





**Figure 3: ForenSeq DNA Signature Prep Kit Forensic Loci and Investigative Workflow**—With ~200 genetic markers in a single workflow, the MiSeq FGx System 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. Furthermore, the low 1 ng DNA input requirement provides additional support for cases with precious samples.

## 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 (iSNPs),<sup>3,4</sup> which are informative for source attribution,

phenotypic-informative SNPs (piSNPs),<sup>5</sup> which provide estimates of eye color (blue, intermediate, brown) and hair color (brown, red, black, blond), and biogeographical ancestry-informative SNPs (aiSNPs).<sup>6</sup> 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  $\leq 125$  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 Illumina sequencing by synthesis chemistry (Figure 5).

## Multiplexing and Rapid Sample Processing

The ForenSeq DNA Signature Prep Kit supports the preparation of up to 384 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 iSNPs. 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 iSNPs
- 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 high throughput and exceptional data accuracy of the MiSeq FGx Forensic Genomics System.



