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Clean Health Safety NEWS
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NGS現場の会 第4回研究会
実験環境の
コンタミネーション対策には、
KOACHが必須
結核に対する院内感染予防対策
病院等の結核の感染対策として
求められているN95マスクについて
興研株式会社
http://www.koken-ltd.co.jp/

NGS Field 4th Meeting

KOACH is indispensable to prevent contamination in experimental environments.

As an innovative technology to allow researchers to read large amounts of genetic codes in DNA/RNA in a short period of time, next generation sequencer (NGS) is used all over the world, contributing to the development in a wide range of fields such as medicine, pharmacy and agriculture.

Researchers who use NGS meet regularly to exchange information at NGS Field meetings. At the 4th meeting in September, 2015, Dr. Hirokazu Takahashi, Japan Science and Technology Agency's CREST researcher of Hiroshima University, made a presentation concerning benefits of contamination control using enzyme free of DNA contamination and ISO Class 1 clean environment.

●Presentation titled: "Impact of and countermeasure against foreign DNA contamination in single cell genome sequencing."

The following are excerpts from his presentation.

Highly reliable data can be stably obtained if contamination-free clean examination environments are arranged

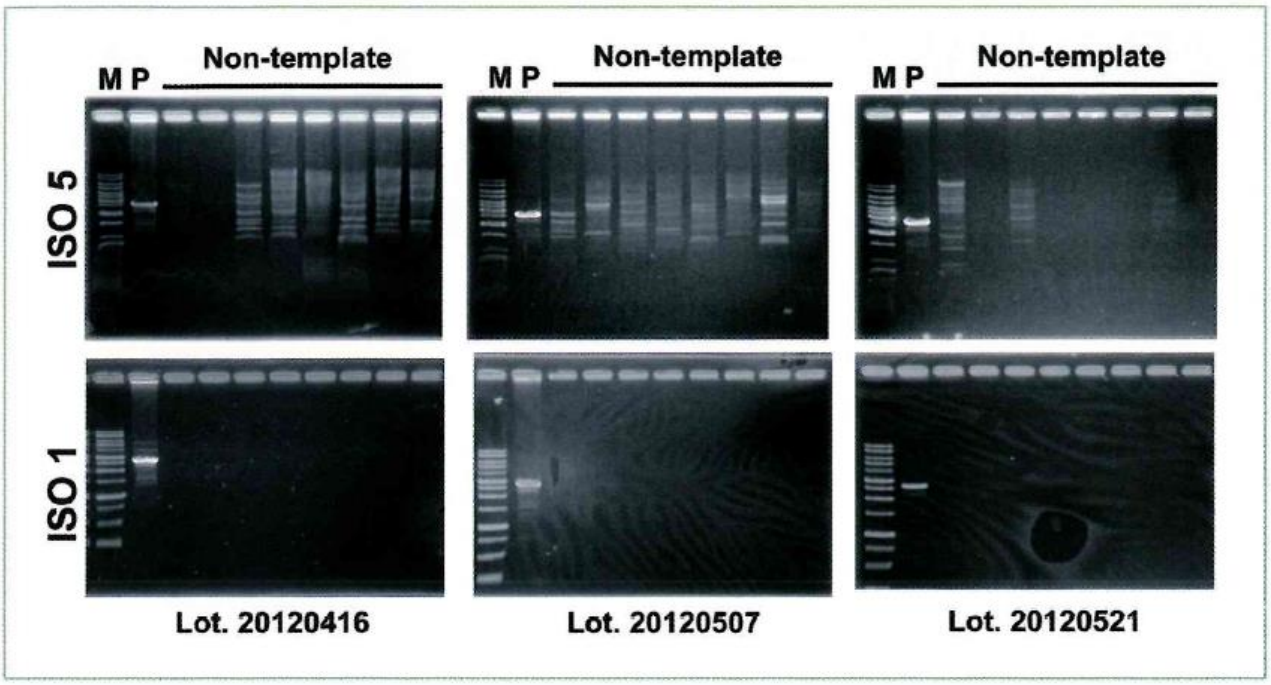
In biochemistry many cases have been reported recently in which contamination caused decrease in reliability of observed data. For example, Kara Longo and other authors warn in their paper that a question is raised about the reliability of genome databases because abundant human DNA contamination (about 25%) were identified in non-primate genome databases.

While in forensic medicine that deals with human life and ethics or in archaeology in which extremely rare samples are handled, researchers have a high level of awareness about contamination prevention, researchers in such fields as microorganism and molecular biology that deals with inexhaustible supply of samples may often show lack of awareness of the importance of contamination prevention. I would like to stress that it is essential to prepare a contamination-free experimental environment in order to obtain reliable outcome of the study at a stable pace.

We addressed three problems that cause contamination.

In order to analyze rare or infinitestimal amount of samples in DNA analysis, a target region of DNA must be amplified before DNA analysis is performed. If, however, samples are contaminated, unnecessary DNA contained in contaminants may be amplified at the same time together with the target DNA. Thus, controlled experiments must be performed to prove that there is no untargeted DNA contamination (Fig.1). At the same time, it is indispensable to prepare a contamination-free experimental environment.

■Fig.1 Results of Controlled Experiments to Prove that Only Targeted DNA is Amplified.
(Comparison is made between two types of environments: ISO Class 1 and ISO Class 5)



In ISO Class 5 environment, you can see 8 negative control bands (Non-template), meaning that the contamination is amplified while in ISO Class 1 environment, negative control bands are not detected, meaning that the contamination is not amplified.

Gel Electrophoresis of DNA products amplified by the same phi29 DNA polymerase (BamHI/EcoRI double digested) M: 1kb maker, P: pCU19 (2.7kb)

■Table 1 Types of Contaminations and Countermeasures

1. Cross Contamination

Cross contamination can occur in a laboratory through filthy pipette or hands of workers.

[Countermeasure] Use dedicated pipettes and filter tips; use consumables that are guaranteed DNA-free.

2. Intrinsic Contamination

DNA residues of microorganisms used for enzymes or reagents may contaminate amplified products.

[Countermeasure] Use low-DNA reagents; use DNA-free reagents.

3. Carry-Over Contamination

Residues of the amplified products generated in the previous PCR cycles may contaminate reaction solutions used in the next cycles.

[Countermeasure] DNA amplification should be performed in separate rooms (literally divided or use a clean bench).

As Table 1 shows, there are several types of contaminations: Since countermeasures are now widely available for cross-contamination, more attention is being paid to “intrinsic contamination” defined as unnecessary amplification of DNA intrinsically contained in enzymes used for an experiment and “carry-over contamination” that may be caused by residues of the amplified products generated in the previous PCR cycles.

While currently I can avoid cross-contamination by using only dedicated experimental instruments for each experimental sample, I have taken measures to prevent intrinsic contamination by developing enzymes in which DNA mixture is reduced to the utmost limit. As for a measure to prevent carry-over contamination, I am focusing my attention on cleanroom facility to avoid airborne particulates based on my belief that carry-over contamination may be caused by airborne particulates including DNA that is diffused in the laboratory.

Previously I used a clean bench that can create a clean environment of ISO Class 5. Even if I used dedicated instruments and the specifically developed enzymes thoroughly, I could not obtain accurate results on a continuous basis. This experience made me re-think about our cleanroom facility to prevent this carry-over contamination.

Once the carry-over contamination was solved, negative controls have produced stable results.

Assuming that there exist one billion airborne particles of 0.1 μm in diameter per cubic meter of air, one estimate is that one hundredth to one thousandth of them are virus like particles which may include DNA.

HEPA filters that are installed in the conventional cleanroom system, however, cannot remove these virus like particles less than 0.3 μm in diameter completely. Similarly, they cannot remove DNA derived from dead cells in the air either. For this reason, it is concluded that a clean bench with HEPA filters is not good enough to prevent contamination in the laboratory environment.

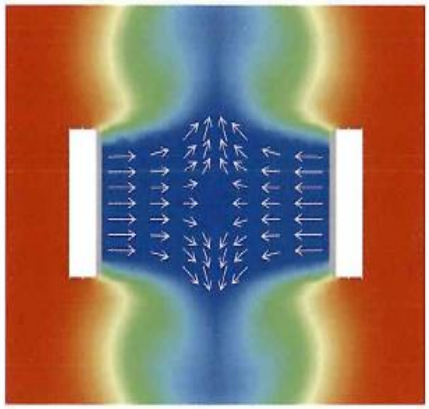
Then, we have paid attention to Koken’s Table KOACH (Fig.2) that is able to create a local environment of ISO Class 1 cleanliness in order to solve the carry-over contamination problem that is caused by airborne particulates in the laboratory. Being equipped with nanofiber filters that can remove airborne particulates of 0.1μm in diameter, Table KOACH can achieve the world’s highest level of cleanliness rated at ISO Class 1,thus preventing virus like particles from entering. Furthermore, because airflows blown by the two opposing push hoods collide with each other in the middle of the space between the two hoods and are pushed outward there continuously, contaminants generated by the movement of worker’s hands will not stay inside and be pushed out of the clean area to the outside.

Thanks to the introduction of Table KOACH, measures to prevent carry-over contamination are strengthened and we are now in a position to enjoy obtaining stable results in our experiments.

Preventive Canopy for Table KOACH T500-F

For biochemical experiments at Dr. Takahashi's laboratory, an ionizer-equipped preventive canopy for Table KOACH T-500 is designed to protect the work space from the falling/flying bacteria and prevent plastic instruments from being charged with electricity.

Fig.2 Table KOACH introduced to prevent carry-over contamination.



Mechanism of KOACH to create a clean space with ISO Class 1 cleanliness

Airflows purified by ultrahigh performance filters are blown from the two push hoods placed opposite to each other. These airflows are uniformed laminar airflows, having common vectors (same direction and same velocity) are destined to move outward after colliding with each other at the middle point, thus creating a super clean zone in the space framed by the two push hoods without encircling that space.

NGS Field 4th Meeting
Conferences and Exhibitions

"Keen interest in contamination control"

About 700 researchers gathered at the 4th meeting of NGS Field in Tsukuba International Congress Center. They are using NGS (next generation sequencer) to study microorganisms, metagenome and epigenome and exchanged information actively during the three-day conference.

Many researchers who think that the conventional contamination control is not good enough visited the Koken exhibition booth where Table KOACH was displayed. They showed a keen interest in the exhibition to learn that the combination of Table KOACH and phi29 DNA polymerase will provide an effective measure to prevent both intrinsic contamination and carry-over contamination.

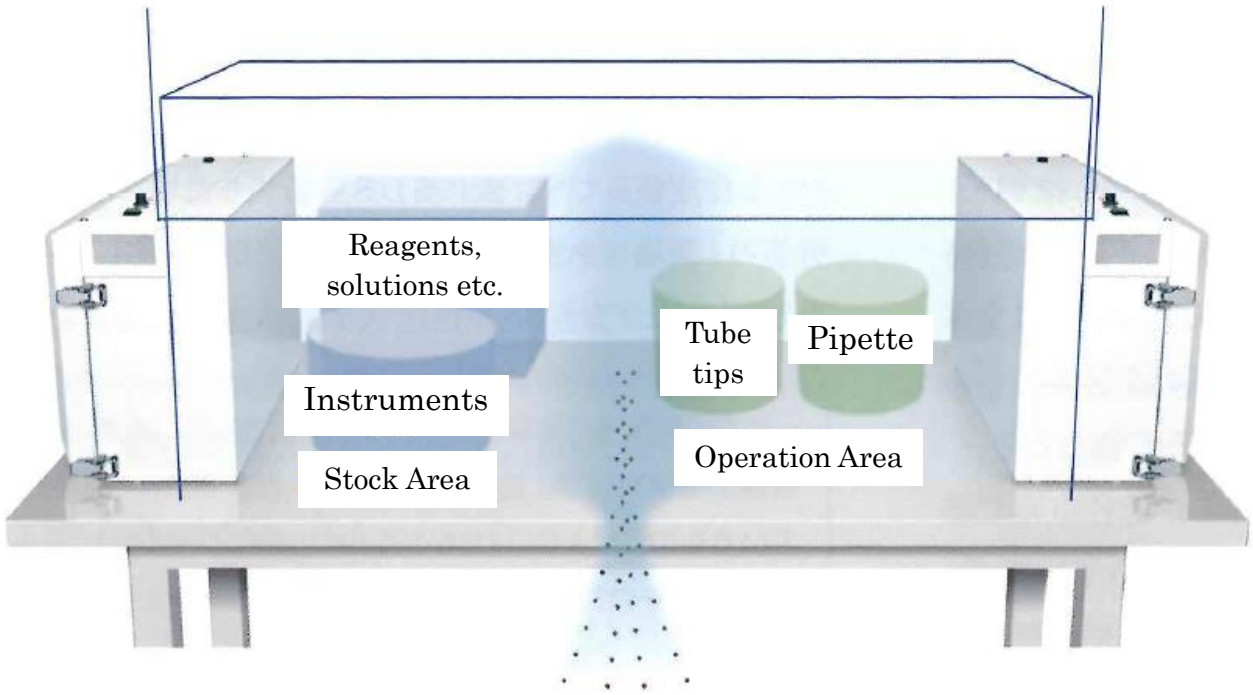
NGS Field 4th Meeting (Epochal Tsukuba)

Features of KOACH No.3 Dividing a space by taking advantage of airflows

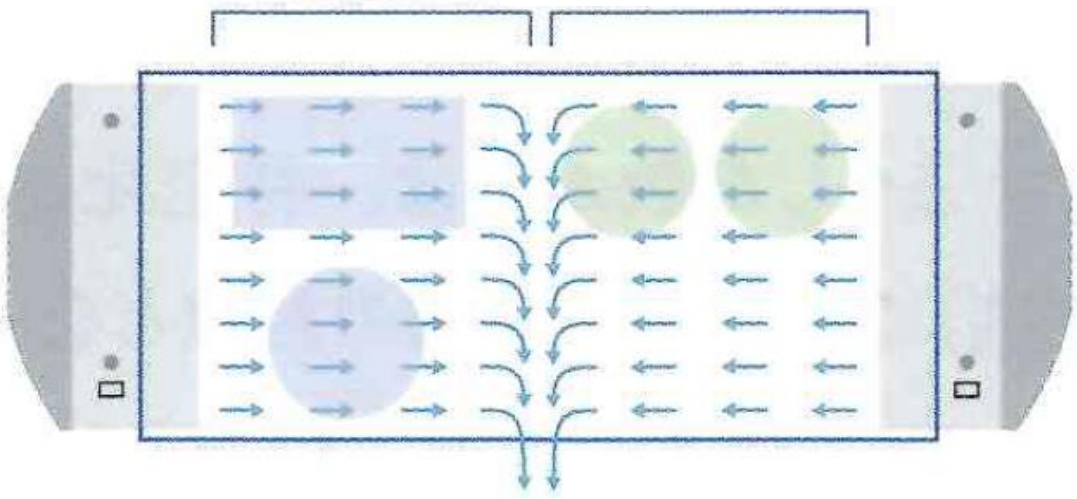
As a clean space is practically divided for different uses by taking advantage of the special features of airflows generated by KOACH, an effective contamination control can be implemented.

The airflows generated by KOACH can create two distinct areas in the same clean space whose airs do not mix together. To make the most use of a clean environment of ISO Class 1 cleanliness provided by KOACH, Dr. Takahashi has designed an innovative work layout for experiments by taking advantage of the KOACH's airflow features.

Fig.1 A example of new work layout
(Two distinct clean areas are used for different purposes)



Stock Area　　Operation Area



Airflows generated by KOACH collide with each other and be pushed outward without mixing together, thus forming two distinct areas in the same clean space.

By making two uniformed laminar airflows having common vectors collide with each other, two distinct clean areas in the same space can be created.

In a clean space formed by KOACH, uniformed laminar airflows having common vectors from opposite directions collide at a right angle in the middle point and be pushed out of the space to the outside, thus creating two distinct areas whose airs are not mixed with each other on the right and left side of the collision of the airflows.

By taking advantage of two distinctive areas for different work purposes, an innovative work layout can be implemented to reduce contamination risk.

An example of work layout

~ National Food Research Institute / National Agriculture and Food Research Organization ~

A Table KOACH was installed at National Food Research Institute, National Agriculture and Food Research Organization to which Dr. Takahashi belonged previously. There, an innovative work layout was implemented to take advantage of two distinct areas in a clean space created by the airflows of KOACH.

Fig.1 shows the work layout design. In the area on the left of the center necessary test samples etc. for an experiment are stored in a clean condition as “stock room,” while the area on the right is used as “operation room” to perform a work that requires a high level of cleanliness.

When getting things like laboratory equipment in and out of the “stock room,” particles may leak into this area. However, the “operation room” on the right can be protected by the clean airflows flowing in the opposite directions from contamination so that a high level of cleanliness is kept properly during work. Similarly, even if particles enter the operation room on the right temporarily during work, they are not allowed to enter the stock room on the left so that stored stocks can be kept clean.