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; 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 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). The ForenSeq mtDNA Whole Genome Kit is 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. The kit offers a simple, cost-effective solution for analyzing mtDNA with the full power of mtGenome sequencing backed by established workflows and QIAGEN 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.

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.

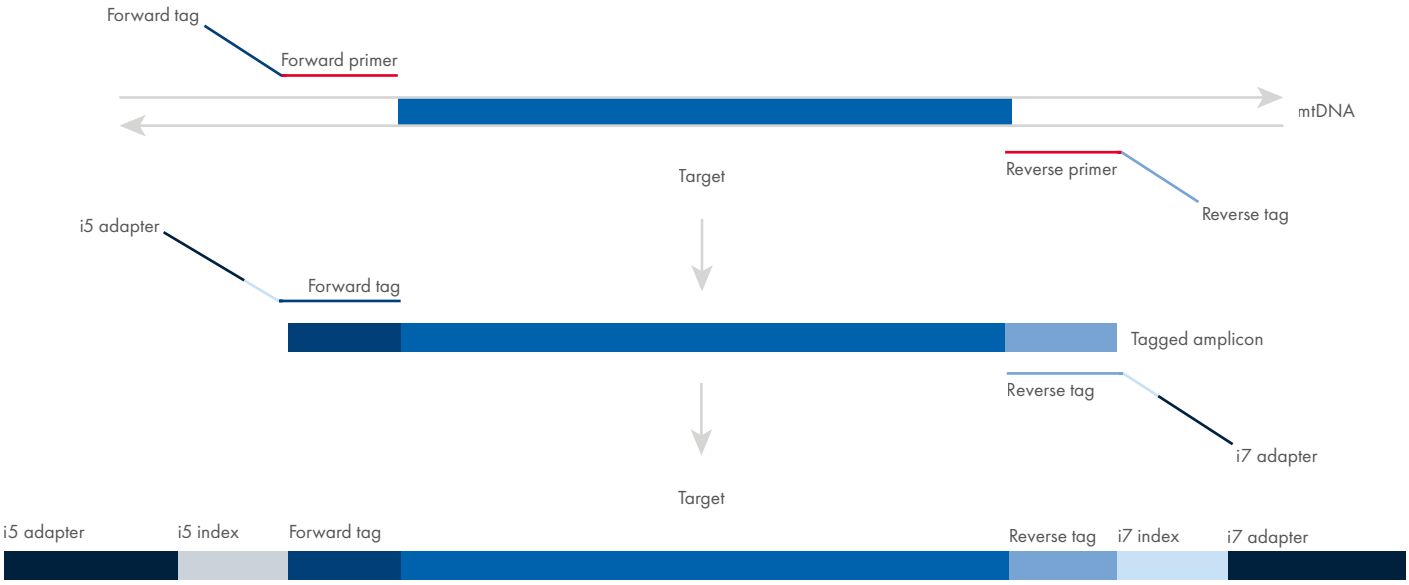

Figure 1. Streamlined ForenSeq chemistry with multiplexing capability.
Tagged oligos for each target sequence mix with samples split across two PCR pools. PCR links the tags to copies of each target, forming DNA templates with regions of interest flanked by universal primer sequences. Index adapters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

Table 1. Specifications of the ForenSeq mtDNA Whole Genome Kit

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 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. The protocol includes an optional second purification that further improves the performance of low-level and complex samples. The second purification is recommended for input gDNA ≤20 pg but 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.

Two purifications show progressive coverage advancements compared to a single purification, culminating with 90% improvement at 2 pg (Figure 3).

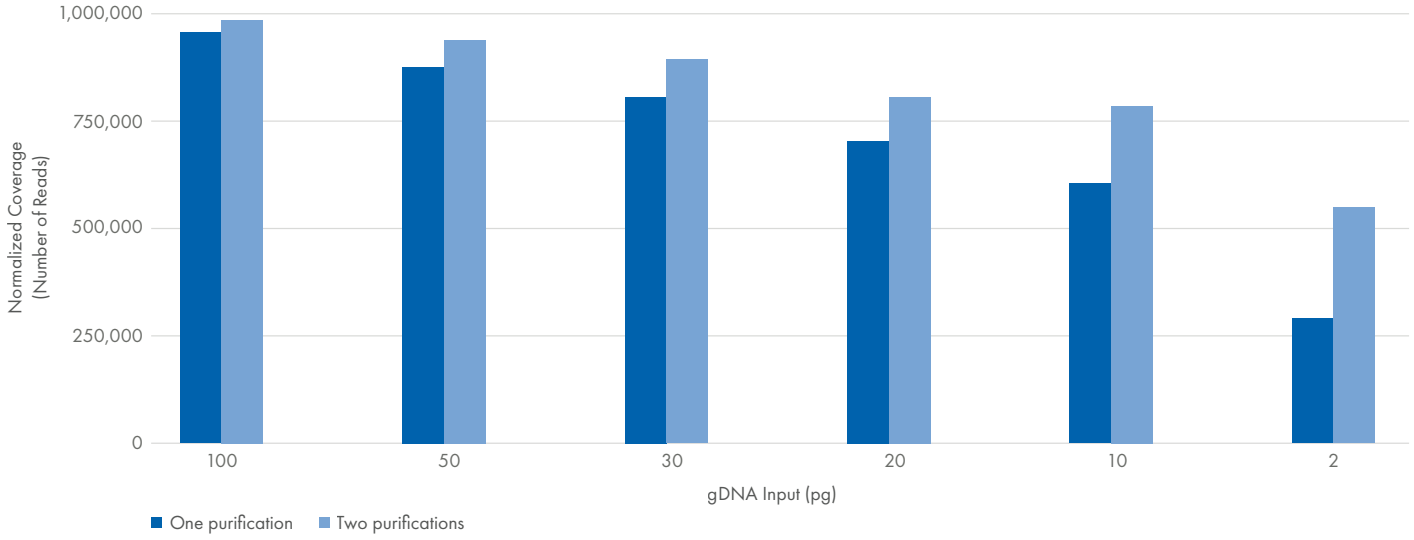

Figure 2. High coverage across a range of input DNA amounts.
The kit delivers consistently high coverage, with a second purification optimizing data quality and extending the lower threshold of input amounts.

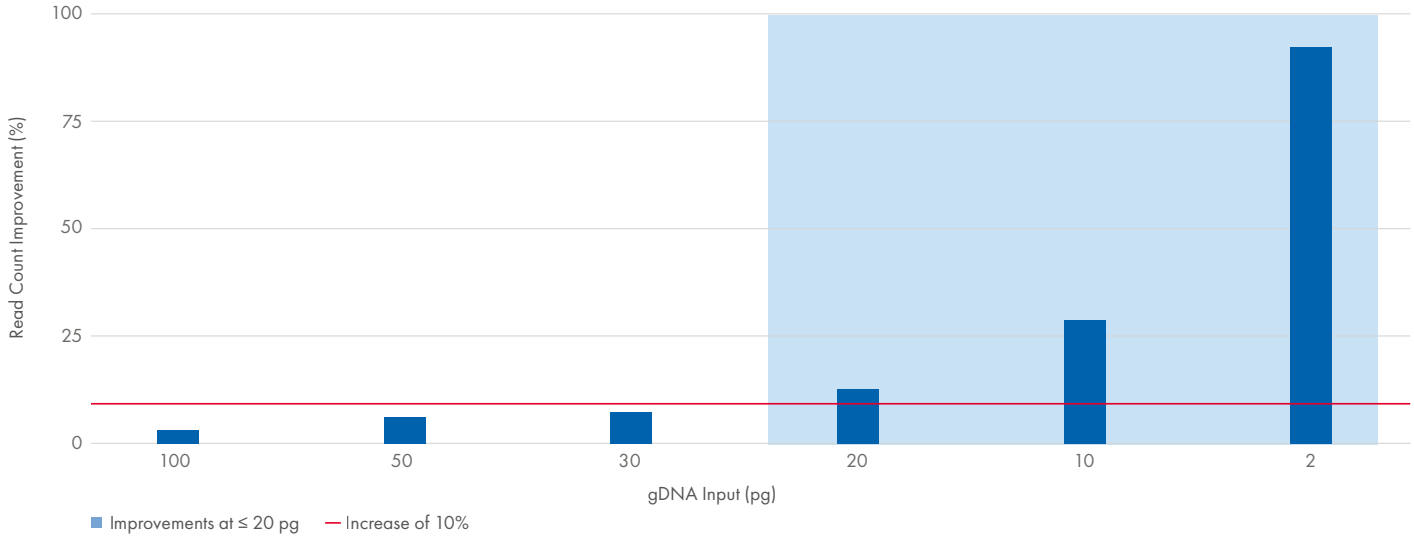

Figure 3. Read count improvement with a second purification.
When compared to one purification, a second purification provides a 30% increase in read counts at 10 pg gDNA input and a 90% increase at 2 pg. Improvements >10% help variant calling.

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, up to 16 of these preplated, dual-index libraries are pooled and sequenced together in one run without increases to cost or time (2).

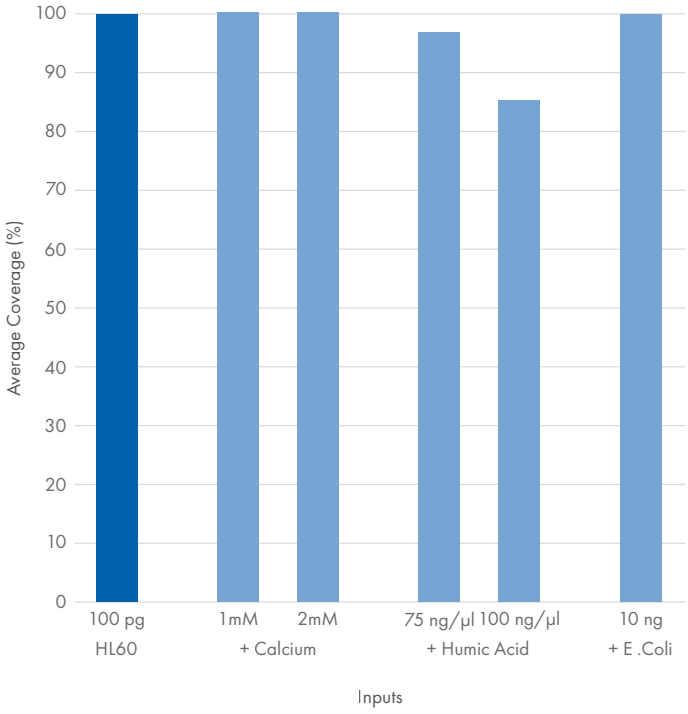

Figure 4. Buffers highly resistant to relevant PCR inhibitors.
The ForenSeq buffer system promotes an environment that tolerates a range of inhibitors at amounts above what is typically observed in forensic samples.

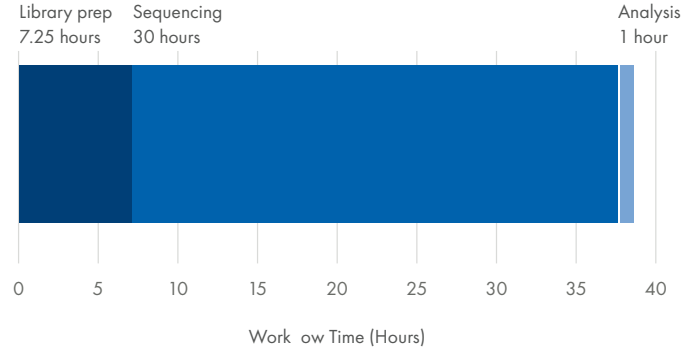

Figure 5. Rapid review of mtDNA whole genome data.
A minimal analysis time of one hour caps the ForenSeq mtDNA sequencing workflow. Total workflow time is 40 hours, with expected variations depending on equipment used, automation procedures, user experience and normalization and purification options.

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 bioinformatic and seamless integration with other QIAGEN 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.

Proven ForenSeq workflow with QIAGEN support

A simple, efficient workflow underpins all ForenSeq 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).

The ForenSeq mtDNA Whole Genome Kit is offered at a competitive list price for a whole genome, short-amplicon commercial assay with zero per-seat license fees for the software. The 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.

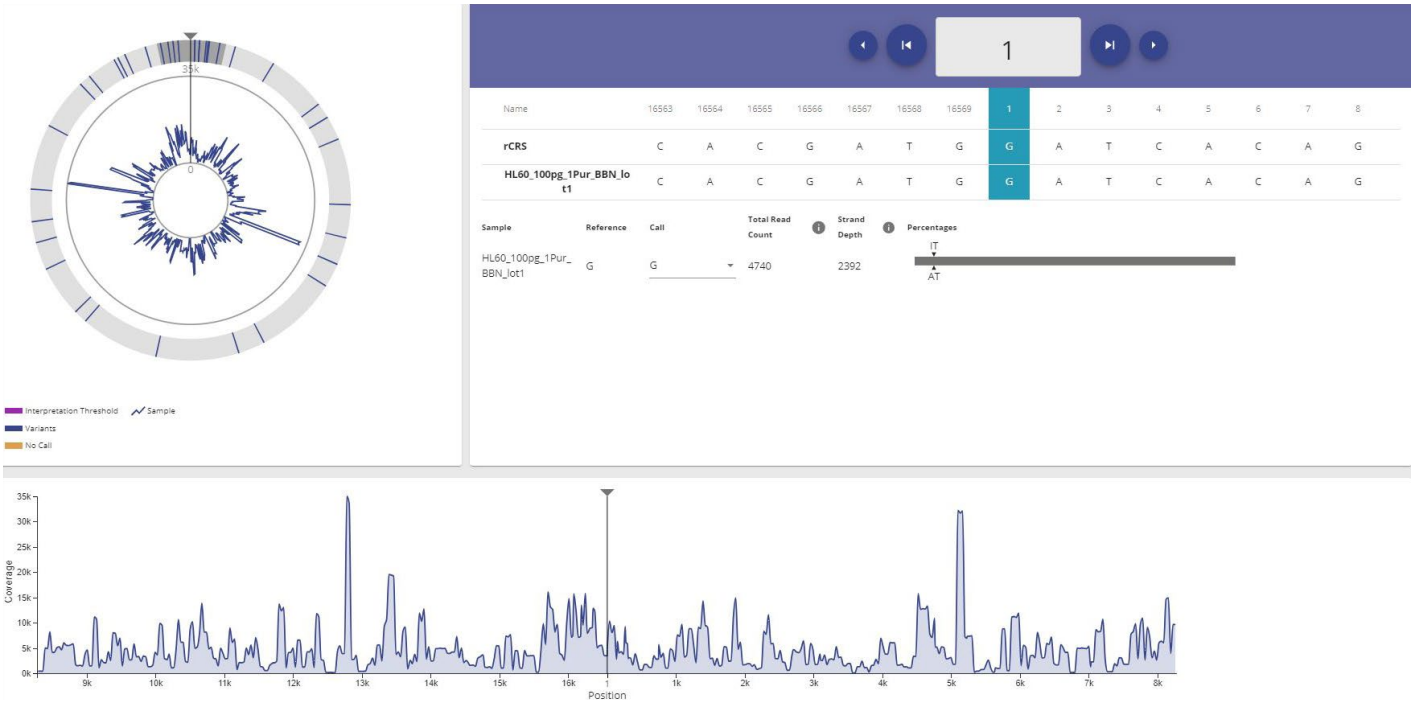

Figure 6. Built for optimal usability and streamlined data review.
ForenSeq UAS v2.0 features an intuitive user interface and comprehensive toolset for in-depth analysis of the whole mtGenome. You can review summarized results or a detailed data display.

Summary

The ForenSeq mtDNA Whole Genome Kit features the highest resolution for mtGenome sequencing in the QIAGEN 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 and various sample types. The kit is suited to a broad range of forensic and database applications, including missing persons, disaster victim identification (DVI) and population studies.

The ForenSeq mtDNA Whole Genome Kit is specifically designed for the flagship forensic NGS solution, integrating 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 ForenSeq mtDNA Whole Genome Kit provides an optimal mtDNA sequencing experience (4).

Ordering Information

Product	Contents	Cat. no.
ForenSeq mtDNA Whole Genome Kit	Includes all the primary reagents necessary for 48 reactions for the preparation of complete whole mtDNA genome libraries	V16000086
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 Kit	Supports up to 12.5 million paired-end reads for deep sequencing or high-throughput sample processing	15066817

Learn more about NGS for HID in your lab. Visit **qiagen.com/ForenSeqmtDNA-WG**

References:

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2. Illumina: Multiplex Sequencing https://www.illumina.com/techniques/sequencing/ngs-library-prep/multiplexing.html (accessed May 13, 2024)
3. 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
4. Illumina: Introduction to SBS Technology https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html (accessed May 13, 2024)