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what's new

Data Quality Worth Sharing – NGM™ and NGM SElect™ Kits

For laboratories preparing to implement the newly expanded European Standard Set (ESS) loci and share data across borders, data quality can make all the difference.

Senior Forensic Scientist Nicola Oldroyd recently presented a webinar entitled "Next Generation STR Kits: A New Generation of Interpretation and Implementation Considerations" that covered the key aspects of data quality to be considered, including:

- Maximizing data recovery from challenging samples
- Establishing interpretation guidelines
- Avoiding data review bottlenecks and redundant amplifications
- Streamlining comparison with legacy data
- Concordance studies: understanding the workflow implications for your laboratory

To view a recording of this webinar, visit www.appliedbiosystems.com/ngm and click on "View Webinars."

To learn more, see the article 'Implementation of Next Generation STR Kits in Europe: Challenges and Solutions' in this edition of *Forensic News*.

SE33 Sequence Discoveries Have Significant Implications for Genotyping Accuracy

During development of the NGM SElect™ kit, our scientists discovered a SNP-containing region that, if contained within the SE33 amplicon, causes a mobility shift and subsequent incorrect allele designation in affected samples. Significant efforts were invested in designing primers to exclude the affected region while maintaining optimal performance.

Senior Forensic Scientist Nicola Oldroyd recently presented a webinar entitled "Development of the AmpFℓSTR® NGM SElect™ Kit: New Sequence Discoveries and Implications for Genotype Concordance" that explains what we found and how we dealt with it. It also demonstrates that when it comes to developing DNA typing systems for forensic applications, faster is not always better. Rigorous developmental validation and concordance studies are required to help ensure confident interpretation and reporting of results.

To view a recording of this webinar, visit www.appliedbiosystems.com/ngm and click on "View Webinars."

To learn more, see the article Development of the AmpFℓSTR® NGM SElect™ Kit: New Sequence Discoveries and Implications for Genotype Concordance in this edition of *Forensic News*.

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Validation of Identifiler® Plus Kit Completed by Multiple U.S. Forensic Laboratories

Offering significant improvements in performance, robustness, efficiency and data quality, the AmpFℓSTR® Identifiler® Plus kit is being validated and implemented by numerous U.S. forensic labs.

Below are links to six validation presentations from the recent 10th Annual Future Trends in Forensic DNA Technology Seminar Series, given by U.S. forensic laboratories that wanted to share their success. These presentations include information and data from validation studies including sensitivity, evaluation of PCR cycle number, inhibition, degradation, reproducibility, precision, specificity, and non-probative case samples to meet SWGDAM guidelines. A common theme is the enhanced ability to recover interpretable results from a wider range of casework samples.

1) SWGDAM Developmental Validation of the AmpFℓSTR® Identifiler® Plus Kit William Frank, Illinois State Police
2) Moving Towards the Future: The Validation of Identifiler® Plus Kellie Fenesan, San Bernardino County Sheriff's Crime Laboratory
3) Identifiler® Plus: Validation and Test Site Results Lynne Burley, Santa Clara Crime Lab
4) Identifiler® Plus Validation Michael Donley, Harris County Crime Lab
5) The NIST Experience with Identifiler® Plus Becky Hill, NIST
6) Identifiler® Plus Validation - Tucson PD Experience Jelena Myers, Phoenix PD

"I told you, call me 'The Hammer'!"

A humorous video demonstrates how the AutoMate *Express*™ Forensic DNA Extraction System can efficiently facilitate high-quality profiles—even with "The Hammer" breathing down your neck.

View Video: (~4 minutes)

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HID in action

Use of the PrepFiler BTA™ Forensic DNA Extraction Kit and the AmpFℓSTR® Identifiler® Plus Kit in Real Case Samples

Irma Ferreira
Human Genetics Laboratory, AMPATH, South Africa

As a laboratory working mainly with paternity cases we occasionally have to deal with some difficult forensic samples such as bones and teeth. Since we are not a forensic laboratory and do not have the equipment necessary to handle different sample types it can be a real challenge to deliver the required results in these cases.

We are currently using QIAamp® DNA Micro kits to isolate DNA and generating STR profiles with the AmpFℓSTR® Identifiler® kit from Applied Biosystems. Having been made aware of the new PrepFiler BTA™ Extraction kit from Applied Biosystems we took up the challenge to compare our current extraction method against the PrepFiler BTA™ extraction method. In addition to trying this new method, we also decided to test the performance of the new AmpFℓSTR® Identifiler® Plus kit against the Identifiler® kit.

We conducted the testing on two case studies that had previously not yielded any DNA results.

Case Study One

The first case study was done on a 16-year-old bone sample. The body was buried in a shallow sand grave and only a few bones were left when discovered. The femur was taken, cleaned with bleach and ethanol and left to dry completely. The femur was sawn in half and the spongy bone inside was scraped out with a scalpel and ground to a powder using a pestle and mortar.

The 50 mg powdered bone sample was then isolated with the QIAamp® DNA Micro kit and the PrepFiler BTA™ kit.

Both extractions were performed exactly according to the manufacturer's protocol. Currently we do not perform quantitation of DNA samples, and thus, had to experiment with various input volumes of DNA extract to get the best possible result. STR amplification was performed with both the Identifiler® and Identifiler® Plus kits (Fig.1-3).

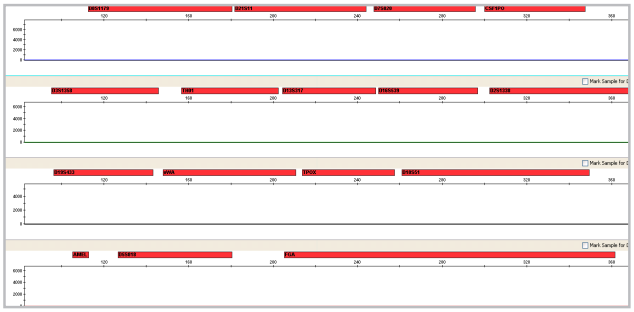

Figure 1: Bone sample extracted with QIAamp® Micro DNA kit and amplified with the Identifiler® kit. Neither the Identifiler® nor the Identifiler® Plus kits produced results.

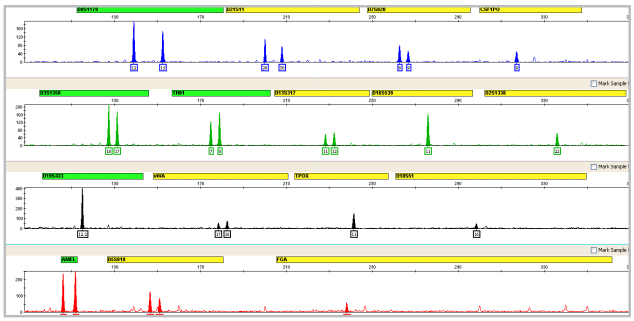

Figure 2: Bone sample extracted with the PrepFiler BTA™ extraction kit and amplified with the Identifiler® kit. Y scales between 200-400 RFU.

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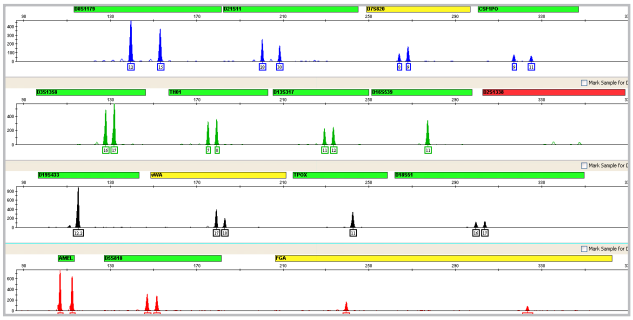

Figure 3: Bone sample extracted with the PrepFiler BTA™ kit and amplified with the Identifiler® Plus kit. Y scales between 600-1000 RFU.

Case Study Two

We received a tooth sample from a burnt, decomposed body that had some bloody tissue attached. The family suspected the body to be the remains of their missing son and wished to perform paternity testing with the tooth specimen. Roughly 50 mg of powdered tooth was isolated using both extraction methods. The bloody tissue (<25 mg) was only isolated using the PrepFiler BTA™ kit due to a limited amount of sample.

The tooth produced no DNA results with the QIAamp® DNA Micro kit but full profiles could be generated with both the Identifiler® and Identifiler® Plus kit when the DNA was extracted with the PrepFiler BTA™ kit (Figures 4 & 5). From the bloody tissue a partial profile could be generated using the Identifiler® kit (Figure 6) but a full profile could finally be obtained following amplification with the Identifiler® Plus kit (Figure 7).

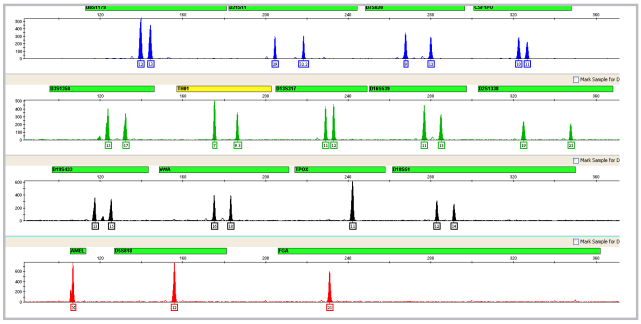

Figure 4: Tooth sample extracted with the PrepFiler BTA™ kit and amplified with the Identifiler® kit. Note that this is a female profile. Y scales between 500-1000 RFU.

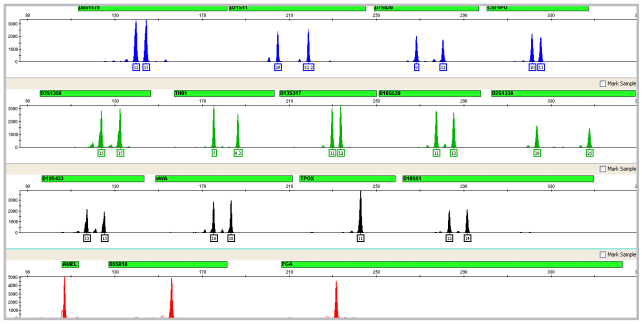

Figure 5: Tooth sample extracted with the PrepFiler BTA™ kit and amplified with the Identifiler® Plus kit. Note that this is a female profile. Y scales between 3000-5000 RFU.

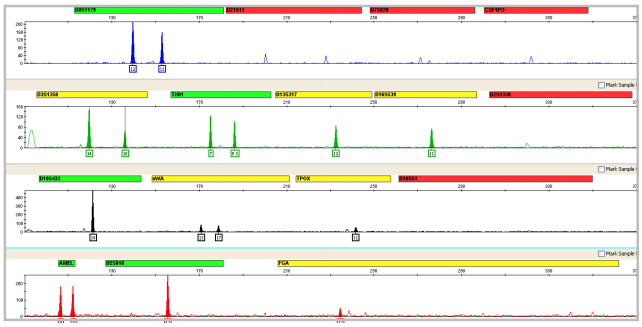

Figure 6: Bloody tissue from tooth extracted with the PrepFiler BTA™ kit and amplified with the Identifiler® kit. Note: compared to the tooth sample, this a male DNA profile. Y scales between 200-500 RFU.

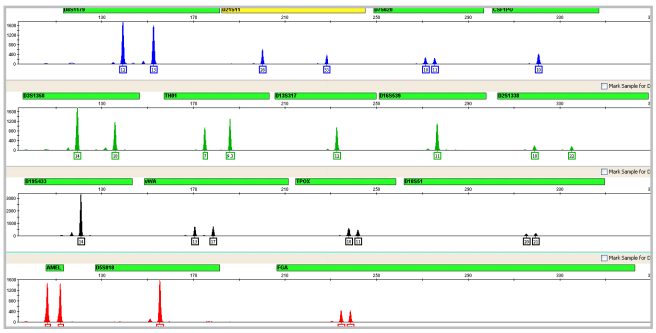

Figure 7: Bloody tissue from tooth extracted with the PrepFiler BTA™ kit and amplified with the Identifiler® Plus kit. Note: compared to the tooth sample, this a male DNA profile. Y scales between 1600-3500 RFU.

We suspected that the female profile obtained from the tooth sample was due to contamination. It did not match any of the laboratory personnel, but we know that the tooth was handled by a family member without the necessary sterile precautions, which could have caused the contamination. Probability of Paternity was then calculated from the Alleged Father DNA profile with the male DNA profile from the bloody tissue and the Probability of Paternity was proven at >99.9%.

Conclusion

From the results obtained in the two case studies mentioned above, the new PrepFiler BTA™ extraction kit for forensic samples, especially developed for samples such as bones and teeth, performed significantly better than the QIAamp® DNA Micro kit, and the Identifiler® Plus kit performed better than the current Identifiler® kit. The Identifiler® Plus kit delivered better amplification of the larger molecular weight markers, higher overall signal intensity, cleaner baselines and improved heterozygous peak height balance.

Previously we were unable to obtain any DNA for these cases, but with the combination of the PrepFiler BTA™ chemistry and the new AmpFℓSTR® Identifiler® Plus kit from Applied Biosystems, we were able to generate full DNA profiles. The combination of both kits provided a powerful tool for the typing of difficult forensic samples.

How to Cite This Article

I. Ferreira (2011) Use of the PrepFiler BTA™ Forensic DNA Extraction Kit and the AmpFℓSTR® Identifiler® Plus Kit in Real Case Samples. *Forensic News* January; [Internet] 2011. [cited: year, month, date]; Available from: http://www.appliedbiosystems.com/ForensicNews-Past

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HID in action

An Efficient Method of Extracting DNA from Bone Remains from the Spanish Civil War—A Comparative Study of Two Methods: PrepFiler BTA™ and DNAzol® Methods

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Introduction

In recent years Spain began the process of recovering the bodies of people who went missing during the Spanish Civil War. This project has been named "Recovery of Historical Memory." The first 24 bodies which were lying in the "Pozo de las Brujas", (Arucas-Gran Canaria) were recovered in December 2008. These remains had been thrown inside a well on March 19, 1937. This well was approximately 50 metres deep and had never produced water (Figure 1).

Figure 1. The excavation site "Pozo de las Brujas".

The DNA identification work was conducted by two laboratories: Department of Genetics (University of La Laguna) and our laboratory, Forensic Genetics Service (University of Las Palmas de Gran Canaria). To obtain the DNA, both laboratories used the same extraction protocol involving DNAzol®/QIAquick® (Qiagen) reagents [1]. The mtDNA control region sequence of the 24 bodies was obtained and we were able to identify 14 unique haplotypes together with five couples who shared haplotypes. However, when comparing these sequences with those obtained from samples of suspected relatives, it was not possible to obtain conclusive identifications because of the limited number of appropriate samples available from relatives for comparison purposes.

Given the difficulties we encountered in establishing the identity using mtDNA, we decided to analyze the nuclear DNA using the new AmpFℓSTR® NGM™ PCR Amplification Kit from Life Technologies. This approach seemed to be promising due to the increased ability of the NGM™ Kit to recover information from severely degraded samples, and the kit's remarkably high tolerance to inhibitors. Moving on to the quantification, using RT-PCR of the DNA extracted, we were able to obtain values between 0.02 ng and 1.6 ng total DNA. However, the amplification and analysis of STRs resulted in only seven partial profiles.

At this time we became aware of a new optimized lysis buffer for difficult forensic samples, PrepFiler BTA™ kit (Bone, Teeth, Adhesive), which is used in combination with the Applied Biosystems® PrepFiler™ Forensic DNA Extraction kit. This extraction method was applied to 20 of the 24 remains detailed above. The results were very satisfactory. Starting with only 50 mg of pulverized bone (10 times less than the previous

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method), we obtained values between 0.05 to 5 ng of total DNA, and the STR analysis of these 20 individuals allowed us to obtain 13 complete profiles and five partial profiles.

Clearly better results were obtained using the PrepFiler BTA™ method, but to make a comparison between both methods, it was necessary to contrast the results based on the same sample from an individual and the same amount of powder. The goals we set for this comparative trial were to compare the DNA extraction efficiency of both methods and compare the quality of extracted DNA, contrasting the results obtained in the quantification by RT-PCR and STR profiles.

Materials and Methods

Prevention of Contamination

All extractions were performed in a dedicated laboratory physically separated from the main genetics department. The laboratory is constantly irradiated with UV lamps and frequently cleaned with bleach. All sample manipulations were performed in a laminar flow cabinet, with dedicated pipettes and sterile filter tips. Solutions were commercially acquired when possible; otherwise they were autoclaved and UV-treated. Lab coats, face shields, hats, and sterile gloves were used at all times. To monitor contamination during extraction, an extraction blank was processed together with the sample. PCR contamination was monitored using a negative control per reaction.

Samples

The samples used were pulverized femur bone samples about 71 years old. We selected five individuals and from each took four aliquots of 50 mg powder. Two aliquots were extracted independently and in parallel by the standard method of the extraction laboratory (DNAzol® reagent) and the other two by the PrepFiler BTA™ method. The pulverized samples were obtained from the less damaged areas of bone. A layer of approximately 1 mm was removed from the surface by sanding with a drilling machine in order to reduce contamination from previous handling. The sample was then ground to a fine powder with the drilling machine.

Extraction

DNAzol® Method:

> **Lysis:** 800 µL of DNAzol® reagent was added to 50 mg of bone powder and left in agitation (900 rpm) at room temperature for two and a half days.
>
> **DNA Purification:** The lysate was purified using the QIAquick® PCR Purification Protocol (Qiagen). The DNA elution volume was 100 µL.

PrepFiler BTA™ Method:

> **Lysis:** 220 µL of PrepFiler BTA™ lysis buffer plus 3 µL of DTT (1M) and 7 µL of Proteinase K (20mg/mL) were added to 50 mg of bone powder and left stirring at 1,100 rpm and 56º C for 18 hours.
>
> **DNA Purification:** The DNA purification was performed following the Standard Protocol of the PrepFiler™ Extraction kit. The DNA elution volume was 50 µL.

Real-Time PCR Quantification

The number of molecules used as a template for PCR amplification was determined by real-time PCR quantification using TaqMan® reagent–based chemistry in an Applied Biosystems® 7500 Real-Time PCR System. Primers, probes, thermal cycling, and analysis conditions were as described by Hudlow – 2008[2]. The quantification results were analyzed using SDS software v 1.4.

This real-time PCR assay consists of four probes that quantify non-degraded DNA (human and male), degraded DNA and the IPC (Internal PCR Control).

Concentration of DNA

DNA samples were concentrated using the Amicon Ultra 0.5 mL 100K centrifugal filter and by following their specifications for DNA/RNA concentration. For each method, the two DNA samples extracted from each individual were concentrated together.

Amplification

The extracted DNA was amplified with the AmpF*ℓ*STR® NGM™ PCR Amplification Kit (Applied Biosystems) in a GeneAmp® 9700 Thermal Cycler.

Electrophoresis and Analysis

PCR products were electrophoresed on an Applied Biosystems® 3130*xl* Genetic Analyzer. The STR profiles were analyzed using GeneMapper® ID Software v 3.2.1. The run modules and conditions and the data analysis were performed according to the NGM™ PCR kit protocol instructions.

Results and Discussion

Quantification

The quantification of DNA samples extracted by both methods (Table 1) reveals that only degraded DNA was obtained using the DNAzol® method. By contrast, using the PrepFiler BTA™ extraction method, it was possible to recover non-degraded DNA as reflected in the data obtained for samples 2, 5, 13, and 16. These results show that the extraction method using the PrepFiler BTA™ lysis buffer delivered better quality DNA than the DNAzol® extraction method.

	BTA Extraction Method			DNAzol® Extraction Method		
	Non-Degraded DNA Yield (ng)		Degraded DNA Yield (ng)	Non-Degraded DNA Yield (ng)		Degraded DNA Yield (ng)
Sample	Human	Male		Human	Male	
1	0.04	0	0.4	0	0	0.6
2	0.10	0	0.5	0	0	0.9
5	0.10	0.4	1.4	0	0	0.5
13	0.04	0.5	1.1	0	0	1.0
16	0.04	0	0.9	0	0	0.3

Table 1. Total DNA yield obtained from the BTA and DNAzol® extraction methods. This data is estimated based on the real-time PCR results.

STR Profiles

The comparison of the profiles obtained for different samples using the NGM™ PCR Amplification kit (Table 2) shows non-complete profiles with DNA obtained by DNAzol® extraction method, while four complete and a partial profile are obtained with the DNA extracted using the PrepFiler BTA™ method.

	Number of STR Loci Amplified using the NGM™ Kit	
Sample	BTA Extraction Method	DNAzol® Extraction Method
1	11/16	12/16
2	16/16	8/16
5	16/16	14/16
13	16/16	8/16
16	16/16	6/16

Table 2. Number of STR loci amplified using the AmpFℓSTR® NGM™ PCR Amplification Kit (Applied Biosystems) from DNA extracts obtained from the PrepFiler BTA™ and DNAzol® extraction methods.

The PrepFiler BTA™ method resulted in a far greater number of loci being recovered for the majority of the samples compared to the DNAzol® method. Even in situations where the DNAzol® method delivered a higher amount of DNA (e.g., Sample 2), the quality of the profile was higher as reflected in the profiles obtained (Figures 2, 3). These results confirmed what we had observed in the quantification results—that the DNA extracted using the PrepFiler BTA™ method was of higher quality than the DNA extracted using the DNAzol® method.

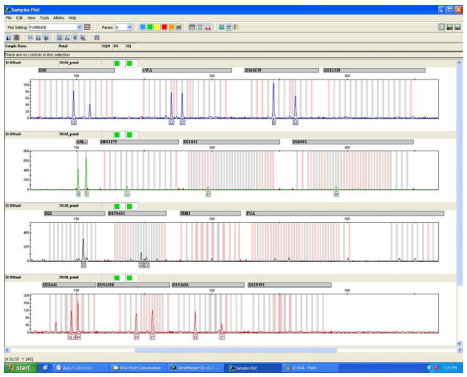

Figure 2. Electropherogram of sample 2 DNAzol® extracts amplified using 0.5ng DNA.

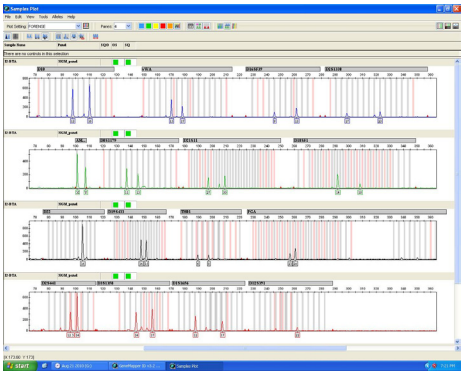

Figure 3. Electropherogram of sample 2 BTA extracts amplified using 0.3ng DNA.

Conclusions

The PrepFiler BTA™ method is a very efficient extraction method for obtaining high-quality DNA from ancient bones. The small amount of starting material required (50 mg) is another of its advantages as well as a reduction in processing time.

Moreover, most laboratories perform DNA extraction of ancient samples using homebrew methods. Using a commercial kit such as the PrepFiler BTA™ method will allow standardization of tests and therefore, standardization of results.

References

[1] Höss M, Pääbo S: DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Res 1993; 21: 3913– 3914.

[2] Hudlow W.R., Chong M.D., Swango K.L., Timken M.D., Buoncristiani M.R. A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A diagnostic tool for STR typing. Forensic Science International Genetics. 2008 2: 108-125.

How to Cite This Article

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HID in action

Optimization of miniSTR Analyses Allows for the Study of Ancient Degraded Samples

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The vertiginous progress in molecular biological techniques has promoted the retrieval and analysis of DNA from very diverse ancient specimens. This has broadened the scope of possibilities for genetic anthropology, enabling the study of a wide range of samples of historical interest and permitting the identification of important historical persons and the establishment of familial relationships as well as population migration studies. The development of new methodologies and the adoption of suitable laboratory practices and approaches makes it possible to obtain results of accreditable authenticity from a number of challenging samples that until recently were not amenable to analysis. However, along with the technical progresses, this new field has revealed certain peculiarities and drawbacks.

The special characteristics of ancient DNA (aDNA) determine the approaches chosen to study it. After overcoming inhibition, if present and possible, the next challenge is the great degree of molecular fragmentation. In this respect, mitochondrial DNA (mtDNA) was readily chosen as the preferred target as its elevated copy number in cells makes it more likely to survive than nuclear DNA. Thus, even in cases where little or no nuclear DNA was present, mtDNA was deemed to be the panacea that permitted genetic information to be obtained from difficult degraded samples.

We have analyzed a set of medieval human remains (carbon dated to 960–1300 AD) where a wide range of conditions were found in terms of quantity of nuclear DNA and presence of PCR inhibitors and contamination. Much effort has been made in obtaining clean, unambiguous mtDNA sequences. In a few cases, the attempts have been successful, although the authenticity of the sequences obtained cannot be guaranteed as molecular damage of DNA occurs inexorably over time and some base changes could have occurred. In other cases, in spite of following the strictest procedures to eliminate sample contamination and to avoid further contamination in the laboratory, contamination in the DNA extracts or in the amplified products could not be fully avoided and endogenous mtDNA sequences of the sample were completely masked and impossible to discern.

To date many research groups have reported successful results on mtDNA from ancient samples from very diverse origins. One of the most outstanding cases is the achievement of the mitochondrial genome of the Neanderthal[10] and some others from extremely well preserved samples such as those kept in permafrost[12]. There is no doubt about the advantages of the study of mtDNA. Nevertheless, it is important to put its applications in perspective in the most general sense. Firstly, when studying animal species different from human, it is generally possible to detect exogenous contamination from human sources, which is not always the case with human samples. Secondly, the excellent degree of preservation conferred by constant low (below zero) temperatures is not the most common situation in the majority of cases of study. Thirdly, many of these exciting reports are the result of laboratory consortiums and the common effort of up to fifty researchers working with cutting-edge, and therefore, highly expensive technology and equipments. This is not the reality of the great bulk of laboratories currently working in forensic genetics today, as in many cases they cannot undertake

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such investments. Moreover, in many cases of study on human ancient DNA the ultimate goal is to establish familial relationships, which may be limited if only a uniparental genetic marker such as mtDNA is used.

Alternatives to mtDNA analysis exist. The analysis of short tandem repeats (STRs), which have the greatest discriminating power, is usually unsuccessful when dealing with degraded and scarce samples. The quest for extracting information from nuclear DNA, even in the most adverse situations, has led to the development of markers of reduced size, such as the miniSTRs, or even to the analysis of single nucleotide polymorphisms (SNPs)[5]. MiniSTRs are considered to be the most effective approach for degraded DNA[5]. Commercial laboratories have readily developed kits containing informative miniSTRs that enable the study of difficult samples while allowing comparisons with the standardized core loci STRs. Moreover, the recently introduced generation of kits not only have reduced the size of amplicons but also improved buffer formulations and primer synthesis processes, resulting in an increase in the sensitivity and success rates in the presence of inhibitors, high-quality profiles, and low baselines.

In our experience with genotyping medieval human remains, we have studied autosomal STR loci using a standard human identification STR kit (AmpFℓSTR® Identifiler® PCR Amplification Kit, Applied Biosystems) in cases where the amount of nuclear DNA retrieved allowed its use. However, in many of them, even when the amount of DNA was adequate for conventional STR analysis, DNA was degraded to the point that the Identifiler® kit would fail. MiniSTR loci were then typed using the AmpFℓSTR® MiniFiler™ PCR Amplification kit (Applied Biosystems). MiniSTRs have proven to be the best approach in most cases, obtaining full or partial miniSTRs profiles when conventional STRs failed. The advent of the AmpFℓSTR® NGM™ PCR Amplification kit (Applied Biosystems) further maximizes the chances of obtaining a result from severely degraded samples, even in the presence of high levels of inhibitors.

Forensic scientists have used different strategies to increase the sensitivity of routine typing systems available in order to augment the molecular information obtained from degraded skeletal remains, which have been grouped under the term "low copy number" (LCN) typing.[6,4,13,9,2]. The MiniFiler™ kit is also amenable to optimization when the recommended working conditions are not successful for typing, especially for valuable samples. An interesting case in our lab was a medieval bone sample that yielded a DNA amount amenable to being analyzed by the MiniFiler™ kit, but no results were generated using either the standard conditions or the same method with increased number of cycles (36 instead of 30; Figure 1A). Nevertheless, seven miniSTRs (out of eight) plus the amelogenin marker were obtained after further optimization of the typing protocol, which consisted of doubling the amount of PCR master mix and primer mix and adding extra magnesium (0.5 µL 50 mM $MgSO^4$) and Taq polymerase (0.5 µL AmpliTaq Gold® DNA Polymerase, Applied Biosystems) to the PCR reaction in a final volume of 38 µL (Figure 1B). Cycling conditions were performed according to the manufacturer's recommendations, but six additional PCR cycles were used (36 cycles instead of 30). The DNA amount per PCR reaction was within the recommended range (0.50–0.75 ng). Another example is presented in Figure 2.

Raising the number of cycles greatly increases PCR sensitivity. Nevertheless, when the input DNA is too low, there is a risk of fluctuation effects due to stochastic sampling of the two alleles present in a heterozygous individual, generally manifested as a peak imbalance of the alleles.[7,2] For this reason, cautious interpretation of LCN STRs results is needed. On the other hand, it is generally possible to produce results in duplicates or triplicates, and ideally, more than one sample from the same individual can be analyzed, thus increasing the confidence and robustness of the analysis.

The use of highly optimized miniSTR typing systems allows for the genotyping of ancient human remains, proves to be a valuable tool for genetic anthropology and in many cases becomes an excellent alternative to mtDNA analysis as a first choice approach in genotyping ancient degraded samples. The possibility of obtaining information from nuclear DNA from compromised samples is very well received, as the STR loci are the most informative genetic markers for identity testing.

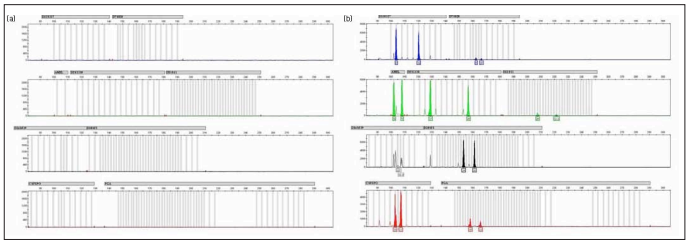

Figure 1: A medieval bone sample was extracted with a silica DNA binding method [8]. STR amplification was performed with the MiniFiler™ kit. (A) Amplification with the MiniFiler™ kit under the recommended conditions produced no results. (B) Seven out of eight miniSTRs plus the amelogenin marker were obtained after optimization of amplification with the MiniFiler™ kit (see text).

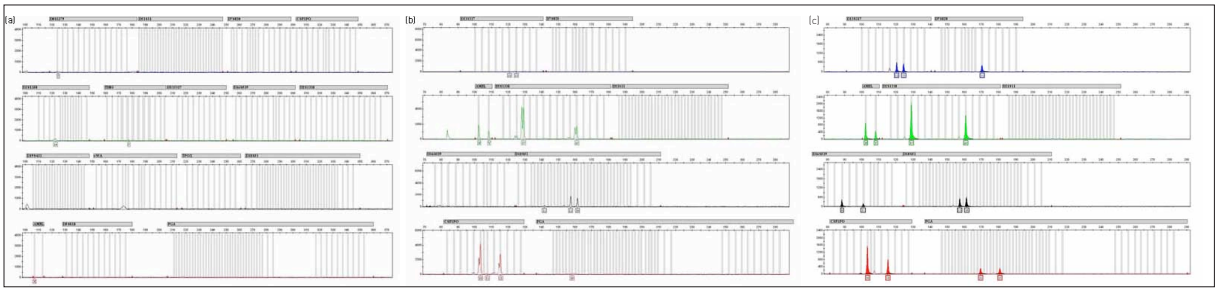

Figure 2: Example of miniSTR analysis on a case study. A medieval bone sample was extracted with a silica DNA binding method [8]. STR amplification was performed with both Identifiler® and the MiniFiler™ kits. (A) Amplification with the Identifiler® kit produced no results. (B) Amplification with the MiniFiler™ kit under the recommended conditions produced a partial profile. (C) Seven out of eight miniSTRs plus the amelogenin marker were obtained after optimization of amplification with the MiniFiler™ kit (the number of cycles was raised from 30 to 35 and 0.8µL Taq polymerase were added per reaction).

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HID in action

Application of the Applied Biosystems PrepFiler™ Forensic DNA Extraction Kit in the Identification of 23 Fire Victims

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In the face of mass disasters, Institutes of Forensic Medicine and Criminology Laboratories are tasked with the problem of victim identification. The problem of identification has recently become one of the most important issues in forensic medicine. Events leading to mass disasters and resulting in numerous victims (fires, air catastrophes, natural disasters, bomb explosions, etc.) are becoming more and more common. Every event that involves victims, in some cases many of them, demands from us an individual attitude. In the case of fire victims, identification based on DNA comparison offers a huge advantage over traditional techniques. Remains of fire victims undergo deep transformation in high temperatures, thus making the use of traditional identification methods impossible. Most often corpses are detected over different time scales (up to several days) and due to this the extent of decay can vary for each corpse. The advantage of using genetic identification methods lies in the fact that the DNA profile of an individual can be obtained from a very small amount of preserved biological material.

Aim:
The aim of the examination was to determine the identity of 23 people, victims of a fire in an apartment building in Kamień Pomorski, using the Applied Biosystems® PrepFiler™ Forensic DNA Extraction Kit as part of the workflow.

Material And Methods:
The accident took place overnight on the 12th of April 2009. The fire enveloped the first and second doors of the apartment block at 11 Wolińska Street. Human remains found at the site of the fire were collected into 22 bags marked with apartment numbers. The number of missing persons was estimated at 23, seven male and 16 female. The group in question was made up of people of different ages – from two to 89 years old. DNA polymorphism examinations were started immediately after evidence material had been delivered to the laboratory. During autopsy, samples of blood were collected on sterile swabs. Liquid blood, muscle fragments, bones and teeth were also collected. The comparative material used was pharyngeal swabs collected from the closest relatives. The samples were extracted with the Applied Biosystems® PrepFiler™ Forensic DNA Extraction Kit. The DNA extracted was subjected to enzymatic amplification (PCR) using the AmpFℓSTR® SGM Plus® and AmpFℓSTR® YFiler® personal identification kits.

The samples for identification were collected during forensic examination and autopsy. The human remains had been seriously changed due to the high temperatures, were incomplete, the soft tissues were burnt and charred to a great extent, whole limbs or parts of limbs were missing, and corpses were in multiple parts and mixed up with bedding (Fig. 1 and 2). In all 120 samples were collected. These samples consisted of:

- 29 soft tissue fragments
- 23 bone fragments
- 26 teeth
- 42 blood samples collected with sterile swabs

Sampling and securing of material for DNA examination took place in the prosectorium of the Forensic Medicine Institute. The process of collecting samples for genetic analysis lasted two days and was undertaken in the presence of a forensic medicine expert in the field of genetics.

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Figure 1: Burned corpse from the scene of accident.

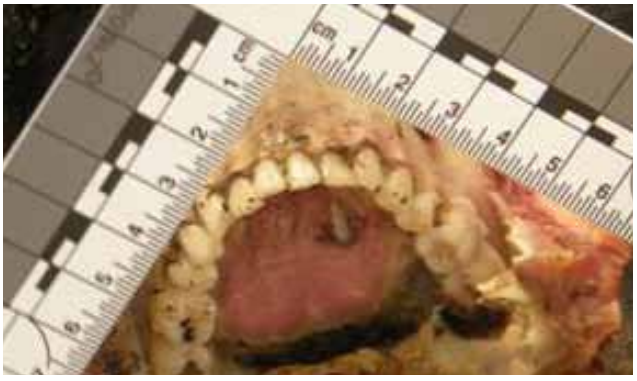

Figure 2: Samples collected at the scene of accident.

Comparative material
In the fire, 23 people lost their lives, including three entire families. The comparative materials used for DNA examination were samples extracted from biological relatives such as swabs from an oral cavity mucosa. The total number of reference samples used was 18.

Extraction
All examined evidence samples (with the exception of bone and teeth fragments) and comparative materials were treated with the PrepFiler™ Forensic DNA Extraction Kit. The kit is characterized by its exceptionally efficient capability for the binding and recovering of DNA using magnetic particle structures. It was specially designed for extracting genomic DNA from a vast scope of forensic samples. Forensic samples often contain chemical compounds which impede the amplification of nucleic acids during PCR reactions. The kit enables the removal of inhibitors and helps improve general efficiency, clarity and concentration of the extracted DNA for both difficult and routine forensic samples.

For these reasons it was decided to use the PrepFiler™ kit for extraction. Standard DNA extraction protocol was used for the process. This protocol is used for most forensic samples.

The extraction process using the PrepFiler™ kit is a gradual one that involves five different stages.

Stage I: The Lysis
Measured muscle fragments were cleaned and placed in the PrepFiler™ Spin Tube. 300 µL of the PrepFiler™ Lysis Buffer and 3 µL of the DTT buffer were added to the materials. This was then incubated in a thermomixer for one hour at a temperature of 70°C.

Stage II: Elimination of substrates from the sample lysate
The sample content together with the substrate was put on the PrepFiler™ Filter Column and then placed in the PrepFiler™ Spin Tube and centrifuged for two minutes at 14000 rpm. After removing the PrepFiler™ Filter Column we had 180 µL of lysate in the PrepFiler™ Spin Tube.

Stage III: Binding of genomic DNA with magnetic particles
After removing the substrates, 15 µL of the PrepFiler™ magnetic particles were added to the tube containing the lysate. After vortexing 180 µL, isopropanol was added and then put into a shaker. The sample was shaken for ten minutes at room temperature at 1000 rpm.

Stage IV: Rinsing of bound DNA

DNA particles, which were bound with magnetic particles, were placed in a magnetic column for two minutes. After this time the visible liquid was carefully removed and rinsed through adding 300 µL PrepFiler™ Wash Buffer. The sample was vortexed and placed in a magnetic column for an additional two minutes and the liquid was removed. This activity was repeated twice more. After removing the liquid three times, the magnetic column was opened and the DNA bound with magnetic particles was dried for seven minutes.

Stage V: Elution of DNA

At this stage DNA eluate was separated from the magnetic particles by adding 50 µl of the PrepFiler™ Elution Buffer to the tube containing cleaned DNA. It was then placed in a thermocycler and incubated for five minutes at 70° C and 900 rpm. The sample was placed in a magnetic column for two minutes and the liquid containing DNA was then transferred to a new tube.

The extracted DNA underwent enzymatic amplification (PCR) with the use of the personal identification kits, AmpFℓSTR® SGM Plus® and AmpFℓSTR® Yfiler® kits, according to manufacturer's recommendations. DNA profile analysis was performed with the use of the Applied Biosystems® Genetic Analyzer 3130 and the results were analyzed using the GeneMapper® *ID-X* v1.1 (Applied Biosystems, USA) software. Biostatistical calculations were made with support of DNA Stat1.0 and PATCAN V.1.1 softwares.

Results:

Determined DNA Profiles Of Fire Victims

While examining the 28 muscle samples with the SGM Plus® kit the following results were obtained:

- 21 full DNA profiles
- Three profiles missing results for one marker
- One profile missing results for two markers
- One profile missing results for four markers
- One profile missing results for seven markers
- One complete amplification failure

On examining 23 samples taken on cotton swabs with the SGM Plus® kit the results obtained were:

- 18 full DNA profiles
- One profile missing result for one marker
- Two complete amplification failures

Samples extracted from the muscle fragments were also examined with the use of the Yfiler® kit. After examining eight samples the following was observed:

- Five profiles missing results for one marker
- One profile missing results for three markers
- One profile missing results for five markers
- One profile missing results for seven markers

Combining the above results, 23 full profiles of fire victims were obtained.

SAMPLE	D3S1358	VWA	D16S539	D2S1338	AMG	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
1	14-16	16-17	11-12	17-18	XX	12-15	27-32.2	12-18	12-15	6-8	22-25
2	14-15	16-18	11-12	17-20	XX	8-15	27-29	14-18	15-15.2	6-8	20-22
3	14-16	16-19	12-13	17-19	XX	8-11	29	12-18	14-15	6-8	22-23
4	17-18	17-18	12	19-24	XX	11-12	29-32.2	17-19	13-15	6-9	20-21
5	15-18	15-17	12	17-24	XX	11-15	32.2-33.2	17	14.2-15	6-7	20-22
6	14-16	17-18	9-13	19-24	XX	13-14	30-33.2	11-21	15-15.2	6-9.3	20-24
7	17-18	17	12-13	16-23	XX	14	31-32.2	13-15	16-16.2	9.3	20-21
8	15-17	15-17	12-13	16-24	XY	14	31-31.2	15-16	16	8-9.3	21-23
9	17-18	17	12	16-23	XX	13-14	31-32.2	13-15	16-16.2	8-9.3	20-21
10	16-18	15-17	12	20-24	XY	11-14	32.2-33.2	15-17	14-15	6	21-25
11	14-15	17-18	12-13	17-20	XY	13-15	29-31.2	14	12-15.2	8-9	20
12	15-17	14-19	11-13	16-24	XX	14-15	30.2-31.2	12-15	13-14	7-8	19-20
13	15-16	15	12	17-20	XY	14-15	30-33.2	15-17	14-14.2	6-7	22-25
14	17-18	17-19	8-9	23	XX	12-13	30	12-16	14	6-9	21-26
15	17	17	12-13	16-25	XX	14	31.2-32.2	13-16	16-16.2	8	20-21
16	15-18	15-17	12	16-23	XX	13-14	31-32.2	13-16	16-16.2	8	20-21
17	16	15	9-10	20	XY	10-12	31.2-32	14-18	13-14	6-9.3	19-25
18	15-16	16-17	11-12	18-20	XX	11-15	29-32.2	12-18	14-15	6-8	22-25
19	15-18	15-17	12	17-24	XY	11-14	29-33.2	15-19	13-14	7-9	20-25
20	17-18	17	12	23-25	XX	13-14	30-32.2	12-13	13-16.2	8-9.3	20
21	15	17-19	9-12	24-25	XX	11-13	28	15-18	14-15	7-8	23-25
22	16-17	15-16	11-14	16-25	XX	12-13	28-29	14-15	14-15	9-9.3	20-26
23	16-17	15-17	12-13	19-24	XY	10-11	28	13-17	14	9.3	23-24

Table 1: DNA polymorphism results obtained for all victims using the personal identification system AmpFℓSTR® SGM Plus® kit.

	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385 a/b	DYS393	DYS391	DYS439	DYS635	DYS392	Y GATA H4	DYS437	DYS438	DYS448
8	16	13	24	29	16	-	11/14	13	11	12	23	13	13	15	12	19
10	-	13	24	30	19	-	17/18	13	10	-	23	11	11	14	10	20
11	-	13	-	-	16	-	10/14	13	10	-	23	-	-	14	11	20
13	17	13	24	30	19	13	17/18	13	10	12	23	-	11	14	10	20
17	17	13	25	-	16	-	9/14	13	10	-	23	-	-	14	11	20
19	17	13	24	30	19	13	17/18	13	10	12	23	-	11	14	10	20
23	16	13	25	29	16	-	11/15	13	10	11	23	11	12	14	11	20

Table 2: DNA polymorphism results obtained for all victims using the personal identification system AmpFℓSTR® YFiler® kit.

Discussion:

120 samples from the remains of 32 corpses were taken during the examination. 23 genetic profiles were obtained and compared to the DNA profiles of the victims' relatives. All 23 victims of the fire were identified during the examination. All of these profiles were determined within 24 hours of the commencement of the genetic examination and the victim identification was finished in a week. The method of DNA extraction used (PrepFiler™ kit) proved to be exceptionally efficient, enabling fast DNA extraction from materials such as blood and soft tissues. In cases of mass disasters the identification of victims and the length of time it takes is of great importance. The application of a universal method for different kinds of biological and reference material considerably reduces the length of time needed for examination. The product obtained during the DNA extraction with the PrepFiler™ kit showed only a small degree of degradation and thus led to a quicker examination.

Conclusions:

- In spite of the fact that the examined remains were greatly charred full DNA profiles were obtained using the SGM Plus® kit
- The extraction method used here led to an exceptionally fast execution of the identification examination
- The PrepFiler™ kit DNA Extraction Kit can be widely used for the identification of victims of mass disasters

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HID in action

DNA Extraction from Casework Samples using the Applied Biosystems® AutoMate *Express*™ Forensic DNA Extraction System

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The Department of Forensic Molecular Biology at the University of Berne, Switzerland, is a medium throughput forensic DNA department with an annual load of approximately 6,000 forensic casework samples and 3,500 offender reference samples, in the form of buccal swabs. All these samples are required to be tested in duplicate before submission to the Swiss national DNA Database. Casework sample profiles are required to be submitted to the National Database within twelve days and offender reference profiles within six days. With a staff of three analysts supported by seven technicians, meeting these deadlines is a somewhat daunting task. We have over many years developed and validated manual DNA extraction methods utilising organic solvents from forensic samples on a variety of substrates. We became very efficient but with increasing workloads found this to be inadequate, so consequently we looked to automate some of our processes.

In 2007 we introduced a cartridge based automated DNA extraction instrument into our laboratory which significantly decreased our turnaround time without a loss of quality. In 2008 our department was approached to take part in the test site studies for the Applied Biosystems® PrepFiler™ manual DNA extraction kit. We found that the chemistry utilized resulted in very "clean" DNA with efficient removal of inhibitors. We eagerly awaited the development of this system onto a small platform and were very pleased at the development of the AutoMate *Express*™ Instrument, and that we were able to test this in January of 2010. After a successful test period, we decided to purchase this instrument and have since introduced it into our routine processing of casework samples.

The AutoMate *Express*™ Forensic DNA Extraction System

The AutoMate *Express*™ Instrument processes up to 13 samples in one run. For our laboratory this is ideal as we can simultaneously extract DNA from 12 samples and one negative control. Downstream this translates conveniently to a single electrophoresis run on a 3130*xl* Genetic Analyzer with PCR positive and negative controls and Allelic Ladder (16 samples).

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We are using both the PrepFiler Express™ and the PrepFiler Express BTA™ Forensic DNA Extraction Kits for the processing of our samples. All reagents (except DTT), tubes and pipettes are conveniently supplied within the individual Kits. The general procedure is the lysis of the samples in special PrepFiler LySep™ Columns after which the lysates is simply centrifuged to separate this from the "carrier" (swab, clothing, cigarette filter etc.). The incubation times are short, 40 minutes for body fluids and two hours for powdered bone.

The PrepFiler Express™ Forensic DNA Extraction Kit has given us reliable results for the extraction of DNA from all types of samples resulting in very "clean" DNA, even from difficult stains such as blood on denim. The PrepFiler Express BTA™ Forensic DNA Extraction Kit has proven to be excellent for the extraction of DNA from samples such as bones and stamps and we prefer to process our cigarette butts using this.

Our one major modification of the process has been to carry out the lysis overnight as this gives us the convenience of processing a variety of samples in a series of twelve the following morning. There have been no undue "side effects" in the quantity or quality of DNA obtained from using this extended incubation time. However, we have to use tubes with screw caps to prevent evaporation of the lysis solution thus preventing crystallization of the high salt content.

In general we have found that the AutoMate *Express*™ Instrument is easy to use, clean and is also fast. However, if we had a wish list to make it even more suitable for our workflow it would be for the following: The processing volume, 500µl for the PrepFiler Express™ and 230µl for the PrepFiler BTA™ kit is relatively small. We often receive two swabs from touch or contact DNA traces and it is simply not possible to combine both swabs into one extraction process. Another "wish" would be to increase flexibility by being able to choose between different final elution volumes, such as 75µl or 100µl in addition to the standard 50µl. We believe these to be resolvable problems involving slight modification in the scripting of the process and would go a long way towards making a very good instrument even better.

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technical focus

Implementation of Next-Generation STR Kits in Europe: Challenges and Solutions

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Life Technologies

Introduction

In 2005, the European forensic community published recommendations on the development of new STR multiplexes to provide greater discrimination power and enhanced performance to facilitate expansion of data sharing initiatives between European countries. The AmpFℓSTR® NGM™ and NGM SElect™ kits have been developed in direct response to these recommendations for high performance, highly discriminating STR kits.

The NGM™ and NGM SElect™ kits contain all 12 loci specified in the recently expanded European Standard Set of Loci (ESSL), together with the remaining markers from the SGM Plus® kit and the SE33 locus (NGM SElect™ kit only). Together, the NGM™ kits provide options that enable all European countries to obtain results for all required loci—including the SE33 locus, if required—from a single multiplex. In addition, reformulated reaction reagents and a greater number of loci concentrated in the low molecular weight region enable enhanced sensitivity and the ability to recover results from degraded and severely inhibited samples.

Successful and efficient analysis of difficult forensic samples does not rely on loci configuration and performance alone, however. That's why Applied Biosystems next-generation STR kits have also been designed to streamline the implementation process, promote a highly optimized laboratory workflow, and enhance data quality for confident and efficient interpretation.

Data Quality Worth Sharing

The expansion of the ESSL was a major step towards establishing routine data sharing between European countries, but the success of such initiatives will also depend on several other key factors. The most critical of these may be the long-term quality of the data contained within each national database once the new loci are introduced.

As a result of the limited size of the previous ESSL, only seven loci are common to all currently operational European DNA databases. Consider now the effect when some of these loci do not produce results due to the compromised nature of the sample. With only three or four loci available for comparison, any search across databases may yield hundreds, if not thousands, of adventitious matches, decreasing significantly the effectiveness of cross-border searches in detecting those responsible for criminal activity.

The expansion of the ESSL to 12 loci will ensure that, in the long term, national databases have more loci in common. But it is the enhanced capability of next-generation multiplexes such as the NGM™ and NGM SElect™ kits that will help ensure that more of those loci produce unambiguous results, maximizing the effectiveness of international data exchange.

Implementation of Next-Generation STR Kits: Challenges

Although our next-generation chemistries have been developed to help combat the performance challenges encountered regularly by forensic laboratories, a number of interpretational and logistical challenges exist that must be addressed before the promise of better performance can become an operational reality.

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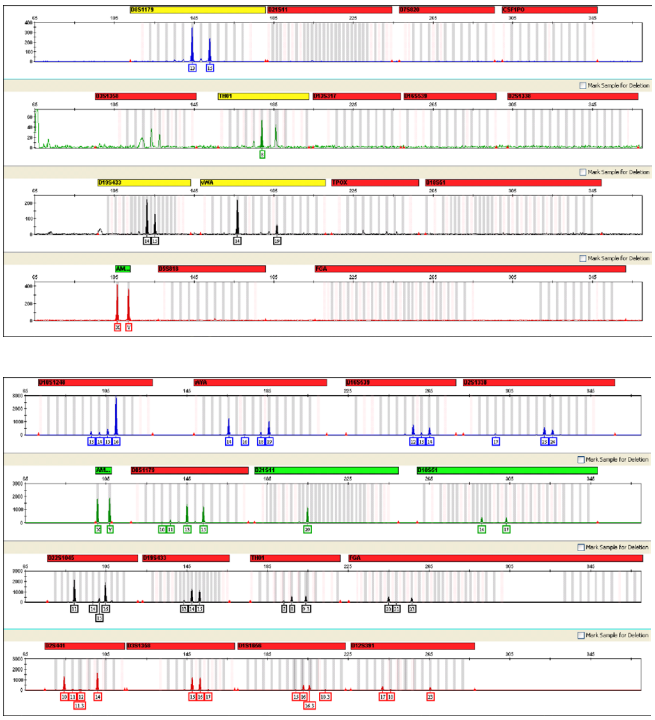

Figure 1. An illustration of how the improved performance capabilities of next-generation kits may result in the detection of more mixtures than with previous kits. An Identifiler® kit amplification of a sample taken from a pair of worn gloves (top panel) yields a weak, apparently single-source profile. An NGM™ kit amplification of the same sample (bottom panel) reveals a mixture originating from at least two contributors. (Results courtesy of Laboratorio de Biologia, Guardia Civil, Madrid, Spain.)

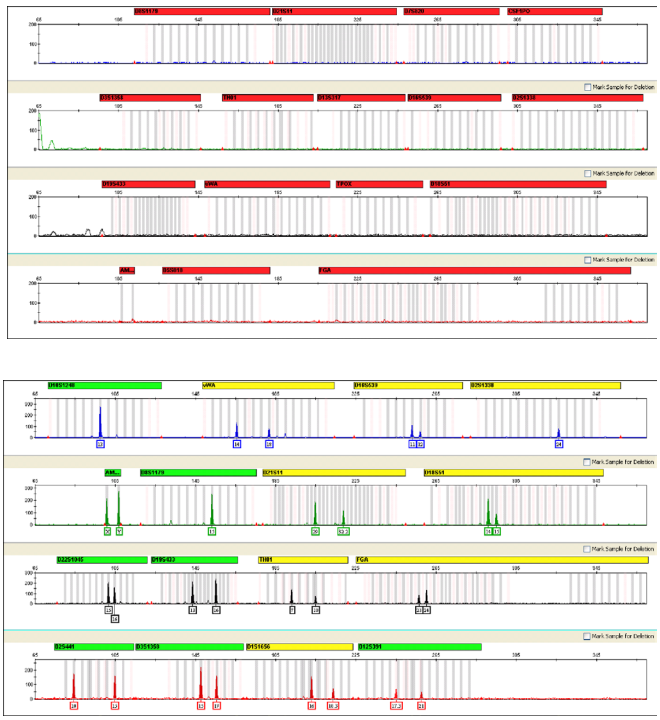

Figure 2. An illustration of how the increased performance capabilities of next-generation kits may result in more samples producing a result after failing to register the presence of DNA when quantitated. A bone sample that failed to produce a quantitation value also failed to produce a result when amplified with the Identifiler® kit (left panel). The same sample, when amplified with the NGM™ kit (right panel) yielded results for all 16 loci. (Data Courtesy of RIS Carabinieri Roma, Italy.)

- *Challenge 1*: The increased sensitivity and robustness afforded by the NGM™ and NGM SElect™ kits will lead to detection of (1) higher overall peak heights, (2) lower levels of input DNA and (3) a greater frequency of mixed samples. Laboratories will need to ensure that their instrument platforms are well maintained and calibrated to cope with the increase in signal. Their interpretation guidelines must also evolve to handle more stochastic results and more results from multi-contributor mixtures (Figure 1).
- *Challenge 2*: The introduction of larger and more capable multiplexes will lead inevitably to increases in the amount of time required for data analysis. This will require laboratories to reassess their resource requirements and distribution.
- *Challenge 3*: The ability to generate results from samples previously classed as "un-amplifiable" (Figure 2) will require laboratories to examine how quantitation results should be interpreted, especially for samples generating a negative quantitation value that may now be more likely to generate an STR result.
- *Challenge 4*: The level of concordance between new and existing kits should be considered in terms of the anticipated frequency of non-concordant events and the impact this may have on the laboratory workflow. This may affect the number of sample re-amplifications that may be required for genotype confirmation, as well as the efficiency of searches against national and international databases.
- *Challenge 5*: The introduction of a new technique often requires a diversion of resources from routine operations to (1) conduct the validation experiments needed to generate appropriate standard operating procedures and interpretation guidelines, and (2) train laboratory staff to become familiar with the new method and its operation.
- *Challenge 6*: The laboratory is not alone in facing implementation hurdles. Database authorities across Europe must ensure their national infrastructure can accommodate the five new loci. Match search algorithms may also require configuration to address the potential for non-concordant results that may arise from the wider use of alternative primer sequences for the same loci.

This list is by no means exhaustive but represents the most common concerns raised by forensic laboratories during discussions regarding implementation of next-generation STR kits. These and other considerations must be addressed before forensic laboratories and law enforcement agencies are able to take advantage of the opportunities afforded by the latest technological developments.

The NGM™ & NGM SElect™ Kits: Designed with Implementation in Mind

Fortunately, the NGM™ and NGM SElect™ kits have been developed to not only deliver enhanced performance and discrimination, but also simplify the whole implementation process as much as possible. Designed specifically to promote an optimized laboratory workflow and enable analysts to interpret results with enhanced confidence, the NGM™ and NGM SElect™ kits address many of the challenges highlighted above.

One Sample, One Amplification

Consistent with recent industry trends and responding to feedback from the ENFSI and EDNAP groups, the NGM™ and NGM SElect™ kits have been designed to enable laboratories to obtain high-quality results from the majority of database and casework samples with one kit and one amplification. A single amplification approach provides numerous benefits, including:

- Reduced time required for validation and analyst training prior to implementation
- Reduced sample processing and analysis time
- Reduced cost associated with the implementation process and the sample processing workflow
- Increased sample throughput capabilities, without the need for additional staff and/or automated system resources

Exceptional Data Quality

Maximizing all aspects of data quality is one of the best ways to promote an optimized workflow and increase the confidence with which data can be interpreted. In the NGM™ and NGM SElect™ kits, highly optimized dye chemistry combines with the latest primer manufacturing techniques to enable superior multiplex system performance and generate a result that is consistently more balanced than ever before.

- *A Good Dye's Work*
  The NGM™ and NGM SElect™ kits utilize the proven, highly optimized G5 dye set already used in the Identifiler®, SEfiler Plus™, MiniFiler™ and Yfiler® kits. This dye set is designed to be fully compatible with the range of Applied Biosystems capillary electrophoresis platforms validated for forensic applications, enabling creation of high-quality matrix and spectral calibrations. Optimizing the spectral calibration file is one of the simplest ways to maximize data quality and simplify the data analysis process. By eliminating pull-up peaks, profiles become simpler to interpret for analysts as well as expert system software packages. Single-source samples can pass more easily through expert system software, while analysts spend less time editing profiles for the superfluous labels that are inevitable if using either a sub-optimal spectral or a chemistry based on less compatible dye systems (Figure 3).

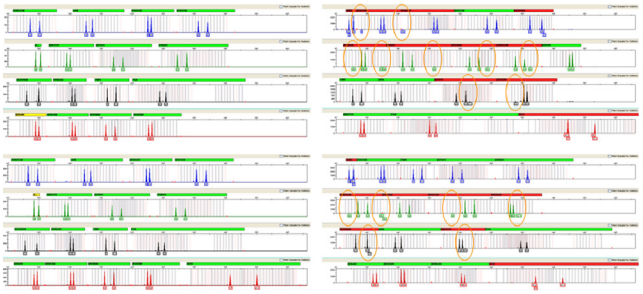

Figure 3. An illustration of the benefits of using a dye set designed to be fully compatible with Applied Biosystems capillary electrophoresis platforms. When analyzed with an optimal spectral calibration for the G5 dye set, the NGM™ (top left) and NGM SElect™ (bottom left) kits produce clean results that require little or no editing from the user. Profiles from other commercially available kits (top and bottom right) show significant interference from spectral pull-up (indicated by the orange circles) resulting from the use of less compatible dye sets (all electropherograms show signal in the 2,000–3,000 RFU range). These profiles require considerable user editing to remove superfluous labels, increasing analysis time and reducing the effectiveness of expert system software analysis.

- *Finely Balanced*
  Balance across all aspects of the profile is a key measure of profile quality. The NGM™ and NGM SElect™ kits have been designed to maximize the balance within and between each dye color and between the two alleles of a heterozygote to enhance the efficiency with which single-source samples can be analyzed using expert system software and assist with the interpretation of mixtures. Heterozygote peak height ratio can be helpful in determining the number of contributors present in a mixed sample and to evaluate the genotype of the minor contributor(s). Studies associated with the development of previous AmpFℓSTR® kits demonstrated that heterozygote peak height ratio is primarily a function of input DNA. Therefore, the NGM™ and NGM SElect™ kits have been optimized for 1 ng input DNA and 29 cycles of PCR to maximize the balance between sensitivity of the system as a whole and the heterozygote peak height ratio of individual loci (Figure 4). Balancing the loci within a color, such that one locus does not outperform another, enhances the ability to detect a full profile for the minor contributor to a mixture, even when the minor contributor may be present at a comparatively low level (Figure 5).

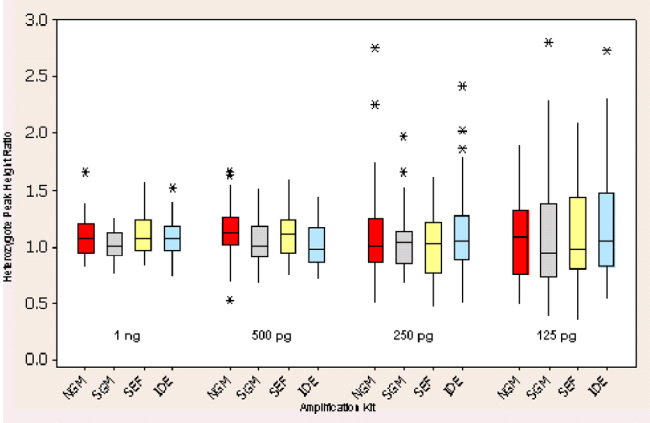

Figure 4. An illustration of the level of heterozygote peak height ratio expected as DNA input concentrations are reduced for the NGM™, SGM Plus®, SEfiler Plus™ and Identifiler® kits. The best levels of balance are achieved using higher input DNA concentrations; therefore, the NGM™ and NGM SElect™ kits are optimized for a combination of 1ng input DNA concentration and 29 amplification cycles.

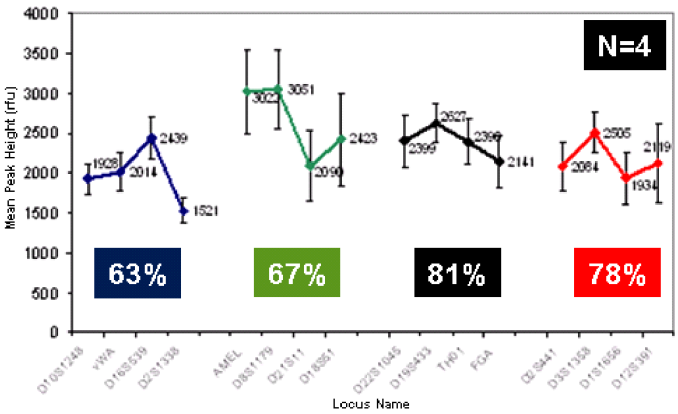

Figure 5. An illustration of the level of balance expected both within and between dye colors for the NGM™ kit. Both the NGM™ and NGM SElect™ kits are designed to maximize balance within a color and take advantage of the variable binning capability of the Applied Biosystems CE platforms to deliver improved balance between dye colors. More balanced profiles render all sample types easier to interpret. Single-source samples pass more efficiently through expert system analysis software and the minor contributors in mixed samples are more likely to be detected in casework samples.

- *Improved Baseline Quality*
  To further enhance profile quality, significant efforts have been invested in improving our manufacturing processes, particularly those relating to primer production. The new methods have resulted in the removal of small, dye-related artifacts in the low molecular weight region, generating significantly cleaner baselines (Figure 6). Cleaner baselines speed up the analysis process by reducing the degree of editing required and simplifying the interpretation process.

Maintenance of Existing Primer Sequences

Whenever a new kit development project commences, there is always the opportunity to modify primer sequences for any of the loci in the system. However, there are significant benefits associated with maintaining, when possible, the primer sequences for loci common to existing AmpFℓSTR® chemistries. These include concordance of results, confidence in the performance of the system and simplified analyst training.

- *Maximized Result Concordance*
  One of the most important considerations when implementing a new STR kit is the level of concordance that may be expected when comparing new data with the vast repositories of historical data currently stored in national DNA databases. By utilizing the same primer sequences for loci common to other AmpFℓSTR® kits, comparisons between new and existing results are simplified. Amplifications with the same primers should be expected to deliver identical results (barring any compromise to the sample that causes a partial profile or other anomaly to occur). This helps reduce the occurrence of mismatches and the need for re-amplification of samples to verify the correct genotype.

If using a sample processing strategy that involves two amplifications of a sample with two kits that use different primer sequences for overlapping loci, there is the possibility of obtaining different results for the two amplifications due to the presence of primer binding site mutations affecting one of the two primer sets. While this is a fairly common and well understood occurrence within the forensic laboratory, questions may arise regarding which result to load to a national database and how to explain the two different results to a non-scientific jury in court. Using a single kit for all amplifications containing primer sequences consistent with those used to generate results already stored in national databases simplifies all aspects of result reporting and comparison. This is one of the significant advantages offered by the NGM™ and NGM SElect™ kits.

Result concordance is further enhanced by maintaining both the allelic ladder allele ranges and the number of physical and virtual bins contained in the panel and bin sets. This is consistent for all loci in the NGM™ and NGM SElect™ kits, which are also common to the SGM Plus®, Identifiler®, SEfiler Plus™, and MiniFiler™ kits (Figure 7). Therefore, the panel and bin sets associated with the new kits genotype exactly the same alleles as many previous kits for the common loci, thus eliminating the possibility of non-concordance arising from the use of different allelic ladders with different ranges in different kits.

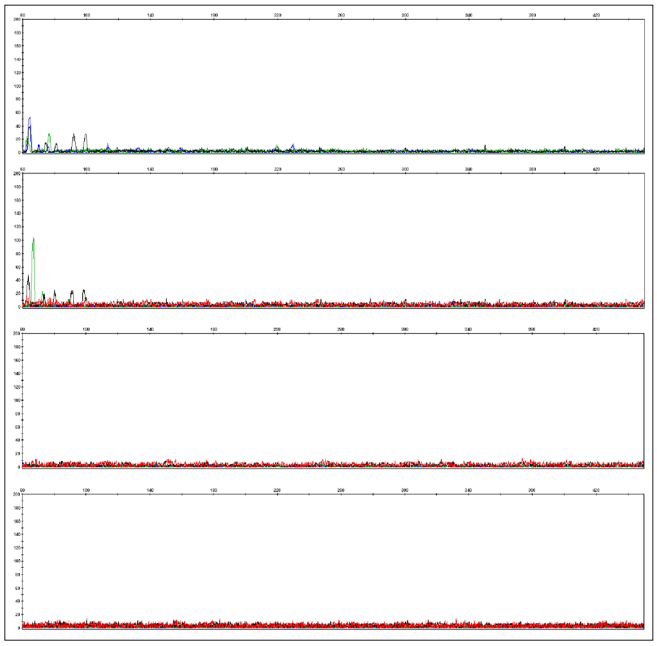

Figure 6. A comparison of negative control reactions for the SGM Plus® (top panel), Identifiler® (second panel), NGM™ (third panel) and NGM SElect™ (bottom panel) kits. The improved primer manufacturing process introduced for the NGM™ and NGM Select™ kits results in significantly cleaner baselines than for previous AmpFℓSTR® kits.

- *Confidence in Multiplex Performance*
  The construction of large multiplex STR systems is a complex process, and the most time consuming and challenging aspect of this is the design and subsequent testing of the primers. A key criterion associated with the construction of large multiplexes is to ensure the specificity of the primers with regard to their interactions with other primers in the multiplex. As multiplexes become larger, the number of primers required also increases, raising the possibility that each primer may interact not just with its own locus partner, but with others in the system, causing non-specific products to form. Non-specific products complicate interpretation and should therefore be avoided whenever possible. Using primer sequences with a long and established history of high-quality performance provides a solid foundation for a new and expanded multiplex, simplifying multiplex construction and minimizing the chances of generating non-specific artifacts. In the case of the NGM™ kit, only the primer sequences for the five new loci required investigation. These were redesigned from those published in the literature to optimize performance and position in the new multiplex.

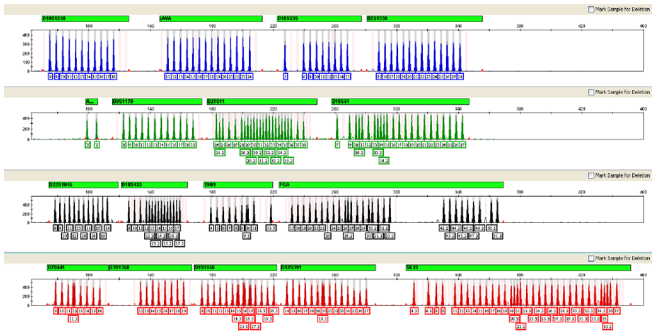

Figure 7. Example of the NGM SElect™ kit allelic ladder. The NGM™ and NGM SElect™ allelic ladders genotype the same range of alleles as the ladders used for loci common to the SGM Plus®, Identifiler®, SEfiler Plus™ and MiniFiler™ kits. This ensures genotype non-concordance does not arise through the use of allelic ladders with different ranges in different kits.

Specific to the NGM SElect™ kit, the primers for the SE33 locus were also redesigned from those contained in the SEfiler Plus™ kit to relocate the amplicon into the available region in the PET (red) dye channel. This project illustrated the challenges associated with the introduction of just one new set of primers into an already established multiplex, as well as the potential impact of changing primer sequences on genotype concordance. (For more details on the introduction of the SE33 locus into the NGM SElect™ kit, please refer to the article "Development of the AmpFℓSTR® NGM SElect™ Kit: New Sequence Discoveries and Implications for Genotype Concordance" in this issue of *Forensic News*).

- *Simplified Analyst Training*
  Maintaining primer sequences also simplifies analyst training in that the position of the core loci in the new multiplex are maintained, reducing the amount of training required to become familiar with the new system. The 11 loci contained in the SGM Plus® kit were used as the backbone of the NGM™ and NGM SElect™ kits, with 10 out of the 11 loci maintaining positions and dye colors in the new multiplexes (Figure 8). The remaining locus, D3S1358, maintained its position but required a change of color from blue to red. When comparing the configuration of the NGM SElect™ kit to the SEfiler Plus™ kit (the only other AmpFℓSTR® kit containing SE33), eight out of the 12 loci contained in the SEfiler Plus™ kit maintain positions and colors while D3S1358, D21S11 and D18S51 maintain position but change color. SE33 is the only locus that changes both position and color to optimize its location in the new multiplex.

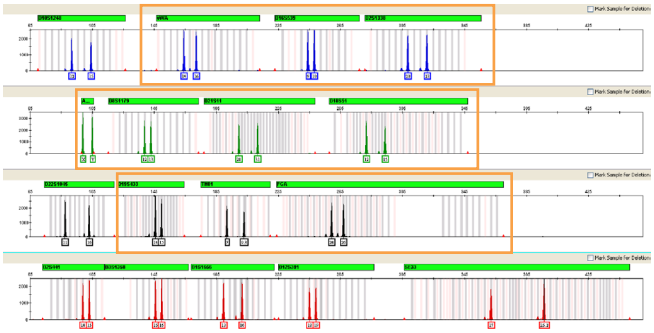

Figure 8. Illustration of how the maintenance of primer sequences from previous kits helps to maintain the position of loci within the new multiplex and reduces the amount of analyst training time required. The orange boxes indicate the loci that maintain position and colors in the NGM™ and NGM SElect™ kits compared to the SGM Plus® kit.

Conclusion

Here we have described how the NGM™ and NGM SElect™ kits have been developed not only to deliver unparalleled performance on challenging samples, but also to ease the burden of implementation faced by laboratories looking to adopt the expanded European Standard Set of Loci. These kits have been designed specifically to address the requests made by the ENFSI and EDNAP groups for improved discrimination and performance in a single amplification, thereby maximizing the efficiency of the laboratory workflow. Radical developments in amplification chemistry enable a significant increase in the amount of information recoverable from inhibited and degraded casework samples when compared to previous AmpF*l*STR® kits, while maintenance of key aspects of our kit portfolio simplify many aspects of implementation and operational processes. We believe the NGM™ and NGM SElect™ kits represent a high quality, uncomplicated way for laboratories to achieve successful and efficient analysis of forensic samples, and participate effectively in data exchange programs.

How to Cite This Article

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technical focus

Development of the AmpFℓSTR® NGM SElect™ Kit: New Sequence Discoveries and Implications for Genotype Concordance

Nicola Oldroyd, Robert Green, Julio Mulero, Lori Hennessy, Jonathan Tabak
Life Technologies, Worldwide

Introduction

One of the most important considerations for laboratories when implementing a new STR kit is the level of concordance that may be expected when comparing new data with previously generated data, including the vast repositories of historical data currently stored in national DNA databases. In recognition of this fact, one of the most important aspects of any new Applied Biosystems STR kit development project is a comprehensive investigation into the impact any changes to existing primer sequences may have on comparison of new and existing results. This investigation was of particular importance in the development of the NGM SElect™ kit, as the primers for the SE33 locus required a complete redesign from the SEfiler Plus™ kit to relocate the amplicon into the vacant region in the new multiplex.

A Brief History of SE33 Primer Sequences

The SE33 locus has been included in three AmpFℓSTR® kits to date. Up until recently, profiling of the SE33 locus was confined mainly to Germany, the only country in Europe to include SE33 in their national standard locus set. To meet the profiling needs of the German forensic community, SE33 was added to the existing SGM Plus® kit loci to create the original SEfiler™ kit in 2001. To maintain concordance as much as possible between the results generated by the SEfiler™ kit and existing data generated in Germany, we utilized the most established primer sequences described in the published literature at the time. The primer sequences slotted into the existing framework of the SGM Plus® kit, requiring adjustments of primer concentrations for several loci to balance the system and a change from the 4-dye set used for the SGM Plus® kit to a 5-dye set as used for the then recently released Identifiler® kit.

Although the SEfiler™ kit met the locus needs of the German forensic community, it bore the same performance limitations as the other kits developed around the same time in terms of the relatively low level of inhibition it was able to overcome on difficult samples. Following the release of the MiniFiler™ kit in 2007 and the demonstration of increased capability to deal with PCR inhibitors, the SEfiler™ kit was redeveloped to become the SEfiler Plus™ kit. The new kit was identical to its predecessor with the exception that the master mix was re-optimized to overcome higher levels of inhibitors than possible with any previous AmpFℓSTR® kit, thereby improving performance with difficult samples.

Around the same time, the European forensic community was holding discussions regarding expansion of the European Standard Set of Loci (ESSL) to support burgeoning cross-border data sharing efforts. SE33 was becoming more widely used as neighboring countries began sharing data with Germany. Although, ultimately, SE33 was not added to the expanded ESSL, the requirement stipulated by the Prüm Treaty for countries to upgrade their profiles to match those contained in the databases of those nations with which they wish to exchange data increased the need for the availability of high-performance systems containing this locus.

The recent development of the NGM™ kit provided a platform from which to build a next-generation STR chemistry containing SE33 and thereby continue to meet the diverse requirements of the European forensic community. Given the strict design

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requirements associated with the NGM™ kit (for more details, see "Implementation of Next-Generation STR Kits in Europe: Challenges and Solutions" in this issue of *Forensic News*) and the limitations associated with the use of mobility modifiers, the existing SE33 primer sequences from the SEfiler™ and SEfiler Plus™ kits could not be used in the new multiplex. Therefore, to create the NGM SElect™ kit, which contains all 16 loci contained in the NGM™ kit plus SE33, the primers for the SE33 locus required a complete re-evaluation.

NGM SElect™ Kit Development: SE33 Primer Design Challenges

Designing primers for SE33 can pose more challenges than for other loci due to the high level of sequence complexity associated with this locus. SE33 has a higher mutation rate than other STR loci in routine forensic use (Table 1). This leads, in part, to the multiple length microvariant alleles associated with this locus and the high discrimination power for which it is renowned. What is perhaps less commonly known is that the mutation rate also leads to multiple sequence variants that cause difficulties when trying to identify stable primer binding sites. Most significantly, our investigations have uncovered the potential for these sequence variants to significantly affect the genotyping accuracy of the locus, if the primers are positioned incorrectly.

We approach all primer design investigations in a similar way. We utilize all aspects of published information to guide our choice of primer site locations and then investigate multiple primer options around those locations to identify primers that deliver the desired combination of amplicon size, performance on difficult samples, balance with the rest of the system, specificity and concordance with previous kit data. As a result, we generally expect to screen multiple sets of primers before establishing the final configuration. However, the number of primers requiring investigation for the SE33 locus far exceeded our expectations due to difficulties in trying to identify primers that met all of our design criteria.

Prototype Version 1 SE33 Primers

During the early stages of the NGM SElect™ kit development project, we identified promising candidate SE33 primers that were included in an early evaluation reagent release intended for use by a small number of test site laboratories. These primers met the majority of our design criteria in terms of performing well on inhibited samples and balancing effectively with the rest of the system. A population study of approximately 1,200 samples showed strong concordance with previous data; however, during evaluation of the multiplex by our test sites, we identified a small PET-labeled dye artifact in the region of the D2S441 locus (Figure 1). One of the main improvements seen in all of our next generation STR chemistry kits is the greater cleanliness of the baseline. The artifacts that affected older AmpF*ℓ*STR® kits have been removed, simplifying allele designation and interpretation for low level or difficult samples. Therefore, the presence of this artifact, unique to this configuration of the NGM SElect™ kit, caused the Prototype v1 SE33 primers to fail our specificity criteria. These primers were therefore rejected.

Prototype v2 SE33 Primers

Following a second round of intensive primer design efforts, the Prototype v2 SE33 primers were identified. Again, these primers were tested against the full range of design criteria that now also included ensuring the absence of artifacts in the low molecular

Locus	Mutation Rate
TH01	0.01%
D19S433	0.11%
D16S539	0.11%
D3S1358	0.12%
D8S1179	0.14%
vWA	0.17%
D21S11	0.19%
D18S51	0.22%
FGA	0.28%
SE33	0.64%

Table 1: Comparison of mutations rates for a selection of loci in common forensic use. Note the significantly higher mutation rate displayed by the SE33 locus.

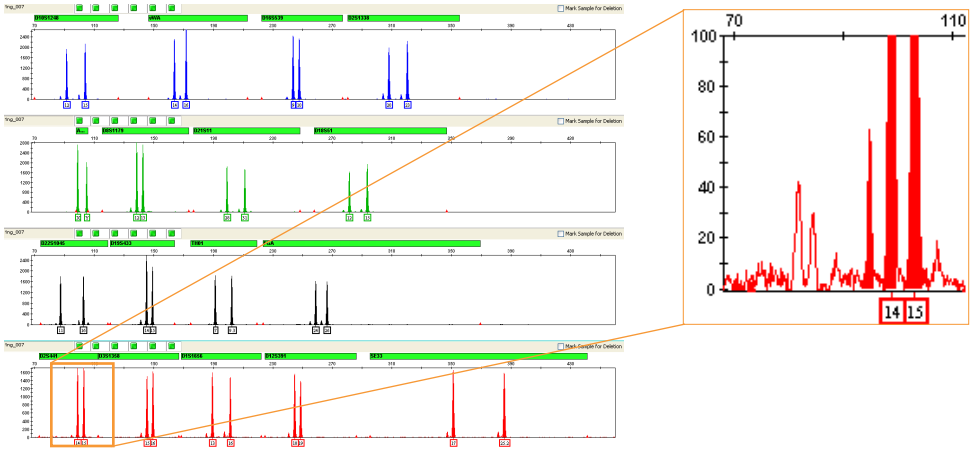

Figure 1. Prototype version 1 amplification of 1ng 007 control DNA for 29 cycles. The expanded insert indicates the artifact related to the SE33 primers visible in the D2S441 region.

weight region of D2S441. When evaluating the anticipated read region of the system, the D2S441 artifact had been eliminated and no new dye-related artifacts could be identified. However, the peak heights for the D16S539 locus appeared to be compromised, thereby affecting the overall balance of the system. On closer inspection, two additional allele peaks were now visible in the high molecular weight range of the FAM (blue) dye channel, close to the end of the electropherogram detected during a standard capillary electrophoresis run at approximately 540 bp (Figure 2). Genome searches revealed an exact match to the Prototype v2 SE33 unlabelled primer, 553 bp away from the D16S539 labeled primer that could not have been predicted prior to conducting the experiments. Once again, these primers therefore failed our specificity criteria and were subsequently rejected.

Prototype v3 SE33 Primers

Having so far failed to establish primer sequences that met all the required design criteria, it was now becoming increasingly difficult to identify new primer options for testing. The main restriction related to the size of the amplicon, which had to be large enough to fit into the available space in the NGM™ kit PET dye channel without becoming too large such that performance became significantly affected.

The SE33 locus exhibits multiple microvariants, with alleles often differing in size by only a single base; therefore, resolution of the system is important to ensure accurate genotyping. As the amplicons become larger, resolution on the CE platform becomes more difficult. This, combined with the obvious challenges posed to larger amplicons by degraded DNA, meant it was critical to ensure that the size of the amplicon did not increase too significantly.

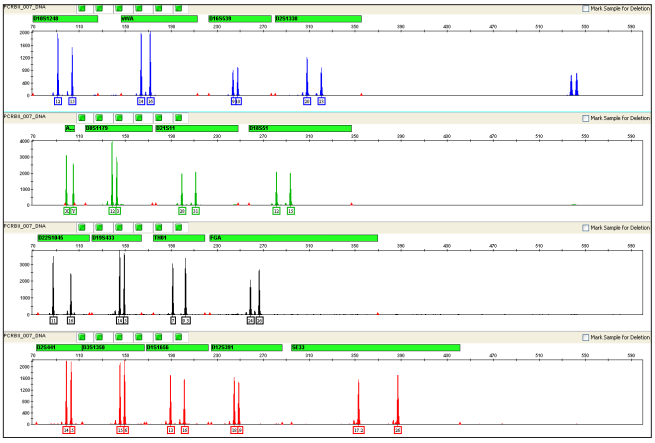

Figure 2. Prototype v2 amplification of 1ng 007 control DNA for 29 cycles. The alleles generated by the interaction of the D16S539 labeled primer and the Prototype v2 SE33 unlabeled primer are visible in the nigh molecular weight region of the FAM (Blue) dye channel of the electropherogram.

While our proprietary mobility modifiers often prove effective in optimizing spacing between loci by slowing down the migration of some of the amplicons, there is a limit to how many of these molecules can be attached to the primers at any one locus and contained in the system as a whole before primer manufacturing becomes too difficult and the multiplex performance suffers. As a result, these must be used prudently and the amplicon position within the system is still dictated predominantly by the location of the primers themselves.

Extensive investigation and manipulation was required to develop the Prototype v3 SE33 primers, which looked promising upon initial investigation. The primers passed all the required specificity and performance tests, removing the artifact in the D2S441 read region and the additional allelic peaks associated with D16S539. The SE33 alleles gave strong signal, were well balanced with the rest of the system and performed well in tests with inhibited samples. As a final check, the population study was repeated to evaluate concordance with SEfiler Plus™ kit data, and it was during these tests that a significant issue was detected.

Identification of Non-Concordance at SE33

When SE33 results were compared between the Prototype v3 SE33 primers and the SEfiler Plus™ kit, 18/350 African American samples (5.1%) and 1/333 U.S. Caucasian samples gave non-concordant results (Table 2). The source of the non-concordance appeared to be a 1 bp shift in one of the alleles in each of these samples, generating an off ladder (OL) call when the sample was genotyped using GeneMapper® *ID-X* v1.1 software (Figure 3). Subsequent interpretation by an analyst according to generally accepted protocols would result in a microvariant genotype being assigned to each of these samples instead of the accurate genotype. The shifts were visible on different CE platforms

Ethnic Group (Self Identified)	Total Number of Samples	Number of Off Ladder Allele Calls	Discordance Rate with SEfiler Plus™ Kit Results
African Americans	350	18	5.1%
Caucasians	333	1	0.3%
Hispanics	376	0	0%
Koreans	148	0	0%

Table 2: SE33 concordance results for the Prototype v3 SE33 primers and SEfiler Plus™ kit. Results displayed indicate *genotype* discordance rates and not *allele* discordance rates to more accurately reflect the operational impact of discordance on forensic result comparison.

including the 3130xl and 3500xL instruments, on multiple injections of the same sample, and did not appear to relate to any one particular allele as the affected samples displayed a range of genotypes (Figure 4).

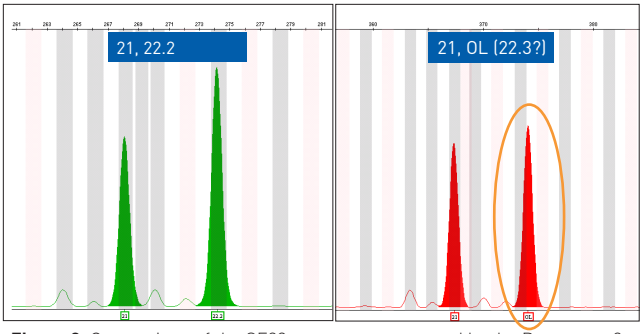

Figure 3. Comparison of the SE33 genotypes generated by the Prototype v3 SE33 primers (red) and the SEfiler Plus™ kit (green) from African American population database sample IBB 115. The larger allele of the heterozygote genotypes one base pair larger with the prototype v3 SE33 primers than with the SEfiler Plus™ kit.

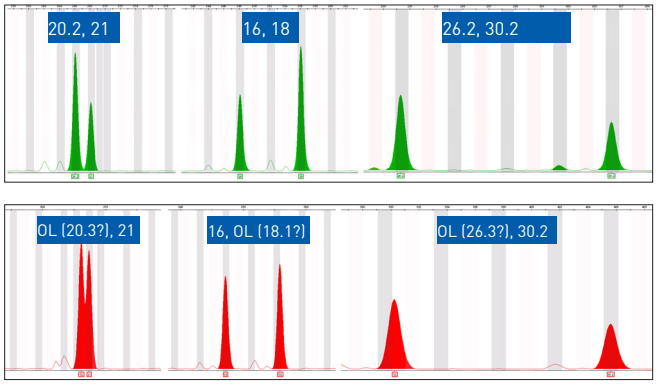

Figure 4. Further examples of genotype discordance between Prototype v3 SE33 primers and the SEfiler Plus™ kit. In every sample, one of the alleles of each heterozygote genotypes 1 bp larger with the Prototype v3 SE33 primers compared to the SEfiler Plus™ kit.

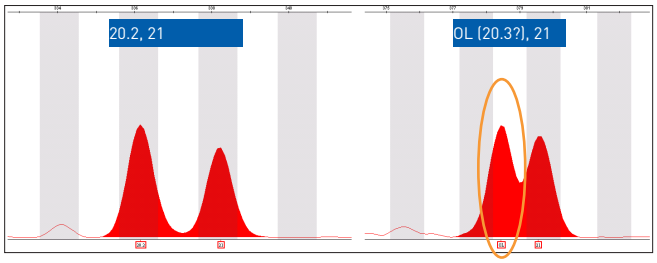

Figure 5. Comparison of the genotypes obtained for African American sample IBB 297 from the ESX 17 (left panel) and ESI 17 (right panel) kits. The lower molecular weight allele of the heterozygote genotypes 1 bp larger with the ESI 17 kit than with the ESX 17 kit.

The same set of samples amplified with both Prototype v1 and v2 SE33 primers did not exhibit the allele shift, pointing to an issue specific to the amplicon generated by these particular primers.

Diagnosing Non-Concordance at SE33: Cause and Effect on Interpretation

Initial diagnostic experiments involved the use of other commercially available primer sequences for SE33 to see if they exhibited the same phenomenon. When the 19 samples exhibiting a shift were analyzed using the PowerPlex® ESX and ESI 17 kits, the ESX 17 kit generated genotypes concordant with the SEfiler Plus™ kit, whereas the ESI 17 kit showed the same allele shift as the Prototype v3 SE33 primers (Figure 5).

The only way to determine categorically the cause of the shift was to investigate the v3 prototype SE33 amplicon at the sequence level. All 19 alleles showing the shift were sequenced together with several "normal" alleles to determine the difference between the two allele species. These investigations revealed a SNP-containing region within the Prototype v3 SE33 amplicon that, when a SNP occurs, affects the mobility of the amplicon on the capillary electrophoresis platform (Figure 6). Several SNPs within this region were identified, each one causing the shift whenever present within the amplicon.

The frequency of this mutation alone would be sufficient cause to redesign the primers to eliminate this effect; however, several other factors combined to make the correction imperative. Non-concordance between results generated using STR primer sequences is a well known phenomenon and most often results from primer binding site mutations affecting one of two or more different primers, leading to the occurrence of null alleles. Null alleles are, in general, well understood, occur at a low frequency in any given population, easy to diagnose by peak height comparison or amplification at a reduced annealing temperature and, should the need arise, easy to fix by the manufacturer using well established techniques.

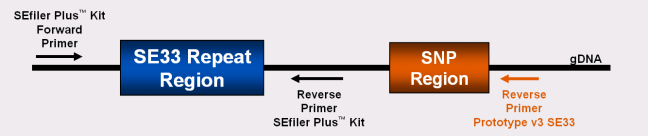

Figure 6. Schematic representation of the SNP-containing region in the Prototype v3 SE33 amplicon. Primers used in the SEfiler Plus™ kit are shown in black and the position of the Prototype v3 SE33 reverse primer, sited further away from the repeat region to lengthen the amplicon, is shown in orange (Prototype v3 SE33 forward primer not indicated; representation not to scale).

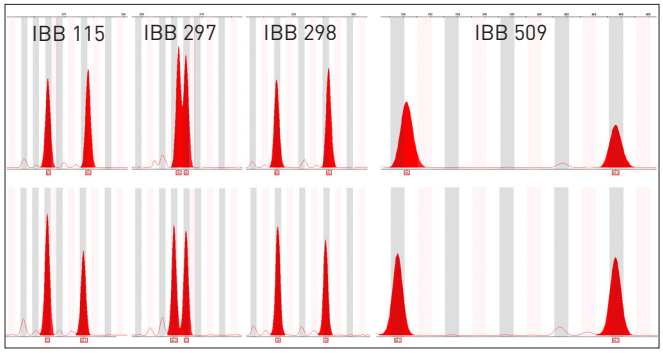

Figure 7. Examples of correct genotypes restored by redesigning the SE33 primers for the final NGM SElect™ kit. Genotypes generated from four population samples using the Prototype v3 SE33 primers (top panel) and final NGM SElect™ kit SE33 primers (bottom panel). The finalized SE33 primers eliminate the SNP-containing region from the amplicon thereby delivering accurate genotypes and restoring concordance with the SEfiler Plus™ kit.

The mobility shift, however, is a different situation altogether. This is a new observation that, according to our investigations, occurs at a high frequency in samples of African descent. At any other locus, the occurrence of microvariant alleles at a relatively high frequency in a population would be sufficient to trigger further investigation; however, because SE33 displays such alleles on a far more regular basis, the occurrence of microvariant alleles would not be viewed with such suspicion and the result more likely accepted at face value. All of these considerations together with the lack of concordance demonstrated with previous SE33 results caused these primers to fail spectacularly with regard to the design criteria specified for the NGM SElect™ kit. A complete redesign was yet again required in order to eliminate the SNP-containing region from the amplicon entirely and restore genotyping accuracy at this locus.

Finalizing the SE33 Primers for the NGM SElect™ Kit

The difficulties experienced with the design of the SE33 primers clearly highlighted the potential implications of introducing new primer sequences into an existing multiplex. In order to minimize these risks as much as possible, we adopted an alternative strategy. Up until this point, we had been attempting to design two new primers for the SE33 locus, one forward and one reverse, each running the risk of causing non-concordant results with the SEfiler Plus™ kit. Our new strategy involved maintaining one of the original SE33 primers from the SEfiler Plus™ kit and designing a new second primer to generate the longer amplicon necessary for the NGM SElect™ kit. This reduces the risks and minimizes the challenges associated with introducing new primers into the multiplex.

The new primer combination avoids the SNP-containing region entirely, thereby eliminating the risk of generating inaccurate genotypes and restoring concordance with the SEfiler Plus™ kit (Figure 7). When the concordance study was repeated with the final primer configuration for the NGM SElect™ kit, all samples gave identical genotypes to the SEfiler Plus™ kit with the exception of one African American sample where a null allele, caused by a mutation under one of the SEfiler Plus™ primers, was recovered by the NGM SElect™ kit (Table 3). By the time the primer sequences for SE33 were finalized, we had evaluated more than 80 different primers to identify what proved to be just the one new primer required to relocate the amplicon and maximize locus performance.

Ethnic Group (Self Identified)	Total Number of Samples	Concordance Rate with SGM Plus® Kit	Concordance Rate with SEfiler Plus™ Kit
African Americans	350	100%	99.7%*
Caucasians	333	100%	100%
Hispanics	376	100%	100%
Koreans	148	100%	100%

Table 3: SE33 concordance results for the SE33 primers contained in the final NGM SElect™ kit. Again, results displayed indicate *genotype* discordance rates and not *allele* discordance rates to more accurately reflect the operational impact of discordance on forensic result comparison. The 99.7% concordance in the African American database represents 349/350 samples generating identical SE33 genotypes for the NGM SElect™ and the SEfiler Plus™ kits. The remaining sample exhibited a null allele with the SEfiler Plus™ kit that was recovered using the NGM SElect™ kit.

Additional Primer Improvements to the NGM SElect™ Kit

The sequential nature of the NGM™ and NGM SElect™ kit projects enabled collection of additional information about the performance of the NGM™ kit in different laboratories and on different populations during the development of the NGM SElect™ kit. As a result, we were able to identify improvements that could be made to the NGM SElect™ kit to improve genotyping accuracy still further.

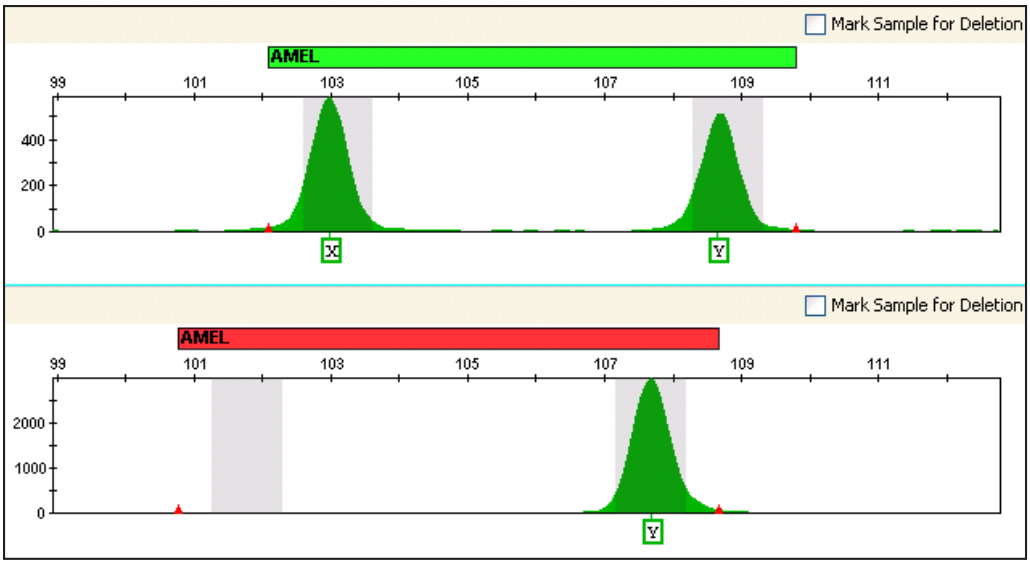

Figure 8. Illustration of the impact of the novel mutation identified at the Amelogenin locus which affects the redesigned primers in the NGM™ kit. This sample when amplified with the SGM Plus® kit (top panel) yields a complete male X,Y genotype at Amelogenin. The same sample when amplified with the NGM™ kit (bottom panel) shows complete dropout of the X allele.

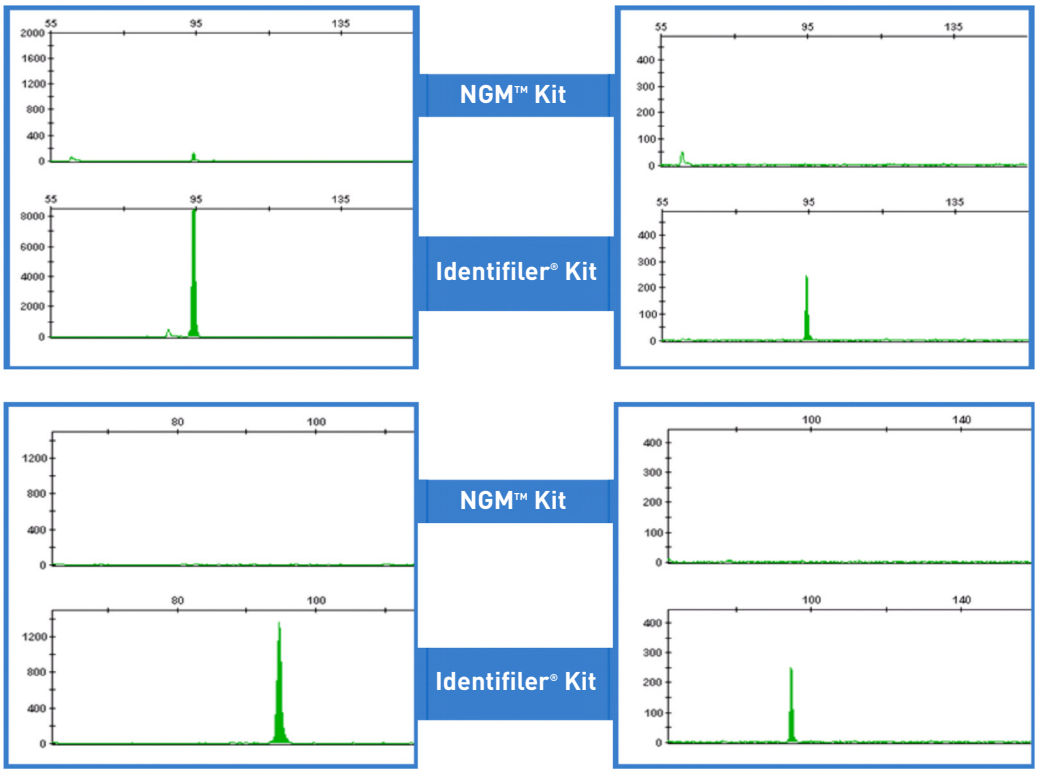

Figure 9. Illustration of the reduction in Amelogenin cross reactivity with common animal species in the NGM™ kit. The electropherograms show Amelogenin results for Identifiler® and NGM™ kit amplifications of Equine (top left), Bovine (top right), Canine (bottom left) and Ovine (bottom right) samples, demonstrating the reduction in cross- reactivity achieved by redesigning the Amelogenin primers.

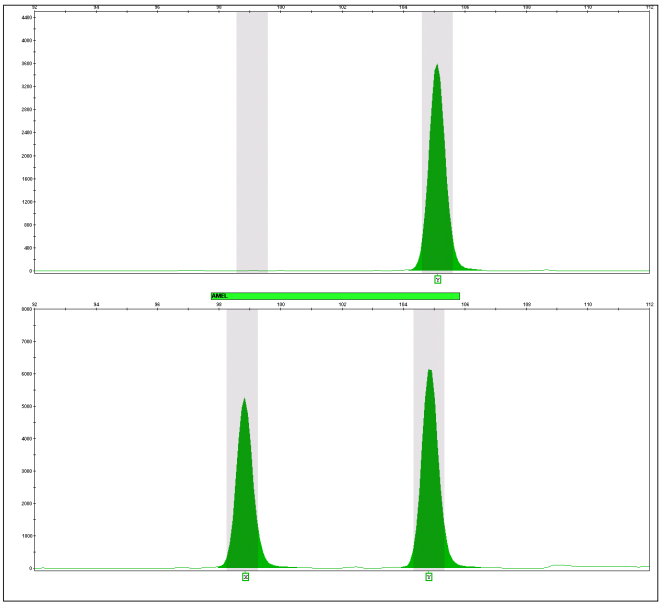

Figure 10. Illustration of the dropout of the X allele when amplified with the Amelogenin primers contained in the NGM™ kit (top panel) and the recovery of the allele when amplified with the NGM SElect™ kit (bottom panel), which contains the additional primer to address the mutation.

Addressing a Novel Mutation at the Amelogenin Locus

During the development of the NGM™ kit, a novel mutation associated with the X allele at Amelogenin was identified that causes dropout of the X allele in affected samples (Figure 8). This mutation only affects the NGM™ kit due to the fact that the Amelogenin primers were redesigned slightly from previous AmpFℓSTR® kits to reduce the level of cross-reactivity with certain common domestic and agricultural animal species. Cross-reactivity at Amelogenin with some animal species has been well documented, but the additional power of the buffer system in the NGM™ and NGM SElect™ kits increases the signal associated with the cross-reactivity. This can potentially reduce the reaction components available for amplification of human DNA in a sample of mixed special origin.

The redesign of the Amelogenin primers virtually eliminated this cross-reactivity (Figure 9), but also caused dropout of the X allele in two samples from our population study. This very low percentage of occurrence, combined with the fact that the samples still type successfully as male (due to the presence of the Y allele) and that Amelogenin is rarely used in database searches, prompted the decision not to address this mutation in the development of the NGM™ kit. However, subsequent reports from customer laboratories have indicated that this mutation occurs at a higher frequency in some regional populations (e.g., aboriginal samples), and that Amelogenin provides a useful guide to contributor ratios during mixture interpretation. Therefore, we decided to introduce a new primer to address this mutation in the NGM SElect™ kit (Figure 10).

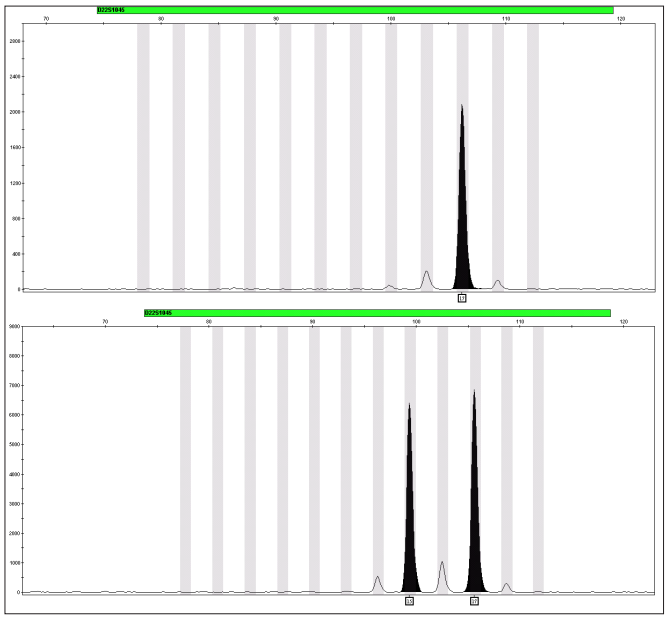

Figure 11. Illustration of the null allele detected at the D22S1045 locus with the NGM™ kit (top panel) and the recovery of the allele when amplified with the NGM SElect™ kit (bottom panel), which contains the additional primer to address the mutation. This mutation was detected in ~1.4% of our African population samples and in each case the null allele genotyped as a 15 upon recovery.

Addressing Mutations at the D2S441 and D22S1045 Loci

Once the decision had been made to address the Amelogenin mutation in the NGM SElect™ kit, thereby introducing changes to the primer complement relative to the NGM™ Kit, the opportunity was available to make further changes as part of the same update. Sequence investigations identified a primer site SNP at the D22S1045 locus in approximately 1.4% of African American genomes and another primer site SNP at the D2S441 locus in approximately 6% of Korean genomes. These mutations were addressed using additional primers, recovering the null alleles encountered with the original primer sets for these loci (Figures 11 and 12).

Upgrading the NGM™ Kit Primer Set

The design brief governing both the NGM™ and NGM SElect™ kits dictated that these kits should be identical with respect to performance, configuration of common loci, and primer complement. This was to ensure complete comparability and concordance between the two kits and enable laboratories to choose whichever kit best meets their locus requirements.

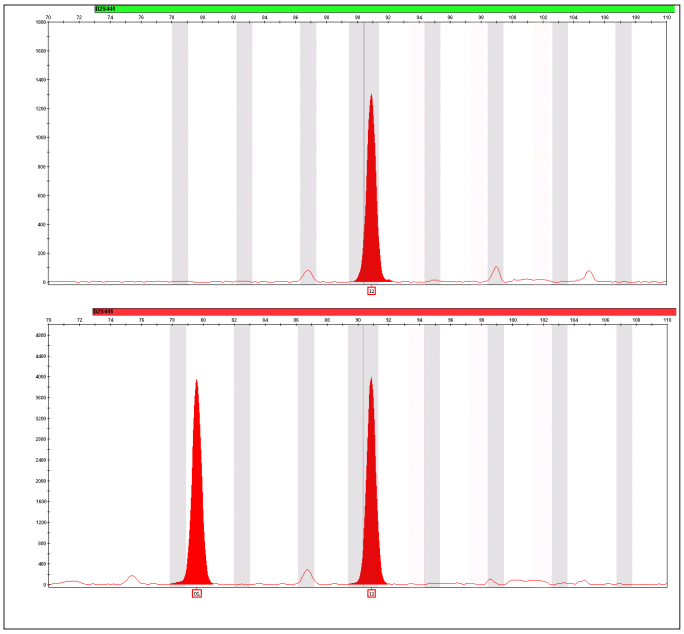

Figure 12. Illustration of the null allele detected at the D2S441 locus with the NGM™ kit (top panel) and the recovery of the allele when amplified with the NGM SElect™ kit (bottom panel), which contains the additional primer to address the mutation. This mutation was detected in ~6% of our Korean population samples and in each case the null allele genotyped as a 9.1 upon recovery.

The changes made to the primer sets for Amelogenin, D2S441, and D22S1045 in the NGM SElect™ kit introduce differences between the two kits; therefore, to address this situation and restore concordance, the same primer improvements will be made to the NGM™ kit.

The additional primers have been tested extensively as part of the NGM SElect™ kit development project, and, due to the similarities between the two kits, the new primers can be introduced simply into the NGM™ kit without impacting kit performance. We anticipate completing this project quickly, with the new configuration of the NGM™ kit available early in 2011. For laboratories already up and running with the NGM™ kit, a full revalidation should not be necessary. A small performance comparison should be all that is required to ensure direct compatibility of results.

AmpFℓSTR® Kits Primer Design Strategy

The development of both the NGM™ and NGM SElect™ kits illustrates the overall primer strategy we apply when developing every AmpFℓSTR® kit. Primer sequences and complements are maintained wherever possible to enable maximum concordance between data generated with different AmpFℓSTR® kits. This helps ensure consistent genotypes between historical and contemporary data sets, and between database results and the majority of casework samples, simplifying the overall analysis, interpretation and reporting of genotype results.

<table>
<tr><th rowspan="2">AmpFℓSTR® Kit</th><th colspan="2">Primer Set Configuration</th></tr>
<tr><th>STR Primers</th><th>Amelogenin</th></tr>
<tr><td>Profiler® Kit</td><td rowspan="3">Identical primer sequences for all common loci</td><td rowspan="4">Identical Amelogenin primer sequences</td></tr>
<tr><td>Profiler Plus® Kit</td></tr>
<tr><td>COfiler® Kit</td></tr>
<tr><td>SGM Plus® Kit</td><td rowspan="4">Inclusion of one additional primer for D8S1179</td></tr>
<tr><td>Identifiler® Kit</td></tr>
<tr><td>Profiler Plus® ID Kit</td></tr>
<tr><td>SEfiler Plus™ Kit</td><td rowspan="2">Amelogenin primers redesigned</td></tr>
<tr><td>NGM™ Kit</td><td></td></tr>
<tr><td>NGM SElect™ Kit</td><td>SE33 primer sequences redesigned</td></tr>
<tr><td>MiniFiler™ Kit</td><td colspan="2">All primers redesigned</td></tr>
</table>

Table 4: AmpFℓSTR® kit family primer set configuration matrix. Green sections indicate kit configurations which use identical primer sequences. Orange sections indicate where small changes have been made to the primer configurations and where those changes would have a small impact on operational result comparisons. Red sections indicate where primers have been redesigned more comprehensively and where the impact on result comparison may be more significant.

Changes are made to the primer sequences or complements only when the result is of significant benefit to the analysis of forensic samples in an operational context. For example, the primer sequences for the loci contained in the MiniFiler™ kit were completely reengineered with the very specific intent of reducing the amplicon size to promote improved performance on degraded samples. The performance goal of this kit could not be met without changing the primer sequences and therefore made logical sense.

New primers can also be introduced into a multiplex system to improve genotyping accuracy for known mutations. The three primers included in the NGM SElect™ kit to address specific mutations represent only the second time degenerate primers have been added following the introduction of loci into the AmpFℓSTR® kit series. The first and only other time this occurred was when an additional primer for the D8S1179 locus was included in the Identifiler® kit, which addressed a rare but high frequency mutation in a population of Chamorros and Filipinos from Guam.

Finally, primers may also need changing when a locus requires repositioning within a new multiplex as in the case of SE33 in the NGM SElect™ kit. As described above, this can be a highly complicated exercise, and therefore is not undertaken unless there is no other way to accommodate all the necessary loci within a single multiplex.

A summary of the primer changes made during the entire AmpFℓSTR® kit development program can be found in Table 4.

Discussion

The events that unfolded during the development of the NGM SElect™ kit clearly illustrate just how complex the process of STR multiplex design and construction can be. The NGM SElect™ kit was based entirely on the already completed NGM™ kit and therefore benefited from the wealth of information and experience gained during not only that project but also from every other AmpFℓSTR® kit development. Despite this, the introduction of the single SE33 locus posed significant challenges, not just relating to multiplex design and construction but also with regard to the behavior of individual loci on the electrophoresis platform. The discovery of the SNP-containing region close to the SE33 repeat region posed new questions regarding concordance and required new answers.

This project demonstrates how unpredictable events can occur during every project and how development of successful multiplexes cannot be rushed. Painstaking evaluation of every aspect of kit performance supported by a comprehensive in-house developmental validation study is required. This helps to ensure that, upon release, the kit is validated for use in operational forensic laboratories—without unpleasant surprises. We take great pride in the quality of our STR multiplex kits and design them to maximize the ease with which they can be used by operational laboratories. We hope that by sharing information on the hurdles we encountered during development of the NGM SElect™ kit, forensic laboratories may more fully understand, and have further confidence in, our ongoing efforts to support their operations.

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How to Cite This Article

N. Oldroyd, *et al*. (2011) Development of the AmpFℓSTR® NGM SElect™ kit: New Sequence Discoveries and Implications for Genotype Concordance. *Forensic News* **January**; [Internet] 2011. [cited: year, month, date]; Available from: http://www.appliedbiosystems.com/ForensicNews-Past

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technical focus

Optimizing GeneMapper® *ID-X* Performance: Maintaining Audit Records

Jeff Sailus, Jacquelyn Benfield
Life Technologies

Introduction

GeneMapper® *ID-X* Software contains an audit trail feature that allows users to monitor user actions and helps satisfy regulatory requirements. Users can utilize the GeneMapper® *ID-X* Software audit system to specify the types of actions included in the audit record and the auditing mode, but as these records accumulate over time, performance of the system can be detrimentally affected. This document is intended to assist users in ensuring that the number of stored audit records does not exceed the recommended level, provide guidance on the removal of excess audit records and promote successful maintenance of the software as a whole.

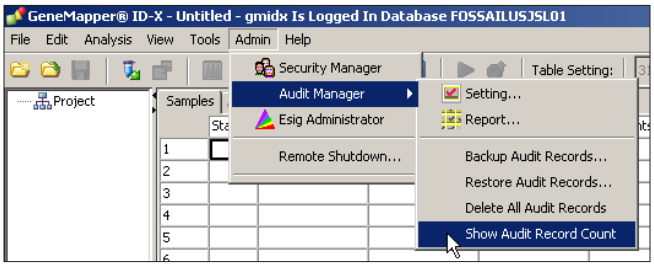
Creation of Audit Records

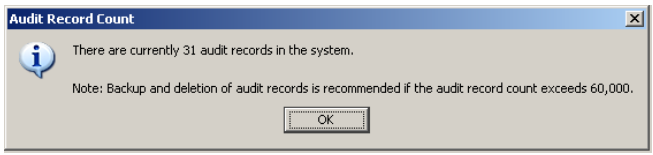
When a user creates, edits, or deletes an item marked for auditing (e.g., makes an allele edit or creates a set of analysis parameters), the change is tracked in the audit record. If auditing is switched *On*. the user must enter a reason for the change in the "Reason(s) for Change" dialog box. If auditing is switched to *Silent*, the change is recorded but the user is not prompted to enter a reason for the change. If auditing is switched to *Off*, no audit records are created. This step is performed for each Audit Map Object in the software.

The default audit settings, applied when the software is installed, are configured to monitor the most frequent and important actions anticipated during operational use of the software using a combination of On, Off, or Silent modes for different actions. Therefore, even if a laboratory has never actively configured the auditing system, the default audit settings will result in audit records being accumulated. Likewise, for those users actively selecting the *Silent* mode for ease of operation, audit records will still accumulate and need to be monitored to avoid reaching a level that may impair the operation of the software. For more information on management of the audit system and setting audit parameters, please refer to Chapter 2 of the GeneMapper® *ID-X* Software v1.0 Administrator's Guide.

Management of Audit Records

One way to prevent deterioration of software performance is to ensure that the number of audit records does not exceed the recommended limit of 60,000. Users can easily check the number of audit records within the system using the "Show Audit Record Count" function. To perform this check, choose the "Show Audit Record Count" option within the "Admin>Audit Manager" menu option as show below:





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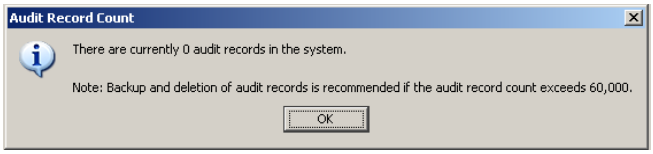
The rate of accumulation of audit records will depend on the audit record settings used in the laboratory and the workload of the system. Therefore, users should periodically check the level of audit records in the system and archive as required. A mechanism for backing up and deleting audit records is contained within Chapter 2 of the GeneMapper® *ID-X* Software v1.0 Administrator's Guide (Managing the Auditing System, Backing Up, Removing, and Restoring Audit Records). Some users may be concerned that deletion of the audit records via this system results in the permanent removal of all audit trails associated with their previously analyzed data. The audit trail for the data contained within any given project is preserved within the project file. Therefore, to protect these records, users should export any projects currently saved within the software database **prior to** performing the record deletion procedure. After the clean-up procedure has been completed, the user will still be able to view the audit trail associated with any archived projects together with the analyzed data by re-importing the project back into the software database. Whenever a project is re-imported into the software database, the audit record count will increase, demonstrating the preservation of the audit record within the individual project.

Re-Import Restores Audit Records – Example

In the following example, the software database contains a single project showing 31 audit records relating to allele calls, recorded according to settings defined by the user.

If the user exports this project and then performs the procedure for deletion of audit records, the Audit Record Count returns to zero.



Upon re-import of the original project to the software, the original Audit Record Count is restored to 31, indicating preservation of the audit trail in the Project. The Audit Records are only restored for the individual Project that is imported. Upon importation of additional Projects, Audit Records related to those Projects will also be restored in the database.

Audit Record Counts in Excess of the Recommended Limit

When audit records are not managed effectively, the number of records has been known to approach 300,000, resulting in significant software performance issues including increases in the amount of time required to save, export and import projects. If the number of audit records exceeds 75,000, the audit record backup and delete operations described above might not operate as expected. It is therefore recommended, wherever possible, to back up and delete audit records before the records exceed 75,000.

Should the number of records exceed 75,000, the user may be required to uninstall and reinstall the Full Installation of the GeneMapper® *ID-X* Software in order to restore communication with the host database. If your records exceed this value and the mechanism described above is insufficient to restore performance of the software install, please contact your local HID Technical Support representative for proper back up and restoration guidance before performing a reinstall of the Full Installation of the GeneMapper® *ID-X* Software. It is very important that when considering a reinstallation of the software for any reason, users ensure they export and save any user-defined settings or projects contained in the software before uninstalling the existing instance. These settings and projects will be used to restore data after reinstall (e.g., items contained in the GeneMapper® *ID-X* Manager or user-specific Panel and Bin settings).

Further information on maintaining the Software and Database Application and Backing Up the Database prior to uninstallation can be found in Chapter 4 in the GeneMapper® *ID-X* Software v1.0 Administrator's Guide.

NOTE: While users should use this procedure to back up the database for historical purposes, the user should NOT restore this database after reinstallation of the server software. Performing a

reinstall of the poor performing database will return the system to its previous inhibited state. This backup should only be maintained for historical purposes and disaster recovery purposes.

The Importance of Software Maintenance

Maintenance of any software system is important to help ensure the proper function and speed of the system, particularly in the type of multi-user environment supported by the GeneMapper® *ID-X* software. Users are encouraged to implement a scheduled regular maintenance plan for GeneMapper® *ID-X* Software, including periodic database back up and audit record deletion schedules. Should you have any questions or concerns regarding proper maintenance of your GeneMapper® *ID-X* Software installation, please contact your local HID Field Applications Scientist who will be able to provide further information on all the operations described in this article.

How to Cite This Article

J. Sailus, *et al*. (2011) Optimizing GeneMapper® *ID-X* Performance: Maintaining Audit Records. *Forensic News* **January**; [Internet] 2011. [cited: year, month, date]; Available from: http://www.appliedbiosystems.com/ForensicNews-Past.

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product updates

Now Available! HID Real-Time PCR Analysis Software v1.1 Update

We are pleased to announce the HID Real-Time PCR Analysis Software v1.1 Update is now available for download. This software update incorporates several new features and addresses several known issues that exist in the HID Real-Time PCR Analysis Software v1.0, while the C_T and baseline algorithms and the core software functionality remain unchanged. The highlights of the software update include fixes to the user interface freeze and amplification plot drop issues sporadically encountered during software operation. In addition, several feature enhancements such as improved result reports and exports, enhanced data analysis capabilities and simplified plate setup have been implemented.

For more detailed information about this software update, please refer to the HID Real-Time PCR Analysis Software v1.1 Release Notes that can be obtained from the HID Real-Time PCR Analysis Software v1.1 download page:

View Software Download Page

Please note that the web downloadable software update is used to upgrade existing HID Real-Time PCR Analysis Software v1.0. For new users, the complete HID Real-Time PCR Analysis Software v1.1 is available in CD format (P/N: 4413973) and can be ordered from the Applied Biosystems Customer Service department.

Before upgrading the HID Real-Time PCR Analysis Software v1.0, please read the HID Real-Time PCR Analysis Software v1.1 Release Notes and the Getting Started Guide. During the software upgrade, please click the .exe file and follow the on-screen instructions to automatically upgrade the HID Real-Time PCR Analysis Software from v1.0 to v1.1.

Visit the HID Real-Time PCR Analysis Software v1.1 Product Page

product updates

Now Available: GeneMapper® *ID-X* Software v1.2 User Bulletin

Click here to download the User Bulletin

product updates

Coming Soon! GeneMapper® *ID-X* Software v1.1.2 and 1.2.1 Update for CODIS Export

GeneMapper® *ID-X* Software v1.1.2 and 1.2.1 are patch releases to expand CODIS export functionality in GeneMapper® *ID-X* Software v1.1.1 and 1.2. These patches were developed to enable users to customize the list of markers and associated data for export in the following CODIS uploadable file formats: CMF v1.0 (.dat), CMF 3.0 (.xml) and CMF 3.2 (.xml).

These patches will be available as a web downloadable software update and in CD format to upgrade existing GeneMapper® *ID-X* Software v1.1.1 and 1.2 and will be available free of charge. The updates will be posted to the GeneMapper® *ID-X* Software download web page within the next several weeks. For additional information, please contact your local technical support representative.

product updates

Notification: Support Retirement of the 3100-*Avant* and 3100 Capillary Electrophoresis Instruments Effective December 31, 2011

In order to help plan for your laboratory's future needs, we wish to inform you that Life Technologies has planned the retirement of the 3100-*Avant* and 3100 capillary electrophoresis instruments ("3100 System") effective on **December 31, 2011**, following the 3100 System sales discontinuance for the human identification market in July 2005. To obtain detailed information about the 3100 System retirement, please visit www.appliedbiosystems.com/lifecycle.

We have several attractive promotion options available to help you continue to run human identification applications on a newer and more advanced capillary electrophoresis platform:

- Discount promotion for trading in your 3100 system towards the new Applied Biosystems 3500 Series Genetic Analyzers for Human Identification. For more information about the 3500 Series, please visit www.appliedbiosystems.com/3500hid.
- Upgrade promotion for upgrading your 3100 System to Applied Biosystems 3130/3130*xl* Genetic Analyzers for Human Identification. For more information about the 3130 system, please visit: https://products.appliedbiosystems.com/ab/en/USadirect/ab?cmd=catNavigate2&catID=601644.

Please note that the 3130 Genetic Analyzers for Human Identification will no longer be available for sale and upgrade from the 3100 System following the discontinuation of the 3130 Genetic Analyzers for Human Identification on June 30, 2011. Life Technologies is committed to providing full support for the 3130 Series for at least five years after the discontinuation.

In addition, we also offer attractive service plans as well as comprehensive validation support and training services to support your transition to our newer platforms. For specific details on these time-limited trade-in and upgrade promotions, please contact your local sales representative.

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legislation corner

U. S. Congress Moves Towards Full Funding for FY 2011 DNA Backlog Grants; Legislative Solutions to Address Rape Kit Backlogs; State Database Expansion

Lisa Hurst
Gordon Thomas Honeywell Governmental Affairs

Fiscal Year 2011 Federal Appropriations

Although the final appropriations package for Fiscal Year 2011 (that began October 1, 2010) is far from complete, it appears that Congress is once again poised to provide full funding of $151 million for the Department of Justice Debbie Smith DNA Backlog Elimination program, as administered by the National Institute of Justice (NIJ). If NIJ follows a schedule similar to previous years, look for the crime lab solicitations in early Spring 2011, with awards issued in late September.

Congressional and State Action on Rape Kit Backlogs

Over the last twelve months, Congress and several state legislatures have paid careful attention to the matter of rape kit backlogs—specifically, to those kits potentially sitting in police evidence rooms, which have never been submitted to crime laboratories for analysis. In some instances, such kits may have been held by law enforcement due to questions of consent or veracity of victim statements. However, a surprising number of rape kits in these backlogs have been from stranger rapes and were held back from the crime lab for no logical reason. This failure to adequately investigate sexual assaults has been a rallying cry for numerous victim-based associations that have been calling for new policies for testing of all rape kits.

In California, a bill was passed by the Legislature to require a significant new reporting regime for local law enforcement regarding rape kit backlog (AB 558). The new law would have required ongoing reporting of figures on the number of rape kits collected, the number submitted for analysis, the number *not* submitted for analysis, the number tested, and the number *not yet* tested. In addition to the new reporting burden, there was also a feeling that the net effect of the law would be a significant increase in the number of rape kits submitted for analysis (as local law enforcement would not want to be held responsible for holding onto large numbers of unsubmitted rape kits). Unfortunately, the Legislations did not consider or attempt to offset the resources impact this would have on law enforcement or laboratory operations. In the Governor's veto message, he indicated this lack of consideration of the limited resources available to crime laboratories.

Crime laboratories in Illinois did not fare as well in the 2010 State Legislative session. Untested rape kits were a matter of particular concern to the State Legislature as well as to other state politicians, including the Attorney General. After considerable study and debate, HB 3269, the *Sexual Assault Evidence Submission Act*, was signed into law. This bill requires all local law enforcement to submit all sexual assault evidence within ten days, requires all old sex assault evidence to be submitted within 180 days of enactment (DATE) and mandates a statewide counting of backlogged rape kits. With this information, the lab is required to develop and submit a strategy (including funding needs) for analyzing all these cases by February 2011. Furthermore, the law requires that the lab shall perform all DNA analysis of sexual assault evidence within six months of receipt *upon sufficient funding*. Not surprisingly, at this time sufficient funding has not been provided but the State Legislature will be considering the funding piece of this law again in 2011.

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In Congress, the Chairman of the Senate Judiciary Committee (Sen. Patrick Leahy) has introduced the *Justice for All Act Reauthorization*, S. 3842. This bill makes some significant changes to the *Debbie Smith DNA Backlog Elimination Act*. Most notably, a new area of funding is added to the DNA grant program that would allow local law enforcement to access the funding in order to develop protocols, provide training, and create communication systems to track DNA evidence. Protocols and new definitions of DNA backlogs would first be developed by NIJ and then various reporting requirements on backlog figures would be mandated for participation in the DNA grant program. If these new reporting requirements (such as figures on the number of unanalyzed rape kits) are not met, then a 50 percent penalty is applied to the next year's funding award. At this time, there is some interest by the Committee in still moving this bill forward in 2010 but with the clock winding down for the year, significant activity is unlikely.

Update on State DNA Database Legislation and Related Federal Legislation

The 2010 State Legislative sessions ended with three additional states passing legislation to require DNA collection before conviction for certain felony crimes (North Carolina, Ohio, Utah)—bringing the total to 24 states that have passed such laws nationwide. Not surprisingly, limited state budgets have become an increasingly significant obstacle in passing arrestee DNA legislation. Each year, a growing number of states choose to fund their DNA database programs through a nominal fee on all criminal convictions and related violations (such as speeding and parking tickets). However, ensuring that fees go directly into a DNA fund and not through a general fund first is of great importance in the viability of this fee as a true funding source for DNA programs.

At the federal level, Congress has been working on H.R. 4614 / S. 3805, the *Katie Sepich Enhanced DNA Collection Act*. This bill would provide significant new funding to states that have enacted laws for collection of DNA upon arrest for felony crimes. The bill passed the House of Representatives in May by an overwhelming majority (357-32) and is currently pending in the Senate.

Please be sure to contact Lisa Hurst at 202-251-8978 or lhurst@gth-gov.com for assistance in any way in determining how best to address concerns with or support of state and international legislative matters pertaining to forensic DNA.

How to Cite This Article

L. Hurst (2011) Congress Moves Toward Full Funding for FY 2011 DNA Backlog Grants; Legislative Solutions to Address Rape Kit Backlogs; State Database Expansion. *Forensic News* January; [Internet] 2011. [cited: year, month, date]; Available from: http://www.appliedbiosystems.com/ForensicNews-Past

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event corner

Event Recap: 10th Annual *Future Trends in Forensic DNA Technology* Seminar Series

Over 560 forensic scientists attended the 10th Annual *Future Trends in Forensic DNA Technology* Seminar Series as it toured across the United States this summer according to the following schedule:

- July 7 — Austin, TX
- July 20 — Berkeley, CA
- July 22 — Anaheim, CA
- July 28 — Burlington, VT
- Aug 3 — Orlando, FL
- Aug 5 — Atlanta, GA
- Aug 17 — Washington, DC
- Aug 19 — New York, NY
- Aug 24 — Chicago, IL
- Aug 26 — Phoenix, AZ

The "HID Roadshow", as it is often called, featured insightful presentations from practicing forensic scientists, crime scene investigators, governmental affairs experts, and Life Technologies scientists, covering a wide range of topics including:

- Optimizing Extraction Efficiency with the AutoMate *Express*™ Forensic DNA Extraction System
- Laboratory Evaluation of Bench-top DNA Extraction Technologies for Forensic Casework Applications
- Laboratory Validation of the AmpFℓSTR® Identifiler® Plus Kit
- Technical Challenges Related to the Development and Use of Large Multiplex STR Assays
- Validation of the 3500 Series Genetic Analyzer for Human Identification
- Interesting Cases
- DNA Policy and Funding Update: the US and Abroad
- Strategies for Efficient Integration of New Technologies into Your Workflow while Enhancing Quality Assurance

Life Technologies would like to extend our sincere thanks to all the speakers and attendees from the forensic community whose participation made this year's *Future Trends in Forensic DNA Technology* Seminar Series a tremendous success. We hope you will join us at next year's Seminar Series and other HID University events as we continuously strive to offer high-quality training opportunities to meet the unique and evolving needs of forensic DNA scientists.

View the presentations from the 10th Annual *Future Trends in Forensic DNA Technology* Seminar Series

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Event Recap: The ANZFSS 20th International Symposium on the Forensic Sciences

Over 950 delegates attended the 20th International Symposium on the Forensic Sciences held at the Sydney Convention and Exhibition Centre in Darling Harbour, Sydney, Australia, September 5–9, 2010. This bi-annual meeting of forensic practitioners covers the fields of forensic DNA, analytical chemistry, toxicology, pathology, anthropology, crime scene and education and more.

The Life Technologies booth was a popular stop for attendees; picking up an Identifiler® Direct Notebook or Identifiler® Plus Magnetic Board and meeting our international support and marketing personnel from offices all across the globe.

The true highlight of the event was the "Debbie Smith Public Lecture on DNA Evidence." Debbie and Rob Smith attended from the United States to share their very personal story on how DNA evidence can change the life of a rape victim. Debbie was the victim of sexual assault in 1989. Her assailant was convicted some time later with the help of the DNA database CODIS (Combined DNA Index System). The audience was greatly moved as Debbie recounted her assault and her subsequent fight for justice. With the support of her husband Rob, Debbie campaigned successfully for U.S. legal reform to improve investigations and services for this crime, resulting in the Debbie Smith Act of 2004.

To learn more about Debbie Smith, visit http://www.h-e-a-r-t.info/.

To learn more about the ANZFSS 20th International Symposium on the Forensic Sciences, visit http://www.anzfss2010.com/.

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Event Recap: 21st Annual International Symposium on Human Identification

Over 600 registrants attended the 21st annual ISHI meeting held in San Antonio, Texas. The Life Technologies booth included a race car game that generated a lot of excitement and product displays featuring The AutoMate *Express*™, Next-Generation AmpFℓSTR® Kits, and the 3500 Series Genetic Analyzer.

Presentations and Posters

Development and Validation of 3500 Series Genetic Analyzers
Jeff Sailus, Life Technologies (Oral Presentation)

Competitive Extraction Robot Audit: A Head to Head to Head Comparison of the Applied Biosystems® AutoMate *Express*™, QIAGEN® EZ1® Advanced XL and the Promega® Maxwell® 16
Meredith A. Turnbough, University of North Texas Health Science Center, Department of Forensic and Investigative Genetics (Poster Presentation)

An Automated Benchtop System for Extraction of High-Quality DNA from Biological Materials
Allison Holt, Life Technologies (Poster Presentation)

Identifiler® Plus Validation for Forensic Casework
Roger Kahn, Harris County Institute for Forensic Sciences (Poster Presentation)

The AmpFℓSTR® NGM SElect™ Kit: A Next-Generation Multiplex STR Kit Containing the SE33 Locus
Bob Green, Life Technologies (Poster Presentation)

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Join Life Technologies at the 31st Stain Workshop of the German Society of Legal Medicine (GEDNAP) in Hamburg, Germany, February 25–26, 2011

This international workshop focuses on quality assurance as well as the most recent developments in forensic DNA analysis technologies. The scientific program is accompanied by a presentation of the results of the GEDNAP Proficiency Test, which has been adopted by the ENFSI group as the officially accepted external trial system.

For more information on the conference, visit:
http://www.r-km.de/Spurenworkshop2011/index.html

Central European Forensic User Meeting

Life Technologies will once again host the *Central European Forensic User Meeting* in connection with the 31st Stain Workshop. The User Meeting will be held in German language and will focus on tools, methods and next-generation solutions that improve efficiency and performance across the forensic DNA analysis workflow. Stay tuned for further information!

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did you know

In California, "Familial DNA Searching" was recently used in the "Grim Sleeper" case—the first high-profile U.S. case cracked by this controversial investigative technique.

Learn more >>

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