Thawing and transfer of cryopreserved mouse embryos
Cryopreservation & Assisted Reproduction Laboratory
LASP
Frederick National Laboratory for Cancer Research
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1. Transfer straw(s) from liquid nitrogen freezer into a small Dewar flask.
2. Using forceps hold the straw near the label for 40 seconds in air and then plunge into water at room temperature until the ice disappears from the media.
3. Remove the straw from the water and gently dry it using a tissue.
4. Holding the straw firmly, cut through the middle of the cotton/PVA plug, leaving half of the cotton plug in place.
5. Cut off the heat seal.
6. Hold the straw vertically over a 35mm Falcon 351008 Petri dish and use a metal rod to push the remaining cotton/PVA plug through the straw and expel 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 or the plug to fall into the drop as these can lead to embryo loss.
7. Keep embryos in the media expelled from the straw for 5 min. During this time the embryos will shrink due to the presence of sucrose. Sucrose is a non-permeating solute that will draw the permeable propanediol (PrOH) out of the blastomeres. Moving embryos prior to end of 5 min can result in blastomere lysis.
8. After 5 minutes, use a glass pipette to transfer the embryos into a 200µl drop of M2 (Millipore; MR-015P-5D; EmbryoMax® M2 Medium (1X), Powdered, with phenol red). The embryos will rapidly take up water and assume normal appearance.
9. Rinse the embryos in an additional 200µl drop of M2.
10. The embryos should be transferred into the oviducts of 0.5 day pseudopregnant recipients. We recommend that 10-12 embryos be transferred per female recipient. Our laboratory uses B6D2F1 females for this purpose.

Please note: We recommend that you prepare to transfer the embryos into pseudopregnant recipient females upon receipt of the dry shipper.

References:
J.P. Renard and C. Babinet. 1984. High survival of mouse embryos after rapid freezing and thawing inside plastic straws with 1-2 propanediol as cryoprotectant. Journal ofExperimental Zoology. 230:443-448.