BioResource now !

June 2005

BioResource now! No.6 is here

Article on silkworm : pg 3 - 4

Download the PDF version of this newsletter at
http://www.shigen.nig.ac.jp/shigen/news/news.jsp

Introduction to a Resource Center No.1

Hiroshi Mizusawa, Leader of JCRB Cell Bank, Head of the JCRB Dept.,
National Institute of Biomedical Innovation (NiBio) http://www.nibio.go.jp/

Objectives of JCRB in Ministry of Health, Labor and Welfare

History of the JCRB Cell Bank

The Ministry of Health, Labour and Welfare established the "Japanese Collection of Research Bioresources (JCRB)" in 1985 in accordance with the "Comprehensive 10-year Strategy for Cancer Control" to support cancer research. At that time, Japan depended heavily on other countries for experimental materials because organizations that supports researches such as cell banks were rare in Japan. As Japan started receiving acknowledgements as a developed country, the growing need for the establishment of an independent research organization urgently necessitated an improvement in infrastructure. In these circumstances, establishing the JCRB was an epoch-making measure at that time but a necessary step nonetheless. As a result, the Japanese Cancer Research Resources Bank consisting of a cell bank and a gene bank was founded. The cell bank was placed in the National Institute of Health Sciences (NIHS) while the gene bank was placed in the National Institute of Infectious Diseases (NIID). The free distribution of accumulated resources during the first 10 years fueled the rapid increase of demand for research bioresources as many researchers began utilizing them. At the end of the "Comprehensive 10-year Strategy for Cancer Control" in 1995, the cell bank had achieved a distribution of 5000 ampoules annually. (Figure 1)

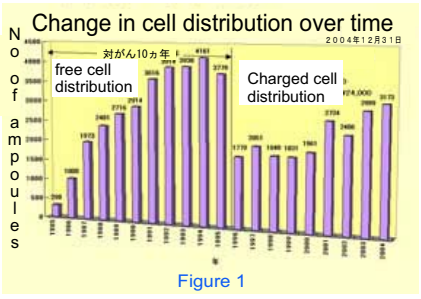


Figure 1

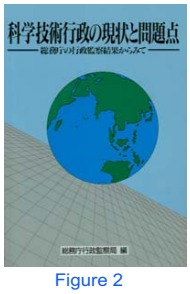


Figure 2

At that time, the cause of cancer was understood to be closely related to genetic functions. Due to that, fundamental researches of molecular biology researches were thriving and along with it, the demand for cancer related genes and cultured cells grew. During this period of time, research resources banks were being established internationally in rapid succession and together with the development of fundamental cancer researches, the relationship between genes and the carcinogenic mechanism were elucidated successively.

JCRB after the "Comprehensive 10-year Strategy for Cancer Control"

In the late 20th century, an international team of researchers achieved a scientific milestone by unraveling for the first time the human genome sequences. This completely revolutionized life sciences researches in the 21st century. Researchers started using genes as blueprints to explain life phenomena. Cultured cells functioning as the smallest unit of life was again basked in attention. Now that human genome sequencing is completed, researchers are focusing on studies to understand the hidden functions of genes, thus proving again the invaluable existence of living cells.

The demand for cultured cells rose continuously since the establishment of the cell bank and although it was apparent that the "Comprehensive 10-year Strategy for Cancer Control" needed to be extended, a solid plan for its continuation was not formed when it came to an end in 1995. Meanwhile in 1991, the Ministry of Internal Affairs and Communications conducted an administrative evaluation of the national Science and Technology Policy and published a report entitled "Science and Technology Policy - Status and Problems" in July 1992. (Figure 2)

The report strongly acknowledges the need for research bioresources and achievements of the JCRB Cell Bank were highly praised. Moreover, the report also recommended that the JCRB Cell Bank be extended and reinforced with proper manpower. As a result, the continuation of the JCRB Cell Bank was decided and the number of staff was increased. The Gene Bank which was under the administration of the National Institute of Infectious Diseases was also extended. It was then considered appropriate to charge for resource distribution and due to the fact that it would be troublesome for a national research organization to handle paid resource distribution, the Japan Health Sciences Foundation established the Health Science Research Resources Bank (HSRRB) to handle cell distribution. Since then, the JCRB Cell Bank and HSRRB collaborated on the undertakings of the cell bank.

Our role in supporting researches

This year marks the 20-year milestone for JCRB Cell Bank. Over the years, we have stayed true to our emphasis on our position in supporting researches because we understand that it is important for the accumulated cultured cells to be utilized as bioresource by as many researchers as possible. As a result, we have adopted a research style that results in studies to be shallow yet extensive. Results obtained from observing a particular cell has to be verified by all the other cells under preservation.

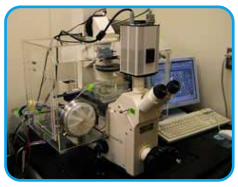
A typical example of this research style is the studies that we have been conducting recently to evaluate the individualities of cultured human cells by using STR analysis. We are using the well-established STR-PCR methods to conduct this experiment. We extracted DNA from cell lines and used the PowerPlex 1.2 System by Promega to analyze 9 different loci to examine if each cultured cell was unique. Comparative studies usually required loci profiles to be recorded and archived in a database so that an analysis program which uses this database to compare cells can be developed. However we have developed the Cell ID system, an original system that runs on the internet and is able to perform the comparative analysis mentioned above quickly. Please take a look at this system as it is publicly available from the JCRB Cell Bank website.

JCRB Cell Bank http://cellbank.nibio.go.jp/

Although it is just a matter of examining if 2 kinds of arbitrary chosen human cells are identical, the results can detect cross-contamination and also verify the cells. For this study, approximately 800 kinds of human cells were examined and the results show that 7.6% of the human cells kept in the JCRB Cell Bank were found to have errors in them. This study will have to be continued permanently because cross-contamination is unavoidable.

If a cell was used in researches to the day that it was confirmed to have an error, this would mean that researchers have published papers based on an erroneous cell. In such circumstances, then identifying an error and notifying researchers about the error should also be considered as an important task for the cell bank.

The task of correcting errors in cells is very important but it is not an easy task. Although cell culture has long become a technology used by everyone, it is no longer taught as a subject in universities, resulting in the diminishing awareness of the need to inherit its scientific research methods. As a result, cases of cell culture carried out without consideration for the importance of contamination or cross-contamination are on the rise. In order to rectify this situation, it is necessary to take measures such as encouraging people who are handling cell culture to look back on its historical progress and creating opportunities to ponder over the meaning of cell culture technology. It is also important to collect references for this purpose.



Left: The latest model of movie camera for cultured cell
Right :The first cell culture movie recording device in Japan invented by Prof. Katuta which utilizes inverted microscopes.

In addition, the cell bank is required to deal with research materials obtained from human specimen and therefore should actively study issues concerning research ethics. Currently, ethical issues on research have stirred intense controversy from the public and should be handled with caution.

Can cells be merchandised?

It is possible for private enterprises to deal with research materials if they have commercial value and their distribution is profitable. As it is, there are many vendors for DNA which has commercial value.

Cell culture maintenance requires a lot of manpower. Moreover, if quality control tests such as inspections for contamination or evaluations for individualities are conducted, they would increase expenditure. As a result, many private enterprises have not been able to make a profit. Due to this, even the Institute for Fermentation, Osaka (IFO), internationally acclaimed as a preservation center for microorganisms and animal cultures, has stopped administrating the preservation of resources. Even in the international scene, private enterprises are withdrawing from cell banks undertakings one after another and it has become common for such businesses to be operating with subsidies offered by either the government or local public organizations. Such businesses are now focusing on developing commercial products with materials obtained from research resources bank instead of commercializing cells.

Please enlarge these photos on your computer to see the details.

It is difficult to commercialize bioresources because it is necessary to preserve and stock a variety of cell cultures even those with little demand. The cell bank is now preserving cells that presently have absolutely no demand. Who can say that cells with no demand should not be preserved? The trends of researches are incomprehensible and it is difficult to accurately predict the utilization of resources. Cells with no demand at present could have a new found use and value in the future. If the success of researches depended on the research methods and the needs of the times, then no one can declare definitively that cell cultures of no value now will not be of great value in years to come. Therefore, the fundamental practice is that every cell culture that a researcher wishes to deposit shall be preserved. Financially speaking, this would be a burden and is not a good way for enterprises to make a profit. This is the main reason why cells are not suitable as products for sale.

A change of location and thereafter

The JCRB Cell Bank, after being housed for 20 years since 1985 at the National Institute of Health Sciences (NIHS) in Yoga has moved to a new facility, NiBio which is located in a town called Saito, a new town established this year in the northern region of Ibaraki City, Osaka and has commenced services on 1st April 2005. The facility in Yoga was cramped and it was difficult to take safety measures. The transfer has helped to solve this problem and it is easier to work now. After the end of the “Comprehensive 10-year Strategy for Cancer Control”, JCRB teamed up with the Health Science Research Resources Bank (HSRRB), established by the Japan Health Sciences Foundation, to administer the JCRB Cell Bank with the support of the Ministry of Health, Labour and Welfare. Now that both institutions are located in the Kansai Area, I think our work efficiency would improve.

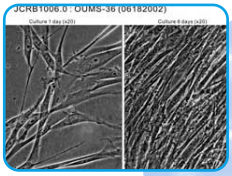
NiBio in Saito

HSRRB in Sennan

The NIHS Cell Bank and the NIID GeneBank moved to Saito too and together with NIID’s Experimental Animals Research Division form a new organization structure called Bioresources Division in NiBio. This division consists of a cell resources laboratory (now called JCRB Cell Bank), a gene resources laboratory (now called JCRB Gene Bank) and an experimental animal research laboratory.

The JCRB Cell Bank has a team of 3 full time staff, 4 part time staff and 2 guest researchers, all whom are very enthusiastic about constructing a new cell bank. They have also successfully started cell deposition services.

In the future, we will strive to contribute to all research activities in Japan by considering the appropriate structure needed by our administration and examining the fields of research which should be carried out in the research resources bank and we hope to develop into a useful cell bank.



Cell image in the online catalog

DNA sequencer for STR analysis

Preserving cells in liquid nitrogen filled tanks

Resource Feature (Silkworm) No. 4

Visiting Kyushu University

We visited the Institute of Genetic Resources, located in the Graduate School of Agriculture, Kyushu University, which is the designated resource center for the National BioResource Project – Silkworm. We are constructing a database of silkworm resources (SilkwormBase) together with the staff there. This was the best season to observe the various phenotypes of the silkworm strains as well as the preservation work carried out.

There are few strains which eggs can be cryopreserved and most stocks have to be maintained by breeding. As we learnt from this visit, many people from professors to technical officers, part-time staff, alumni, volunteers and students have contributed to the success in strains conservation. We were given a great opportunity to observe the busiest stage of their strain conservation undertakings.

The Institute of Genetic Resources

We took the subway airport/ Hakozaki line from Hakata station, got off at Kaizuka station and walked for 5 minutes to arrive at the Institute of Genetic Resources. It was located in a quiet area, interspersed with fields and houses, giving out a warm atmosphere. The sight of bamboo boxes used to breed silkworms (right) lined out in the sun to dry by a mulberry field made us feel quite nostalgic. Upon entering the institute, we heard silkworms chomp on mulberry leaves as everyone was busy going about their manual work.

Up to the age of 5 (feeding and larvae inspection)

The silkworms were kept in rearing boxes, sorted by their strains and fed mulberry leaves until they reach age 5, a process that takes about 2 - 3 weeks after the eggs hatch (Photo 1). During this process, the silkworms were fed 5 times a day in accordance to their growth, which was quite an ordeal for the staff. Mountains of mulberry leaves were kept in storage to feed the two hundred thousand silkworms which consume about 500 kg of mulberry leaves daily (Photo 2).

When the silkworms reach age 5, the larvae go through an inspection. About 500 strains are examined for visible mutation features. This inspection is carried out at each stage as the silkworms go through the egg, larva, pupa and adult stages (Photo 3).

● Photo 1

Feeding in rearing boxes

● Photo 2

Mountains of mulberry leaves

● Photo 3

Larvae inspection

Mounting

Once the silkworms reach age 5, they stop feeding on mulberry leaves and move away from the center of the rearing box to search for a corner. Once they find a good spot, they stop moving and face upwards to spin their cocoons. This was a very fascinating sight to observe (Photo 4). Matured silkworms were transferred onto the mountage or cocoon frames for cocooning, a process referred to as “mounting” and moved from the rearing room to the mounting room (Photo 5).Soon, the silkworms started secreting impurities from their bodies, causing their bodies to shrink. Then they attached themselves to the mountage and started spinning their cocoons (Photo 6).

● Photo 4

Spinning cocoons

● Photo 5

Mounting room

● Photo 6

In the mountage

Cocoon inspection

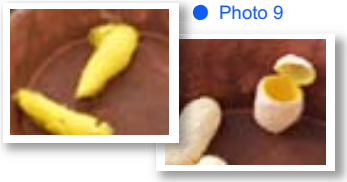
During our first visit, we were able to observe the larvae at age 5 and the mounting process. We visited the center again after a week to observe the cocoons. During our second visit, the larvae had fully cocooned themselves and the staff was in the midst of inspecting the cocoons and pupae. The larvae that we last saw attached to the frames in the mounting room had turned into yellow, white or rose-colored cocoons (Photo 7). The cocoons were first removed from the mountage, tagged with dates and their stock name and sorted out in containers (Photo 8). Then inspections of color, size and strain contamination were conducted. This is an important task because strain conservation would not be possible if they were contaminated by invasive species. It is difficult for a novice to differentiate the patterns and texture of the cocoons and therefore it was task that would be greatly influenced by experience.

Furthermore, there were cocoons from Europe and China (Photo 9) and also cocoons from different regions which showed significant differences in color and size. We were able to view the cocoons of about 500 strains and were surprised by the diversity of the strains.

● Photo 7: Cocoons in the mountage

● Photo 8 : Cocoons in containers

● Photo 9



● Photo 10
Extracting pupae from cocoons

Next, the pupae were extracted from the cocoon by hand (Photo 10) and their size, shape and wing formation were inspected. After the inspection, the pupae were examined with the naked eye one by one and separated according to gender.

After that, pairs of pupae were put into a special container (Photo 11) to breed and produce eggs. This is to ensure that eggs will be laid following the round shape of the container, a state in which they will be preserved. (Authors: Mutsumi SAITO, Miharu YOSHIOKA)

● Photo 11 : Special containers to make silkworm lay eggs

Our kind hosts

Prof. Fujii

Prof. Banno

Women Workers during Wartime

We were shown records of strain inspection carried out continuously for 1 - 4 times a year since 1912 (left). They show important strains’ phenotype data recorded during inspection. In 1944, women played an important role in this as men were drafted into the war.

First volume of 1944

Records during the war

Reporters

Info on silkworm resources

Silkworms no longer exist in the wild. They are kept and conserved by humans and have become fully domesticated. Japan is the only country to systematically collect and preserve silkworm strains. They are a genetic resource unique to Japan but an asset to the world.

Silkworms are widely used by researchers to study genetics, physiology, biochemistry, and pathology. Recently, along with the progress of the "silkworm genome project", studies of silkworm genes with special functions ranging from feeding habits and taste, to the resistance and sensitivity to pathogens such as viruses; fungi; bacteria; and mold are performed intensively. These results make silkworm, a Lepidoptera insect, a premier model for studies of pest control and will facilitate the production of new pesticides.

The Institute of Genetic Resources is currently pursuing the following undertakings under the National BioResource Project (NBRP).

1. Establishing a system to supply highly reproducible and stable materials
2. Accumulating and conserving mutants related to functional genes
3. Accumulating, conserving and supplying silkworm genome resources

Further information on resources such as how to search for resources and resource distribution is available from the website below.

Details of silkworm resource

SilkwormBase (test version) offers a search engine for silkworm resources and further details are as follows.

SilkwormBase is composed of
1. Genetic resource stock information
2. Mutant genetic information
3. Reference information (focused on references related to the gene name)
4. Distribution request information
5. Related site links, etc.

SilkwormBase : http://shigen.lab.nig.ac.jp/silkwormbase/

(test version)

Public information on resource

● Classification by phenotype

Symbol	Phenotype	No. of related strains
X	new mutant in analysis	13
a	lethal: embryo & larvae	18
b	cocoon: shape & quality	17
c	cocoon: color	26
d	egg: shape & chorion	30
e	egg: serosa color	28
f	larvae: appendage & marking	37
g	larvae: marking	16
i	larvae: eye-, head-, & tail spot	13
k	larvae: body color	23
l	larvae: body color	28
m	mosaic & malformation	16
n	larvae: body shape	26
o	larvae: translucent skin	35
p	local cultivar(landrace)	18
r	chromosomal aberration	15
t	growth, voltinism, moltinism	22
u	pupa & adult	20
w	multi marker line	27

This is resource information released publicly at present. Silkworm resources are available all year long.

■Breeding Schedule

2005 Silkworm Breeding Schedule

Phase	Beginning of breeding	Fifth instar larva	Pupal stage
1	5/6	5/22 - 26	5/26 - 6/4
2	6/24	7/9 - 14	7/14 - 24
3	8/12	8/27 - 9/2	9/2 - 12
4	9/30	10/14 - 19	10/19 - 29
5	11/18	12/3 - 9	12/9 - 19

A reference for user to request strains

Information Technology Vol. 6

"About Search Engines"

It is said that the number of websites available through the Internet exceeds a few billion. Search engines are indispensable when searching through this enormous heap of information to obtain the required information efficiently. Many end users are using search engines like Google, Yahoo, Infoseek and MSN Search daily.

Search engines are also very important to us, website administrators because most users arrive at our website via search engines. Whether or not our website is listed as a search result will greatly influence the access to our website and the number of end users.

Although I mentioned in my previous article on access analysis that including access counts from robotic search engines during analysis will result in a miscalculation of the correct access total, we should not totally ignore robotic search engines because doing that might greatly reduce access to our website. We once made a mistake in denying robotic search engines access because the intensity of their access was a burden to our servers. That resulted in a loss of many end users and we had to quickly remove the access denial.

For upcoming issues, I plan to introduce the type of search engines and their features and also talk about how to use them to our advantage in increasing our access and end users.

(Genetic Informatics Lab., Center for Genetics Resosurce Info. Takehiro YAMAKAWA)

Editor's notes: Dr. Mizusawa has kindly contributed an introductory article on the recently relocated JCRB Cell Bank. An article truly filled with his passion for his 20 years' work. Since the establishment of the Genetic Resource Committee in 1999, he has given me guidance in many ways as a senior. I sincerely thank him. (Y.Y)

We were surprised by the silkworm strains of over 500 varieties, tools used since the Taisho era and intact records of strain inspection conducted over the span of 94 years. Thank you for taking the time to explain the process to us in spite of your busy schedules. We would like to thank Prof. Fujii, Prof. Banno and the entire staff of the institute. (Y.M, S.M.)

translated by Sharoh Yip

Contact: Yata 1111, Mishima, Shizuoka 411-8540, JAPAN
Center for Genetic Resources Information, National Institute of Genetics
TEL 055-981-6885 (Yamazaki)
E-mail BRnews@chanko.lab.nig.ac.jp