

## Splitting iPS cells on MEFs:

(Thomson Lab, January 2008)

*based on splitting onto one plate*

1. Warm collagenase IV split media to 37 °C in a water bath.
2. Aspirate media off of cell culture plate.
3. Add 1ml of collagenase split media to 1 well of 6 well plate
4. Incubate at 37 °C with 5% CO<sub>2</sub> for 10 minutes. The cells are ready when the edges of the colony are rounded up and curled away from the MEFs on the plate.
5. Gently wash cells 3 times with sterile DMEM/F12 while colonies are still attached to plate.
6. Using a 5ml pipet, scrape and wash the colonies off of the plate.
7. Break up the colonies by pipetting up and down against the bottom of the tube until just before there appears to be a fine suspension of cells. iPS cells do best with slightly bigger colonies than ES cells.
8. Plate onto fresh feeder layers (previously washed with PBS). Plate cells by adding 0.4ml per well of a 6 well plate until the last 0.5–0.6ml remain. Add the last remaining volume dropwise to each well.
9. Make sure the cells are evenly distributed across the entire well.
10. Place gently in incubator. Again make sure the cell are not disturbed.
11. Let cells settle overnight in incubator.

*Notes:*

*\* iPS cells are very sensitive to the quality and quantity of the MEFs – too thick or too sparse can lead to differentiation.*

*\* iPS cells, especially early passaged cells, are prone to differentiation. It is not uncommon to have to pick off bad colonies every single passage. Luckily, the undifferentiated iPS cells are very robust, and recover nicely, even when you pick to keep.*

*\* Regardless of your previous ES culturing skills, we recommend you **FREEZE DOWN ALIQUOTS OF YOUR CELLS AS SOON AS POSSIBLE**. Protocols for freezing and thawing iPS cells are identical to those for ES cells.*