Dispase Splitting Protocol	Berggren, Travis tberggren@salk.edu
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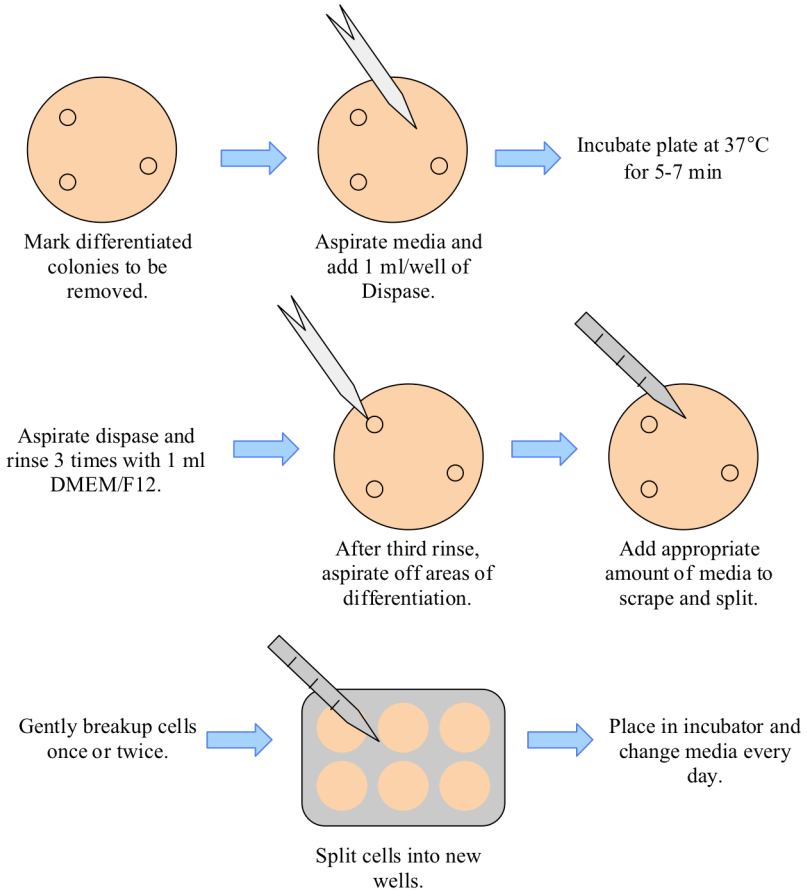
Submitted by	Berggren, Travis tberggren@salk.edu
Adapted from	Salk STEM Cell Core in-house protocols
Contributors	Lutz, Margaret. Modesto, Veronica.
Affiliation	The Salk Institute

Introduction:

This protocol is used for general maintenance and passaging of hES and iPS cells. It assumes that the cells are grown in 6 well format. The well to be split should be close to confluence. The colonies should be large and touching each other.

Flow Chart:



Protocol Steps:

Prepare the Matrigel Plate:

1. Thaw a 0.5mg vial of Matrigel on ice.

2. Bring up the thawed 0.5mg of Matrigel in 12 mls of ice cold DMEM/F12.

3. Immediately plate 2 mls per well of diluted Matrigel on a 6 well plate.

4. Plate must sit at room temperature for 1 hour or in a 37C incubator for at least 20 minutes before it can be used.

5. Matrigel plates can be made up to one week ahead of time and stored in the 4C refrigerator wrapped in Parafilm. Note: After removing from the 4C refrigerator, the plates must sit at room temperature for one hour or in a 37C incubator for 20 minutes before being used.

6. After Matrigel plate has sat for appropriate amount of time, aspirate off DMEM/F12.

7. Place 1.5 mls of warm hES media into each well to be split into. Note: We recommend using mTeSR1 but any media formulated for use in feeder free conditions can be used.

Prepare the Dispase Solution:

8. Dissolve 2mg of Dispase into 1 ml of DMEM/F12.

9. Alternatively, make a 10x stock of Dispase (20mg/ml) and store in 1 ml aliquots in the -20C freezer. These aliquots can be thawed and diluted with 9mls of DMEM/F12.

10. Once in solution, Dispase can be stored in the 4C refrigerator for up to 2 weeks.

Passage the hES cells:

11. Mark areas of differentiation on the well to be split using the microscope objective marker.

12. Aspirate off spent media.

13. Place 1 ml of warm Dispase in each well to be split.

14. Incubate at 37C for 5-7 min. Note: After incubation, the edges of the colonies should be starting to curl up.

15. Aspirate off the Dispase.

16. Rinse the well gently 3 times with 1 ml warm DMEM/F12. Aspirate after each wash.

17. After the third rinse, aspirate off previously marked spots of differentiation with a Pasteur pipette.

18. Add desired amount of media for scraping off cells. For example: For a 1:4 split, add 2mls of media.

19. While holding pipette at a 90° angle, scrape a glass pipette back and forth across well while slowing expelling media to rinse off colonies.

20. Repeat this until at least 90% of colonies are detached from the well.

21. Pipette cells gently up and down twice to break up the colonies into smaller pieces.

22. Place appropriate amount of cell/media mixture into each well of the previously prepared Matrigel plate. For example: For a 1:4 split, scrape off in 2mls of media and plate 0.5mls into each well of the previously prepared plate.

23. Place in 37C incubator.

24. Give the plate a gentle shake back and forth and side to side to help distribute the colonies evenly in the wells. Tip: Remember to feed the wells not split in the old plate. These wells can be used to split the next day if this split did not go well.

25. Change the media the next day.

Materials:

Product	**Supplier**	**Catalog #**
Growth Factor Reduced Matrigel	BD	354230
Dispase	Life Technologies	17105041
DMEM/F12	Life Technologies	11330057
mTeSR1	Stemcell Technologies	05850
6 well plate	USA Scientific	
Microscope Objective Marker	Nikon	MBW10020

Serological Pipettes	Various Sources	
5 ml Glass Pipette	Fisher Scientific	13-678-27E
Pasteur Pipettes	Fisher Scientific	13-678-20D