

EVALUATION OF THE VERIFILER™ EXPRESS PCR AMPLIFICATION KIT WITH REFERENCE BLOOD AND BUCCAL SAMPLES FOR DATABASE AND PATERNITY TESTING

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Abstract

Short tandem repeat (STR) analysis of buccal and blood samples are often used by forensic database and paternity laboratories to generate DNA profiles. Buccal samples are often used for forensic DNA databases and paternity analysis. This noninvasive collection procedure provides a good source of DNA for samples that incorporate into the direct amplification workflow with minimal effort. With a growing number of single-source reference samples collected for processing, direct amplification is a fast and effective method to increase efficiency in a single-source reference sample workflow. By eliminating the extraction and quantitation steps, the direct amplification protocol saves time and resources.

The new VeriFiler™ Express kit is a 6-dye, direct amplification STR chemistry designed for use with single-source, blood and buccal reference samples collected on treated (Copan NUCLEIC_CARD™ or Whatman FTA® cards) and untreated paper, cotton swabs, Copan FLOQSwabs™, and Bode DNA Buccal Collectors™. The VeriFiler™ Express kit amplifies all Globalfiler™ Express markers except SE33 and DYS391 and includes two sex-discrimination markers, Amelogenin and Y-indel. For customers who demand a streamlined workflow that supports increased throughput and reduced processing time, the new VeriFiler™ Express PCR Amplification Kit—with 25 STR markers including all CODIS core loci and two highly discriminating Penta markers—provides superior genotyping results.

In this presentation, we evaluate first pass success rate and chemistry performance when the VeriFiler™ Express PCR amplification kit is used with various sample collection devices. The 'Kinship and Paternity' module on the Converge™ software was used to analyze the paternity samples genotyped using the VeriFiler™ Express Kit. Summarized data from internal and external studies is presented.

VeriFiler™ Express Kit Configuration

Figure 1. VeriFiler™ Express Kit Loci Comparison

Locus	VeriFiler™ Express	Identifiler™ Kits	Globalfiler™ Kits	NGM™ Select Kits
D3S1358	✓	✓	✓	✓
VWA	✓	✓	✓	✓
D16S539	✓	✓	✓	✓
CSF1PO	✓	✓	✓	✓
TPOX	✓	✓	✓	✓
Y Indel	✓	✓	✓	✓
Amelogenin	✓	✓	✓	✓
D8S1179	✓	✓	✓	✓
D21S11	✓	✓	✓	✓
D18S51	✓	✓	✓	✓
Penta E	✓	✓	✓	✓
D2S441	✓	✓	✓	✓
D19S433	✓	✓	✓	✓
TH01	✓	✓	✓	✓
FGA	✓	✓	✓	✓
D22S1045	✓	✓	✓	✓
DS818	✓	✓	✓	✓
D13S317	✓	✓	✓	✓
D7S820	✓	✓	✓	✓
D6S1043	✓	✓	✓	✓
D10S1248	✓	✓	✓	✓
D181656	✓	✓	✓	✓
D12S391	✓	✓	✓	✓
D2S1338	✓	✓	✓	✓
Penta D	✓	✓	✓	✓
DYS391	✓	✓	✓	✓
SE33	✓	✓	✓	✓

Figure 2. The VeriFiler™ Express Kit amplifies all Globalfiler™ Express markers in addition to the highly discriminating Penta E and Penta D with the exception of D6S1043¹, SE33¹ and DYS391¹.

¹ These markers are not part of the core CODIS loci.

Figure 2. VeriFiler™ Express Kit Configuration

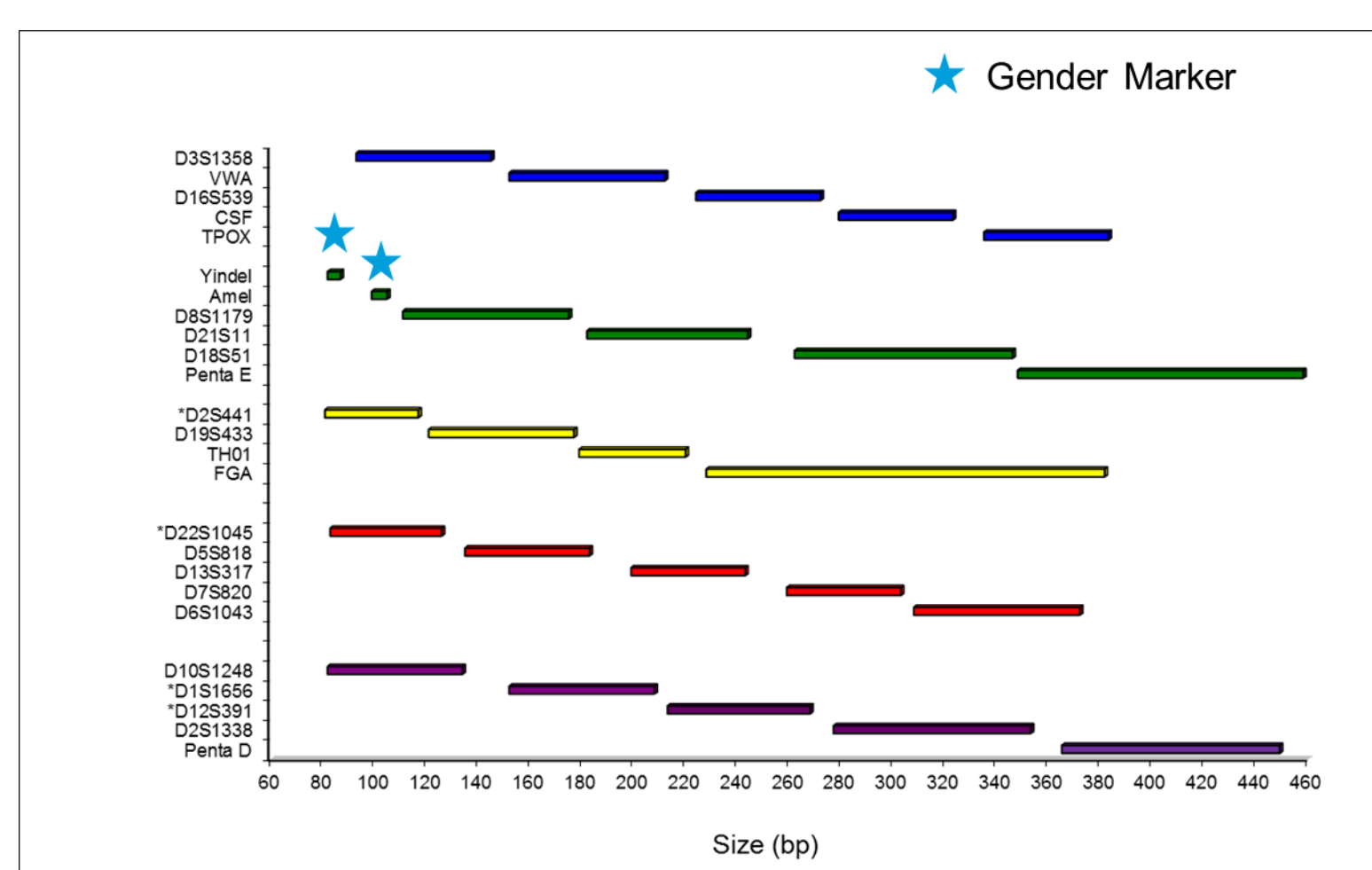


Figure 2. The VeriFiler™ Express Kit utilizes a 6-dye configuration and consists of 23 autosomal STR loci along with 2 gender markers, Amelogenin and a Y-indel (insertion/deletion polymorphic marker). The primer sequences for the common loci in the kit are shared with the Globalfiler™ Express kit.

VeriFiler™ Express Kit Instrument Compatibility and Workflow

Figure 3. VeriFiler™ Express Kit Instrument Compatibility



Figure 3. The VeriFiler™ Express Kit is validated on the 96-Well GeneAmp™ 9700 Thermal Cycler with Silver or Gold-Plated Silver 96-well Block, 3500 Series Genetic Analyzers, and GeneMapper® ID-X Software v1.4. The performance of the chemistry was also verified on the VeriFiler™ 96-Well Thermal Cycler, Proflex™ 1x96-well Block Thermal Cycler, 3130xl Series Genetic Analyzers, and 3730 Genetic Analyzers.

Figure 4. VeriFiler™ Express Kit Workflow

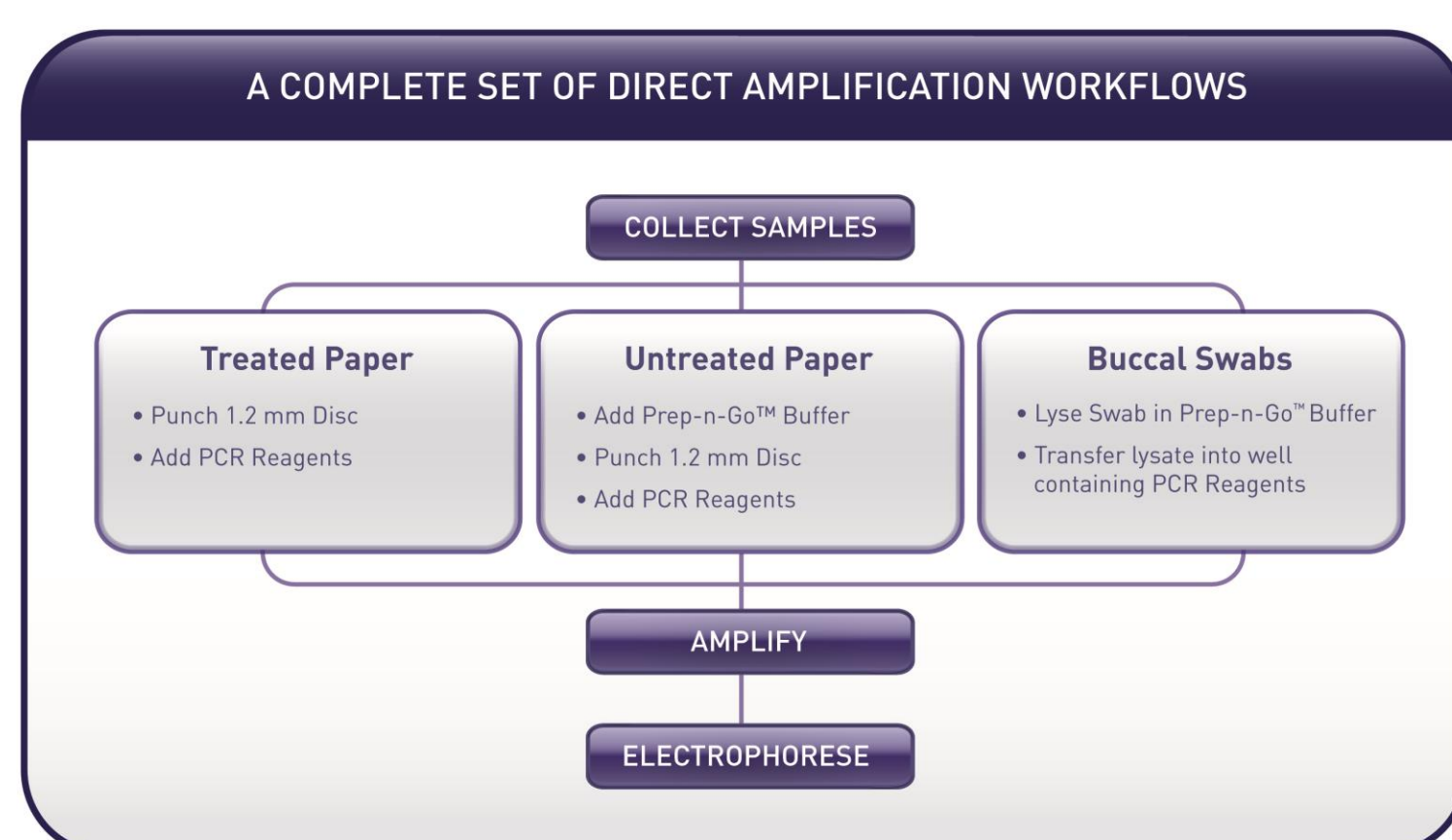


Figure 4. Overview of the workflow for processing blood or buccal samples deposited on treated paper (e.g. FTA® cards), untreated paper (e.g. Filter paper), and swab substrates (e.g. Copan FLOQSwabs™) using the VeriFiler™ Express Kit.

Figure 5. VeriFiler™ Express Kit Supported Sample Types

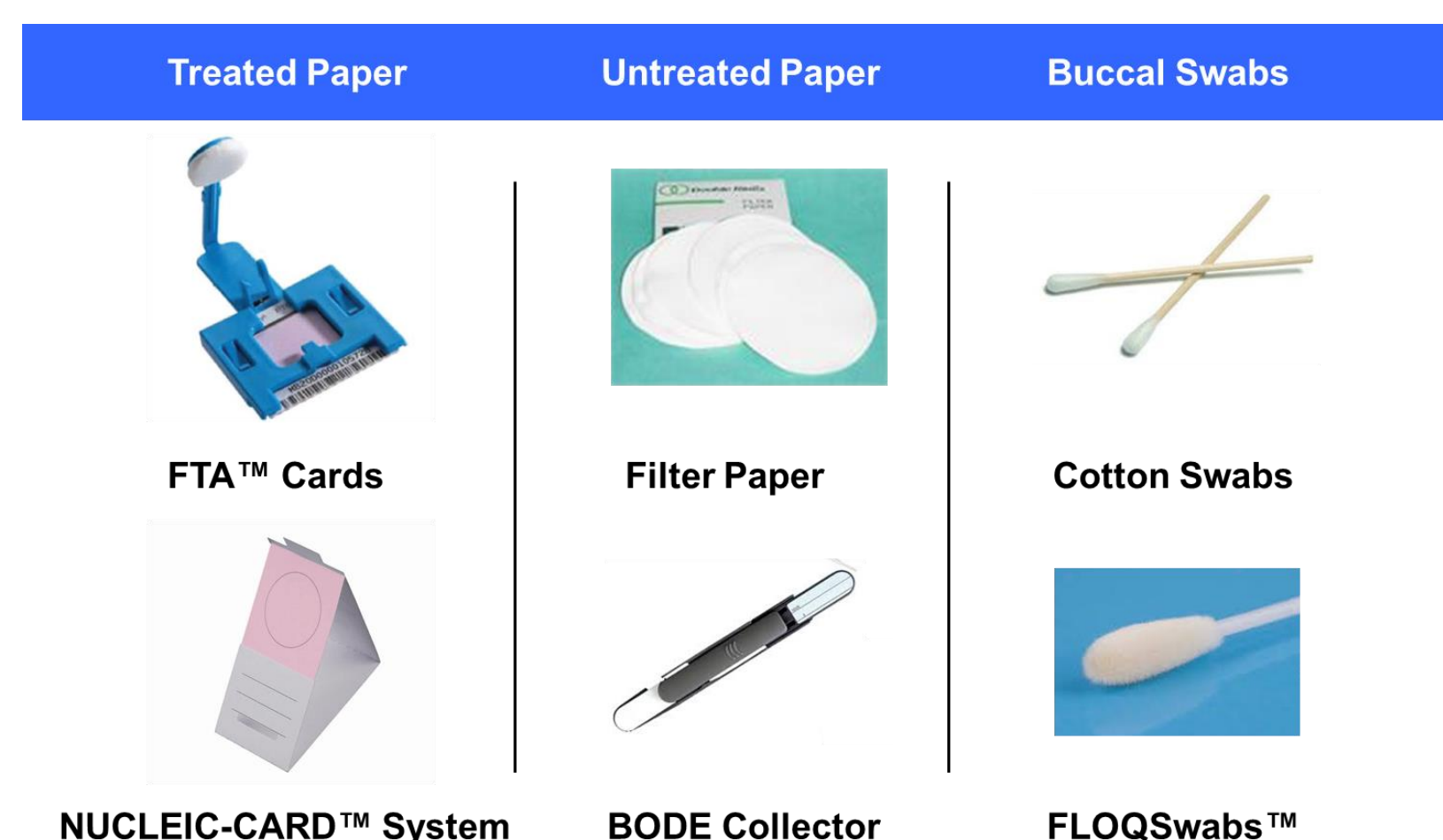


Figure 5. Overview of the sample types validated with the VeriFiler™ Express Kit through internal and external testing.

VeriFiler™ Express Kit Validation Results

Figure 6. VeriFiler™ Express Kit Allelic Ladder

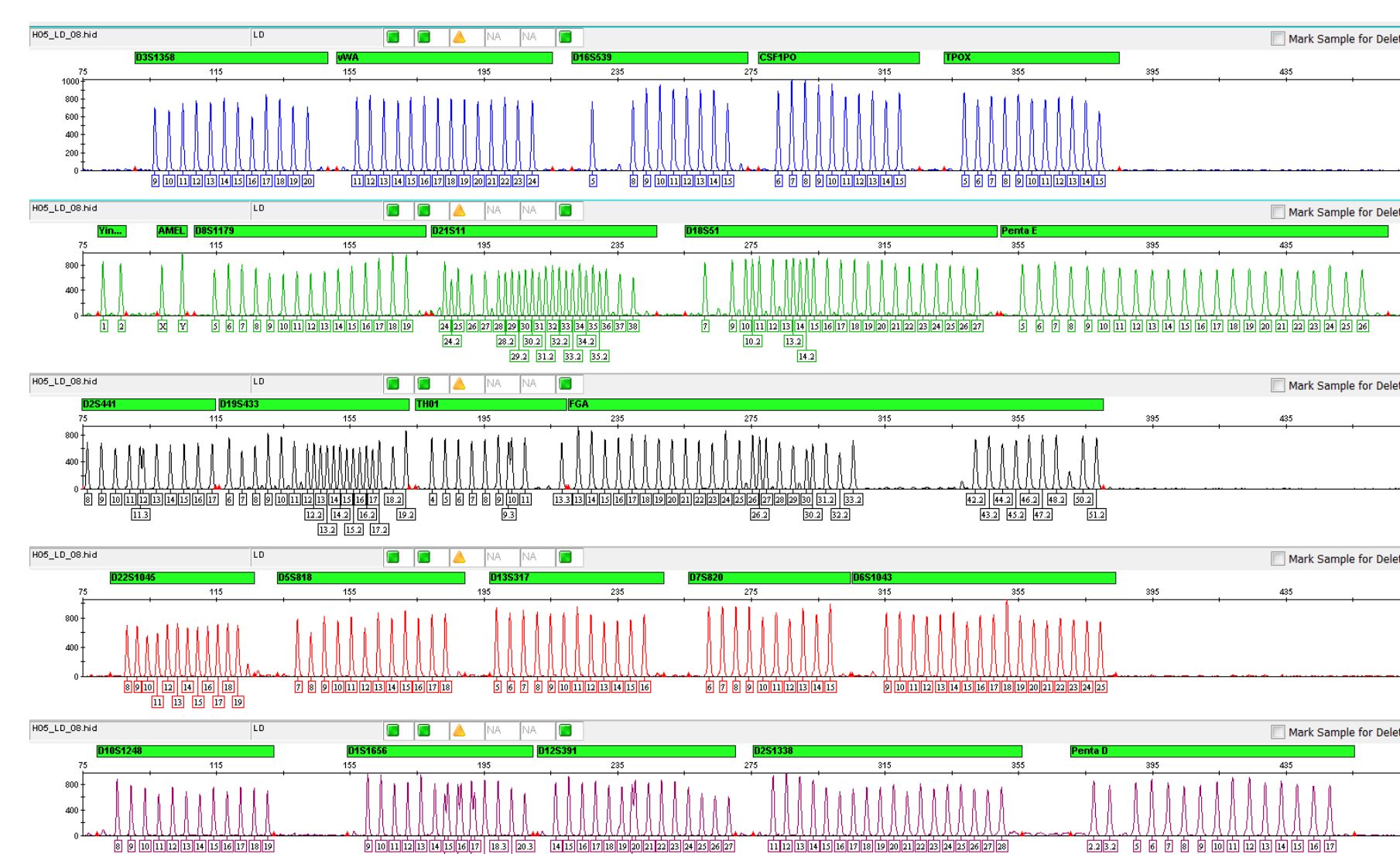


Figure 7. The VeriFiler™ Express Kit allelic ladder consists of a total of 356 alleles. In addition, the VeriFiler™ Express Kit bin file contains 265 virtual bins to allow for accurate genotyping. Y-axis scale is 0 to 1000 RFU.

Figure 7: Direct amplification with VeriFiler™ Express Kit

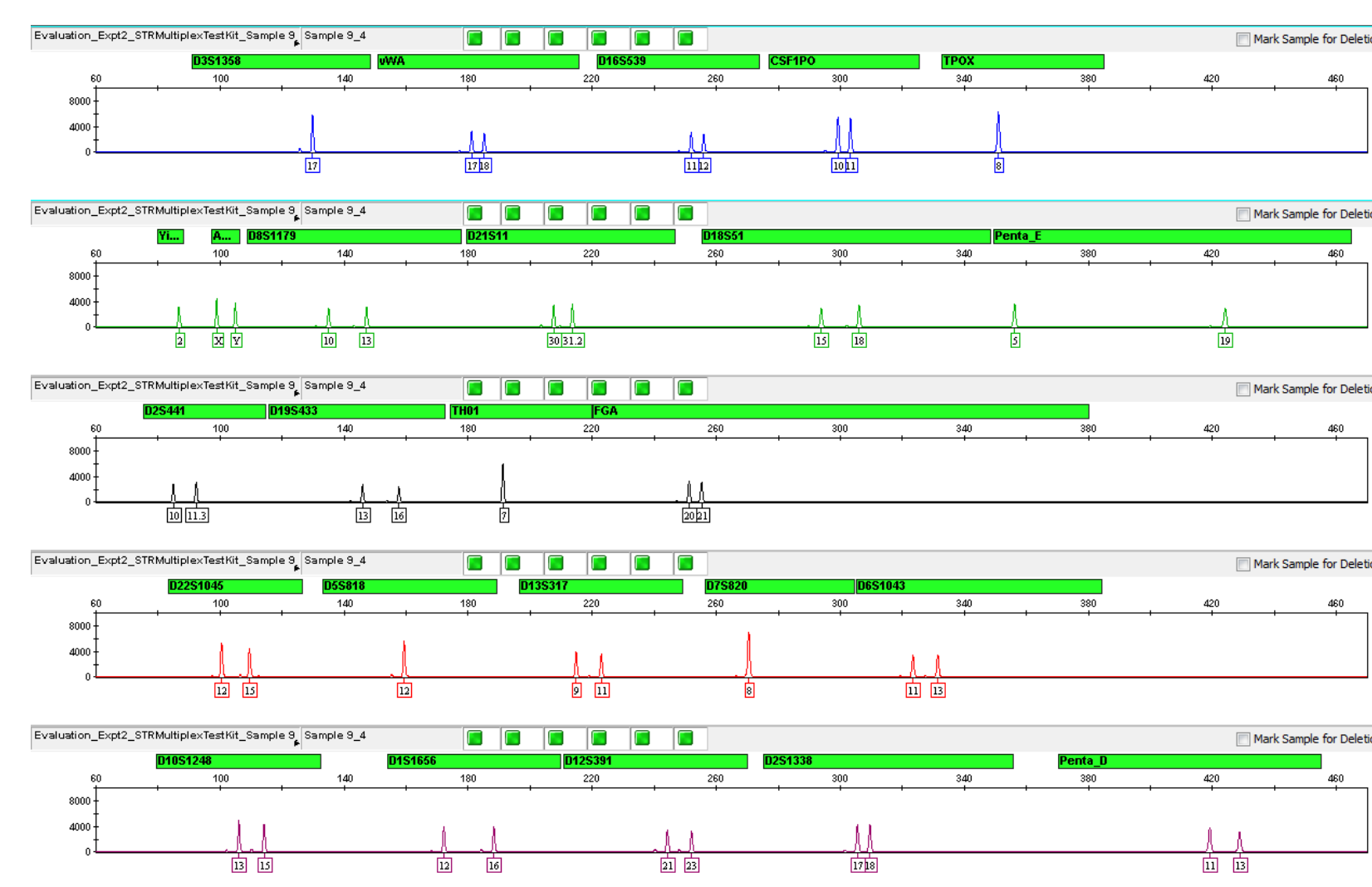


Figure 8: Buccal sample on Copan NUCLEIC-CARD™ paper (1.2 mm punch) was directly amplified at 27 PCR cycles with the VeriFiler™ Express Kit and analyzed on the Applied Biosystems 3500xL Genetic Analyzer. Y-axis scale is 0 to 10,000 RFU.

Figure 8: VeriFiler™ Express Kit First Pass Success Rate

Sample Type	Sample Size	Cycle Number	First Pass Success Rate	Prep-n-Go (Y/N)
Blood on Filter Paper	>100	24-27	98%	Y
Blood on FTA	>100	24-27	100%	N
Buccal on FTA	70	27	94%	N
Buccal on Copan NUCLEIC-CARD	>100	26-27	100%	N
Bode Buccal Collector	98	26	100%	Y
Copan FLOQSwab	>100	26-27	100%	Y
Puritan Cotton Swab	46	28	94%	Y

Figure 9. Internal and external testing of the VeriFiler™ Express Kit showed high first-pass success rate with minimal number of off-scale allele calls for all supported sample types.

Figure 9: Kinship Analysis using the VeriFiler™ Express Kit and the Converge™ Software

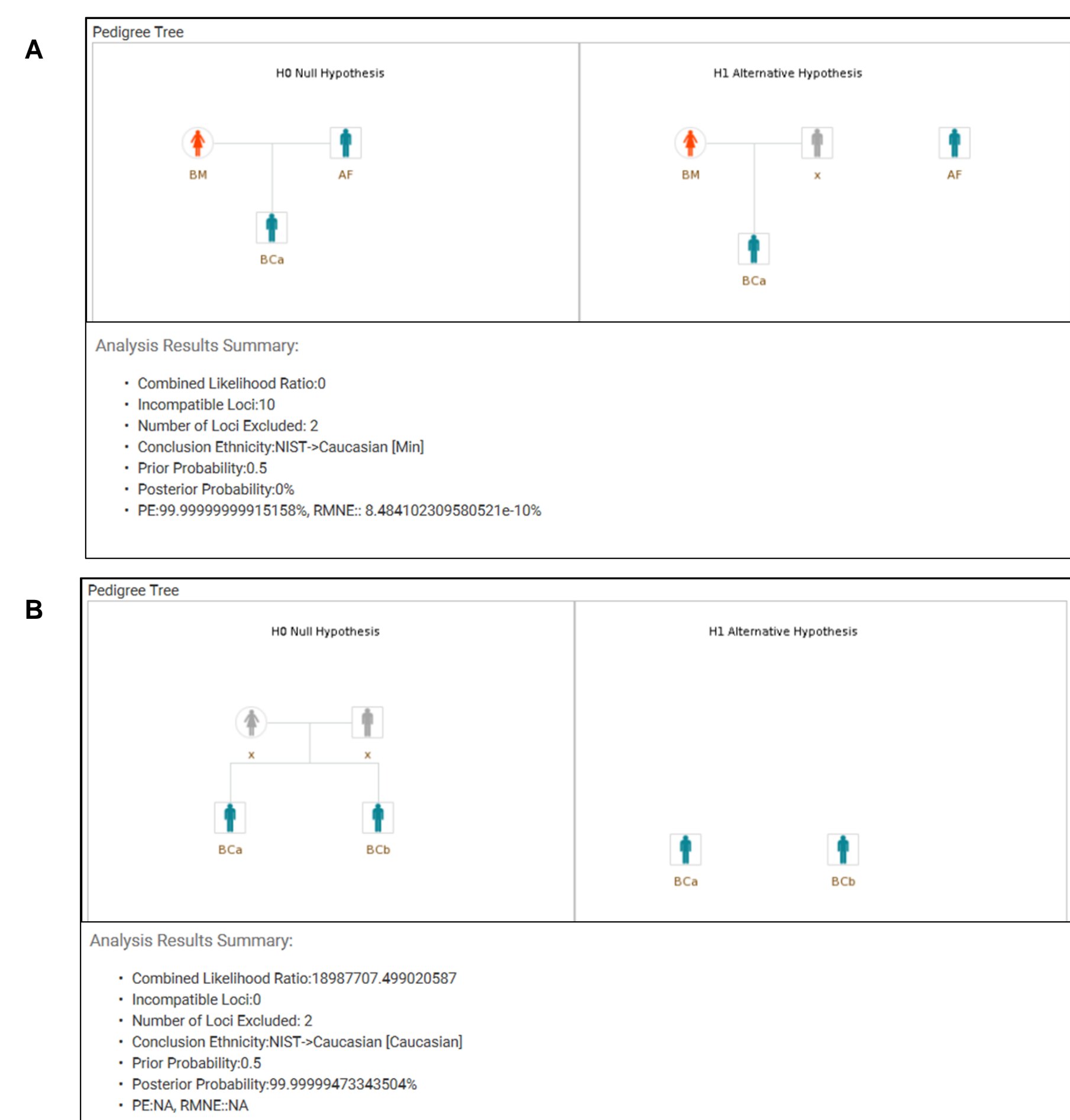


Figure 9. The STR profiles generated for known family trio's with VeriFiler™ Express Kit was analyzed using the Converge™ software 'Kinship Analysis' platform. A) A standard trio exclusion case with a known unrelated male tested as the alleged father showed low posterior probability, confirming the unlikelihood of the male being biologically related to the child. B) Kinship analysis of known siblings showed high posterior probability, confirming the likelihood of the two individuals being related.

Conclusions

- The VeriFiler™ Express Kit enables direct amplification of multiple sample types with minimal processing.
- The VeriFiler™ Express Kit has been optimized for a fast PCR time of ~45 minutes at 25 cycles with a full reaction volume of 25 µL.
- The VeriFiler™ Express Kit includes an expanded allelic ladder for accurate genotyping.
- The data demonstrates robust performance with high first pass success rate for all sample and substrate types tested.
- Integration of the VeriFiler™ Express Kit with the Converge™ 'Kinship Analysis' platform, with population frequency tables for 4 ethnicities (African-American, Asian, Caucasian, and Hispanic) seeded in the software, delivers a simple workflow for paternity testing.

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