## 1. PURPOSE

To describe the procedure for manual passaging of iPSCs maintained on Matrigel using the StemPro EZ Passage Tool

## 2. SUPPLIES

Complete mTeSR Plus Medium (StemCell Technologies, Cat # 05825)

Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)

StemPro® EZPassage™ Disposable Stem Cell Passaging Tool (Life Technologies, Cat # 23181-010)

5ml and 10ml sterile serological pipettes

## 3. PROCEDURE

**NOTE:** You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, **the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting**.

3.1 Prior to passaging your cells, check colonies in a microscope and using a cleaning tool to remove any areas of differentiation from the culture.

3.2 Aspirate spent media and add desired amount of fresh mTeSR Plus media to each well. **For example:** Add 3mls of media for 1:6 split (0.5ml of cells into 6 wells)

3.3 Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 2mls of complete mTeSR Plus media to each well (for a final volume of 2.5mls per well after cells have been added).

3.4 Using the EZPassage™ tool cut colonies into small squares.

3.4.1 Hold the culture vessel in one hand and **pull (roll)** the StemPro® EZPassage™ Toolacross the entire plate in one direction (left to right). Apply enough pressure so the entire roller blade touches the plate and maintain uniform pressure during the rolling action.

3.4.2 Continue to pull the tool parallel until you have covered the entire plate in one direction.

**NOTE:** Do not move the tool back and forth throughout the well; only move in the left to right direction.

3.4.3 Rotate the plate 90° and repeat step 3.4.1

**OPTIONAL:** View the plate in the microscope to visually confirm that the colonies have been properly cut. Colonies should have a “checkered” look to them.





8687 Melrose Ave. STE B227, West Hollywood, CA 90069
groupipscore@cshs.org • 310-423-3400 • www.csbiomfg.com

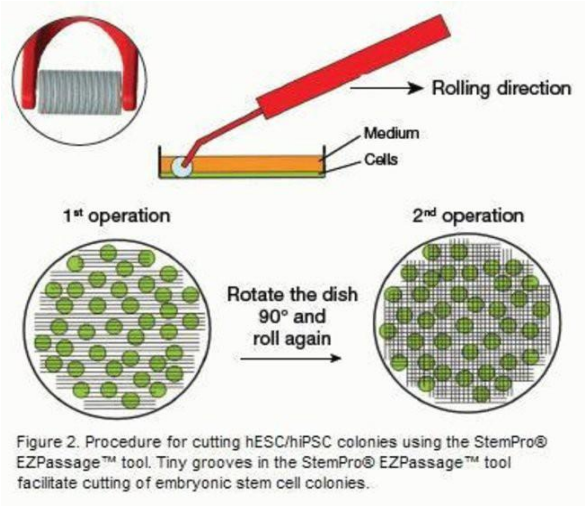
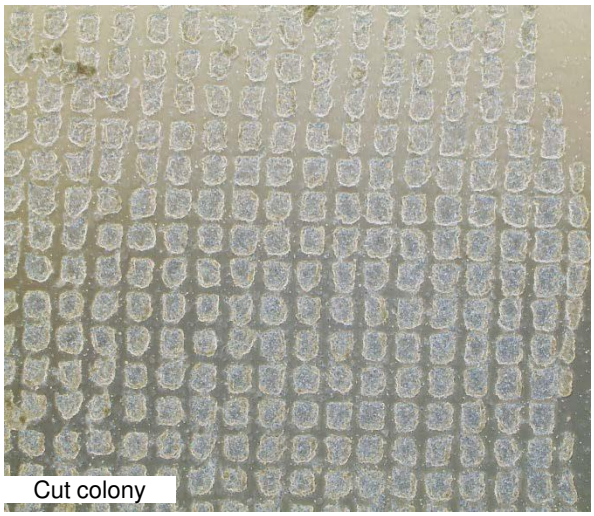



Figure 2. Procedure for cutting hESC/hiPSC colonies using the StemPro® EZPassage™ tool. Tiny grooves in the StemPro® EZPassage™ tool facilitate cutting of embryonic stem cell colonies.




3.5 Using 5ml serological pipette, pull up the media from the well.

3.6 Hold the serological tip perpendicular to the bottom of the well and then gently scrape the well to dislodge the colonies from the plate while simultaneously releasing medium.
**NOTE:** It is important to continuously have medium coming out of the pipette. **Do not scrape the wells "dry" (no media).** This will cause the cut colonies to smear and the cells will die.

3.7 Continue to pipette up and down gently to completely dislodge the cut colonies. Avoid causing bubbles.

3.7.1 Try not to repeat steps 3.5 – 3.7 more than 5 times because this will cause more cell death because it will break up the colony sizes too small.

3.8 Add 0.5ml of cell suspension to each well of the new 6-well plate.

3.9 Place the plate in the 37°C incubator with 5% $CO_2$ and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.10 Do not move the plate for 24 hours.

3.11 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate.

3.12 Change the media every day until ready to be used or passaged again.





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## 4. Troubleshooting

| PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| Colonies are still whole after rolling EZ Pass tool over them | Did not exert the proper amount of pressure when rolling the tool over the well<br><br>The EZ pass tool is not touching the bottom of the well | Place a greater amount of pressure onto the tool (like the amount of pressure you would place on a pen when writing)<br>Ensure that the EZ Pass tool is touching the bottom of the well. You may have to adjust the angle at which the tool is entering the well |
| Colonies appear smeared after rolling the EZ Pass tool over them | Too much pressure was applied to the EZ Pass tool when rolling the tool over the well | Use less pressure when using the tool (like the amount of pressure you would place on a pen when writing). The handle of the tool should not bend while in use |
| Colonies are still stuck to the bottom of the plate | Did not scrape the colonies properly with a serological pipette | Repeat step 3.5 – 3.7, applying more pressure when scraping the cells |
| Colonies appear smeared after scraping the wells (step 3.6) | Did not have medium coming out from the pipette while scraping | These cells are unusable. Perform the protocol on a different well of cells. Make sure to continuously be releasing medium from the pipette during the scraping process |
| Colonies have all attached to the center of the plate | Colonies did not evenly spread across the plate | Make sure to perform step 3.9. Perform this step in the incubator so after the plate is rocked back and forth it won't be disturbed<br>Place plates in the back of the incubator to ensure they are not bumped after passing<br>Open and close the incubator door gently to prevent any dislodging |
| Colonies did not attach to the plate after passaging | Matrigel plates were old or died out<br><br>Cell line may have difficulties attaching | Ensure that you are using a Matrigel plate that is no more than 2 weeks old and that every well is properly coated<br>Cell line may require a double concentration of Matrigel. Coat a new plate. |





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