University of Basel

Centre for Transgenic Models
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CTM

ULTRA-RAPID EMBRYO FREEZING PROTOCOL

Material:

Straws:

- Insemination straws (cristal) from Cryo Bio Systems Ref. 006578

Cryoprotectant:

- Sucrose 1.026 g
- Ficoll 70.000MW 1.8 g
- Ethylene glycol 4.0 ml
- M2 medium with Pen/Strep added to 10 ml

Prepare Cryoprotectant:

- Dissolve the three ingredients and adjust volume to 10ml with M2.
- Filter through a 0.45 µm Millipore filter.
- Aliquot into sterile tubes and store at -20°C.

KSOM Culture dish:

- 30 – 60 minutes before culture, prepare a tissue culture plate with several droplets of 20 µl KSOM (for ~ 20 embryos per drop). Layer just enough mineral oil over the top of the drops that they completely covered, and place in incubator at 37°C, 5% CO_2.

Freezing:

1. Collect oviducts and somewhat of the top of the uterus of females and flush each with M2 to recover embryos.
   - Use a needle that has had the sharp tip filed down.
   - Collect 2-cell embryos using drawn glass capillaries with flame polished tips, and place the embryos carefully in new M2 medium.
2. Label straws with type of embryo, date and straw number on appropriate labels for liquid nitrogen.
3. Centrifuge the cryoprotectant for eliminate air bubbles. Push the PVP plug with a needle more inside the straw. Load needed straws with each ~ 50 µl cryoprotectant solution (room temperature).
4. Transfer (~20) embryos into the cryoprotectant solution with a MINIMAL volume of M2 medium taken.
   - Place the embryos at the end of the cryoprotectant solution that is furthest away from the end of the straw.
   - Watch under the microscope when you pipette the embryos, to avoid introducing bubbles.
5. Incubate each loaded straw at room temperature for 1 to 3 minutes.
6. Heat-seal the end of the straw with bag-sealer.
7. Immerse the straw directly into liquid nitrogen, sealed end first.