

# ForenSeq™ mtDNA Control Region Solution

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

## Highlights

- State-of-the-Art Assay**  
 State-of-the-art amplicon design enables optimal tiling strategy and assay performance
- Integrated Workflow**  
 Designed concurrently, the assay, bioinformatics, and software work as a supported, integrated solution
- Scalability**  
 With a flexible throughput, you can run a few samples or an entire kit in less than two working days

## Introduction

The Verogen ForenSeq™ mtDNA Control Region Solution is a targeted assay for analyzing the human mitochondrial DNA control region of interest using a tiled, targeted amplicon approach.

Libraries are:

- Prepared using the ForenSeq mtDNA Control Region Kit, which includes the option of either bead-based or quantitation-based normalization.
- Sequenced using sequencing-by-synthesis technology on the MiSeq FGx®.
- Analyzed using the new ForenSeq Universal Analysis Software v2, which is specifically designed for ForenSeq mtDNA data.

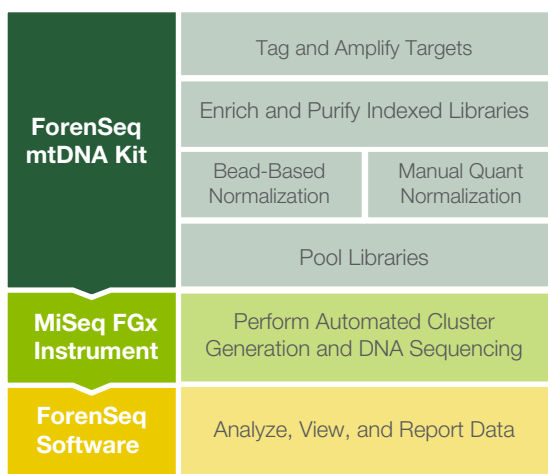


Figure 1: ForenSeq mtDNA Control Region Workflow includes library preparation using the ForenSeq mtDNA Control Region Kit, sequencing on the MiSeq FGx, and analysis using the ForenSeq Universal Analysis Software v2.

## State-of-the-Art Assay Optimized for Performance

The ForenSeq mtDNA Control Region Kit uses more than 120 primers designed against the most recent and well-curated mtDNA variant and frequency data to generate 18 primary amplicons that span the complete mtDNA control region.

All primary amplicons are < 150 bp in length, which maximizes performance on degraded samples. To facilitate data analysis and ensure no gaps in sequence as a result of bioinformatic trimming, all amplicons have a minimal overlap of  $\geq 3$  bp.

To complement the state-of-the-art primer design, the buffer system for the ForenSeq mtDNA Control Region kit has been optimized to combat the inhibitors commonly encountered in forensic samples and provide a robust environment for successful generation of ForenSeq mtDNA amplicons.

## Supported, Integrated Workflow

The ForenSeq mtDNA Control Region Solution was developed as a fully supported sample-to-result workflow, including library preparation, sequencing on a MiSeq FGx, and data analysis with ForenSeq Universal Analysis Software v2. All components of the workflow were developed concurrently and designed to work as an integrated solution to guarantee as many base positions as possible are accurately and confidently called. The software analysis tools are based on the mtDNA BaseSpace applications, which emphasize usability in data review and provide an approachable and efficient means of data analysis.

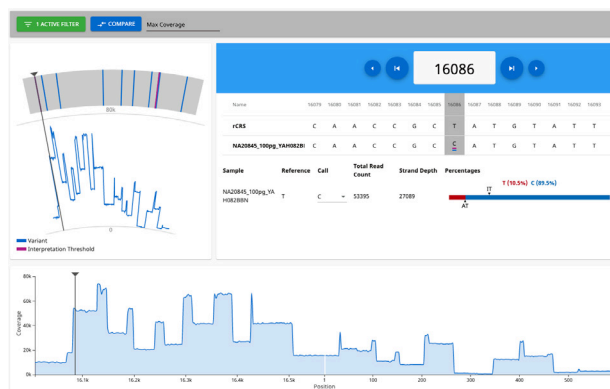


Figure 2: ForenSeq Universal Analysis Software provides an intuitive user experience to facilitate a detailed and rapid result review

Because the ForenSeq mtDNA product line was developed as an integrated solution, all components of the ForenSeq mtDNA Solution are fully supported by the Verogen team. The Verogen applications team offers dedicated, skilled support to help forensic laboratories successfully navigate this powerful technology.

## Highly Scalable Solution

To support a wide range of laboratory throughput requirements, the ForenSeq mtDNA Control Region Solution easily scales from a single library preparation of a few samples, which is ideal for managing daily operations, to an entire kit of 48 samples, which is perfect for project work or database operations.

The ForenSeq mtDNA Control Region Solution uses the industry standard sequencing-by-synthesis technology, which offers unparalleled accuracy with fast, efficient data review. Even for large sample batches, library preparation is uncomplicated and reportable results are available within two working days.

With a shelf life of at least 12 months from date of shipment, even laboratories with the lowest throughput needs are able to take advantage of the system before reagents expire. Laboratories with higher throughput needs can run an entire kit using one MiSeq FGx Reagent Micro Kit, which minimizes cost per sample and maximizes workflow efficiency.

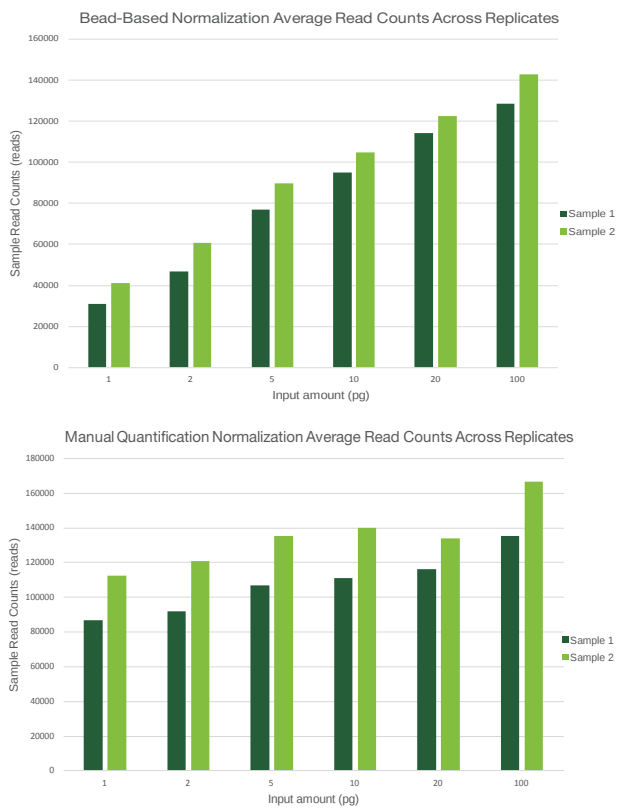


Figure 3: Sensitivity and reproducibility for both bead-based normalization and manual quantification of samples

## Part of the ForenSeq Ecosystem

The same trusted ForenSeq chemistry powering the ForenSeq DNA Signature Prep Kit underpins the ForenSeq mtDNA product line, vastly reducing training hurdles for laboratories that would like to perform both nuclear and mitochondrial analysis. Additionally, the Universal Analysis Software v1.3 required for ForenSeq DNA Signature Prep Kit data analysis is included on the new server hardware without any changes to the software or data analysis.

This expanded capacity enables greater utility from NGS platforms, promotes sample insourcing, and improves ease of automation.

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

### 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

## Learn More

For more on the ForenSeq mtDNA Control Region Solution, visit: [www.verogen.com/products/forenseq-mtdna-control-region](http://www.verogen.com/products/forenseq-mtdna-control-region).

## Ordering Information

Product	Catalog No.
ForenSeq™ mtDNA Control Region Kit (48 reactions)	TG-650-1001
MiSeq FGx® Reagent Micro Kit	TG-143-1002
ForenSeq™ Universal Analysis Software and Server*	SE-550-1002
ForenSeq™ Universal Analysis Software Upgrade Kit*	SE-550-1003

\* Includes Universal Analysis Software v2 with mtDNA analysis functionality

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