

ForenSeq® mtDNA Control Region Solution

Analyze the mitochondrial DNA control region from high-quality or highly degraded samples

Highlights

- **State-of-the-art assay**
Short mean amplicon size, designed for maximum variant detection and optimized for inhibitor tolerance
- **Flexible and scalable throughput**
Results for up to 48 samples simultaneously in less than two working days
- **Integrated workflow**
Powerful assay, industry leading sequencing and intuitive software integrate seamlessly for optimum laboratory efficiency

Introduction

The ForenSeq mtDNA Control Region Solution is a complete DNA to Data workflow. The workflow comprises a high-quality assay system, a preprogrammed and automated sequencing platform and an intuitive software analysis program (Figure 1).

Designed by and for forensic DNA scientists the start-to-finish workflow includes:

- **ForenSeq mtDNA Control Region Kit**
A total assay kit containing all primary reagents necessary for the preparation of complete mtDNA control region libraries
- **MiSeq FGx® Sequencing System**
The only NGS system specifically designed and validated for forensic applications, based on sequencing-by-synthesis technology
- **ForenSeq Universal Analysis Software v2.0**
A three-part software module specifically designed for concurrent analysis of the mtDNA control region data.

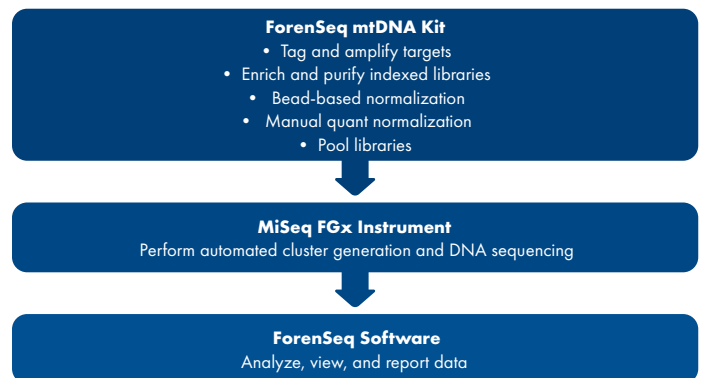


Figure 1.

The ForenSeq mtDNA Control Region Workflow, including the ForenSeq mtDNA Control Region Kit for library prep, the MiSeq FGx Sequencing System, and the ForenSeq Universal Analysis Software is familiar to existing ForenSeq users and easy to learn for those new to the system.

Optimized for forensic sample performance

The high density of mutations concentrated in the mtDNA control region and the degraded nature of most forensic samples in which mtDNA analysis is used make optimal primer design challenging. The ForenSeq mtDNA Control Region Kit is optimized to generate complete and actionable coverage, even for very low input amounts (Figure 2).

The ForenSeq mtDNA Control Region Kit uses more than 120 primers to improve performance on highly degraded material. The primers are designed from recently curated mtDNA variant and frequency data and generate 18 primary amplicons in the mtDNA control region. All primary amplicons are <150bp in length, with an average amplicon size of 118 bp, the lowest of any commercial mtDNA assay systems currently available.

All amplicons have a minimum overlap of 3 bp to avoid gaps in sequence following post run, bioinformatic trimming. The buffer system is optimized for inhibitors to create a robust amplification environment (Figure 3).

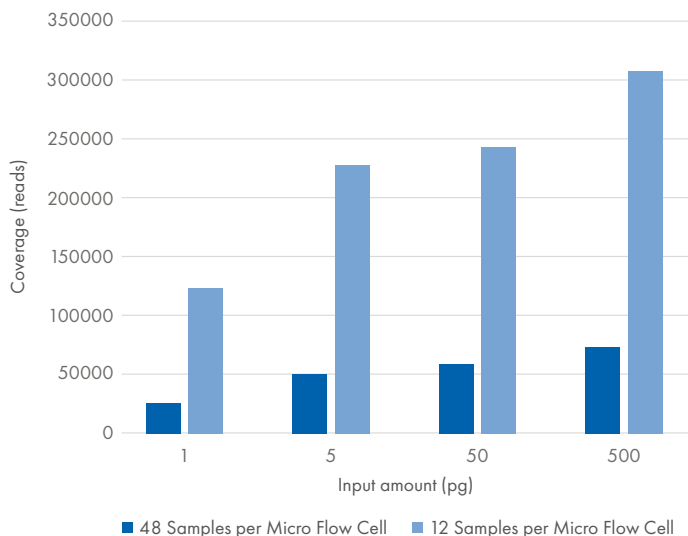


Figure 2. The ForenSeq mtDNA Control Region system delivers extensive coverage across a wide range of DNA input amounts, even for large sample batches, supporting both small casework sample batches and large reference or population runs.

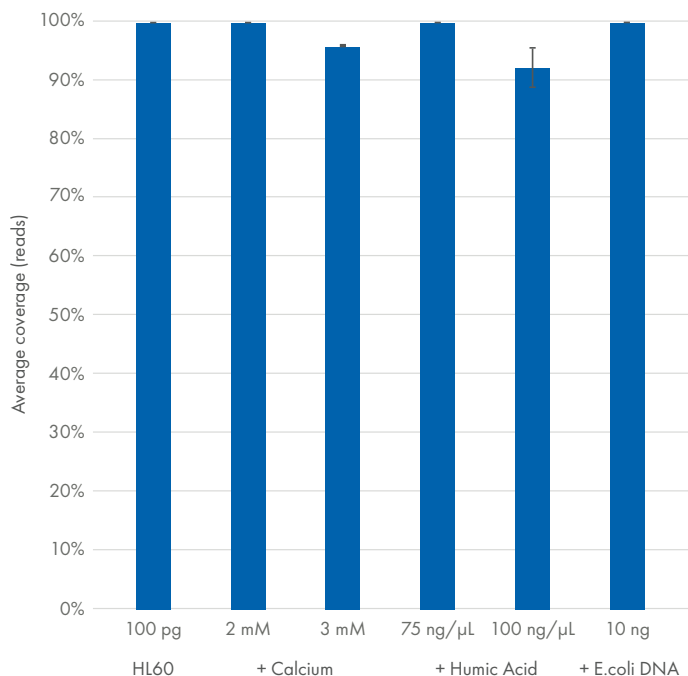


Figure 3. An improved ForenSeq buffer system maximizes performance in the presence of a range of common inhibitors at levels above those typically found in forensic samples.

Flexible and scalable throughput

The ForenSeq mtDNA Control Region workflow is completely flexible and scalable for all throughput requirements, allowing laboratories to generate complete mtDNA control region results for 3-48 samples simultaneously in less than 2 working days. For low throughput requirements, the kit's 48 reactions maintain a 6-month shelf-life. For higher demand laboratories, a similar library preparation routine makes automation easy.

Familiar and simple ForenSeq workflow

The ForenSeq mtDNA Control Region Solution is based on the tried and trusted ForenSeq workflow, originally developed for the ForenSeq DNA Signature Prep Kit. Preprogrammed protocols are provided for the MiSeq FGx Sequencing System including a new Universal Analysis Software (UAS) tool designed specifically for simple analysis of forensic mtDNA Control Region data, regardless of batch size. Leveraging the same fundamental workflow reduces training requirements for labs performing multiple ForenSeq assays and lays the foundation for future applications, maximizing the utility of a single platform and workflow infrastructure.

Fast and intuitive data analysis

ForenSeq UAS v2.0 has been developed to provide powerful bioinformatic tools in an easy-to-use interface. This is an advantage to laboratories already analyzing mtDNA data and assists laboratories looking to reintroduce in-house mtDNA testing or adopt for the first time. UAS v2.0 displays coverage plots and mutation positions relative to the revised Cambridge Reference Sequence (Figure 4) and allows comparison of up to 8 samples simultaneously. Batches of up to 48 samples can be analyzed quickly and efficiently, then exported into EMPOP format for direct upload into the EMPOP database for comparison to wider data sets and evaluation of haplogroup assignment.

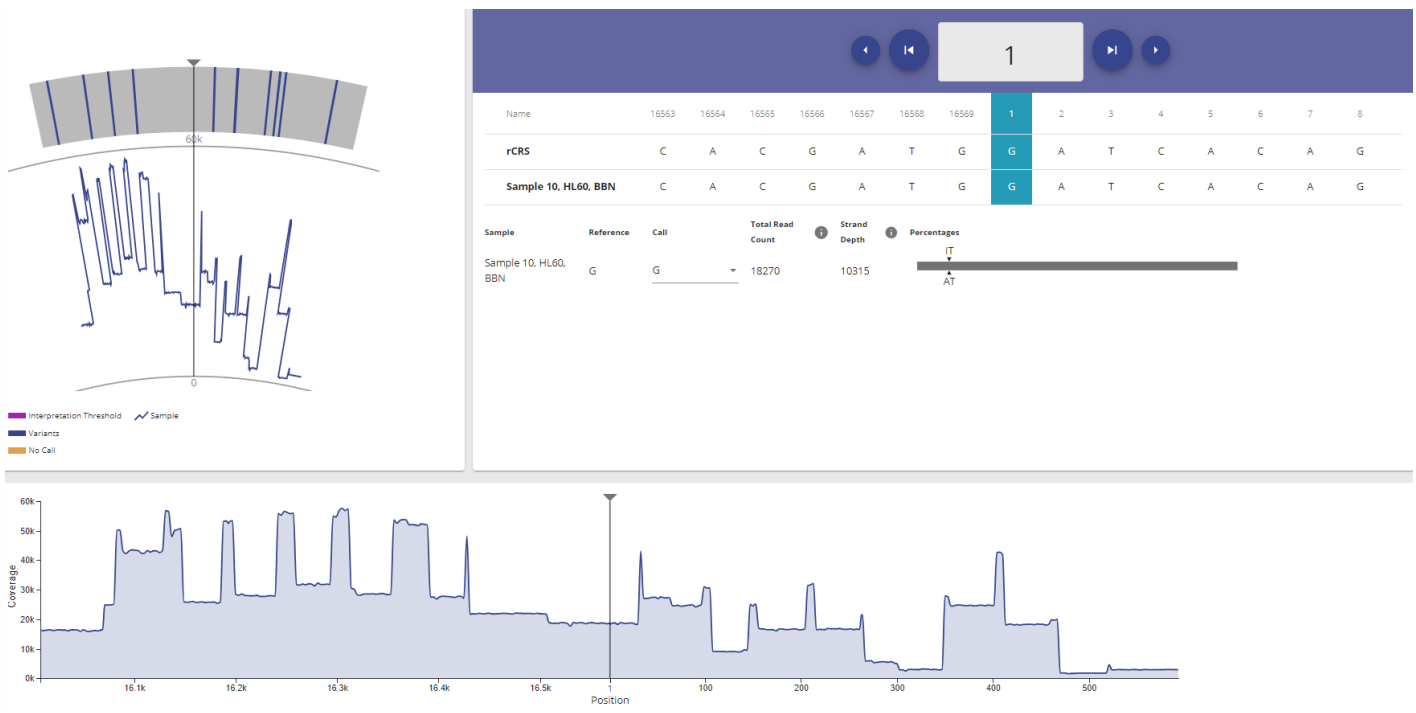


Figure 4. ForenSeq UAS v2.0 provides an intuitive user interface focusing on the control region, coverage levels and call positions.

Table 1. Specifications of the ForenSeq mtDNA Control Region Kit

Specification	Value
Sample type	DNA extracted from a range of degraded samples, including bone, teeth and hair shaft
Recommended input per reaction	50 pg genomic DNA (two reactions per sample)
Multiplexing capacity per run	3–48 samples
Degraded sample detection	All primary amplicons ≤150 bp Average primary amplicon size 118 bp
Efficient time to answer	Analyzed results in <2 working days Approximately 75 minutes hands-on time
Primer set	18 primary amplicons; 122 primers

Fully integrated and supported

All components of the ForenSeq mtDNA Control Region Solution have been designed, developed and tested concurrently to work as an integrated system and maximize data quality and utility. The ForenSeq mtDNA Control Region Solution is fully supported by QIAGEN. Our manufacturing operations work diligently to provide a fully quality controlled and tested, forensically enabled system. Our applications team offer dedicated, skilled support to help forensic laboratories successfully leverage this powerful technology.

Table 2. ForenSeq Universal Analysis Software server specifications

Components and specifications
Intel® Core i7-7700 3.6 GHz, 4 core, Kaby Lake processor
2 x 16 GB 2666 MHz DDR4 memory
8405E 12 Gbps, 4 port RAID Adapter
256 GB 7 mm Solid State Drive
2 x 2000 GB 7 mm Solid State Drives
300 W power supply
Windows® 10 Pro operating system
Keyboard and mouse combination
24" 1920 x 1080 LED 1080p monitor

Ordering Information

Product	Contents	Cat. no.
ForenSeq mtDNA Control Region Kit	Includes all the primary reagents necessary for 48 reactions for the preparation of complete mtDNA control region libraries	V16000085
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	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 Micro Kit	Supports up to 5 million paired-end reads for small batch sizes and faster turn-around times.	20021681



Learn more about NGS for HID in your lab. Visit [qiagen.com/ForenSeqmtDNA-CR](https://www.qiagen.com/ForenSeqmtDNA-CR)

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