Forensic News

October 2006

What's New

The next generation of STR technology begins with the AmpFℓSTR® MiniFiler™ PCR Amplification kit, available soon from Applied Biosystems.

MiniFiler™, the world's first commercially available 9-plex miniature STR amplification kit, is expected to revolutionize the way forensic scientists process casework samples by significantly increasing the ability to obtain information from DNA evidence, specifically inhibited and/or degraded samples. By combining innovative primer design, improved PCR amplification conditions, and a proprietary mastermix, the MiniFiler™ kit provides increased sensitivity, robust results in the presence of inhibition, and improved discrimination for casework samples. Working in conjunction with other AmpFℓSTR® kits, the MiniFiler™ kit can recover more complete DNA data from challenging samples, enabling more crimes and missing person cases to be solved.

The figures below display data from test sites, including the University of Innsbruck, University of Copenhagen and others, showing compromised DNA samples analyzed with Identifiler® or SGM Plus® and then with the MiniFiler™ Kit. (Missing loci recovered with the MiniFiler Kit are circled.)

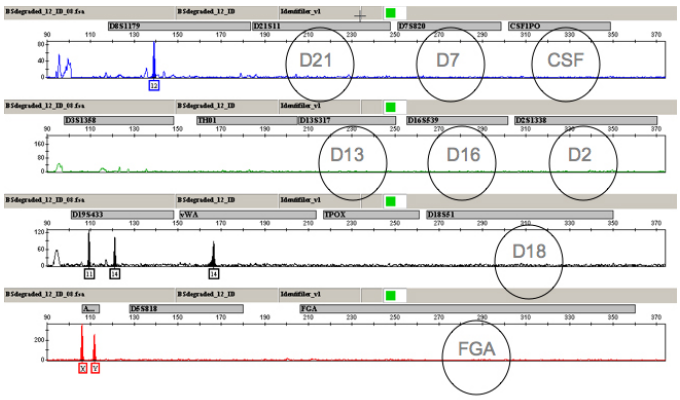

Figure 1. Degraded buccal swab sample amplified with Identifiler® kit-30 cycles.

Figure 2. Degraded buccal swab sample amplified with MiniFiler™ Kit.

Continued next page

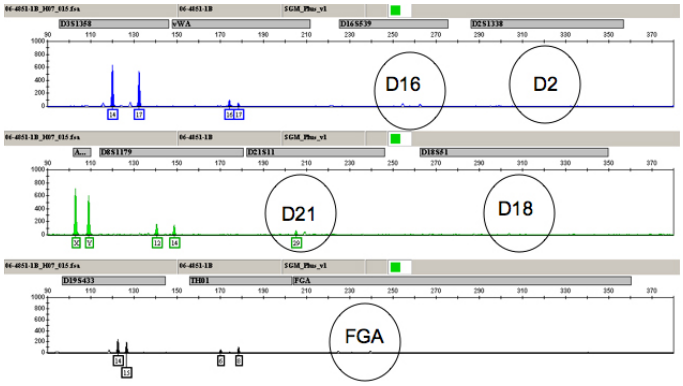

Figure 3. Decomposed muscle sample amplified with SGM Plus® Kit.

Figure 4. Decomposed muscle sample amplified with MiniFiler™ Kit.

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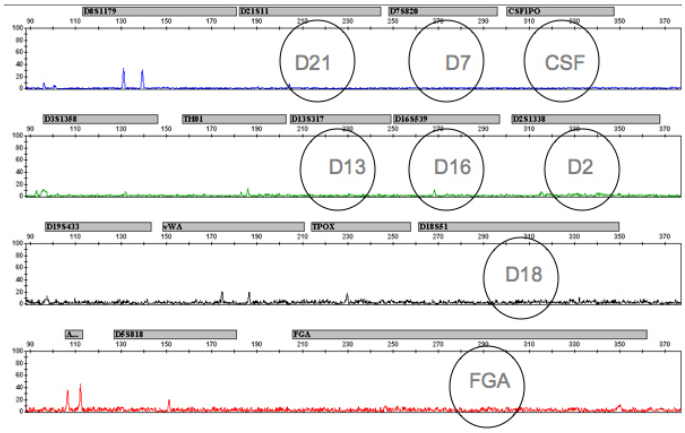

Figure 5. Bone sample amplified with Identifiler® Kit.

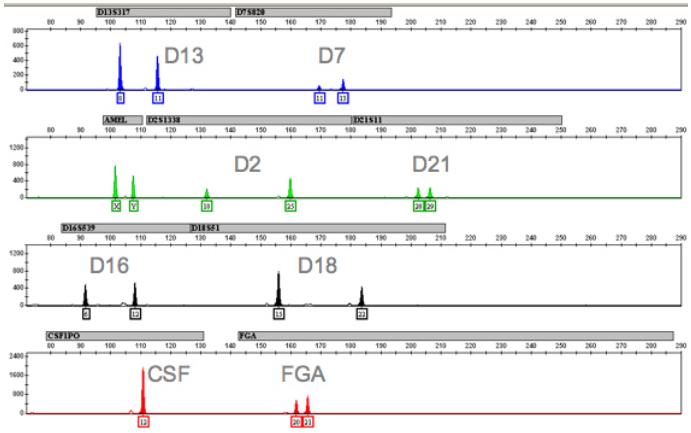

Figure 6. Bone sample amplified with MiniFiler™ Kit.

AmpFℓSTR® MiniFiler™ PCR Amplification Kit – Fact Sheet

- A single amplification providing information on the eight largest and most difficult to amplify loci in the AmpFℓSTR® Identifiler and SGM Plus® PCR Amplification Kits including D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA
- Loci are compatible with CODIS and international databasing standards
- Includes amplification of amelogenin allowing sex determination
- Utilizes proven 5-dye technology allowing compatibility of workflows with Identifiler®, SEfiler™ and Yfiler™ kits
- Optimized for use with the most difficult types of samples including those that are degraded and contain inhibitors
- Ability to overcome the inhibitory effects of substances such as heme and humic acid which are commonly encountered in forensic samples
- Can be used as an adjunct to other commercially available amplification kits to recover the larger loci which cannot be amplified or that drop out
- Recovery of full profiles with 125 pg input DNA
- Highly discriminating results with Probability of Identity values of less than 1×10^{-10} for US populations
- Developmental validation studies performed according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines

Please see the AmpFℓSTR® Kit Product Portfolio on the following page, showing loci amplified by kit, dye label and probabilities of identity.

Continued next page

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AmpFℓSTR® Kit Product Portfolio

Autosomal Loci Guide:

AmpFℓSTR kit loci reference table showing loci amplified by kit, dye label, and probabilities of identity.

Locus	Chromosome Location	Identifiler® kit	SEfiler™ kit	SGM Plus® kit	Profiler Plus® kit	COfiler® kit	Profiler® kit	MiniFiler™ kit
CSF1PO	5q33.3-34	6-FAM™				JOE™	JOE™	PET®
D2S1338	2q35-37.1	VIC®	6-FAM™	5-FAM™				VIC®
D3S1358	3p	VIC®	6-FAM™	5-FAM™	5-FAM™	5-FAM™	5-FAM™	
D5S818	5q21-31	PET®			NED™		NED™	
D7S820	7q11.21-22	6-FAM™			NED™	NED™	NED™	6-FAM™
D8S1179	8q24.13	6-FAM™	VIC®	JOE™	JOE™			
D13S317	13q22-31	VIC®			NED™		NED™	6-FAM™
D16S539	16q24-qter	VIC®	6-FAM™	5-FAM™		5-FAM™		NED™
D18S51	18q21.3	NED™	PET®	JOE™	JOE™			NED™
D19S433	19q12-13.1	NED™	NED™	NED™				
D21S11	21q11.2-q21	6-FAM™	PET®	JOE™	JOE™			VIC®
FGA	4q28	PET®	NED™	NED™	5-FAM™		5-FAM™	PET®
TH01	11p15.5	VIC®	NED™	NED™		JOE™	JOE™	
TPOX	2p23-2per	NED™				JOE™	JOE™	
vWA	12p12-pter	NED™	6-FAM™	5-FAM™	5-FAM™		5-FAM™	
SE33	6q14		VIC®					
Amelogenin	X: p22.1-22.3 Y: p11.2	PET®	VIC®	JOE™	JOE™	JOE™	JOE™	VIC®
Probability of Identity								
African American *		1.31×10^{-18}	6.47×10^{-15}	7.91×10^{-14}	1.48×10^{-11}	3.15×10^{-7}	1.23×10^{-10}	6.52×10^{-11}
US Caucasian *		5.01×10^{-18}	7.46×10^{-14}	2.99×10^{-13}	1.04×10^{-11}	1.19×10^{-6}	2.79×10^{-10}	8.21×10^{-11}
US Hispanic *		7.65×10^{-18}	NA	NA	NA	NA	NA	1.05×10^{-10}
Native American *		3.62×10^{-17}	NA	NA	NA	NA	NA	2.08×10^{-10}

Y Chromosome Loci Guide:

Locus	Yfiler™ kit
DYS456	6-FAM™
DYS389I	6-FAM™
DYS390	6-FAM™
DYS389II	6-FAM™

Locus	Yfiler™ kit
DYS458	VIC®
DYS19	VIC®
DYS385 a/b	VIC®

Locus	Yfiler™ kit
DYS393	NED™
DYS391	NED™
DYS439	NED™
DYS635	NED™
DYS392	NED™

Locus	Yfiler™ kit
Y GATA H4	PET®
DYS437	PET®
DYS438	PET®
DYS448	PET®

*Refer to appropriate User's Manual for additional population information.

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Frequently Asked Questions Regarding the AmpFℓSTR® MiniFiler™ PCR Amplification Kit

Q: Which loci are included in the MiniFiler™ kit?
A: The MiniFiler™ kit is a 9-plex which includes: D13S317, D7S820, Amelogenin, D2S1338, D21S11, D16S539, D18S51, CSF1PO, and FGA.

Q: How do I integrate the MiniFiler™ kit into my current sample workflow?
A: The MiniFiler™ kit is an adjunct kit to the Applied Biosystems autosomal STR kits that are currently being used for forensic casework. Loci that drop out in DNA samples which produce partial profiles using currently available STR kit technology may be recovered using the MiniFiler™ kit. The MiniFiler™ kit has been optimized to produce results from degraded and/or inhibited samples.

Q: Can the profiles produced with the MiniFiler™ kit be uploaded into CODIS?
A: At the present time the MiniFiler™ kit is still in development and therefore has not been accepted by NDIS. When the kit is available in the final manufactured format the appropriate experiments will be conducted and submitted to NDIS for approval.

Q: Will the MiniFiler™ kit gain acceptance by ENFSI and GEDNAP?
A: An ENFSI / GEDNAP ring trial is in the planning phase. When the MiniFiler™ kit is available in the final manufactured format a formal ring trial will proceed. A journal article will be authored and published in a peer reviewed journal once complete.

Q: I am interested in bringing the MiniFiler™ kit into my lab when available. What do I need to prepare for integration?
A:
- Ability to run 5-dye applications – The MiniFiler™ kit uses the same size standard, matrix standard and capillary electrophoresis modules as Identifiler®, Yfiler™ and SEfiler™.
- GeneMapper® *ID* v3.2 – Data from the MiniFiler™ kit can not be analyzed with GeneScan® / Genotyper® software.
- New panel and bin set for GeneMapper® *ID* software.

Q: When will the MiniFiler™ kit be available for purchase?
A: The kits will be commercially available early in calendar year 2007.

Q: What types of samples can I analyze using the MiniFiler™ kit?
A: Any casework, missing person or disaster victim sample which is degraded, inhibited or otherwise compromised, producing an incomplete profile using traditional STR technology. This includes: bone, hair, blood, saliva, swabs, tissue, "touch" samples, etc.

The AmpFℓSTR® MiniFiler™ PCR Amplification kit - Inhibition Study

With the introduction of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit approaching there is an increased focus on the ability to overcome the effects of inhibitors on forensic samples.

Applied Biosystems tested the performance of the MiniFiler™ kit against the Identifiler® kit and PowerPlex® 16 kit to evaluate the effect of inhibitors on each kit. Because the MiniFiler™ kit has been developed and optimized to overcome the effects of sample inhibition, it demonstrated the best performance with inhibitors present. However, the following data demonstrates that the Identifiler® kit also has capacity to overcome low levels of inhibitors that may be present in a sample.

Kits tested in parallel:
- AmpFℓSTR® Identifiler® Kit
- AmpFℓSTR® MiniFiler™ Kit
- PowerPlex® 16 Kit

Inhibitors tested:
- Hematin: Main oxygen carrier in blood
- Calcium Phosphate: Component of bone
- Indigo: Dye from denim
- Melanin: A pigment present in skin and hair
- Collagen: Connective protein in bone
- Humic Acid: Polyphenolic compound in soil

DNA sample used: Male DNA 007

The number of alleles that will be seen using DNA 007 and the associated kits.

	Identifiler® Kit	MiniFiler™ Kit	PowerPlex® 16 Kit
DNA Input	1 ng	0.5 ng	1 ng
# PCR Cycles	28	30	30
# Alleles for 007	29	17	29

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Representative data from experiments performed:

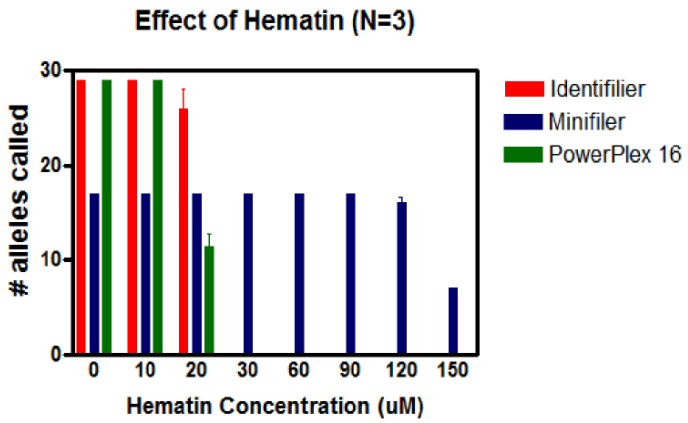

Figure 1: The effect of hematin on the three kits tested. The MiniFiler™ kit did not exhibit any affect of inhibition until 120uM of heme was added to the reaction. The Identifiler® kit did not have any loci drop out until 20uM of heme was added to the reaction.

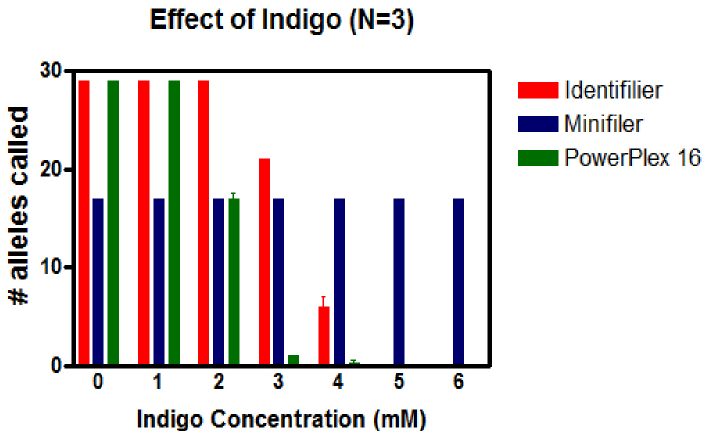

Figure 2: The effect of indigo dye on the three kits tested. The MiniFiler™ kit did not have any loci drop out even when 6mM of dye was added into the reaction. The Identifiler® kit did not have any loci drop out until 3mM of indigo was added to the reaction. However, the PowerPlex® kit did show inhibition at 2mM of dye present.

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As a follow up to the data produced in the inhibition study, the MiniFiler™ kit was run directly against the Identifiler® kit and the combined performance was evaluated.

The male DNA 007 was used in this experiment. Please note that the Male DNA 007 is heterozygous for 13/16 loci when using the Identifiler® Kit. Both reactions were inhibited by adding Calcium Phosphate at 1.25 mM into the reaction. As expected, the larger loci in the Identifiler® kit dropped out; however, the MiniFiler™ kit amplified all alleles. When the data is combined a full profile is obtained.

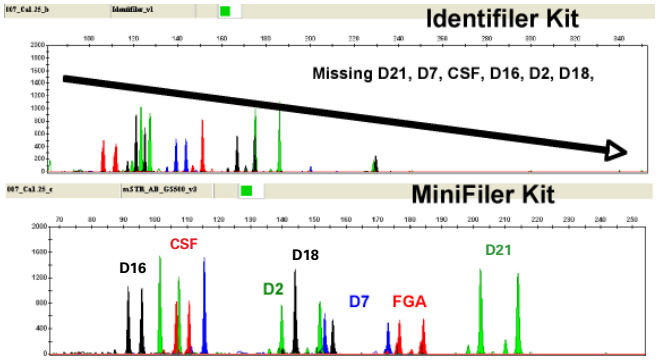

Figure 3. This data demonstrates that the MiniFiler™ kit is an excellent adjunct to the Identifiler Kit.

Additional data has been submitted in an abstract to the American Academy of Forensic Science meeting 2007.

Applied Biosystems

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Customer Corner

MiniSTRs: Past, Present, and Future

By John M. Butler, National Institute of Standards and Technology

DNA molecules that are exposed to water and/or heat will over time begin to break down into smaller pieces. This degradation occurs due to bacterial, biochemical or oxidative processes. A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or compromised forensic evidence improves with smaller sized PCR products. For example, in 1994 the Forensic Science Service noted that smaller STR loci worked more often on biological remains recovered from the Branch Davidian fire. The first major effort to purposefully reduce STR amplicon sizes was for use in time-of-flight mass spectrometry, where detection sensitivity improved dramatically with PCR products less than 100 bp in size. Later many of these "miniSTR" primers were labeled with fluorescent dyes and used to aid identification of World Trade Center victims. A timeline covering the development of miniSTRs may be found at http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm.

Using their 5-dye chemistry and mobility modifier technology, Applied Biosystems has developed a miniSTR kit capable of amplifying 8 core STR loci and amelogenin with reduced PCR product sizes relative to current commercial kits. This kit, which includes an improved PCR master mix, should greatly aid efforts to recover results from degraded DNA samples. However, it is important to keep in mind that because different PCR primers are in use with the miniSTR kit relative to previous AmpFℓSTR® kits, discordant results may occur due to primer binding site mutations that cause allele dropout.

Unfortunately, amplicon size and the ability to amplify extremely degraded DNA molecules was not considered when the 13 CODIS core STR loci were selected, and thus several of them have a large number of repeats (e.g., D21S11) or wide allele ranges (e.g., FGA) that are not optimal for generating small amplicons. An additional 26 polymorphic STR loci, which contain narrow allele ranges and when PCR-amplified are less than 150 bp, have been recently characterized at NIST. Equally important, all of these miniSTR loci are located on separate chromosomes or are genetically unlinked from the widely used 13 CODIS markers and thus may be used in conjunction with them. Several of these new miniSTR loci—namely, D2S441, D10S1248, and D22S1045—have been recommended by leaders in the European forensic DNA community for adding to their core genetic systems used in human identity testing. More information on these new miniSTR loci is located at http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm.

The utility of miniSTR assays has been confirmed in intra- and inter-laboratory studies involving degraded bone samples and aged blood and saliva stains. In all cases, success rates in recovering information from compromised DNA samples improve with miniSTR systems compared to conventional STR kits.

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Applied Biosystems

Forensic News

October 2006 Customer Corner

Evaluation of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit for Use with Compromised DNA Samples

Arthur J. Eisenberg, Ph.D., Xavier G. Aranda, M.S., and John V. Planz, Ph.D.
Department of Pathology and Human Identification, University of North Texas Health Science Center

The DNA Identity Laboratory at the University of North Texas Health Science Center (UNTHSC, Fort Worth Campus) in partnership with the Laboratory for Forensic Anthropology at the University of North Texas (UNT, Denton Campus) jointly comprise the University of North Texas System, Center for Human Identification (UNTSCHI). The mission of the Center is to conduct forensic analysis for the identification of human skeletal remains; to facilitate the collection and analysis of family reference samples in order to establish a basis for the identification of persons reported missing; and to provide educational and professional training to the medico legal community. The DNA Identity Laboratory is accredited under the requirements of ISO 17025 for DNA Analysis by Forensic Quality Services (FQS) and offers both Short Tandem Repeat (STR) and mitochondrial DNA (mtDNA) analytical methodologies. The director of the Laboratory for Forensic Anthropology is board certified by the American Board of Forensic Anthropology and provides the analysis of skeletal remains in order to determine cause and manner of death. For the past several years the UNT System, Center for Human Identification has received significant funding from the National Institute of Justice under the President's DNA Initiative for a national missing persons program (2004-DN-BX-K212). These funds allow the Center to provide, at no charge, both the molecular (mtDNA and STR) analysis of human remains and family reference samples as well as the forensic anthropological analysis of skeletal remains.

The identification of the individual whose remains have been recovered is typically dependant upon the amount of genetic data that can be obtained from these challenging and often compromised samples. The most informative genetic data is provided through the analysis of the traditional STR markers used in forensic DNA analysis. The amount and condition of the DNA recovered from skeletal remains is often variable. We and others have reported varying degrees of success in obtaining genetic profiles for the 13 Core STR loci from skeletal remains. UNTSCHI evaluated its success in obtaining DNA profiles for the 13 core STR loci plus the Amelogenin marker using the AmpFℓSTR® Profiler Plus® *ID* and COfiler® kits with 133 compromised and challenging unidentified human remain (UHR) samples. The results represented in figure 1 demonstrate that for approximately 55% of the samples, DNA profiles from less than 10 loci were obtained.

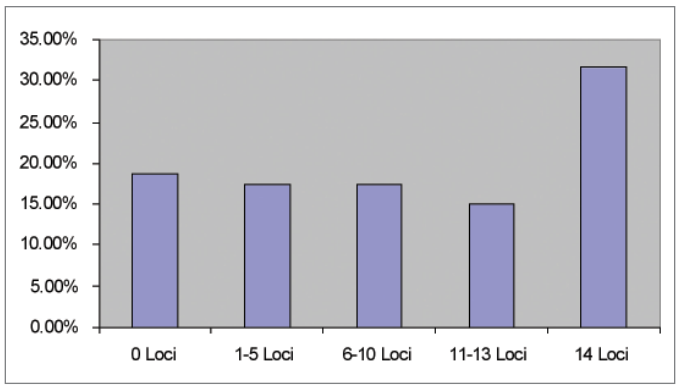

Figure 1. Number of loci successfully amplified from UHR samples with the Profiler Plus® *ID* and COfiler® multiplex kits.

Figure 2 depicts the success rate of the individual loci obtained using the Profiler Plus® *ID* and COfiler® multiplex kits. In compromised samples, the largest molecular weight loci, including FGA, D21S11, D18S51, D13S317, D7S820, D16S539, and CSF1PO most often fail to amplify.

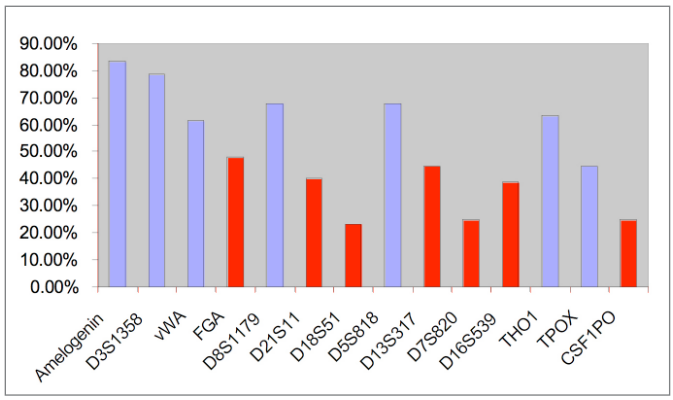

Figure 2. Success rate of the individual loci obtained using the Profiler Plus® *ID* and COfiler® kits from UHR samples.

These results demonstrate a clear need for new systems and alternate testing methods designed to maximize the information contained within compromised samples. Further, they indicate the specific loci that repeatedly fail to amplify.

UNTSCHI has received another grant from the National Institute of Justice under the President's DNA Initiative (2004-DN-BX-K212) to investigate a variety of newer technologies to aid in the identification of UHRs by increasing the efficiency and amount of genetic information obtained from compromised samples. A major component of this grant is the investigation of the miniSTR primer sets developed by NIST and others to increase the successful amplification of the STR loci currently accepted within CODIS, and to investigate the use of mini primer sets with other non CODIS STR loci. As part of this study we have evaluated the NC01 and NC02 loci characterized by NIST and for the past several months we have been working with Applied Biosystems on their development of the AmpFℓSTR® MiniFiler™ PCR Amplification kit. The MiniFiler™ kit was designed to amplify eight autosomal STR loci (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, and FGA) and the sex determining marker Amelogenin. With the exception of the D2S1338 locus, the other seven loci are a subset of the core CODIS STR loci and are the specific loci that repeatedly fail to amplify with compromised human skeletal remains.

UNTSCHI recently has participated in a multi laboratory study to evaluate the performance of the MiniFiler™ kit, and more specifically to evaluate its performance on skeletal remains and other compromised samples. The objectives of the study were to evaluate the reproducibility, sensitivity, utility with mixture samples, and the performance with challenged casework samples. The reproducibility was tested by amplifying the DNA from four samples along with a negative and positive control (DNA 007). The sensitivity of the kit was assessed using a 2-fold serial dilution of the control DNA ranging from 1ng to 31.25pg. The mixture study utilized two DNA samples with a target of 1ng of total input DNA for the following mixture ratios: 0:1, 15:1, 10:1, 7:1, 3:1, 1:1, 1:0. The reproducibility, sensitivity, and mixture experiments were run in triplicate using a 25µl amplification reaction and the following amplification conditions: 95ºC hold (11 min.); 30 cycles 94ºC (20 sec.), 59ºC (2 min.) and 72ºC (1 min.) and a 60ºC (45 min.) final extension. The samples were run on an Applied Biosystems 3130*xl*

Genetic Analyzer and the profiles evaluated using GeneMapper® *ID* Software v3.2. Single amplification reactions were done for casework samples and run using the identical amplification and analysis parameters.

The MiniFiler™ kit provided highly reproducible profiles with sensitivity to 125pg (figure 3). Partial profiles were obtained at 62pg with each of the replicates showing some allelic drop-out and peak imbalance. Limited genetic data was obtained at 32pg. The MiniFiler™ kit appears to be at least twice as sensitive as the Profiler Plus® *ID* and COfiler® kits which we have routinely utilized in our lab. The MiniFiler™ kit detected mixtures at the 15:1 ratio, with the alleles from the major and minor contributors seen at all loci (figure 4).

Figure 3. Amplification of 125pg of control DNA007 with the MiniFiler™ kit.

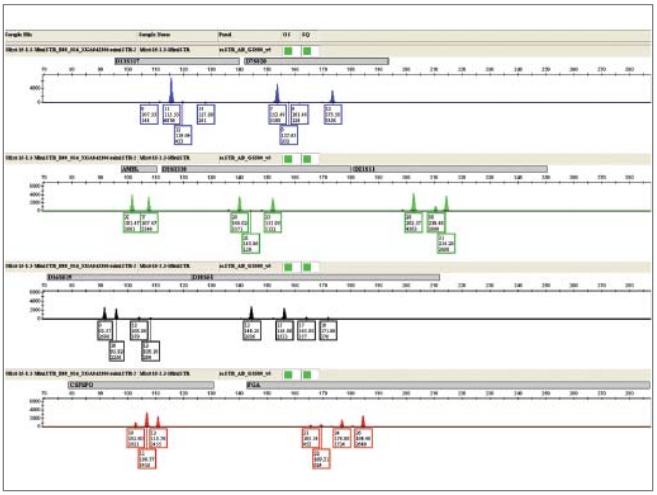

Figure 4. Amplification of the 15:1 mixture with the MiniFiler™ kit.

In addition to the samples provided for the multi laboratory study, our lab was asked to evaluate the MiniFiler™ kit on compromised DNA samples obtained from skeletal remains that had been previously analyzed with the Profiler Plus® *ID* and COfiler® kits. Samples were selected that provided limited genetic data resulting from degradation and/or inhibition. The results from one of the compromised samples are shown in figure 5. The loci FGA, D21S11, D18S51, D5S818, D13S317, and D7S820 failed to amplify with Profiler Plus® *ID*, and CSF1PO and D7S820 failed to amplify with COfiler®.

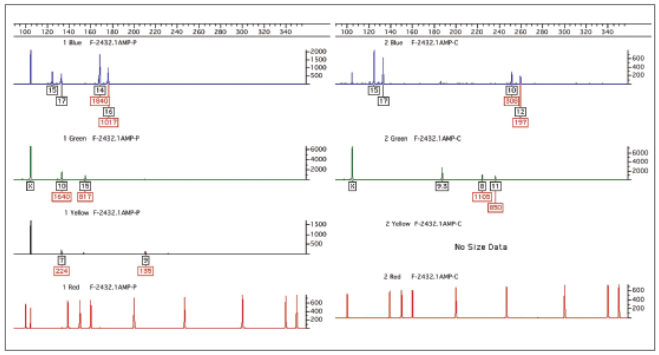

Figure 5. Partial profile obtained from the amplification of casework sample with Profiler Plus® *ID* kit (left) and with the COfiler® kit (right).

The same casework DNA sample was amplified with the MiniFiler™ kit using the cycling conditions previously described and a complete profile was obtained (figure 6). DNA profiles were clearly obtained for the loci FGA, D21S11, D18S51, D13S317, D7S820 and CSF1PO. The addition of these 7 loci would provide valuable genetic data that could greatly assist in the identification of the individual whose human remains were recovered.

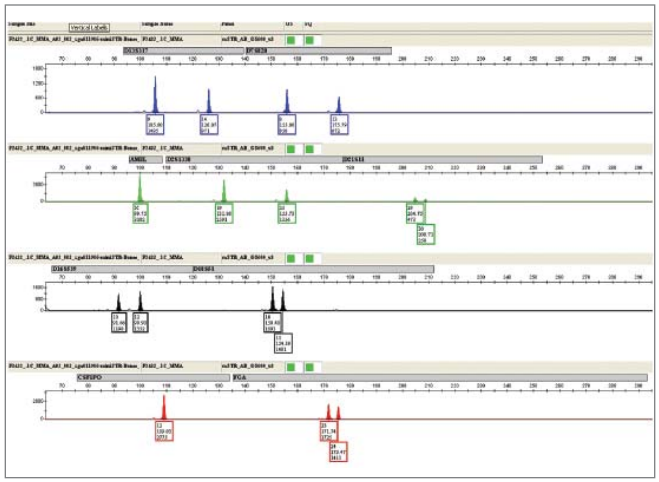

Figure 6. DNA profile obtained from the amplification of casework sample with the MiniFiler™ kit.

In the vast majority of samples analyzed with the MiniFiler™ kit, additional genetic data was obtained in comparison to the current commercially available amplification systems. Although our evaluation of the MiniFiler™ kit is not complete, the results shown clearly demonstrate the success of using the miniSTR technology with compromised samples. We believe these results indicate that the use of the MiniFiler™ kit and other miniSTR systems will provide valuable genetic data from compromised casework samples and unidentified human remains and ultimately enable the resolution of cases previously unobtainable.

For more information on the services provided by UNT System, Center for Human Identification, please call 800-763-3147 or e-mail: MissingPersons@hsc.unt.edu.

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Applied Biosystems

October 2006 FAS Corner

FAQ's on Challenging Samples: Strategies for improving results from inhibited/degraded samples.

Q: What are common misconceptions about handling inhibited/degraded samples?
A: Inhibited samples are often confused with degraded samples (and vice versa), and although the two types of samples present difficulty to the analyst, they can often be distinguished after close examination of the quantification results, DNA profiles and a consideration of the sample's origin. The Quantifiler® Human DNA Quantification Kit contains an internal PCR control (IPC) that can serve as a useful indicator of potential inhibition.

Q: What information about the sample may assist in identifying challenging samples?
A: Careful consideration should be given to the totality of the information regarding a sample:
- What is its origin, if known? Whole blood sample, bone, buccal swab, black denim, etc.
- What type of substrate was it extracted from? Cotton tipped applicator, dyed fabric, leather, plant material, etc.
- What natural elements has this sample been exposed to? Heat, sunlight, moisture/humidity, etc.
- Does the sample contain possible inhibitors of DNA polymerase such as textile dyes, heme or humic acid?

Q: How do you tell the difference between inhibited and degraded samples?
A: The presence of inhibitors that co-extract with sample DNA may be detected using the Quantifiler® Human DNA Kit through an evaluation of the IPC results. Inhibited samples exhibit an IPC with a higher than expected Ct value or an IPC that does not produce a signal which crosses the threshold. Samples exhibiting potential inhibition may result in lower than expected quantification results requiring further sample clean-up or dilution in order to obtain accurate quantification. Neglecting to re-quantitate an inhibited sample after clean-up or dilution may result in off-scale data for smaller fragments when run on a Capillary Electrophoresis instrument. A degraded sample (with no inhibitors) would produce an expected and consistent IPC value. Quantification of degraded DNA may result in an overestimate of the amount of DNA present with preferential amplification of smaller amplicons relative to the larger amplicons. Addition of higher than normal amounts of input DNA may assist in recovery of information from the longer amplicons.

Q: How can extraction/purification methodology impact results from compromised samples?
A: The presence of PCR inhibitors that are co-extracted with sample DNA can result in allele dropout or imbalances in heterozygous loci. A number of techniques exist to overcome inhibition of amplification including:
- Use of extraction methods which effectively remove inhibitors from the sample
- Dilution of extracted DNA which consequently dilutes the inhibitor, thereby minimizing the effect
- Addition of BSA to the PCR reaction

Continued next page

Q: What indicators of degradation and/or inhibition should I look for in sample data?

A: The classic "ski slope" effect is typical of a degraded sample. In such samples, the ability to amplify the longer amplicons is diminished relative to the ability to amplify the shorter amplicons. Samples containing inhibitors may exhibit partial profiles similar to those generated from degraded samples, resulting in complete loss of all alleles or resulting in drop-out or imbalance at select loci.

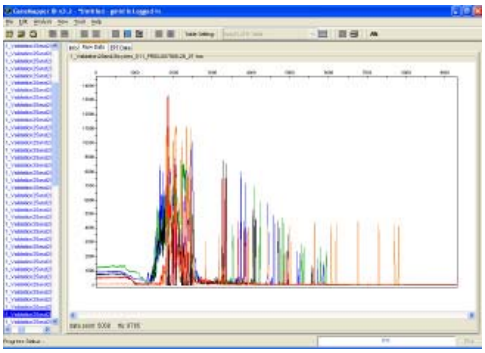

Figure 1. The classic "ski slope" effect typical of degraded samples.

Until recently, the ability to recover information from degraded samples was limited. Technological improvements have been implemented in the MiniFiler™ PCR Amplification kit that include moving the primers in closer to the locus repeat regions. This has allowed the generation of smaller amplicons, thus increasing the probability of obtaining a full profile from a degraded sample.

Q: What is the best strategy for recovering information from degraded and/or inhibited samples?

A: The MiniFiler™ PCR Amplification Kit has been demonstrated to yield the greatest amount of information from samples that have previously produced partial profiles or no profile at all using existing commercially available autosomal amplification kits. In the presence of PCR inhibitors, the MiniFiler™ kit outperforms other commercially available multiplex kits with regard to recovery of genetic information. The MiniFiler™ kit contains 8 autosomal markers and the sex-determining marker amelogenin. Use of a dual-amplification strategy using MiniFiler™ and Identifiler® kits is the best strategy for recovery of all 15 autosomal markers from compromised samples.

Applied Biosystems

Forensic News

October 2006 FAS Corner

Tube Problem Identified After Extensive Troubleshooting

By Matthew Gamette, Forensic Scientist
Washington State Patrol Crime Laboratory – Spokane

We often load runs on the ABI PRISM® 310 Genetic Analyzer that go for several days, and the sensitivity is usually maintained throughout the run. A few months ago we noticed that the samples would start to lose sensitivity and then just totally drop out. This started to happen around 24-30 hours after the ROX™ dye/formamide/DNA was loaded and the run began. Not all the samples would lose sensitivity or drop out at the same time, but after 30-40 hours, all the samples would flatline (including the ROX™ dye peaks).

The results were looking like something I had seen before when we switched from a -20 freezer to a -4 freezer for snap cooling (we do heat denature at 95 degrees and snap cool in our laboratory), although the drop out became apparent much earlier in the run. That problem was resolved with the purchase of a new -20 freezer. In this case, I checked all the freezer temps and logs and they appeared functional and without problems. We also checked the temperature to heat denature and found no problems. My immediate thought was that it was something with the formamide, but I had no proof of that. We requested a new lot from Applied Biosystems. Three lots of HI-DI™ formamide later, and with no other labs reporting problems with the same lots, we knew it could not be the formamide itself. We then turned to the 310 instrument, the confounding variable being that when the samples were reloaded in new formamide/ROX™ dye from the exact same amp tube (and same 310 System setup), the sample would run strong on the instrument again.

I was sure it had to be something in the formamide mixture breaking down over time. We checked our water systems because we have a brand new lab, and our water was excellent with no service calls reported. We have three purification/filtering systems in the lab including treatment with UV twice before it is delivered at our sinks. We determined it was not a water problem. We ruled out our loading tubes because the same effect was seen in 0.2 strip tubes or 0.5ml tubes. We changed the buffer, POP-4™ polymer, and other 310 system consumables with no change. We examined our TE amplification buffer and other Applied Biosystems amplification products and they all checked out fine. We also threw away any unused formamide aliquot to avoid repeated freeze/thaw cycles. We changed out the syringes because we thought it might be a pumping problem and that also had no effect.

Through this process we could load runs for less than 24 hours to get the data off, but this made it hard to get cases out the door. We could also reload samples and get the sample sensitivity back, but only for the 24-30 hour window. Redenaturing the samples had no effect on the sensitivity problem. During this entire process, we were in contact with Applied Biosystems, and they continued to recommend things to change out or try. They asked us to clean all our blocks and even sent us a new block, capillaries, vials, septa, and new consumables. We were all at the end of our patience with this problem. It seemed that we had changed every variable in the process, one variable at a time, but nothing worked. This was both time consuming and detrimental to case delivery productivity.

Generally, when our new HI-DI™ formamide comes into the lab, it is divided into 1ml aliquots. We used colored tubes so that we could distinguish lot changes by a change in color. This color change reminded us to update our data sheets with the new lot of formamide. I was in the amp room one day looking at the labels on the bags of tubes. The full bags of tubes from USA Scientific appeared different than the previous (older) lot. The dye appeared darker. The bags also had polypropylene written on them, whereas the type of plastic was not noted on the older lot bags. I asked another scientist if we just received a new lot of tubes and she noted that we had back stocked them some time ago. I did not

know exactly when we started using the newer tubes for aliquots, but based on the number of tubes used, I think it was around the time we started to see the problem.

After months of switching every component in the process, we switched to clear 1.5ml tubes (the same type we use for extraction and DNA storage) with a newly ordered lot of formamide. Immediately, we saw the problem resolve and the loaded racks were good on the instruments for days and even worked for a week in QC related follow-up work. We concluded that it must have been the dye (or some chemical from the colored tubes) leaching into the formamide aliquots. We will now be using all clear 1.5ml tubes. It should be noted that we switched tube colors with each new formamide lot ordered to solve this problem, and we had the same issue with each lot until we switched to the clear tubes.

We have been informed that other labs are now seeing similar problems with clear tubes, and I wonder if a switch in the type of plastic or some chemical from the manufacturing process might be to blame rather than the dye of the tube. We did not do further research to pinpoint the problem with the tubes due to a casework bottleneck that needed to be resolved at the end of this process.

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Julio J. Mulero, PhD
Staff Scientist
Applied Markets

Julio joined Applied Biosystems in 2003. His work focuses on developing Human Identification reagents and he participated in the research and development of the AmpFℓSTR® Yfiler™ and MiniFiler™ PCR Amplification Kits. Previously, Julio spent five years as a Senior Scientist in the functional genomics group at Hyseq Inc. Julio completed his Ph.D. at Cornell University in the department of Biochemistry and Molecular Biology. You can reach Julio at mulerojj@appliedbiosystems.com

Maurice Padilla
HID Field Applications

Maurice joined Applied Biosystems as a HID Field Application Specialist in July 2006. In his current role, Maurice trains forensic DNA analysts on HID applications including real-time PCR, GeneMapper® *ID* Software, and 3130 instruments. Previously, Maurice worked as a forensic analyst for approximately five years at the Texas Department of Public Safety Austin laboratory and at the City of Austin Police Department Forensic laboratory. Maurice completed his bachelor's degree at Purdue University and his master's degree at Texas A&M University. Maurice is based in Austin, TX. You can reach Maurice at Maurice.Padilla@appliedbiosystems.com

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Applied Biosystems

Forensic News

October 2006

AB Corner

Rebecca Clifton, PhD
Account Manager - Forensics
Australia & New Zealand

Rebecca Clifton joined Applied Biosystems in June 2003 after completing her PhD at Prince Henrys Institute of Medical Research (Monash University, Australia). For the past three years, Rebecca has worked as a general sales specialist for the Molecular Biology division at Applied Biosystems, and recently took on a new role as the Australasian Forensic Key Account Manager for Applied Markets. In her new role Rebecca ensures that regional human identification laboratories, organizations and governing bodies benefit from a refocus of Applied Biosystems resources on Australian, New Zealand & Fijian forensic communities. Rebecca is based in Melbourne, Australia. You can reach Rebecca at cliftorj@appliedbiosystems.com

Applied Biosystems

Forensic News

October 2006 Legislation Corner

David Scoville, long-time advocate for DNA database expansion, joins Governor Pataki at a press conference. David's daughter Patty was killed in Vermont by a man who was eventually identified by a database match.

New York Makes Significant Expansion to the State DNA Database

By Lisa Hurst, Smith Alling Lane

In the final moments of the 2006 New York state legislative session, Governor Pataki successfully pushed through legislation requiring DNA samples to be collected from all convicted felons (SB 8446). This was accomplished after a highly coordinated effort involving criminal justice interests from every corner of the state. For several years, a proposal to include DNA samples from all convicted felons in the database has been convincingly passed by the State Senate; however, the State Assembly consistently blocked the measure. The opposition came primarily from the powerful Assembly Speaker's Office, which was concerned with civil liberty and privacy issues, and, in particular, with the existence of local suspect indexes.

As one of his last legislative efforts as New York's Governor, Pataki made DNA legislation enactment his priority in 2006. He hosted numerous press conferences and authorized the Director of the Criminal Justice Services Division, Chauncey Parker, to begin a "whistle-stop" tour, garnering vocal support from every District Attorney, Police Chief, Sheriff, and multiple victims groups. Moreover, New York's Mayor Michael Bloomberg began to publicly urge for an expansion of the DNA database. In fact, the Mayor held a press conference addressing the issue at the City's nearly-completed $250 million DNA laboratory. The Mayor was joined by the District Attorneys from the five Burroughs, the Police Commissioner, Medical Examiner, and several victim advocates. Throughout the state, the issue received positive media coverage – in many instances victims of preventable crimes stepped forward to tell their stories. A true ground-swell of support arose, pressuring the Assembly to take action.

The enacted legislation requires DNA to be collected upon conviction for all felons and for a variety of misdemeanors, including petty theft. The New York Division of Criminal Justice Services (which oversees the operation of the state's DNA index system) estimates that prior statutes captured approximately 14% of convicted criminals in New York for inclusion in the DNA database. Now, with the passage of SB 8446, New York expects to include 50% of all convicted criminals in the database. This dramatic increase will add an estimated 50,000 offenders each year to the state and national database system.

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Additionally, unlike many other states that have passed similar database expansion legislation, New York included $20 million in the state budget for implementation of the new DNA law. The money is to be divided among the following purposes:

- State Police laboratory to expand capacity for offender samples and casework: $9.1 million.
- Local DNA laboratories (seven) for casework capacity enhancement (funding will primarily support new staff): $6 million
- SUNY-Albany to support specialized technical training for DNA lab personnel ("DNA Academy"): $2 million
- DNA training for law enforcement and other criminal justice personnel including medical examiners, coroners and SANE/SAFE medical staff: $2 million
- Collection of DNA offenders by probation agencies: $1 million

Thanks to this funding, the state hopes to complete all DNA analysis in-house by the spring of next year. With the new expansive law (applying not only to convicted felons but also to a broad range of misdemeanants), and with the demonstrated dedication to provide state funding, New York is currently poised to reap considerable public safety benefits from its forensic DNA programs.

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Forensic News

October 2006

Legislation Corner

Global Trends in DNA Databasing – Connecting Across Borders

By Chris Asplen, Smith Alling Lane

As more countries establish and expand their forensic DNA databases, the potential to successfully share data between countries has also expanded. Universally, countries continue to move towards the passage or expansion of legislative authority for forensic DNA databases. Even countries which lack DNA specific legislation have begun the process of databasing under other criminal justice authority. And as databases increase in size and effectiveness, the value of sharing data across borders becomes readily apparent.

Increased capacity to share DNA profiles across borders has also been mirrored by an accelerated need for such connectivity. The advent of European unionization has made cross border travel in Europe an unmonitored activity. People can move freely between countries absent the expectation that they will be questioned or checked against any database before entering the country. That applies not only to law abiding citizens but also to those predisposed to commit crime. As such, an experienced criminal with an extensive criminal record can travel freely between countries, free from fear that his criminal record will follow him. This is particularly true in the case of DNA profiles contained in individual country databases. The most heinous child sexual predators, once paroled, can travel from the country which maintains his DNA profile across the border to a neighboring country where the power and potential of DNA is rendered useless. In other words, a perpetrator can be convicted in the United Kingdom, (where DNA is used extensively to solve crime,) move to Spain, (which maintains no offender database,) and commit numerous crimes undetected for lack of sharing vital data. The weaker a country's database, the more attractive to the criminal.

There are however, significant efforts to recognize DNA's full potential across borders. In May, 2005 seven countries signed the Prum Treaty, an agreement designed to facilitate greater data transfer between countries, including forensic DNA profiles. The signatories to the treaty were Belgium, Germany, Spain, France, Luxembourg, the Netherlands and Austria. The stated goal of the treaty is the "stepping up of cross-border cooperation, particularly in combating terrorism, cross-border crime and illegal migration." While the treaty addresses several kinds of data exchange, its primary focus is on DNA data exchange.

Initial, bi-lateral tests of cross-border data sharing have already proven the immense value of database interconnectivity. In a project between Austria and Germany, initial comparisons yielded in excess of 1200 person to person hits, 257 German person to Austrian stain hits, 387 Austrian person to stain hits and 871 stain to stain hits.

Interpol has also established the Interpol DNA Gateway which is designed to facilitate the comparison of DNA profiles between Interpol member countries. A member state may submit DNA information for inclusion on the DNA gateway at the General Secretariat via Interpol's secure telecommunications system, or as hardcopy. The system is indexed into four main categories: crime scene samples, reference samples, missing persons and unknown deceased. Importantly, Member States which submit DNA profiles to Interpol in accordance with their legislative requirements may limit access by naming specific countries to be prevented from accessing their information. When member states are notified of a match, the contributing countries may then determine whether they release further information pertaining to the specific DNA profile.

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Applied Biosystems

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International connectivity of forensic DNA databases is the next logical progression in the effort to maximize the crime fighting potential of DNA technology. Eventually, countries will possess the ability to use DNA to track down perpetrators anywhere in the world, validating law enforcement's favorite credo, "You can run, but you can't hide."

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October 2006

Event Corner

Join us at the 17th International Symposium on Human Identification

Applied Biosystems invites you to join us at the 17th International Symposium on Human Identification in Nashville, Tennessee, October 9-12, 2006. Drop by booth #306 to receive your "Don't be a Drop Out" T-shirt*, and learn more about the AmpFℓSTR® MiniFiler™ PCR Amplification Kit, the world's first commercially available 9-plex miniature STR kit. The symposium is a great opportunity to obtain additional information about this next generation STR technology which significantly increases your ability to obtain information from compromised DNA evidence. See you in Nashville!

Wear your "Don't be a Drop Out" T-shirt* on Wednesday, October 11th and receive a $20 Gaylord Opryland gift certificate* good for resort dining, gift shops, spa, lodging, tours and more!

Schedule of Events

Oral Presentations:

Title: *Validation of the AmpFℓSTR® Minifiler™ PCR Amplification Kit: A 9-Plex MiniSTR Assay for the Analysis of Compromised DNA Samples*
Author: Julio Mulero, PhD, Applied Biosystems
Date/Time: Wednesday, October 11

Title: *Development of New Tools to Aid in the Identification of Missing Persons and Unidentified Human Remains*
Author: Arthur Eisenberg, PhD, University of North Texas Health Science Center
Date/Time: Wednesday, October 11

Title: *GeneMapper® ID Software v3.2 : Expert System Solutions*
Author: Lisa Calandro, M.P.H., and Ravi Gupta, Applied Biosystems
Date: Wednesday, October 11 - Expert System Breakout Session

Posters:

Title: *Evaluation of the Applied Biosystems AmpFℓSTR® MiniFiler™ PCR Amplification Kit*
Author: Jennifer L. Zimdars, MFS, Armed Forces DNA Identification Laboratory

Title: *A Streamlined Approach to Validating New Forensic DNA Technologies*
Author: Jacki Benfield, Applied Biosystems

Title: *Validation of GeneScan™ 600 LIZ® Size Standard for Forensic Applications*
Author: Liwei Qi, Applied Biosystems

Title: *Implementation of the Applied Biosystems 3130 Genetic Analyzer and GeneMapper® ID Software v3.2 in Forensic Casework*
Author: Karen Mills, Centre of Forensic Sciences

Title: *A Multiplexed System for Quantification of Y-DNA and Total Human DNA*
Author: Jaiprakash Shewale, PhD, Applied Biosystems

*Terms and conditions: Limited to persons directly involved in Life Sciences research, as determined at the sole discretion of Applied Biosystems. Limited to one per person. Available while quantities last. No purchase necessary. Void where prohibited. Other restrictions may apply.

Applied Biosystems

October 2006

Event Corner

EAFS Events Emphasize Need for Interconnectivity of National DNA Databases

On June 13th, as part of the European Academy of Forensic Sciences meeting in Helsinki, Finland, Applied Biosystems hosted a workshop featuring Mr. John Dickinson. John is the father of a young girl who was murdered in France while on a school trip and whose case was successfully prosecuted because of DNA. John spoke about the role that DNA technology played in solving his daughter's murder as well as the need for the interconnectivity of national databases. Kees van der Beek from the Netherlands Forensic Institute also presented on the current efforts underway to facilitate DNA data exchange between countries. A special reception was also hosted by Applied Biosystems for the Country directors of the European Network of Forensic Science Institutes (ENFSI).

Applied Biosystems

Forensic News

October 2006

Product Corner

GeneMapper® *ID* Software v3.2.1 Patch Released

Applied Biosystems has released the v3.2.1 patch for GeneMapper® *ID* Software which provides four changes to improve functionality. To download the patch, please visit: http://www.appliedbiosystems.com/genemapperid_3.2.1. Installing the upgrade is a simple process, and does not require the user to uninstall the previous version. All new licenses will include the 3.2.1 patch; therefore, no upgrade will be required.

About the GeneMapper® *ID* v3.2.1 Patch

The GeneMapper® *ID* Software v3.2.1 patch provides four changes to existing GeneMapper® *ID* v3.2 software that will improve functionality. The v3.2.1 patch provides the following changes:

1. Update for 'Overlapping bins'.

In some rare instances, a pattern resembling two or more bins overlapping one another may be observed and/or allelic ladder peaks labelled in an unexpected manner. These effects would be easily recognized during the examination of the allelic ladder for correct allele assignments. The patch incorporates an additional sorting step so that bins are sorted by both the user-interface and analysis code thus ensuring that bins are sorted as expected. Figure 1 shows an example of the overlapping bin pattern obtained prior to implementation of the change to the sorting protocol.

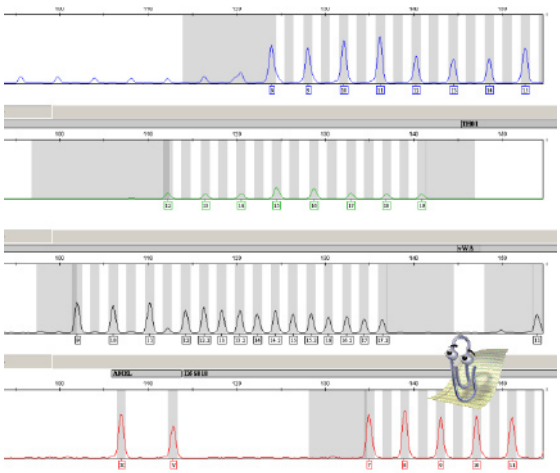

Figure 1. Demonstration of overlapping bin pattern

2. Update for 'Error found in combined table'.

In one reported instance, the message "An Internal Error has Occurred" is displayed when trying to export a combined table. To address this, the software now performs a check, which has eliminated the error message and allows export of the combined table.

3. Update for 'Marker Specific Stutter ratio'.

The values in the user interface under the 'Marker Specific Stutter ratio' column of the Panel Manager were updated to display four digits after the decimal point. Previously, the stutter value was truncated at 2 digits after the decimal point whereas the underlying panel contained four digits after the decimal point. For example, the Identifiler®_v1 stutter value for D8S1179 in the underlying panel is 0.082 whereas in the user interface the stutter value is listed as 0.08. The v3.2 software applied the stutter filter based on the value in the underlying panel and not based on the value displayed in the user interface. The v3.2.1 patch aligns the value in the user interface with that in the underlying panel.

4. Updated Panel and Bin files.

The following D2S1338 bin designation has been corrected in the Identifiler_v1 panel.

Original bin designations:

Marker Name	D2S1338	
14 305.31	0.5	0.5
15 309.31	0.5	0.5
16 313.31	0.5	0.5
17 317.31	0.5	0.5
18 319.31	**0.5**	**0.5**
19 325.31	0.5	0.5
20 329.31	0.5	0.5

…etc.

Modified bin designations:

Marker Name	D2S1338	
14 305.31	0.5	0.5
15 309.31	0.5	0.5
16 313.31	0.5	0.5
17 317.31	0.5	0.5
18 321.31	**0.5**	**0.5**
19 325.31	0.5	0.5
20 329.31	0.5	0.5

…etc.

We have verified through testing of Identifiler® allelic ladders and samples that the offsets were correctly applied to the original bin designation for the DS21338-18 alleles and allelic ladders were correctly genotyped both prior to the bin modification and using the modified bin.

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Performance Validation

The changes listed above resulted in modifications that DO NOT affect the analysis algorithms in the GeneMapper® *ID* Software v3.2, which include peak detection, size standard matching, sizing, allele calling, bin offsetting, and filtering. According to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines, modifications of this nature require a performance check rather than a complete performance validation. Internal testing was performed to verify the above described functionality and to demonstrate that these minor modifications have not affected any other functionality within the GeneMapper® *ID* v3.2.1 Software patch. Nevertheless, each human identification laboratory analyzing forensic, paternity, databasing, and single-source samples using GeneMapper® *ID* Software v3.2 for data analysis should perform their own appropriate performance verification after such modifications are implemented.

October 2006

Product Corner

QuantiBlot® Kit Human DNA Quantitation Kit Discontinuation

With the successful worldwide adoption of the Quantifiler® Human and Quantifiler® Y DNA Quantification Kits and the validation of the Applied Biosystems 7500 Real Time PCR System, Applied Biosystems has determined that the QuantiBlot® Human DNA Quantitation Kit will be discontinued on June 30th, 2007.

Throughout the next several months, we will provide guidance on how to implement the Quantifiler® kits into your sample workflow by recommending validation experiments, providing on-site support, and extending special offers for instrumentation and reagents.

Applied Biosystems is committed to supporting your laboratory's successful transition from QuantiBlot® to real time quantitation using the Quantifiler® Human DNA and Quantifiler® Y Human Male DNA Quantification Kits with the Applied Biosystems 7500 Real Time PCR System.

If you have questions regarding the obsolescence of the QuantiBlot® Kit or any other item related to this notice, please contact your local Applied Biosystems office, or in North America, call 1-888-821-4HID.

We appreciate your loyalty and business and look forward to working with you.