Thawing Cells

1. Make sure tissue culture hood fan is on and running for at least 20 minutes before working in the hood.

2. Wash hands and arms up to elbows thoroughly with hot water and soap. Wear gloves if you want.

3. Spray bench surface with ethanol and wipe down with a paper towel.

4. Look up position of the vial of the desired cells in the LN inventory on Google Docs file: https://docs.google.com/spreadsheets/d/1ge9ayyZVuSRdl35X8IuKF4diiJ-5q_ge/edit?gid=888735165#gid=888735165

5. Take the vial out of liquid nitrogen freezer and place in hot water bath in a small beaker of clean water. Do not let the water level be high enough in the beaker to go over the lid of the vial, and make sure the water temperature is not > 37C.

6. Let media thaw. Then, in the tissue culture hood, transfer to a 15 mL centrifuge tube and centrifuge for 5-7 minutes.

7. In the tissue culture hood, take the old media off of the cells using a water aspirator tube with a glass pipette at the end.

8. Add the new media 5 mL at a time, gently mixing in the tube to re-suspend cells. Then transfer this to an T75 flask. Do this twice so that cells are in 10 mL of fresh media in the new flask.

9. Write cell name, thaw, new cell passage number, the date, and your initials and put into the incubator.

10. Within the next 6-10 hours change the cell media. Take off the media they have started in, rinse very gently with sterile PBS, and add 20 ml of new media.

11. Check the cell for a few days and when cells are confluent, begin regular passing routine.