iPS Cell Culture

v1-1
11/30/17

Coat wells with hESC-qualified Matrigel

1. Remove an aliquot of Matrigel from -80C, sized for the number of plates that need to be coated (an aliquot labelled with "2" on the lid is enough to fully coat (2) full 6-well plates. Temporarily place at 4C until ready.
2. Multiply the number on the lid by 6.25, pipette this amount of DMEM-F12 into a 50ml conical tube. (eg. a tube labelled "2" needs 12.5ml of DMEM-F12)
3. Remove the aliquot of matrigel from 4C, and transfer into the DMEM-F12 using a pipette. If the Matrigel is still frozen, it is acceptable to quick-thaw the Matrigel by taking a portion of the DMEM-F12 and adding to the Matrigel aliquot. Pipette up-and-down until thawed, and transfer back into the DMEM-F12.
4. Add 1ml of diluted Matrigel per single 6-well (ie. 6 ml are required per 6-well plate). Scale as needed for the desired well size.
5. Parafilm the plate to ensure the coated wells do not dry out. Incubate the plate for 1hr at room temp.
6. The plate is now ready to be seeded with cells. Alternatively, store the plate at 4C for up to 2 weeks. After removing a stored plate from 4C, repeat 1hr incubation at room temp.

Passage iPS Cells

Important note: passaging cells with EDTA (0.5mM EDTA in PBS) is a clump-passage method. Plating iPSCs as aggregates of 100-300 cells (see example pictures) is critical for healthy attachment and minimal death or differentiation. When plated correctly, it is not necessary to supplement with a ROCK inhibitor (eg. Y-27632) for routine passaging. EDTA has the additional benefit of selectively lifting iPS cells, fibroblasts/feeders and fibroblast-like differentiation will remain adhered to the well if manual scraping can be avoided during passaging

7. Before passaging, manually scrape as much differentiation from the well as possible. Determine the split ratio based on overall confluency (see example pictures).
8. Aspirate the culture media along with any scraped differentiation. Rinse once with 1ml of EDTA, aspirate.
9. Add 1 ml of EDTA per 6-well. Incubate for approximately 5min at 37C. Exact incubation time is cell line dependent (typically 3-7min). Incubation is done when colonies appear to be breaking apart in the center (see picture).
10. While the cells are incubating, aspirate the Matrigel from the prewarmed plate and replace with mTeSR (final volume after plating cells should be 2-3 mls).
11. **GENTLY** remove the plate from the incubator and place in the hood. At this point, cells are very loosely adhered and easily dislodged.
12. Carefully aspirate the EDTA.
13. Add ~2ml (can vary depending on desired split ratio) of pre-warmed mTeSR to neutralize the EDTA. To dislodge and pool cells, draw up the mTeSR and quickly eject back onto the bottom of the well. **IMPORTANT**, **STOP PIPETTING** if all the cells cannot be easily pooled with 2-3 rounds of rinsing the well. Further pipetting excessively breaks up the cell clumps and is counter-productive. Instead, use a cell-scraper to dislodge the remaining colonies. (Make a note that the incubation time with EDTA can be increased for future passaging)
14. If necessary, pipette to break up colonies to the target 100-300 cell-per-clump size (see picture for reference).
15. Gently pipette a fraction of the iPS clumps into a new well(s) to achieve the predetermined split ratio.
16. Move the plate into a 37C incubator. To evenly distribute cells, shake forward-backward then left-right. Do not swirl.
17. The day after passaging is the ONLY day it is acceptable to skip a media change. Beyond that, replace media daily with 2ml per 6-well.

Freeze iPS Cells

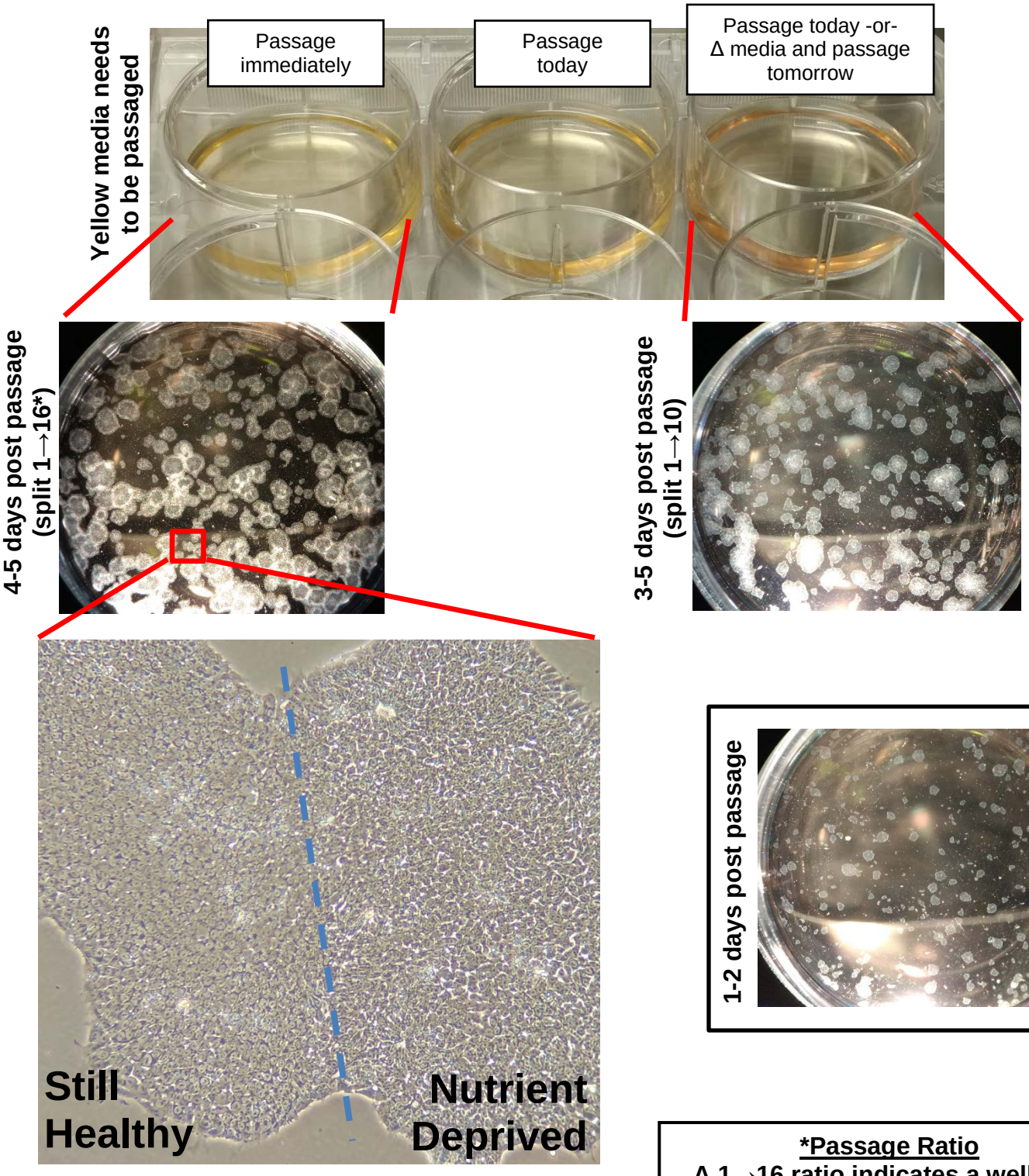
Before starting: ensure there is an available freezing container, print labels and label cryovials. One confluent 6-well is sufficient to freeze two vials (ie. a full 6-well plate yields 12 frozen tubes). Cell viability post-thaw is best if ice-cold Cryostor is used (step 21-23)

18. Passage cells as described in steps 7-13. **IMPORTANT**: maintain as large of cell clumps as possible. It is advisable to use a cell scraper rather than pipetting to dislodge cells (step 13).
19. Combine all wells of a single cell line into a 15 ml conical tube.
20. Centrifuge for 3min at 300g to pellet cells.
21. During centrifugation: Remove Cryostor from 4C and place in hood, remove caps from cryovials and set aside.
22. Aspirate mTeSR from pelleted cells. Leave 100-200µl to ensure the pellet is not disturbed.
23. Carefully add Cryostor to the cell pellet (1ml per well collected). Gently pipette to mix. Only pipette enough to break up the pellet while maintaining as large of individual clumps as possible.
24. Gently pipette 0.5ml of resuspended cells per cryovial.
25. Move cryovials into freezing container. Leave overnight at -80C. Transfer into liquid nitrogen the following day.

Thaw iPS Cells

Before starting: Prepare plate, warm media to 37C. A frozen sample prepared as described above will be plated into two 6-wells

26. Remove cryovial from liquid nitrogen. Ensure cap is fastened tightly (can loosen during storage). Quick-thaw in 37C water bath. Keeping the cap above water level minimizes contamination risk. As soon as the last ice crystal thaws, spray liberally with ethanol and move into hood.
27. Gently transfer thawed cells into 15ml conical containing 4ml mTeSR, maintain as large of cell clumps as possible.
28. Centrifuge for 3min at 300g to pellet cells.
29. Aspirate mTeSR from pelleted cells. Leave 100-200µl to ensure the pellet is not disturbed.
30. Gently resuspend pellet in 4 ml of mTeSR while maintaining cell clumps.
31. Transfer resuspended cells into two 6-wells, 2mls per well.
    a. Antibiotics can be added to a final concentration of 1x if desired. Adding the ROCK inhibitor Y-27632 at a final concentration of [10µM] can increase viability. Remove ROCK inhibitor the following day.
32. Move the plate into a 37C incubator. To evenly distribute cells, shake forward-backward then left-right. Do not swirl.
33. The following day, replace media with 2ml per 6-well.

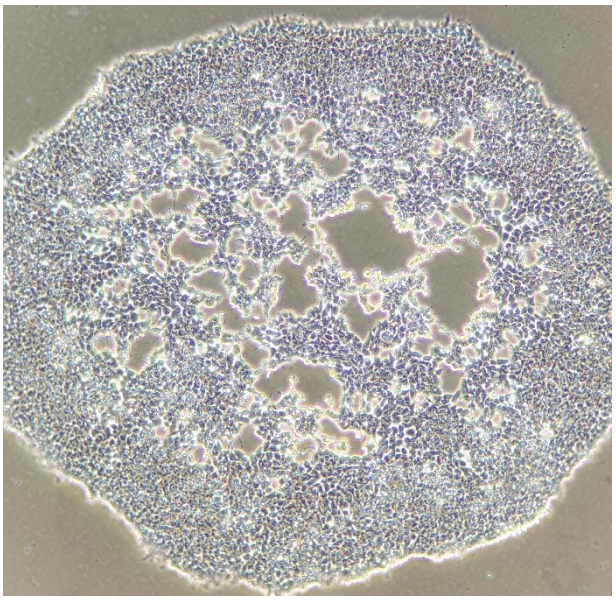


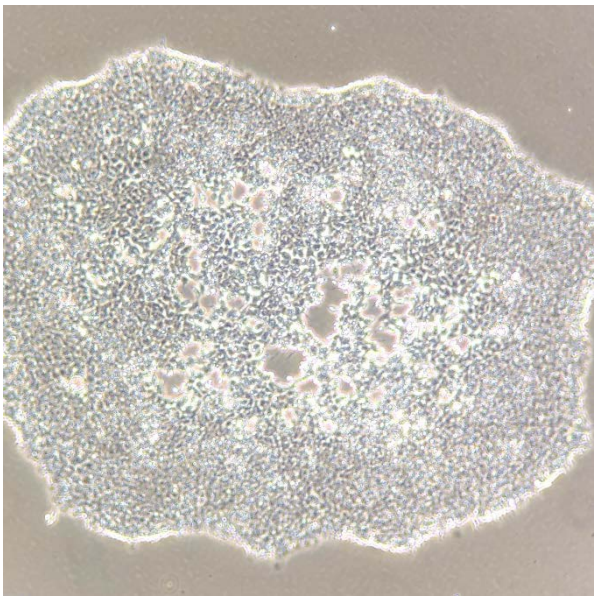
***Passage Ratio**

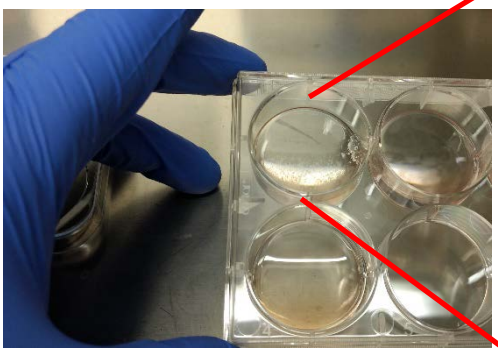
A 1→16 ratio indicates a well that should be split into 16 new wells.

(eg. When passaging, neutralize EDTA with 3.2 mls of mTeSR. Plate 0.2mls into each new well)

Correct amount of EDTA treatment

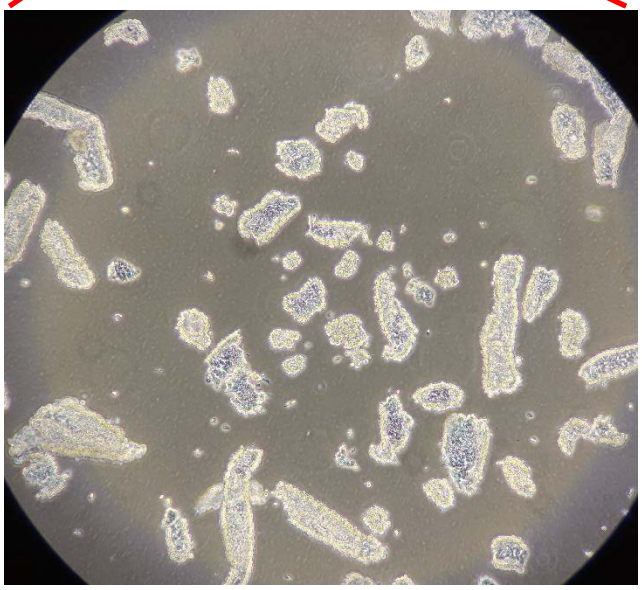






Pipette or scrape to lift cells

Plate clumps containing approx. 100-300 cells



Reagent	Company	Catalog Number
6-well tissue culture plates	Corning	#3516
mTeSR	Stem Cell Technologies	#85850
Cryostor	Stem Cell Technologies	#7930
hESC-Qualified Matrigel	Fisher	#8774552
Y-27632	Stem Cell Technologies	#72307
DMEM-F12	Gibco	#11320-033