

Mouse Bank at CARD Kumamoto University, Japan

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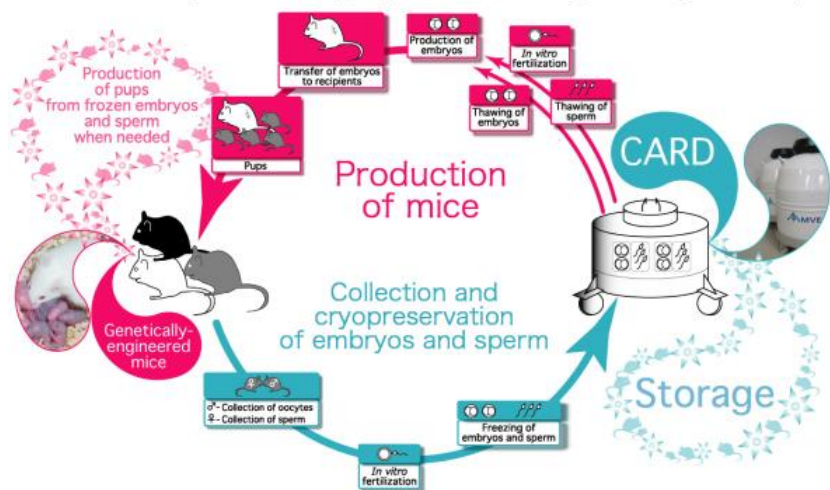
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SYNOPSIS

Cryopreservation of mouse embryos and spermatozoa has become the foremost technique for preserving large numbers of different strains of mice with induced mutations. In 1998, our mouse bank was established in the Center for Animal Resources and Development (CARD), Institute of Resource Development and Analysis, Kumamoto University, Japan, based on the Preservation, supply and development of genetically engineered animals report published by the Ministry of Education, Culture, Sports, Science and Technology. We cryopreserve mouse embryos and sperm, supply these resources, organize training courses to educate people and form part of a domestic and international network of both mutagenesis and resource centers. We currently have over 1,500 mouse strains, 842,000 frozen embryos and 26,000 straws containing frozen sperm. Moreover, we disclose information about 1,300 deposited strains. Furthermore, over 400 strains of frozen embryos or mice produced from frozen embryos and sperm are being supplied to the requesters both domestically and internationally. Additionally we hold training courses on the cryopreservation of mouse germplasm 2-3 times a year, both domestically and internationally. In the course, we teach basic reproductive engineering techniques to trainees on a man-to-man basis. We have already held 28 training courses on the cryopreservation of mouse germplasm at our center and at other institutes.

Mouse bank system using reproductive engineering techniques



Keywords: resource, genetically engineered mouse, cryopreservation, embryo, sperm

Introduction

The Center for Animal Resources and Development (CARD), Kumamoto University was established in 1998 based on the "Preservation, supply and development of genetically engineered mice" report published on July 10th, 1997 by the Subdivision of Resource Study, Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology (available at <http://card.medic.kumamoto-u.ac.jp/card/english/index.html>).

In 2000, a new facility for this purpose was completed and we started to cryopreserve, supply and develop genetically engineered mice. This paper attempts to introduce our mouse bank.

1) Mouse Bank¹

1)-1 Mouse bank system

Figure 1 shows the outline of the mouse bank at our center. When the researchers deposit their mouse strains, they send the mice to our center. In our center, we carry out *in vitro* fertilization using sperm and oocytes collected from the mice, then freeze the embryos produced and sperm in a liquid nitrogen tank. As a quality control measure, a number of frozen embryos are thawed to check 1) if the frozen embryos develop into live young, 2) whether the developed mice are microbiologically clean, and 3) whether transgenes are successfully transmitted to the young. We also thaw one straw containing frozen sperm to check the sperm motility. Thereafter, information regarding the deposited mice is put on our website (CARD R-BASE).

1)-2 CARD R-BASE

Figure 2 shows the website (CARD R-BASE: <http://cardb.cc.kumamoto-u.ac.jp/transgenic/index.jsp>) concerning mouse strains deposited in our center. Mouse strains are classified as Inbred, Spontaneous/Chemical induced mutant, Transgenic, Targeted mutant, Gene trap and Insertion mutant. If you click on individual group of mouse strains, for example, 'Transgenic', you can obtain a list of transgenic mouse strains. Moreover, if you click on each strain name, you can see more detailed information, such as strain, gene and reference.



Figure 2. CARD R-BASE website. Only part of the website is shown in this figure. Both Japanese and English versions are available.

1)-3 Supply of mouse strains (Figure 1)

First, requesters access CARD R-BASE. If they would like to obtain certain mouse strains, they order them from our center. If the requesters have adequate facilities to carry out reproductive engineering techniques, we send frozen embryos. On the other hand, if they do not have such facilities, we produce pups from frozen embryos or sperm, and the pups produced are sent to the requesters.

Using reproductive techniques, we manage the mouse bank very efficiently (Figure 3). We currently have over 1,500 mouse strains (Table 1), 842,000 frozen embryos and 26,000 straws containing frozen sperm. Moreover, we disclose information about 1,300 deposited strains (Figure 2). Furthermore, over 400 strains of frozen embryos or mice produced from frozen embryos and sperm are being supplied to the requesters both domestically and internationally (Table 1).

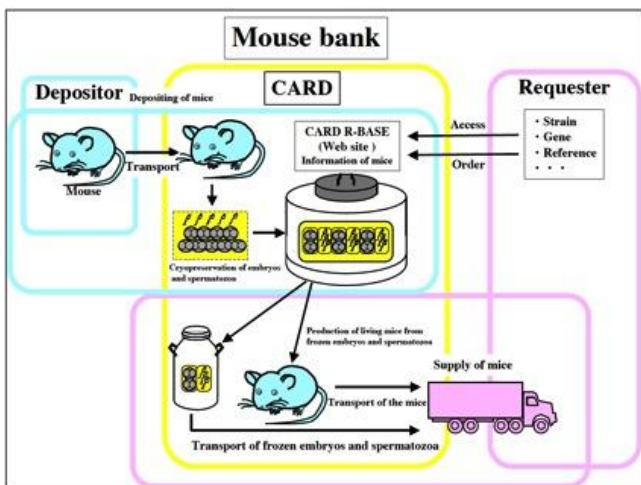


Figure 1. Outline of mouse bank at our center. Information on the application procedure and necessary documents are available from our website. Mouse embryos or sperm obtained from mice sent from depositors are cryopreserved and stored in liquid nitrogen tanks. These frozen embryos, or live mice obtained by transfer of thawed embryos into foster mothers, are sent to requesters.

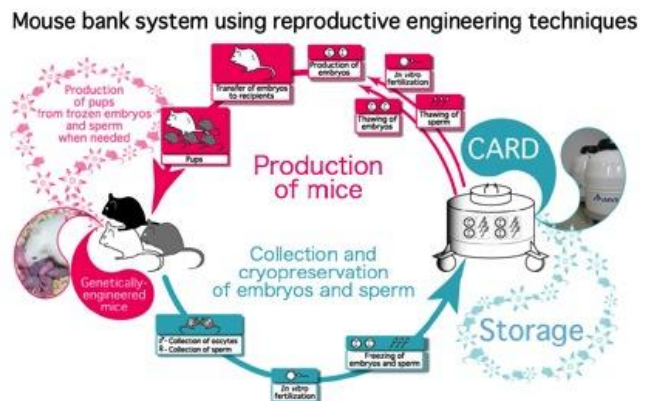


Figure 3. The mouse bank system. The mouse bank system offers efficient and regular delivery of genetically engineered mice using reproductive engineering techniques.

Table 1. Cryopreservation and supply of genetically engineered mice at CARD

	No. of strains cryopreserved	No. of strains supplied
2000	144	4
2001	97	10
2002	67	34
2003	89	33
2004	116	58
2005	109	39
2006	151	42
2007	169	35
2008	159	58
2009	231	68
2010	221	61
Total	1,553	438

2) Training Course on the Cryopreservation of Mouse Germplasm

We hold training courses on the cryopreservation of mouse germplasm 2-3 times a year, both domestically and internationally. In the course, we teach basic reproductive engineering techniques to trainees on a man-to-man basis.

We have already held 28 training courses on the cryopreservation of mouse germplasm at our center and at other institutes. Figure 4 shows the schedule for a training course that took place at Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Chungbuk in April, 2008. Among other things, we taught the trainees how to freeze sperm and embryos, carry out *in vitro* fertilization, and transfer embryos.

3) CD Manual Regarding Reproductive Engineering Techniques in Mice

The first CD edition of the manual for reproductive engineering techniques in mice was released in 2005. The second edition was

Program of Training Course on Cryopreservation of Mouse Germplasm
Place: Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Chungbuk
Date: April 2 (Wed) – 4th (Fri), 2008

Course Schedule

Wednesday, April 2th
10:00 – 10:45, Presentation
Mouse Embryo Bank and Reproductive Engineering Techniques Naomi Nakagata
11:00 – 12:00, Making glass capillary for embryo manipulation.
12:00 – 1:00, Lunch
1:00 – 3:00, Sperm freezing
3:00 – 3:30, Coffee Break
3:30 – 5:00, Flushing of 2cell embryo from oviduct

Thursday, April 3th
9:00 – 12:00, *In vitro* fertilization (IVF)
12:00 – 1:00, Lunch
1:00 – 3:00, Vasectomy of male mice
3:00 – 3:30, Coffee Break
3:30 – 5:00, Selection and Collection of fertilized eggs after IVF

Friday, April 4th
9:00 – 12:00, 2-cell embryo freezing and thawing
12:00 – 1:00, Lunch
1:00 – 3:00, 2-cell embryo transfer (into oviduct)
3:00 – 3:30, Coffee Break
3:30 – 5:00, Morula embryo transfer (into uterus)
End of course

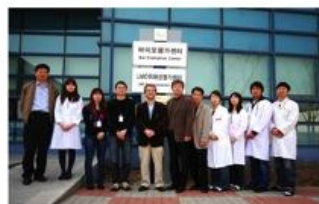


Figure 4. Training course at Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB). This figure shows the course schedule and a ceremonial photograph.

then released in 2007. This CD contains lots of pictures, photos and movies to illustrate each topic item, and so is a useful tool for beginners.

Contents

- Preparing and Assembling Pipettes for Embryo Handling
- *In Vitro* Fertilization
- Simple Vitrification of Mouse Embryos
- Vitrification and Transplantation of Mouse Ovary
- Cryopreservation of Mouse Spermatozoa
- *In Vitro* Fertilization using Cryopreserved Spermatozoa
- Partial Zona Dissection (PZD)
- Collecting Two-Cell-Stage Embryos
- Transportation of Mouse Oviducts Containing 2-Cell Embryos at Low Temperature (0°C)
- Vasectomy for the Creation of Sterile Males
- Embryo Transfer into the Oviduct
- Production of Chimeric Mice by 8-Cell Aggregation
- Embryo Transfer into the Uterus
- Caesarean Section and Fostering

Up to now, we have released a CD manual regarding reproductive engineering techniques in mice in Japanese, English, Chinese and Korean (<http://www.funakoshi.co.jp/export/product/TRG/RETM-E.pdf>). We plan to release revised versions containing improved and new methods sequentially.

Conclusion and Prospects

Recently, we have developed a cryopreservation method for mouse sperm to obtain a relatively high fertilization rate^{2,3}. If we freeze mouse sperm using this method, we can obtain over 1,000 pups derived from frozen-thawed sperm collected from just one male mouse *via in vitro* fertilization and embryo transfer techniques. We also have demonstrated that the frozen-thawed 2-cell embryos and the 2-cell embryos produced by frozen-thawed sperm and transported from our center to many institutes at a refrigerated temperature have the ability to develop well into live young^{4,5} (Figure 5). The advantages of this method are that the receivers do not have to master thawing techniques, and special containers (e.g., dry shippers) are not required for the transport of embryos. Therefore, I strongly believe that the transport of embryos at a cold



Figure 5. Live young derived from 2-cell embryos transported at a refrigerated temperature. Pups obtained from 2-cell embryos produced by frozen-thawed sperm transported at a refrigerated temperature from CARD, Kumamoto University to Asahikawa Medical College (a distance of over 2000 km) are shown.

temperature will become an established method for the exchange of various strains of genetically engineered mice.

Since the establishment of our center, one of our goals has been to set up a domestic and international network to promote biological sciences throughout the world. To accomplish this goal, we became a founding member of the Federation of International Mouse Resources (FIMRe: <http://www.fimre.org/>)⁶. The FIMRe is a collaborating group of Mouse Repository and Resource Centers worldwide whose collective goal is to archive and provide strains of mice to the research community as cryopreserved embryos and gametes, ES cell lines, and live breeding stock. In addition, we organized the Asian Mouse Mutagenesis and Resource Association (AMMRA: <http://www.ammra.info/>) in 2006. The AMMRA is a collaborative group of Mouse Mutagenesis and Resource Centers in Asia. Its mission is "To promote mouse mutagenesis projects and to facilitate access to mouse resources in Asia". Its goals are "the use of mouse models for understanding the genome function and improvement of human health".

Looking to the future, we would like to use new reproductive engineering techniques in collaboration with the network described above in order to further develop our mouse bank and to provide various genetically engineered mouse strains promptly to research communities all around the world.

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