

STANDARD OPERATING PROCEDURE

 CEDARS-SINAI BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE	INDUCED PLURIPOTENT STEM CELL CORE	IPSC PASSAGING PROTOCOL	
	THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY	STEMPRO EZ PASSAGING TOOL	SOP Number: SOP-iPSC-003
			Version: A

1. PURPOSE

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

2. SUPPLIES

Complete mTeSR Medium (Basal medium + 5x Supplement) (StemCell Technologies, Cat # [05850](#))

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

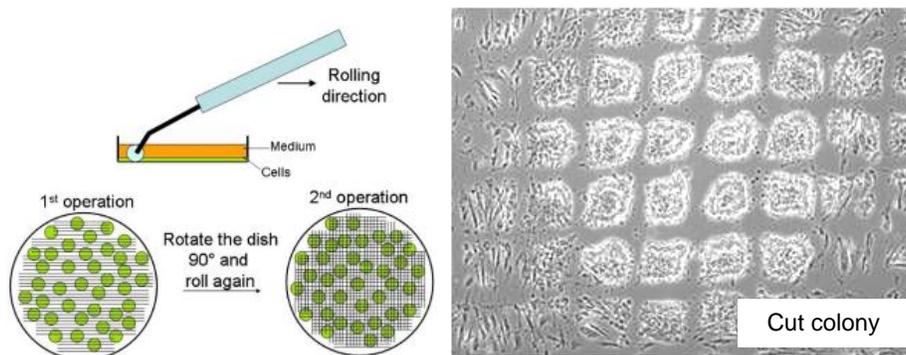
3. PROCEDURE

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 pulled glass pipette or colony marker, remove any areas of differentiation from the culture.
- 3.2 Aspirate spent media and add desired amount of fresh mTeSR + Supplement 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 media to each well (for a total 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™ Disposable Stem Cell Passaging Tool across 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 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.



- 3.5 Using 5ml serological pipette, aspirate medium from the well. Hold the serological tip perpendicular to the bottom of the well and then gently dislodge the colonies from the plate while simultaneously releasing medium.

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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.

- 3.6 Continue to pipette up and down gently to completely dislodge the cut colonies. Avoid causing bubbles.
- 3.7 Add 0.5ml of cell suspension to each well of the new 6-well plate.
- 3.8 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.
- 3.9 Place the plate in 37°C incubator with 5% CO₂. Do not move the plate for 24 hours.
- 3.10 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate. Change medium.

4. Troubleshooting

PROBLEM	POSSIBLE	CAUSE SOLUTION
Colonies are still whole after rolling EZ Pass tool over them	<p>Did not exert the proper amount of pressure when rolling the tool over the well</p> <p>The EZ pass tool is not touching the bottom of the well</p>	<p>Place a greater amount of pressure onto the tool (similar to the amount of pressure you would place on a pen when writing)</p> <p>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</p>
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 (similar to the amount of pressure you would place on a pen when writing)
Colonies are still stuck to the bottom of the plate	Did not scrape the colonies properly with a serological pipette	Repeat step 3.5, applying more pressure when scraping the cells
Colonies appear smeared after scraping the wells (step 3.5)	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 3.8. Perform this step right before placing the plate into the incubator
Colonies did not attach to the plate after passaging	<p>Matrigel plates were old or died out</p> <p>Cell line may have difficulties attaching</p>	<p>Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated</p> <p>Cell line may require a double concentration of Matrigel. Coat a new plate.</p>