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, 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 2013, IBBP Center stored 3,598 384-well plates consisting of 1,381,632 clones as cDNA/BACs clones, 1013 tubes for plant and animal samples and 618 tubes for microorganisms.

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 cryopreservation 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 technology 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 2013 we had eleven applications and accepted nine proposals. We are also working to establish a research center for cryo-biological study thorough this Collaborative Research Project.

Research activity by D. Tanaka

Specially Appointed Assistant Professor: *TANAKA, Daisuke*
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Cryopreservation

Cryopreservation protocols contain components which are usually developed empirically using each biological resource's specific strategy to enhance survival (Benson 2008). The theory of cryopreservation encompasses several interconnected disciplines ranging from the physiological to cryophysical. Water status and cryopreservation, in combination with physiological factors, are the most influential determinants of survival (Figure 1).

So far cryopreservation has been considered in terms of the liquid and solid (ice) phases of water. It is also possible to cryopreserve tissues by the process of "vitrification", the solidification of liquids without crystallization. This comprises a "glassy state" as the system is amorphous, lacks organized structure but possesses the mechanical and physical properties of a solid (Taylor *et al*. 2004).

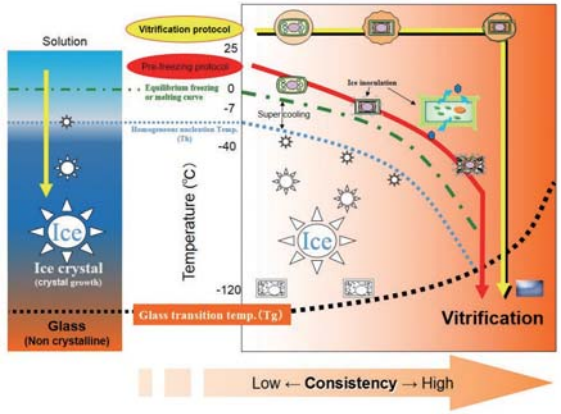

Figure 1. Phase diagram of vitrification of a solution.
Vitrification, a physical process, can be defined as the phase transition of an aqueous solution from a liquid into an amorphous glassy solid, or glass, at the glass transition temperature (Tg), while avoiding ice crystallization.

Vitrification-based protocols are known to be effective for long-term, stable preservation of plant germplasm; this protocol can reduce the cost and manpower for maintaining a large number of germplasm lines and keep many valuable genetic lines for a long term under genetically stable conditions. However, it is still not widely employed as a reliable long-term preservation protocol due to the lack of basic knowledge on the cellular and water behavior in tissues when immersed in liquid nitrogen.

In the present study, electron microscopy combined with freeze-substitution was employed to examine the ultrastructure of cells of shoot apices of a Chrysanthemum plant that were cooled to the temperature of liquid nitrogen after exposure to various steps of the Cryo-plate protocol (Figure 2-3).

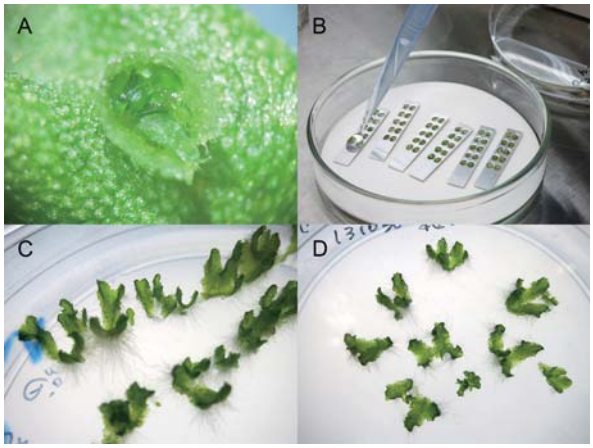

Figure 2. Cryopreservation protocol using aluminum cryo-plate.
A: Preparation of *in vitro* grown gemmae of liverwort. B: Placing precultured gemmae in a cryo-plate's wells. C: Regrowth of cryopreserved livewort line 'Takaragaike-1' 30 days after rewarming. D: Control (without cooling in liquid nitrogen).

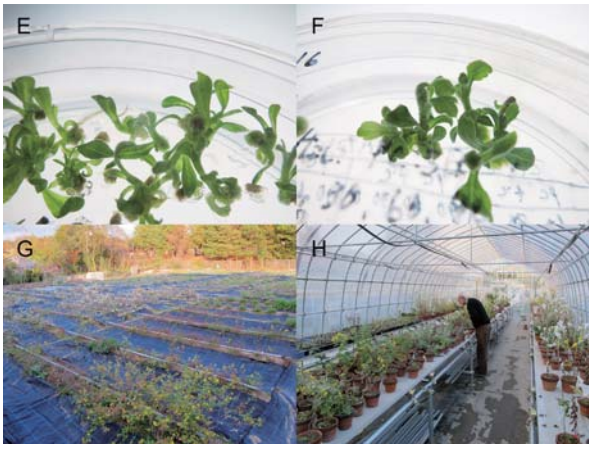

Figure 3. Plantlets regenerated from shoot apices of chrysanthemum cryopreserved by the D-Cryo-plate protocol. Shoot apices were on the post-thaw medium for 30 days. E, cryopreserved. F, control (without cooling in liquid nitrogen). G-H, Field preservation of chrysanthemum biological resources. About 2,000 races/lines are cultivated in the fields of Hiroshima University, Japan.

Publication List

〔Original papers〕

- Matsumoto, T., Akihiro, T., Maki, S., Mochida, K., Kitagawa, M., Tanaka, D., Yamamoto, S., and Niino, T. (2013). Genetic stability assessment of wasabi plants regenerated from long-term cryopreserved shoot tips using morphological, biochemical and molecular analysis. Cryo Letters *34*, 128-136.
- Niwa, N., Akimoto-Kato, A., Sakuma, M., Kuraku, S., and Hayashi, S. (2013). Homeogenetic inductive mechanism of segmentation in polychaete tail regeneration. Dev. Biol. *381*, 460-470.

〔Original paper (E-publication ahead of print)〕

- Kondo, T., Sakuma, T., Wada, H., Akimoto-Kato, A., Yamamoto, T., Hayashi, S. TALEN-induced gene knock out in Drosophila. Dev. Growth Differ. 2013 Oct. 31.

Research activity by T. Kimura

Specially Appointed Assistant Professor:
KIMURA, Tetsuaki

Analysis of median fin-rays development

The vertebrate body plan has evolved by the acquisition of structures projecting from the body axis. The original structures have transformed, as an environmental adaptation, into appendages such as fins, limbs, and wings. Median fins are the oldest of such evolved structures. In order to better understand the mechanisms by which the median fins developed, we crossed two inbred lines of medaka (*Oryzias latipes*). From the results, we found that the number of anal fin-rays was determined by two genetic traits, the anteroposterior length of the anal fin and interval between the anal fin-rays. The 19-ray fish has a longer anal fin than the 17-ray fish (Figure 1A). The 19-ray fish has the same anal fin length as the 17-ray fish (Figure 1B). This indicates that the 19-ray fish has narrower intervals between fin-rays than the 17-ray fish.

Further, the pattern of rays was independent of the pattern of the somites and vertebrae. Thus, the pattern formation of fin-rays proposes a new model of bone patterning.

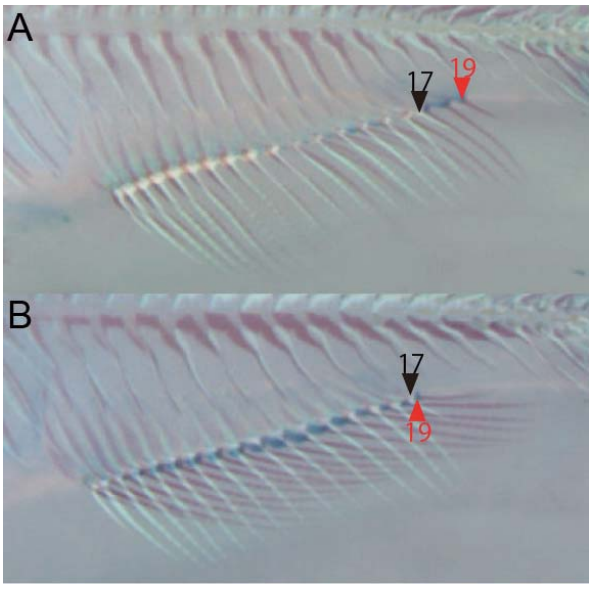

Figure 1. Superimposition of F_2 anal fin-rays. (A) Superimposed images of F_2 fish with 17 and 19 rays. (B) Superimposed images of F_2 fish with 17 (the same fish as in A) and 19 rays (a different fish from that in A). All three fish have 29 vertebrae. Note the pattern of the vertebrae is the same for both A and B. Arrowheads indicate the posteriormost ray. Numbers indicate total number of fin-rays.

Publication List

〔Original paper〕

- Ohshima, A., Morimura, N., Matsumoto, C., Hiraga, A., Komine, R., Kimura, T., Naruse, K., and Fukamachi, S. (2013). Effects of body-color mutations on vitality: an attempt to establish easy-to-breed see-through medaka strains by outcrossing. G3 *3*, 1577-1585.