Next-generation sequencing (NGS), also known as massively parallel sequencing (MPS), is potentially game changing for forensic labs and the criminal justice system. However, putting NGS into place requires labs to adopt new technologies, processes and methods.

The Battelle Applied Genomics team provides technology translation services to help government agencies and forensic laboratories integrate NGS into their existing workflows. Over the past year, Battelle researchers and technologists have been heavily involved in helping labs select or develop NGS test kits, develop and validate laboratory workflows, set up analysis pipelines and train laboratory personnel on NGS methods. Here are answers to some of the most common questions that come up during the process.

**Q. Does the NGS workflow decrease turnaround time for DNA samples? How long would it take for a rush case to be completed?**

A. Yes, the turnaround time is decreased due to the ability to multiplex, or parallel process, a large number of markers into one run. For example, if a rush case requires autosomal STRs, YSTRs and mtDNA to be typed, all of the analyses can be completed in a single run. Currently, that processing time is about four days, including analysis. NGS forensic kits are continually undergoing development and optimization, much of which is aimed at reducing sample preparation time.

**Q. How much will NGS cost compared to current workflows?**

A. While it depends on the exact workflow and reagents used, traditional capillary electrophoresis (CE)-based workflows cost approximately $25 for autosomal STRs, $25 for YSTRs and $65 for mtDNA CR, for a total of $115 per sample. These estimates do not include extraction or labor costs. An NGS workflow for all of these markers would cost approximately $85, with additional savings realized in labor costs due to the combined workflows.

**Q. How much new equipment will be required for NGS?**

A. The only new equipment necessary is the sequencing instrument itself. While not mandatory, labs may also want one or two ancillary instruments for quality checking. Sample prep requires standard laboratory equipment such as a thermal cycler, centrifuge and pipettes. NGS is also amenable to automation, so users can implement such devices if desired.

**Q. How long will it take to train analysts in interpreting the results and generating statistical calculations?**

A. Much of an NGS workflow consists of standard molecular biology procedures, so training will take about as long as standard forensic DNA training in an accredited lab. Since all of the STR and YSTR markers currently used in forensic DNA can also be found in NGS, statistical calculations will be performed in the same manner. The only difference is that statistics may need to be performed on SNPs, in which case more research should be performed to create a high-quality database for SNP frequencies.

**Q. How does one deal with all the data that is generated and what role does informatics play in the analysis of the samples?**

A. Data storage and security issues are real, and laboratories must plan accordingly. Fortunately, many forensic laboratories have skilled IT staff, and therefore should be able to address these issues and ensure data integrity, retrievability and security. A laboratory that is planning to implement NGS into casework or database applications should start the planning process early. Analysis of the samples requires specialized software, which is subject to rigorous testing and evaluation procedures to ensure quality.
Q. How sensitive is the process with forensic/degraded samples?

A. NGS is beneficial to forensic samples because of the ability to sequence shorter pieces of DNA (i.e., degraded samples) than the standard technology. Another indication of the sensitivity of NGS is the ability to easily distinguish mixtures, as well as distinguishing stutter from a true allele, based on sequence.

Q. Are the results going to be searchable in a national database?

A. Ultimately that is the goal. Much developmental work and initial validation have been completed to demonstrate backwards compatibility of NGS-derived genotype results with those through legacy CE. More extensive studies are underway to support this objective.

Q. What advantages does NGS offer in terms of mixture analysis? Can identical twins be differentiated with this new technology?

A. The amount of information obtained from each sample using NGS is vast. Hence, if properly modeled, mixtures can be more easily deconvoluted than with current technologies. Genetic or epigenetic markers that can differentiate between identical twins have been shown to be feasible, but would require much more validation before this capability is used routinely.

Q. What new validation issues may arise with NGS?

A. Many of the validation requirements presently categorized within the FBI’s DNA Quality Assurance Standards will apply directly as written. These will include noise determinations, interpretation of controls, establishment of stutter values etc. Additional requirements will be needed to define and subsequently evaluate performance parameters that are specific to the NGS technology. These will include analytical process determinations such as flow cell capacities, sequence variant identification and frequency assessments, as well as broader concepts within bioinformatics such as genotype concordance (NGS to CE), nomenclature and data management.

Q. How much information is too much for the forensics field?

A. It’s not necessarily a matter of too much information, but the proper interpretation of the information. The increase in information gained by moving from traditional DNA analysis to NGS methods is similar in scale to the increase in information gained by moving from an optical microscope to a scanning electron microscope. Although the resolution is much higher, interpreting the images requires placing the information into the right conceptual perspective.

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If you have additional questions, please contact us at 800.201.2011 or solutions@battelle.org to speak with an Applications Specialist.