BioResource Now !

Issue Number 8 June 2012

Hot News〈NO.40〉

Ten years of dedication to a single subject
—Path to the development of a freeze-drying method for sperm preservation

National BioResource Project "Rat"
Takehito Kaneko
Lecturer, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

In August 2001, I alighted at the Honolulu airport in Hawaii. This was the beginning of my research in developing a freeze-drying method for sperm preservation. A research group led by Dr. Ryuzo Yanagimachi at the Hawaii University successfully bred mice from freeze-dried sperm in 1998. My objective was to join in the NIH project led by the Dr. Yanagimachi's group and improve the efficiency of this sperm preservation method. Over a 2-year period, I conducted research on improving sperm tolerance against freeze-drying and the efficiency with which freeze-dried sperms could be produced (all this while resisting the temptations of Hawaii, a tourist's paradise). My effort was finally rewarded when I elucidated that addition of metal chelates to the preservative solution, pH, preservation temperature, and maturity of sperms greatly affected the fertilizing capacity of freeze-dried sperms.

I prefer using the freeze-drying method even though cryopreservation using liquid nitrogen is considered a more "mainstream" approach because I realized the following significant applications of this technique for the bioresource project that I have been involved in:

- Special cryoprotective agents or preservatives are unnecessary (Tris-EDTA solution can be used to preserve sperms).
- Periodical purchase of liquid nitrogen and replenishment of liquid nitrogen tanks are unnecessary (freeze-dried sperms can be preserved in a refrigerator).
- Facilities and maintenance costs can be reduced greatly.
- Management and replication of preserved samples are easy.
- Freeze-dried sperms can be shipped internationally at room temperature without dry shipper or dry ice (freeze-dried sperms are viable for 3 months at room temperature).

Therefore, even after returning home, I could continue my research aimed toward improving the efficiency of the freeze-drying sperm preservation method, while I was engaged in the bioresource project. It is our priority as a part of the bioresource project to conserve precious genetic resources reliably for the next generation. Hence, mouse and rat strains have been preserved using frozen sperms and fertilized eggs to alleviate potential risks such as infectious diseases and genetic contaminations that may occur if individual animals are bred. However, this requires considerable running costs in order to expand facilities for the increasing numbers of preserved strains, increased liquid nitrogen usage, and periodical facility maintenance.

In addition, we recently experienced natural disasters, the Tohoku earthquake and the tsunami in March 2011. These events compelled us to reconsider the safety aspect of future bioresource projects. The disaster dramatically changed my earlier view—the development of the freeze-drying sperm preservation method is still underway, at the research level, and practical application is futuristic. Many research samples preserved in refrigerators and freezers were lost after the disaster because of long-term electric outages. The liquid level inside a liquid nitrogen tank can be maintained unless the tank is damaged, even following electric outage; thus, genetic resources were saved initially, right after the disaster. However, the depleting liquid nitrogen, evaporating at a rate of 5-10 L/day, was a serious threat as a result of devastated production factories and termination of supply lines caused by road closures and disruption to the transportation system.

It was then that I realized we urgently need to develop a new, liquid nitrogen-free, long-term preservation method by which genetic resources can securely be preserved even during natural disasters.

Under such circumstances, we successfully developed a long-term, freeze-drying method for sperm preservation. Fertile offspring were born from freeze-dried rat and mouse sperms that had been preserved in a refrigerator at 4°C for 5 and 3 years, respectively (Photo 1). The litter sizes in both rats and mice were not different from those produced from the sperms that were used immediately after freeze-drying; therefore, any deterioration of sperms or a decrease in their fertilization capacities were undetected. The result thus highlighted a practical application of the freeze-drying sperm preservation method: genetic resources can be preserved and transported more safely, easily, and inexpensively than the previous cryopreserved method using liquid nitrogen. In addition, this method will be an effective tool for preserving transgenic and congenic strains that can pass on genes to the next generation only with sperms and for replicating the preserved strains in an easy and inexpensive manner. Thus far, there have been no reports showing successful freeze-drying of a fertilized egg. Inbred lines need to be preserved as fertilized

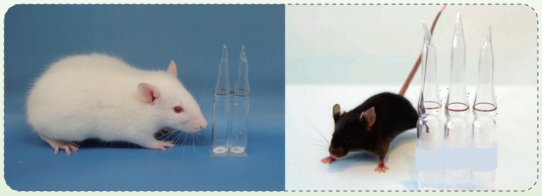

Photo 1: A rat (left) and a mouse (right) produced from freeze-dried sperms that had been preserved in ampules and stored in a refrigerator at 4°C for 5 years.

↳ To the next page

eggs; hence, it is impossible to freeze-dry all rat and mouse strains. However, significant advantages can be gained in cost and safety aspects simply by preserving sperms using a freeze- drying method, whereas fertilized eggs are stored using the liquid nitrogen method.

The successful development of the freeze-drying sperm preservation method required 10 years. This is indeed "ten years of dedication to a single subject."

This sperm preservation method will likely find applications for protecting wild animals and preserving elite livestock. With this in mind, I would like to take a new role in developing a long-term preservation of sperms at room temperature and imple- menting a freeze-drying preservation method of fertilized eggs; these techniques are expected to be adopted for successful long-term preservation of other animal species.

References:

- *PLoS One* 7(4): e35043, 2012
- *Cryobiology* 64: 211–214, 2012

Related websites:

- http://www.anim.med.kyoto-u.ac.jp/reproduction/sperm_freeze-drying.aspx
- http://www.anim.med.kyoto-u.ac.jp/reproduction/home.aspx
- http://www.kyoto-u.ac.jp/ja/news_data/h/h1/news6/2012/120410_1.htm (Japanese only)

Installation of BLAST+

In 2009, NCBI released BLAST+, the improved version of the conventional BLAST (Legacy BLAST). Primary improvements of BLAST+ include a division of a very long query sequences and an introduction of partial match search in the BLAST Trace-back operations; therefore, processing of a large-scale search, which was extremely time consuming in the previous version, can be executed in a relatively shorter time.

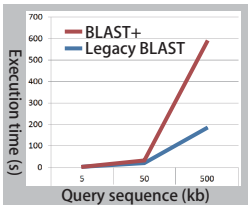

Fig. 1: Execution time of tblastx in BLAST+ and in Legacy BLAST

Fig. 1 shows the difference between the search time in BLAST+ and Legacy BLAST. We measured a processing time of executing tblastx with the filtering turned off, using sequences of 5 kb, 50 kb, and 500 kb as queries. The sequences were obtained arbitrarily from the whole genome sequence of Escherichia coli (4.6 Mb) as a database.

※The execution environment is listed in Table 1.

As a result, it was found that the longer the query sequence, the greater the difference between the execution time in BLAST+ and in Legacy BLAST. Similar to Legacy BLAST, BLAST+ can be used in a local environment upon installation. The next step I will introduce here is to install BLAST+ in a Linux environment as an example and then execute the program.

Table 1: Specifications of the machine

OS	CentOS 6.2 (32bit)
CPU	AMD Athlon™ 64 X2 Dual Core Processor 3800+(2.0GHz)
Memory	2.0Gb
HDD	1Tb, SATA 3.0, 7200rpm

Installation procedure of BLAST+

① Open the download page of the newest version of BLAST+ from the download page of BLAST on the NCBI website:
ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/
② Download the newest version of the file as of June 2012:
ncbi-blast-2.2.26+-x64-linux.tar.gz, to an arbitrary directory.
Attention:The download may take time depending on the specifications of the computer.
③ Double click and unzip the gz file.
④ Input a command from the terminal (Table 2-a), and make a path through the bin folder containing the BLAST execution file.

Ongoing Column [No 70]

⑤ Finally, input "blastn -version" and execute the program. Installation is successful if the BLAST+ version is displayed (Fig. 2).

```
$ blastn -version

blastn: 2.2.26+
Package: blast 2.2.26, build Feb 9 2012 16:01:19
```

Fig. 2: Example of version information

Format of database

① Open the download page of the database from the BLAST download page on the NCBI website: ftp://ftp.ncbi.nlm.nih.gov/blast/db/
② Access the FASTA directory and download the target file to an arbitrary directory.
③ Input and execute the commands shown in Table 2-b or 2-c from the terminal.
Attention: Name of an output database and construction of hash index are unnecessary.
④ Upon execution of the program, database formatting is successful if the constructed database information is displayed in the terminal.

Execution of BLAST

① Prepare a query in FASTA format.
② Input and execute commands listed in Table 2-d and 2-e from the terminal.
③ Check whether the result file is output in the current directory, which indicates that the process was successful.

Table 2: Command list

Item No.	Objective	Command
a	Make a path	export PATH=$PATH : absolute path to the designated directory
b	Format of base sequence DB	makeblastdb -in FASTA file -dbtype nucl -out database -hash_index
c	Format of amino acid sequence DB	makeblastdb -in FASTA file -dbtype prot -out database -hash_index
d	blastn	blastn -db database -query query file -out output file
e	blastp	blastp -db database -query query file -out output file

We highly recommend BLAST+, which exercises the full potential for a large-scale search.

(Kyohei Matsuno, Genetic Resource Center)

Recommended Book ! 〈NO.6〉

"Cellular slime molds—their astonishing intelligence"

Toshiyuki Nakagaki (PHP Science World, 2010)

The author is famous for receiving the Ig Nobel Prize twice. The Ig Nobel Prize is awarded to research achievements that "first make people laugh, and then make them think."

One of the impressive abilities of true slime molds, which are unicellular organisms, is the ability to solve a maze puzzle; this discovery lead to the award of an Ig Nobel Prize in 2008. This book describes how, if a slime mold is left in a maze and baits are placed at the entrance and exit of the maze, the slime mold extends itself from the entrance to the exit with the shortest path, trying to eat the 2 baits simultaneously. In other words, the slime mold wants to eat both baits, but without dividing itself into 2, and thus maintains its single body with the shortest tube or protoplasm.

Another story is about the "slime mold representing the railroad network of the Kanto area of Japan," for which the author was awarded the Ig Nobel Prize in 2010. If baits are placed on primary cities on the map of the Kanto area portrayed on an agar plate and a slime mold is set on the position of Tokyo, the cell extends itself by protoplasmic streaming and reaches the baits. The cell attempts to eat many baits simultaneously and thus forms a network of tubes, the shape of which resembled an actual Japan Railways (JR) route map. In doing so, the cell optimized the tube extension process in 2 ways: by minimizing the total extension of the tubes and by assuring the connection of the body in case of tube breakage (connection assurance). The experimental evidence gained from these and other studies leaves one to conclude that cellular slime molds surely exhibit "astonishing intelligence." (K.N.) (This book is only in Japanese)

Contact Address

Genetic Resource Center,
National Institute of Genetics
1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan
Tel.: 055-981-6885 (Yamazaki)
E-mail : brnews@shigen.info

Editor's Note

Innovation in resource preservation technology greatly contributes to the administration of resource centers. In addition, the influence of the innovation is immeasurable if the technology will expectedly be widespread to laboratories throughout the world in future. By any measure, the persisting endeavors of Dr. Kaneko for 10 years from the discovery of a new technology to its practical application are nothing short of remarkable.

(Y.Y.)

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

BioResource Now !
Issue Number 8 June 2012

"translated by ASL translation service"