BioResource Now !

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Cell Bank

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Introduction

Following the advancements in genetic engineering technologies such as gene cloning by polymerase chain reaction (PCR) techniques and the generation of mutant mice by fusing embryonic stem (ES) cells and homologous recombination, gene function analysis has rapidly developed since the end of the 20th century. Techniques such as cell reprogramming by nuclear transfer methods and ES cell culturing, which were developed in the 20th century, have blossomed into induced pluripotent stem (iPS) cell technologies in the 21st century. The iPS cell technologies have revealed that somatic cells can easily be reprogrammed into ES cell-like pluripotent stem cells and even into other cells without forming pluripotent stem cells. I believe that the "era of freely manipulating cells" has emerged from the "era of freely manipulating genes." In such an environment, the number of cell line types has increased extensively.

Quality Control

As mentioned earlier, genetic engineering technologies have advanced greatly and the number of cell types used by researchers has also increased rapidly. With an increase in the number of cultured cell types, a quality control system common to all cell lines has been established.

The first step in cell culturing is microbial decontamination. In a cell culture system, contamination with microorganisms such as bacteria, fungi, viruses, and mycoplasmas may occur. Bacterial contamination can be prevented by adding antibiotics (generally penicillin and streptomycin) to the culture medium. Fungal contamination can also be prevented to a certain extent by adding antibiotics to the culture medium. Nevertheless, contamination with bacteria or fungi is not a major scientific issue because results obtained using a contaminated cell culture system are generally not published.

On the other hand, a viral contamination is slightly more complex. Contamination with a certain virus may occur in the cells during the culture process. Viral contamination may depend on the characteristics of the cells and diseases. It is not economically feasible to test for all possible viral contaminations in a sample. RIKEN BioResource Center (BRC) Cell Bank tests for only hepatitis B and C viruses in hepatocytes and human immunodeficiency virus and human T-lymphotropic virus in hemocytes considering the viral tropism*1. These viruses are important, because they are biological hazards.

Mycoplasma Contamination

Mycoplasma*2 contamination is one of the most difficult problems amongst various microbial contaminations, because, in such cases, the cultured cells continue to grow along with the contaminants. Moreover, the proliferation rate of the contaminated cells is sometimes faster than that of the non-contaminated cells. It is not possible to detect a mycoplasma contamination by only observing cells through a phase-contrast microscope in the routine evaluation. The only solution to this problem is periodic monitoring of the cultured cells for contamination with mycoplasmas during an experiment. However, most researchers consider this to be troublesome and often omit this step. As a result, cultured cells contaminated with mycoplasmas are often used in experiments. The recent issue of Nature strikes a warning note regarding this matter (Fig. 1).

Several methods exist for determining mycoplasma contamination, of which the sensitivity of the DNA staining method is the highest. RIKEN BRC Cell Bank has adopted the DNA staining method for routine examination (Fig. 2).

Commercial kits are now available, which facilitate the detection of mycoplasma DNA by PCR, and the identification of mycoplasma-specific enzymes. Simple methods that utilize these kits are recommended for general research purposes.

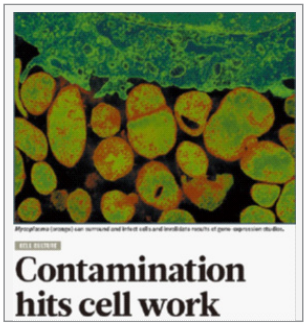

Fig. 1: *Nature* 511, 518 (2014)

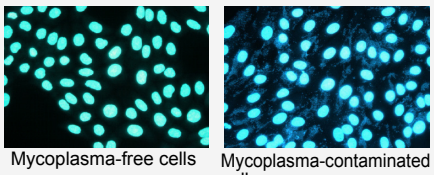

Fig. 2: Examination of mycoplasma contamination
Target cells were cultured in a medium and added to the Vero cell*3 culture system. The Vero cells were cultured for approximately 1 week, the cultured cells were stained with a DNA-staining agent, and the cells were photographed. The cells in which only nuclei were stained were considered mycoplasma-free. The cells in which both the cytoplasm and the nuclei were stained were considered to be contaminated with mycoplasma; the existence of microorganism-derived DNA in these cells was attributed mainly to mycoplasma.

Cell-Line Misidentification

Often, a cell line that is believed to be derived from stomach cancer specimens may actually be derived from a uterine cancer specimen; this is known as a cell-line misidentification and is an important issue among research communities. Similar to mycoplasma contamination, researchers are often unable to discern cell-line misidentification during the observation of cells under a phase-contrast microscope in routine examinations. Cell lines cannot be identified without analyzing the cells at the genetic level. Microsatellite*4 (short tandem repeat: STR) polymorphism analysis is a highly accurate examination method, which is used in criminal investigations. Since this method was reported to be applicable to cultured cell lines, major cell banks worldwide have adopted STR polymorphism analysis for routine cell line examinations (Fig. 3).

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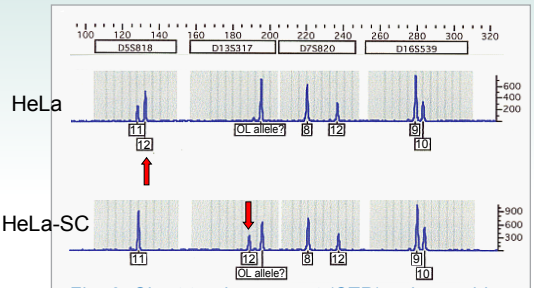
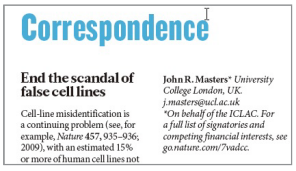


Fig. 3: Short tandem repeat (STR) polymorphism analysis to determine cell-line misidentification
This figure shows the results of an STR analysis of HeLa and HeLa-SC cells. HeLa-SC is a substrain of HeLa. Although polymorphisms of eight loci were analyzed, this figure shows the results of only four loci. Two peaks derived from the father's and the mother's alleles were detected on each locus. However, when the length of the STR derived from the father was the same as that from the mother, only one peak was detected on each locus. The peaks of HeLa cells are not always similar to those of HeLa-SC cells due to mutations, which occurred during long-term culturing.

The method to detect cell-line misidentification includes specificity that cannot be completed in a general laboratory. A general laboratory may use a cell line after ensuring that it is free from mycoplasmas by performing the DNA staining method, which is the most sensitive method for the same. However, for detecting cell-line misidentification, the cell line to be tested must be compared with all available cell lines in the world, considering that cell lines have been freely exchanged among researchers worldwide. Moreover, determining where exactly a particular cell line has been used in the previously would be difficult.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End the scandal of false cell lines

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Cell-line misidentification is a continuing problem (see, for example, *Nature* 457, 935–936; 2009), with an estimated 15% or more of human cell lines not

Fig. 4: *Nature* 492, 186 (2012)

There is an ardent need of a database that lists the results of STR polymorphism analysis on all cell lines available. Major global cell banks have recognized the importance of such a database and have therefore established the International Cell Line Authentication Committee (ICLAC), which jointly launches awareness campaigns to combat cell-line misidentification. In line with this, recently, an article for educational purposes was published in Nature (Fig. 4). However, the awareness campaigns have only just begun.

Disease-Specific iPS Cells

Cultured cells have been generally used as model for studying normal and pathological cells. In this context, typical cancer cell lines have mainly been used as models for cancer research. However, for diseases other than cancer, no disease model cells exist. The newly emerged human iPS cell establishment technology is expected to be applied to regenerative medicine as well as for the establishment of novel disease model cells. Namely, iPS cells can be established from a somatic cell derived from a patient (the established cells that are disease-specific iPS cells), disease model cells can then be generated from such disease-specific iPS cells, and these disease model cells can subsequently be used for experiments.

For example, it is impossible to collect brain cells for research purposes from a patient suffering from degenerative encephalopathy. However, iPS cells that are established from patient's skin or blood cells can be used to induce cranial nerve cells, which may in turn be used as disease model cells. In clinical practice, biopsy is performed to collect tissues from the heart, liver, or kidney, but the risk of hemorrhage in such cases is high. If a target tissue can be differentiated and induced from iPS cells, the disease-specific iPS cells could replace conventional biopsies; the potential for this application is tentative as the research on disease-specific iPS cells has only just begun, and it remains to be ascertained whether disease-specific iPS cells can be used as a platform in certain fields.

Genome-Editing Cells using CRISPR/Cas9

Genome-editing technologies have rapidly advanced. In particular, the recently developed CRISPR/Cas9*5 technology has attracted the attention of numerous researchers and its application is being studied in all fields of the life sciences, including cell culture. In order to establish disease-specific iPS cells, the presence of the corresponding disease is mandatory. For a disease whose causative gene has already been specified, even when multiple causative genes exist, disease-specific iPS cells can be artificially established by introducing a gene mutation that causes the disease in normal iPS cells using CRISPR/Cas9 technologies. Various types of disease-specific iPS cells with different genetic backgrounds can be established by using a large number of normal iPS cells derived from different individuals.

Conclusion

For the new cell lines discussed in this paper, it is important to establish a new quality control method that differs from the conventional quality control methods. We need to develop our cell bank projects while considering the maintenance of harmony between the advancement of science and socio-economic conditions

"For users of RIKEN BRC Cell Bank"

The general instructions for submission to all journals published by the American Association for Cancer Research (AACR) describe the policy regarding authentication of cell lines. Other journals have also begun to adopt similar instructions. These general instructions are available in HTML format at

http://www.shigen.nig.ac.jp/shigen/news/n_letter/2014/n8/aacr_contribution_rule.html

*1 : The determination of a cell type that can multiply based on the virus type.
*2 : A parasite with a eukaryotic host.
*3 : A cell line derived from the renal epithelial cells of Cercopithecus aethiops.
*4 : A repetitive sequence existing on a cell nucleus or organelle genome consisting of simple sequence repeats with multiple bases.
*5 : A genome-editing tool based on advanced technologies.

Database of this Month

National BioResource Project "Chrysanthemum"

- Number of wild strains:204
- Number of experimental strains: 36
- Number of other strains: 20

(As of August 2014)

DB name : Chrysanthemum
URL : http://www.shigen.nig.ac.jp/chrysanthemum/
Languages : Japanese, English
Original contents :
- Information on the localities, phenotypes, polyploidy, and photographs of wild, experimental, and other strains are available to the public.
- Details of the role of Chrysanthemum seticuspe as a model organism, the structures of chrysanthemum flowers, autopolyploidy-enhanced evolution, endangerment of wild chrysanthemums due to genetic pollution, and the development of DNA markers that allow differentiation between species are included.

Features :
- In the resource lists, resources have been classified in an easy-to-understand manner.
- Strains with available photographs are labeled with a camera icon.
- The core institution can upload strains data in Microsoft Excel format to publish them.

Cooperative DB : Research Resource Circulation (RRC)
DB construction group : NBRP Chrysanthemum NBRP Information
Management organization: Genetic Resource Center, NIG
Year of first DB publication: 2004 Year of last DB update: 2013

Comment from a developer: The NBRP Chrysanthemum database consists of a content management website and a public website. The core institution can make any database related changes, including uploading and publishing information about strains and related image files. When uploading strains data, the "Strains" menu on the public website will be created dynamically according to the numbers of strains and subclasses, enabling customization of the order of items in the menu.

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Editor's Note

Dr. Yukio Nakamura kindly wrote about the RIKEN BRC Cell Bank two times, once in 2005 and once in 2008. The article of the month revealed that the field of cell culture, including disease-specific iPS cells and genome-editing cells, has rapidly advanced within a short period of time. Dr. Nakamura also introduced the latest information about quality control of cell lines worldwide, which is a sensitive issue among researchers. Quality control is the essence of resource projects required to secure reproducibility in experimental science. The ripple effect of quality control is expected to influence other resources (Y. Y.).

BioResource Information

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