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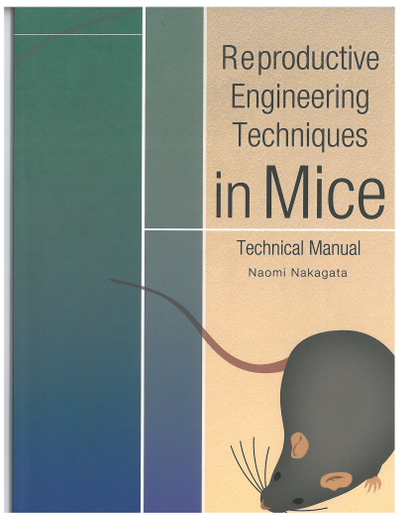
Reproductive Engineering Techniques **in Mice**



Technical Manual
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Preface

In recent years, the number of genetically engineered mice being produced has increased dramatically. Moreover, the rapid progress in the development of genome-editing (TALEN and CRISPR/Cas9) techniques for molecular biology research has been remarkable, so much so that a genetically engineered mouse strain can be produced easily in a few months. Production has been supported by reproductive engineering techniques such as *in vitro* fertilization, embryo and sperm cryopreservation, and embryo transfer techniques. Such techniques have become invaluable peripheral technologies, and their use is expanding rapidly.

This rapid expansion has led to the publication of many technical manuals relating to reproductive engineering techniques in mice hereto. However, mouse reproductive engineering techniques mainly involve delicate operations under a stereoscopic microscope, which means that a sufficiently detailed technical manual has not yet been published.

With that in mind, in this book we aim to create a mouse reproductive engineering technique manual that can be easily understood by anyone. We included a generous number of diagrams, photographs and videos in our manual, and explained each step of each reproductive engineering technique as clearly and thoroughly as we could. We sincerely hope that our manual will become the definitive guide for students, technicians, researchers and other people wishing to study mouse reproductive engineering techniques.

Naomi Nakagata

CONTENTS

* Please see page 90 for details.

5-1 Transport of 2-Cell Embryos at Cold Temperature

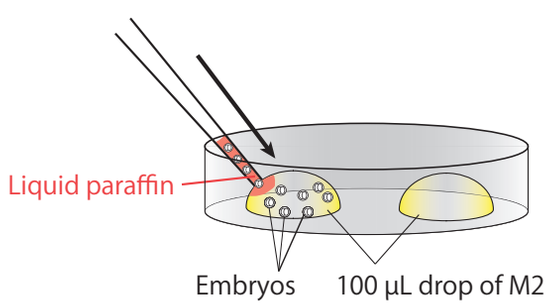
Materials and Equipment

1. 2-cell embryos (adaptable for fresh and frozen/thawed embryos)
2. Plastic dishes (35 mm X 10 mm Cat. No. 430588; CORNING)
3. Gel-loading tip (Cat. No. 010-R204S; Bio Medical Instrument)
4. M2 (Cat. No. M7167; Sigma)
5. 0.5 mL tube (Fisherbrand Flip Cap Microtubes 0.5 mL; Fisher Scientific Cat. No. FS-MCT-060 -C)
6. Transfer pipettes
7. KSOM/AA
8. Liquid paraffin
9. Temperature data logger (Thermochron iButton Cat. No. DS1921G; Maxim Integrated Products)
10. CARD Cold Transport Kit (Cat. No. KYD-006-EX, Cosmo Bio Co., Ltd.)
    - Thermos bottle (Cat. No. JMK-501; Thermos K.K.)
    - Paper box (in which a 0.5 mL tube can stand)
    - Cotton wool
    - Cold packs (small and large)
    - Polystyrene foam transport box (Cat. No. KC-3, KARUX)

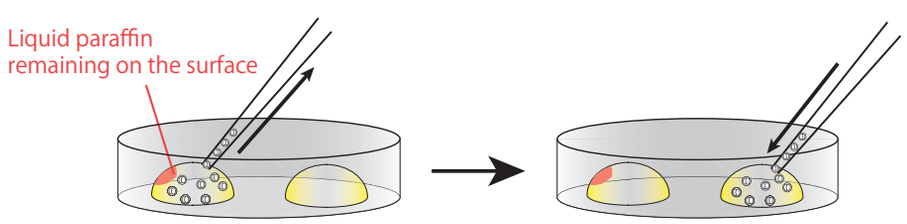
Procedures

Cold Storage of 2-cell Embryos

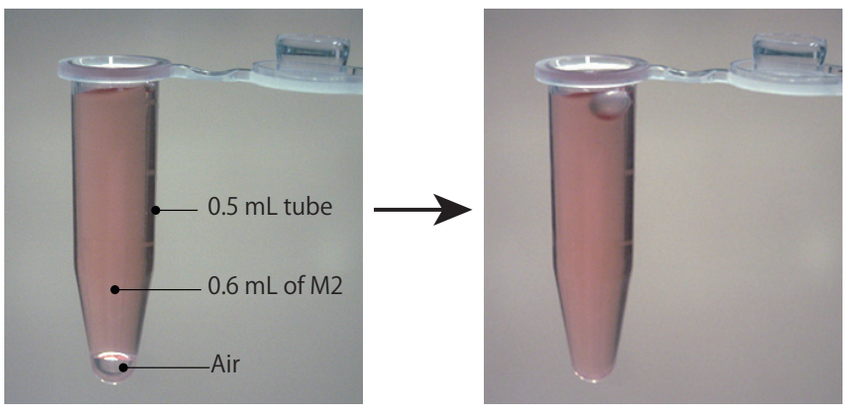
1. Place two 100 µL drops of M2 on a plastic dish.
2. Transfer the 2-cell embryos from the culture medium to the drop of M2.



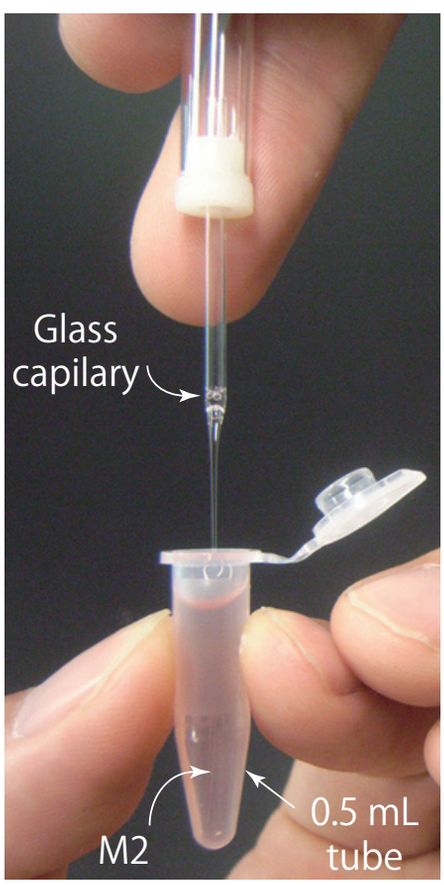
3. Exchange the capillary for embryo manipulation and aspirate the embryos into the new capillary, making sure to avoid dropping liquid paraffin onto the drop of M2. Transfer the embryos into the drop of M2 made in step 1.



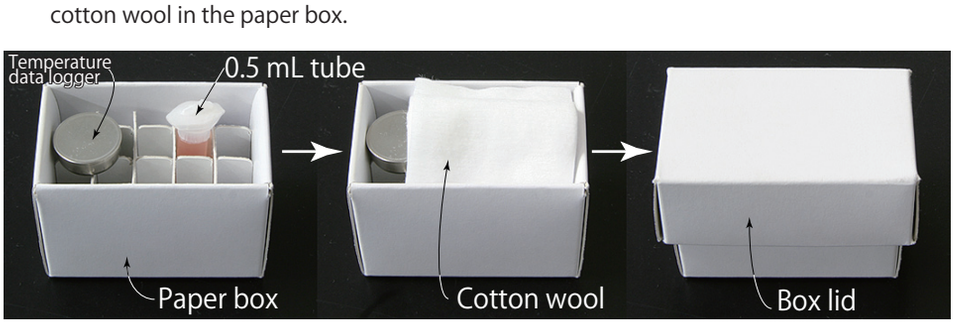
4. Fill a 0.5 mL tube with 0.6 mL of M2 at room temperature. If there is a bubble in the bottom of the tube, tap the tip of the tube to release the bubble.



5. Collect and transfer the embryos into the bottom of the tube (40 embryos/tube).



6. Place the tube containing the embryos, a temperature data logger and a piece of cotton wool in the paper box.



7. Store the paper box in the refrigerator (4-8℃).

Comment

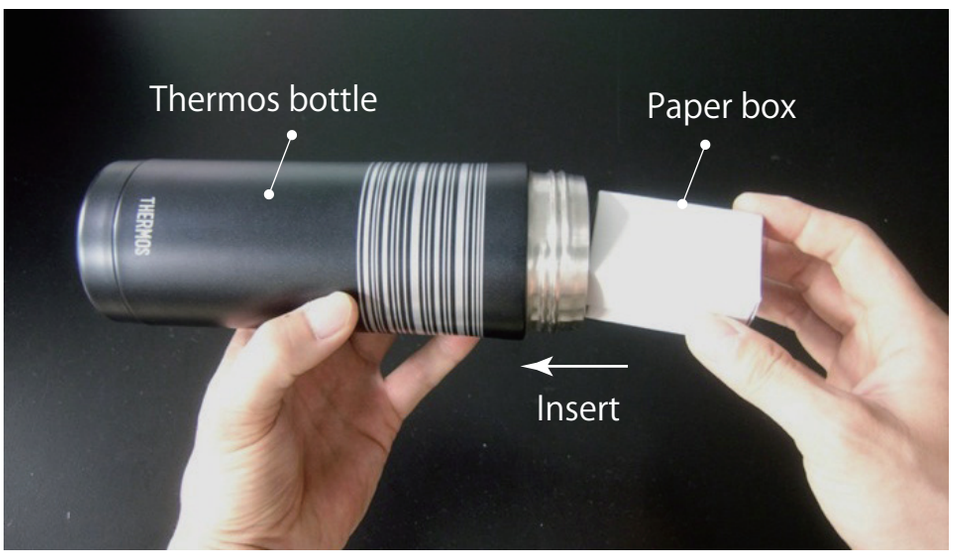
The embryos will maintain developmental ability for up to 72 hours.

Package and Transport of 2-cell Embryos

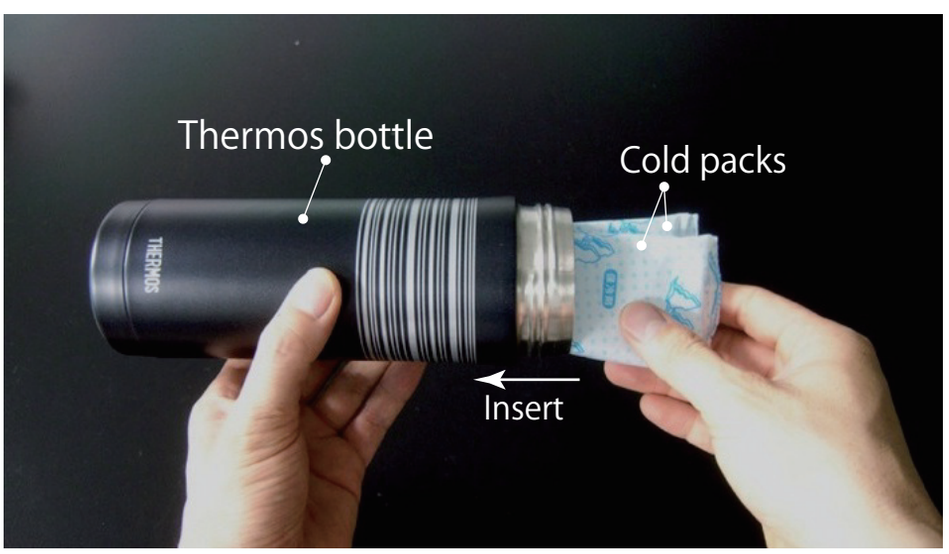
Prepare a paper box containing 2-cell embryos in the same manner as described before (Cold Storage of 2-cell Embryos).

The cold packs (large) and a foam transport box must be precooled to 4-8℃ before use. Use the cold packs (small) and a thermos bottle at room temperature.

1. Insert the paper box containing the embryos into a thermos bottle.

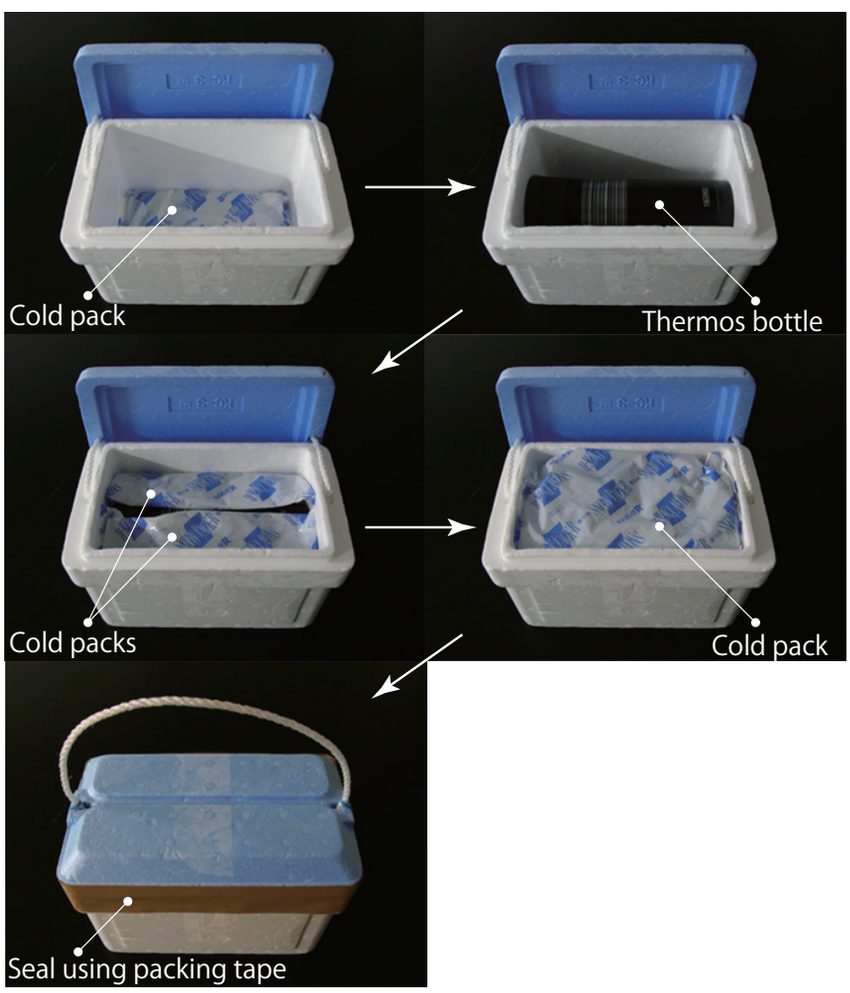


2. Insert two cold packs (small) into the thermos bottle.



3. Close the bottle cap.

4. Put a cold pack (large) in the bottom of a foam transport box, then put the thermos bottle on top of it.
5. Pack one cold pack (large) on either side of the bottle, then set a further pack (large) on top and close the lid.
6. Seal the lid of the foam transport box using packing tape.



7. Keep the foam transport box in the refrigerator until a courier comes to pick it up.
8. Send the samples via regular courier service.

Note

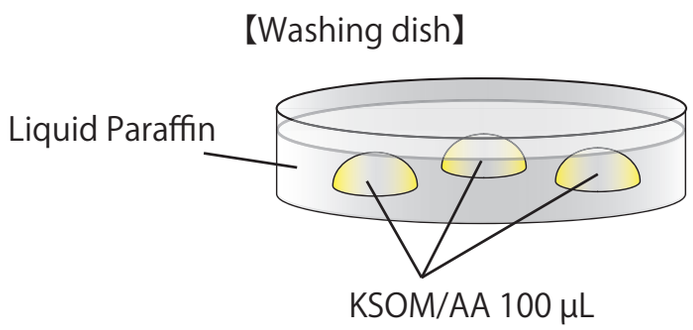
Take care not to place the paper box upside down.

Note

It is only possible to place the thermos bottle in the center of the foam transport box and not the actual bottom, because the length of the thermos bottle is the same as that of the inner length of the foam transport box. This is to protect the thermos bottle during shipping.

Collection of 2-cell Embryos from the Transport Box

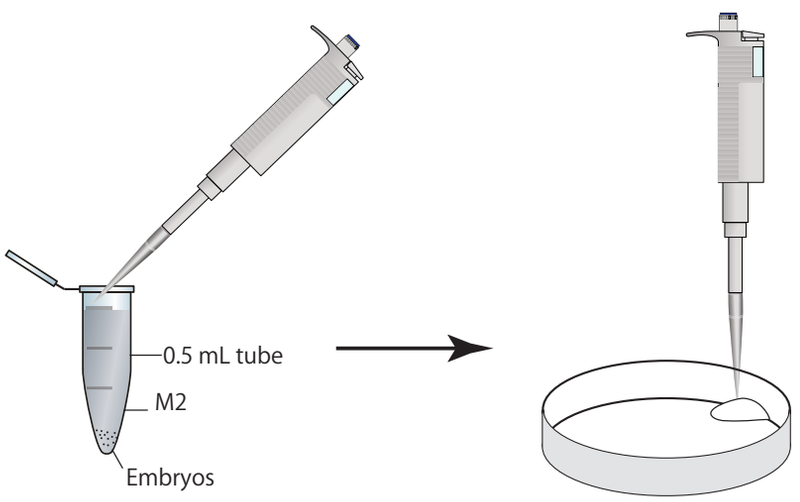
1. Put 3 drops (100 µL / drop) of KSOM/AA into a dish and cover them with liquid paraffin. Place the dish in an incubator (37℃, 5% CO_2 in air) for at least 30 minutes.



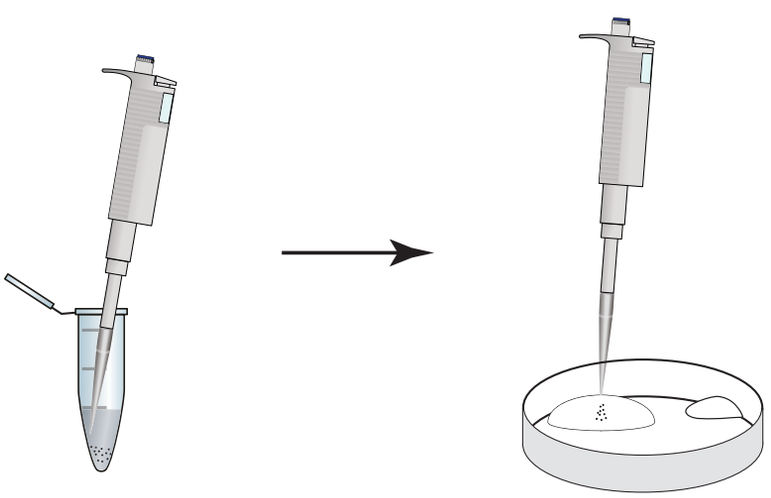
2. Retrieve the paper box containing the samples from the thermos bottle.
3. Leave the paper box at room temperature for 30 minutes.

[Removing the sample] No. 11-01

4. Open the paper box and gently remove the cotton. Once removed, pick up the tube containing the embryos and open it.
5. Collect an upper layer of 200 µL M2 from the tube using a gel-loading tip, then transfer the aliquot to the edge of the plastic dish.



6. Carefully retrieve all M2 containing the embryos from the bottom of the tube using a gel-loading tip, then transfer the aliquot to the center of the plastic dish.



Note

The sample must be transferred at a refrigerated temperature. Please ask the courier service directly about conditions during transport.

Comment

The embryos will maintain developmental ability for up to 72 hours.

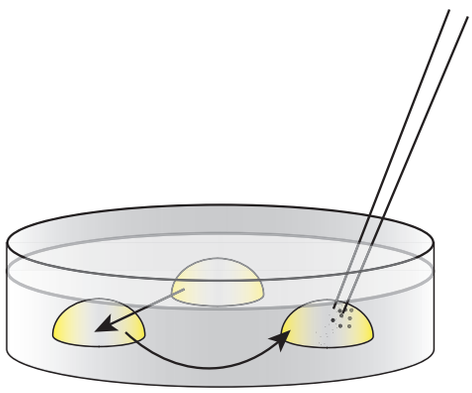
Comment

The embryos will sink to the bottom of the tube during those 30 minutes.

Note

For easy manipulation, take care to avoid aspirating air bubbles in the gel-loading tip.

7. Collect the embryos from the M2, then transfer and wash them in each of the three drops of 100 µL KSOM/AA (washing dish).



8. Transfer the embryos into the oviducts of a pseudo-pregnant mouse.

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Note

If you cannot retrieve all stored embryos, rinse the inside of the tube using the 200 µL M2 at the edge of the plastic dish.

Comment

Ideally, embryo transfer to pseudo-pregnant mice should be performed immediately upon the arrival of embryos.
(Please refer to the chapter of Embryo Transfer into the Oviduct on page 66.)

5-2 Transportation of Mouse Oviducts Containing 2-Cell Embryos at Low Temperature (0℃)

Materials and Equipment

1. 0.8 M Sucrose
2. PB1
3. KSOM/AA
4. Plastic bag
5. Thermos bottle
6. Crushed ice
7. Conical bottom cryotubes (Cat. No. 366656; NUNC)
8. Plastic dish (35 mm X 10 mm Cat. No. 430588; CORNING)

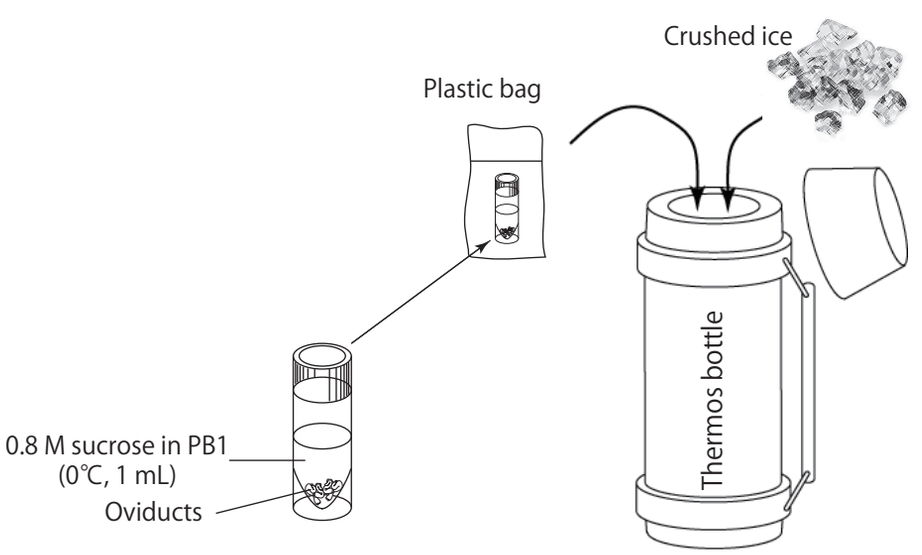
Procedures

Dissection of Oviducts from Superovulated, Plugged Females

1. Inject females i.p. (8-12 weeks old) with 7.5 IU of PMSG (14:00-18:00).
2. Inject females i.p. with 7.5 IU of hCG 48-52 hours after giving them the PMSG injection, and allow the male and female mice to mate overnight.
3. Check the females for vaginal plugs from early the next morning until noon. (Please refer to the chapter of Embryo Transfer into the Oviduct on page 66.)
4. At 44-46 hours after administering the hCG, sacrifice the plugged females.
5. Remove the oviducts of the females, and put them into a 100-200 μL drop of 0.8 M sucrose (0℃). (Please refer to the chapter of *In Vitro* Fertilization on page 9.)

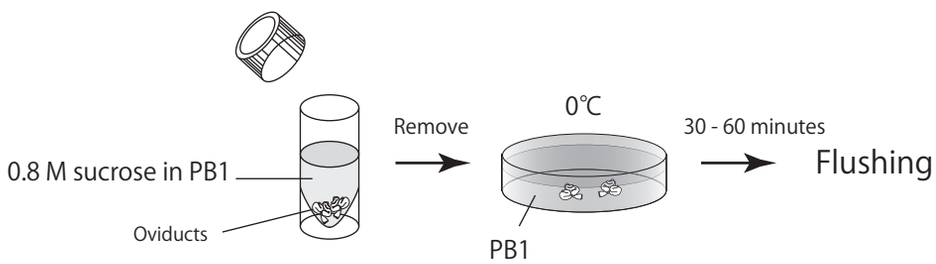
Transportation of Mouse Oviducts

1. Transfer the oviducts into a cryotube containing 1 mL of 0.8 M sucrose (0℃).
2. Enclose the tube in a plastic bag and seal it using a sealer.
3. Transfer the plastic bag to a thermos bottle containing crushed ice and transport it using a door-to-door delivery service.



Collection of Embryos

1. Remove the tube from the thermos bottle.
2. Remove the oviducts from the tube and keep them in PB1 (0℃) for 30-60 minutes.
3. Flush the oviducts with PB1 (0℃). (Please refer to the chapter of Collecting 2-Cell Stage Embryos on page 42.)
4. Wash the embryos with 3 cycles of fresh KSOM/AA (37℃).



Note

Embryos will rapidly degenerate if step 2 is skipped.

Note

The oviducts should not remain in the tube longer than 48 hours or the embryos will degenerate.
Embryos should be frozen if they will not be used immediately.
(Please refer to the chapter of Simple Vitrification of Mouse Embryos on page 54.)

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