FREEZING and THAWING IPS CELLS ON A 96-WELL PLATE

Freezing

1) Remove medium from wells
2) Rinse wells with 100 ul 1X D-PBS (without calcium and without magnesium)
3) Add 30 ul Accutase, incubate at 37C.
4) Using a 12-channel pipette, add 150 ul Freeze medium (10% DMSO in FBS) per well. If you use a half of the cells for other purposes such as gDNA extraction and only have 15 ul of the cell suspension, reduce the amount of Freezing medium to 75 ul. Pipet up and down to dislodge the cells from the plate. Avoid creating too many bubbles.
5) Layer 75 ul sterile filtered mineral oil (Sigma-embryo grade)
6) Parafilm around the edge of plate and place on bed of ice
7) Transfer plate to -80˚C freezer. Do not put plate directly on surface of -80˚C freezer. Before freezing the 96-well plate, I place an empty Styrofoam container in -80˚C. This way the plate and cells cool and freeze a bit more slowly which helps the survival

Thawing

1) Place the frozen plate in a 37˚C CO2 incubator and wait for 10 to 15 minutes.
2) Prepare 500 ul Essential 8 media (E8) + Rock inhibitor (Ri) in a 1.5 ml tube.
3) Transfer all the cell suspension to the 1.5 ml tube with 500 ul E8 + Ri and mix.
4) Spin the tube at 6000 rpm for 5 min.
5) Using a micropipette, carefully remove oil in the top layer first. Next, remove the media.
6) Resuspend the cells in E8 + Ri and plate them on Matrigel-coated wells.