Maintenance of iPSC

Plates are coated with Geltrex (diluted in DMEM/F12 solution) and placed in incubator 37ºC 5%CO_2 for 30 minutes. Geltrex solution is aspirated before the cells are plated in E8 flex medium. We use 6-well plates and coating volume 1ml per well.

For media change use prewarmed room temperature (place it outside of fridge for at least 15 minutes) E8 Flex media (#A2858501, Gibco™).

1. Aspirate old media with floating cells using aspirator or 5ml serological pipette.
2. Add 2ml fresh media daily, using a 5ml serological pipette.
3. Place cells in the incubator at 37ºC and 5% CO_2.

Passaging of iPSC using EDTA

Prepare 0,05M EDTA solution, by diluting 0.5M EDTA in PBS w/o Ca2+, Mg2+ (Thermo Fisher, 14190250).

Based on the growth characteristics of a particular cell line, cells should be passaged every 3 to 5 days to keep them in the log phase of growth and prevent spontaneous differentiation, especially if the cell density gets too high. For established cultures, a typical split ratio is between 1:10 and 1:20.

1. Aspirate medium from the culture and rinse once with 1 ml of 0.5 mM EDTA.
2. To detach cells, add 1 ml of 0.5 mM EDTA to the culture vessel and place for 2-3 minutes into incubator until colonies will appear with holes in them.
3. Aspirate EDTA gently and add 3 ml of culture medium to one well. Firmly tap the side of the plate for approximately 5 - 10 seconds or use a scraper to gently dislodge the cells from the vessel. Pipet cell suspension one or two times up and down using a 5 ml pipette.
4. Seed the cells at an appropriate cell density by transferring the required volume of cell suspension to the prepared plate/well prefilled with medium.
5. Rock the plate forth and back to guarantee an equal distribution of cell cluster throughout the whole well. Place the plate into the incubator (37°C, 5% CO2).

Freezing of iPSC

Detach cells with EDTA the same way as describet above. As freezing media use 10% DMSO and 90% of Knockout Serum (Life Technologies, 10828028).