

Verogen MiSeq FGx™ Forensic Genomics Solution

Solve more cases and generate more leads with the power and accuracy of the Verogen massively parallel sequencing solution.

Highlights

- Fully Validated, Sample-to-Answer Solution**
 All-inclusive system ties library preparation, sequencing, data analysis, and reporting into a single integrated protocol
- Simple, Streamlined Workflow**
 Interrogate 200 genetic markers in a single, streamlined workflow eliminating the need for multiple STR kits
- Access a Wider Range of Information**
 Biogeographical ancestry, phenotypic-informative SNPs, and sequence variants provide valuable information not widely available with current technology
- Superior Analysis of Challenging Samples**
 Advanced capacity to handle challenging samples such as complex mixtures or degraded DNA



Figure 1: Illumina MiSeq FGx Instrument—The Illumina MiSeq FGx instrument is a compact, fully validated next-generation sequencing platform for forensic genomics applications.

Introduction

The Verogen MiSeq FGx Forensic Genomics Solution is the first fully validated¹ sequencing workflow specifically designed for use in forensic genomics applications (Figure 1). With the high resolution and unmatched accuracy of massively parallel sequencing technology, the MiSeq FGx Solution can transform the most fragile, degraded, or mixed samples into powerful results.

A Complete Sample-to-Answer Solution

The MiSeq FGx Solution delivers a complete DNA-to-Data workflow for the analysis of forensic DNA samples. The solution begins with the ForenSeq™ DNA Signature Prep Kit, which includes all reagents required to prepare DNA libraries for sequencing (Figure 2). The MiSeq FGx Reagent Kit provides sequencing reagents, an RFID labeled reagent cartridge, and wash solution, which are then loaded onto the Illumina MiSeq FGx instrument along with the sequencing-ready DNA libraries. The intuitive touch screen interface provides simple, step-by-step guidance through each stage of the sequencing run including library and reagent loading, run configuration, and run monitoring. ForenSeq Universal Analysis Software delivers a powerful suite of forensic analysis capabilities including automatic detection of mixed DNA samples, generation of population statistics, and CODIS-compatible reports, as well as an optional off-line report to enable flanking region analysis. The software also enables estimation of visible traits and biogeographical ancestry markers that can provide crucial investigative leads in “no suspect” cases. Built-in automation of the on-board cluster generation and analysis initialization means minimal hands-on time is required.



Figure 2: ForenSeq DNA Signature Prep Kit—The ForenSeq DNA Signature Prep Kit includes all reagents required to prepare DNA libraries for sequencing, including PCR reagents, index adapters, and purification and normalization beads.

Simple, Streamlined Workflow

With current forensic capillary electrophoresis (CE) methods, limited DNA quantities may require forensic analysts to choose between available fragment length-based short tandem repeat (STR) tests. These trade-offs can result in lengthy, complex decision trees as technical limitations are weighed against potentially informative genetic data.

The MiSeq FGx solution eliminates the need to choose between relevant STR tests by providing all currently available STR typing tests in a single workflow (Figure 3). One run on the Illumina MiSeq FGx instrument simultaneously interrogates ~200 genetic markers, including a combination of autosomal, X-, and Y-STRs. Furthermore, the ability to obtain highly discriminating data from less than 100 pg of DNA¹ enables testing of a broad range of samples where DNA quantity is limited.

Access a Wider Range of Informative SNPs

Another casework challenge occurs when an autosomal STR profile is established, but no suspect is available for direct comparison, or no hits are found in national or local criminal databases. In addition to providing a complete set of autosomal STR markers currently utilized around the world for casework and criminal DNA databasing, the ForenSeq DNA Signature Prep Kit also contains marker sets not routinely available with traditional CE methods. These include a dense set of identity informative single nucleotide polymorphisms (iiSNPs),²⁻³ which are informative for source attribution, biogeographical ancestry-informative SNPs (aiSNPs),⁴ and phenotypic-informative SNPs (piSNPs),⁵ which provide estimates of eye color (blue, intermediate, brown) and hair color (brown, red, black, blond). In generating tactical investigative leads from “no suspect” cases that may otherwise have reached dead ends, aiSNPs and piSNPs can be critical.

Superior Analysis of Challenging Samples

The challenges presented by degraded DNA, low quantity DNA, or complex DNA mixtures can potentially complicate or derail an investigation. The MiSeq FGx Solution offers an enhanced capacity to analyze these types of challenging samples.

When analyzing partially or highly degraded DNA, the SNPs in the ForenSeq DNA Signature Prep Kit are targeted by amplicon sizes ≤ 125 bp, making them extremely well-suited for analysis of degraded DNA.⁶ With complex DNA mixtures, the large number of markers included in the kit—many of which are highly polymorphic—coupled with the ability to look into the flanking regions of many loci, enable an improved ability to detect low-level minor components that may go undetected by traditional methods.⁶

These capabilities, combined with the inherent sensitivity of Illumina sequencing by synthesis (SBS) chemistry and the low DNA input requirement, provide superior resolution of a broad range of cases compared to conventional STR and CE analysis.

Easy, Optimized Software Solution

The MiSeq FGx Solution includes ForenSeq Universal Analysis Software: a fully optimized, software solution designed for forensic genomics applications (Figure 4). The software features a comprehensive suite of library management and analytical tools. These tools include sample search, data visualization at library and locus levels, STR and SNP allele genotype calling, visualization of intra-STR sequence variation, statistical estimates of combined genotype frequency, statistical estimates of biogeographical ancestry and phenotype, and quality flagging. In addition, automated report generation includes separate reports on flanking region variation and CODIS-compatible sample-level or project-level reports. ForenSeq Universal Analysis Software is delivered pre-installed on a dedicated, standalone server. The server is sold separately to maximize flexibility and enable remote data review and analysis, independent of the MiSeq FGx instrument.

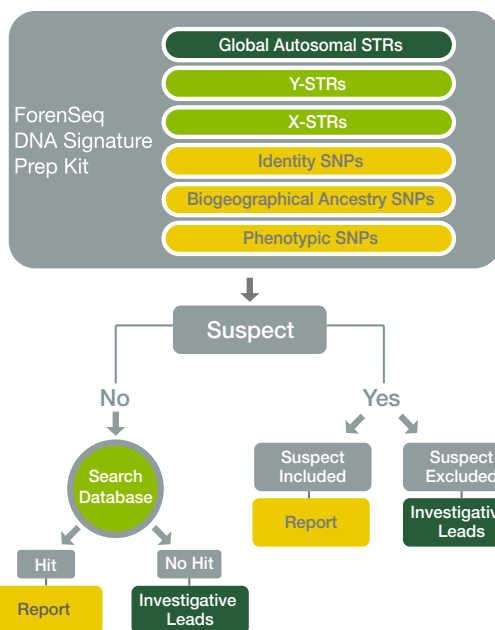


Figure 3: MiSeq FGx Solution Investigative Workflow—From a dedicated library preparation kit to automated data reports, the MiSeq FGx Solution offers the most complete, integrated workflow in the industry.

Exceptional Data Quality

The MiSeq FGx Solution leverages Illumina SBS chemistry—the most widely adopted next-generation sequencing technology in the industry.⁷ Exceptional data quality is achieved by a proprietary, reversible terminator-based method that detects single bases as they are incorporated into massively parallel DNA strands. Fluorescent terminator dyes are imaged as each dNTP is added and then cleaved to allow incorporation of the next base. With all 4 reversible, terminator-bound dNTPs present during each sequencing cycle, natural competition among bases minimizes incorporation bias.⁸ Base calls are made directly from signal intensity measurements during each incorporation cycle, reducing raw error rates compared to other technologies.⁹⁻¹² The result is highly accurate base-by-base sequencing that virtually eliminates sequence context-specific errors, even within repetitive sequence regions or homopolymers.

Summary

The Verogen MiSeq FGx Forensic Genomics Solution is a fully validated sequencing workflow specifically designed for use in forensic genomics applications. The complete sample-to-answer solution includes the ForenSeq DNA Signature Prep Kit, the Illumina MiSeq FGx Instrument, and the ForenSeq Universal Analysis Software package. With the speed and accuracy of Illumina massively parallel sequencing technology, combined with the unprecedented focus of Verogen forensic genomics applications, criminal justice has a powerful new ally.

Learn More

To learn more about the Verogen MiSeq FGx Forensic Genomics Solution, visit: www.verogen.com/products

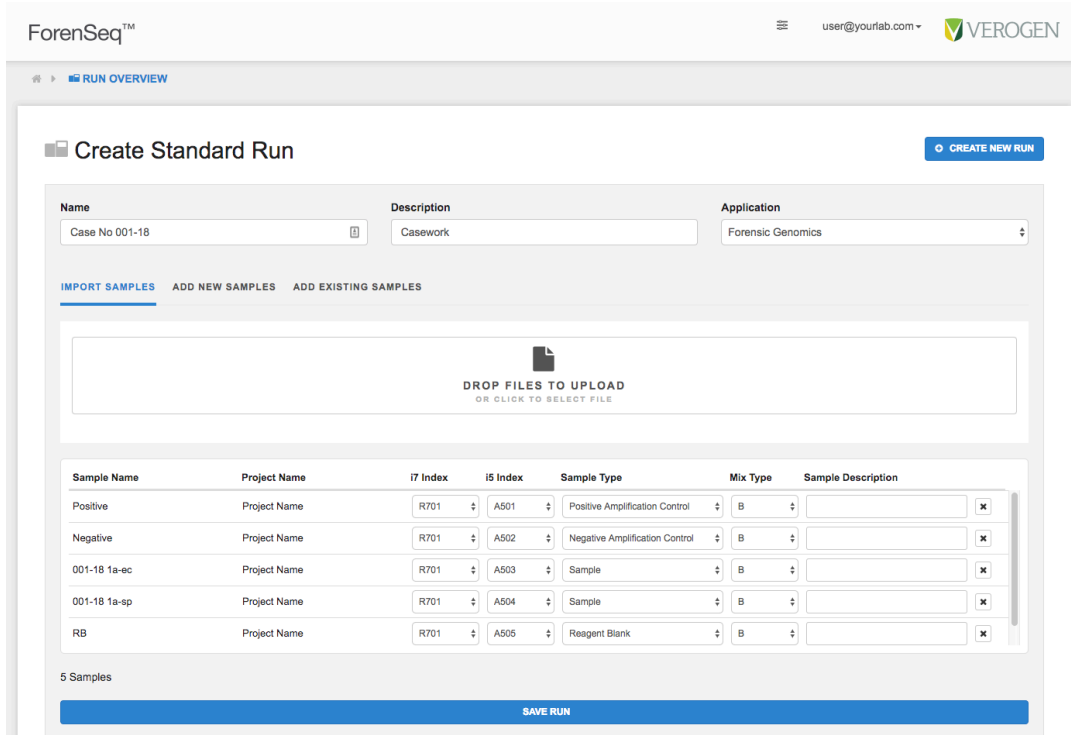


Figure 4: ForenSeq Universal Analysis Run Overview Screen—ForenSeq Universal Analysis Software features DNA library management tools, data analysis and visualization, and automated reporting. The Run Overview screen features easy data entry fields for Run Name and Run Description. Sample information, index combinations, and application options can also be imported with a tab delimited .txt file. Sample types include Known Sample, Forensic Sample, Reagent Blank, Negative Amplification Control, Positive Amplification Control, and Human Sequencing Control.

References

- Jäger AC, Alvarez ML, Davis CP, et al. Developmental validation of the MiSeq FGx Forensic Genomics System for targeted next generation sequencing in forensic DNA casework and database laboratories. *Forensic Sci Int.* 2017;28: 52-70.
- Kidd KK, Pakstis AJ, Speed WC, et al. Developing a SNP panel for forensic identification of individuals. *Forensic Sci Int.* 2006;164(1): 20-32.
- Sanchez JJ, Phillips C, Børsting C, et al. A multiplex assay with 52 single nucleotide polymorphisms for human identification. *Electrophoresis.* 2006;27(9): 1713-1724.
- Kidd KK, Speed WC, Pakstis AJ, et al. Progress toward an efficient panel of SNPs for ancestry inference. *Forensic Sci Int Genet.* 2013;10: 23-32.
- Walsh S, Liu F, Wollstein A, et al. The HirisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Sci Int Genet.* 2013;7(1): 98-115.
- Verogen (2018) ForenSeq DNA Signature Prep Kit Data Sheet (www.verogen.com/products).
- Nakazato T, Ohta T, and Bono H. Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS One.* 2013;22;8(10): e77910.
- Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature.* 2008;456(7218): 53-9.
- Ross MG, Russ C, Costello M, et al. Characterizing and measuring bias in sequence data. *Gen Biol.* 2013;14: R51.
- Liu L, Li Y, Li S, Hu N, He Y, et al. Comparison of next-generation sequencing systems. *J Biomed Biotechnol.* 2012: 251364.

Table 1: MiSeq FGx Solution Performance Parameters

Feature	Performance
Low Input DNA	highly discriminating profiles < 100 pg robust performance ≥ 100 pg optimal input 1 ng
Locus Multiplexing Capability	~200 loci
Accurate Low-level Mixture Detection	detects minor contributors at < 5% of major
Sample Multiplexing Capability	up to 96 samples maximum
Deep Coverage	12.5 million reads per run
Short Amplicon Detection	≥ 65 bp

- Sebastian J, Fritz JS, Karola P, et al. Updating benchtop sequencing performance comparison. *Nat Biotechnol.* 2013;31: 294-296.
- Quail MA, Smith M, Coupland P, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics.* 2012;13: 341.

Table 2: Illumina MiSeq FGx Instrument Specifications

Instrument Configuration
RFID tracking for consumables MiSeq FGx Control Software
Instrument Control Computer (Internal)
Base Unit: Intel Core i7-2710QE 2.10 GHz CPU Memory: 16 GB RAM Hard Drive: 750 GB Operating System: Windows 7 embedded standard
Operating Environment
Temperature: 22°C ± 3°C (66–77°F) Humidity: Noncondensing 20%–80% Altitude: Less than 2,000 m (6,500 ft) Air Quality: Pollution degree rating of II Ventilation: Maximum of 1,364 BTU/h For Indoor Use Only
Light Emitting Diode (LED)
530 nm, 660 nm
Dimensions
WxDxH: 68.6 cm × 56.5 cm × 52.3 cm (27.0 in × 22.2 in × 20.6 in) Weight: 54.5 kg (120 lbs) Crated Weight: 90.9 kg (200 lbs)
Power Requirements
100–240V AC @ 50/60Hz, 10A, 400 W
Radio Frequency Identifier (RFID)
Frequency: 13.56 MHz Power: 100 mW
Product Safety and Compliance
NRTL certified IEC 61010-1 CE marked FCC/IC approved
a. Computer specifications are subject to change.

Ordering Information

Product	Verogen Catalog No.
Illumina MiSeq FGx Instrument	SY-411-1001
MiSeq FGx Reagent Kit	TG-143-1001
MiSeq FGx Reagent Micro Kit	TG-143-1002
ForenSeq DNA Signature Prep Kit (384 reactions)	TG-450-1001
ForenSeq DNA Signature Prep Kit (96 reactions)	TG-450-1002
ForenSeq Index Adapter Fixture	FC-451-1001
ForenSeq Universal Analysis Software and Server	SE-550-1001