies for passaging.
5. With yellow tip or other tool roundabout colony and cut it for several pieces. Scrape them/Flow them into media and pipette them into new dish
6. During cultivation remove differentiated or bad-looking colonies by scraping daily.

II. Passaging with EDTA

1. Check the cells under the microscope, they should be 80-90% confluent.
2. Prepare new Matrigel dish with fresh media (CM/E8/mTeSR1). You can use 5-20uM ROCK inhibitor (stock 100mM; 1:5000).
3. Wash cells with PBS.
4. Add ½ volume of dish of 0,5mM EDTA in PBS (stock 0,5M).
5. Keep 3-5 min at RT or 37°C.
6. Aspirate EDTA.
7. Add 1mL of media and wash the cells.
8. Seed cells into new dish in ratio based on plating efficiency (1:5-1:20).

III. Passaging with TrypLE

1. Check the cells under the microscope, they should be 80-90% confluent.
2. Prepare new Matrigel dish with fresh media (CM/E8/mTeSR1). You can use 5-20uM ROCK inhibitor (stock 100mM; 1:5000).
3. Wash cells with PBS.
4. Add ½ volume of dish of TrypLE.
5. Keep 2-3 min at 37°C.
6. If cells are still attached, aspirate TrypLE. If majority of cells is already floating keep TrypLE.
7. Add 1mL of media and wash the cells by pipetting.
8. Move cells into 15mL tube and centrifugate them at 200g 4 min.
9. Aspirate supernatant. If TrypLE was not aspirated, pellet is not stable, be careful.
10. Add fresh medium and pipette cells into single-cell suspension.
11. Seed cells into new dish in ratio based on plating efficiency (1:5-1:20).

IV. Freezing cells

1. Cells from 3cm dish in 80-90% confluency for 2-3 vials.
2. Enzymatically or manually passage cells and centrifugate them at 200g 4 min.
3. Prepare cryovials and label them (name, clone, passage number, cultivation conditions, date).
4. Aspirate supernatant and add 500uL of Synth-a-freeze per vial, gently resuspend.
5. Quickly insert into Cryo-boxes and move to -80°C.
6. Keep Cryo-boxes at -80°C for 24-48h and move vials into liquid nitrogen for long term storage.

V. Thawing cells

1. Prepare coated dish with fresh media with 5-20uM ROCK inhibitor.
2. Move cryovial from liqid nitrogen or -80°C into box with dry-ice or ice.
3. Thaw cryovial in 37°C water bath until small ice piece remains.
4. Gently transfer into 15mL tube.
5. Gently pipette drop by drop 2mL of cold media.
6. Centrifuge at 200g for 3-4min.
7. Aspirate supernatant and resuspend in prewarmed media, immediately seed into dish.

VI. Embryoid bodies – manual scraping

1. Prepare fully confluent 3cm dish (cells have to be in monolayer).
2. Scrape monolayer with yellow tip into square-like pieces and seed them into non-adhesive (bacteriological) 6cm dish with 5mL of MEF media
3. Change media 2x week (Mo; Th)
4. Cultivate for 15 Days
5. Analyze spontaneous differentiation (Western blot, qPCR)

VII. Embryoid bodies - polyhema 96W

1. Prepare polyhema coated 96 well plates.
2. Seed 9.000-18.000 cells/well in MEF media with 20uM ROCK inhibitor.
3. Centrifugate 200g 4min.
4. Change media 3x week (Mo; We; Fr).
5. Cultivate for 15 Days.

VIII. Bürker chamber

1. After passage, resuspend cells in 1mL of media (in higher cell counts add more media.
2. Pipette 10uL into Bürker chamber.
3. Count 3 big squares (9 in Bürker chamber), make average, multiply by 10,000 = cells/mL