NIBB Center of the Interuniversity Bio-Backup Project (IBBP Center)

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In order to realize a life sciences community resilient to natural disasters and calamities, the National Institutes for Natural Sciences (NINS) and Hokkaido University, Tohoku University, University of Tokyo, Nagoya University, Kyoto University, Osaka University, and Kyushu University concluded an agreement on June 1st 2012 to launch a system to 'back up' the biological resources essential to the work being done at universities and research institutions nationwide, called the 'Interuniversity Bio-Backup Project (IBBP)'.

The IBBP Center was established as a centralized backup and storage facility at NIBB, while IBBP member universities set up satellite hubs and work closely with the IBBP center to put in place reciprocal systems for backing up important biological resources that have been developed by researchers residing in the area each university satellite hub is responsible for.

Figure 1. IBBP Center.

Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

The IBBP Center includes earthquake proof structures capable of withstanding even very large scale quakes (equipped with emergency backup power generators), cryopreservation facilities equipped with automatic liquid nitrogen feeding systems, deep freezers, and refrigerated storage (mainly for seed stocks), as well as all manner of automated laboratory equipment, cell culture tools, and the latest equipment necessary to back up the genetic resources in a collaborative manner. The specific methods of preservation are freezing of the sperm and eggs of animals, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

University satellite hubs receive preservation requests of biological resources from researchers and report to the Managing Project Committee of IBBP (constituted of faculty of NIBB and satellite institutes), where the relevance of the request is reviewed. When the request is sustained, biological resources to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated) and their particular information will be registered into a database. In the event of a disaster leading to the loss of a researcher's own biological resources, preserved samples will be promptly supplied back to the researcher so they can quickly resume their work.

Through the development of this backup system biological resources that had only been stored at individual research institutes can now be preserved at the state of the art facilities of the IBBP Center, and Japan's research infrastructure has been significantly strengthened.

I. Current status of back up for the biological resources

In 2017, IBBP Center stored 4,478 384-well and 69 96-well plates consisting of 1,726,176 clones as cDNA/BAC clones, 12,225 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 1769 133mm-straw tubes for sperm and 654 seed samples. In total 1,728,599 samples are stored.

Figure 3. Cryotube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

II. Collaborative Research Project for the development of new long-term storage technologies and cryo-biological study

As the IBBP Center can only accept biological resources which can be cryopreserved in liquid nitrogen, researchers cannot backup those biological resources for which cryo-preservation methods are not well established, to increase the usability of IBBP, we started a collaborative research project for the development of new long-term storage technologies and cryo-biological study in 2013. This collaborative research includes two subjects 1) Establishment of new storage technologies for biological resources for which long-term storage is unavailable. 2) Basic cryobiological research enabling us to improve low temperature storage for biological resources. In 2017, we also worked to establish a research center for cryo-biological studies thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2017 on November 1-2, 2017 at the Tsukuba Center for Institutes, Tsukuba, Ibaraki, Japan. We had 132 participants from several fields covering physics, chemistry, biology, and technology.

Figure 4. Group photo of Cryopreservation Conference 2017.

Research activity by H. Taketsuru

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Development of rat vitrified embryos in each developmental stage

Vitrification of embryos is a simple method and is used in various animals. Although vitrification of 2-cell embryos is possible, only a few reports were published about vitrification of embryos in various developmental stages. Vitrified pronuclear and morula stage embryos are used for producing genetically engineered rats and knockout/in rats. This study examined the development of rat embryos in pronuclear, 2-cell and morula stages after vitrification.

Wistar female rats aged more than 8 weeks were injected with 150 IU/kg PMSG and 75 IU/kg hCG. Females were then mated with mature male rats of the same strain. Pronuclear and 2-cell stage embryos were collected from oviducts. Morula embryos were collected from the uterus. Pronuclear, 2-cell and morula stage embryos were vitrified using a solution containing 10% propylene glycol, 30% ethylene glycol, 20% Percoll and 0.3 M sucrose in PB1.

Figure 5. Vitrification and transfer of rat embryos.

Each embryo was warmed using a solution containing 0.3 M sucrose in PB1. Morphologically normal embryos were transferred into the oviducts of the pseudopregnant females (Figure 5). Pronuclear embryos were vitrified after culturing for 0, 4 and 7 hours. After warming, the embryos that developed to the 2-cell stage were transferred into the oviducts of the pseudopregnant females.

The development to offspring of vitrified-warmed pronuclear, 2-cell and morula stage embryos were 16%, 33% and 52%, respectively (Figure 6). When the pronuclear embryos were vitrified after culturing for 0, 4 and 7 hours, the developmental rates to offspring were 12%, 25% and 24%, respectively. These results indicated that tolerance to vitrification was increased with embryo development. Vitrified embryos can be used for producing genetically engineered rats and knockout/in rats.

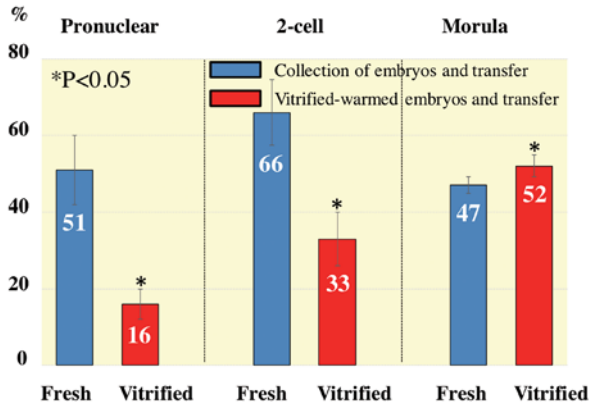

Figure 6. Development to offspring of vitrified embryos.

The level of ovulation in various rat strains and development of embryos after vitrification

In rats, it is now possible to produce genetically engineered strains, not only as transgenic animals but also using gene knockout techniques. Reproductive technologies have been used as indispensable tools to produce and maintain these novel valuable strains. Here we studied the sensitivity to PMSG/hCG for oocyte collection, and the development of embryos after cryopreservation in some strain rats (Figure 7).

Figure 7. Rat strains.

The response to pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) was examined by injection of 150IU/kg PMSG + 75IU/kg hCG or 300IU/kg PMSG + 300IU/kg hCG. The numbers of 2-cell embryos collected from F344/Stm female rats were high (31.0). On the other hand, the number of 2-cell embryos collected from BN/SsNSlc female rats were low (2.4) in these strains. Of cryopreserved 2-cell embryos collected from these strains, more than 65% of embryos survived after warming. After transfer, offspring were obtained from cryopreserved 2-cell embryos in all strains. However, the offspring rate was different for strains, F344/Stm and Jcl: Wistar were more than 50%, and ACI/Nkyo was 20% (Figure 8).

In summary, there are differences in sensitivity to superovulation and the development to offspring rate from cryopreserved embryos in these strains.

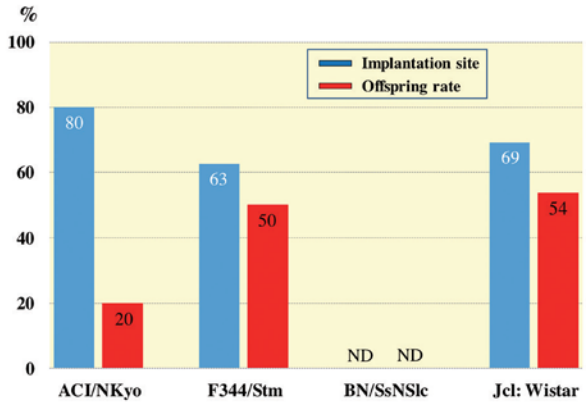

Figure 8. Development to offspring of vitrified embryos in some strains.