

VIC® dye-labeled artifact at ~120 bp

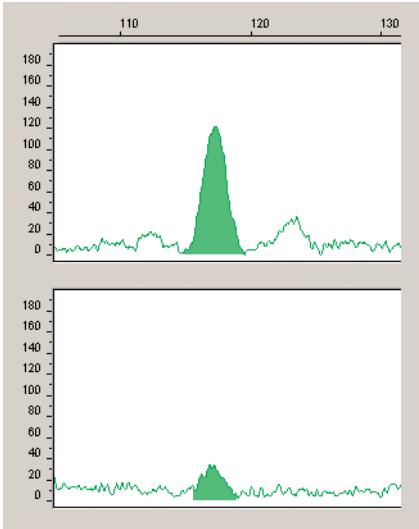
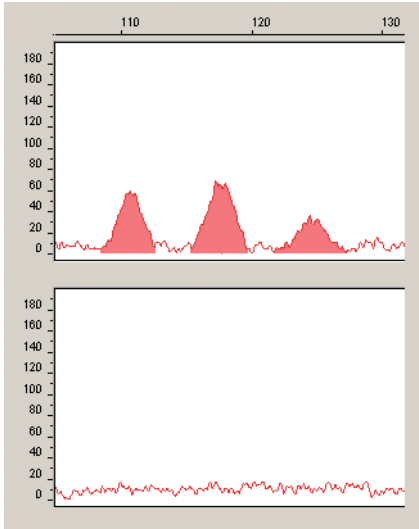


Figure 4. Comparison of the observed VIC® dye-labeled and PET® dye-labeled artifacts for negative control amplifications with a previously released Identifiler® Kit, lot number 0301011, and lot number 0310018. DNA was generated using the ABI Prism® 310 Genetic Analyzer with the Windows NT® OS using the 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 pane.

PET® dye-labeled artifacts observed between the Amelogenin and the D5S818 loci



Previously released Identifiler® Kit, lot number 0301011 (representative of lots 0103001 to 0308017)

Identifiler® Kit, lot number 0310018

The PCR reaction components, primer sequences, and amplification protocols have all been developed, optimized and validated together with Applied Biosystems instrumentation and software to provide specific, robust amplification. Validation experiments have been performed according to “Quality Assurance Standards for Forensic DNA Testing Laboratories” (DNA Advisory Board). For information on Applied Biosystems developmental validation of the Identifiler® kit please reference the article “Developmental Validation of a Single-Tube Amplification of the 13 CODIS Loci, D2S1338,

D19S433, and Amelogenin: The AmpF/STR® Identifiler® PCR Amplification Kit,” Journal of Forensic Sciences, November 2004.

The Identifiler® Kits are subjected to rigorous quality control testing to ensure consistent 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 each lot meets quality assurance testing specifications.

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/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/STR® Identifiler® PCR Amplification Kit.

ORDERING INFORMATION

Description	Quantity	P/N
AmpF/STR® Identifiler® PCR Amplification Kit	200 tests, 25 µL/test	4322288
AmpF/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 system	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
AmpF/STR® MiniFiler™ PCR Amplification Kit	100 tests, 25 µL/test	4373872

AmpF/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/STR® Kits
- Loci consistent with major worldwide STR databasing standards
- Five-dye fluorescent DNA technology enables high-throughput analysis and maintains desirable small amplicons
- Validated and optimized integrated systems including AmpF/STR® reagents, genetic analysis instrumentation, genotyping software and technical support.

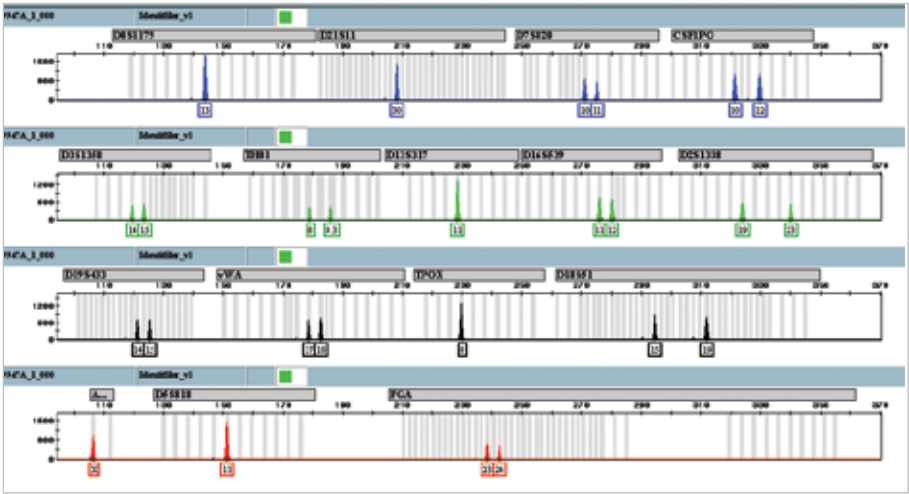


Figure 1. GeneMapper® software electropherogram showing the AmpF/STR® Identifiler® PCR Amplification Kit results for fifteen STR loci and the Amelogenin locus analyzed on the ABI Prism® 3130 Genetic Analyzer. DNA fragments are labeled in 6-FAM™ dye (blue), VIC™ dye (green), NED™ dye (yellow, depicted in black), and PET™ dye (red). The GeneScan™-500 size standard is labeled with LIZ™ dye (orange).

High-Throughput Casework and Database Multiplex

As the most trusted world-wide leader in DNA typing reagents, instrumentation, and software, Applied Biosystems delivers its single most discriminating STR based technology for human identification applications. The AmpF/STR® Identifiler® PCR Amplification Kit simultaneously amplifies 15 STR loci plus the Amelogenin gender-determining marker in a single, robust PCR assay. Well characterized 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. Data generated from these loci also satisfy the recommendations of the European Network of Forensic Science Institutes (ENFSI) and Interpol organizations.

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

Increase Throughput, Obtain Trusted Results

Previously, submission of samples to CODIS required two separate PCR amplifications, such as the AmpF/STR® Profiler Plus® and AmpF/STR® COfiler® PCR Amplification Kits. To simplify this process and increase sample throughput, the Identifiler® Kit enables a robust, 16-locus multiplex PCR assay, which reduces labor required for sample preparation, amplification, and analysis of STR results. After amplifying forensic

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NOTICE TO PURCHASER: LIMITED LICENSE

Use of the AmpF/STR® Identifiler® PCR Amplification Kit is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, and 5,773,258, and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product solely in forensic and paternity testing, including reporting results of purchaser’s activities for a fee or other commercial consideration, and also for the purchaser’s own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The AmpF/STR® Identifiler® PCR Amplification Kit is covered by U.S. Patent No. 5,364,759 owned by Baylor College of Medicine and is sold under license from Baylor College of Medicine. Not for re-sale.

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samples using the Identifiler® Kit, only one capillary electrophoresis injection is required to obtain complete STR results.

The AmpF/STR® Identifiler® Kit provides trusted STR results for human identification applications. It has been validated and optimized to ensure robust and reliable PCR amplification when used with Applied Biosystems genetic analyzers and software. The AmpF/STR® Identifiler® Kit is designed to provide:

- Identical primer sequences utilized in all AmpF/STR® Kits¹
- Amplicon allele size ranges between 100 to 360 base pairs for robust PCR amplification of all loci
- An integrated system that enables flexibility—choose the genetic analysis system combination appropriate for your laboratory’s needs

The Identifiler® Kit is validated for world-wide use in database, forensic, and paternity laboratories. Each laboratory using the Identifiler® Kit should perform appropriate validation studies.

Five-Dye Technology

Throughout the last decade, the use of four fluorescent dyes (5-FAM™, JOE™, NED™, and ROX™ dyes) to label DNA fragments for automated STR analysis has provided a significant increase in throughput over more traditional methods including silver staining, radiolabeling, or chemiluminescence. Today, an increase in the demand for genotypic information requires a more sophisticated and higher throughput solution. Applied Biosystems introduced five-dye technology for automated DNA fragment analysis, enabled by the

TABLE 1. THE AmpF/STR® IDENTIFILER® KIT LOCI			
Locus Designation	Chromosome Location	Alleles included in Identifiler® 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	NED™
D19S433	19q12-13.1	9-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	
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	PET®
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	
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	

* All STR loci included in the AmpF/STR® Identifiler® 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.

TABLE 2. POPULATION GENETICS OF THE AmpF/STR® IDENTIFILER® KIT LOCI			
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

development of two proprietary fluorescent dyes. By adding additional dyes to the AmpF/STR® Identifiler® 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 Applied Biosystems 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 while collecting additional information. 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. The AmpF/STR® Yfiler®, SEfiler®, and MiniFiler™ PCR Amplification kits utilize the same five-dye technology, allowing for compatible workflows.

Trusted AmpF/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 oligo-nucleotide synthesis of the Identifiler® Kit primers, the same robust primer sequences developed for previous AmpF/STR® Kits are used without modification. Maintaining primer sequences ensures that current

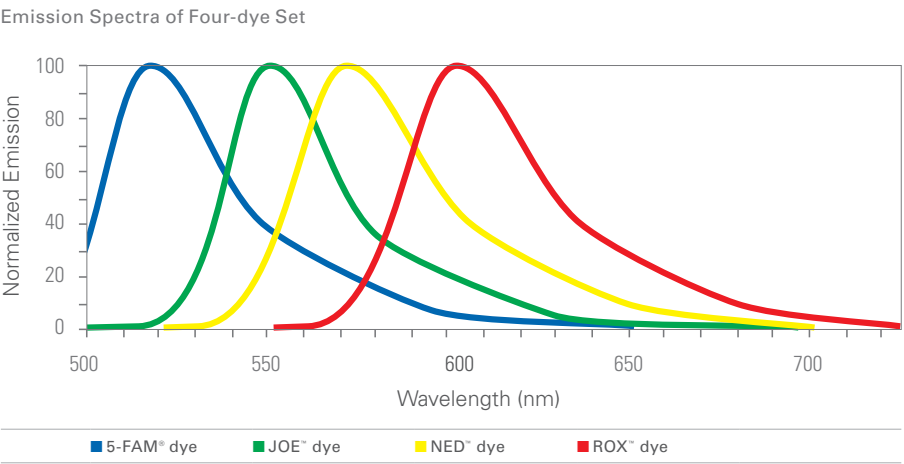


Figure 2a. Shown above are the emission spectra of 5-FAM™, JOE™, NED™, and ROX™ dyes used with the AmpF/STR® Profiler Plus, COfiler®, and SGM Plus® kits.

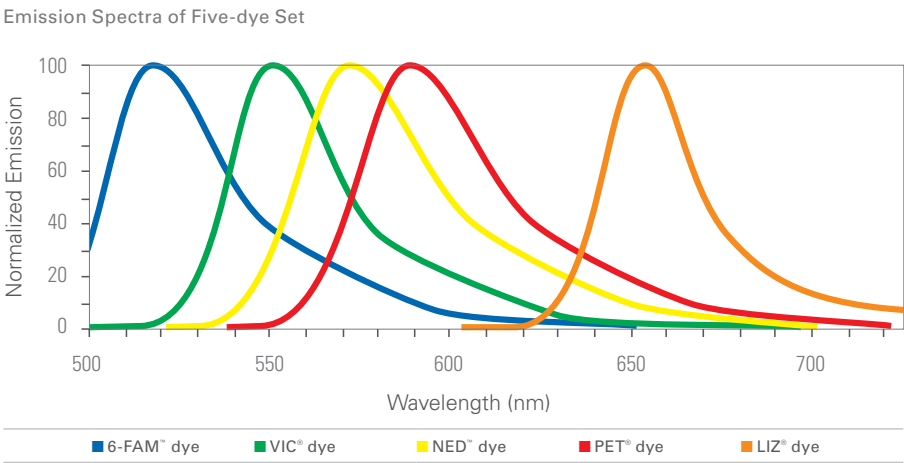


Figure 2b. Shown above are the emission spectra of 6-FAM™, VIC™, NED™, PET™, and LIZ™ dyes used with the AmpF/STR® Identifiler® Kit. This dye set was specifically designed to expand the spectral detection range on ABI Prism genetic analysis instrumentation.

TABLE 3. EXAMPLES OF SAMPLE THROUGHPUT FOR ABI PRISM® SYSTEMS BASED UPON A 9-HOUR WORKDAY.				
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

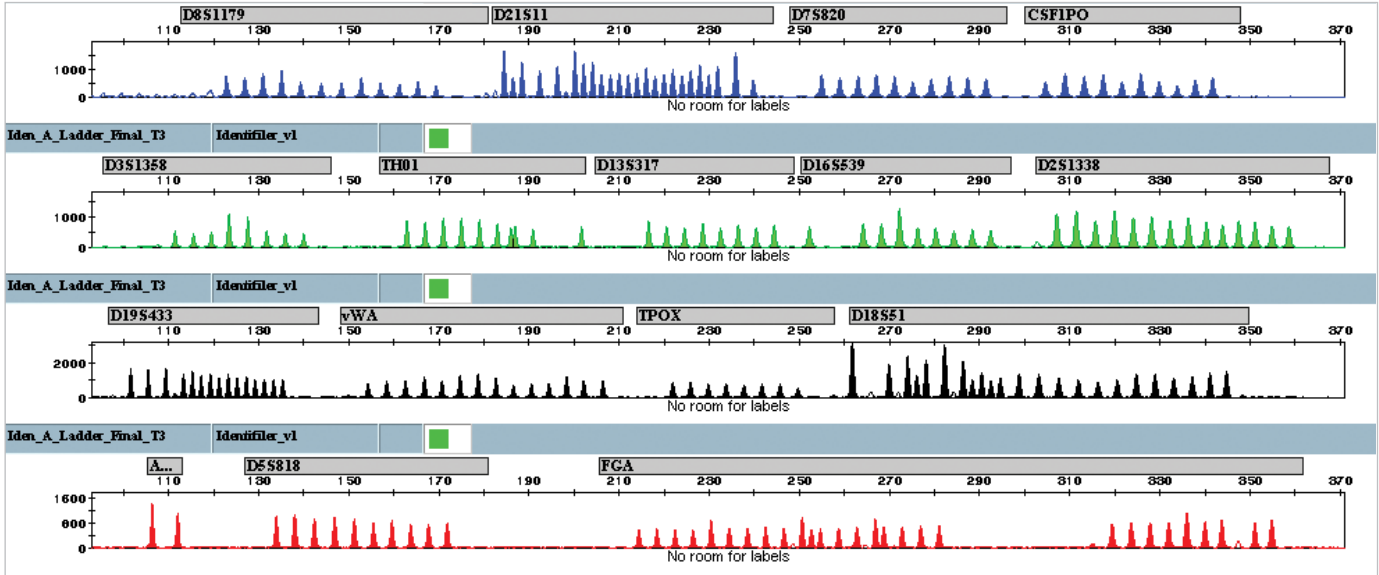


Figure 3. GeneMapper® ID Software plot of the AmpF/STR® Identifiler® Allelic Ladder analyzed on the 3100 Genetic Analyzer. The new PET® dye (red) used to label PCR products increases in-lane multiplexing throughput capabilities when used along with 6-FAM™, VIC®, and NED™ dyes. The AmpF/STR® Identifiler® Allelic Ladder includes additional alleles for D18S51, D21S11, FGA, TH01, and vWA loci as compared to the AmpF/STR® Profiler Plus and Cofiler® Allelic Ladders.

users of the AmpF/STR® Profiler Plus, COfiler®, and SGM Plus® kits can quickly adopt the Identifiler® Kit with complete confidence.¹ In addition, small amplicons less than 360 base pairs are maintained, ensuring robust PCR amplification with low-quantity DNA samples.

Reduction of Artifacts

In Applied Biosystems continual efforts to maximize product quality, we have modified our manufacturing processes to reduce a VIC® primer-related artifact observed at approximately 120 bp, and a PET® primer-related artifacts observed between the Amelogenin and the D5S818 loci. These process modifications are reflected in both the Identifiler® Primer Set and the Identifiler® Allelic Ladder.

Rely on the world’s most trusted systems

Applied Biosystems offers fully integrated reagents, instruments, and software for processing STR data generated in human identification laboratories. DNA samples amplified with the Identifiler® Kit are analyzed with the Applied Biosystems genetic analysis instrument that accommodates an individual laboratory’s throughput needs. Each Applied Biosystems 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.

Experienced Field Application Support Scientists are available to answer questions and to provide on-site assistance in the use of Applied Biosystems AmpF/STR® kits,

instrumentation, and software. In addition, Applied Biosystems also offers forensic training courses designed to assist with the implementation of AmpF/STR® and DNA quantitation technology, software, and instrumentation.

Product Specifications

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