Standard Operating Procedure
for
Subculture IPS Cells

1.0 PURPOSE

This procedure describes how to subculture IPS cells.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who wish to subculture IPS cells.

3.0 PROCEDURE

1. Prepare MEF culture 6-well dishes one day before subculture iPSC.
2. Before splite the iPSC, wash each well of MEF culture dishes with 2 ml DMEM/F12 medium. Add 1.5 ml hES medium containing 10 μM Y-27632/Rock inhibitor into each well.
3. Splite iPSC.

   a. Aspirate hES medium from iPSC culture dishes. Wash each well of 6-well dishes with 1 ml DMEM/F12 medium.

   b. Add 0.5ml dipase into each well and incubate in 37 °C incubator for 3-5 mins.

   c. Wash each well with 2 ml DMEM/F12 medium.

   d. Add 2 ml hES medium containing 10 μM Y-27632/Rock inhibitor into each well.

   e. Detach iPSC colonies from the dishes with a glass pipette and put the cells into a 15-ml tube. Rinse the well with 1 ml hES medium containing 10 μM Y-27632/Rock inhibitor and transfer it into the same tube. Gently cut colonies into small pieces with a pipette.

   e. Add a suitable amount of iPS cells into each well and make sure that the total volume of each well is not less than 2 ml. Note: usually one well of iPS colonies is splited to six wells.

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