Micro-technology

Gene Measuring System capable of safe and reliable diagnoses

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Many genome annotations have been accumulated through the Human Genome Project, heralding a new era of healthcare. In the medical area, personalized medicine is being introduced in hospitals and clinics, while in the areas of living, the environment, and industry, gene measuring systems are expected to help achieve healthy lives and environmental hygiene in terms of the safety of foods and the preservation of nature and water. This paper describes the current situation in the medical area which will soon be revolutionized, as well as the on-going application of biological information to industry. It then examines the technical issues of the gene measuring system required, and how Yokogawa is working to solve them by using micro-technology and photonics technology.

INTRODUCTION

Many gene annotations have been decoded through the Human Genome Project by the International Human Genome Sequencing Consortium. This enormous amount of digitalized biological information paves the way for a new era of health-care where "personalized medicine" as well as preventive medicine are expected to be practiced in hospitals and clinics.

Meanwhile, in the areas of living, the environment and industry which are continually changing, biological information is expected to help ensure healthy lives and environmental hygiene including the safety of foods and the preservation of nature and water.

Breakthroughs in the living environment have always been brought by technological innovations. This paper predicts what the ongoing revolution in the medical industry might lead to, and investigates industrial applications of biological information. It then examines the technical challenges required for gene measuring systems, and introduces how Yokogawa is addressing the challenges using micro-technology and photonics technology.

APPLICATION AND DEVELOPMENT OF GENE MEASURING TECHNOLOGY

There are two promising areas of application for gene measuring technology: medicine and industry. Figure 1 shows the areas that Yokogawa is targeting for gene measuring systems.

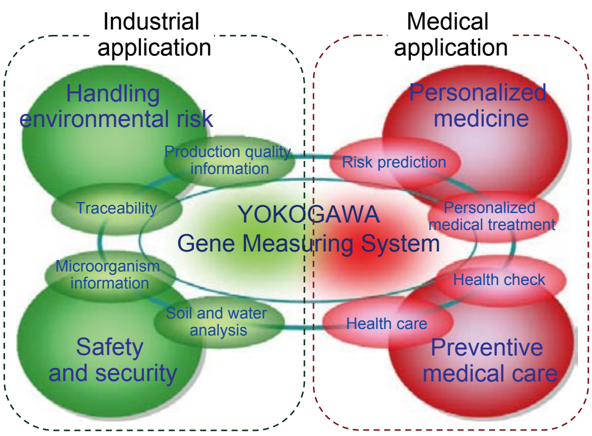

Figure 1 Application of gene measuring systems in respective area

Medical application … "Personalized medicine and preventive medicine"

Medical practice begins with measurement. Diagnosis is the first step in medicine and starts with measuring the human body, ranging from measuring body temperature and blood pressure to biochemical screening and various screenings with medical images. To use the measured results to make an accurate diagnosis, the medical industry is working on

*1 Biomolecule Analysis Center, Corporate R&D Headquarters standardizing the diagnosis by setting guidelines. This approach will provide a general and universal solution for diagnoses.

Once all genes had been completely decoded by the Human Genome Project, the work on identifying their functions started, and the causes of diseases are being clarified by identifying pathomechanisms at the molecular level of genes. Identification of the functions of genes has clarified genetic differences among individuals, leading to the new field of personalized medicine. In this approach, medicines and treatments are selected according to the individual's constitution or treatments with few side-effects are chosen by examining the patient's genomic typing and gene expression.

To achieve personalized medicine, individual gene analysis is crucial. Personalized medicine using gene analysis has already emerged in the U.S. where DNA chips (DNA: Deoxyribo Nucleic Acid) are used in the healthcare area to predict the effectiveness or side effects of medicines, with the approval of the Food and Drug Administration (FDA). In addition, in view of the need for preventive medicine, which focuses on preventing a disease before it occurs or treating a disease before it is manifested in a patient, systems such as for monitoring a person's condition need to be developed.

Industrial application … "The safety of foods and the preservation of nature and water"

As shown in Figure 1, the use of biological genetic information in industry is not confined to hygiene inspections and food safety inspections in production lines such as identifying contaminant microorganisms and ensuring quantity control, but is expanding to encompass securing safety and security and evaluating environmental risks.

In the food industry and in agriculture, forestry and fishery, genetically modified pest-resistant crops are being promoted to help stabilize or boost food production. [1] As an evaluation technology to identify such modified breeds and evaluate their safety, stable gene measuring technology is desired. Also, it is becoming possible to use genetically modified plants or microorganisms to purify the environment by absorbing hazardous substances such as heavy metals. Although chemical pesticides have been widely used for extirpation and weeding, microbial pesticides are now being developed to reduce the environmental load of chemical substances. Regarding the use of microbial pesticides, which is a new technology for environment-friendly agriculture, gene measuring systems are expected to be used to help control the quality and quantity of pesticides used, track how they work and evaluate their influence on other biological environments. Safe systems that can easily be used on-site are desired.

Figure 2 shows inspections and technologies where measurement of genes, control of production and creation of quality information are required. These are important in any industrial area as well as in medical area.

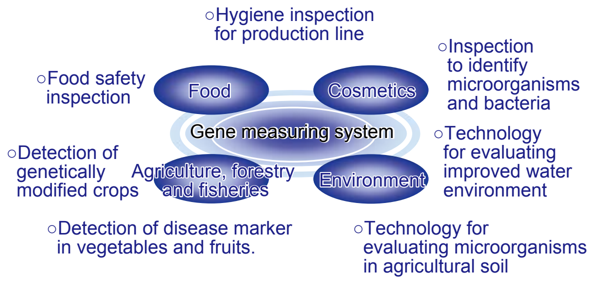

Figure 2 Use of gene measurement in industry

DEVELOPMENT OF A GENE MEASURING SYSTEM

We have investigated the requirements of gene measuring systems for the areas shown in Figure1. Based on the investigation, we are now prototyping and improving a system.

Needs and issues required for gene measuring systems

Specific needs required for gene measuring systems can be roughly categorized into the following five, though the degree of importance varies depending on its application.

1) Accuracy: This is mandatory but has not been officially standardized yet. The progress of standardization must be monitored.
2) Safety: In the medical area, the process for burning and disposing of used medical kits to curb the risk of infectious diseases has been established.
3) Swiftness: In the medical area, if the results of diagnosis can be provided within one hour, diagnosis during an operation or at the point of care (bedside examination) will rapidly spread, and current diagnosis procedures may change dramatically. In industry, shortening the measuring time reduces the tact time.
4) Simplicity: Conventional manual gene analysis is complex and requires skills. There is a strong need to be able to obtain the same results easily regardless of the operator's skill.
5) Reasonable price: There is pressure on price in the medical area due to the trend of curbing medical expenditures, and reasonable prices are important especially for diagnoses for identifying infectious diseases. For repeated measurements in industry such as measurements to identify microorganisms or species, reasonable prices are indispensable.

Basic concept and prototyping of a gene measuring system

We have developed a prototype of a gene measuring system for use in clinical sites and inspection and research sites.

Concept of the gene measuring system

Figure 3 shows the concept of the gene measuring system. The measuring targets of this system are samples such as blood, specimens and bacteria from a patient. The sample is inserted first into a dedicated integrated cartridge. Cells are lysed and genes such as DNA are extracted in the cartridge. The genes extracted are detected and analyzed by fluorescent measurement on a DNA chip using a dedicated biochip reader. The results of the analysis are fed back to physicians and used for diagnosis and treatment.

This gene measuring system provides safe and reliable automatic analysis at clinical sites that used to require experienced operators and a dedicated laboratory. Furthermore, it is possible to link up with the latest medical or genetic information, electronic medical charts and other information by accessing the network if necessary.

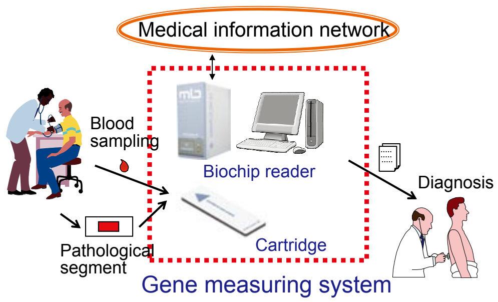

Figure 3 Concept of gene measuring system

Configuration of the gene measuring system

The prototype of the gene measuring system consists of an integrated cartridge and a biochip reader. The former automatically executes all phases of genomic diagnosis from pre-processing to detection which used to be conducted manually, while the latter reads genomic data.

Integrated cartridge … "**Safe and reliable measurement**"

Figure 4 shows processes executed in the integrated cartridge. All processes from pre-processing of a specimen to DNA binding on a DNA chip are executed using technologies of fluid process and reaction in the cartridge.

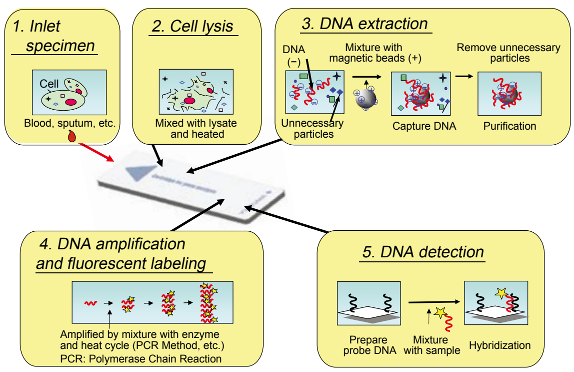

Figure 4 Processes in integrated cartridge

On the DNA chip (micro array) substrate, many types of known DNA (probe DNA) are attached to separate sites respectively. DNA is extracted from the specimen sample, to which a fluorescent label is attached. When this sample fluid is mixed with DNA on the DNA chip, genes in the specimen (DNA in sample fluid) bind with DNA of the corresponding nucleic acid array on the chip to form a double strand. This formation is called hybridization. The hybridization makes specific spots light up due to fluorescence, which are scanned by the biochip reader, and finally genes in the sample are identified.

Figure 5 shows features of the integrated cartridge. With the integrated structure where reagents are sealed in advance and waste fluid is never leaked, the internal processes are isolated from the external environment. Thus leakage of dangerous bacteria or viruses is prevented, while internal contamination with external biomolecules causing noise is prevented. It also helps to ensure a stable reaction regardless of the operator's skill. When the measurement is completed, the cartridge with specimen and waste solution inside can be burned and disposed of. These features enable safe, reliable and simple measurement suitable for all clinical sites.

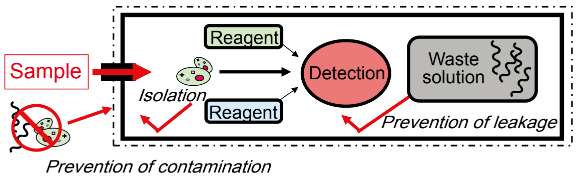

Figure 5 Features of integrated cartridge

Biochip reader … "**High sensitivity and wide dynamic range**"

Figure 6 illustrates the biochip reader. The reader reads fluorescent light on the chip with a laser and identifies genes. By applying the multi-beam method, which reads multiple spots simultaneously by irradiating probes on top of the DNA chip with several laser beams at a time, measurement can be performed without having to move the chip. As a result, vibration of the reader is suppressed and stable measurement at clinical sites is possible.

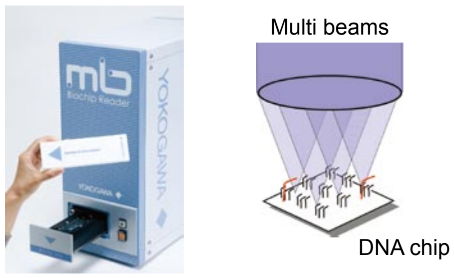

Figure 6 Biochip reader

By exploiting the supersensitive fluorescent measurement technology developed for confocal scanners, we could reduce background light. Furthermore, we improved the optical system of the light source and light-receiving part and its image processing, and achieved high sensitivity and wide dynamic range beyond 16 bits.

The performance of the system is sufficient not only for research purposes but also in medical and industrial applications.

Development toward commercialization of a fully automatic clinical genomic diagnosis system

This R&D was conducted under the technology development project (2002-2005), "Project for Developing Biotechnology IT Integration Equipment / Development toward commercialization of fully automatic clinical genomic diagnosis system for *mycobacterium tuberculosis*, *acid fast bacteria*, *tuberculosis drug-resistant strain of bacteria*," by the New Energy and Industrial Technology Development Organization (NEDO).

The research was carried out through an academia-industry alliance among Tokyo University of Agriculture and Technology, National Institute of Genetics, PropGene Inc. and Yokogawa Electric Corporation. Yokogawa was in charge of developing the fully automated integrated cartridge and reading and analyzing system for the genomic diagnosis system platform for clinical use. Figure 7 shows the deliverables upon completion of the project in March 2006.

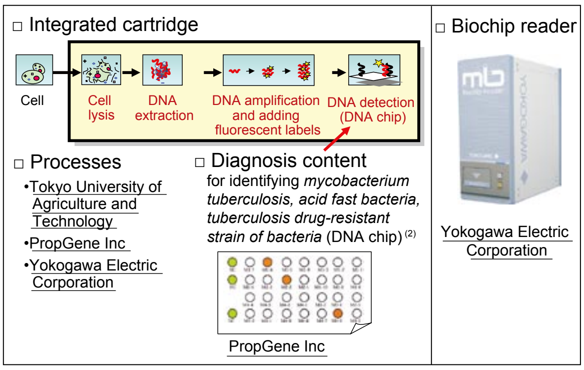

Figure 7 Deliverables of the project

The project, using living bacteria (10^8 pieces) such as *E. coli* and *M. marinum* as samples, reduced the measuring time to just four hours from extraction to detection. Figure 8 illustrates the measurement results. The left side shows the results of detection of *E. coli* and the right side shows that of *M. marinum*, and both detections were successful. This is the first successful sequence of actions in the world achieved by an integrated cartridge in which DNA is extracted from living bacteria and detected on a DNA chip.

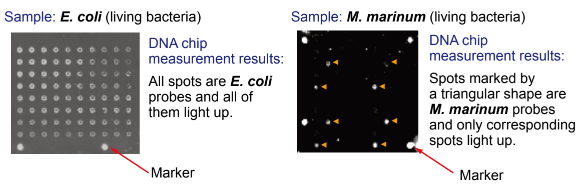

Figure 8 Measurement results in the project

Continual improvement

Though the NEDO project has already finished, efforts to improve the devices are continuing. As Figure 9 shows, in 2007 we successfully shortened the analysis time to approximately two hours by improving processes of the cartridge. To make further improvements, in the same year we started evaluation tests of the biochip reader at users' research sites.

Figure 9 Cartridge for *mycobacterium tuberculosis* and *acid fast bacteria*

Future development

The reading and analyzing system and the integrated cartridge described here are expected to be used widely in the medical area, together with contents requiring safe, reliable and simple handling. One typical application is for infectious disease epidemics expected after devastating damages caused by natural disasters such as major earthquakes and typhoons around the world. Our system offers the analytical functions required for taking effective countermeasures quickly to prevent secondary damage due to these diseases from spreading.

Meanwhile, in industry, monitoring systems using gene measurement technology for investigating food safety and environmental conditions are being developed. In industries related to crop, soil and water quality, monitoring systems are needed to guarantee safety and security. The gene measuring system introduced in this paper is applicable to these areas, and we intend to work on immediate involvement in these areas.

CONCLUSION

As biological information becomes digitized and industrialized, the knowledge gained should be used in various areas such as personalized medicine, healthy living, food safety, preservation of nature and water, improvement of energy efficiency for a sustainable society, reduction of medical expenditures and others. Utilizing micro-technology and photonics technology accumulated so far, Yokogawa intends to contribute to society by providing the mother tools needed for the social and industrial infrastructure to achieve a sustainable global society.

REFERENCE

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(2) Masao Fukushima, Kenichi Kakinuma, et al., "Detection and Identification of Mycobacterium Species Isolates by DNA Microarray," Journal of Clinical Microbiology, Vol. 41, No. 6, 2003, pp. 2605-2615