Universität Heidelberg- IBF/ Biotechnologie Labor
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Thawing of "Mouse" Embryos from the IBF- BTL

Materials

- Media (M2 + M16)	- Liquid Nitrogen Dewar
- Sucrose (Sigma: S-1888)	- Timer
- 1ml Syringe	- Embryo handling pipette
- Yellow pipette tip	- ø3cm culture dishes
- Scissors	- Forceps

Revitalisation media : 1ml M2 + 0,035g Sucrose

1. Cut off the ends of the straw in LN2 vapor phase, otherwise there is a great risk that the straw can explode.
Remember the side (metal ball) where the embryos are.

2. Thaw the straw at room temperature for 60-120 second (hold it always horizontally).

3. Cut off a bit from the yellow pipette tip. The serrated end of the tip should also be cut off, so that it will fit better on the syringe. Take the syringe with prepared pipette tip and flush your straw (**Air only!**) into a ø3cm culture dish. Always flush it from the side, where the glass bulb was inserted.

4. Add immediately 100µl revitalisation media to the drop and incubate it for 5 minutes at RT (1.dilution).

5. Add additional 100µl revitalisation media to the drop and incubate again for 5 minutes at RT (2.dilution).

6. Add additional 100µl revitalisation media to the drop and incubate again for 5 minutes at RT (3.dilution).

7. After the last dilution step, take up all morphological intact embryos and wash them in M2 media (3 x 150µl).

8. Store the washed embryos in preincubated M16 (incubation time M16 >1 hour) in the CO2 incubator prior to the transfers.

9. Transfer the embryos at the same day of thawing into the oviduct of 0.5d pseudopregnant females.