ForenSeq® Kintelligence Kit

The only forensic investigative 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 PRO™, with application-specific support.

Introduction

Forensic investigative genetic genealogy (FIGG) is a powerful tool for lead generation that includes or excludes people as possible contributors of biological samples in a variety of investigations, such as cases of missing persons, unsolved violent crimes and innocence projects. FIGG combines 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).

Forensic samples are often degraded or inhibited due to source material age or environmental exposure, resulting in low-quality data that limits FIGG. Methods of DNA profile generation such as microarray genotyping and whole-genome sequencing (WGS) cannot support the full spectrum of biological samples associated with forensic cases.

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, QIAGEN offers the ForenSeq Kintelligence Kit, the only FIGG assay fit for forensic purpose (Table 1).
The ForenSeq Kintelligence Kit is designed for sequencing on the MiSeq FGx® Sequencing System, data analysis in Universal Analysis Software (UAS) and long-range kinship analysis in GEDmatch PRO.

The ForenSeq Kintelligence Kit has been internally validated, optimized and operationalized by the Australian Federal Police National DNA Program for Unidentified and Missing Persons (AFP Program) for coronial casework (2).

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 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. The fully integrated NGS workflow 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, the ForenSeq chemistry offers a high degree of flexibility for sample sources such as buccal swabs, blood, bones and teeth. 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.

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 to 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 FIGG (Figure 1, 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.

The ForenSeq Kintelligence design was influenced by the ForenSeq DNA Signature Prep Kit. The ForenSeq Kintelligence Kit 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, informative Y-SNPs and X-SNPs are also included (3).

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 as well as variable extraction and storage procedures. Accordingly, the ForenSeq Kintelligence Kit is designed to deliver high recovery rates from compromised samples, performing studies analyzing degraded and inhibited casework-type

Table 1.
Specifications of the ForenSeq Kintelligence Kit

Specification	Value
Sample types	Blood, bone, buccal swabs, hair, teeth, semen and any other tissue source with sufficient nuclear DNA
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

Table 2.
SNP content of the ForenSeq Kintelligence Kit

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

samples and conducting a sensitivity assessment with control samples. The ForenSeq Kintelligence Kit reproducibly generates SNP calls across a range of input DNA amounts.

Mock casework studies (Table 3) 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.

The sensitivity assessment (Figure 2) evaluated control DNA at inputs ranging from 50 pg to 5 ng. The inputs ranging from 250 pg to 5 ng all achieved a call rate of >99.8% (corresponding to >10,212 loci). Performance was similarly high at 50 pg and 100 pg, at 87.8% and 99.4%, respectively. High average coverage of ~1500× across target sites allows these high call rates and ensures confident calls.

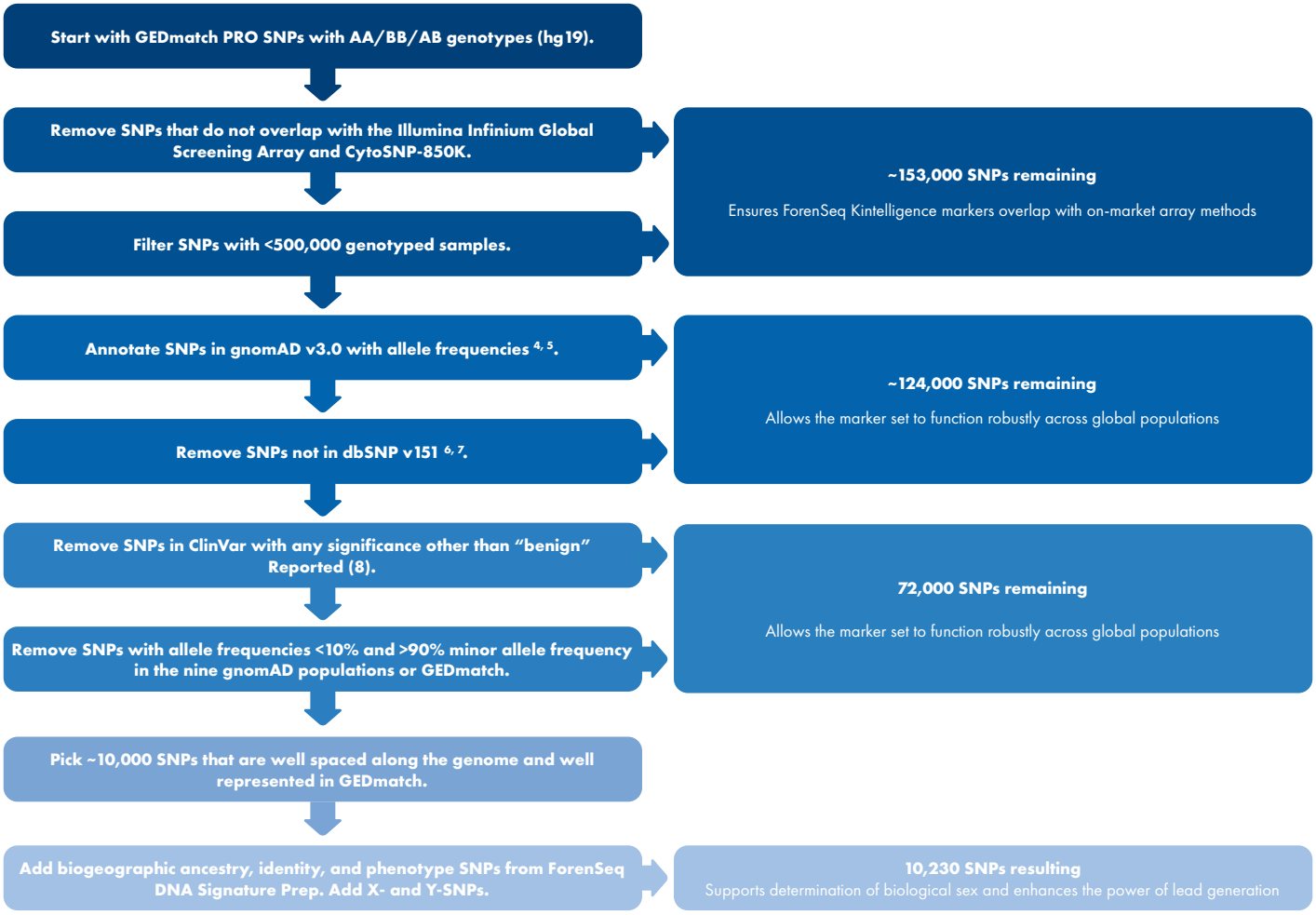

Figure 1.
A series of filters retain established kinship markers while removing those that do not meet stringent criteria. The result is a forensically curated content design uniquely suited for FIGG.

Table 3. Call rates for casework type samples with ForenSeq Kintelligence

Sample type	Input amount (ng)	DI range	Average call rate (%)
Blood	1	1–158*	93.5
Bone	1	1–14†	97.5
Teeth	1	0.5–0.8‡	99.1

* Calculated using the InnoGenomics® InnoQuant® HY Kit according to manufacturer's instructions.
† Calculated using the Applied Biosystems® Quantifiler™ Trio DNA Quantification Kit according to manufacturer's instructions.
‡ Calculations based on short and long quantification values from InnoGenomics.

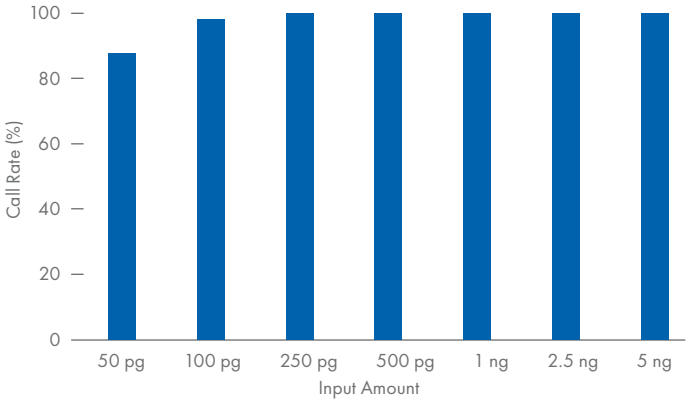

Figure 2.
Duplicate libraries were sequenced over a sensitivity titration of control DNA across a range of inputs and the average call rate is plotted. The assay demonstrates high call rates that are balanced across SNP categories.

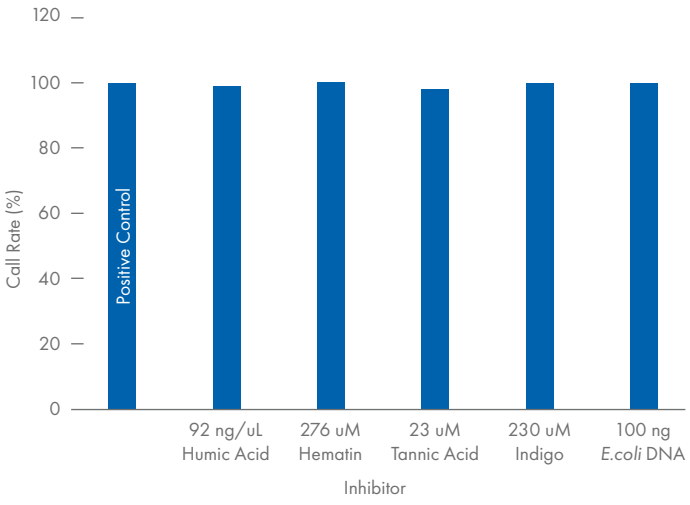

Figure 3.
An evaluation of inhibitor performance demonstrated an average call rate of 98.8% from 1 ng DNA input, demonstrating powerful resistance to above-average amounts of many common inhibitors.

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. These conditions can result in low DNA recovery and may impact 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 100 ng microbial DNA, we observed a SNP recovery rate of 99.9%. Control samples inhibited with spike-ins of significant amounts of common forensic inhibitors demonstrated a high average call rate of 98.8%, comparable with control samples (Figure 3). The high average call rate of enables deep coverage of samples regardless of low input.

Secure, seamless solution

The ForenSeq Kintelligence Kit is fully kitted to deliver an end-to-end FIGG 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 4).

Secure environment for making comparisons

Purpose-built kinship tools in GEDmatch PRO promote efficient comparisons of ForenSeq Kintelligence data with opted-in GEDmatch kits (violent crime cases) or all kits (unidentified human remains). 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 (9).

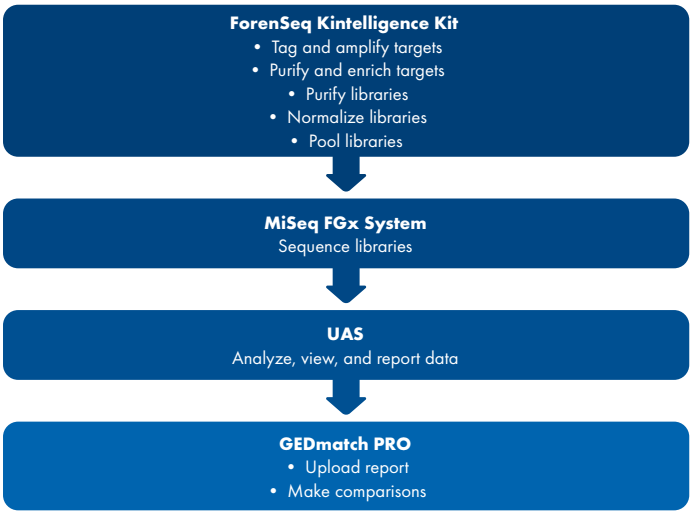

Figure 4.
The ForenSeq Kintelligence Kit is the first step of an integrated FIGG workflow. Long-range kinship estimation in GEDmatch PRO informs the construction of a family tree. Testing with an STR assay confirms results.

Support and expertise for forensic applications

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

Summary

The ForenSeq Kintelligence Kit is an integrated solution for FIGG. Libraries are prepared, sequenced and analyzed in a single, streamlined workflow for efficient assessment of forensically relevant SNPs. The ForenSeq Kintelligence Kit was developed according to existing STR-based methods, with input from on-market methods and established research and databases. The kit provides laboratories with 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.

Ordering Information

Product	Contents	Cat. no.
ForenSeq Kintelligence Kit	Includes all the required reagents to prepare up to 12 low-quality forensic samples for forensic genetic genealogy	V16000120
MiSeq FGx Sequencing System	Desktop instrument with two run modes for a range of forensic genomics applications within a validated NGS workflow	15048976
ForenSeq Analysis Software Server, Monitor	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 FIGG for HID in your lab. Visit **qiagen.com/Kintelligence**

References:

1. Tillman A, Sjölund P, Lundqvist B, Klippmark T, Älgenäs C, Green H. Whole-genome sequencing of human remains to enable genealogy DNA database searches – a case report. *Forensic Sci Int Genet.* 2020; 46: 102233. https://doi.org/10.1016/j.fsigen.2020.102233
2. Watson J, McNevin D, Grisedale K, Spiden M, Seddon S, Ward J, Operationalisation of the ForenSeq Kintelligence Kit for Australian unidentified and missing persons casework. *Forensic Sci Int Genet.* 2024; 68: 102972. https://doi.org/10.1016/j.fsigen.2023.102972
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. https://doi.org/10.1016/j.fsigen.2017.01.011
4. Karczewski KJ, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020; 581: 434– 443. https://doi.org/10.1038/s41586-020-2308-7
5. Francioli L, MacArthur D, gnomAD v3.0 genomAD blog 2019; October 16. https://gnomad. broadinstitute.org/blog/2019-10-gnomad-v3-0/
6. Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 151). https://www.ncbi.nlm.nih.gov/snp/
7. Sherry ST, et al. dbSNP: The NCBI database of genetic variation. *Nucleic Acids Res.* 2001; 29: 308–311. https://doi.org/10.1093/nar/29.1.308
8. Landrum MJ, et al. ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* 2018; 46: D1062–D1067. https://doi.org/10.1093/nar/gkx1153
9. AWS Cloud Security https://aws.amazon.com/security/ (accessed April 07, 2024)