Standard Operating Procedure
for
Thawing Human Embryonic Stem Cells

1.0 PURPOSE

This procedure describes the method of thwing of human embryonic stem cells (hESCs).

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all laboratory personnel.

3.0 PROCEDURE

1. Prepare a well of six-well plate for thawing by either plating mouse embryonic fibroblasts (mEFs) and arresting cell growth (Mitomycin C) or plating Matrigel, depending on whether mEF or feeder cell-free culture is desired, respectively. For mEFs, refer to SOP protocol 'Culturing hESCs on mEFs', and for feeder-free culture refer to SOP protocol 'Culturing hESCs in conditioned medium or in chemically defined medium (including preparation of Matrigel-coated plates)'.
2. Wear eye protection, and use autoclave gloves to remove a vial of hESCs from liquid nitrogen. Warning: vials can explode after removing from liquid nitrogen. Minimize holding the vial directly and place it on a tube rack.
3. Quickly place the vial in a 37^{o}C water bath. Swirl gently without submerging the cap.
4. When only an ice-crystal remains remove vial from water bath. Immerse vial in 75-95% ethanol to avoid contamination and transfer to sterile tissue culture hood.
5. Gently pipet cells into a 15-mL conical tube using a 1- or 5-mL serological pipette.
6. Slowly and drop-wise add hESC/mTeSR media (mEF/feeder cell-free culture respectively) while shaking tube gently to mix hESCs. This reduces osmotic shock to the cells and dilutes DMSO concentration immediately.
7. Centrifuge cells for 5 minutes at 200xg. Remove and discard the supernatant, and gently resuspend cell pellet in 2 ml of hESC/mTeSR media (mEF/feeder-free culture respectively).
8. Transfer cells to the prepared well of six-well plate using the relevant mEF or feeder-free culture protocol. Change media the next day and culture the cells using the standard method.

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