ForenSeq™ DNA SIGNATURE PREP KIT TESTING DEGRADED DNA AT VARIOUS DEGREES

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Typing of short tandem repeats (STRs) is the basis for human identification in current forensic testing. The standard method uses capillary electrophoresis (CE) to separate amplicons by length and fluorescent labeling. In recent years new methods, including massively parallel sequencing (MPS), have been developed. MPS offers the opportunity to test more genetic markers in a run than possible with standard CE technology. Further, MPS separation depends on sequences rather than lengths, therefore amplicons can be small or, even of the same lengths. These improvements are advantageous when testing challenging forensic samples that could be severely degraded.

This study tested the ForenSeq™ DNA Signature Prep kit (Verogen) on successions of degraded DNA samples, ranging from mild to severe degradation in repeated experimental runs. The degree of degradation was measured by Quantifiler™ Trio DNA quantification kit (Thermo Fisher) and made visible using gel electrophoresis.
The series of degraded DNA samples revealed that dropped-out loci were primarily loci with low read numbers (coverage) as well as long amplicons (e.g. PentaE, DXS8378, and rs1736442). However, severely degraded DNA could still result in 90% of the expanded 20 CODIS loci, while only 35% were obtained using standard CE technology (PowerPlex® Fusion 5C kit from Promega).

This study followed the manufacturer's recommendations for casework (32 samples per run including positive and negative controls, at 1 ng DNA input per sample). Despite passing the manufacturer's quality metrics, positive controls (2800M) lacked some outputs, mostly at SNPs.