Thawing ES cells

Materials:

E14 media

Methods:

1. Quickly transfer one vial of ES cells (2 X 10^6 cells/vial), stored in the gas phase of liquid nitrogen into a 37C water bath.
2. Take the vial out of water bath BEFORE the cells have been completely thawed. You should be able to see a small ice clump floating on top.
3. Carefully, transfer the cells into a 15ml Falcon tube that contains pre-warmed medium using 1ml pipette. Rinse out the cells with medium to make sure all the cells have been transferred.
4. Pellet the cells by spinning for 5 minutes at 1000 rpm.
5. Aspirate off medium and gently resuspend cells in 5ml of pre-warmed medium.
6. Transfer the cell suspension to a 10cm gelatinized dish with 5ml of medium (total volume of ~10ml) and grow in a 37C humidified 5% CO_2 incubator.
7. Change medium daily until 80% confluent (approx. 10-12 million cells). Usually, it takes 2-3 days.
8. When confluent, split 1:3 to 1:6 depending on its confluency.