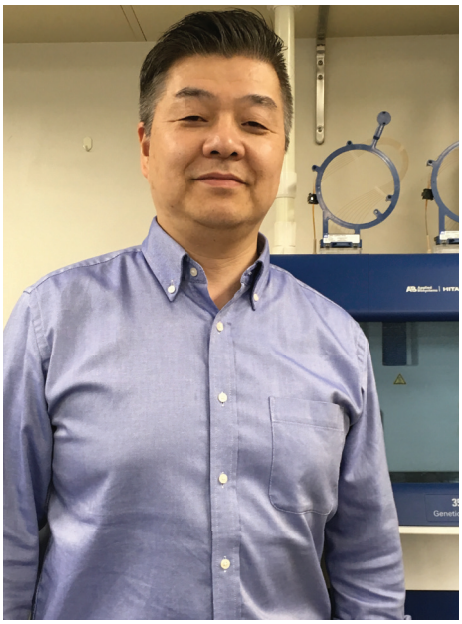
Human identification

Maximizing results with the right tools for the job

Capillary electrophoresis (CE) and next-generation sequencing (NGS) applications in forensic DNA analysis



We recently spoke with Dr. Masaki Hashiyada, associate professor in the Department of Legal Medicine at Kansai Medical University. His laboratory utilizes the Applied Biosystems™ SeqStudio™ Genetic Analyzer and the Applied Biosystems™ Precision ID NGS System for human identification in their programs. The instruments are used across a broad range of forensic applications, including many challenging sample types and in collaboration with multiple users.

Thermo Fisher Scientific: Tell us about yourself and your forensic program.

I have been in the forensic community for the past 20 years and my research focus is forensic genetics. There are three pillars I work on: education, research, and expert services. I manage the forensic genetics program in Kansai Medical University, including classroom lectures and lab exercises for medical students. The exercise section is open for highly motivated students who are interested in forensics regardless of their grades, as well as for visiting fellows to learn forensic genetics methodology deeply through a series of experiments.

Share with us what projects and types of cases you are working on.

Our expert services include two major areas: forensic autopsy and DNA testing based on CE. In Japan, Prefectural Police Laboratories handle most of the DNA testing in criminal investigation activities, so most of the DNA samples sent to my laboratory are really challenging ones that Prefectural Police Laboratories are unable to interpret, such as cold cases, old bone samples, or low-template DNA.

An example of a cold case we processed was a homicide case from 20 years ago with blood stain samples that were collected from the crime scene. The stains were a possible mixture of victim and suspect, and other blood stains were from the presumed escape route of the suspect. Another example of low-template DNA was a body fluid sample collected from a bed at the murder scene.

As I am a member of the World War II victim identification project, led by the Ministry of Health, Labor and Welfare, we test old bone samples on a regular basis using autosomal STR, Y-STR, and mitochondrial DNA sequencing.

Recently, an increasing number of cases have been submitted for whole mitochondrial genome (mtDNA) sequencing and ancestry SNPs analysis through NGS. Ancient Jomon bone samples, which are 16,000 years old, are a good example from which to show the power of NGS. mtDNA whole genome sequencing enables us to discriminate mitochondrial DNA haplogroup using SNPs analysis when it is impossible in conventional CE-based mtDNA sequencing, because it can detect one SNP per run. This result was presented at the Human Identification Symposium (HIDS) conference in 2018.

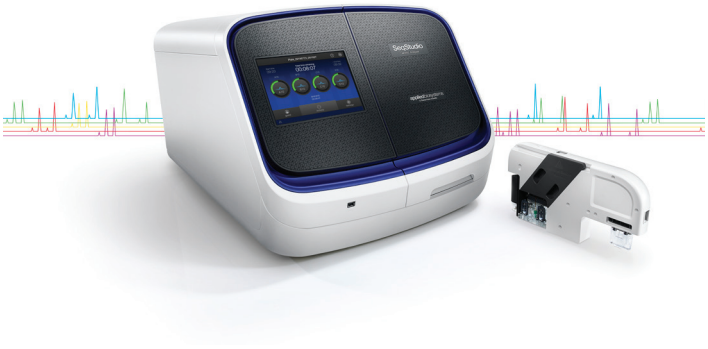
NGS is also a powerful investigative tool. We ran the Applied Biosystems™ Precision ID Ancestry Panel for blood stains in the cold case I mentioned earlier. Especially in cold cases, law enforcement officers need to maximize the amount of information from a very limited casework sample.

My research focus is on different NGS applications. The platform we are using is the Precision ID NGS System for human identification, which consists of the Ion Chef™ System, Ion GeneStudio™ S5 System and Applied Biosystems™ Converge™ Software. Recently many of my projects have been for metagenomics for human gut microbiome, age prediction by methylation analysis, ancestry SNPs, mitochondrial DNA whole genome sequencing, and molecular autopsy, primarily relating to heat disease, including mutation detection, methylation status, and miRNA analysis.

Describe some of the challenges you face in your lab.

My samples are challenging. They are samples that police laboratories decided were too difficult to analyze themselves. In other words, the DNA amount is very low, or the DNA is degraded. Here are some examples I was asked to analyze recently:

- A touch DNA sample, collected from a shoe sole, used to recover suspect DNA from mixtures
- A touch DNA sample from a brick, which was a weapon in the case
- A decades-old body fluid stain taken from a cold case



We also analyze old bone samples very frequently. It is essential for us to quickly implement new technologies to upgrade our DNA testing and interpretation operations so we can deal with such challenging samples. DNA extraction and purification also matter. In 2020, we shifted to the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit after implementing the SeqStudio Genetic Analyzer to upgrade from the Applied Biosystems™ ABI PRISM™ 310 Genetic Analyzer and the 5-dye chemistry of the Applied Biosystems™ AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit.

What are the benefits of the SeqStudio Genetic Analyzer that made it the best choice for your lab?

First of all, it is very cost-efficient. There is a single-polymer cartridge for both sequencing and fragment analysis, so I don't need to change the polymer when I switch between STR and sequencing projects. It is much easier to use than before because it is cartridge-based and I don't have to worry about bubbles—just insert the cartridge and walk away. I can also leave the cartridge on the instrument for up to 6 months. I also like the touch panel control on the SeqStudio instrument compared to previous systems where I had to control the instrument with data collection software on a separate computer. Last, but not least, it is compact and does not take up much room in our lab. It has high-end features with a small footprint.

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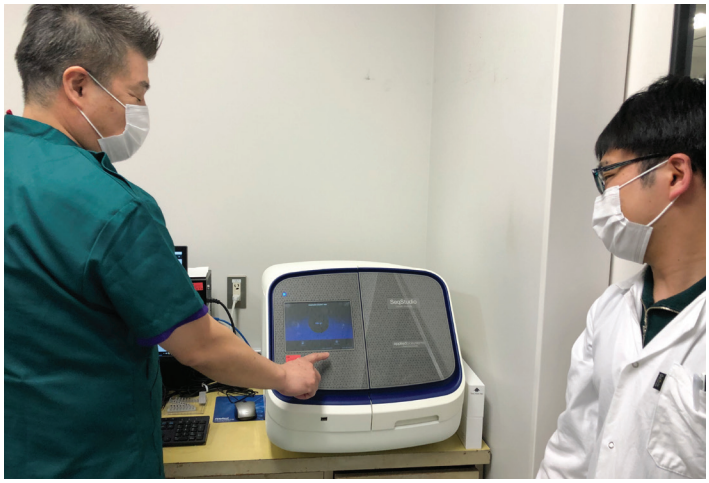
Why did you choose the SeqStudio system?

I am always dealing with challenging samples, so it is essential for me to implement the most updated methods. Since around 2016, it has been a really pressing issue to upgrade our STR amplification chemistry by implementing the GlobalFiler kit. Although Thermo Fisher Scientific colleagues recommended the Applied Biosystems™ 3500 Genetic Analyzer, its throughput was too high for us, so the SeqStudio analyzer's throughput better met the usage of our lab. After the Research Use Only (RUO) version of the system was launched, I waited for the human identification (HID) validation of the SeqStudio system. I do believe the Applied Biosystems SeqStudio Genetic Analyzer for HID was the right decision for our lab because it is validated for forensic use and combines well with validated Applied Biosystems™ STR chemistry and GeneMapper™ ID-X Software v1.6. Recently, the Thermo Fisher Human Identification Professional Services (HPS) team carried out all of our GlobalFiler validation studies and experiments on the SeqStudio system. They went through all of this in just one week and were able to provide accurate, well-presented results.



The SeqStudio system is very valuable in processing the difficult case samples very quickly. We can run the GlobalFiler kit, Applied Biosystems™ Yfiler™ Plus PCR Amplification Kit, and mitochondrial sequencing applications on the same instrument without changing the cartridge or polymer. And it is very easy for students and fellows to use in their forensic genetic studies, including complex mixture analysis. Recently, due to increasing demands and more samples submitted, we are considering purchasing one more SeqStudio system for personal use in our lab.

To learn more about the SeqStudio Genetic Analyzer and the Precision ID NGS System for human identification, please visit **thermofisher.com/ce-ngs**

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