

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 Verogen ForenSeq™ mtDNA Control Region Solution is a complete DNA to Data workflow including a high quality assay system, a pre-programmed and automated sequencing platform, and an intuitive software analysis program (Figure 1). Designed by and for forensic DNA scientists as a start-to-finish ecosystem, the workflow is highlighted by:

- 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®:**
 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.

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, however the ForenSeq mtDNA Control Region kit is optimized to generate complete and actionable coverage, even for very low input amounts (Figure 2).

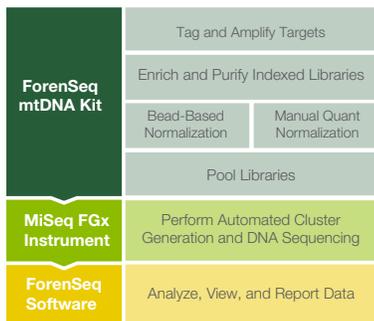


Figure 1: The ForenSeq mtDNA Control Region Workflow, including the ForenSeq mtDNA Control Region Library Prep kit, the MiSeq FGx System, and the ForenSeq Universal Analysis Software will be very familiar to existing ForenSeq users and easy to learn for those new to the system.

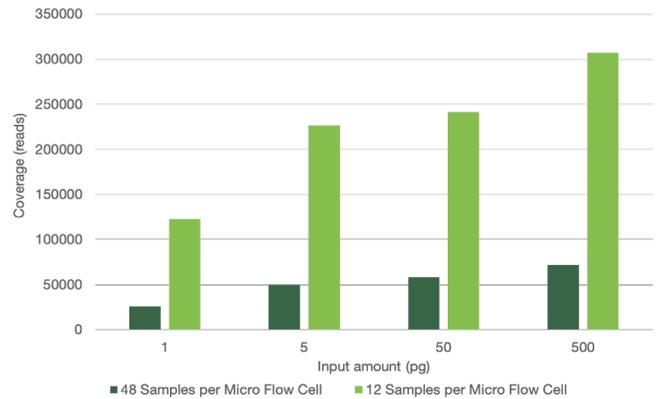


Figure 2: The ForenSeq mtDNA Control Region system delivers extensive coverage across a wide range of DNA input amounts, even for large sample batches, supporting both small casework sample batches and large reference or population runs.

To improve performance on highly degraded material the ForenSeq mtDNA Control Region kit uses more than 120 primers, designed from recently curated mtDNA variant and frequency data, generating 18 primary amplicons in the mtDNA control region. All primary amplicons are <150 bp in length, many < 100 bp – the lowest average amplicon size of any current commercial mtDNA assay system.

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

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 12-month shelf-life and for higher demand laboratories, a similar library preparation routine makes automation easy.

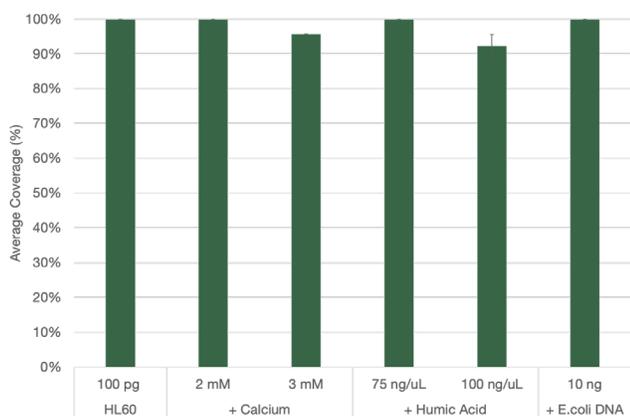


Figure 3: An improved ForenSeq buffer system maximizes performance in the presence of a range of common inhibitors at levels above those typically found in forensic samples.

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. Pre-programmed protocols are provided for the MiSeq FGx 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 & Intuitive Data Analysis

ForenSeq UAS v2.0 has been developed to provide powerful bioinformatic tools in an easy-to-use interface, offering advantages to laboratories already analyzing mtDNA data and assisting 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



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

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.

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 maximise data quality and utility. Fully supported by Verogen, our manufacturing operations work diligently to provide a fully quality controlled and tested, forensically enabled system and our applications team offer dedicated, skilled support to help forensic laboratories successfully leverage this powerful technology.

Table 1: ForenSeq mtDNA Control Region Kit—Specifications

Specification	Value
Sample Types	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

Table 2: ForenSeq Universal Analysis Software Server Specs

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	Part No.
ForenSeq™ mtDNA Control Region Kit (48 reactions)	V16000085
MiSeq FGx® Reagent Micro Kit	20021681
ForenSeq™ Universal Analysis Software and Server*	SE-550-1002
ForenSeq™ Universal Analysis Software Upgrade Kit*	SE-550-1003



Learn More

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