Monterotondo Cryopreservation Course 1999-2011

Campus "A. Buzzati-Traverso"
Consiglio Nazionale delle Ricerche

The Jackson Laboratory

Laboratory Course on
CRYOPRESERVATION OF MOUSE GERMPLASM

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Consiglio Nazionale delle Ricerche
Campus "A. Buzzati-Traverso"
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METHOD TAUGHT AT THE ANNUAL JAX-EMMA CRYOCOURSE ORGANIZED AT CNR-MONTEROTONDO, BY, MARCELLO RASPA

METHOD FOR FREEZING 2-CELL EMBRYOS COLLECTED FROM IVF IN PLASTIC SEMEN STRAWS

(This method can also be used for freezing up to blastocyst stage of embryo development)

Equipment

Instruments, etc. for embryo collection.
Plastic semen straws (0.25 ml, 133 mm in length from IMV)
Straw labels. (Brady part LAT-17-361-2.5)
Monoject syringe (Atlantic Healthcare, Portland. SHA501400, box 100)
Freezing apparatus (controlled rate freezer)
Cristaseal (Fisher part number 02-676-20)

Solutions

M2 media

Cryoprotective solution: 1.5 M PROH
Use Analar grade PROH (Sigma, M.W. 76.1, Cat. No. P-1009)

- Pipette 4.4 ml M2 into a Falcon tube.
- Add 0.6 ml PROH.
- Filter sterilize into a 35 mm Falcon dish. Label dish.

Dilutent: 1.0 M Sucrose

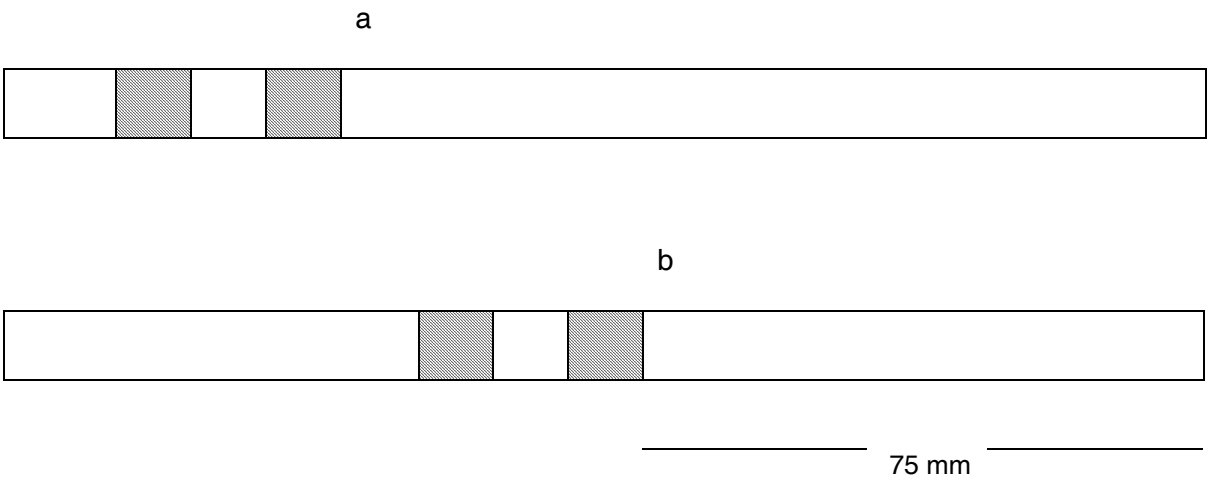
- Dissolve 1.71 g sucrose in 5 ml M2.
- Filter sterilize into a 35 mm Falcon dish. Label dish.

1. Prepare the cooling apparatus: Alcohol filled controlled rate freezer cool and hold at –7°C
2. Collect the embryos, screen carefully for abnormalities, and hold at room temperature in M2 until ready to freeze

Prepare straws

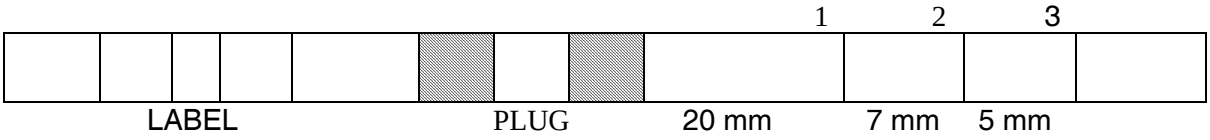
The straws are 133mm in length. By marking the straw at various intervals we can load it with the correct amounts of cryoprotectant and diluent.

- Take a straw (133mm in length) and using a metal rod with a stop, push the plug from *a* to *b.*



Attach a label to identify the straw. (Brady part LAT-17-361-2.5)

With a marker pen, make three marks on each straw. You can use a piece of graph paper as a guide.



1st: mark 20 mm from the plug.

2nd: mark 7 mm from mark 1.

3rd: mark 5 mm from mark 2.

3. Fill the straws: Attach the straw to a monoject syringe (Atlantic Healthcare, Portland) to load it with cryoprotectant and diluent.

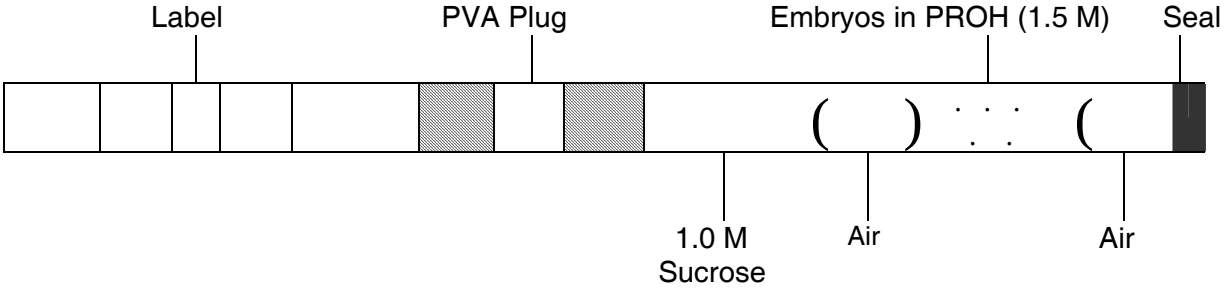
Attach the 1 ml monoject syringe to the end of the straw

- Aspirate sucrose to mark 3.
- Aspirate air so that the sucrose meniscus reaches mark 2
- Aspirate 1.5 M PROH so that the sucrose meniscus reaches mark 1.
- Aspirate air until the column of sucrose reaches halfway up the plug and forms a seal with the polyvinyl alcohol.
- Remove the 1ml syringe

The straws are now ready for loading

4. Pipette the embryos into a dish with a large drop of 1.5 M PROH and equilibrate for 15 minutes.

5. Move embryos into groups of about 20-30, or whatever number is going into each straw
6. To load the embryos into the straw insert the pipette into the 1.5 M PROH fraction of each straw. Try to spread embryos evenly along the length of the fraction—this can be visualized quite well under the microscope. Do not put more than 3 bubbles in with the embryos
7. Seal the straws using "Cristaseal."
8. Place the loaded straws into the cooling apparatus and hold at -7°C for 5 minutes
9. Seed the sucrose fraction by touching it near the plug with the tips of forceps cooled in liquid N_2.
10. After the ice migrates to the embryo fraction, wait 3 minutes, then cool at –0.5°C/minute to -30°C.



11. AT -30^{0} C remove the straws from the controlled rate freezer and plunge the straws directly into liquid N_2.

12. Transfer the straws to the appropriate LN_2 refrigerator, taking care at all times to keep the embryo fraction submerged in liquid nitrogen.

Thawing

A. Transfer the straw from the LN2 storage freezer to a smaller container of liquid nitrogen.

B. Using forceps, hold the straw near the label and hold in air for 40 seconds then hold in water at room temperature until the ice disappears.

C. Wipe the straw dry.

D. Holding firmly cut off the seal and cut through the PVA plug, leaving about half the cotton plug in place to act as a plunger.

E. Using a metal rod, expel the entire liquid contents of the straw into a 35 mm Falcon dish. Do not let the plug drop into the dish

F. Wait 5 minutes. The embryos will shrink considerably.

G. Transfer the embryos to a drop of M2 . They will rapidly take up water and assume normal appearance

H. Wash the embryos in a fresh dish of M2 and then either transfer to oviducts of a day 1 pseudopregnant recipient, or culture to the blastocyst stage in KSOM and transfer to the uterus of a day 3 recipient.

References

Renard, J.P. and Babinet, C. (1984). High survival of mouse embryos after rapid freezing and thawing inside plastic straws with 1-2 propanediol as cryoprotectant. *Journal of Experimental Zoology* 230, 443-448.