Thawing and use of cryopreserved mouse embryos

A. Rapid thawing of straws

1. Transfer the appropriate straw(s) from the storage refrigerator to a small dewar of liquid nitrogen.

2. Using forceps, hold the straw near the label for 30 seconds in air, and then place it in water at room temperature until the ice disappears from the media. Handle it gently and do not agitate it in the water bath.

3. Wipe the straw with a tissue, then holding the straw firmly and horizontally, cut through the middle of the cotton/PVA plug.

4. Cut off the plug of capillary sealant or the heat sealed end of the straw, then holding the straw vertically over a 60mm Falcon 353004 Petri dish, use a metal rod to push down the remaining cotton/PVA plug, expelling the contents of the straw into a single drop in the Petri dish. Do not allow the tip of the straw to touch the expelled media as the embryos will stick to the straw.

5. Set a count-down timer for 5 minutes. The embryos will shrink considerably due to the presence of sucrose. Sucrose is a non-permeating solute and has the effect of drawing the ProH, a permeable solute, out of the blastomeres.

6. After 5 minutes, transfer the embryos to a 200µl drop of M2 using an embryo handling pipette. They will rapidly take up water and assume normal appearance.

7. Transfer the embryos to a fresh drop of M2 for a further 5 minutes.

8. Examine the embryos closely under the microscope. Discard any that appear grossly abnormal or have more than 2 lysed blastomeres.

B. Culturing embryos for developmental assessment

1. Using a clean embryo handling pipette, transfer the embryos from M2 to a 60mm (353004) Petri dish containing KSOM under silicone fluid or mineral oil. Note the developmental stage of the embryos.

2. Place the dish in the incubator at 37˚C, 5% CO_2 overnight.

3. The following day, examine the embryos under the microscope to assess their developmental stage. Note which stage the embryos have reached (4-cell/8-cell/morula/blastocyst/hatched).

4. Repeat daily for up to 3 days. By the third day, the 4-cell and 8-cell embryos should have developed to the expanded blastocyst stage and started to hatch from the zona pellucida.

C. Washing embryos before transfer to pseudopregnant recipients

To minimise the risk of transmitting infectious agents during the embryo transfer procedure, we advise that the standard embryo washing procedure described by the International Embryo Transfer Society (http://www.iets.org) is followed. This procedure is described in detail in the IETS's Manual (4th Edition). (http://www.iets.org/pubs_educational.asp)

The essential elements of the IETS's embryo washing procedure are as follows:

1. All embryos should have an intact zona pellucida before (and after) washing.

2. Only embryos free of adherent debris should be taken through the washing procedure. Embryos may be cleaned before they are

washed.

3. Take the embryos through at least 10 separate wash dishes containing culture medium (e.g. M2). The wash dishes should contain 2-3ml of medium so that the each wash represents a **>100 fold** dilution of the previous wash.

4. A new embryo handling pipette should be used each time the embryos are moved from one dish to the next. The pipette should be filled with medium from the clean dish before aspirating the embryos and placing them in the clean dish.

5. To minimise the risk of transferring pathogens to the next dish, aspirate the embryos in the smallest amount of medium possible.

6. Procedure can be used for either *in vivo* or *in vitro*-derived embryos.