STANDARD OPERATING PROCEDURE

CEDARS-SINAI BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE	INDUCED PLURIPOTENT STEM CELL CORE THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY	**THAWING IPSCS FOR MAINTANENCE AND EXPANSION**	
		SOP Number: SOP-iPSC-006	**Version: A**

1. PURPOSE

To describe the procedure for thawing iPSC colonies for maintenance and expansion.

2. SUPPLIES

Complete mTeSR Medium (Basal Medium + 5x Supplement)

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

5ml and 10ml sterile serological pipettes

Sterile 15ml conical tube

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 Remove cells from the LN2 tank.

3.2 Thaw cells quickly in a 37°C water bath using a "figure 8" motion until you see a pea sized ball of ice.

3.3 Using a 2ml pipette, move frozen cells into a sterile 15ml conical

3.4 Slowly add mTeSR medium to conical drop by drop to dilute CryoStor CS10 (1:10 ratio is recommended).

3.5 Centrifuge the conical/cell mixture for 1 minute at 1000rpm.

3.6 While cells are spinning, aspirate Matrigel from dish and add an appropriate volume of mTeSR to the well.

3.7 Aspirate the medium from cells and re-suspend cells to desired volume with mTeSR.

3.8 Plate the cells into the new well.
NOTE: 1 cryovial will typically thaw into one well of a 6-well plate.

3.9 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.

3.10 Place the plate in a 37°C incubator with 5% CO_2. 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. Change medium.