

AmpF ℓ STR[®] Identifiler[®] PCR Amplification Kit

- **Fifteen STR (short tandem repeat) loci and Amelogenin co-amplified in a single tube**
- **Incorporation of the same proven, reliable primer sequences previously used in all AmpF ℓ STR Kits¹**
- **Loci consistent with all major worldwide STR databasing standards**
- **Five-dye fluorescent DNA technology enables high-throughput analysis and maintains desirable small amplicons**
- **Complete system including reagents, ABI PRISM[®] genetic analysis instrumentation, software, technical support, and specific limited license rights for forensic, paternity, and research use**
- **Significant reduction in artifacts now observed in both PET[®] and VIC[®] dye results**

High-Throughput Databasing Multiplex

Drawing from the technology supporting the reliable, trusted AmpF ℓ STR PCR Amplification Kits, Applied Biosystems delivers its single most discriminating fluorescent STR-based assay for human identification

applications. The AmpF ℓ STR Identifiler PCR Amplification Kit simultaneously amplifies 15 STR loci plus the Amelogenin gender-determining marker in a single, robust PCR. The widely accepted tetranucleotide loci co-amplified in the Identifiler Kit include the thirteen core STR loci as required for sample entry into CODIS (Combined DNA Index System): CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, and vWA. The data generated from these loci also satisfy the recommendations of the European Network of Forensic Science Institutes (ENFSI) and Interpol organizations.

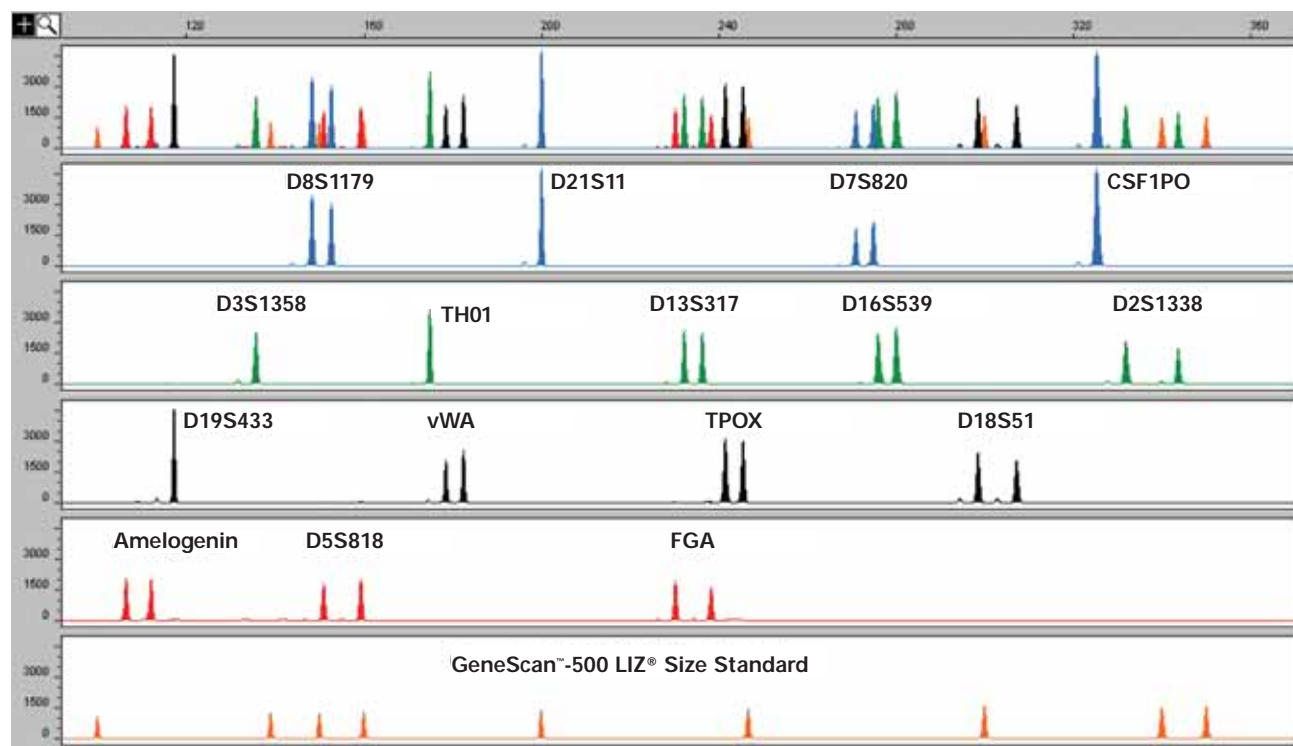


Figure 1. GeneScan software electropherogram showing the AmpF ℓ STR Identifiler PCR Amplification Kit results for fifteen STR loci and the Amelogenin locus analyzed on the ABI PRISM 3100 Genetic Analyzer. DNA fragments are labeled in 6-FAM[™] dye (blue), VIC dye (green), NED[™] dye (yellow, depicted in black), and PET dye (red). The GeneScan-500 size standard is labeled with LIZ dye (orange).

Two additional tetranucleotide loci, D2S1338 and D19S433, provide concordance with the AmpF ℓ STR[®] SGM Plus[®] PCR Amplification Kit, which was developed in collaboration with the Forensic Science Service (FSS). The combination of loci included in the Identifier Kit renders it the most powerful AmpF ℓ STR Kit currently available.

Speed and Throughput

Previously, submission of samples to CODIS required genetic information generated from two separate PCR amplifications, such as the AmpF ℓ STR[®] Profiler Plus[®] and AmpF ℓ STR[®] COfiler[®] PCR Amplification Kits. To simplify this process and increase databasing throughput, the Identifier Kit features a robust, 16-locus multiplex PCR, which cuts the preparation and labor of amplification and analysis in half. Now with the Identifier Kit, a single multiplex PCR, followed by one capillary electrophoresis injection or gel lane analysis, is all that is necessary to provide complete databasing information for an individual sample.

While enhancing throughput with a single amplification is a key feature of the Identifier Kit, other factors are important to the human identification laboratory, including robustness and reliability of the PCR amplification. The AmpF ℓ STR Identifier Kit was designed as a single amplification with these important design goals in mind:

- Identical primer sequences among all AmpF ℓ STR Kits¹
- Amplicon allele size ranges between 100 to 360 base pairs for robust PCR amplification of all loci
- A complete system approach designed for flexibility—choose the ABI PRISM system appropriate for your laboratory's analysis needs

Locus Designation	Chromosome Location	Alleles included in Identifier [®] Allelic Ladder	Dye Label
D8S1179	8	8-19	6-FAM [™]
D21S11	21q11.2-q21	24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38	
D7S820	7q11.21-22	6-15	
CSF1P0	5q33.3-34	6-15	
D3S1358	3p	12-19	VIC [®]
TH01	11p15.5	4-9, 9.3, 10, 11, 13.3	
D13S317	13q22-31	8-15	
D16S539	16q24-qter	5, 8-15	
D2S1338	2q35-37.1	15-28	
D19S433	19q12-13.1	9-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED [™]
vWA	12p12-pter	11-24	
TPOX	2p23-2per	6-13	
D18S51	18q21.3	7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27	
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	PET [®]
D5S818	5q21-31	7-16	
FGA	4q28	17-26, 26.2, 27-30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	

Table 1. The AmpF ℓ STR Identifier Kit Loci. All STR loci included in the AmpF ℓ STR Identifier PCR Amplification Kit are co-amplified in a single PCR and analyzed simultaneously in a single gel-lane or capillary electrophoresis injection with ABI PRISM systems.

Population	Average Probability of Identity	Power of Discrimination	Average Probability of Paternity Exclusion
African American n = 357	1.31 x 10 ⁻¹⁸	1 in 7.64 x 10 ¹⁷	0.9999996
US Caucasian n = 349	5.01 x 10 ⁻¹⁸	1 in 2.00 x 10 ¹⁷	0.9999992
US Hispanic n = 290	7.65 x 10 ⁻¹⁸	1 in 1.31 x 10 ¹⁷	0.9999990
Native American n = 191	3.62 x 10 ⁻¹⁷	1 in 2.76 x 10 ¹⁶	0.9999527

Table 2. Population Genetics of the AmpF ℓ STR Identifier Kit Loci.

These features make the Identifier Kit suitable for database, forensic, and paternity laboratories. Each laboratory using the Identifier Kit should perform appropriate validation studies.

Five-Dye Technology

Although the use of four fluorescent dyes (5-FAM, JOE™, NED, and ROX™ dyes) to label DNA fragments for automated STR analysis provides a significant increase in throughput over more traditional methods (such as those based on silver staining, radioactivity, or chemiluminescence), an increasing demand for genotypic information requires even higher-throughput solutions. Applied Biosystems introduced a five-dye system for automated DNA fragment analysis, enabled by the development of two proprietary fluorescent dyes. By adding additional dyes to the AmpF/STR® Identifier® Primer Set, more loci are multiplexed in a single gel lane or capillary injection. The new PET and LIZ dyes (depicted as red and orange, respectively, by GeneScan Software version 3.1 or higher) are specifically designed to expand the spectral detection range on ABI PRISM genetic analysis instrumentation. Together with 6-FAM, VIC, and NED dyes, the spectral emission for this five-dye set extends to 660 nm. By comparison, the four-dye set consisting of 5-FAM, JOE, NED, and ROX dyes (dye set “F”) yields a spectral range extending to 610 nm.

The expanded spectral range of the five-dye set allows color separation comparable to the four-dye set, minimizing spectral overlap. In this five-dye set, 6-FAM, VIC, NED, and PET dyes are used to label PCR fragments, while LIZ dye is used to label the GeneScan-500 size standard.

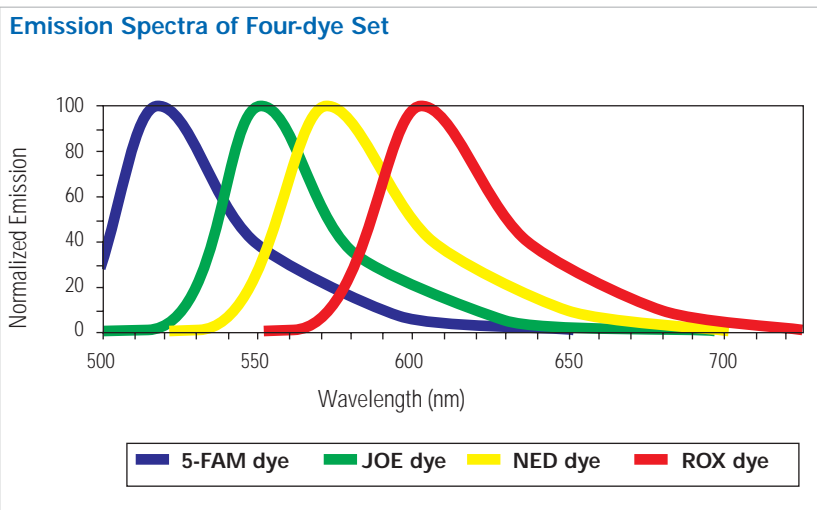


Figure 2a. Shown above are the emission spectra of 5-FAM, JOE, NED, and ROX dyes used with the AmpF/STR Profiler Plus, COfiler, and SGM Plus kits.

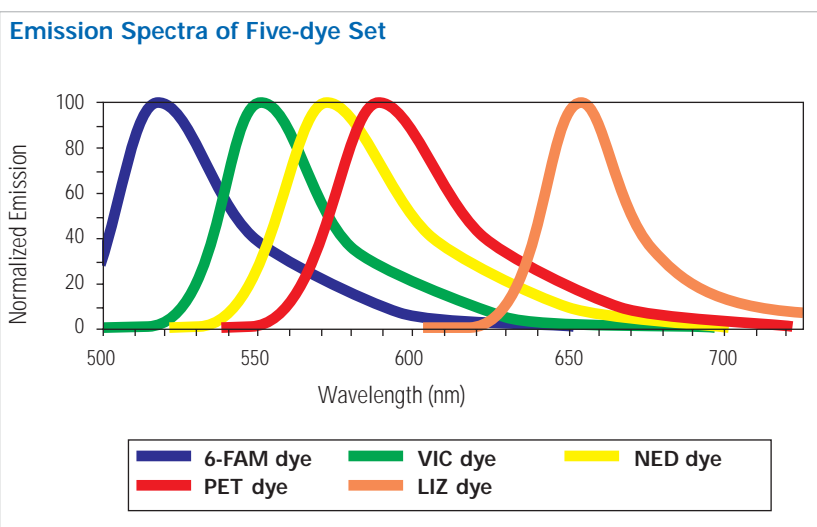


Figure 2b. Shown above are the emission spectra of 6-FAM, VIC, NED, PET, and LIZ dyes used with the AmpF/STR Identifier Kit. This dye set was specifically designed to expand the spectral detection range on ABI PRISM genetic analysis instrumentation.

ABI PRISM System	Samples per Run	Number of Runs per Day	Days in Operation	Samples per Year
310	48	1	335	16,080
377 (34-lanes)	34	3	240	24,480
377 (96-lanes)	96	3	240	69,120
3100	16	24	240	92,160

Table 3. Examples of sample throughput for ABI PRISM systems based upon a 9-hour workday.

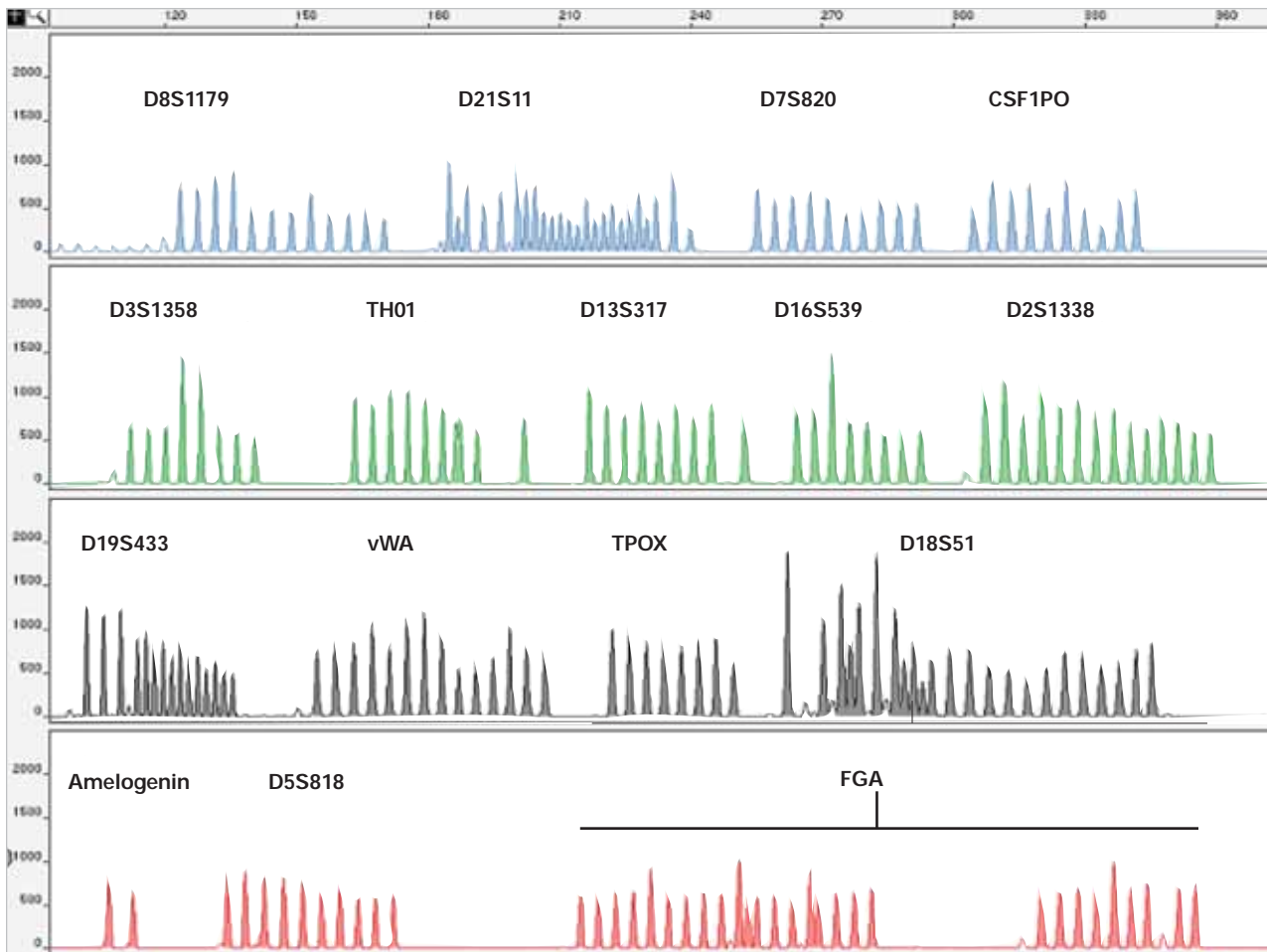


Figure 3. GeneScan software plot of the AmpF ℓ STR Identifier Allelic Ladder analyzed on the 310 genetic analyzer. The new PET dye (red) used to label PCR products increases in-lane multiplexing throughput capabilities when used along with 6-FAM, VIC, and NED dyes. The AmpF ℓ STR Identifier Allelic Ladder includes additional alleles for D18S51, D21S11, FGA, TH01, and vWA loci as compared to the AmpF ℓ STR Profiler Plus and Cofiler Allelic Ladders.

Trusted AmpF ℓ STR Kit Primer Sequences Remain Unchanged

By combining the five-dye system with carefully selected, non-nucleotide linkers placed between the primer and the fluorescent dye during oligonucleotide synthesis of the Identifier Kit primers, the same robust primer sequences developed for previous AmpF ℓ STR Kits are used without modification. The non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing.

Maintaining primer sequences means that current users of the AmpF ℓ STR Profiler Plus, COfiler, and SGM Plus

kits may quickly adopt the Identifier Kit with complete confidence.¹ In addition, small amplicons less than 360 base pairs are maintained, ensuring robust PCR amplification.

Reduction of Artifacts

In Applied Biosystems continual efforts to improve the quality of our products, we have made some modifications to the manufacturing process of the Identifier Kit. For samples amplified with the Identifier Kit, these modifications reduce the artifacts that are observed in both PET dye and VIC dye results.

A VIC dye labeled artifact was observed at approximately 120 bp. In order to address this artifact, an additional step has been introduced in the purification process of the VIC dye labeled PCR primers in the Identifier Kit. PET dye labeled artifacts have also been observed between the Amelogenin and the D5S818 loci. A modification to the manufacturing process of the PET dye labeled primers has been introduced, while maintaining the primer sequences, resulting in a reduction in the artifacts visualized. As noted in the Identifier Kit User's Manual (PN 4323291), non-nucleotide linkers are

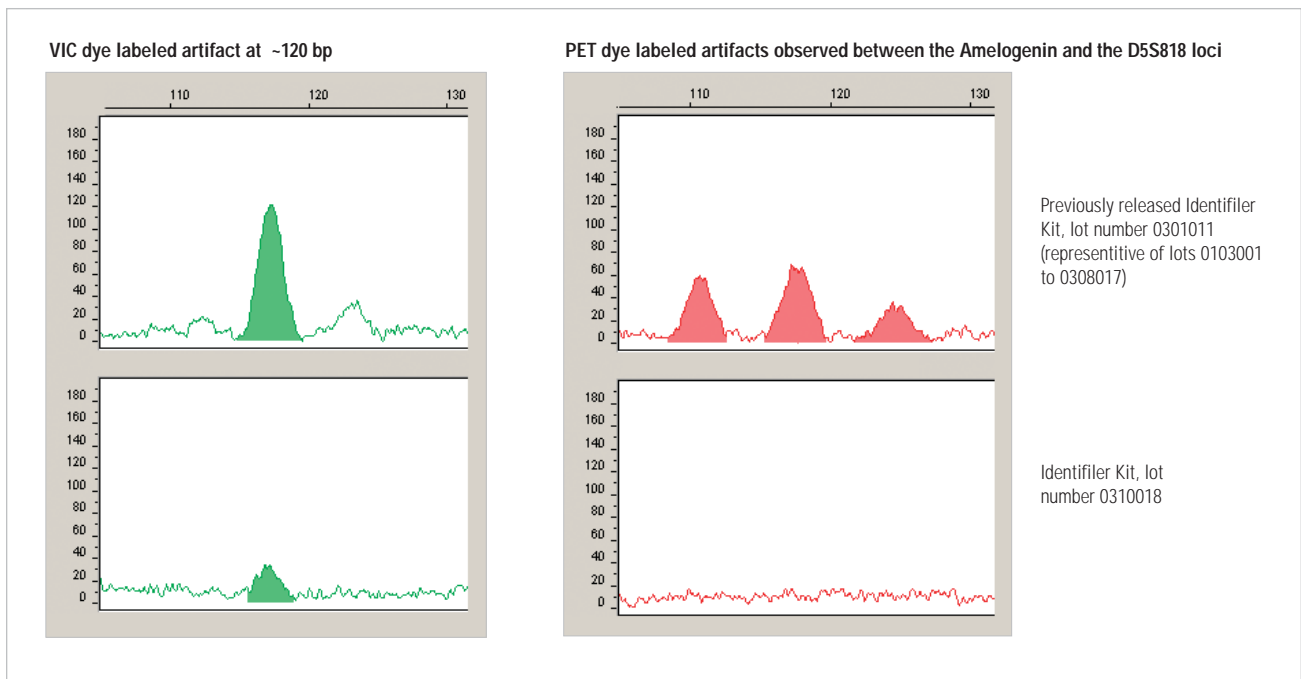


Figure 4. Comparison of the observed VIC dye labeled and PET dye labeled artifacts for negative control amplifications with a previously released Identifiler Kit, lot number 0301011, and lot number 0310018. DNA was generated using the ABI PRISM® 310 Genetic Analyzer with the Windows NT® OS using the G5 module. The artifacts have been highlighted for illustrative purposes. Both the VIC dye labeled and PET dye labeled artifacts are greatly reduced in the bottom panels.

used in primer synthesis between the primer oligonucleotides and the dye. The non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing. The PET dye artifacts were observed to be correlated with the use of a particular non-nucleotide linker.

By using a different non-nucleotide linker during synthesis of the PET dye labeled primers in the Identifiler Kit primer set for this kit, the PET dye artifacts are now observed to be significantly diminished. The modifications made in the production of the PET and VIC dye labeled primers are reflected in both the Identifiler primer sets and the Identifiler Allelic Ladder.

Complete System Approach

Applied Biosystems offers fully integrated reagents, instruments, and software for processing data generated in human identification laboratories. DNA samples amplified with the Identifiler Kit may be analyzed with the ABI PRISM genetic analysis

instrument that accommodates an individual laboratory's throughput needs. (Each ABI PRISM genetic analysis instrument must be equipped with a version of data collection software capable of five-dye analysis, and the instrument calibrated with the appropriate matrix standards.) For post-collection software packages, choose from Applied Biosystems analysis software products. Sample tracking and data management requirements for databasing laboratories can be met with Applied Biosystems SQL*GT™ Laboratory Information Management System (LIMS).

Experienced technical application specialists are available to answer questions you may have, including field specialists who provide on-site assistance in the use of our entire product line. We also offer forensic training courses to implement STR technology in your laboratory to help you develop complete competence and confidence in its use.

Product Specifications

Each AmpFℓSTR Identifiler Kit contains pre-formulated AmpFℓSTR PCR Reaction Mix, blended AmpFℓSTR Identifiler Primer Set, and AmpliTaq Gold® DNA Polymerase sufficient for 200 tests. Each kit also contains AmpFℓSTR® Control DNA 9947A of a known genotype and AmpFℓSTR Identifiler Allelic Ladder. The AmpFℓSTR Identifiler Allelic Ladder includes additional alleles for D18S51, D21S11, FGA, TH01, and vWA loci, as compared with the AmpFℓSTR Profiler Plus and COfiler Allelic Ladders.

The PCR reaction components, primer sequences, and amplification protocols have all been developed to provide specific, robust amplification. Validation experiments have been performed according to "Quality Assurance Standards for Forensic DNA Testing Laboratories" (DNA Advisory Board).

The Identifiler Kits are subjected to rigorous quality control testing to ensure reliable performance. Applied Biosystems develops and manufactures its products in accordance with ISO 9001 quality system requirements. Additionally, a Certificate of Analysis is available upon request that confirms that the specific combination of components within a lot meets quality-assurance testing specifications.

The AmpF ℓ STR Identifiler Kit conveys certain PCR rights to perform paternity testing services. When used in conjunction with an Authorized Thermal Cycler, the Identifiler Kit also conveys certain PCR license rights for forensic and research use.

Ordering Information

Description	Quantity	P/N
AmpF ℓ STR [®] Identifiler [®] PCR Amplification Kit	200 tests, 25 μ L/test	4322288
AmpF ℓ STR [®] Identifiler [®] PCR Amplification Kit User's Manual	1 manual	4323291
GeneScan [™] -500 LIZ [®] Size Standard	800 reactions	4322682
Matrix Standard Set DS-33 (6-FAM [™] , VIC [®] , NED [™] , PET [®] , LIZ [®] dyes) for use with 310 and 377 systems	1 tube with 5 dyes	4318159
Matrix Standard Set DS-33 (6-FAM [™] , VIC [®] , NED [™] , PET [®] , LIZ [®] dyes) for use with 3100 and 3100-Avant systems	1 tube with 5 dyes	4323016

References

¹ In order to address a mutation observed in a population of Chamorros and Filipinos from Guam, a degenerate unlabeled primer for the D8S1179 locus has been added to the AmpF ℓ STR Identifiler Primer Set. The addition of the degenerate primer allows the amplification of those alleles in samples containing this mutation without altering the overall performance of the AmpF ℓ STR Identifiler PCR Amplification Kit.



iScience. To achieve accurate, reproducible results, life scientists are taking advantage of advanced analysis systems that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible.

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Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

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