Recovery Thaw Protocol for Vials

Supplies

- Dewar containing the sample to thaw
- Vial rack
- 1ml pipetter
- Forceps
- 10 - 35mm Falcon dishes containing 1 ml of DPBS
- Media

Thaw Protocol

The procedure used for freezing and thawing in The Jackson Laboratory Frozen Embryo Repository is based on that described by Whittingham, et al. [Whittingham, D., S. Leibo, et al. (1972). Survival of mouse embryos frozen to -196°C and -269°C.Science. 178:411-14.]

Embryos require a slow thaw:

- To thaw, remove vials from liquid nitrogen and place in a rack at room temperature until completely thawed (this will take about 15-20 minutes).
- SLOWLY pipette 800ul of DPBS, drop wise into each vial to dilute the DMSO.
- Using a 1ml pipetter set at 1ml, suck up about half the solution and then expel slightly (to dislodge any embryos that may be stuck to the side of the vial) and then continue removing all the solution and placing it into a small petri dish.
- Wash the embryos through 10 dishes of DPBS, using a new pipette for each wash (each dish should contain 1ml of media).
- Your embryos should be transferred into your clean unit as soon as possible.