The Future of Forensic Genomics is Here

King's College London researchers are finding that the MiSeq FGx™ Forensic Genomics System provides more information-rich data than CE-based forensic DNA testing methods.

Introduction

Those who watch popular television crime scene investigation shows know that DNA testing results are part of the modern detective's toolkit. Forensic DNA analysis can help in identifying missing persons, victims of mass disasters, unidentified remains, complex familial relationships, and promising investigative leads.

Next-generation sequencing (NGS) now gives forensic testing the potential to deliver extended capabilities, higher resolution, and greater efficiency—leading to more reliable results. David Ballard, PhD, a postdoctoral scientist, and Laurence Devesse, a forensic research associate, are members of the Forensic Genetics Group at King's College London led by Denise Syndercombe-Court, PhD. The team is at the forefront of forensic genomics and through establishment of a Knowledge Transfer Partnership (KTP), their work with the MiSeq FGx Forensic Genomics System is leading the way in the maturation of modern forensic identification technologies.

Moving Beyond CE-Based Methods

In mid-2015, Boston police discovered the remains of a preschool aged girl on Deer Island Beach.[1] For months, the police, with the help of forensic scientists, worked tirelessly to identify "Baby Doe"—to date, they have been unable to do so. When unidentified human remains are discovered, in varying states of decomposition, genetic testing can provide investigators with valuable information about the decedent. Today, most forensic labs use capillary electrophoresis (CE)-based analysis methods to attempt such identifications, the forensic DNA standard for the last ~20 years. Ballard says that such methods are a bit like a barcode—where one can look at a few very specific areas of the genome.

"These are just size-based markers," Dr. Ballard said. "If a full result is developed, you look at how many repeats of a particular DNA sequence there are and that gives you a barcode that is unique to each individual. This is challenging with complex forensic samples. There have been a lot of advances in the field over the past 20 years, and although CE-based methods are very good in terms of sensitivity and accuracy, they possess limitations that just cannot be overcome. NGS advances have surpassed size-based CE detection."

Ms. Devesse agrees. "Because CE-based methods separate DNA based on size, markers of the same length cannot be differentiated easily," she added. "With CE the forensically relevant areas of DNA must be designed to be different sizes. That is a problem when the forensic sample is partially degraded, because you need to look at the smallest fragments possible. You can do that only to a limited extent using CE.

CE-based methods have several other limitations beyond degradation. They also require that tests be performed serially rather than concurrently. In an era when forensic labs are being asked to do more DNA-related testing on a single sample, this can be a significant drawback.

"If you want to analyze polymorphisms on the Y-chromosome and also at autosomal loci with CE-based systems, you'd have to perform them separately in iterative fashion," Dr. Ballard stated. "You can't perform both assays at the same time."

Those CE limitations aren't specific to identity testing. Dr. Ballard says that multiple tests would also be required for intelligence gathering requests, to garner more information about biogeographical ancestry or phenotyping. When it comes to more complex questions, the number of markers in CE-based forensic STR systems are often not enough to aid the case.

"If you are looking at disaster victim identification, or a complex familial relationship regarding an immigration case, you often need to analyze many more markers to obtain enough information to prove whether people are related or not," Dr. Ballard said.

Hence, the group's interest in contemporary NGS using the MiSeq FGx System. It enables forensic scientists to ask a wider range of questions about a sample in a single, targeted assay—providing a more powerful intelligence tool for themselves and associated law enforcement organizations.

"When you are working with a complex sample, where you might have to run an STR test, then a SNP test, and then other tests as well, it's a lot simpler to use a complete NGS solution like the MiSeq FGx System," Ms. Devesse said. "You can perform a single run and get all that information right from the start, which saves a lot of time."

Dr. David Ballard is a postdoctoral scientist and Laurence Devesse is a forensic research associate in the Forensic Genomics Group at King's College London.

MiSeq FGx System Results Are Impressive

Under the KTP agreement, Ms. Devesse works in collaboration with Illumina on a part-time basis. She is performing forensic validation studies using real-world (and rather complex) DNA samples.

"It's a very useful project for King's College and Illumina," Ms. Devesse stated. "I was able to use the MiSeq FGx System at Illumina and was involved in some of the last stages of development. I brought samples from King's to help Illumina test and evaluate protocols. This kind of partnership is important in helping us get what we want out of the technology, as well as establishing criteria that are important across the forensic DNA community."

The results have been striking. Dr. Ballard says that the number of markers that can be used with the MiSeq FGx System is a significant improvement over older methods.

"The fact that the MiSeq FGx System gives you so much information at one stage with both identity SNPs and routine STRs, is hugely important," he says. "It's also very useful that you can get all the information at one time instead of doing one set of tests, and then another set, and so on..."

He is excited about the possibility of testing mixed DNA samples. In the United Kingdom, gun control is very tight, so there are very few guns in the country. According to Dr. Ballard, when a gun is recovered as evidence in a criminal case, you can all but guarantee it's been handled by multiple people—which makes testing difficult.

"You tend to find that any gun that's been used in any sort of crime has been used by several people," Dr. Ballard said. "So if you take a swab from a gun grip, DNA from multiple people will likely be detected. One of the potential beauties of the MiSeq FGx System is that we can try to separate out the different people that might be in a single swab sample to help the police make their case or refute the suspicion."

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Dr. Ballard is also enthusiastic about the system's short amplicon lengths—and the ability to reduce the amplicon size down to 70–120 bases. "In very poor quality samples, the DNA is fragmented," Dr. Ballard stated. "With traditional STR tests, amplicons must be designed to be longer, and so poor quality samples often produce poor quality results. With SNPs in the ForenSeq™ DNA Signature Prep Kit, you use much shorter amplicons—and get much better results."

In fact, Ms. Devesse has demonstrated this in her forensic validation studies. "Using shorter amplicons is very useful in forensic casework, especially when you don't know exactly what you're dealing with or how degraded a sample might be," Ms. Devesse said. "For a fragment of DNA that is quite small, you might get a value for your quantification that appears to be within an optimal range and yet not have many fragments large enough to type enough STRs to draw a conclusion. The ForenSeq kit maximizes the chances of developing a useful profile from samples such as bone, teeth, and unidentified remains."

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ForenSeq Universal Analysis Software Delivers Easy-to-View Data

After working with the MiSeq FGx System for nearly a year, Dr. Ballard and Ms. Devesse agree that although the switch to such a system can be a bit of a "culture shock," it's made easier by how simple the system is to use.

"It has a straightforward interface," Ms. Devesse said of the ForenSeq Universal Analysis Software. "It's very easy to follow the guide and go through the steps. Illumina really focused on the software. It is extremely user-friendly. You can access the initial information that you need easily."

She likes that the initial screen of the ForenSeq software provides quality information about the MiSeq FGx System run—including metrics about cluster density and clusters passing filter. "You see green if the quality is good, orange if user intervention may be helpful—and you have information from the start about the overall health of your run," Ms. Devesse said. "When you see the green, you can start to look at other controls and evaluate sample quality. For each sample, you can see all of the ForenSeq autosomal STR, X STR, and Y STR allele calls, the SNP calls, including identity SNPs and, if desired, phenotypic SNPs and biogeographical ancestry SNPs. For each locus, the intensity (read count) of the allele is displayed. If there's a DNA mixture, the software indicates that there are more alleles than you should have for a particular marker. So you get a lot of information right away—and from there, you can start looking at phenotypic and biogeographical ancestry estimations and perform direct comparisons to other samples, such as reference samples."

Dr. Ballard believes the MiSeq FGx System will be great for developing investigative leads for different cases. "With the MiSeq System, we have the potential to generate information regarding what part of the world a person comes from, as well as physical traits including hair and eye color. As development of these predictive techniques continues, they will become increasingly useful. They provide law

enforcement agencies with direction as to what avenue they should follow first, which leads they should prioritize, or which scenarios to exclude from their primary focus. We are very interested in continuing the forensic validation process."

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Currently, the Forensic Genetics Group at King's College is validating the MiSeq FGx System for criminal work with mixture and concordance studies—obtaining population allele frequencies and examining data for accuracy, sensitivity, and reproducibility. In parallel, they will continue to use the MiSeq FGx System for complex immigration and relationship cases.

MiSeq FGx System Provides Answers in Complex Immigration Cases

Dr. Ballard believes that the future is bright. Ten years ago, he would never have imagined himself one day taking a small sample of DNA, the same sample he would have put into a 10 loci STR kit, and be able to get the immense number of sequence reads that offer so much information, from biogeographical ancestry to eye color.

"We think it will work much better with partially degraded DNA, enabling a lot more samples to be interpreted," Dr. Ballard said. "I think you'll find, especially in the human identification field, that the use of the MiSeq FGx will be taken up quickly. If it works as well as we hope for mixtures, I imagine there will soon be labs that are dedicated to running samples for those kinds of case samples across countries."

> "When we ran these cases through the MiSeq FGx System, we were able to prove that they were related as parent and child, thanks to the breadth of markers within the ForenSeq DNA Signature Prep Kit."

The laboratory has already successfully used the MiSeq FGx System for complex immigration cases. In the United Kingdom, individuals can apply to have family members from abroad come join them to live in the country. If they are turned down due to doubt of a familial relationship, then the individual can appeal, and request for genetic testing.

"We've now had 2 cases where we've seen multiple exclusions with standard forensic STRs," Dr. Ballard stated. "Normally, labs will determine that 2–3 exclusions between a parent and a child means that there is no parental relationship. When we ran these cases through the MiSeq FGx System, we were able to prove that they were related as parent and child, thanks to the breadth of markers within the ForenSeq DNA Signature Prep Kit. Without the MiSeq FGx System, proving that would have been very complex, if not impossible." The superior ability of the MiSeq FGx System to discern relatedness could also help in resolving paternity and missing persons cases, and assist in disaster victim identification.

Ms. Devesse is the daughter of a forensically trained police officer and has been fascinated with forensic science since childhood. She agrees that the MiSeq FGx System shows great promise in forensic DNA analysis. "More and more, we are seeing changing attitudes about this kind of testing," Ms. Devesse said. "When we show forensic scientists who haven't experienced the system how it works, for example on highly degraded remains, you can see these people's faces change—they want to know more. We're at the stage where we are really showing people what the MiSeq FGx System can do."

References

1. Cook L, Ansari A, and Botelho G. Experts: 'Baby Doe' perhaps placed on Boston shoreline. CNN. www.cnn.com/2015/08/12/us/boston-baby-doe-investigation/. August 12, 2015. Accessed August 25, 2015.

Learn more about the Illumina products and systems mentioned in this article:

- MiSeq FGx Forensic Genomics System
  www.illumina.com/systems/miseq-fgx.html
- ForenSeq Universal Analysis Software
  www.illumina.com/informatics/research/sequencing-data-analysis-management/forenseq-universal-analysis-software.html
- ForenSeq DNA Signature Prep Kit
  www.illumina.com/products/forenseq-dna-signature-kit.html

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