MiSeq FGx® Sequencing System

The first and only fully validated next-generation sequencing instrument designed for forensic genomics

Highlights

- *Easy-to-use instrument*
  Simple operation with an intuitive touch-screen interface and load-and-go reagents.
- *Superior analysis of challenging samples*
  Advanced capacity to process complex DNA mixtures, degraded DNA and other challenging samples.
- *Integrated sequencing workflow*
  End-to-end solution interrogates thousands of markers in one run, consolidating library prep, sequencing and analysis into a single workflow.
- *Applications for human identification*
  Adjustable read lengths, dual run modes and two flow cells optimize flexibility across a growing range of capabilities.

Introduction

The MiSeq FGx Sequencing System is the first and only next-generation sequencing (NGS) instrument designed and validated for forensic genomics applications. The MiSeq FGx System is key to the National DNA Index System (NDIS)-approved MiSeq FGx Forensic Genomics Solution, an end-to-end platform for analyzing forensic DNA samples.

The MiSeq FGx System packages a simple workflow and tailored data output into a compact desktop instrument that fits into virtually any laboratory. Integrated software facilitates run setup, sample tracking, user management, audit trails, results interpretation and reporting. The MiSeq FGx System leverages the most widely adopted NGS technology in the industry and, together with Illumina® sequencing-by-synthesis (SBS) chemistry, delivers superior resolution and unmatched accuracy for a variety of casework, from the everyday to the complex (1, 2).

Figure 1.
The compact MiSeq FGx System pairs a simple workflow and user-friendly interface with power and accuracy to solve more cases and generate more leads.

NGS advantage for human identification

NGS detects the full spectrum of genetic variation in a DNA sample compared to only size-based analysis of short tandem repeats (STRs) using capillary electrophoresis (CE). Inherently sensitive, NGS technology goes beyond fragment size to identify underlying sequence variation. Comprehensive results are delivered quickly and clearly, displaying base-by-base sequences for easy interpretation with utmost confidence.
Building on STR capabilities, NGS brings other advantages to modern forensic genomics. These include analysis of nuclear single nucleotide polymorphisms (SNPs), mitochondrial DNA (mtDNA), messenger RNA (mRNA) and epigenetic markers, bolstering cases that require human identification while eliminating the need to weigh technical limitations against potentially informative data. Achieve superior results in a single sequencing run (3).

Simple, streamlined workflow

All libraries sequenced on the MiSeq FGx System follow the same core NGS workflow: library prep, sequencing and analysis (Figure 2).

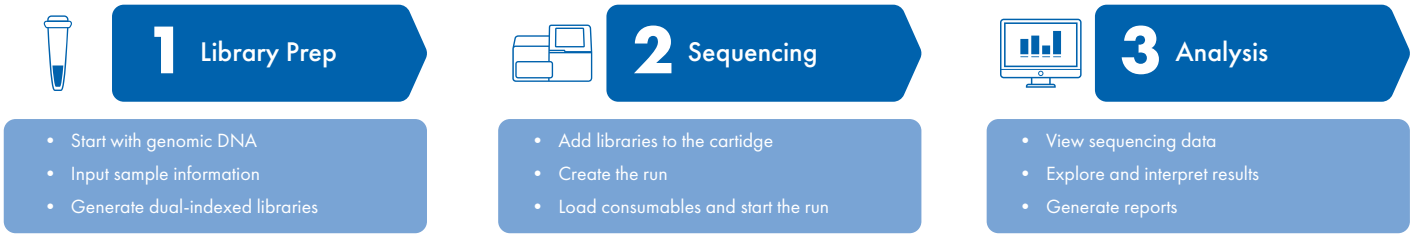

Figure 2.
The NGS workflow takes you from sample to answer in three simple steps.

The first step uses a library prep kit to add primers to genomic DNA (gDNA), mtDNA or mRNA extracted from forensic samples. Simultaneous capture and amplification of hundreds of target regions generates dual-indexed libraries ready for sequencing. After library prep, load-and-go reagents simplify run setup. Thaw the prefilled reagent cartridge, add libraries to the cartridge then insert the cartridge into the instrument. The system software accepts run parameters and starts sequencing with the push of a button (4).

Integrated software solution

The MiSeq FGx System features an intuitive touch-screen interface that provides step-by-step guidance through each stage of a sequencing run, from consumables loading through run configuration and monitoring. Onboard cluster generation and automated analysis initialization minimize hands-on time. Complementing the onboard software, the system integrates with Universal Analysis Software (UAS).

UAS is a fully optimized analysis platform that delivers a powerful suite of forensic-tuned capabilities, including automatic detection of mixed samples, generation of population statistics, database-compatible reports, and more. UAS ships preinstalled on a dedicated server. The server is independent of the instrument, eliminating the need for auxiliary hardware and computing resources and maintaining the minimal instrument footprint of only 0.4 square meters.

Exceptional data quality

The MiSeq FGx System achieves exceptional data quality by employing 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 deoxynucleotide triphosphate (dNTP) is added and then cleaved to allow incorporation of the next base. With all four reversible, terminator-bound dNTPs present at each sequencing cycle, natural competition among bases minimizes incorporation bias. The software makes base calls directly from signal intensity measurements during each incorporation cycle, reducing raw error rates compared to other technologies. The result is highly accurate, base-by-base sequencing that minimizes sequence context-specific errors, even within repetitive sequence regions or homopolymers (5).

By applying this chemistry to forensic genomics, the MiSeq FGx System delivers an enhanced capacity to analyze degraded DNA, low-quantity DNA, complex DNA mixtures and other challenging samples that can complicate or derail an investigation. Small amplicon sizes are well suited for interrogation of degraded DNA. Large numbers of markers—including many that are highly polymorphic—improve system ability to discern low-level minor components that CE might not detect. Additionally, one sequencing run interrogates thousands of forensically relevant genetic markers, eliminating the need to choose between fragment length-based STR kits or otherwise make tradeoffs to accommodate challenging samples.

Growing suite of applications

In partnership with the forensic community, QIAGEN is continuing to expand the menu of forensic applications optimized for the MiSeq FGx System. With faster turnaround times and simplified workflows, the MiSeq FGx System offers an extensible alternative to CE. The instrument enables capabilities across many sample sources and a growing number of applications, including both short- and long-range kinship analysis.

For added functionality, the system features dual modes: Forensic Genomics mode for ForenSeq® libraries only and Research Use Only (RUO) for other chemistry. Two run modes promote a range of forensic genomics applications while retaining the flexibility to work in a research environment. Easily switch between two sequencing kits to tune output for the current application or sample batch size. Adjustable read lengths, flow cell options and choice of run modes allow unprecedented flexibility for matching data output to an ever-increasing range of human identification needs.

Summary

The MiSeq FGx System is integral to a validated workflow designed specifically for forensic laboratories. Fully supported by QIAGEN, the system has available performance qualifications and regular maintenance aligned to forensic laboratory requirements. Building on the speed and accuracy of Illumina SBS chemistry, QIAGEN brings the unprecedented focus of forensic genomics to NGS, granting criminal justice a powerful ally.

Table 1. Features of the MiSeq FGx Sequencing System

Feature	Details
Configuration	RFID tracking for consumables MiSeq FGx Control Software
Instrument computer (internal)	Base unit: Intel® Core i7-2710QE 2.10 GHz CPU | Memory: 2 × 8 GB DDR3 Hard drive: 1 TB | Operating system: Windows® 10 Enterprise LTSC 2019 (embedded)
Light Emitting Diode	530 nm, 660 nm
Throughput	1–384 samples per run, depending on assay
Performance parameters	Maximum read length: 2 x 300 bp, depending on assay | Output (2 x 150 bp run): ≥5 Gb Reads passing filters: 12.5 million | Q30 score (at read length of 2 x 150 bp): ≥80% Total overall accuracy: ≥99.66% | Total overall reproducibility: 99.7%

Table 2. Specifications of the MiSeq FGx Sequencing System

Specification	Details
Operating environment	Temperature: 19–25°C (66–77°F) | Humidity: Noncondensing 30–75% Altitude: <2000 m (6500 ft.) | Air quality: Pollution degree rating of II Ventilation: Maximum 1364 BTU/h | For indoor use only
Dimensions	W×D×H: 68.6 cm × 56.5 cm × 52.3 cm | (27 in. × 22.2 in. × 20.6 in.) Weight: 54.5 kg (120 lb.) | Crated weight: 90.9 kg (200 lb.)
Power requirements	100–240 V AC @ 50/60Hz, 10A, 400 W
Radio Frequency Identifier (RFID)	Frequency: 13.56 MHz; Power: 100 mW
Safety and compliance	NRTL certified IEC 61010-1 | CE marked FCC/IC approved

Ordering Information

Product	Contents	Cat. no.
MiSeq FGx Sequencing System	Desktop instrument with two run modes for a range of forensic genomics applications within a validated NGS workflow	15048976
ForenSeq Universal Analysis Software (UAS)	Software pre-installed as a dedicated server specific for forensic genomics for run setup, sample management, analysis and report generation. This product includes server, mouse, keyboard and monitor.	9003364
MiSeq FGx Reagent Kit	Supports up to 12.5 million paired-end reads for deep sequencing or high-throughput sample processing	15066817
MiSeq FGx Reagent Micro Kit	Supports up to 5 million paired-end reads for small batch sizes and faster turnaround times	20021681

Learn more about NGS for HID in your lab. Visit **qiagen.com/MiSeqFGx**

References:

1. Jäger, AC, 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 Genet.* 2017; 28: 52–70. doi.org/10.1016/j.fsigen.2017.01.011.
2. Nakazato T, Ohta T, Bono H. Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS One.* 2013; 8 (10): e77910. doi.org/10.1371/journal.pone.0077910.
3. Ballard D, Winkler-Galicki J, Wesoły J. Massive parallel sequencing in forensics: advantages, issues, technicalities, and prospects. *Int J Legal Med.* 2020; 134: 1291–1303. doi.org/10.1007/s00414-020-02294-0.
4. Ingold S, et al. Body fluid identification using a targeted mRNA massively parallel sequencing approach – results of a EUROFORGEN/EDNAP collaborative exercise. *Forensic Sci Int Genet.* 2018; 34: 105–115. doi.org/10.1016/j.fsigen.2018.01.002.
5. Bentley, DR, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature.* 2008; 456: 53–59. doi.org/10.1038/nature07517.