1. PURPOSE

To describe the procedure for pelleting iPSC colonies for molecular work.

2. SUPPLIES

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

DPBS without Ca & Mg, sterile

5ml and 10ml sterile serological

pipettes Sterile 0.6ml Eppendorf tubes

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

Sterile 15ml conical tube

3. PROCEDURE

3.1 Prior to pelleting 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 the 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 DPBS without Ca & Mg to obtain 1ml per Eppendorf tube

NOTE: The size of the pellet is based on the need, for DNA/RNA extraction large cell pellets aren't usually necessary but for protein, large pellets are usually better to work with.

3.9 Add cells to Eppendorf tubes

3.10 Use cells immediately or for long term storage keep in a -80°C freezer

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