

# Splitting Human ES cells on Matrigel: *based on splitting onto one plate*

Thomson Lab, 2004

1. Warm collagenase media to 37°C in a water bath.
2. Aspirate media off of cell culture plate.
3. Add the following amount of collagenase
4. 0.5ml/well of 4 well plate
5. 1.0ml/well of 6 well plate
6. Incubate at 37°C for 5-10 minutes; stop incubation when edges of colonies begin to pull away from the plate.
7. Aspirate the collagenase and add appropriate volume (3ml) of conditioned media (CM) to the plate (see notes).
8. Collect cells off of the plate by scraping and washing with the CM, transfer to a 15ml tube.
9. Aspirate matrigel off of 6 well plate (see matrigel aliquoting and plating procedure)
10. Add 2ml of CM per well of 6 well plate.
11. Add 5ul bFGF.
12. Plate 0.4ml/well into each well until there is approximately 0.6 ml remaining.
13. Add the remaining 0.6ml dropwise to each well until done.
14. Make sure the cells are evenly distributed across the plate.
15. Place gently into incubator.
16. Let settle overnight.

## Notes:

- *High density MEFs for CM last about 2 weeks after plating.  
Be sure to check them regularly for cell death.*
- *bFGF is stable at 4 C for one month.*