1. PURPOSE
To describe the procedure for freezing iPSC colonies for cryopreservation.

2. SUPPLIES
- Complete mTeSR Medium (Basal Medium + 5x Supplement)
- CryoStor CS10 (Stemcell Technologies, Cat # 07930)
- 5ml and 10ml sterile serological pipettes
- Nalgene Cryovials (Fisher Scientific, Cat # 03-337-7Y)
- BD Falcon Cell Scraper (VWR, Cat # 15621-005)
- Sterile 15ml conical tube

3. PROCEDURE
3.1 Prior to freezing 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.
3.3 Add 1ml of fresh mTeSR to each well.
3.4 Using a cell scraper, gently lift the colonies from the plate.
   **NOTE:** It is important that you do not exert too much pressure when using the cell scraper. Too much pressure can cause the cell scraper to “smash” or smear the colonies, rendering them unusable.
3.5 Collect the cells in a sterile 15ml conical.
3.6 Centrifuge the cells for 1 minute at 1000rpm
   **Optional:** You may also allow the cells to settle via gravity by standing the conical tube upright for 5-7 minutes.
3.7 Aspirate the supernatant without disturbing the cell pellet.
3.8 Re-suspend the cell pellet in an appropriate volume of CryoStor CS10 to obtain 1ml per cryovial.
   **NOTE:** Typically, one confluent well of a 6-well dish can be distributed into 2 cryovials.
3.9 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.
3.10 Add cells to cryovials and freeze using an isopropanol freezing vessel at -80°C overnight.
3.11 Transfer frozen vials to an LN2 tank.