Standard Operating Procedure
for
Freezing Human Embryonic Stem Cells

1.0 PURPOSE
This procedure describes the method of freezing/cryopreservation 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

3.1 Prepare hESCs for freezing. The hESCs should be in a healthy state for several passages before freezing, and it is preferred that they are in exponential growth phase.

1. Grow hESCs on six-well plates on the desired platform using the respective SOP protocol: For culture utilizing mouse embryonic fibroblasts (mEFs) use protocol 'Culturing hESCs on mEFs', and for feeder cell-free culture use protocol 'Culturing hESCs in conditioned medium or chemically defined medium (including preparation of Matrigel-coated plates)'. The number of individual wells needed for growing hESCs is the number vials desired for freezing.
2. Let the cells grow to a 60-70% confluence. Do not let them grow for the typical duration until they are ready for passaging, but for shorter time, for example, let them grow only for about 4 days.

3.2 Freeze hESCs.

1. On the day of freezing, prepare freezing medium as follows. For mEF culture, prepare half freezing volume of 2× freezing medium comprising of 60% defined or ES-certified FBS, 20% sterile cell culture grade DMSO, 20% hESC media. For feeder-free culture, use full freezing volume of mFreSR (Stemcell Technologies, Vancouver, BC). The medium volume is 1 ml × the number of wells of six-well plates that have been cultured for freezing.
2. Replace isopropanol in the cryo-freezing container if needed: The isopropanol in the cryo-freezing container should be replaced after every five cell freezing/cryopreservation cycles.
3. Label cryopreservation vials with initials, date, passage number, cell-type, cell-line and indicate one-well of a six well plate. Replace isopropanol in the cryo-freezing container if needed: The isopropanol in the cryo-freezing container should be replaced after every five cell freezing/cryopreservation cycles.
4. Follow the standard method for passaging hESCs pertaining to either mEFs or feeder cell-free culture. Passage all the wells together, and combine contents in a 15-mL conical tube after scraping colonies on collagenase/dispase.
5. Centrifuge cells in 15-mL conical tube for 5 minutes at 200xg.
6. Gently reconstitute cell pellet in half freezing volume of hESC media (for mEF culture), or full freezing volume of mFreSR (for feeder-free culture). Try to have a slightly larger clump size than regular passaging.

7. For mEF culture, add equal volume of 2× freezing medium drop-wise to the cells. Mix quickly, but gently and transfer 1 ml cell suspension to each cryopreservation vials. Place the vials in the cryo-freezing container and immediately freeze in -80^{o}C (overnight).
8. The next morning, transfer vials to liquid nitrogen storage.

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