ForenSeq® DNA Signature Prep Kit

Achieve high resolution and exceptional accuracy, even with complex mixtures, or degraded DNA

Highlights

- *Eliminate the need for multiple STR kits*
  Interrogate 200 genetic markers using a single, streamlined workflow.
- *Access a wider range of informative SNPs*
  A dense set of forensically relevant SNPs provide valuable information not widely available with current technology.
- *Superior analysis of challenging samples*
  Obtain highly discriminating data from less than 100 pg of DNA, even with complex mixtures, or degraded DNA (1).
- *Multiplexing and rapid sample processing*
  Prepare up to 96 libraries simultaneously using a simple plate-based format and standard lab equipment.
- *Approved for upload to the National DNA Index System (NDIS)*
  The first NGS-based STR sequencing chemistry approved for upload to the NDIS for casework.

Introduction

The ForenSeq DNA Signature Prep Kit is part of the MiSeq FGx® Forensic Genomics Solution, a complete, fully validated (1) DNA-to-data workflow specifically designed for forensic genomics applications (Figure 1). The ForenSeq DNA Signature Prep Kit includes all the required reagents to prepare sequencing libraries* from forensic DNA samples. With a simple plate-based workflow, prepare up to 96 DNA samples for Illumina next-generation sequencing (NGS), the most trusted and widely adopted technology in the industry (2).

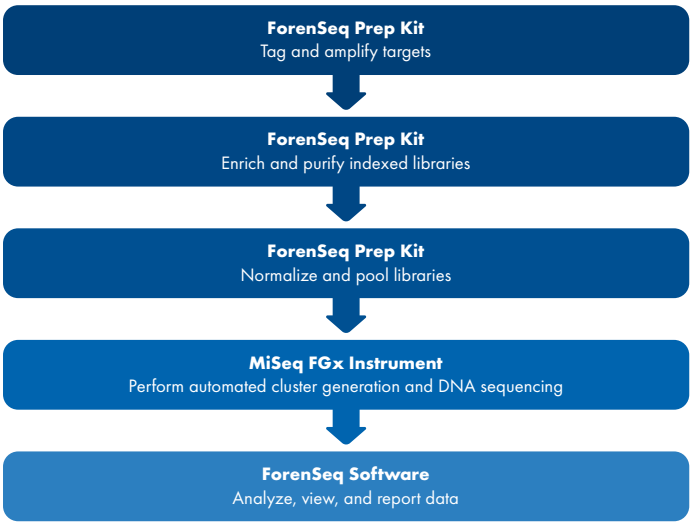

Figure 1.
ForenSeq DNA Signature Prep Kit Workflow—The ForenSeq DNA Signature Prep Kit is part of a fully integrated, sample-to-answer solution, including library preparation, DNA sequencing platform, and data analysis software specifically designed for forensic genomics.

Eliminate the need for multiple STR kits

The ForenSeq DNA Signature Prep Kit consolidates all autosomal short tandem repeat (STR) markers currently used around the world for casework and criminal DNA databasing into a single, streamlined workflow, eliminating the need to run multiple STR tests (Figure 2). The ForenSeq DNA Signature Prep Kit is the first NGS-based STR sequencing chemistry approved for upload to the National DNA Index System (NDIS) for casework. More importantly, in cases where DNA quantity is limited, the difficulty may come from having to choose between relevant STR tests and risk the oversight of potentially informative genetic data. The ForenSeq DNA Signature Prep Kit delivers over 200 genetic markers in a single test when running primer set B, removing the tradeoffs and risk imposed by technical limitations such as low DNA quantity.

* A sequencing "library" is a collection of amplified DNA fragments from a single DNA sample.

Access a wider range of informative SNPs

Significant casework challenges occur when no suspect is available for direct STR profile comparison and no hits are found in local or national criminal databases. In addition to autosomal, X-, and Y-STRs, the ForenSeq DNA Signature Prep Kit offers marker sets not routinely available with traditional capillary electrophoresis (CE) methods.

These include a dense set of identity-informative single nucleotide polymorphisms (iiSNPs) informative for source attribution (3, 4), phenotypic-informative SNPs (piSNPs) for estimates of eye color (blue, intermediate, brown) and hair color (brown, red, black, blond) (5), and biogeographical ancestry-informative SNPs (aiSNPs) (6). The aiSNPs and piSNPs can be critical in generating investigative leads from "no suspect" cases that may have otherwise gone cold (Table 1).

Superior analysis of challenging samples

Many cases are complicated and sometimes unresolved due to the presence of highly degraded DNA, low-quality DNA or complex DNA mixtures. The ForenSeq DNA Signature Prep Kit provides an enhanced capacity to process these types of challenging samples.

Most SNPs included in the ForenSeq DNA Signature Prep Kit contain amplicon sizes <125 bp, making them extremely well-suited for analysis of degraded DNA or even highly inhibited DNA extracts (Figure 3). The ForenSeq DNA Signature Prep Kit also displays an improved ability to detect low-level minor components in mixtures that may otherwise go undetected with conventional STR and CE analysis.

This increased power is due to the large number of markers included in the kit, many of which are highly polymorphic, coupled with the inherent sensitivity of the MiSeq FGx instrument (Figure 4).

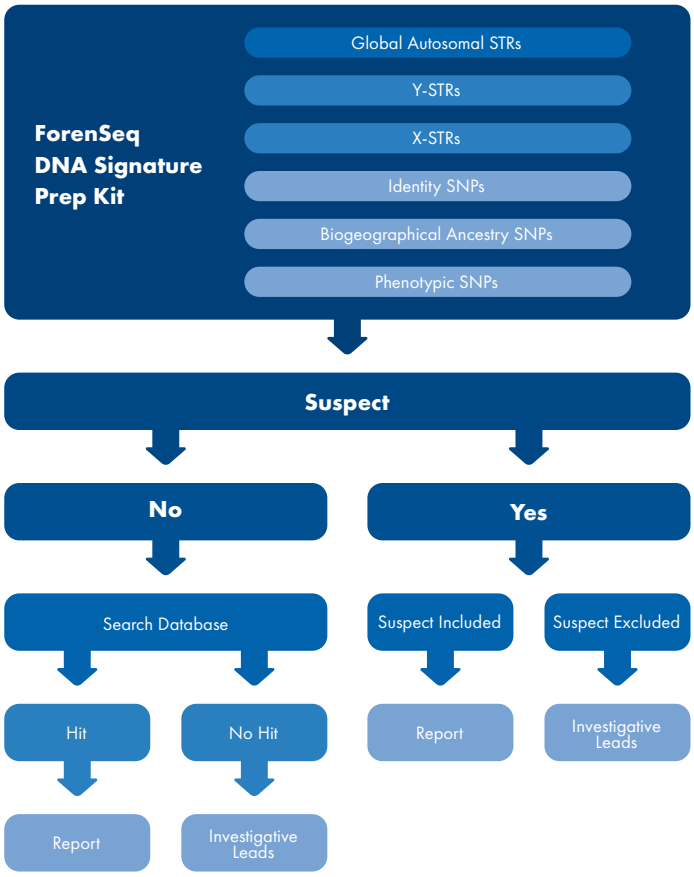

Figure 2.
ForenSeq DNA Signature Prep Kit forensic loci and investigative workflow. With ~200 genetic markers in a single workflow, the MiSeq FGx Solution offers a comprehensive multiplex of STRs and SNPs and a straightforward path to human identification.

Figure 3.
Analysis of High Molecular Weight vs. Degraded DNA. Comparison of high molecular weight gDNA and partially degraded gDNA performance in ForenSeq DNA Signature Prep Kit. Most SNP amplicons (black circles) in the kit are <125 bp in length, allowing the most information to be extracted from an unknown sample. Powerful population statistics are generated, even when all or most STRs (blue circles) are lost. When no suspect is available for comparison, investigative genetic leads can be generated from additional classes of SNPs—even from partial STR profiles.

Table 1.
Forensic loci covered by the ForenSeq DNA Signature Prep Kit

Feature	Number of markers*	Amplicon size range (bp)	Included in DNA Primer Mix A	Included in DNA Primer Mix B†
Global autosomal STRs	27	61–467	Yes	Yes
Y-STRs	24	119–390	Yes	Yes
X-STRs	7	157–462	Yes	Yes
Identity SNPs	94	63–231	Yes	Yes
Phenotypic SNPs	22	73–227	No	Yes
Biogeographical ancestry SNPs	56	67–200	No	Yes

* SNP and STR chromosome locations can be found in the ForenSeq DNA Signature Prep Kit Reference Guide. † Over 200 markers analyzed when running primer set B.

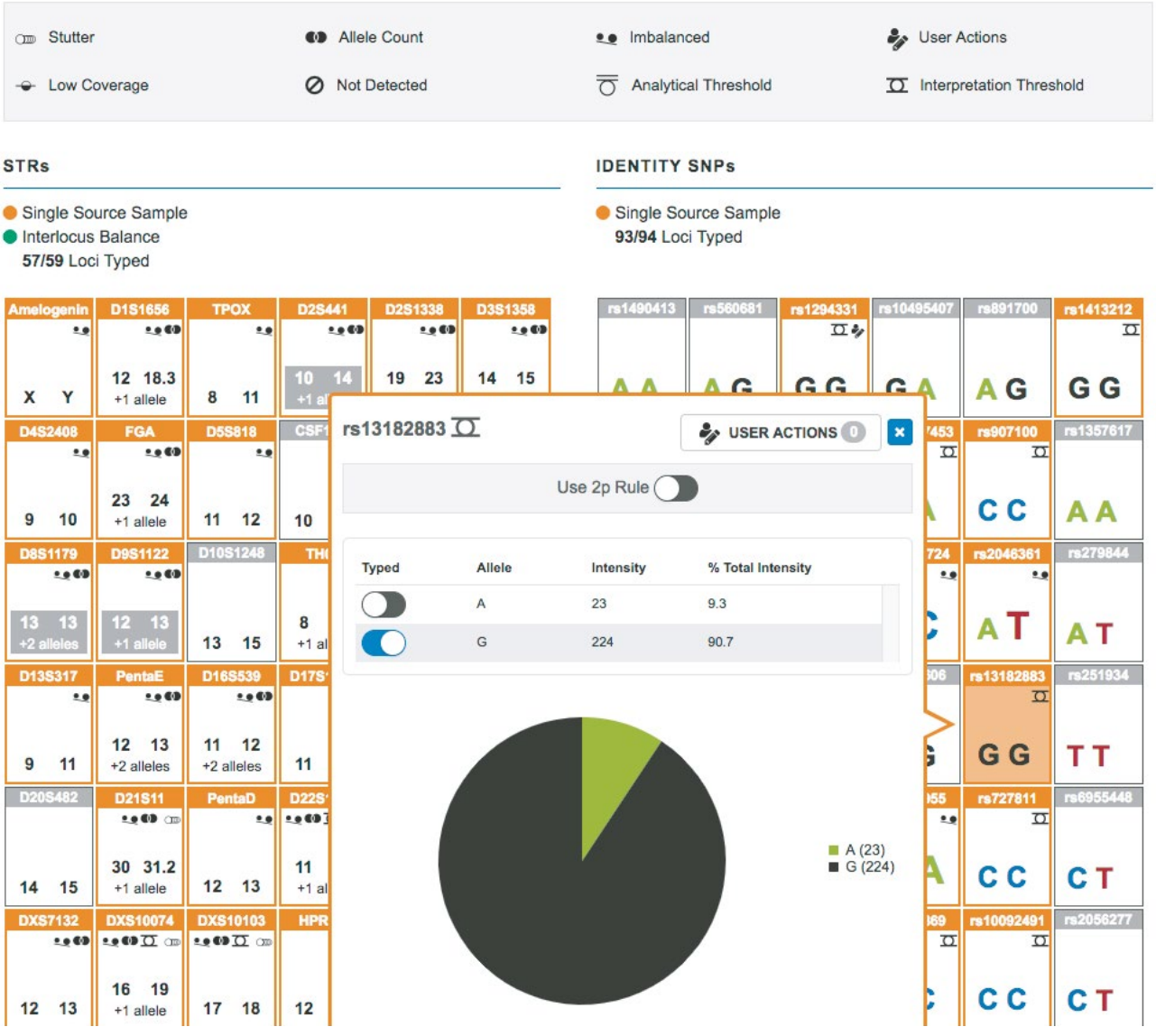

Figure 4.
ForenSeq DNA Signature Prep Kit DNA Mixture Detection. Detect DNA mixtures using quality icons such as Stutter, Allele Count, Imbalanced, etc. (gray panel) and Single Source Sample color indicators (green/orange dots). Drill deeper into loci data with pop-up panels to verify and interpret the sample mixture. For example, click on a SNP locus box to view a pop-up panel with a circle graph showing quantitative signal balance between alleles.

Multiplexing and rapid sample processing

The ForenSeq DNA Signature Prep Kit supports the preparation of up to 96 libraries simultaneously using a simple plate-based format and standard lab equipment. In a single reaction, Primer Mix A enables testing of all autosomal, X-, and Y-chromosome STR targets and the full set of iiSNPs. Primer Mix B also includes the aiSNPs and piSNPs (Table 1).

The ForenSeq DNA Signature Prep Kit includes:

- Multiplexing—Amplify STR and SNP amplicons in a single reaction and sequence up to 96 samples in one sequencing run
- DNA Primer Mix A—Contains primer pairs for 58 STRs (including 27 autosomal STRs, 7 X, and 24 Y haplotype markers) and 94 iiSNPs
- DNA Primer Mix B—Contains all markers in DNA Primer Mix A, plus primer pairs for 56 biogeographical aiSNPs and 22 piSNPs (2 aiSNPs are also used for phenotype estimation)

The easy, integrated workflow maximizes laboratory efficiency by simplifying training programs, validation procedures, proficiency testing and other quality assurance/quality control measures required by external certification and accreditation programs. The solution is backwards compatible with current CE allele calling.

Summary

The ForenSeq DNA Signature Prep Kit is part of a complete, end-to-end next-generation sequencing solution specifically designed for use in Forensic Genomics applications. The ForenSeq DNA Signature Prep Kit leverages the high throughput and exceptional data accuracy of the MiSeq FGx Forensic Genomics Solution.

Table 2.
Specifications of the ForenSeq DNA Signature Prep Kit

Specification	Value
Sample types	gDNA, buccal swabs, FTA® card
Recommended input for human gDNA per sample	1 ng
Recommended input for FTA card punch per sample	1.2 mm
Multiplexing capacity per run	8–96 samples
Short amplicon detection	≥65 bp
Accurate low-level mixture detection	Detects minor contributors at <5% of major
Locus multiplexing capability	Simultaneous analysis of ~200 genetic markers

Ordering Information

Product	Contents	Cat. no.
ForenSeq DNA Signature Prep Kit (96)	Includes all the required reagents to prepare sequencing libraries from forensic DNA samples; part of the MiSeq FGx Forensic Genomics Solution	V16000023
ForenSeq DNA Signature Prep Kit (384)	Includes all primary reagents necessary for 384 reactions to prepare sequencing libraries from forensic DNA samples	15066151
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.	9003364
MiSeq FGx Reagent Kit	Supports up to 12.5 million paired-end reads for deep sequencing or high-throughput sample processing	15066817
MiSeq FGx Reagent Micro Kit	Supports up to 5 million paired-end reads for small batch sizes and faster turnaround times	20021681

Learn more about NGS for HID in your lab. Visit **qiagen.com/DNASignaturePrep**

References:

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6. Kidd KK, et al. Progress toward an efficient panel of SNPs for ancestry inference. *Forensic Sci Int Genet.* 2013; 10: 23–32. doi: 10.1016/j.fsigen.2014.01.002