ForenSeq mtDNA Whole Genome Kit

A scalable, integrated workflow to obtain full mitochondrial DNA profiles from challenging samples.

Highlights

- **Greater power of discrimination**
  Improve data recovery from degraded samples with short amplicons designed against current mtDNA population databases for optimum variant detection.
- **Optimized library prep performance**
  Maximize coverage with a tiled approach that overlaps the amplicons to prevent sequence gaps and data loss.
- **Simple workflow with flexible throughput**
  Scale for all throughput levels, from small manual sample sets to large automated batches, and easily transition between ForenSeq kits for other applications.
- **Integrated solution that maximizes results**
  Leverage a commercial-grade, all-in-one kit designed, tested, and developed with dedicated instrumentation and software, backed by full Verogen support.

Introduction

The ForenSeq™ mtDNA Whole Genome Kit offers a next-generation sequencing (NGS) solution to prepare forensic samples for analysis of the entire mitochondrial genome (mtGenome). Grounded in the same chemistry and workflow as the ForenSeq mtDNA Control Region Kit, which targets the traditional reporting region, the ForenSeq mtDNA Whole Genome Kit improves haplotype resolution and grants laboratories access to the genetic diversity of the full mtGenome sequence. Workflow enhancements simplify complex bioinformatics and support automation for parallel processing of up to 48 samples.

When nuclear DNA analysis fails to produce an actionable outcome, laboratories require an alternative but familiar means of obtaining a DNA profile. The ForenSeq mtDNA Whole Genome Kit meets this challenge, offering a simple, cost-effective solution for analyzing mtDNA with the full power of mtGenome sequencing backed by established workflows and Verogen support. A tiled assay design maximizes results on degraded samples while facilitating fast, easy library prep and integrated, semi-automated analysis. A highly scalable library prep protocol and all-inclusive reagent system facilitate transitions between ForenSeq kits and support a range of throughput requirements, batch sizes, and input amounts. In concert with the MiSeq FGx® Sequencing System and ForenSeq Universal Analysis Software (UAS) v2.0, the ForenSeq mtDNA Whole Genome Kit enables NGS analysis of mitochondrial DNA (mtDNA) with consistent performance and reliable results.

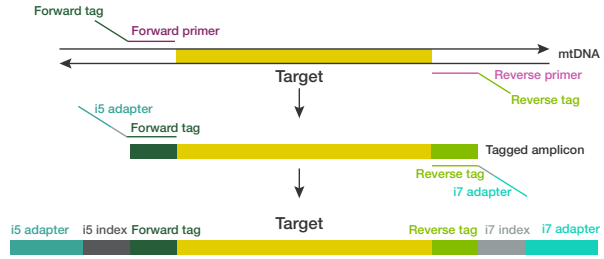

Figure 1: Streamlined ForenSeq chemistry with multiplexing capability— Tagged oligos for each target sequence mix with samples split across two PCR pools. PCR links the tags to copies of each target, forming DNA templates with regions of interest flanked by universal primer sequences. Index adapters then attach to the tags for sequencing.

Expanded Library Prep Chemistry

The ForenSeq mtDNA Whole Genome Kit is a sensitive, PCR-based assay that delivers data from the complete 16,569 bp sequence of the human mtGenome. A low input recommendation of 100 pg genomic DNA (gDNA) ensures reliable results that are reproducible both within and between samples despite the limited availability of input DNA. The ForenSeq mtDNA Whole Genome Kit has the same core primer set as the ForenSeq mtDNA Control Region Kit but quadruples the number of primers to over 500 for efficient amplification of degenerate bases. This expanded primer set is designed against current mtDNA databases for optimum variant detection. Library prep reagents amplify the mtGenome in two reactions, generating over 200 short overlapping amplicons that are then combined for sequencing. Figure 1 summarizes the assay and Table 1 provides a complete list of kit specifications.

Table 1: Kit Specifications

Specification	Value
Target size	16,569 mtGenome
Sample type	gDNA and mtDNA extracted from hair, bone, teeth, and buccal swabs
DNA input recommendation	100 pg gDNA per sample*
Kit configuration	48 reactions
Multiplexing capacity	16 samples per run
Number of primers	> 500
Number of amplicons	245
Amplicon size	Maximum 209 bp Mean 131 bp
Amplicon overlap	≥ 3 bp
Total time	~7 hours and 15 minutes
Hands-on time	~1 hour and 45 minutes

* Each 100 pg sample is divided into two 50 pg reactions.

High Coverage, Powerful Inhibitor Resistance

The ForenSeq mtDNA Whole Genome Kit delivers 100% amplicon coverage across a spectrum of throughputs and input amounts. To further improve the performance of low-level and complex samples, the protocol includes an optional second purification. Although recommended for input gDNA ≤ 20 pg, the second purification also demonstrates utility for greater input amounts. Figure 2 presents a comparison of coverage results for libraries with and without the second purification, revealing improved coverage from inputs as varied as 2 pg and 100 pg. Compared to a single purification, two purifications show progressive coverage advancements, culminating with 90% improvement at 2 pg (Figure 3).

Another challenge of forensic sample analysis is the frequent presence of calcium, humic acid, *E. coli*, and other PCR inhibitors that can compromise library prep. Humic acid, for example, disturbs the DNA polymerase and impairs amplification by binding to template DNA. The ForenSeq mtDNA Whole Genome Kit features an optimized buffer system that overcomes forensically relevant PCR inhibitors to provide a robust environment for amplification. When varying amounts of three inhibitors common in forensic mtDNA samples were added to 100 pg of Control DNA HL60, results demonstrated high resistance with consistent coverage across all inputs (Figure 4).[1]

Streamlined Sequencing Solution

A user-friendly workflow with enhanced data quality uniquely positions the ForenSeq mtDNA Whole Genome Kit as a comprehensive library prep solution for mtGenome sequencing applications. Fully kitted library prep reagents—including sample purification beads, normalization beads, and index adapters—provide a comprehensive assay calibrated for high performance on the MiSeq FGx System. Two normalization options, bead-based and manual quantification, grant additional flexibility for scalable study design.

The assay starts with ForenSeq-based library prep to convert input gDNA into adapter-tagged, sequencing-ready libraries. Fast and easy, the prep requires less than one day to process a single plate of 96 samples split into two 48-reaction sets, a strategy that promotes efficient amplification of overlapping amplicons to ensure complete coverage. Using a simple procedure with the ForenSeq Index Plate Fixture for guidance, Index 1 (i7) and Index 2 (i5) adapters are combined and added to DNA fragments. For greatest operational efficiency and scalable multiplexing without increases to cost or time, up to 16 of these preplated, dual-index libraries are pooled and sequenced together in one run.[2]

After sequencing, ForenSeq UAS v2.0 finishes automated data analysis in only one hour (Figure 5). This sophisticated software uses the index adapter sequences to demultiplex the data and accurately assign reads to the appropriate libraries in a pool. When analysis is complete, the software provides a comprehensive suite of visualization tools for intuitive exploration of and reporting on mtDNA data using VCF and BAM formats that are also compatible with third-party tools (Figure 6). Data export formats tailored to EMPOP, CODIS, and IGV ease downstream analysis. Between the adaptable workflow, simplified bioinformatics, and seamless integration with other Verogen products, the ForenSeq mtDNA Whole Genome Kit offers a streamlined library prep method that delivers high-quality sequencing data in an easy-to-use format.

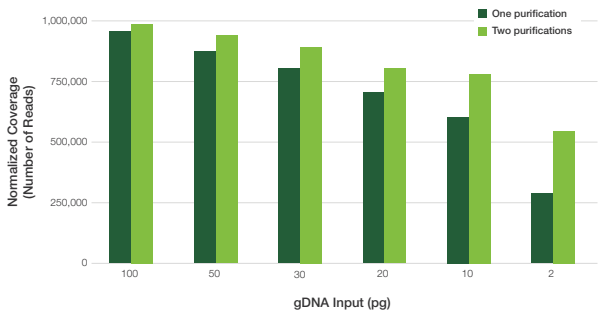

Figure 2: High coverage across a range of input DNA amounts—The kit delivers consistently high coverage, with a second purification optimizing data quality and extending the lower threshold of input amounts.

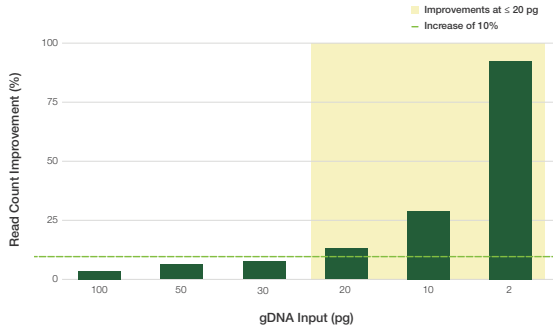

Figure 3: Read count improvement with a second purification—When compared to one purification, a second purification provides a 30% increase in read counts at 10 pg gDNA input and a 90% increase at 2 pg. Improvements > 10% help variant calling.

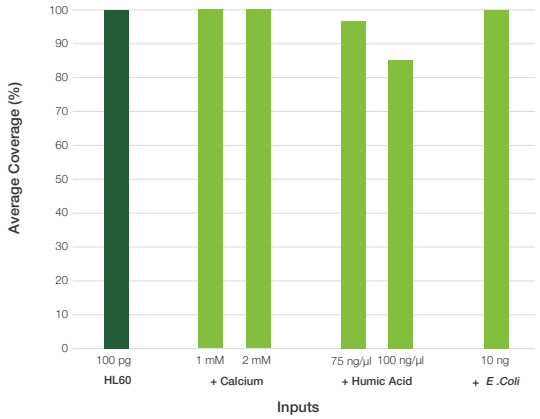

Figure 4: Buffers highly resistant to relevant PCR inhibitors—The ForenSeq buffer system promotes an environment that tolerates a range of inhibitors at amounts above what is typically observed in forensic samples.

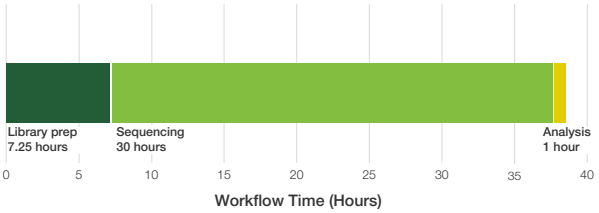

Figure 5: Rapid review of mtDNA whole genome data—A minimal analysis time of one hour caps the ForenSeq mtDNA sequencing workflow. Total workflow time is 40 hours, with expected variations depending on equipment used, automation procedures, user experience, and normalization and purification options.

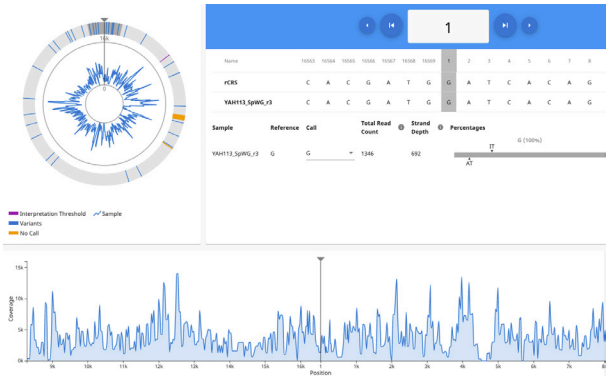

Figure 6: Built for optimal usability and streamlined data review—ForenSeq UAS v2.0 features an intuitive user interface and comprehensive toolset for in-depth analysis of the whole mtGenome. You can review summarized results or a detailed data display.

Proven ForenSeq Workflow with Verogen Support

A simple, efficient workflow underpins all Verogen chemistry, minimizing training and implementation hurdles and facilitating the transition from traditional methods. Originally developed for the ForenSeq DNA Signature Prep Kit, a trusted solution for interrogating forensic STRs and SNPs, the ForenSeq workflow provides best-in-class NGS tools for forensic genomics applications. All workflow components were designed, developed, and tested together as an end-to-end system, integrated from library prep through analysis and beyond for exceptional performance on forensic samples that is backed by extensive support capabilities.[3]

Offered at the lowest list price of any whole genome, short-amplicon commercial assay with zero per-seat license fees for the software, the ForenSeq mtDNA Whole Genome Kit is not only state-of-the-art, but cost-effective and convenient. Laboratories already processing ForenSeq libraries can leverage the same instrument, foundational protocols, and infrastructure to quickly and easily insource mtDNA whole genome capability. Laboratories that are new to NGS will find that the ForenSeq mtDNA Whole Genome Kit offers a straightforward access point.

Summary

The ForenSeq mtDNA Whole Genome Kit features the highest resolution for mtGenome sequencing in the Verogen library prep portfolio. This user-friendly, automation-compatible solution provides a common workflow for a variety of study designs spanning multiple applications. Tiled amplicons, fit-for-purpose buffers, and a thoughtfully designed protocol enable support for the lower end of DNA input amounts, various sample types, and a broad range of forensic and database applications, including missing persons, disaster victim identification (DVI), and population studies. Furthermore, the ForenSeq mtDNA Whole Genome Kit is specifically designed for the flagship forensic NGS solution, which integrates MiSeq FGx System sequencing with ForenSeq UAS v2.0 sample management, analysis, and reporting. Underscored by the power of Illumina sequencing-by-synthesis (SBS) chemistry, the innovative ForenSeq mtDNA Whole Genome Kit provides an optimal mtDNA sequencing experience.[4]

Learn More

To learn more about ForenSeq mtDNA Whole Genome, visit verogen.com/products/forenseq-mtdna-whole-genome-kit/.

Ordering Information

Product Name	Part #
ForenSeq mtDNA Whole Genome Kit (48 reactions)	V16000086
ForenSeq Index Plate Fixture	15055269

References

1. Sidstedt, Maja, Linda Jansson, Elin Nilsson, Laila Noppa, Mats Forsman, Peter Rådström, and Johannes Hedman. "Humic substances cause fluorescence inhibition in real-time polymerase chain reaction." *Analytical Biochemistry* 487 (October 2015): 30–37. https://doi.org/10.1016/j.ab.2015.07.002.
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3. Jäger, Anne C., Michelle L. Alvarez, Carey P. Davis, Ernesto Guzmán, Yonmee Han, Lisa Way, Paulina Walichiewicz, et al. "Developmental validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories." *Forensic Science International: Genetics* 28 (May 2017): 52–70. https://doi.org/10.1016/j.fsigen.2017.01.011.
4. "Sequencing by Synthesis (SBS) Technology," Science and Education, Illumina, accessed June 24, 2020, https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html.

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