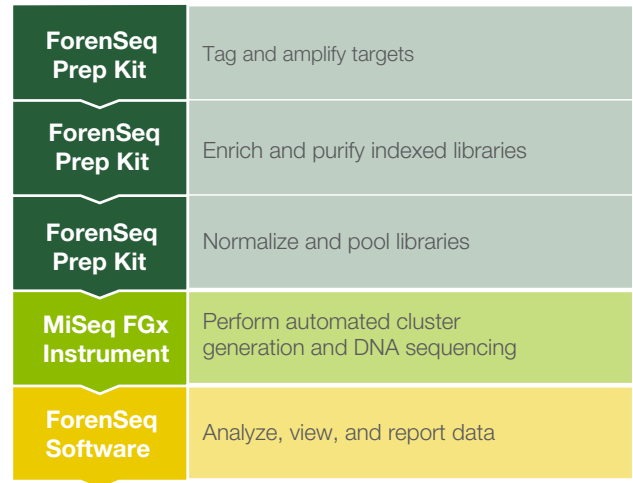


ForenSeq™ DNA Signature Prep Kit

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 DNA¹
- Multiplexing and Rapid Sample Processing**
 Prepare up to 96 libraries simultaneously using a simple plate-based format, and standard lab equipment



Introduction

The ForenSeq DNA Signature Prep Kit is part of the Verogen MiSeq FGx™ Forensic Genomics Solution, a complete, fully validated¹ 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 (Figure 2). 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.

Table 1: ForenSeq DNA Signature Prep Kit—Forensic Loci

Feature	Number of Markers ^a	Amplicon Size Range (bp)	Included in DNA Primer Mix A	Included in DNA Primer Mix B ^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

a. SNP and STR chromosome locations can be found in the ForenSeq DNA Signature Prep Kit Reference Guide.

b. Over 200 markers analyzed when running primer set B.



Figure 2: ForenSeq DNA Signature Prep Kit—The ForenSeq DNA Signature Prep Kit includes all reagents required to prepare DNA libraries for sequencing, including PCR reagents, index adapters, and purification and normalization beads.

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

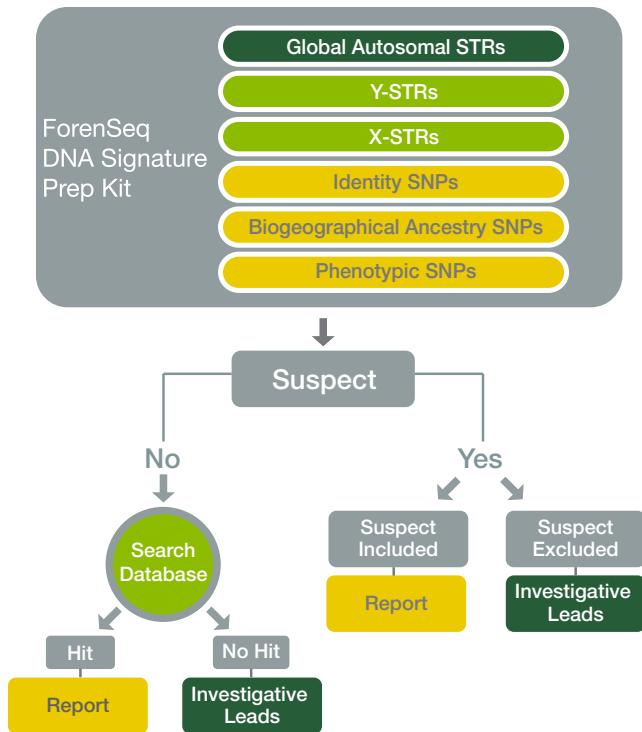


Figure 3: ForenSeq DNA Signature Prep Kit Forensic Loci and Investigative Workflow—With ~200 genetic markers in a single workflow, the MiSeq FGx Solution offers the most comprehensive multiplex of STRs and SNPs and the most straightforward path to human identification.

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 3). 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 approximately 200 genetic markers in a single test, removing the tradeoffs and risk imposed by technical limitations such as low DNA quantity.

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),^{3,4} which are informative for source attribution, phenotypic-informative SNPs (piSNPs),⁵ which provide estimates of eye color (blue, intermediate, brown) and hair color (brown, red, black, blond), and biogeographical ancestry-informative SNPs (aiSNPs).⁶ 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 4). 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 Illumina MiSeq FGx instrument (Figure 5).

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 includes the aiSNPs and piSNPs.

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 massively parallel sequencing solution specifically designed for use in Forensic Genomics applications. The ForenSeq DNA Signature Prep Kit leverages high throughput and exceptional data accuracy of the Verogen MiSeq FGx Forensic Genomics Solution.

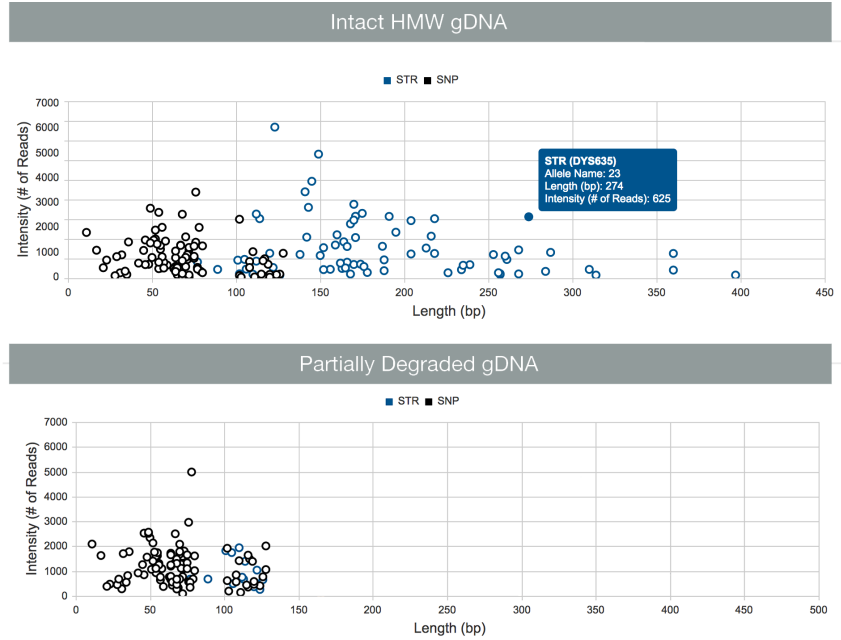


Figure 4: Analysis of High Molecular Weight vs. Degraded DNA—Comparison of high molecular weight gDNA and partially degraded gDNA performance in ForenSeq DNA Signature Prep Kit. Most ForenSeq SNPs (black circles) are < 125 bp in length, allowing the most information to be extracted from an unknown sample. 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 DNA profiles.

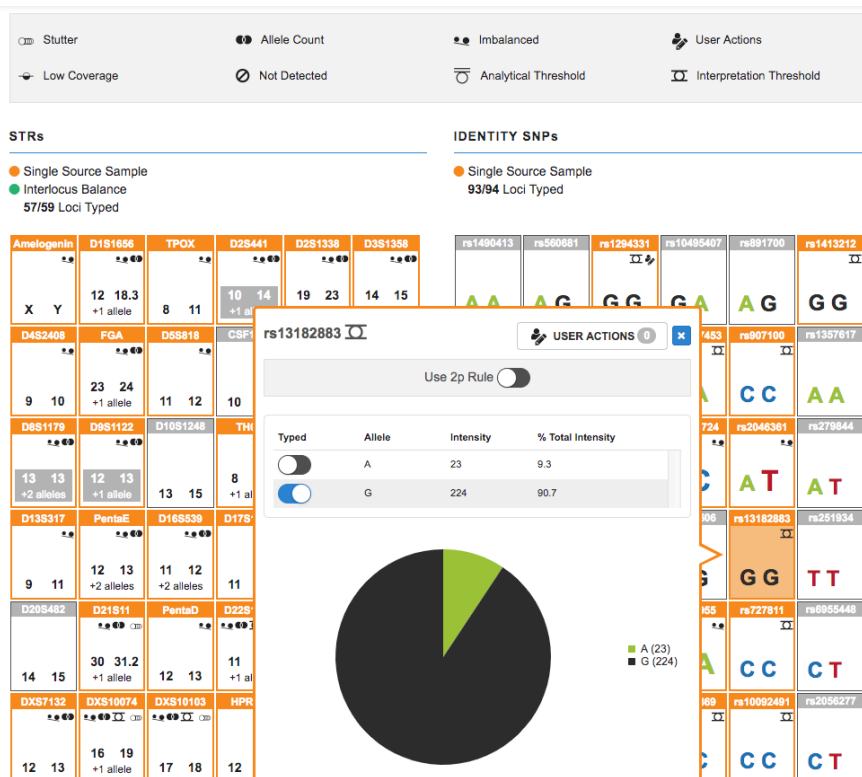


Figure 5: ForenSeq DNA Signature Prep Kit DNA Mixture Detection—Detect DNA mixtures using quality icons such as Slutter, Allele Count, Imbalanced, etc. (gray panel) and Single Source Sample color indicators (green/orange dots). Drill deeper into loci data with pop-up panels to verify and interpret the 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.

Table 2: ForenSeq DNA Signature Prep Kit—Specifications

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

Learn More

For more on the ForenSeq DNA Signature Prep Kit, visit:
www.verogen.com/products

References

- Jäger AC, Alvarez ML, Davis CP, 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.* 2017;28: 52-70.
- Nakazato T, Ohta T, and Bono H. Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS One.* 2013;22;8(10): e77910.
- Kidd KK, Pakstis AJ, Speed WC, et al. Developing a SNP panel for forensic identification of individuals. *Forensic Sci Int.* 2006;164:20–32.
- Sanchez JJ, Phillips C, Borsting C, et al. A multiplex assay with 52 single nucleotide polymorphisms for human identification. *Electrophoresis.* 2006;27:1713–1724.
- Walsh S, Liu F, Wollstein A, Kovatsi L, et al. The HlrPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Sci Int Genet.* 2013;7: 98–115.
- Kidd KK, Speed WC, Pakstis AJ, et al. Progress toward an efficient panel of SNPs for ancestry inference. *Forensic Sci Int Genet.* 2013;10:23–32.

Table 3: ForenSeq DNA Signature Prep Kit—Kit Contents

Reagent	Description
Box 1	
2800M	ForenSeq Control DNA
PCR1	ForenSeq PCR1 Reaction Mix
DPMA	DNA Primer Mix A
DPMB	DNA Primer Mix B
FEM	ForenSeq Enzyme Mix
Box 2	
PCR2	ForenSeq PCR2 Reaction Mix
HP3	2N NaOH
LNA1	Library Normalization Additives 1
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
HSC	Human Sequencing Control
A501-A508	i5 Indexed Adapters
R701-R712	i7 Indexed Adapters
Box 3	
SPB	Sample Purification Beads
RSB	Resuspension Buffer
LNB1	Library Normalization Beads 1

Ordering Information

Product	Catalog No.
ForenSeq DNA Signature Prep Kit (384 reactions)	TG-450-1001
ForenSeq DNA Signature Prep Kit (96 reactions)	TG-450-1002
ForenSeq Index Adapter Fixture	FC-451-1001

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