**Freezing Protocols**

**Method 1: normal tissue culture cells**

- Prepare filtered FBS and filtered freeze medium #1 (80% growth medium for the cells of interest plus 20% DMSO).
- Trypsinize cells if necessary, spin down and resuspend at $6X10^6$/mL in pure filtered FBS on ice.
- Add an equal volume to the cells in FBS of freeze medium #1 slowly, drip by drip, with careful swirling in between drops to slowly introduce the DMSO into the cells. This step should be performed on ice.
- When the freeze medium is all added and mixed, 1 mL of cells/medium should be distributed into pre-labeled, cooled cryovials. This results in $3X10^6$ cells per vial, which we write on the cryovials also, along with the date and the person freezing.
- Cryovials are transferred to the Mr. Frosty container (Fisher #15-350-50 Thermo Scientific No.:5100-0001) which was stored at 4°C then the cells in the container transferred to -80°C for 24 hrs then to liquid nitrogen for long-term storage. Alternatively, Styrofoam racks can be used to insulate the vials while freezing. Log cells into N2 book to record.

**Method 2: sensitive primary cells and ES cells**

- Prepare filtered FBS and filtered freeze medium #2 (80% FBS of the type used for the cells of interest plus 20% DMSO).
- Trypsinize cells if they are adherent. Spin down cells and resuspend at $6X10^6$/mL in pure filtered FBS on ice.
- Add an equal volume to the cells in FBS of freeze medium #1 slowly (the entire process should take 10 minutes if you are patient), with careful swirling in between to slowly introduce the DMSO into the cells. This step should be performed on ice.
- When the freeze medium is all added and mixed, 1 mL of cells/medium should be distributed into pre-labeled, cooled cryovials. This results in $3X10^6$ cells per vial, which we write on the cryovials also, along with the date and the person freezing. The cell density may be adjusted to different densities depending on the experiment, but the
- Cryovials are transferred to the Mr. Frosty container (Fisher #15-350-50 Thermo Scientific No.:5100-0001) which was stored at 4°C then the cells in the container transferred to -80°C for 24 hrs then to liquid nitrogen for long-term storage. Log cells into N2 book to record.

Note: cell number or density in the tube is sort of arbitrary and people often don't count before they freeze cells-but you do have to know what sized well/dish is appropriate for each freeze, so standardizing this or making a note on the tube is important.

To thaw: thaw quickly at 37°C, transfer to 15 mL conical, add 9 mL growth medium, mix, centrifuge, resuspend in fresh growth medium and culture.