KOKEN LTD.

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"KOACH" is expected to become a standard countermeasure against contamination in DNA amplification.

The 7th Conference on Society of Genome Microbiology, Japan was held at Nagahama Institute of Bio-Science and Technology in Shiga Prefecture, Japan, from March 8 to 10, 2013.

Koken's Table KOACH "KOACH T 500-F" was introduced in the presentation made by Dr. Toshiro Kobori, Researcher at National Agriculture and Food Research Organization, National Food Research Institute, and Dr. Hirokazu Takahashi, Researcher at Graduate School of Advanced Sciences of Matter, Hiroshima University.

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Could you tell us what National Food Research Institute is studying now?

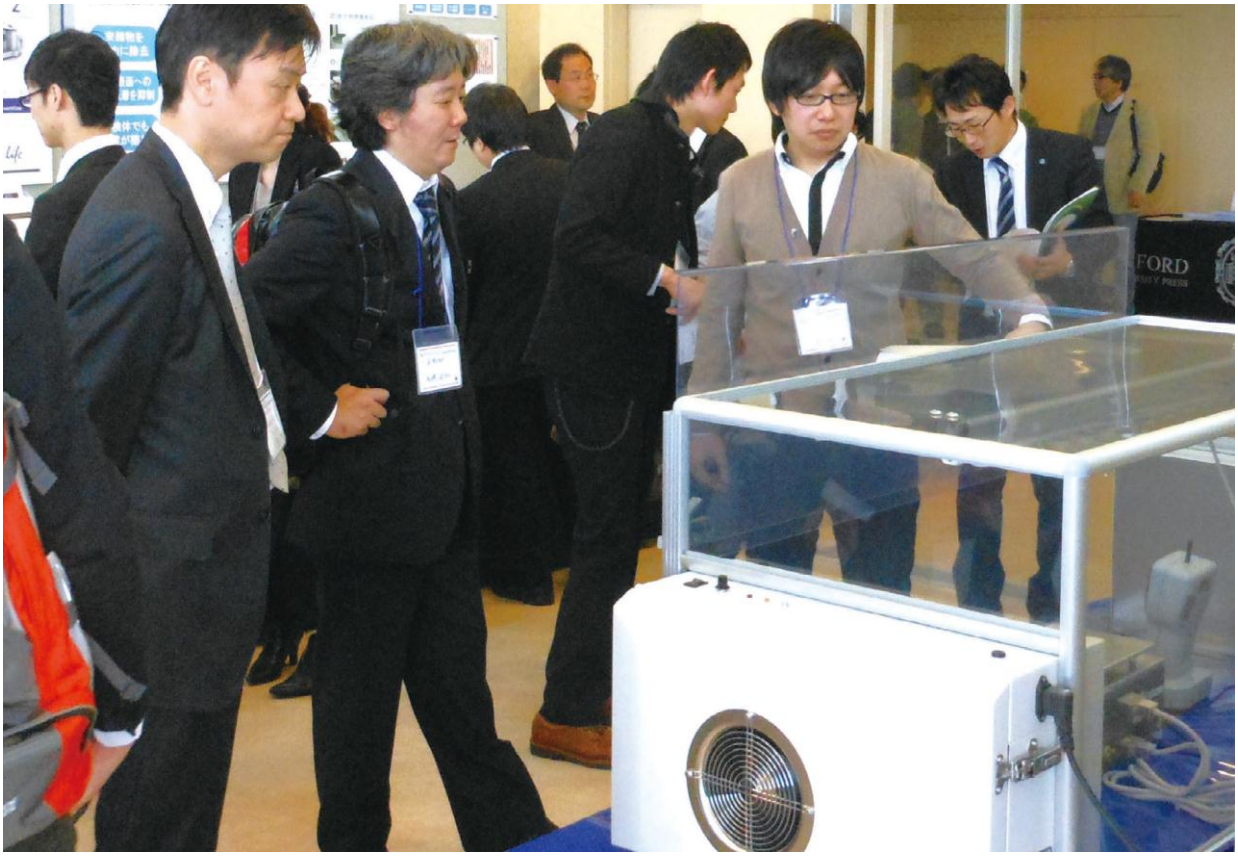
National Food Research Institute is engaged in a fundamental and foundational research such as scientific analyses of food and health, food safety, and development of innovative food distribution and processing technologies. Among them, our research field is DNA analysis of processed foods. Dramatic progress has been made in the DNA analysis for the last few years and it has been applied to various fields of not only foods but also agriculture and medical treatment.

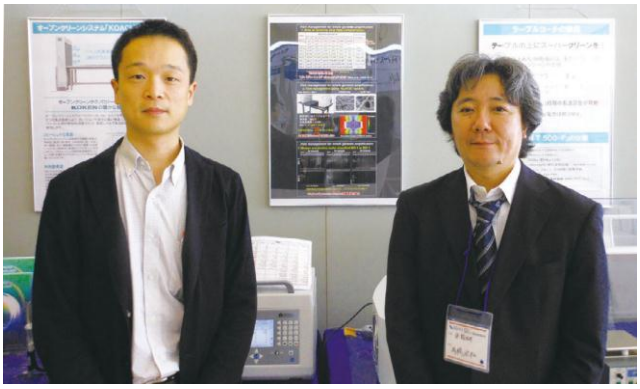
What did you present at the 7th Conference?

In order to produce enough DNA to be analyzed, it is generally necessary to amplify the number of copies of a specific region of DNA.

In recent years in particular, there is an increasing number of cases to handle extremely small amount of samples. A special technique to amplify a tiny amount of all DNA in a specific sample is needed before analyzing DNA.

Such a technique is called "Whole Genome Amplification." At the conference, we presented our research on "Whole Genome Amplification from a Single Cell." It is about how to make the high-fidelity reproduction of genomic DNA for DNA analysis from precious DNA samples like a single cell.





Dr. Kobori (left) and Dr. Takahashi (right)

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How does contamination of the sample affect DNA amplification?

To verify that only the targeted DNA sample is amplified, a control experiment must be made. If the reaction solution is contaminated in the experimental environment, DNA contained in the contaminant is reproduced, resulting in the failure in achieving the reproduction of only the targeted DNA. In a negative control experiment, in particular, if contamination develops to produce an amplification product, it will be impossible to verify that there exists no contaminated DNA in the sample, resulting in the loss of validity of the experimental outcome.

“Whole Genome Amplification” that we are working on is a special technique to improve the efficiency of DNA amplification to the utmost extent. This, unfortunately, will amplify those DNA derived from contaminant viruses at the same instant if viruses floating in the air etc. are contaminated into the reaction solution.

What were major shortcomings in the past countermeasure against contamination?

A clean bench that can generate ISO Class 5 cleanliness is commonly used as a countermeasure against contamination when Polymerase Chain Reactions (PCR method) are carried out. The PCR method is a most commonly used DNA amplification technology. We have utilized a clean bench with ISO Class 5 for our whole genome amplification processes until now. The success ratio in the accurate DNA reproduction has been only about 50 percent.

During a clean bench operation for a PCR experiment, for example, a member of staff only passing behind the clean bench operator may cause the room air to flow into the clean area. Although a real cause is not certain, it may be judged by the operator that the failure in the experiment is attributable to the very act of walking behind him. Such trivial matter may have deteriorated a personal relationship between the staff members.

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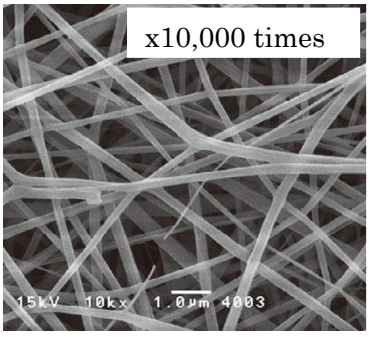
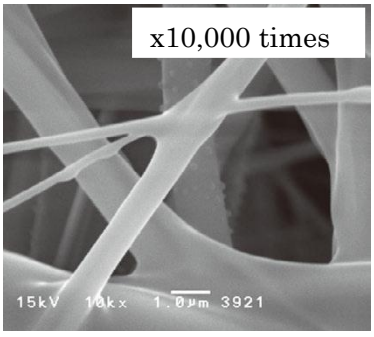
Another concern was about the high cost of reagents. Because the DNA amplification requires the multiple use of high cost reagents, we have no choice but to dispose of them if the experiment does not produce a desired outcome. This is not good to our mental health.



FERENA



Conventional ULPA filter

FERENA (left) has an ULPA-level filtering efficiency with a pressure drop that is the same level of HEPA filters.

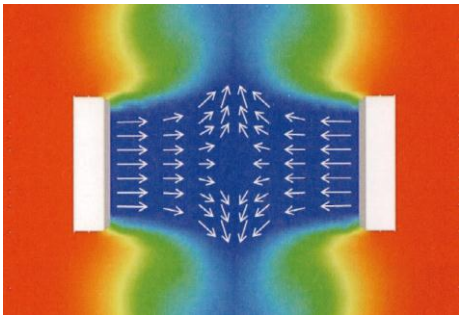


Image of airflows that effectively push contaminants out of the area formed between the pair of facing push hoods.

What made you pay attention to "KOACH" as a countermeasure against contamination?

I thought it necessary to install a clean room in order to achieve a higher level of cleanliness than a clean bench with ISO Class 5 cleanliness. However, we did not enough budget to install it. Two years ago we went to the Convertech Japan Exhibition to look for something better. There "KOACH" attracted my attention.

We saw a demonstration of "Floor KOACH" at the exhibition with interest. We noticed that the number of particles over 0.3 μm were shown zero on a monitor despite that the demonstration area is connected to the outside air.

We wished to be able to conduct our experiment under the circumstances. However, compact-sized equipment which is suitable for our experiment was not available then. When we went to the Convertech Japan Exhibition the following year, we noticed a compact table-top model, "Table KOACH." I thought that this might be the best solution to solve the contamination problem for our Whole Genome Amplification. With this in mind, I asked a staff from Koken for a special favor and used a just-released Table KOACH for experimental purposes.

I was surprised at a better-than expected performance during our trial. We completed our experiments which might take about six months to complete under ordinary circumstances during a two-week trial period. Normally, it takes a very long time to investigate the cause of the experimental failure because verification is required on the multiple items. Thanks to the Table KOACH, our experiments proceeded smoothly without a hiccup. I really appreciate very much your giving us a great favor at that time (smile).

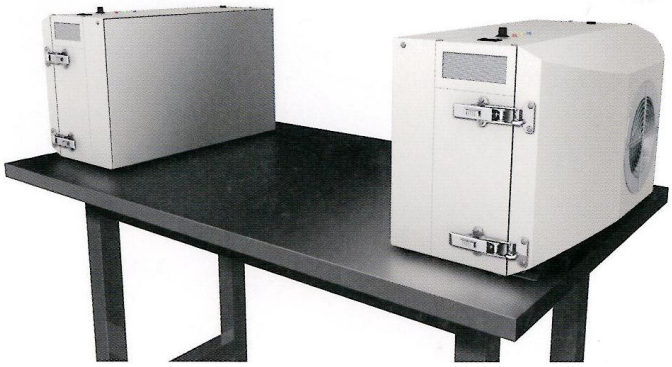


Table KOACH "KOACH T 500-F" creates ISO Class 1-rated air cleanliness. Due to this new measure against contaminants, duration of experiments, that used to take 6 months, can be shortened to only 2 weeks.

KOACH T 500-F (with FERENA filter)

Air cleanliness rating : ISO Class 1
Time required to achieve ISO Class 1:110 seconds
(flow rate : 0.4m/sec)
Size : W: 541mm × D: 289mm × H: 334mm
Size of air-blowing surface : W: 494mm × H: 306mm
Maximum distance for clean zone : Within 700mm*2
(Maximum distance between the pair of push hoods)
Weight : Approx. 14kg/unit
Power : Single phase, 100V 50/60Hz
Power consumption*3 : 35-110W/unit

*2 Flow rate at 0.4m/sec
*3 Varies depending on pressure drop on filter and flow rate.

With the use of the Protective Canopy, which has been used by National Food Research Institute, power consumption can be reduced down to 24Wh in sleep mode while maintaining ISO Class 1-rated air cleanliness in clean zone.

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What were the decisive factors for choosing “KOACH”?

KOACH effectively prevents outside contaminants from entering into a clean space and if they enter into the clean space, they are immediately exhausted out of it. Although this is a common problem for clean bench operations, room air always contaminates the clean zone when materials are taken in and out of the work area.

We used a particle counter when conducting the experiment during our trial. What surprised us was that the reading of the particle counter returned to zero soon after it detected particles due to the placement of the hands on the work area.

How was the KOACH running after the installation?

I wish I could say “no problem,” but we encountered a situation where contaminated DNA were amplified in a negative control experiment despite that the particle counter showed no detection of particles. Having suspected that contamination has developed in the reagents or the procedure, we paid close attention to the procedure by replacing the reagents for new ones. But, we could not obtain an expected outcome.

We were at a complete loss what to do and consulted with the technical staff from Koken, who came back to us with a solution proposal of countermeasure against static electricity on the very same day. Then and there, the cause for the experimental failure was identified and the necessary countermeasure was taken. I remember vividly the day when this happened.

Because the KOACH provided the ISO Class 1 cleanliness, it did not take long to identify the cause for the failure in performance. We did not realize that the Nitrile gloves worn outside the clean zone were charged with static electricity and the contaminants of about 100,000 particles per cubic foot drawn from the outside air were attached on them. We could not believe that we had conducted our experiments under the circumstances.

After this, Koken staff continued to put forward a proposal for improvement in our experimental procedures such as placing possible contamination sources at the downstream side of the airflow from the KOACH. Thanks to their effort, we now feel comfortable staying with the KOACH. I can say in conclusion that the battle to contain contamination, headache suffering from school days, has ended finally with the successful introduction of KOACH. Thank you very much.

Note: Control experiment

Controls are important in the DNA amplification experiment as in all biological disciplines. It proves that the amplified DNA has not come from the contaminated sample.

A positive control, where a targeted region of DNA is present, should assure us that the experimental results are valid provided that the all reagents and equipment are functioning properly by observing the reproduction of DNA. While a negative control, where a targeted DNA is not present, should be designed to not give the desired outcome of the experiment. If the reproduction of DNA is observed, it can be interpreted that something was wrong with the experiment due to the contamination, for example.

About Electrophoregram

Amplified DNA fragments (Amplicons) can be separated easily by Electrophoresis using agarose gels.

Fig.1 shows the results of the amplification of DNA fragments derived from the reaction solutions in one positive control experiment and 8 negative control ones. They were processed under ISO Class 5 experimental environment. As various bands (lines) are shown in the gels in 8 lanes on the right side (negative control), it can be interpreted that the DNA fragments contained in the contaminants were also amplified. On the other hand, Fig. 2 shows the results of the Amplicons under ISO Class 1 experimental environment. No images were visualized in 8 lanes on the right side (negative control). This means that environmental contamination in the DNA samples was prevented.

Fig. 1 ISO Class 5

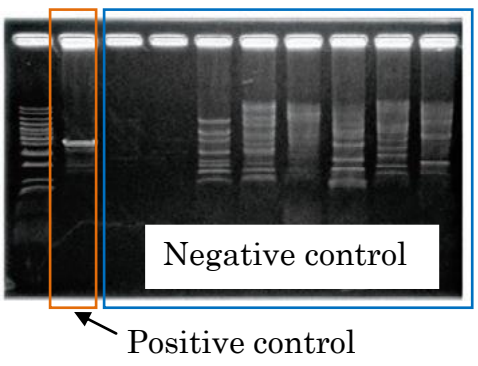
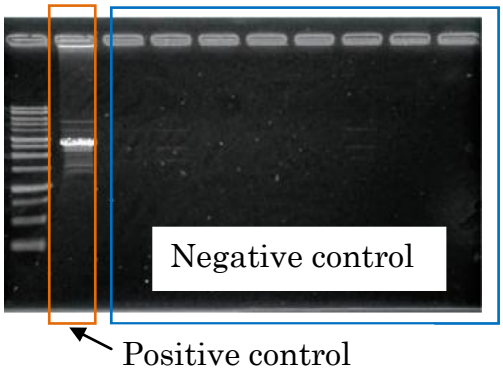


Fig. 2 ISO Class 1



Please note that under both levels of cleanliness, Class 5 or Class 1, amplification reaction itself was successfully processed since correct sized DNA can be identified in the 1st lane on the left side in positive control experiment.