KOKEN LTD.

Clean Health Safety NEWS

2016 3

KOACH INFORMATION

KOACHのような排出力が
クリーンベンチの作業では必要だった

沖縄科学技術大学院大学（OIST）

大気汚染・アレルギー 対策

PM2.5、花粉、ハウスダストなどの
吸入を防ぐためのマスク

空・SORA

No.686

クリーン、ヘルス、セーフティで社会に
興研株式会社

No. 686, March, 2016

KOACH INFORMATION

The Exhaust power of the KOACH is indispensable for clean bench operation.

“The Okinawa Institute of Science and Technology Graduate University (OIST)”

DNA research such as Genome sequencing was a difficult research theme on which only a small number of limited research institutes could work until a few years ago because it required a very high level of clean environment and technology. However, at a laboratory which deals with DNA sequencing at OIST, Mr. Manabu Fujie has succeeded in achieving an environment where he is able to obtain reliable verification results in DNA whole genome amplification by forming a clean zone having “an actual cleanliness” with the introduction of the KOACH.

OIST aims at creating world-class researchers.

The Okinawa Institute of Science and Technology Graduate University (OIST) is an interdisciplinary graduate school offering a 5-year PhD program in Science. With 5 central concepts of “best in the world,” “flexible,” “ international,” “global networking” and “collaboration with industry,” OIST was established in 2005 in order to contribute to the sustainable development of Okinawa and to advance science and technology in Japan and the rest of the world by conducting internationally outstanding education and research in science and technology.

At the University, all education and research is conducted entirely in English as it is

the international language of science used in peer-review journals and international conferences. A wide range of research fields such as physics, chemistry, mathematics, marine science, neuroscience and life science are conducted in an interdisciplinary academic environment at laboratories equipped with state-of-the-art equipment. Students can work under supervision by world leading researchers and by the time they graduate, they have accumulated not only academic knowledge and practical skills but also the kind of professional connections necessary to launch their careers as world-class researchers.

INTERVIEW

We spoke with Mr. Manabu Fujie, technical staff at the DNA Sequencing Section.

KOKEN: What kind of work are you doing?

Mr. Fujie: We at the DNA Sequencing Section provide nucleic acid (DNA or RNA) sequencing services using a DNA sequencer*1. Because OIST is located in Okinawa as the only subtropical region in Japan, we are engaged in quite a few research themes peculiar to Okinawa. For example, we are conducting a study sequencing the genome of the coral life forms living in Okinawa at a brisk pace that enables us to finish sequencing all of them soon. Not only that, we have started to sequence de novo sequencing of a wide variety of species in the world and so far have finished sequencing the genomes of a few hundred species of microorganisms with several million bases and a few dozen of species with hundreds of millions of bases.

Because we occasionally carry out sequencing using a tiny amount of DNA samples for about 1 microgram, we use several sequencers each of which has a different characteristics in order to conduct an analysis by using the optimal experiment method for each sample.

Besides de novo sequencing we are providing sequencing applications for specific research purposes and the number of such applications is increasing year by year.

*1 Sequencer: It is a scientific instrument to determine DNA/RNA base sequences. By determining the sequence of about 3 billion base pairs in human DNA, we may find causes for human diseases or predict future risk of sickness.

KOKEN: What are you as an expert in sequencing expected to do?

Mr. Fujie: In this field of study, even if you said to yourself, “you made it today!” you would despise yourself by realizing that what you have done becomes a walk in the park next day. You feel in your bones how fast innovation occurs.

It is true that what we are expected to do in the research project is to provide services ranging from library preparation to sequencing, but a task should not be handled only for your self-satisfaction. What is critical is to make a zero-based combination of currently available technologies and to always think if it will lead to the opening up of further possibilities. It is ideal to deny before anyone else the experiment technique that you have established and to develop a new and better experiment technique.

As a result of carrying out our job in this way, we successfully reduced the amount of DNA samples from tens of micrograms to 1 microgram to be used for de novo sequencing, which was satisfactory in its own way. If I may use an extreme case, suppose you will carry out an analysis based on the sample of 100 pg. The solution cannot be found simply through the accumulated efforts in the past but it requires a new experiment technique that has incorporated a completely different technology and idea.

*The area where a cell sorter[*2] is in operation for sequencing genomes is locally purified by KOACH 900.

[*2] Cell sorter is used to measure the distribution of a given cell. It is also used for determining a cell’s progress through the cell cycle with a fluorescent staining method.

KOKEN: What is required to be successful in sequencing analysis with a small amount of sample?

Mr. Fujie: DNA amplification technique is the answer to solve the problem of a small amount of DNA sample for sequencing analysis. To use it in the experiment, however, you have to pay attention to the matter of contamination. Just recently, it was reported that as a result of the genome analysis of a certain life form, a horizontal transmission of bacteria-derived foreign DNA has occurred at a high rate. We hear some saying that a contamination is its main cause. Since we were not directly involved in the experiment, we are not in a position to draw a conclusion. However, this incident is sufficient enough to make us understand how significant the impact of contamination on a research is.

Reduction of the amount of DNA samples inevitably necessitates the establishment of a contamination avoidance measure. An ultimate microscale sample is a cell, the smallest unit of life. To create a clean environment for sequencing, we have come to the conclusion that the establishment of an ultimate clean environment in which single cell sequencing can be carried out without additional improvement in the future should be considered.

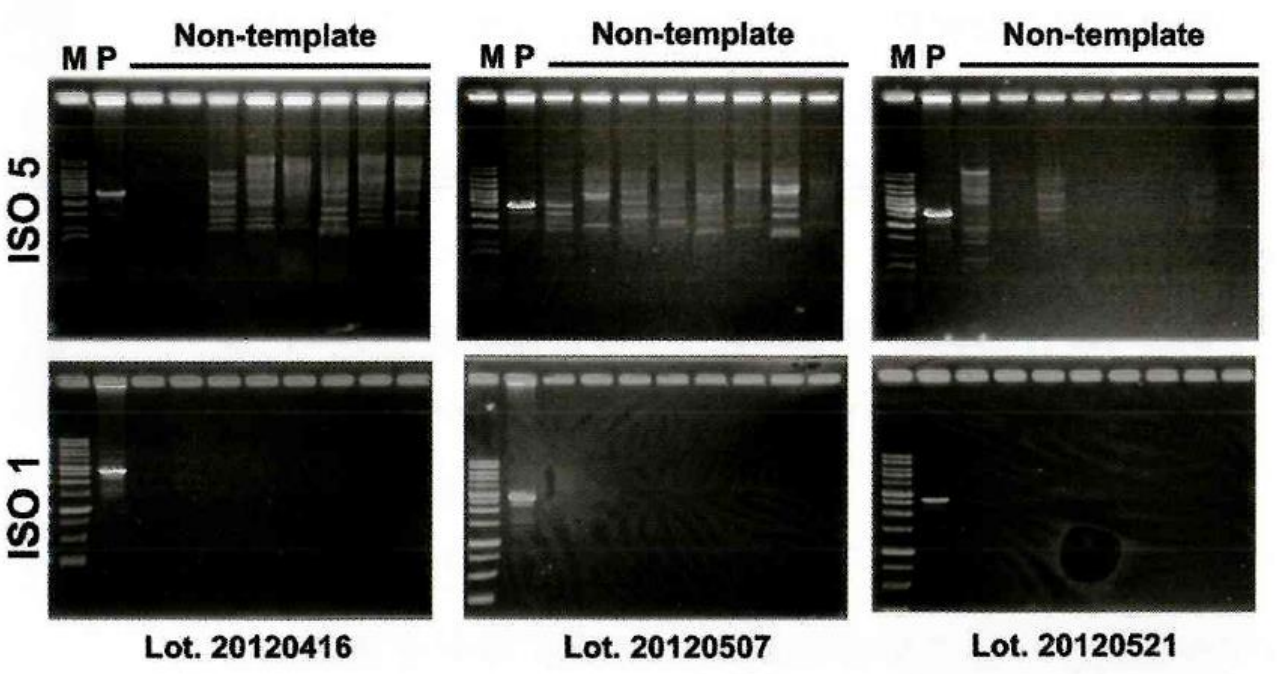
*According to Mr. Manabu Fujie, what he is doing now can be called as a job that makes use of the possibilities to arise from technologies that advance day by day to achieve results in science and industry.

KOKEN: How did you know about KOACH?

Mr. Fujie: I learned about it in a mail from NGS Field*3. Frankly speaking, however, I did not pay attention to the information about the device which can analyze a small amount of DNA samples for picograms because we had been living in a world of micrograms at that time.

About one year after that mail, as I was beginning to understand the necessity of analyzing ultra-microscale samples, I asked KOKEN to send the information on KOACH. I found in the brochure sent by them that a certain famous research institute which was taking a leading position in the single cell research had introduced a KOACH. I was surprised to learn that it argued that the single cell experiment could not have been possible if not for a KOACH since a clean bench was not sufficient enough to achieve the necessary cleanliness (Ref.1).

*3 NGS Field is a community for researchers who use next-generation sequencers. They gather at a regular interval and at the 4th meeting in July, 2015 over 700 participants reported and exchanged information.



* Gel Electrophoresis of DNA products amplified by the same phi29 DNA polymerase

(Ref.1)
Carry-over contamination means that air-born particles including DNA in the residues of the amplified products generated in the previous PCR cycles may diffuse in the

laboratory and contaminate the reaction solutions to be used in the next cycles. Based on my belief that carry-over contamination is thought to be one of the main causes that may hinder experimental results, we examined the necessity of having a cleanroom facility to reduce airborne particulates by comparing ISO Class 5 and ISO Class 1 environments. In ISO Class 5 environment, 8 negative control bands (non-template) were detected, meaning that DNAs in the contamination are amplified, while in ISO Class 1 environment, negative control bands were not detected, meaning that there is no adverse effect derived from contamination.

From a brief summary of Dr. Hirokazu Takahashi's presentation at the 4th meeting of NGS Field titled "Impact of and countermeasure against foreign DNA contamination in single cell genome sequencing"

KOKEN: That led you to check the real thing?

Mr. Fujie: Soon afterward I contacted KOKEN for a permission to visit their Super Clean Technical Center.

I was impressed most of all by the capability of the KOACH which can maintain a high level of cleanliness at all times throughout the operation in addition to its ability to create an ISO Class 1 clean environment necessary for the single cell research. KOACH adopts an open structure without encircling walls. Very gentle laminar airflows, which you may not feel, are blown from a pair of push hoods which are set opposite to each other, collide with each other at the midpoint between the opposing hoods and will be pushed out horizontally as well as vertically. A combination of "open structure" and "airflow principle" creates a strong exhaust power to discharge airborne particles generated internally out of a clean space, thus always keeping the space free from contamination. This is a great advantage for KOACH which has not been achieved by a conventional clean bench.

At the Super Clean Technical Center, we could verify the specifications of the KOACH by evaluating cleanliness with a particle counter using a real machine. Looking at the numbers shown there finally has convinced me why the institutions which are achieving satisfactory results have introduced the KOACH.

KOKEN: How do you use the KOACH in the laboratory?

Mr. Fujie: We use multiple units of the KOACH (Bench type C900 / Pic. 1). Because it can purify a larger area, it is useful to purify a variety of instruments.

Furthermore, it is highly portable for layout change since it has casters. Even if contaminants are brought into the clean zone when taking laboratory instruments or hands in and out, I am pleased to know that the original level of cleanliness can be recovered for a short period of time, which is proved by a particle counter used all times to monitor the clean zone within the KOACH.

(Pic.1) A pair of units of the KOACH are placed across the table on the left and right. Purified airflows blown by these units keep the work area at high level of cleanliness all times.

KOKEN: You have introduced an ionizer, too?

Mr. Fujie: Experimental results vary partly due to seasonal factors. It was said that contamination tends to occur in dry periods. If electrostatically induced contaminants remain adhering to materials used for an experiment, you may not achieve a correct experimental result despite that the KOACH is in operation. To avoid this, we have installed a dedicated ionizer exclusive for the KOACH (Pic.2) as a countermeasure to reduce contamination risk due to static electricity.

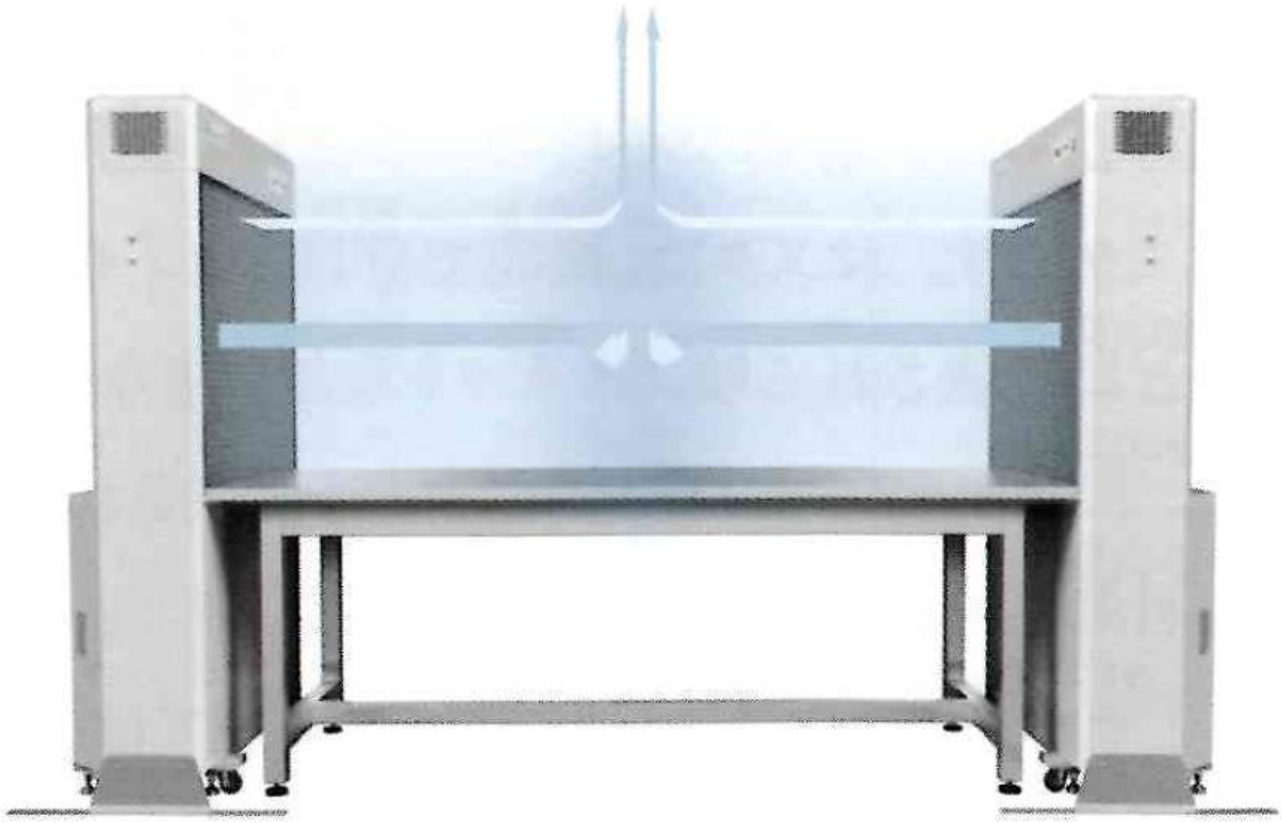
(Pic.2) Ionizer BF-X2MB exclusive for KOACH (Optional)
It is a windless ion generator. It will not disturb airflows blown by the KOACH.

KOKEN: What is needed to conduct cutting-edge research?

Mr. Fujie: To conduct cutting-edge research including ours, the laboratory should not only have a high cleanliness but also keep that high level of cleanliness "all times throughout the actual operation and work*4." In this respect, the KOACH can easily maintain a high level of cleanliness in the area around a worker, which is a great advantage no other products can offer. We have achieved a super clean environment at low cost that cannot be normally realized unless a cleanroom is physically constructed. KOACH will be a strong ally when it comes to whatever experiment or requirement we will encounter in the future in order to achieve the best result.

We would like to prove based on the experimental data that KOACH will become an indispensable tool in any research from now, for it would be fantastic that all the research efforts in the world could progress in a good direction.

*4 Cleanliness during actual work is called "Actual cleanliness."



KOACH that can realize an "actual cleanliness."

Laminar airflows that are blown from a pair of push hoods set opposite to each other collide with each other at the midpoint between the opposing hoods and will be pushed out constantly toward the outside. This "airflow principle" becomes a strong exhaust power to discharge internally generated contaminants out of a clean zone in a short time, thus always keeping the space free from contamination (Achievement of "actual cleanliness").

OIST
OKINAWA INSTITUTE
OF SCIENCE AND TECHNOLOGY
GRADUATE UNIVERSITY

Okinawa Institute of Science and Technology Graduate University (OIST)
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Opened: in September, 2012
President: Dr. Jonathan Dorfan