Freezing Cells

1. Warm cell media and trypsin
2. Prepare the appropriate volume of 10% DMSO/FBS solution (freezing media):
    - 3 mL per confluent plate to be frozen
    - If premade freezing media is being used, ensure that it was made within roughly a month of the current date to ensure quality of media
3. Obtain the correct number of cryo-tubes
    - One cryo-tube contains 1 mL of freezing media/cell mixture
4. Label each tube with the following:
    - Cell type
    - Generation
    - Date
    - Initials
5. Take 10 cm plate(s) of cells out of incubator
6. Aspirate out cell media
7. Trypsinize each plate of cells with 3 mL of trypsin for the appropriate time given the cell
8. Grab a centrifuge tube
    - The size of the centrifuge tube depends on the number of cryo-tubes that the are being frozen. Later on, the cells will be resuspended in $x + 0.5$ mL of freezing media so pick the correct size w.r.t. that step.
9. Add trypsinized cells to the centrifuge tube
10. Add at least 2X media to the centrifuge tube from last step
11. Centrifuge at 1000 rpm for 5 minutes
12. Aspirate the supernatant, being careful not to disturb the cell pellet
    - This can be done easily by just tilting the centrifuge tube to avoid having to get too close to the cell pellet
13. Resuspend with 10% DMSO/FBS, 1 mL per tube to be frozen plus 0.5 mL to act as buffer
    - 3 mL of freezing media added to the centrifuge tube per confluent plate of cells that make up the cell pellet
14. Add 1 mL of the freezing media/cells per cryo-tube
15. Place cryo-tubes in -80° freezer
16. If cells are meant for long term storage, place cells in liquid nitrogen the next day