Freezing Plates

Materials & Reagents required:

- Dry Ice
- EmbryoMax ES Cell Qualified Freezing Medium, 2x (Cat. No. ES-002-D)
- ES Cell Medium:
  - DMEM (Cat. No. SLM-220-B)
  - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
  - 1% Nucleosides,100x (Cat. No. ES-008-D)
  - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
  - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
  - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
  - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
  - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Parafilm® Film
- Pipette
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)

Procedure:

1. Wash ES cell colonies with DPBS and add 35 µL of Trypsin. Incubate for 10 minutes at 37 °C, and then add 65 µL of ES Cell Medium.
2. Disperse the cells with a pipette and transfer into a replica plate containing 65 µL of cold EmbryoMax ES Cell Qualified Freezing Medium (2x).
3. Wrap each plate with Parafilm film and place on dry ice for 20 minutes. Transfer to –80°C to freeze. Plates can keep for a number of months.
4. To thaw, add 150 µL of ES Cell Medium plus selection agent to each well. Thaw plate quickly by placing in a 37 °C incubator.
5. Transfer the thawed plates to a hood and manually pipette each well. Transfer the contents to a fresh 24-well plate with PMEF feeders (if required).
6. The next day, change the media to remove DMSO. Incubate the plate for up to 2 weeks to allow colonies to establish.