Battelle Whitepaper:

# Moving MPS to the Mainstream

## An Implementation Roadmap for Massively Parallel Sequencing in Forensic Labs

Battelle
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## An Implementation Roadmap for Massively Parallel Sequencing (MPS) in Forensic Labs

### Introduction: The New DNA Revolution in Forensics

MPS is moving into the mainstream for forensic investigation. Is your lab ready?

MPS technology has been previously utilized primarily in research environments, but a growing number of internationally accredited DNA forensic laboratories are in the process of validating the technology for forensic typing applications. The implementation of any MPS system in a forensic laboratory will require a careful selection and design of an MPS workflow to support the laboratory's mission and priorities. This process may appear to be daunting, but the benefits MPS delivers for forensic applications are well worth the effort. We'll take a closer look at these advantages and outline an implementation roadmap informed by experience our laboratory has gained through numerous testing projects.

### The Power of Parallel Processing

MPS—sometimes known as next generation sequencing, or NGS—uses massively parallel processing technology to vastly increase the speed and processing power of DNA sequencing devices. Instead of processing amplicons that generate a consensus sequence, MPS technologies can process up to millions of reads, or sequence of nucleotides, thus increasing the resolution of every sample.

For DNA forensics, this data processing power provides several critical benefits for forensic labs:

- Information: Unlike CE technology, a single sequencing run may generate data that encompasses a wide range of genetic markers, such as short tandem repeats (STRs), single nucleotide polymorphisms (SNPs) and even mitochondrial DNA (mtDNA). This may be a significant advantage, especially when dealing with a sample of limited quality, to gain probative information.
- Speed: Parallel sequencing allows MPS to generate more data in less time, allowing for high throughput of DNA samples.
- Sample types: Because MPS generates millions of DNA sequences at a time, its potential level of success with severely challenged forensic samples, such as degraded and/or mixed samples, is significantly increased.

If you think of the human genome as a book, current forensic CE technologies for STR-typing tell you the page length for several of the book's chapters. MPS technology can not only tell you how many pages are in these chapters, it can also report every single word and letter that make up each chapter, opening up new possibilities for DNA forensics.

### Forensic Applications of MPS

DNA typing improves on the ability to make comparisons between samples, for example, to determine whether or not a sample taken from a crime scene and a reference sample are potentially from the same source. Both CE and MPS facilitate this type of comparison, with the former also supporting Combined DNA Index System (CODIS) DNA database searches. Studies have been performed since 2012 that demonstrate the back-compatibility of STR genotyping developed through MPS compared with corresponding CE derived STR truth data. Comprehensive formal validation is underway to further demonstrate concordance of MPS STR typing with CE STR data and provide a foundation for future MPS-based CODIS applications.

However, while both a powerful and invaluable resource, DNA database searches only provide successful results on a percentage of samples queried, with limitations imposed by either the database composition (matching sample not in the database) or sample constraints (inability to develop a DNA profile suitable for database searching).

MPS will enable forensic laboratories to take the analysis beyond such limitations. Because we are now reading the sequence of the DNA instead of evaluating fragment length, forensically relevant information may be obtained from

evidentiary samples for which a DNA database search was unsuccessful or unable to be attempted. This information might include statistical prediction for:

- Ancestry/geographic origin
- Potential familial relationships
- Physical characteristics/phenotypes, such as hair and eye color, skin tone and even certain facial characteristics

This type of information can provide investigators with important clues to narrow down lists of suspects or suggest new avenues for investigation. As we learn more about the human genome and how genes influence phenotypes, this information will become even more powerful.

In addition, the ability of MPS to generate additional information from highly degraded or mixed samples gives investigators a powerful new tool for complex cases, such as analysis of DNA from skeletal remains, old bloodstains or contaminated crime scenes. MPS could be used to help solve cases that have reached a dead end using older DNA technologies.

One of the most promising applications of MPS today may be missing persons' cases. Currently, due to a combination of reasons including technical specialization, many forensic laboratories do not analyze highly decomposed skeletal remains. Using traditional sequencing technologies, investigators may not be able to give families the definitive answers they want. MPS has the potential to provide additional information that may be critical for supporting such difficult investigations. This may be the result of the expanded panel of genetic markers for prediction of physical characteristics, family relationships and increased identification capabilities. It may also provide new leads in other types of cold cases.

## Implementing MPS in Forensic Labs

Implementing MPS will require changes in instrumentation, workflow, quality control, validation and training. Overall, you can expect the implementation process to take about one year. Here are the basic steps you will follow:

1. Selecting an MPS platform
2. Preparing physical laboratory space
3. Training laboratory staff
4. Adapting workflows for lab-specific MPS needs
5. Validating new equipment and workflows

### Selecting an MPS Platform

The first step is deciding which platform to use. There are a variety of commercially available MPS platforms that vary in their laboratory-processing workflow, detection chemistries, data output, read length and error rates. In choosing the right platform for your lab, you will want to consider:

- What kind of samples do you want to run? What type of markers do you want to evaluate from those samples? Different instruments may be more or less suitable for particular sample or marker types. Some platforms even allow for the user to customize their own sample preparation kits with markers they select for a particular sample type.
- How expensive are the reagents and consumables? Equipment cost should be considered using a "total cost of ownership" model that includes these ongoing expenses.
- What is the total number of samples that you will run? If you are anticipating large volumes, you may want to consider automation. If so, you will want to evaluate for compatibility with robotic sample preparation equipment.
- Are the markers you want to target backward compatible? Some platforms provide both SNP and STR data, allowing compatibility with existing STR databases (e.g., CODIS/ National DNA Index System (NDIS). Others may only provide the SNPs, and are not backward compatible with STR-based databases.
- What kind of training does the vendor provide? Make sure that you are comfortable with their training model. Keep in mind that most equipment vendors offer training that concentrates on how to use their equipment and software, rather than how to interpret results or integrate MPS into a forensics laboratory.
- Will it fit in your lab? Physical dimensions may not be the biggest consideration in your purchase, but if you have limited laboratory space make sure you are aware of the footprint of the equipment and the bench space needed.

In addition to purchasing the physical sequencer, you may also want to look into software options. All device manufacturers have software specific to their instruments. However, other software programs, such as Battelle ExactID®, can provide more in-depth analysis of the raw sequencing data.

### Preparing Physical Laboratory Space

Before you set up your new instrument, you need to prepare your physical space. For the most part, ancillary equipment (thermocyclers, consumables, etc.) will be the same as in the current CE processing, so you won't need

to make huge changes to the physical setup of your lab. But there are a few things you may want to consider:

- Make sure you have enough bench space for the sequencer itself. Choose a location that will not interfere with other workflows. If you elect to include automation, you may want to consider space for this as well.
- You may need additional lab bench and hood space to accommodate changes to the post-amplification workflow. Most labs already separate workspace for pre-amplification and post-amplification workflows; however, MPS requires more post-amplification processing than the current technology. Battelle recommends including separation during post-amplification between pre-indexing and post-indexing steps to prevent cross contamination.
- You may need additional freezer or biosafety cabinet space to accommodate larger kits and additional reagents.
- Make sure you have adequate data storage space. MPS generates much more data per run than CE methods. You may require additional hard drive or server space to maintain these large data sets.

**Training Laboratory Staff**

Before you integrate MPS into your lab, laboratory staff will need to be trained. This training must include both procedural training focused on use of the new instruments and workflows, and theoretical training in the applications and analysis of MPS data. The training program should be onsite in your laboratory and tailored to your specific workflows and sample types. Training should include:

- Analytical methods specific to your equipment and software (classroom training seminars)
- Laboratory-specific new workflows and processes
- Software and data analysis training
- Pertinent quality assurance measures (controls, thresholds,etc.) as well as their application within the system validation

Part of this training must include hands-on practice exercises using training sets designed to help staff become comfortable with the equipment, software, workflows and analytical methods. Trainers should also have appropriate assessments in place to evaluate the readiness of individual staff members and the laboratory as a whole. Training may take between four and six weeks.

**Adapting Workflows for Lab-specific MPS Needs**

The workflows for MPS aren't radically different from those for CE, and none of the processes should be unfamiliar to experienced forensic laboratory staff. However, there are some modifications of which you should be aware. While the pre-amplification workflow will be largely the same, you will most likely need to add steps to your post-amplification workflow. These may include:

- Molecular biology techniques, such as a ligation step for library indexing
- An additional purification step
- An additional QPCR step after library indexing

Your individual workflow will vary depending on the equipment you have selected, the types of samples you are running and the setup of your laboratory. You may want to consider bringing in an outside expert to help define the workflows and set up quality control systems for your MPS processes. Battelle offers turnkey implementation support and technology integration services to get you up and running with MPS, from start to finish.

**Validating New Equipment and Workflows**

The FBI's Quality Assurance Standards (QAS) for Forensic DNA Laboratories is universally relied upon by forensic laboratories, as well as accreditation agencies, as the technical requirements necessary to guide a laboratory's validation process. It includes key quality practices for both developmental and internal validation of a DNA technology, with the purpose of identifying limitations and subsequent demonstration of system reliability.

The QAS were developed largely with legacy CE applications in mind, and have not yet been updated specifically for the MPS technology. However, many of the fundamental elements of the existing QAS include quality practices that would still apply in principle to the MPS technology (reproducibility, sensitivity, etc.). Once you have set up your laboratory space and defined your workflows, it will be necessary to design studies that correspond to such internal quality practices. In particular, your validation process must include validation of the new or modified portions of your workflow, including library preparation and bioinformatic processing.

**Support Services**

A lot of information and not quite sure the best way to get started? Don't worry. Battelle provides a variety of outsourcing service options for forensic DNA Laboratories using massively parallel DNA sequencing, ranging from consultation support to complete turn-key operational solutions. This service is developed from Battelle's extensive core experience base with both MPS and forensic applications.

Presently, Battelle and the Ohio Bureau of Criminal Identification (Ohio BCI) are conducting joint MPS validation studies, which includes the traditional frame-work of criterion recommended by the FBI's Scientific Working Group on DNA Analysis and Methods (SWGDAM) as well as the FBI's Quality Assurance Standards (QAS). Additionally, the Battelle/Ohio BCI collaboration has identified and developed validation studies with quality-affecting considerations specific for the MPS technology, augmenting the current elements of SWGDAM and QAS.

## Preparing for the Future of DNA Forensics

As more forensic labs adopt MPS, it is rapidly changing the landscape for DNA forensics. However, there is still work to be done to optimize the technologies, workflows and analytical methods for forensic applications of MPS. Battelle has been working with the U.S. Department of Defense (DoD) and National Institute of Justice (NIJ) to evaluate MPS in the field and develop optimized workflows and validation methods. The Battelle Applied Genomics team led a five-year project to implement MPS technology in the field for the DoD and was chosen to lead an NIJ project evaluating NGS methods in forensic labs around the country. This work has informed development of recommendations and training programs for forensic labs wishing to implement MPS.

Battelle is also collaborating with the Ohio BCI to implement MPS in an Ohio BCI lab. This work, along with Battelle's previous five years of developmental work, has informed the development of Battelle's technology transfer methods in several key areas:

- Validation: As part of the technology transfer program, Battelle and Ohio BCI worked collaboratively on validation test designs and the resulting data and interpretations.
- Training: The training program for the Battelle/Ohio BCI technology transfer was developed by Battelle and administered by Ohio BCI. Building upon Battelle's developmental work in the MPS area, it includes a progressive series of technical lectures, hands-on laboratory instruction and comprehensive training sets.
- Method Advancement: A key attribute of forensic DNA analysis has been the continual advancement of technologies for further optimization. The MPS technology is no exception. Ongoing internal research in the areas of front-end automation, streamlined sequence analysis/interpretation, and efficient data management processes are examples of areas progressing through further development.

Implementing MPS will take some time, but it doesn't have to be difficult. Battelle is working with forensic labs across the country to integrate MPS technologies, optimize workflows and develop new quality control and validation methods. As more labs implement MPS and the technology matures, the industry can expect to see implementation timelines condense and validation methods standardize. We are only just beginning to see how MPS will change the forensic landscape.



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