

NIBB CENTER FOR THE INTERUNIVERSITY BIO-BACKUP PROJECT (IBBP CENTER)



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In order to realize the vision of a life science community that can withstand 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 finalized 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 have set up satellite hubs and work closely with the



Figure 1. IBBP Center.

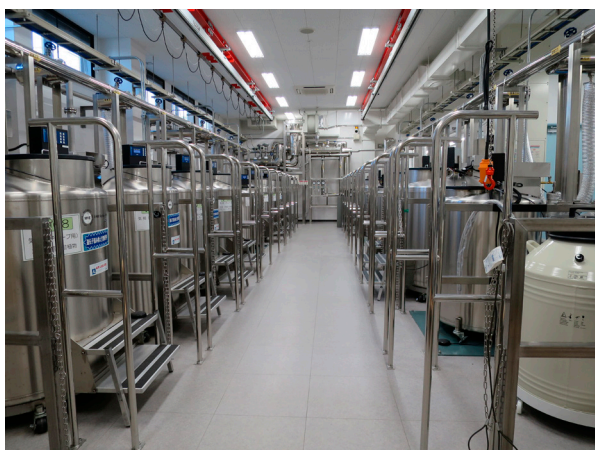


Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

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.

The IBBP Center includes: earthquake proof structures capable of withstanding even very large scale quakes which are 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 preservation methods used are the freezing of animal sperm and eggs, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

When university satellite hubs receive preservation requests involving biological resources from researchers, they report to the Managing Project Committee of IBBP (which is comprised of NIBB faculty members and other satellite institutes), where the relevance of the request is reviewed. If the request is approved, the biological resources that are to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated), and their particulars 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 returned 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 IBBP Center’s state of the art facilities. As a result of this, Japan’s research infrastructure has been significantly strengthened.

I. Current status of back up available for biological resources

In 2018, the IBBP Center stored 5,468 384-well and 112 96-well plates consisting of 2,110,464 clones as cDNA/BAC clones, 15,960 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 4,503 133mm-straw tubes for sperm and 654 seed samples. In total 2,110,464 samples were stored.



Figure 3. Cryo tube 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 biological resources for which cryopreservation methods are not well established. In order 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 focuses on two goals: 1) the 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 2018, we have conducted 18 collaborative research projects aimed at achieving these goals. We also worked to establish a research center for cryo-biological study through this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2018 on October 25-26, 2018 at the Okazaki Conference Center in Okazaki, Japan. We had 108 participants from several fields covering physics, chemistry, biology, and technology.



Figure 4. Group photo of Cryopreservation conference 2018.

RESEARCH ACTIVITY BY H. TAKETSURU

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Vitrification of rat embryos at various developmental stages

Numerous genetically engineered rat strains have been produced via genome editing. Although the freezing of embryos is helpful for the production and storage of these valuable strains, the tolerance to freezing of embryos varies at each developmental stage of the embryo. This study examined the tolerance to freezing of rat embryos at various developmental stages, particularly at the pronuclear stage. Embryos that had developed to the pronuclear, 2-cell, and morula stages were frozen via vitrification using ethylene glycol- and propylene glycol-based solutions (Figure 1). More than 90% of the embryos at all developmental stages survived after warming. The developmental rates of offspring



Figure 1. Vitrification and transfer of rat embryos.

of thawed embryos at the pronuclear, 2-cell, and morula stages were 19%, 41%, and 52%, respectively. Pronuclear stage embryos between the early and late developmental stages were then vitrified. The developmental rates of offspring of the thawed pronuclear stage embryos collected at 24, 28, and 31h after the induction of ovulation were 17%, 21%, and 23%, respectively. These results indicated that the tolerance to vitrification of rat embryos increased with the development of the embryos. The establishment of a vitrification method for rat embryos at various developmental stages is helpful for improving the production and storage of valuable rat strains used for biomedical science.

Figure 2 shows the developmental ability of embryos at various developmental stages. The developmental rates of offspring of fresh embryos collected at pronuclear, 2-cell, and morula stages were 54%, 53%, and 45%, respectively. More than 90% of embryos survived freezing, and 19%, 41%, and 52% of the surviving embryos developed to normal offspring after their implantation at the pronuclear, 2-cell, and morula stages, respectively. The rate of implantation and development to offspring was significantly lower in the pronuclear stage embryos than in the 2-cell and morula stage embryos after freezing.

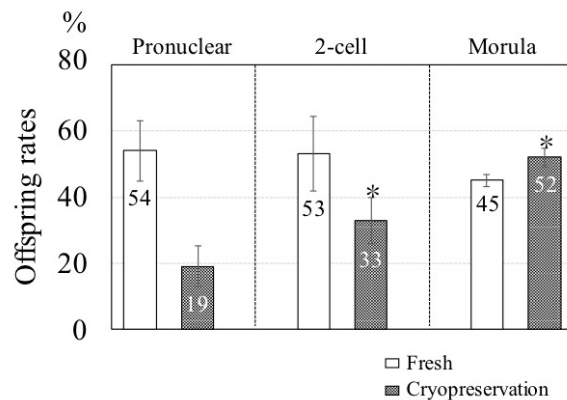


Figure 2. Development of rat embryos at various developmental stages.

The developmental ability of pronuclear stage embryos after various developmental periods are shown below (Figure 3). Although embryos collected at 24h after hCG injection (control) showed high survival rates (93%) after freezing, these embryos showed low rates of development (28%) when compared to 2-cell embryos. The survival of frozen embryos collected at 28 and 31h after hCG injection was high (both 95%), and they showed a significantly higher (45% and 67%) development to the 2-cell stage compared with the control embryos. The development to offspring (21% and 23%) of these embryos was slightly increased, although no significant difference was seen compared with control embryos. The ability of embryos to survive and develop to the 2-cell stage when cultured in vitro for 4

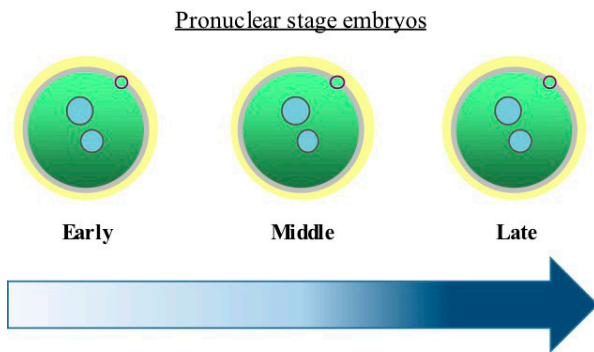


Figure 3. Increase the tolerance to vitrification with embryo development?

or 7h after collection at 24h after hCG injection was also significantly higher than that of control embryos. However, the rate of implantation sites and development to offspring of these embryos were similar to those of embryos collected at 28 and 31h after hCG injection.

Embryo freezing is useful in the production and maintenance of valuable genetically engineered rat strains. Vitrification is helpful because embryos can be rapidly and easily frozen without any specialized equipment, such as a programmable freezer. Vitrified pronuclear stage embryos are required for introducing endonucleases during genome editing. Further studies are needed to establish the effects of vitrification on rat embryos at all developmental stages.

Publication List:

[Original paper]

- Taketsuru, H., and Kaneko., T. (2018). Tolerance to vitrification of rat embryos at various developmental stages. *Cryobiology* 84, 1-3.