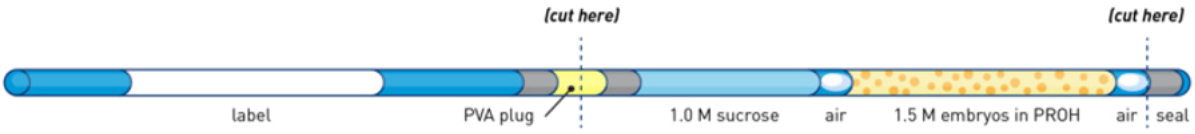
Recovery Thaw Protocol for Straws

Supplies

- Liquid nitrogen Dewar
- 25° Celsius water bath
- Scissor
- Kim Wipe
- Metal plunger (enclosed)
- Timer
- Forceps
- Pipetter
- 10 - 35mm Falcon dishes containing 1 ml of M2
- M2 - See media preparation protocol sheet

Thaw protocol

The procedure used for freezing and thawing in The Jackson Laboratory Frozen Embryo Repository is based on that described by Renard, J.P. and Babinet, C. (1984). High survival of mouse embryos after rapid freezing and thawing inside plastic straw with 1-2 propanediol as cryoprotectant.*Journal of Experimental Zoology* 230, 443-448



1. Transfer the straw from the liquid nitrogen storage freezer to a smaller container of liquid nitrogen as quickly as possible.
2. Using forceps, hold the straw near the label and hold in air for 40 seconds.
3. Place straw, label end up in to the room temperature water bath (25 degrees Celsius) until the ice disappears (~5 - 10 seconds).
4. Carefully wipe the straw dry with a Kim Wipe.
5. Holding the straw firmly on the embryo side of the PVA plug (yellowish color) cut through the middle of the PVA plug.
6. Holding the straw near the bottom seal, cut off the seal.
7. Using your index finger and thumb, round off the end of the straw near the embryos.
8. Using the plunger, expel the entire liquid contents of the straw as one drop into a 35mm Falcon dish. Do not let the plug drop into the dish.
9. Wait 5 minutes. The embryos will shrink considerably.
10. Transfer the embryos to 1ml of M2. They will rapidly take up water and assume normal appearance.
11. Wash the embryos through 10 dishes of M2, using a new pipette for each wash (each dish should contain 1ml of media).
12. Your embryos should be transferred into your clean unit as soon as possible.