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 2016, IBBP Center stored 4,478 384-well and 69 96-well plates consisting of 1,726,176 clones as cDNA/BAC clones, 11,792 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 541 133mm-straw tubes for sperm and 654 seed samples. In total 1,727,371 samples are stored.

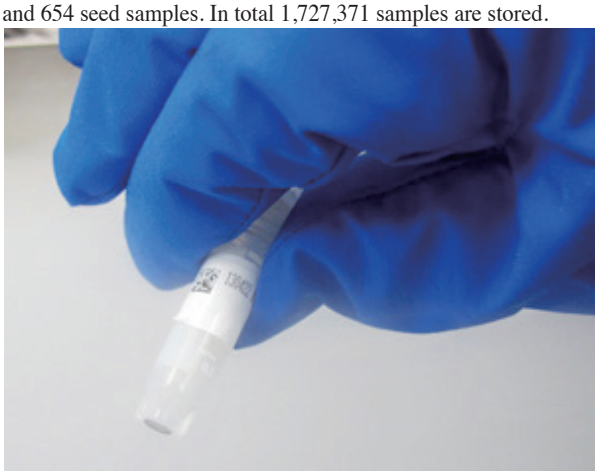

Figure 3. Cryo tube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

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 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 2016, we also worked to establish a research center for cryo-biological study thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2016 on November 10-11, 2016 at the Okazaki Conference Center, Okazaki, Japan. We had 128 participants from several fields covering physics, chemistry, biology, and technology.

Research activity by H. Taketsuru

Specially Appointed Assistant Professor:
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In vitro maturation of immature rat oocytes

Rat oocytes can be produced artificially by superovulation. Because some strains show low sensitivity to superovulation treatment, *in vitro* maturation is an alternative method to produce numerous matured oocytes. Furthermore, establishment of an *in vitro* maturation system with simple culture conditions is cost effective and leads to easy handling of oocytes. This study examined developmental ability of rat germinal vesicle (GV) oocytes maturing *in vitro* under simple culture conditions. Significantly different numbers of ovulated oocytes reached the second metaphase of meiosis (MII) among Jcl:Wistar (17.0), F344/Stm (31.0), and BN/SsNSlc (2.2) rats in whom superovulation was induced by pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (Figure 1). However, similar numbers of GV oocytes were obtained from ovaries of PMSG-injected

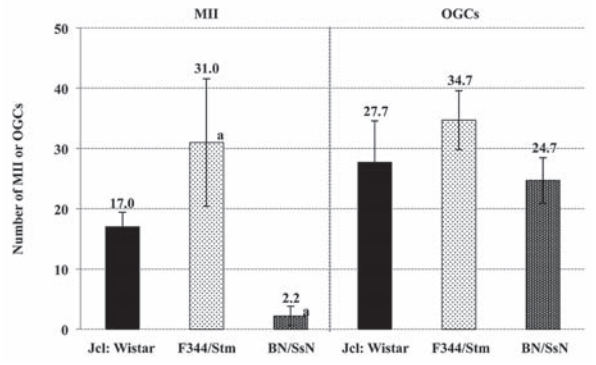

Figure 1. Number of MII oocytes or OGCs collected from ovaries of females. [a]Significantly different at $P < 0.05$.

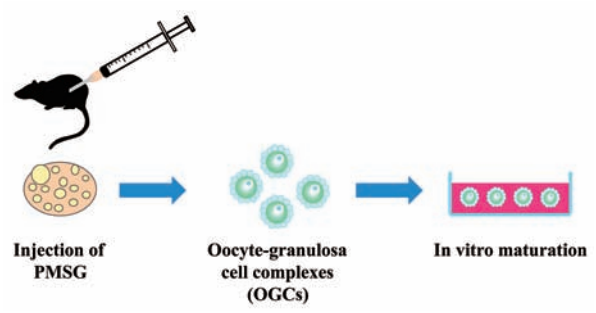

Figure 2. In vitro maturation of immature rat oocytes.

Wistar (27.7), F344 (34.7), and BN (24.7) rats (Figure 1). These GV oocytes were cultured *in vitro* in HTF, αMEM, and a 1:1 HTF+αMEM or TYH+αMEM mixture (Figure 2). High proportions of Wistar and F344 oocytes that matured to MII in αMEM were parthenogenetically activated by strontium chloride treatment (78% and 74%, respectively). Additionally, 10% of matured oocytes of both strains developed into offspring after intracytoplasmic sperm injection and embryo transfer to foster mothers (Figure 3). Although BN oocytes cultured in αMEM could be parthenogenetically activated and developed into offspring, the success rate was lower than that for Wistar and F344 oocytes. This study demonstrated that numerous GV oocytes were produced in rat ovaries by PMSG injection. This simple *in vitro* maturation system of immature oocytes could be further developed to maintain valuable rat strains experiencing reproductive difficulties.

Figure 3. Offspring were obtained from IVM oocytes.

Publication List:

〔**Original paper**〕

● Taketsuru, H., and Kaneko., T. (2016). In vitro maturation of immature rat oocytes under simple culture conditions and subsequent developmental ability. J. Reprod. Dev. *62*, 521-526.

Research activity by T. Kimura

Specially Appointed Assistant Professor:
KIMURA, Tetsuaki

Positional cloning of iridophores mutant

See-through medaka lines are suitable for the observation of internal organs throughout life. They were bred by crossings of multiple color mutants. However, some of the causal genes for these mutants have not been identified. The medaka has four pigment cell types: black melanophores, yellow xanthophores, white leucophores, and silvery iridophores. To date, causal genes of melanophore, xanthophore, and leucophore mutants have been elucidated, but the causal gene for the iridophore mutant remains unknown. Iridophore mutant *guanineless* (*gu*) exhibits a strong reduction in visible iridophores throughout its larval and adult stages. The *gu* locus was previously mapped on chromosome 5, but was located near the telomeric region, making it difficult to integrate into the chromosome. To circumvent this, I perfomed synteny analysis using the zebrafish genome and found a strong candidate gene, *pnp4a*. Gene targeting and a complementation test showed that the candidate gene is a causal gene of gu. This result will allow the establishment of inbred medaka strains or other useful strains with see-through phenotypes, without disrupting the majority of the genetic background of each strain.

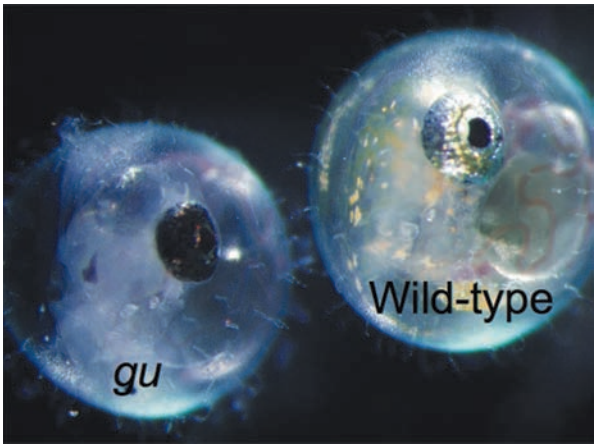

Figure 1. *gu* and wild-type.