

By Digging Deeper into the Genome, Next-Generation Sequencing May Yield More Forensic Clues

Dr. Bruce Budowle at the University of North Texas Health Science Center discusses how next-generation sequencing will transform forensic genomics.

Bruce Budowle, Ph.D., is Executive Director of the Institute of Applied Genetics at the University of North Texas Health Science Center (UNTHSC). Founded in 2009, the institute brings together a number of well-known scientists focused on refining genetic approaches to enhance several fields of study, including forensic DNA. Students enrolled in UNTHSC's forensic science program (1 of only 14 in the United States) also participate in the institute's forensic DNA program, where Dr. Budowle leads a team that balances case work and human identity testing with research and development into new technologies, such as next-generation sequencing (NGS). Their goal is to determine how best to apply and optimize NGS and other methods, and develop protocols that streamline their use in forensic analysis.

We spoke to Dr. Budowle about his thoughts on NGS and its potential impact on forensic genomics.

Q: What forensic DNA R & D efforts are being conducted by your team at the Institute?

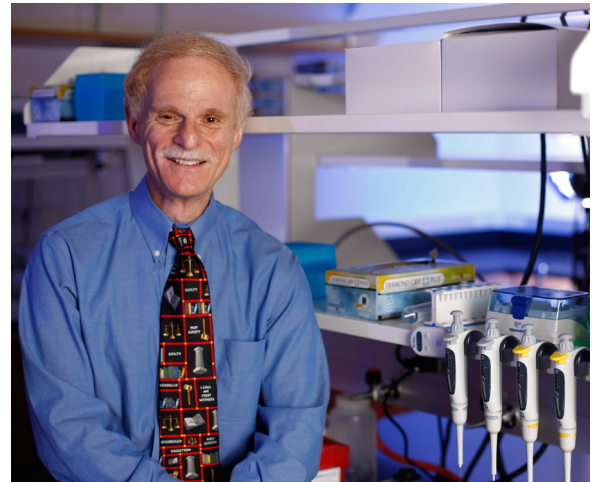
Bruce Budowle (BB): We're developing methods for better DNA recovery and removal of inhibitors from the environment that may affect our analyses. We're also investigating new genetic markers that will allow us to look deeper into forensic and human genetics questions, and identifying new technologies that will expand our capabilities in forensics. We're using a variety of new methods, from synchronous coefficient of drag alteration methods (SCODA) to extract, purify, and concentrate DNA from challenging forensic samples, to NGS technologies that can analyze those samples and help us retrieve the most information possible.

Q: Do you think that NGS will be transformational in forensics?

BB: I think it's going to be another transformational tool that will greatly benefit forensics. We've had a number of them over the years, from restriction enzyme digestion and Southern blotting to the polymerase chain reaction (PCR) that improved molecular biology across the board. Now we have new NGS tools that allow us to look at things in more depth and analyze many more samples in a parallel, multiplex fashion. NGS will also enable us to be more flexible in addressing the forensic challenges we face.

Q: Are there limitations in the current forensic workflow that NGS will alleviate?

BB: Our biggest gap in forensics is getting quality results from the limited information that we can obtain from low quantity, and often poor quality, DNA samples. NGS will enable us to obtain higher quality results from much smaller DNA samples.



Bruce Budowle, Ph.D., is Executive Director of the Institute of Applied Genetics at the University of North Texas Health Science Center (UNTHSC) where he leads a team of researchers in investigating forensic applications, such as next-generation sequencing.

The other limitation is the limits of our core set of markers. There are situations where having more genetic markers would help us answer a forensics question. For instance, when there's a blood stain found on the floor and we're making a comparison between it and a suspect's sample (reference sample), we're directly comparing genetic profiles. We're looking for a 1:1 correspondence of the evidence to the reference sample. In missing persons cases, we're performing kinship analysis, where we're looking at part of a genetic profile of an individual that may be shared with a potential or alleged relative. That means we don't have as much information to compare profiles. It's a little easier to compare first-degree relatives, such as a parent, offspring, a brother, or brother-brother-sister scenario. Identifying half-brothers or cousins, or comparing grandparents to grandchildren, becomes much more complicated. With the power of full genetic marker sets created using NGS, we're going to be able to answer kinship questions we could not even conceive of solving just a few years ago.

Q: How do you see the forensic laboratory using NGS?

BB: As I said earlier, one of the real benefits of NGS is that we'll be able to analyze more markers. We'll be able to apply NGS to reference samples where it will give us a lot more latitude on what we may be



codeine through sequencing, where it was identified that she had a double dose of a particular gene. Since codeine metabolizes into morphine, the child was receiving morphine through the mother's milk. That information took the investigation in a totally different direction, because we had strong evidence that the manner of death was an accident and not an intentional homicide. With NGS, we can sequence a large number of genes at one time and look objectively at all the variants that may be there, providing us with information that could help determine the cause of death in challenging forensics cases.

Q: *The Combined DNA Index System (CODIS) contains DNA profiles contributed by federal, state, and local forensic laboratories in the United States. How do you see CODIS evolving in the near term?*

BB: CODIS needs to start preparing its system to better accommodate NGS data. CODIS is currently fixed on its core markers and everything outside that core is not well addressed in its database. With NGS, we may see in the near future 30, 40, 50, or 200 markers added that will increase our ability to search for portions of those markers against a reference set. Most people are driven by the ability to upload things into CODIS. So in the future, if data from NGS systems can't be uploaded into CODIS, it will slow down the database's evolution. That's why it's so imperative for CODIS to start thinking about how to accommodate the markers NGS can identify and put it into their builds for the next version.

“In the future we'd have one technology that everybody could rely on, with all markers based on that technology. That's what NGS offers.”

Q: *How can NGS be used for bioancestry?*

BB: There are markers that have a higher frequency in one population as opposed to another population. If you see one variant you might be able to infer something about the bioancestry of that individual. With enough markers, population affinity can reach high probabilities. There is tremendous value for us to find more of those markers for our missing persons work. Currently, when law enforcement finds human remains and a skull is located, we give that to an artist who reconstructs the skull and provides us with a drawing of the deceased. Sometimes the drawing is amazingly identical to the individual and other times it's not even close.

In the phenotype area, where we're trying to figure out what a person looks like, a lot of work has been done in determining pigmentation—hair color, eye color, skin color. We now want to focus on markers associated with more specific attributes, such as the shape of the nose, the ears, the lips, the soft tissues, maybe curly versus straight hair. These features might be enough to trigger someone's memory in recognizing the reconstructed individual and would enable us to move forward with a traditional DNA kinship analysis. I think that's the real value of NGS in bioancestry marker discovery.

One other area that might be of interest is microbial forensics. We have a history of microbial forensics dating back to the days of the anthrax letter attack and we are continuing that biosafety and biosecurity work. I think the tremendous depth of coverage that NGS can provide will benefit metagenomics studies that can be performed on the human microbiome, on challenging samples, and on potential threats. It's the ideal technology for that.

Q: *What other benefits will NGS provide?*

BB: NGS will enable a lot more of our analyses to be automated and done with software as opposed to an individual looking at data, verifying, and a second person looking and verifying. We'll rely more on the semi-expert software system capability to work out what markers are there with quality scores and other bits of information to give us confidence in the results. Because that will be done behind the scenes in the computer, it will improve our throughput capabilities.

Learn more about Forensic Genomics at www.illumina.com/forensics



