Freezing / Thawing mammalian cells (AG/2-96)

Freezing:
1. Grow cells to confluency in 100 mm plates.
2. Trypsinize (if necessary) and triturate cells in media.
3. Transfer to 50 ml tube (pool cells from several plates).
4. Spin for 5 mins (Ginty centrifuge, room temp, 850-900 RPM).
5. Resuspend in media with 10% DMSO (sterile) to a final volume of 1 ml per 100mm plate.
6. Aliquot into cryo-vials (1 ml per vial), seal, place between styrofoam tube holders, and freeze at -70 C.
7. Transfer tubes to LN2 for long term storage.

Thawing:
1. Remove cryo vial from -70 freezer (or LN2).
2. Add 0.5 ml warm media to vial (in TC hood) and hold vial in fingers to thaw rapidly.
3. Transfer cells to 10 mls warm media in 15 ml orange cap tube.
4. Triturate well, centrifuge 5 mins (850-900 RPM in Ginty lab centrifuge at room temp).
5. Remove media and repeat as above 3X to remove DMSO.
6. Resuspend in 10 mls media and plate on 100 mm TC plate (collagen coated if PC12 cells) and place in incubator.