CReM
CENTER FOR REGENERATIVE MEDICINE

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Thawing and Plating ES Cells (in feeder free conditions)

Preparation:

Coat 6-well plate with 0.1% gelatin in water (EmbryoMax ES cell Qualified 0.1% Gelatin solution, Chemicon International #ES-006-B)

- 2mL gelatin/well
- sit RT, 20-30min, aspirate off gelatin immediately before plating cells

1. Retrieve vial from liquid nitrogen; open cap to release pressure.
2. Agitate in 37 degree C water bath
3. Alcohol off when small ice pellet remaining
4. Pipette up and down once; transfer the entire 1mL to a 15 cc conical
5. Add drop by drop IMDM on the top of the 1 ml cells and swirl the tube frequently until 4-5 ml of IMDM have been added.
6. Then add an additional 5mL IMDM and pipette up and down to mix at least 5 times
7. Spin 300g, 4C, 5m, and discard supernatant
8. Wash pellet again with 10 mL IMDM (serum free), and spin again.
9. Resuspend pellet in 1mL working media + cytokines (complete SFES media) to obtain a single cell suspension, then add another 2 mL of complete SFES media and plate in 1x well of a 6-well plate pre-coated with gelatin.

Freezing ES Cells

Freezing medium: IMDM with final concentration of 10%DMSO/50%HIFBS/40% IMDM

For 6x wells of a 6-well plate: Label all freezing vials and pre-cool in a -20C freezer

1. Trypsinize cells with 1ml of 0.05% Trypsin/EDTA per well incubate for 1 min at 37C
2. Inactivate trypsin by adding 500ul of IMDM+15%FBS (IMDM+50% serum as per Gouon-Evans, *Nature Biotechnology.* 2006).pipet few times
3. Transfer to a 15 mL conical on ice and top off with IMDM +15% FBS
4. Spin 300g, 4C, 5m
5. Resuspend pellet in 500mcl cold IMDM+15%FBS to obtain single cell suspension; count the cells. you should have ~ 2x106 cells/vial
6. Add 5.5 mL ice-cold freezing medium (10% DMSO + 50% HIFBS + 40% IMDM); pipette up and down 5x.
7. Aliquot 1mL cell suspension/cryovial into 6 pre-cooled vials ***for best results it is essential that the cells are kept cold from the moment they touch DMSO—so pre-cooling the vials helps. Transfer the capped vials immediately into a freezing chamber (pre-cooled to -20C) and place in -80C overnight and then the gas- phase of a liquid nitrogen freezer the next day for long-term storage.