QIAGEN

Reimagining human identification

The benefits of next-generation sequencing for human identification

Sample to Insight

Seek answers, not profiles

Over the last 30 years, DNA profiling has revolutionized forensic science, but the basic concept remains unchanged of obtaining a profile using capillary electrophoresis (CE) followed by comparison with a reference profile or upload to a database.

A direct match isn't always available. How, then, can you move your investigation forward?

Next-generation sequencing (NGS) with the MiSeq FGx® Sequencing System gives you the power to generate much more than just a basic DNA profile. In addition to analyzing STRs, NGS can be used to analyze mitochondrial DNA and detect SNPs useful to establish identity, estimate externally visible characteristics and provide both short- and long-range kinship information. Combine trusted QIAGEN tools for sample collection, preparation and quantification with the MiSeq FGx Sequencing System and data analysis by the ForenSeq® Universal Analysis Software. This suite of capabilities delivers everything you need to make identifications with greater impact.

Whether you are working in forensic casework, databasing, relationship testing or collecting data on missing persons cases, QIAGEN provides a solution for every step of your DNA workflow from sample to insight.

Figure 1. 4N6FLOQSwabs® represent a breakthrough to guarantee that even smallest amounts of DNA can be collected and remain available for testing.

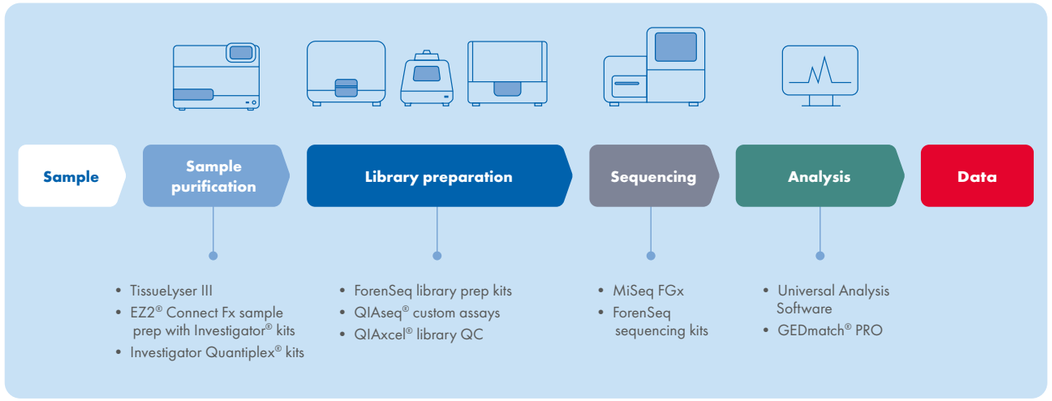


Figure 2. Forensic NGS workflow from sample collection at the crime scene to complete operational sequencing workflows.

STR analysis with NGS

Short tandem repeat markers (STRs) are the foundation of most human identification applications. STRs are traditionally analyzed using length-based comparisons on capillary electrophoresis (CE) machines. The performance of this technology is well-characterized and interpretation methods have evolved to accommodate the specific idiosyncrasies of CE. Amplicons must be designed to accommodate the size limitations of CE dye channels, and CE-based artefacts complicate interpretation. Looking at STR repeat lengths alone masks valuable allele variation and this can lead to inconclusive results.

NGS on the MiSeq FGx Sequencing System eliminates many of these challenges with a base-by-base approach that allows you to obtain more detailed information from your STR analysis.

- The higher resolution of NGS reveals variation within the STRs themselves and informs on a wider range of alleles per locus.
- Data on isoalleles are invaluable in mixture deconvolution and for determining the number of potential contributors.
- Smaller NGS amplicons compared with CE-based targets improve results for degraded samples.
- Interpretation is simplified with the absence of CE-based artefacts such as pull-up, dye blobs, off-ladder alleles, primer peaks or mystery peaks.

The **ForenSeq DNA Signature Plus Kit** targets autosomal, X- and Y-STRs, and includes identity-informative SNPs in a single amplification. Targeting all these markers at once conserves the sample and provides more information for kinship cases where first-degree relatives are not available. Optional ancestry and phenotypic-informative SNPs can help generate additional investigative leads for no-suspect cases. The ForenSeq DNA Signature Plus Kit can generate full profiles from less than 100 pg of DNA. The ForenSeq Signature Plus Kit is the next generation of the ForenSeq DNA Signature Prep Kit and introduces a new positive amplification control and UDI plate. All other components of the kit remain unchanged. The ForenSeq DNA Signature Prep Kit was the first NGS-based short-tandem repeat (STR) sequencing chemistry approved for upload to the United States National DNA Index System (NDIS).

The **ForenSeq MainstAY Kit** is also approved for NDIS upload. This kit focuses on the core autosomal STRs in the European and expanded CODIS loci sets, as well as Y-STRs. The combination of autosomal and Y-STR markers generates highly discriminating data for sexual assault samples, mainstream casework and forensic genetic genealogy confirmatory testing. The cost per sample is comparable to traditional CE analysis but provides significantly more information in a single amplification. This saves analyst time and increases efficiency while conserving valuable sample. The proven ForenSeq workflow can generate comprehensive DNA profiles for up to 96 evidence samples with less than 2 hours of hands-on time from as little as 62.5 pg of DNA.

Part of the ForenSeq MainstAY Kit product line, the **ForenSeq MainstAY SE Kit** targets the same autosomal and Y-STRs, while also including the highly polymorphic SE33 marker.

Externally visible characteristics (EVCs)

While STRs remain the predominant forensic genetic marker for identity testing and kinship analysis, single nucleotide polymorphisms (SNPs) have emerged as an additional powerful tool. SNPs can be used to estimate a range of EVCs such as hair, eye, skin color or biogeographical ancestry.

- Information from EVCs is valuable in no-suspect cases and in cases with no matches to profiles in DNA databases.
- The small amplicon size of SNP analysis is particularly suitable for degraded biological samples.

The **ForenSeq Imagen Kit** incorporates up to 111 EVC SNPs for both biogeographical ancestry inference and phenotype estimation. The kit is an optimized solution for operational forensic labs considering implementing an NGS-based lead generation workflow for no-suspect cases.

The **ForenSeq DNA Signature Plus** and **Kintelligence** kits combine 76 SNPs for eye colour, hair colour and biogeographical inference with STRs and/or identity SNP markers respectively.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a robust source of genetic information and can be a plentiful alternative to nuclear DNA. The higher copy number of mtDNA per cell increases the likelihood of obtaining a DNA profile from challenging samples such as skeletal remains, rootless hairs and teeth. These sample types are frequently the only kinds available for missing persons investigations and disaster victim identification.

A unique inheritance pattern through the maternal line makes mtDNA analysis a useful tool in establishing kinship and identity. In the past, mtDNA analysis has been confined mainly to the hands of specialists with quality and scaling issues restricting broader adoption of this complementary forensic technique.

The **ForenSeq mtDNA Whole Genome Kit** and **ForenSeq mtDNA Control Region Kit** make library preparation for interrogating mtDNA convenient and efficient with optimum sensitivity from minimal DNA input. The kits are a cost-effective solution for analyzing mtDNA when nuclear DNA is unavailable or fails to produce an actionable outcome.

- Simplified workflows relative to the Sanger sequencing approach facilitate routine adoption.
- Highly curated, extended primer sets generate the lowest mean amplicon size of any commercial workflow.
- User-friendly software and plug-and-play sequencing reagents maximize outcomes on degraded and challenging samples.

Kinship analysis with NGS

Kinship analysis covers a broad range of applications. These include paternity testing, forensic investigative genetic genealogy and identification of recovered remains in missing persons inquiries or mass disasters. Currently, the CE-based kinship testing landscape is made up of many different tests, technologies and procedures that impact laboratory efficiency and may not ultimately provide a conclusive answer.

Kinship testing using only STRs to determine relationships is limited to the identification of first-degree relatives. This is problematic in cases where close relatives may not be available as confirmatory references. If more statistical power is required, additional kits including Y- or X-STR markers must be run. If more distant kinship determination is required then additional SNP testing must be performed. This rapidly escalates the cost, time and amount of sample required to establish an identification. Furthermore, the sensitivity of length-based genotyping is not reliable enough to detect very low-level minor contributors in mixed samples and other complex kinship scenarios.

QIAGEN offers many kits to simplify, streamline and expand your kinship testing operations.

- Streamline mainstream paternity testing by targeting the most common autosomal and Y-STR markers in a single analysis with the **ForenSeq MainstAY product line**.
- Detect circulating cell-free fetal DNA in maternal blood. Noninvasive prenatal paternity testing (NIPPT) early in gestation is more accessible compared to CE-based assays with the shorter amplicons and higher sensitivity of ForenSeq MainstAY and MainstAY SE and the identity-specific SNPs in the DNA Signature Plus Kit.
- For the most complex kinship scenarios, reach third-, fourth- and fifth-degree relatives with the focused power of 10,230 SNPs in the **ForenSeq Kintelligence Kit** and **Kintelligence HT Kit**.

Forensic investigative genetic genealogy (FIGG)

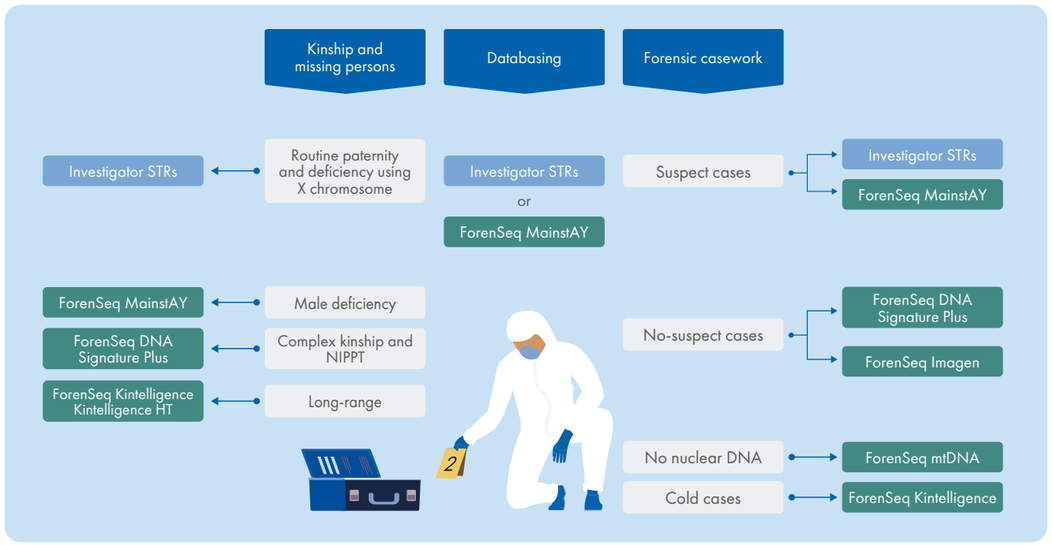
Forensic investigative genetic genealogy (FIGG) combines consumer DNA testing with traditional genealogical research to generate investigative leads in violent crime and unidentified persons and remains cases. Unlike traditional forensic DNA typing, which focuses on STRs, FIGG analyzes single nucleotide polymorphisms (SNPs) and compares these against a database of genetic data from genealogy websites to identify potential relatives. The first and most publicized example of this approach was the 2018 arrest and subsequent conviction of Joseph DeAngelo, also known as the Golden State Killer. Since then, over a thousand cases have been resolved using FIGG.

Traditionally, FIGG used microarrays or whole genome sequencing to generate SNP profiles for samples in question. However, neither microarray technology nor whole genome sequencing was designed for forensic samples. Their use in forensic investigations can create problems around genetic privacy and chain of custody. Both technologies were designed for large amounts of clean, high-quality DNA from a single source. Forensic samples are frequently degraded, inhibited or low quantity, resulting in incomplete data or very low coverage that requires bioinformatic imputation to create a profile that can be used in genetic genealogy databases.

GEDmatch PRO is a dedicated portal designed to support law enforcement and forensic teams with investigative comparisons to GEDmatch data. The portal separates law enforcement comparisons of GEDmatch data from standard genealogy activities and offers a range of tools most relevant to help further investigations.

GEDmatch PRO accepts data from the ForenSeq Kintelligence Kit, the QIAGEN forensic genetic genealogy assay designed specifically for forensic sample analysis. It will also accept uploads from all the data sources GEDmatch supports.

The right kit for your application – analysis, kinship, casework, research or operations



The **ForenSeq Kintelligence Kit** workflow is a sequencing solution that supports FIGG and is designed to address data privacy concerns in a forensic laboratory.

- Kintelligence targets 10,230 SNPs which are human-specific and have no known medical relevance, focusing on kinship determination.
- As a result of optimized algorithms (1), the ForenSeq Kintelligence Kit can confidently uncover genetic associations through the GEDmatch PRO portal out to at least fifth degree, and sometimes beyond that.
- The focused panel provides fewer adventitious matches or false positives compared to whole genome sequencing to minimize the amount of investigative work needed for the average FIGG case.
- The assay is robust to PCR inhibitors and can generate usable profiles with less than 50 pg of DNA, making it ideal for samples that are low-input or contaminated with microbial DNA.
- Up to 12 postmortem, or 36 antemortem samples can be sequenced simultaneously, to reliably reach third-degree relatives with the help of a dedicated UAS kinship database.

Begin your NGS journey today

The MiSeq FGx Sequencing Solution is the first and still the only fully validated NGS system designed for the challenges of forensic laboratories. Dedicated library prep kits, reagents and integrated software, backed by QIAGEN support and services, create a solution ready for immediate adoption into operational laboratories.

With the system in place, your laboratory can realize the full suite of NGS capabilities including STR and mtDNA analysis, kinship determination and estimation of externally visible characteristics. Drive better outcomes from your forensic casework, databasing, missing persons investigations, relationship testing, and more.

Repurposing FIGG tools for unidentified human remains

The **ForenSeq Kintelligence HT Kit** is the first forensic assay to combine high throughput targeted SNP sequencing and a local kinship analysis database for the large-scale identification of human remains.

- ForenSeq Kintelligence HT targets the same explicitly curated 10,230 SNP markers as the ForenSeq Kintelligence Kit for identification of unidentified persons and remains.
- An optimized PCR buffer overcomes challenges with bone samples.
- The unique dual index (UDI) plate facilitates simple manual or automated transfer of the index adapters.

Ordering Information

Product	Contents	Cat. no.
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	V16000084
MiSeq FGx Reagent Micro Kit	Supports up to 5 million paired-end reads for small batch sizes and faster turnaround times	20021681
ForenSeq DNA Signature Plus Kit (96) ForenSeq DNA Signature Plus Kit (384)	Includes all the required reagents to prepare sequencing libraries from forensic DNA samples; part of the MiSeq FGx Forensic Genomics Solution	V16000213 V16000214
ForenSeq MainstAY Kit (96) ForenSeq MainstAY Kit (384)	Includes all the required reagents for 96 or 384 reactions to prepare sequencing libraries generating data for mainstream casework and forensic genetic genealogy confirmatory testing	V16000142 V16000128
ForenSeq MainstAY SE Kit	Includes all the required reagents for 96 reactions to prepare sequencing libraries generating data for mainstream casework and forensic genetic genealogy confirmatory testing including the same markers as in ForenSeq MainstAY Kit + SE33	V16000183
ForenSeq Imagen Kit	Includes the required reagents to process up to 96 samples simultaneously for phenotypic and biogeographical ancestry information to generate comprehensive DNA profiles	V16000189
ForenSeq mtDNA Control Region Kit	Includes all the primary reagents necessary for 48 reactions for the preparation of complete mtDNA control region libraries	V16000085
ForenSeq mtDNA Whole Genome Kit	Includes all the primary reagents necessary for 48 reactions for the preparation of complete whole mtDNA genome libraries	V16000086
ForenSeq Kintelligence Kit	Includes all the required reagents to prepare up to 12 low-quality forensic samples for forensic investigative genetic genealogy	V16000120
ForenSeq Kintelligence HT Kit	Includes all the required reagents to prepare libraries from unidentified persons and remains samples with sequencing capabilities up to 12 PM libraries and 36 AM libraries	V16000190

Reference:

1. Snedecor J, et al. Fast and accurate kinship estimation using sparse SNPs in relatively large database searches. Forensic Sci Int Genet. 2022; Nov:61:102769. doi:10.1016/j.fsigen.2022.102769

Learn more about NGS for HID. Visit **www.qiagen.com/HIDandNGS**