Casework Genetics Uses Illumina Technologies to Decipher Forensic DNA Mixture Evidence

High-throughput arrays enable forensic laboratories to unravel mixed samples and obtain high-quality results from severely degraded genetic material.

Introduction

The application of DNA technology to forensic testing has transformed the justice system, with DNA evidence now routinely used to solve crimes. Yet, the analysis tools available in most forensic laboratories are not as sophisticated as those found in today's genomic facilities. The current state-of-the-art is almost two decades old and there are still samples that defy analysis using current techniques.

Leveraging the ability of the Illumina Infinium® HumanOmni1-Quad BeadChip to genotype over one million single nucleotide polymorphisms (SNPs) per evidence sample, Casework Genetics is bringing the genomic revolution to forensic identification. The company's innovative tools are enabling forensic scientists to move investigations forward, overcoming the roadblocks created by low-quality and mixed evidence samples.

Genetics Enters the Forensics Laboratory

Since the mid-1990's, short tandem repeat (STR) polymorphism analysis has been used for genetic fingerprinting in forensic cases. The STRs used for forensic testing are most commonly tetranucleotide repeats, where a pattern of four bases is repeated multiple times. STR analysis is performed by extracting DNA from cells, amplifying specific regions using PCR, and separating and detecting those regions using gel or capillary electrophoresis. The variable nature of the STR regions enables the discrimination between one DNA profile and another. In the United States, 13 core STR loci are typically used to generate individual profiles, which are stored in local, state, and national DNA databanks for use by law enforcement and government agencies, such as the Federal Bureau of Investigation (FBI).

Kevin McElfresh, Ph.D., Laboratory Director and CEO of Casework Genetics

STR analysis was a significant improvement over earlier DNA profiling methods and has been used successfully to solve criminal cases and identify perpetrators. Yet, it is slow and limited in its ability to generate DNA profiles from mixed or degraded samples often found at the scenes of violent crimes.

SNP Array Tool Provides Comprehensive Genomic Coverage at Higher Throughput

SNPs are highly conserved within a population and make excellent genetic markers. Due to the efforts of the Human Genome Project, the International Hap Map Project, and 1000 Genomes Project (1kGP) the number of known human SNPs now exceeds 35 million. An essential tool of basic research, high-density SNP arrays are routinely used in genome-wide association studies (GWAS) to identify genes or regions involved in heritable disease. Their ability to determine the genetic differences between individuals makes them ideal candidates for forensic testing. With DNA array multiplexing capacity increasing from the simultaneous interrogation of 1,000 to > 5 million SNPs per sample, SNP panels now deliver the high-throughput, accuracy, and specificity needed by forensics laboratories.

Casework Genetics is leveraging these advances, creating tools to answer complex forensic questions that previously have gone unresolved. According to Kevin McElfresh, Ph.D., Laboratory Director and CEO, "The powerful data set obtained from parallel SNP processing overshadows the ability of the 13 or so STRs currently used in crime labs."

Believing that such ultra-high–density SNP panels would be more effective in analyzing complex DNA mixtures—the result of co-mingled body fluids or multi-person handled evidence, such as gun grips—Dr. McElfresh and his team compared a number of platforms for suitability in forensic testing. They chose the HumanOmni1-Quad BeadChip and the Illumina iScan system to perform initial proof-of-concept studies. "In a forensic laboratory the assay has to be well-defined and robust since the starting material is often of poor quality," Dr. McElfresh said. "We were impressed with the high accuracy and data quality of the HumanOmni1-Quad BeadChip. After looking at a number of platforms, we found Illumina's to be the most automated and organized assay; meaning that it would be the easiest to adapt for robust forensic testing."

The four-sample HumanOmni1-Quad BeadChip contains more than one million carefully selected markers derived from the 1kGP, all three HapMap phases, and recently published studies; delivering genome-wide coverage with minimal redundancy. Incorporating high-performance lasers, optics, and detection systems, the iScan System can scan BeadChips in just a few minutes, enabling processing of up to 384 samples per day. "I spoke with laboratory technicians who had the option of using the Illumina or a competitor's platform and they

emphatically said that they preferred the iScan System," Dr. McElfresh added. "With the Experienced User Cards and standard operating procedures already developed, the Illumina platform was an easy choice to make."

Proof-of-Principle Studies Demonstrate Robust Assay Performance

Casework Genetics conducted proof-of-principle studies using mock evidence samples to perform concordance, sensitivity, and preliminary mixture studies. The company was so impressed with the data generated that it went on to complete a full developmental validation of the HumanOmni1-Quad assay according to the ISO 17025 Standard for international accreditation, and the FBI Quality Assurance document[1], which provides the guidelines for forensic operations throughout the United States.

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"Using the HumanOmni1-Quad BeadChip, we accurately detected trace amounts of genomic DNA—down to less than 1% of the total mixture DNA concentration—at overall DNA concentrations of approximately 0.06 ng," Dr. McElfresh said. "While this is in line with STR systems for overall sensitivity, it extends the limit of detectable mixture contributors." One particular advantage of BeadChip technology is the redundant assay of each locus. Coupled with the simple bi-allelic structure of SNP loci, this provides a level of sensitivity that allows analysis of data that would fall under the stochastic threshold of STR systems.

Casework Genetics has used the technology to successfully unravel mixed forensic DNA samples, reviving items that failed to yield interpretable results using STR analysis. "For critical DNA mixtures, STRs often lead to a dead-end of inconclusive data," Dr. McElfresh said. "SNPs are the light at the end of the tunnel in these cases and can lead directly to solving a crime, getting a violent offender off the streets or innocent persons out of the system."

Resolution of mixed samples has been an issue for the forensic community, even since the shift from restriction fragment length polymorphism (RFLP) to STR analysis, and the situation has only worsened as sensitivity has increased, making analysis of touch samples routine. According to forensic DNA expert Martin Tracey, Ph.D., Professor of Biological Sciences at Florida International University, "there have been no advances in STR methodologies or interpretive algorithms offering sufficient improvement to resolve complex mixtures. Casework Genetics' SNP approach using the Illumina BeadChip appears to be the resolution of the mixture comparison dilemma in forensics."

The assay has also been found to be especially applicable to severely degraded genetic material, including crime scene samples found in less than ideal conditions and environments that are hostile to DNA preservation. The HumanOmni1-Quad BeadChip is able to redundantly assay 50 bp fragments, resulting in more DNA available for analysis and increasing the statistical power of the results. In contrast, DNA fragments of miniSTRs range between 70 bp and ~300 bp. "Given the results we are achieving, we believe it is possible to obtain high-quality results from DNA on commonly handled objects in criminal investigations," Dr. McElfresh said.

Proprietary Software Analyzes Raw SNP Data

To keep up with the scale of data that Casework Genetics is generating, Dr. McElfresh and his team developed Forensic SNP Analysis Software, an automated analysis tool to handle the large data set produced by this type of sample processing. It was built upon fundamental advancements made by a team of researchers at the Translational Genomics Research Institute (TGen)—led by David Craig, Ph.D., Associate Director, Neurogenomics Division—which provided a straightforward solution to rapidly and sensitively exploit raw SNP allele intensities from the iScan System[2]. According to Dr. Craig, "the same technologies that sparked a revolution in our understanding of disease have the potential to fundamentally change forensics."

Based on the data generated by the HumanOmni1-Quad BeadChip, the software targets and compares thousands of SNPs on each of the 23 autosomes, on the X and Y chromosomes, and in the mitochondrial genome. It does not require *a priori* knowledge of the number of individuals in the mixed sample or assume the relative amounts of DNA present from each contributor.

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To speed processing time, the software breaks the massive data set into subsets that are organized by chromosome, then analyzes those sets individually. This enables the software to be installed and run on portable notebook computers. The end result is a comparison of all samples and a complete forensic analysis of the data required to interpret the relationship of the samples found in the mixture. "Our software is successful in taking the massive amount of data generated by Illumina GenomeStudio® software and getting it into the forensic format that officers of the court can use effectively" Dr. McElfresh said.

Expanding Forensic Analysis Applications

Confident in their ability to produce high-quality data and compare DNA from multiple contributors to persons of interest, Casework Genetics has also begun looking at more complex problems related to mixtures. For example, comparing mixture samples to other mixture samples in order to determine common contributors.

Dr. McElfresh sees a future where, "it will be possible to bring much more complex mixtures into play and determine whether an individual has been present at public places and transportation hubs. Applications for tracking fugitives and understanding the dynamics of criminal groups such as gangs are each amenable to this technology."

Successfully Delivering Genomics into the Forensics Laboratory

Leveraging the accuracy, sensitivity, and high-throughput of the HumanOmni1-Quad BeadChip and the iScan System, Casework Genetics is positioning itself as a provider of 21st century, fully validated genomic technology for forensic operations. Dr. McElfresh believes the company's strength lies in the fact "that we are first and foremost forensic professionals. We have the courtroom experience that matches our scientific acumen and we are very familiar with the rigors of establishing new technology in the justice system." Thanks to Casework Genetics, forensic DNA reports that describe DNA mixtures, but can't draw conclusions regarding persons of interest, or provide underwhelming population statistics, may become a thing of the past.

References

1. www.fbi.gov/about-us/lab/codis/qas_testlabs
2. Homer N, Szelinger S, Redman M, Duggan D, Tembe W, et al. (2008) Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. PLoS Genet 4: e1000167.

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Pub. No. 370-2011-022 Current as of 07 October 2011

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