Thawing Cryopreserved Cells

NOTE: Be sure to wear a full-face shield during the handling of frozen specimens out of the liquid nitrogen. Spillage of liquid nitrogen can be dangerous!

1)- Transfer cryovials containing frozen cells from liquid nitrogen to ice for transportation to the culture room.

2)- Quickly thaw the cells (< 1 minute) by gently swirling the cryovial in a 37°C water bath until there is just a bit of ice left in the vial. Do not leave the cryovial unattended during the thawing process. (It is important for cell viability that the cells are thawed and processed quickly – thawing only takes a few seconds).

3)- Transfer the vial it into a laminar flow hood. Dry off the outside of the cryovial and wipe with a 70% ethanol solution before opening the vial to prevent contamination.

4)- Transfer the content of the cryovial of cells to an empty 50ml centrifuge falcon tube.

5)- Add up to 20ml complete growth medium (with 10 to 15 % FCS) one **drop at a time** into the tube containing the thawed cells, while shaking it by hand.
(It is important to dilute a cryoprotectant DMSO present in the cryovial at least 20-fold at this point to avoid cell toxicity.)

6)- Centrifuge the cell suspension at approximately 1500rpm for 5 minutes to wash off the DMSO.

7)- After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Remove the supernatant without disturbing the cell pellet.

8)- Gently resuspend the cells in complete growth medium, and transfer them into the appropriate culture flask or plate.
NOTE: To keep the cell line merely in culture resuspend the pellet in 2ml and make 2 times serial dilutions (1ml cells in 1ml complete medium), between 4 and 8 depending on the growth rate of your cells, in 24 well plates.