Freezing MEF's:

Thomson Lab

Resupension Medium

80 % DMEM
20 % FBS
1 % NEAA

Cryopreservation Medium

60% DMEM
30 % FBS
20 % DMSO

1. Wash cells once with PBS w/o CaMg.
2. Add Trypsin/EDTA to cells for approximately 5 minutes at 37∞C.
3. Detach cells from the plate by pipetting off or tapping against the heel of your hand.
4. Neutralize Trypsin/EDTA with an equal volume of culture medium.
5. Pipet to break up chunks. If clumps remain, add suspension to a 50 ml tube and allow the chunks to settle out.
6. Take the supernatant and divide it amongst conical tubes and spin 5 minutes at 1000 rpm.
7. Resuspend pellet in 0.5 ml Resuspension Medium per vial (This is one half the final volume required for freezing).
8. Dropwise, add an equivalent volume (0.5 ml per vial) of Cryopreservative Medium and mix. Your DMSO concentration is now 10%.
9. Place 1 ml of cells in each freezing vial.
10. Rapidly transfer the cells to a freezing container and place at -70∞C overnight (cells don't like to be in DMSO at room temperature for long periods of time).
11. Transfer cells to liquid nitrogen the next day for long-term storage.

Notes:

Pre-label all cryovials appropriately with the following information:

Cell line
Passage number
Number or surface area of cells frozen
Date
Initials

Fill out a freeze/thaw form