Cryopreservation of hiPSCs

Equipment:

- Cryovials

Reagents:

- DMSO
- iPSC Cell Culture Media (e.g. Essential 8, mTeSR, etc.)
- Freezing Media
    - Information on how to make iPSC Freezing Media is in the Preparation Step

Procedure:

Preparation Step:

- Label cryovials:
    - Line Name
    - Passage Number
    - Date frozen down
    - Cell Count (if applicable)
- Prepare Freezing Media (volume depends on how many vials you plan to freeze down):
    - 10% DMSO
    - 90% iPSC Cell Culture Media

Procedure:

1. Aspirate media from cells
2. Add 1 mL of ReleSR per well, shake to cover the well, and aspirate off the ReleSR.
3. Place plate in 37C and let incubate for 3-5 minutes
4. After cells have finished incubating, tap the plate against the hand to dislodge cells and break up cells with 1 mL of iPSC Cell Culture Media.
    a. Be careful to not triturate cells too much. Single-cell suspensions usually have a very low recovery after thaw.
5. Spin cells down at 200g for 5 minutes
6. Aspirate the media and resuspend cells gently with 1mL of freezing media and gently break the pellet apart.
7. Add the remaining volume of Freezing Media and resuspend.
    a. Volume to add:
        i. Freezing ½ well per vial:
            - ((# of wells freezing down) x 2) – 1mL = Volume to add to cells
        ii. Freezing 1 well per vial
            - (# of wells freezing down) – 1mL = Volume to add to cells
8. Place vials in a Mr. Frosty that's **at room temp** (not one that came right from the freezer) and place vials in a -80C freezer**.**
    a. Cells will freeze at approximately 1K/min
    b. Do not place cells in a Mr. Frosty that has come directly from a -80C freezer! Cells will freeze too quickly which will lead to cell membrane damage.

9. Leave cells in Mr. Frosty overnight and transfer cells to a Liquid Nitrogen Dewar 24 hours afterward for long-term storage.

Protocol Updates:

Date	**Update Notes**
9/19/23	Lexi – Made a change to the Cryopreservation media (now 90% E8 & 10% DMSO). Also making a change to passaging with ReleSR instead of EDTA
10/23/23	Lexi – To make the protocol more accessible for cells growing with other types of media, I changed E8 to iPSC Cell Culture media