Datasheet

VEROGEN

ForenSeq Kintelligence Kit

The only forensic genetic genealogy assay that targets the relevant markers in degraded and low-input DNA samples

Highlights

- Fit for forensic purpose
  Sequence compromised samples on a forensically validated system and easily access results.
- Curated content design
  Ensure data privacy with a SNP set hand-selected for forensic relevance.
- Physical control of evidence
  Keep evidence and associated data in your laboratory and retain full control.
- Fully integrated and supported
  Pair two familiar workflows, ForenSeq and GEDmatch, with application-specific support.

Introduction

Forensic genetic genealogy (FGG) is a powerful tool for lead generation in a variety of investigations, such as cases of missing persons, unsolved violent crimes, and innocence projects, that includes or excludes people as possible contributors of biological samples. FGG combines microarray genotyping or whole-genome sequencing (WGS) methods of DNA profile generation with comparisons of genetic relatives in genealogy databases such as GEDmatch®, the largest database of voluntarily submitted DNA profiles for forensic comparisons. Comparisons made in a database allow genealogists to construct a family tree using census records, vital records, obituaries, and newspaper archives, then trace the source of the DNA through the family tree. Short tandem repeat (STR) typing using next-generation sequencing (NGS) or capillary electrophoresis (CE) then confirms the identity of the DNA source.[1]

However, microarray and WGS-based methods cannot support the full spectrum of biological samples associated with forensic cases. These samples are often degraded or inhibited due to source material age or environmental exposure, resulting in low-quality data that limits FGG. Microarrays and WGS also produce large amounts of medically relevant single nucleotide polymorphism (SNP) data that are not applicable to forensic applications, raising concerns about genetic data privacy and usage. To help forensic laboratories address these challenges, Verogen offers the ForenSeq® Kintelligence Kit, the only FGG assay fit for forensic purpose (Table 1). Designed for sequencing on the National DNA Index System (NDIS)-approved MiSeq FGx® Sequencing System, data analysis in Universal Analysis Software (UAS), and long-range kinship analysis in GEDmatch PRO, ForenSeq Kintelligence and a Verogen STR assay take you from DNA to a confirmed identity.[2]

Table 1: Kit specifications

Specification	Value
Sample types	Blood, bone, buccal swabs, hair, teeth, and semen
Recommended input	1 ng gDNA per sample
Multiplexing capacity	3 libraries per run
Kit configuration	12 reactions
Number of SNPs	10,230
Mean amplicon size	< 150 bp
Total library prep time	8 hours
Hands-on library prep time	1 hour and 55 minutes
Sequencing time	28 hours

Fast, flexible workflow

ForenSeq Kintelligence inputs are compatible with DNA extraction methods common in forensic laboratories. Library prep leverages the ForenSeq chemistry backbone that is foundational to the Verogen library prep portfolio. ForenSeq Kintelligence reagents enable preparation of up to 12 dual-indexed, human-specific libraries in 8 hours with only 1 hour and 55 minutes of hands-on time. Sequencing is completed in 28 hours and analysis in 1 hour for a fully integrated NGS workflow that delivers results, including a GEDmatch PRO report that users can directly upload to the database, in fewer than 40 hours. If a match exists, GEDmatch PRO produces results in fewer than 2 days.

In addition to providing rapid library prep that includes normalization, the ForenSeq chemistry offers a high degree of flexibility for sample sources such as buccal swabs, blood, bones, and teeth, while an input volume of 25 µl enhances support for degraded and inhibited samples. The 12-reaction kit includes master mixes for amplification, purification beads for cleanup, and straightforward protocols featuring six safe stopping points and minimal cleanup. A robust polymerase chain reaction (PCR) and primer design enables a single SNP multiplex, eliminating the need for multiple tests. The small average amplicon size of < 150 bp improves amplification efficiency and facilitates recovery and analysis of degraded DNA. Additionally, the ForenSeq Kintelligence Kit includes six unique dual index (UDI) adapters, which attach unique identifying sequences to each end of the sample for optimum data recovery.

Forensic-curated content design

ForenSeq Kintelligence queries 10,230 SNPs that have been carefully selected to support targeted sequencing for FGG (Figure 2, Table 2). These SNPs overlap with relevant markers in the Illumina Infinium CytoSNP-850K BeadChip and Infinium Global Screening Array and are cross-referenced against the Genome Aggregation Database (gnomAD) v3.0 and Single Nucleotide Polymorphism database (dbSNP) v151 for robust performance across global populations. Importantly, ForenSeq Kintelligence excludes the SNPs with known medical associations or low minor allele frequencies to limit privacy concerns and protect genetic health data.

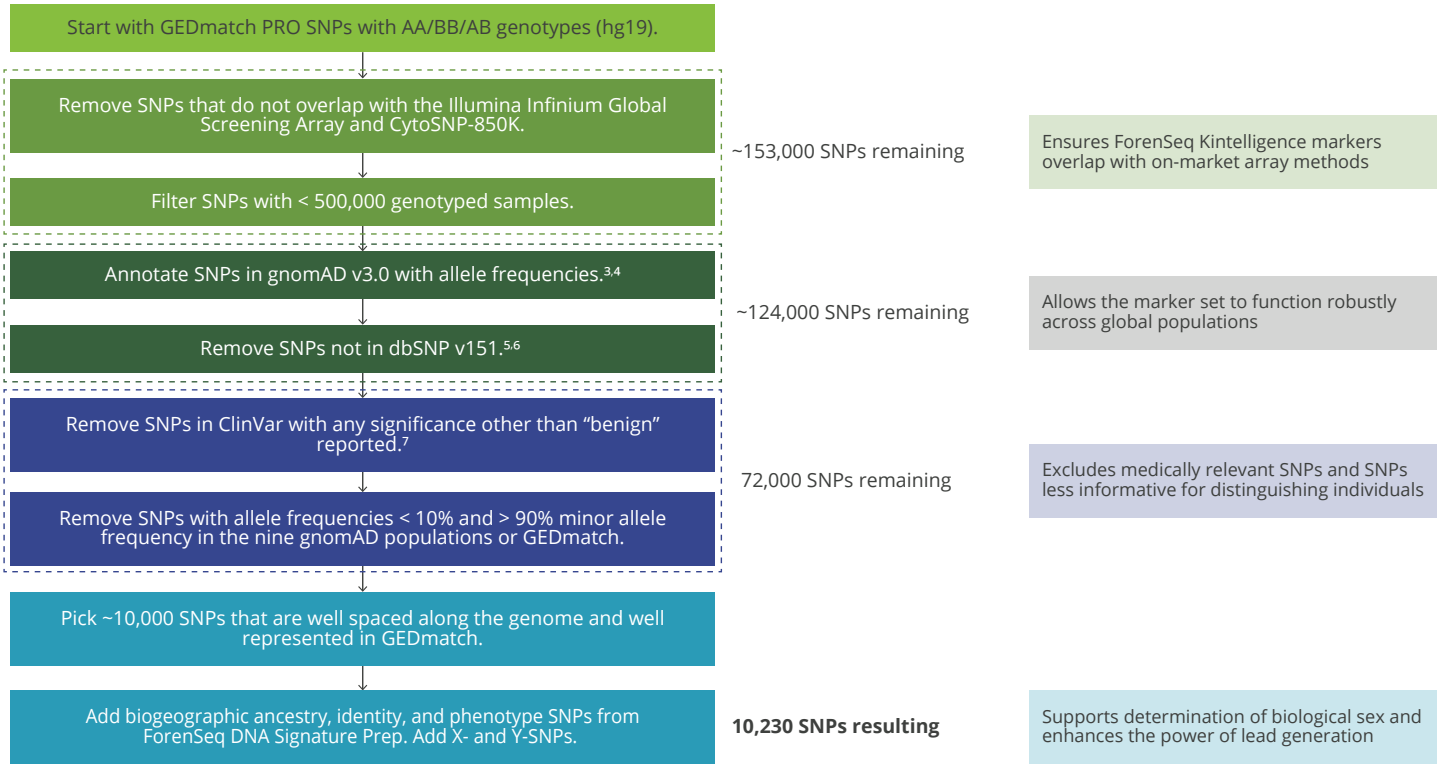

Figure 2: A series of filters retain established kinship markers while removing those that do not meet stringent criteria. The result is a forensically curated content design uniquely suited for FGG.

ForenSeq DNA Signature Prep also influenced the design of ForenSeq Kintelligence, which includes all biogeographical ancestry, identity, and phenotype SNPs validated for investigative lead generation as part of the ForenSeq DNA Signature Prep Kit. To help with lineage and biological sex determination, Verogen also included informative Y-SNPs and X-SNPs, respectively.[2]

Reliable SNP calling and alignment

Cold case samples and missing persons samples are often degraded due to advanced age, exposure to environmental elements and contaminants, and variable extraction and storage procedures. Accordingly, Verogen designed the ForenSeq Kintelligence Kit to deliver high recovery rates from compromised samples, performing studies analyzing degraded and inhibited casework-type samples and conducting a sensitivity assessment with control samples so that the kit reproducibly generates SNP calls across a range of input DNA amounts.

The mock casework studies generated call rates from degraded blood samples, teeth samples inhibited by calcium, and contemporary bone samples subjected to the insults of cremation, burning, and embalming. ForenSeq Kintelligence delivered high performance across all three sample types. Even exceptionally challenging bone samples demonstrated high-quality results, regardless of extraction method or insult (Table 3). The sensitivity assessment evaluated control DNA at inputs ranging from 5 ng to 50 pg. The inputs ranging from 5 ng to 250 pg all achieved a call rate of 100%. Performance was similarly high at 100 pg and 50, at 99.9% and 99.6%, respectively (Figure 3). High average coverage of ~1500× across target sites allows these high call rates and ensures confident calls.

Table 2: Kit SNP content

Category	Number of SNPs	Percentage of Total
Ancestry SNPs	56	0.5%
Identity SNPs	94	1%
Kinship SNPs	9867	96%
Phenotype SNPs*	22	0.2%
X-SNPs	106	1.2%
Y-SNPs	85	0.9%

* Two SNPs overlap the ancestry and phenotype categories and are counted in the phenotype category only.

Table 3: Call rates for casework-type samples

Sample Type	Input Amount (ng)	DI Range	Average Call Rate (%)
Blood	1	1–158[a]	94.6
Bone	1	1–14[b]	99.2
Teeth	1	0.5–0.8[c]	99.5

[a] Calculated using the InnoGenomics InnoQuant HY kit[8]
[b] Calculated using the Applied Biosystems Quantifiler Trio DNA Quantification Kits[9]
[c] Calculations based on short and long quantification values from InnoGenomics

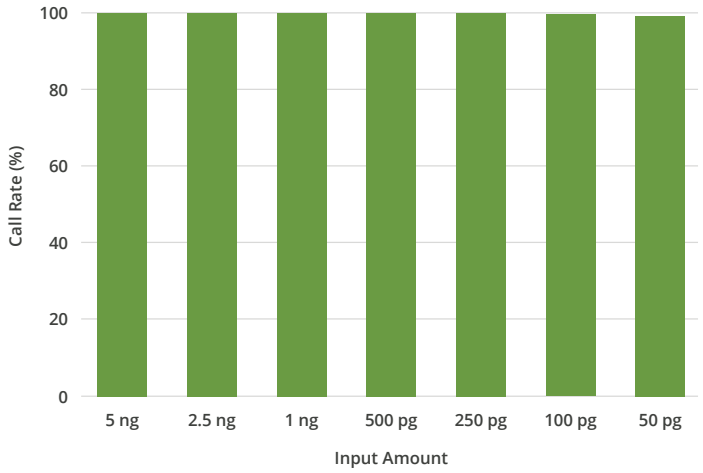

Figure 3: A sensitivity titration of control DNA across a range of inputs demonstrates high call rates that are balanced across SNP categories.

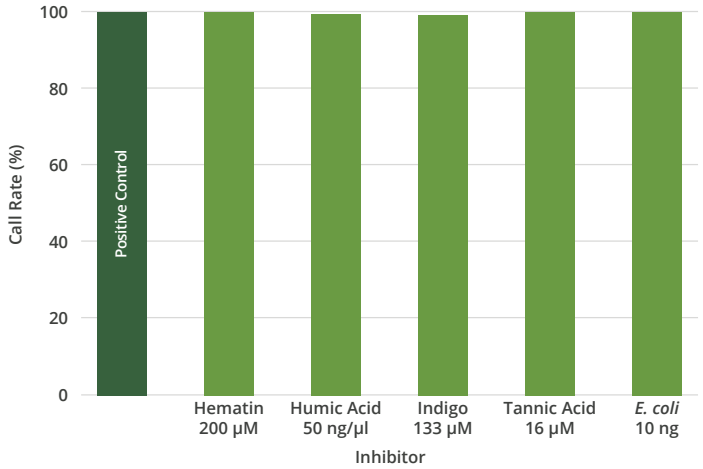

Figure 4: An evaluation of inhibitor performance demonstrated an average call rate of 98% from 1 ng DNA input, demonstrating powerful resistance to above-average amounts of many common inhibitors.

Robust inhibitor resistance

Given exposure to natural elements and prolonged degradation age, cold case samples are prone to failing quality control checks and yielding poor coverage, which results in low DNA recovery and impacts allele call rates. To maximize recovery, ForenSeq Kintelligence buffers tolerate many common inhibitors, including hematin, humic acid, indigo, and tannic acid, and microbial contaminants such as *E. coli*. For 1 ng gDNA samples spiked with 10 ng microbial DNA, Verogen observed a SNP recovery rate of 100%. Control samples inhibited with spike-ins of significant amounts of the aforementioned common forensic inhibitors demonstrated a high average call rate of 99.2%, which is comparable with control samples (Figure 4). The high average call rate of enables deep coverage of samples regardless of low input.

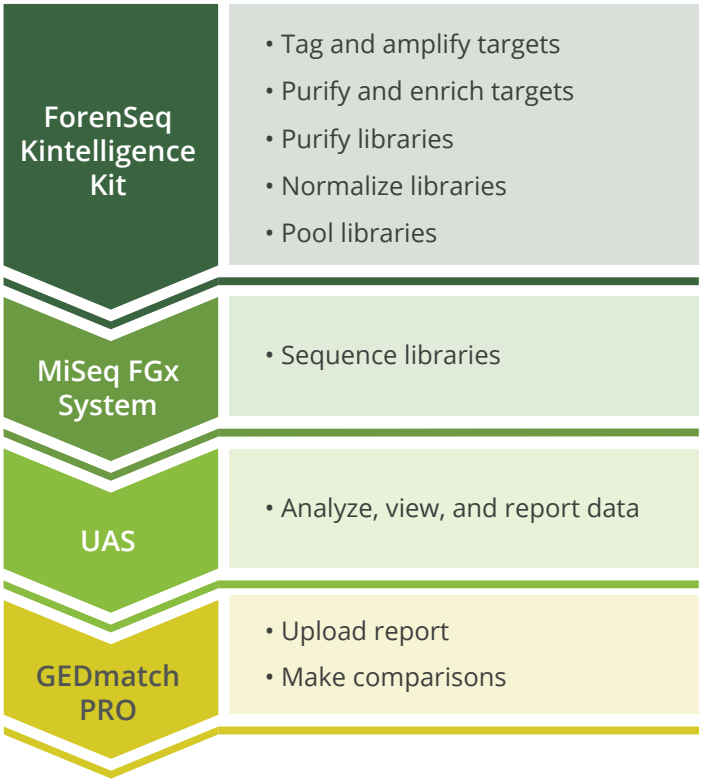

Figure 5: The ForenSeq Kintelligence Kit is the first step of an integrated FGG workflow. After long-range kinship estimation in GEDmatch PRO informs the construction of a family tree, testing with a Verogen STR assay confirms results.

Secure, seamless solution

The fully kitted ForenSeq Kintelligence Kit delivers an end-to-end FGG solution optimized for performance on the MiSeq FGx System with the MiSeq FGx Reagent Kit. A dedicated ForenSeq Kintelligence Analysis Module in UAS starts analysis when sequencing is complete. UAS automatically imports and demultiplexes the data, then assigns reference and alternate alleles based on coverage thresholds. Any imbalanced or low coverage calls are flagged for user review. During communication between the instrument and the dedicated server that hosts UAS, several security measures protect data in transit. Single-click reporting then generates a GEDmatch PRO-compatible report in text file format. The seamless integration of workflow components, from the ForenSeq Kintelligence Kit through GEDmatch PRO, ensures that results are easy to access and upload without breaking chain of custody (Figure 5).

Secure environment for making comparisons

Purpose-built kinship tools in GEDmatch PRO promote efficient comparisons of ForenSeq Kintelligence data with opted-in GEDmatch kits. A combination of physical, electronic, and administrative security measures underpins all assessments in classic GEDmatch, providing a secure environment that is compliant with EU General Data Protection Regulation (GDPR) and other best practices for data handling. Upon upload to GEDmatch PRO, data are encoded and the report is deleted, rendering DNA data inaccessible. Moreover, Amazon Web Services (AWS) hosts the GEDmatch platform, so the platform inherits the industry-standard security, privacy, and compliance capabilities of the underlying infrastructure.[10]

Support and expertise for forensic applications

ForenSeq Kintelligence is backed by the extensive support capabilities of Verogen forensic experts. Laboratories already processing ForenSeq libraries can leverage the same instrument, foundational protocols, and infrastructure to quickly and easily insource FGG capability. Laboratories that are new to NGS will find that the ForenSeq Kintelligence Kit offers a straightforward access point. In either case, Verogen offers onboarding guidance and support that enables laboratories to operationalize the FGG workflow.

Summary

ForenSeq Kintelligence is an integrated solution for FGG. Libraries are prepared, sequenced, and analyzed in a single, streamlined workflow for efficient assessment of forensically relevant SNPs. Developed according to existing STR-based methods, with input from on-market methods and established research and databases, the kit provides laboratories targeted sequencing and accurate analysis from forensic DNA samples, including those that are low-input and highly degraded. By applying a curated SNP set, a low input requirement, and a forensically validated workflow while building on established methodology, ForenSeq Kintelligence offers the opportunity to advance beyond dead ends and find resolution for cold cases, missing persons, and unidentified human remains.

Learn more at **verogen.com/products/forenseq-kintelligence-kit**.

Ordering information

Product	Part #
ForenSeq Kintelligence Kit (12 Reactions)	V16000120

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