Thawing iPSCs

Equipment:

- Pre-Coated Plates (e.g., vitronectin, Matrigel, Geltrex, Cultrex, etc.)
    - We commonly use 3 wells of a 6-well plate when culturing and subculturing iPSCs.
    - For new lines from collaborators (non-commercialized), it might be ideal to thaw iPSCs into a single well if you're unsure of the viability and/or the number of cells that were frozen down.
- Bead bath set at 37C.
    - Put a 50mL conical tube filled with water (Optional: Add 2% conflikt (disinfectant) to tube to help keep cell vials sterile) in the bead bath and allow the water to get up to temperature (takes at least 30 minutes to an hour).
        - This tube can be kept in the bead bath, but the water should be replaced monthly to ensure sterility.
- Dry Ice
    - Used for transferring the tube of cells to the lab so they remain frozen

Reagents:

- iPSC Cell Culture Media
- 10 mM Rock Inhibitor (Tocris, Cat. 1254)

Prep:

- Aliquot the appropriate amount of iPSC Cell Culture Media and allow it to warm to room temperature.
    - Read through the procedure to determine how much media is needed.
- Add Rock Inhibitor to a final concentration of 10uM to the media.
    - Note: Rock inhibitor is typically reconstituted as a 1000X stock.
- Aspirate coating solution from the plate add 1mL of pre-warmed iPSC cell culture media and keep the plate at room temp.

Procedure:

1. Take frozen iPSC vial and thaw in 37C Bath for 2 – 3 minutes. Swirl often and only start next step if there is a small piece of ice left.
    a. Note: If iPSCs are stored far from a water bath, make sure to transport the vial on dry ice
2. Remove iPSC vial from water bath and spray down thoroughly with 70% Ethanol before bringing into a Biological Safety Cabinet (BSC). Make sure to wipe down the vial with a Kimwipe to make sure cells aren't exposed to excess ethanol.
3. Remove contents from vial with P1000 pipette, or 5mL serological pipette, and add to a 50 mL conical tube slowly.
    a. **Make sure to only pipette once as to not add any additional stress to the cells or to inadvertently break them up.**
4. Add 9 mL of iPSC cell culture media drop wise to the 50mL conical tube of cells.
    a. Note: It is fine to add the rest of the hiPSC media at a regular speed after first adding 5 mL dropwise
5. Centrifuge cells at 200g for 5 minutes.

6. Aspirate media from tube (making sure to not to aspirate the cell pellet, which can be faint) and gently resuspend cells in 1 mL of media using a P1000.
    a. **Limit the number of times that cells are resuspended, maybe 2 to 3 times.**
7. Plating: After gently resuspending the cell pellet you can either:
    a. Dilute the cells 1:3 by adding an additional 2mL of media to the cell suspension and add 1mL per well into a total of 3 wells of a 6-well plate.
    b. Add the cells directly to one well of a 6-well plate.
8. Once cells are added to the plate **immediately** mix cells by moving the plate back and forth and side to side
    a. Do **not** swirl the plate to mix as all the cells can collect in the center of the plate and not distribute evenly.
9. Place cells in 37C incubator at 5% CO2
10. Observe the cells the next day and feed cells daily.
    a. Depending on cell viability it is likely that cells might need to be passaged 2-5 days after thawing.
    b. If thawing cells directly into 1 well, it's possible the cells might need to be passaged the next day.

Protocol Updates:

Date	**Update Notes**
10/23/23	Lexi – Changing the use of Rock Inhibitor from Optional to required. Updated the cell dilution from 1:3 to also include adding cells directly into the well if one is unsure of the cell viability and/or number of cells that were frozen down.