1. PURPOSE

To describe the procedure for freezing iPSC colonies for cryopreservation.

2. SUPPLIES

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

CryoStor CS10 (StemCell Technologies, Cat #07930)

5ml and 10ml sterile serological pipettes

Thermo Scientific™ Nalgene™ General Long-Term Storage Cryogenic Tubes (Fisher Scientific, Cat #03-337-7Y)

Corning™ Falcon™ Cell Scraper (Fisher Scientific, Cat #08-771-1A)

Sterile 15ml conical tube

3. PROCEDURE

3.1 Prior to freezing your cells, check colonies in a microscope and using a cleaning tool, 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 and pipet up and down 3 – 4 times to break up colonies

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 plate can be distributed into 2 cryovials.

3.9 Add cells to cryovials and freeze using an isopropanol freezing vessel at -80°C overnight

3.10 Transfer frozen vials to an LN_2 tank

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