EMBRYO CRYOPRESERVATION PROTOCOL

Superovulate females with PMS (5 IU per female);
48 hours later, inject females with HCG (5 IU per female) & mate;
Embryo collection must be scheduled in 72 hours.

Embryo Collection and Cryopreservation:

1- Dissect the oviducts.
2- Flush the embryos using a dull needle by injecting Modified D-PBS (Specialty Media CAT# MR-006-C) through the infandibulum and Oviduct.
3- Collect the 8-cell embryos into cryovials containing 0.1 ml of M-DPBS, 25-30 embryos per vial.
4- Set on ice bath at 0°C.
5- Add 0.1 ml CPA (containing DMSO) and let the vials sit on ice for 30 min.
6- Place vials in –6°C ice bath for 2 min.
7- Seed vials (media in Pasteur pipettes for seeding must be -10°C).
8- Transfer vials to the controlled-rate freezer already set at -6°C & freeze to -80°C (approximate freezing time 2 ½ hours, -1°C every 2 min).
9- Transfer vials to LN2 (liquid phase) for long-term storage.

Thawing Procedure:
1- Thaw each vial at room temperature for 10 min.
2- Slowly add 0.8 ml of M-DPBS to the vial containing the embryos (this process must be done gently, drop by drop). Mix the volume in the cryovial by pipetting the sample.
3- Draw up total volume containing the embryos and place in a 60mm. organ culture dish.
4- Place the dish on ice and transfer the embryos immediately.
5- Embryos should be transferred into the oviduct of the pseudopregnant recipients. We recommend that 10-12 embryos be transferred per female recipient. Our laboratory uses B6D2F1 females for this purpose.

Cryopreservation & Assisted Reproduction Laboratory
LASP/NCI at Frederick
March 2000