Freezing down cells

Grow your cells in a large plate (150 mm) to a confluent monolayer.

Before you start:

1. Make sure the Mr. Frosty is available and at room temperature.
2. Label 10 cryotubes with the cell strain, passage number, your initials, and the date. Place them in the blue rack in the hood.
3. Prepare 10 ml of cryo media. This is just 95% FBS and 5% DMSO. In a 15 ml tube, add 10 ml FBS and 0.555 ml DMSO. Mix well.

Trypsinize as you normally would, adjusting for the larger plate.

For example: You will need to wash with ~20 ml of media, and use 5 ml of trypsin (3 ml media, 2 ml trypsin). Collect the trypsin/cells into a 50 ml tube (instead of 15 ml, using 5 ml serological pipette), and wash the plate with 20 ml of serum-containing media

After you remove the media from your cells, resuspend the pellet in the 10 ml of cryo media.

Aliquot 1 ml of this into each of the 10 cryotubes.

Put the tubes into the Mr. Frosty and place in the -80ºC freezer.

*** Carry out the following steps if liquid nitrogen is available ***

Keep in the freezer for 24 – 48 hours.

Remove the tubes, clip them into canes (you'll need 2) and put them in the liquid nitrogen tank.

Make sure the canes are numbered. If not, number them. Write down all the relevant information in the log book.

Put the Mr. Frosty on the shelf in the lab so it will be ready for the next person.