

Splitting Human ES cells on MEFs:

Thomson Lab, 2004

1. Warm collagenase IV split media to 37°C in a water bath.
2. Aspirate media off of cell culture plate.
3. Add the following amount of collagenase:
0.5ml/well of 4 well plate
1.0ml /well of 6 well plate
4. Incubate at 37°C with 5% CO₂ for 5-10 minutes. The cells are ready when the edges of the colony are rounded up and curled away from the MEFs on the plate.
5. Using a 5ml pipet, scrape and wash the colonies off of the plate.
6. Transfer cell suspension to a 15ml conical tube.
7. Break up the colonies by pipetting up and down against the bottom of the tube until there appears to be a fine suspension of cells (no clumps of cells remain).
8. Spin cells at 1000rpm for 5 minutes.
9. Aspirate collagenase off and wash cells with 3 ml human ES media.
10. Spin 1000rpm 5 minutes.
11. While the cells are spinning prepare the feeder layers by aspirating off the MEF media and washing one time with Ca/Mg free PBS and adding human ES media to the feeder layers (2 ml/well of 6 well plate).
12. Once cells are done spinning, aspirate off wash media.
13. Resuspend cells in an appropriate volume (see notes).
14. 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.
15. Make sure the cells are evenly distributed across the entire well (see notes).
16. Place gently in incubator. Again make sure the cell are not disturbed.
17. Let cells settle overnight in incubator.

Notes:

- Always check your MEFs for viability and contamination before you split onto them.
- MEFs are usually good to split onto for about 10 days after plating.
- Make sure that the human ES cells are evenly distributed throughout the plate, if cells all wind up in the middle they could differentiate or need to be split sooner.
- Be very gentle to evenly distribute the cells. When using quick motions, you will most likely wind up with your cells in the middle of the plate. When you are moving your cells back and forth in the incubator to disperse them, be sure to only move in one direction at a time or a “whirlpool like” effect will happen and all of your cells will swirl in the middle.
- It is a good idea to learn human ES cell culture on MEFs before moving to matrigel.