Recovering cells from liquid nitrogen

It is important to thaw cells as quickly as possible and to remove the DMSO from the culture as soon as possible.

1. For adherent cells, remove one vial from the liquid nitrogen tank and place immediately in the 37oC water bath.
2. Put 9mls prewarmed, complete medium (ie medium + serum + antibiotics) in a 10cm tissue culture dish.
3. When the cells have thawed wash the outside of the vial with 70% ethanol, wipe off, then pipette the contents into the dish. Swirl gently to distribute the cells and place in the incubator.
4. After 3 to 4 hours the viable cells should have stuck to the plastic so remove the medium and replace with 10mls fresh complete medium. This removes the DMSO from the culture.
5. Grow until confluent and away you go!
6. For suspension cells, remove and thaw one vial of cells as above.
7. Transfer contents to 5ml prewarmed, complete medium in a 15ml conical tube, spin at #2.5 in the bench top centrifuge for 5mins.
8. Remove the medium and resuspend the pellet in 10mls fresh, prewarmed complete medium and transfer to your culture vessel. Place in the incubator.

It is not unusual to observe some cell death (maybe 5 to 10%) after thawing frozen cells, hopefully it should not exceed 25% of the cells. Excessive cell death, eg 50% or more, should be investigated. Were the cells frozen down correctly? Was the right freezing mix used? Did you thaw them correctly? Were they healthy when they were frozen? Freezing and thawing cells and mantaining viable frozen stocks is an essential part of any tissue culture work.