Thawing Human ES cells:

Thomson Lab, 2004

1. Remove Human ES cells from liquid nitrogen storage tank.
   Fill out a freeze/thaw form.
2. Thaw cryovial by gently swirling in waterbath until only a small ice pellet remains, being careful not to completely submerge the cryovial under water.
3. Completely submerge cryovial in 95% ethanol.
4. Very gently, pipet cells from the vial into a 15 ml conical centrifuge tube.
5. Slowly, add 9.5ml media dropwise to reduce osmotic shock. While adding media, gently mix the cells in the tube (by gently tapping the tube with a finger.)
6. Centrifuge 1000rpm for 5 minutes.
7. Wash cells by resuspending with 3ml media.
8. Centrifuge 1000rpm for 5 mintutes.
9. Resuspend in 2ml and add 0.5ml per well of a 4 well plate that has MEFs already plated on it.
10. Change media daily, however it may take 2 weeks before cells are ready to be expanded.

Note:

Wear safety glasses when removing cells from liquid nitrogen until the cells are completely thawed.