ForenSeq® Kintelligence HT Kit

The first forensic assay to combine high throughput targeted SNP sequencing with a local kinship database for the identification of human remains

Highlights

- *High throughput, fit for purpose workflow*
  Extend the multiplex sequencing capability of the ForenSeq Kintelligence assay for unidentified human remains samples.
- *Application appropriate kinship power*
  Reliably determine relationships out to the third degree, even with degraded samples.
- *Sensitive solution for low input, low quality samples*
  Sequence compromised samples on a forensically validated system and access results easily.
- *A local database for kinship analysis*
  Pair two familiar workflows, ForenSeq and GEDmatch PRO™ algorithms for kinship testing.

Introduction

The identification of human remains can provide answers to those searching for missing family members and justice when death is not accidental. In the case of historical remains identification or mass fatality events such as natural disasters, terrorist incidents or major conflicts, this process can be challenging given the typically poor quality of the biological material and the need to process many samples.

Short tandem repeat (STR) markers have been the traditional markers of choice for human remains analysis. The utility of STR markers is limited to scenarios involving direct or first-degree family references and where DNA integrity allows for the amplification of sufficient STR fragments for a strong enough statistical outcome. When the DNA is more degraded and/or where references from direct relatives are not available, single nucleotide polymorphism (SNP) markers offer the ideal alternative to STRs.

SNP amplicons are shorter than the majority of STR amplicons with lengths typically less than 150 bp. This makes them well-suited for the analysis of degraded samples. They also have a lower mutation rate than STRs. This is useful for kinship analysis where decreased mutation rate over the course of generations will lead to a reduced chance of false exclusion (1). The ability to multiplex 1000's of markers simultaneously with targeted next-generation sequencing (NGS) provides enhanced kinship power to reach out to more distant relatives.

The ForenSeq Kintelligence Kit was developed to interrogate 10,230 forensically relevant SNPs for the emerging application of Forensic Investigative Genetic Genealogy (FIGG) (2). Up to 3 libraries can be analyzed per MiSeq® FGx run allowing enough SNPs to be typed for the detection of long-range relationships in genealogy databases (3). Unidentified human remains projects often require higher throughput capabilities due to the high numbers of postmortem (PM) samples and family reference antemortem (AM) samples involved. These projects may not need to reach the most distant relationships detectable by the full Kintelligence SNP set.

The ForenSeq Kintelligence HT Kit leverages the same SNP set as Kintelligence but benefits from chemistry, throughput and software enhancements specifically developed for human remains identification programs. The fully integrated workflow includes optimized chemistry for highly degraded and contaminated samples, higher level multiplexing that balances throughput with closer kinship determinations, and a fit-for-purpose analysis software and kinship database housed on a local server.

High throughput, fit for purpose workflow

The fully kitted ForenSeq Kintelligence HT Kit forms part of an end-to-end solution for unidentified persons and remains analysis. Kintelligence HT is optimized for performance on the MiSeq FGx Sequencing System using the MiSeq FGx Reagent Kit and comes with a dedicated analysis pipeline in the Universal Analysis Software (UAS). Library preparation is compatible with the type of samples typically encountered in forensic laboratories and in the context of human remains identification (Table 1).

Reagents enable preparation of up to 96 dual-indexed, human specific libraries in 10 hours, with under 3 hours of hands-on time. Multiplexing studies show that up to 12 PM samples or up to 36 AM samples can be sequenced simultaneously, while still detecting up to third degree relationships. Sequencing is completed in 28 hours, at which point analysis starts automatically in the dedicated UAS module and completes in approximately 1 hour. Samples can then be compared for kinship testing in the ForenSeq Kintelligence HT database.

Table 1. Specifications for the ForenSeq Kintelligence HT Kit

Specification	Value	
Sample types tested	Blood, bone, buccal swabs, rooted hair, teeth and semen	
	AM samples	**PM samples**
Recommended input	1 ng	500 pg
Multiplexing capacity	36 libraries per run	12 libraries per run
Kit configuration	96 reactions	
Number of SNPs	10,230	
Mean amplicon size	< 150 bp	
Total library prep time	10 hours	
Hands-on library prep time	< 3 hours	
Sequencing time	28 hours	

Optimized relationship detection

It is possible to resolve a mass disaster situation such as a plane crash using Rapid or CE-based STR analyses due to the immediacy of the event, a known list of victims and the availability of direct, first-degree references. In situations where more time has passed or the mass fatality event involves multiple generations of families, references from second- or third-degree relatives are far more likely to be the norm.

The 10,230 SNPs included in the ForenSeq Kintelligence HT kit can identify out to fifth degree relatives when sequenced at low plexity (3 libraries per run). Feasibility studies have shown that increasing sequencing plexity combined with algorithms repurposed from GEDmatch PRO can still identify first, second, third and most fourth degree relationships without needing to recover the full SNP set (4). Data has shown that sequencing up to 12 PM samples or 36 AM samples provides a sufficient SNP overlap to reach third degree relationships and allows for a higher throughput approach to address the needs of unidentified human remains programs.

Sensitive solution for low input, low quality samples

Unidentified remains are often subject to multiple insults including age, environmental exposure and contaminating substances. Additional variation can be introduced by a wide range of DNA extraction methods resulting in low quantity, low quality DNA samples. The ForenSeq Kintelligence HT kit reproducibly generates SNP calls across a range of input DNA amounts from sample types commonly encountered in these investigations.

Sensitivity assessments were performed on mock AM and PM samples, evaluating control DNA at varying inputs. With a run plexity of 36 libraries, an average of 5600 SNP loci could be detected in AM samples at 100 pg input (Figure 1A). PM samples were sequenced at a plexity of 12, with an average of 3000 SNP loci detected at 12.5 pg input (Figure 1B).

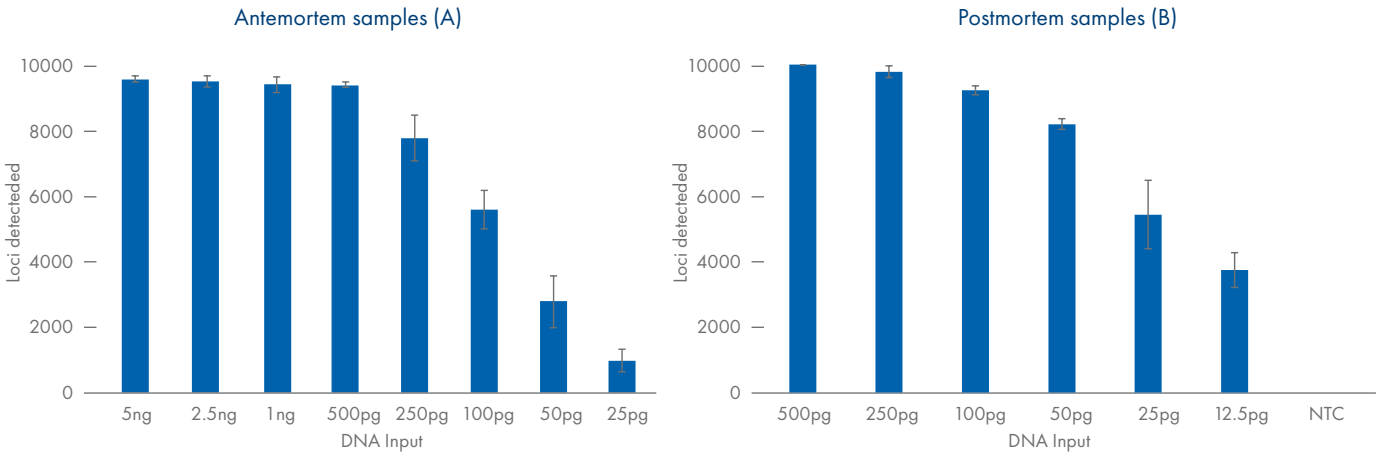

Figure 1.
Sensitivity studies performed using a titration of control DNA (NA24385) demonstrate high call rates across a range of input DNA. The DNA was titrated at each input in triplicate and sequenced on a MiSeq FGx at (A) a plexity of 36 when replicating antemortem samples and (B) at a plexity of 12 when replicating postmortem samples.

The ForenSeq Kintelligence HT kit was tested on a range of sample types typically encountered in human remains testing: interred bones, embalmed bones, teeth and hair. An artificially degraded DNA series (extracted from blood) purchased from InnoGenomics® Technologies (New Orleans LA, USA) was also tested. The ForenSeq Kintelligence HT kit delivered high performance across all sample types. Call rates of over 6,000 SNPs were obtained for most samples (Figure 2).

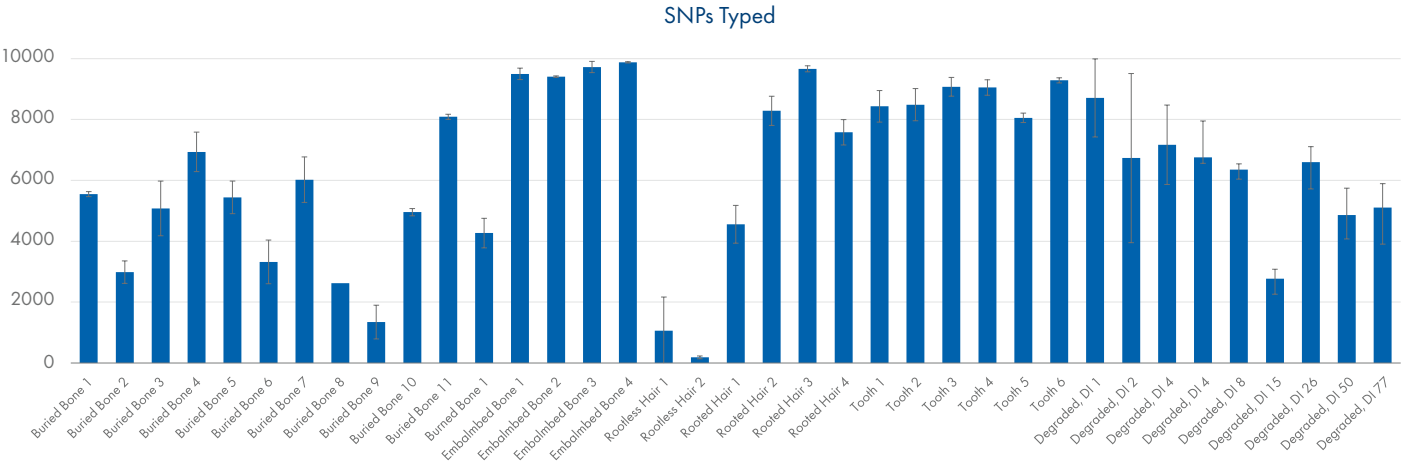

Figure 2.
Call rates for different degraded sample types typically encountered in cases of unidentified human remains. Data shown from runs sequenced at a plexity of 12 libraries.

A local database for kinship analysis

Protection of genetic data is important with all human identification scenarios, and any database resources should be secure, with controlled access. The ForenSeq Kintelligence HT workflow includes the ability to create and maintain a private kinship database within the UAS.

Once a sample has been prepared, sequenced and analyzed with the ForenSeq Kintelligence HT workflow (Figure 3), it will automatically be added to the ForenSeq Kintelligence HT database. A user has the choice to remove a sample from the database at any time and can select samples to perform a query for kinship analysis using built-in analysis tools repurposed from those developed for GEDmatch PRO (Figure 3). One or more unknown samples are compared to one or more reference samples in a pairwise manner. The software generates likelihood ratios and user-generated pedigree trees when a specific relationship has been selected (Figure 4).

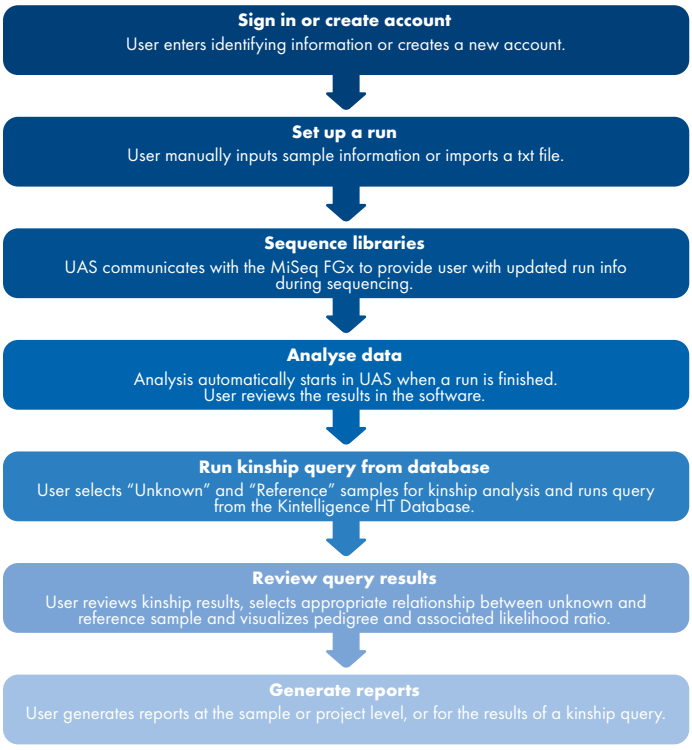

Figure 3.
Overview of workflow steps in the ForenSeq Kintelligence HT UAS.

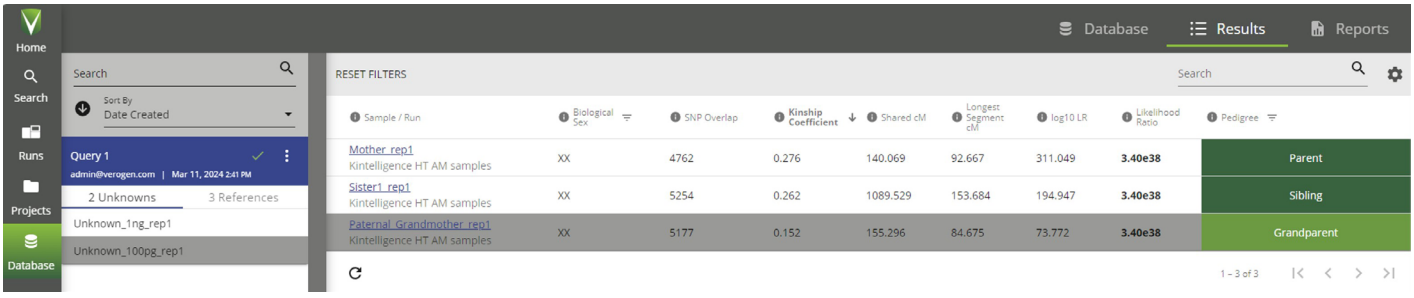

Figure 4.
An example of a query result in the ForenSeq Kintelligence HT Database page. For each pairwise comparison between an unknown sample and a reference sample, a user can select from a predetermined pedigree. This will cause the software to generate a likelihood ratio and a pedigree tree.

The ForenSeq Kintelligence HT kit combines the kinship power of the original Kintelligence kit SNP set with specific throughput, performance and analysis enhancements to improve outcomes for unidentified persons and remains investigations.

- A new PCR additive maximizes performance for bone samples.
- Sequencing of up to 12 postmortem or 36 antemortem samples balances throughput with relationship detection.
- Complex kinship analysis uses algorithms repurposed from those developed for GEDmatch PRO and is conducted in the local, secure Universal Analysis Software environment.

The ForenSeq Kintelligence HT workflow combines higher throughput targeted SNP sequencing with local kinship analysis tools. The workflow goes beyond current capabilities and offers a powerful, unique and integrated way to give names to unidentified human remains.

Notes:

Ordering Information

Product	Contents	Cat. no.
ForenSeq Kintelligence HT Kit	Includes all the required reagents to prepare libraries from unidentified persons and remains samples	V16000190
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 (UAS)	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 and is suitable only for repeat or replacement server purchases by existing customers.	9003364
MiSeq FGx Reagent Kit	Supports up to 12.5 million paired-end reads for deep sequencing or high-throughput sample processing	15066817

Learn about high-throughput kinship testing solution for your lab.
Visit **qiagen.com/KintelligenceHT**

References:
1. Phillips C, García-Magariños M, Salas A, Carracedo A, Lareu MV. SNPs as supplements in simple kinship analysis or as core markers in distant pairwise relationship tests: When do SNPs add value or replace well-established and powerful STR tests? *Transfus Med Hemother.* 2012; 39(3): 202-210. https://doi.org/10.1159/000338857
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://dx.doi.org/10.2139/ssrn.4551904
3. Snedecor J, et al. Fast and accurate kinship estimation using sparse SNPs in relatively large database searches. *Forensic Sci Int Genet.* 2022; 61: p. 102769. https://doi.org/10.1016/j.fsigen.2022.102769
4. Radecke SM, et al. Evaluation of a high-throughput dense single-nucleotide polymorphism PCR multiplex next generation sequencing application for human remains identification. *Forensic Genomics* 2023; 3(3): 75-93. https://doi.org/10.1089/forensic.2023.0005