Universität Heidelberg- IBF/ Biotechnologie Labor
Frank Zimmermann, INF 347, D-69120 Heidelberg, Phone: +49-6221-546883, Fax: +49-6221-545705,
E-Mail: frank.zimmermann@urz.uni-hd.de

Stand:11/2012fz

Thawing of "Rat" Embryos from the IBF- BTL

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

Materials

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

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 seconds (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 at RT no longer than 30 minutes in M2 prior to the transfers.
Do not Incubate the Embryos!!

9. Transfer embryos into the oviduct of 0.5d pseudopregnant females.
(BTL Transfers for each Spraque Dawley Recpient 20 Embryos Unilateral maximum 40 Bilateral in)