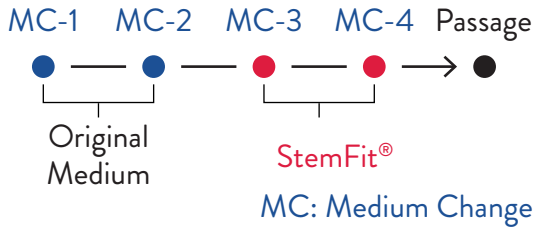
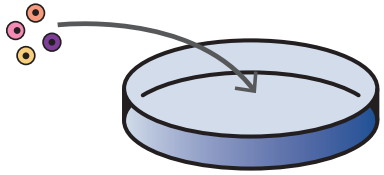
TIPS ON TRANSITIONING CELLS TO STEMFIT® MEDIUM

- Switch culture medium to StemFit® 2-3 days prior to passage



- Seed the cells at higher density (>1.0 x 10^5 cells per well (6-well plate))



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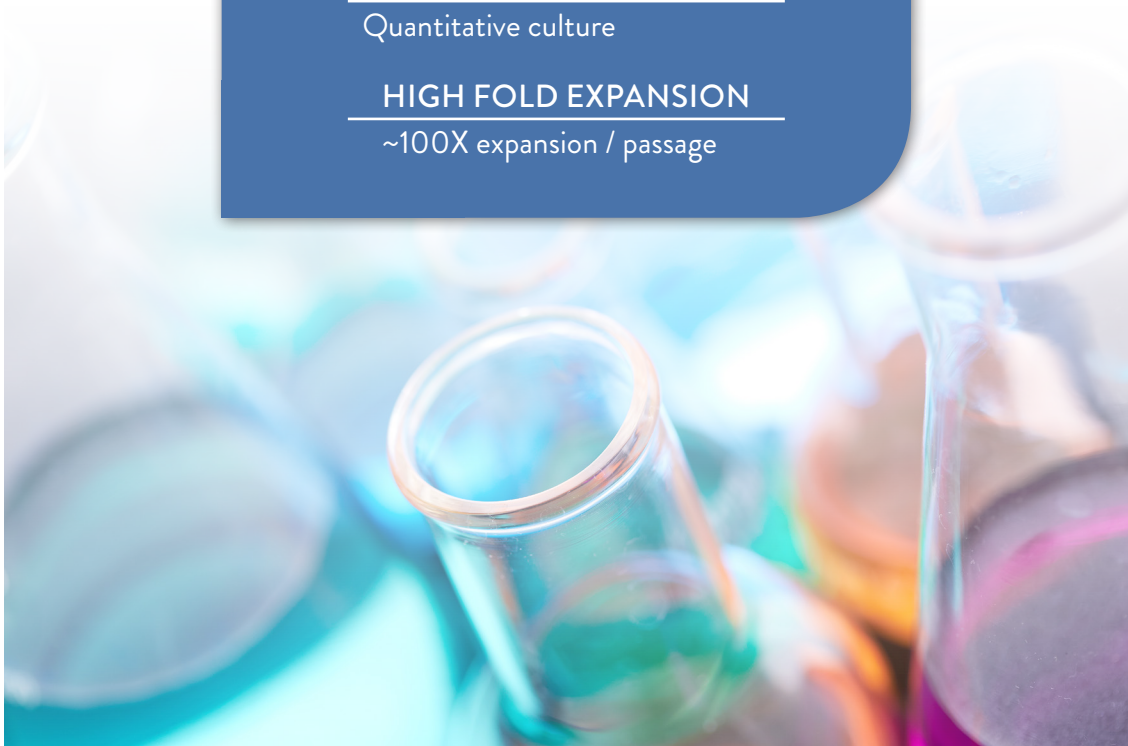
FEEDER FREE MEDIUM FOR ES/IPS CELLS

Key points for successful single-cell passage

StemFit® Technical Tips

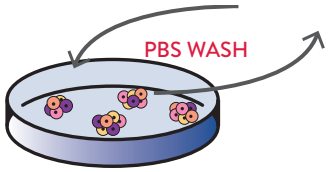
ROBUST AND REPRODUCABLE CULTURE
Quantitative culture

HIGH FOLD EXPANSION
~100X expansion / passage



SINGLE-CELL PASSAGE BRIEF PROTOCOL EXAMPLE (6-WELL PLATE) AND TIPS

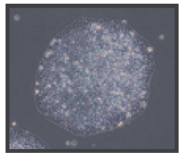
1. Aspirate the medium and wash once with 2ml of PBS



2. Add 500 µl/well of Accutase and incubate at 37°C for 10 minutes

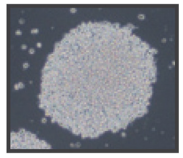
- TrypLE™ can also be used for cell dissociation
- Incubation time may vary depending on the matrix

- Before incubation with Accutase



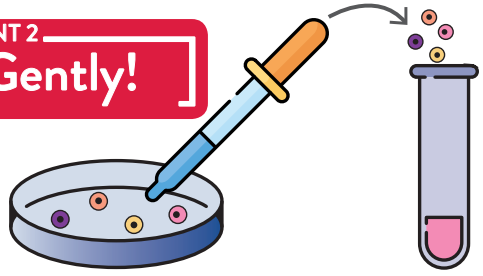
POINT 1
10 min!

- Gaps in the colonies appear and dissociation of colonies is apparent



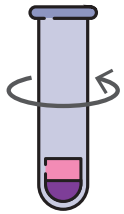
3. Gently pipette the cells to fully dissociate and transfer cells to a 15ml tube filled with 500 µl of culture medium containing 10 µM Y-27632

POINT 2
Gently!



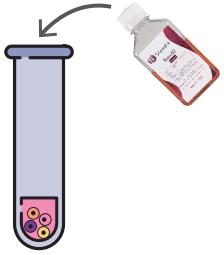
4. Count the cells and centrifuge the tubes

300g RT 4 min



Aspirate the medium and resuspend cells with culture medium containing 10 µM Y-27632

1000 cells / µl

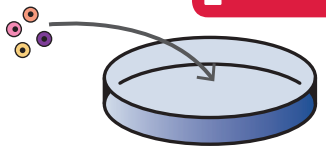


5. Add 10-20 µl (1.0-2.0 x 10^4 cells) of resuspended cells per well in 1.5 mL of culture medium containing 10 µM Y-27632

- It is important to adjust the plating cell number for different lines of hPSCs
- Try higher seeding density when cell or colony quantity is insufficient

POINT 3
Adjust the cell number

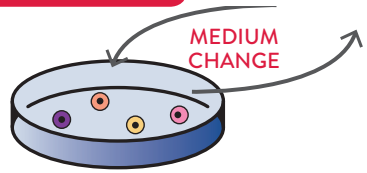
POINT 3
Distribute evenly!



- Immediately distribute the cells evenly over the plate surface to avoid uneven attachment

6. After >24 hours of cultre, replace with fresh culture medium without Y-27632

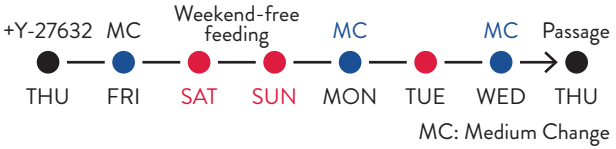
POINT 5
>24 hours!



- It is critical that cells are cultured in Y-27632 containing medium for more than 24 hours

7. Perform medium change

< Passage Schedule Example>



MC: Medium Change

POINT 6
*** If the colour of the medium turns orange or yellow it should be changed every day**

POINT 7
*** Do not allow cells to become confluent**