The AmpFℓSTR® NGM™ PCR Amplification Kit: The Perfect Union of Data Quality and Data Sharing

Since the establishment of the first National DNA Database in the United Kingdom in 1995, forensic DNA databases have become one of the most powerful tools used by law enforcement agencies to facilitate forensic investigations. These databases have been extremely successful in tracing criminals within country borders, but unfortunately crime does not respect national boundaries, and it is becoming increasingly necessary for different countries to share data efficiently and effectively in order to trace those responsible for criminal activity. Efforts to drive this process within Europe have focussed around the Prüm Treaty, to which all member nations of the European Union must commit by 2011. The treaty has opened the door for much wider exchanges of data between the European nations, but this brings with it significant implications for how DNA profiles are generated. Currently, the profiling of only 7 loci (the European Standard Set of loci or "ESS") is required by each European country. More loci will be required in order to prevent significant numbers of adventitious matches once data exchange becomes a regular and widespread reality.

To address this situation, the European Network of Forensic Science Institutes (ENFSI) and European DNA Profiling Group (EDNAP) published recommendations on new multiplex developments. These recommendations were designed to encourage manufacturers to develop a next-generation chemistry capable of producing more discriminating DNA profiles on a wider number of samples. Such a chemistry would facilitate data sharing initiatives through the inclusion of new, highly discriminating, shorter STR loci to maximize performance on degraded and challenging samples. Pending the necessary political approval, these new loci will form part of an expanded ESS, increasing the number of loci available for comparison between databases and minimizing the number of adventitious matches arising when data is compared internationally.

NGM™—The Next Generation Multiplex

The AmpFℓSTR® NGM™ PCR Amplification Kit represents a breakthrough in forensic DNA analysis technology. Leveraging Applied Biosystems recent advances in amplification chemistry development, the NGM™ Kit has been designed specifically to address the twin requirements stipulated by the ENFSI/EDNAP groups for a chemistry that can deliver unparalleled data quality and a powerful level of discrimination to support European data sharing initiatives. The NGM™ Kit simultaneously amplifies the 10 SGM Plus® Kit loci (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 & FGA), which include the 7 ESS loci, together with the 5 additional loci recommended by ENFSI and EDNAP (D10S1248, D22S1045, D2S441, D1S1656 & D12S391). These additional loci have been recommended due to their discrimination power and ability to be engineered to produce amplicons in the shorter size range to facilitate analysis of degraded samples.

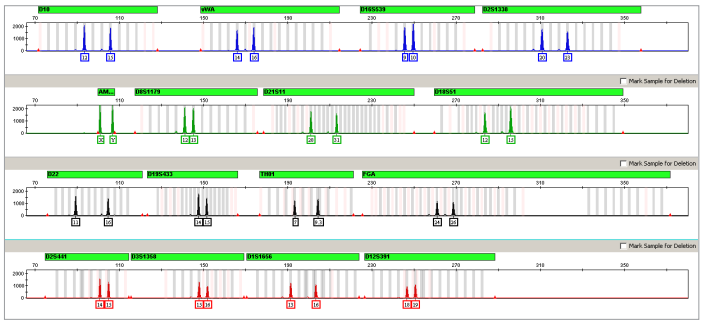

Figure 1: An example of a profile generated from 1ng of Control DNA 9947A using the NGM™ Kit.

Improved Performance on Degraded Samples

Engineering a single multiplex containing 15 STR markers plus Amelogenin, optimized to support the very different demands of databasing and casework samples, is a complex process. Database samples are collected according to regionally standardized processes and generally yield high quantities of high quality DNA. Casework samples, in contrast, are highly varied in nature, frequently compromised by degradation or the presence of inhibitors, and limited in amount—all of which can significantly impact the success of the analysis process. The NGM™ Kit takes advantage of Applied Biosystems demonstrated expertise in utilizing 5-dye chemistry and mobility-modifier technology to maximize the number of loci contained within a multiplex, while minimizing the size of the amplicons. This simultaneously addresses the requirements for overall discrimination power on database samples and performance on degraded casework samples. Three of the new loci (D10S1248, D22S1045 and D2S441) occupy the shortest position in each of three dye colors, maximizing the potential for data recovery from even the most degraded of samples. The two remaining new loci are located in the middle range of the multiplex, and add significantly to the discriminating power of the system as a whole (Figure 2).

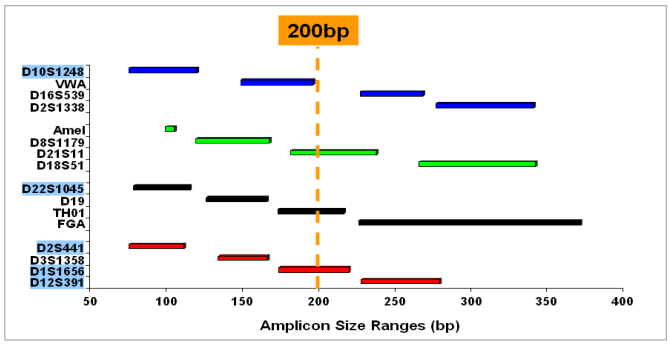

Figure 2: Configuration of the NGM™ Kit. Amplicon size ranges depicted indicate the run length on an Applied Biosystems capillary electrophoresis platform, including the effect of the mobility modifiers used to optimize the spacing between loci, and prevent overlap of alleles between adjacent markers. The names of the 5 new loci included, according to the recommendations of the ENFSI/EDNAP groups, are highlighted in blue.

The combination of loci contained within the NGM™ Kit delivers the highest level of discrimination of any AmpFℓSTR® Kit (Table 1). By concentrating the majority of the loci below 200 bp, a greater number of alleles can be recovered from degraded samples (Figure 3). This means that even partial profiles from highly degraded samples can provide a level of discrimination very close to that possible from a full SGM Plus® Kit profile.

Kit Configuration	Populations		
	US Hispanic	African American	US Caucasian
SGM Plus®	Not Available	7.9×10^{-14}	2.99×10^{-13}
SEfiler™ Plus	Not Available	6.47×10^{-15}	7.46×10^{-14}
Identifiler®	7.65×10^{-18}	1.31×10^{-18}	5.01×10^{-18}
MiniFiler™	1.05×10^{-10}	6.52×10^{-11}	8.21×10^{-11}
Identifiler® ≤200 bp	7.32×10^{-08}	2.01×10^{-08}	6.10×10^{-08}
NGM™	1.60×10^{-19}	4.61×10^{-20}	2.21×10^{-19}
NGM™ ≤200 bp	3.31×10^{-12}	8.75×10^{-13}	2.64×10^{-12}

Table 1: Comparison of Pi values of different AmpFℓSTR® Kit & locus configurations. As shown in yellow, the NGM™ Kit delivers the highest level of discrimination. Even highly degraded samples that yield only partial NGM™ profiles for loci ≤ 200 bp provide strong discrimination (shown in green) close to that possible from a full SGM Plus® profile.

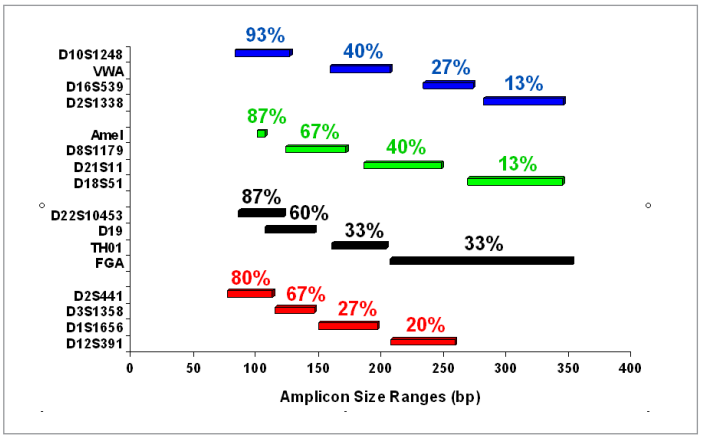

Figure 3: Analysis of the distribution of allele recovery in partial profiles generated using the NGM™ Kit from a selection of challenging casework samples. The size ranges of the loci are depicted without the influence of mobility modifiers, and therefore reflect the actual length of each amplicon. Of particular note is the very high level of recovery obtained using the new miniSTR loci D10S1248, D22S1045, and D2S441.

Maximum Performance on Inhibited Samples

Forensic samples frequently contain inhibitors derived either from the substrate on which the sample is found or, on occasion, from the extraction techniques employed to extract DNA from complex sample matrices. Inhibitors can have a very detrimental effect on the PCR reaction, and interfere with the ability of an amplification kit to generate high-quality results. In 2007, Applied Biosystems® released the MiniFiler™ Kit, the first chemistry designed to address specifically the challenges posed by degraded and inhibited samples. Amplicons engineered in a very short size range combined with a more powerful reaction mix supported the analysis of compromised and difficult samples, recovering data from samples which previously yielded very little or no result. Later that year, the SEfiler™ Plus Kit was released, which built on the developments of the MiniFiler™ Kit, and further enhanced the performance of a large multiplex in the presence of inhibitors. The NGM™ Kit represents yet another step forward. Containing our most powerful reaction mix to date, the NGM™ Kit is capable of recovering data from samples containing unprecedented levels of inhibitors, increasing the number of samples yielding a full profile at the first attempt (Figure 4).

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The Perfect Union of Data Quality and Data Sharing *(continued)*

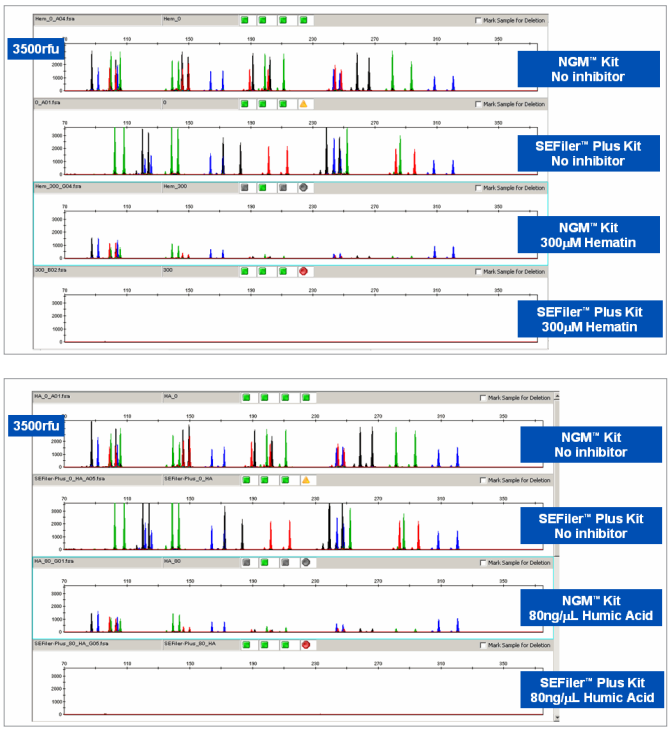

Figure 4: An illustration of the increased capability of the NGM™ Kit to amplify samples in the presence of very high levels of inhibitors. In this example, the NGM™ Kit is compared against the SEfiler™ Plus Kit, the AmpFℓSTR® Kit previously considered the most robust in the presence of inhibitors. When challenged with significant amounts of hematin and humic acid, the NGM™ Kit out performs the SEfiler™ Plus Kit, yielding profiles at levels of inhibition not previously possible.

A Greater Level of Sensitivity

The proven utility of DNA in forensic investigations has driven laboratories to attempt the amplification of low amounts of DNA. The NGM™ Kit has been optimized for 29 cycles of PCR, which, when combined with the highly optimized reaction mix, delivers enhanced sensitivity compared to previous AmpFℓSTR® Kits such as the Identifiler® or SGM Plus® Kits (Figure 5).

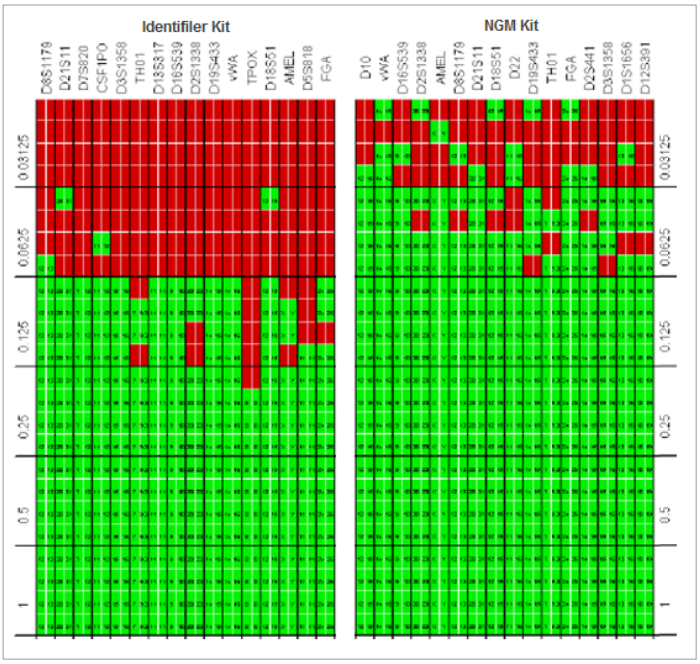

Figure 5: Sensitivity comparison between the Identifiler® and NGM™ Kits conducted by an NGM™ Kit test site. Loci contained within each kit are depicted across the x-axes, and input DNA amount in ng is shown on the Y-axes. The red squares on each diagram indicate amplification replicates where allelic dropout has occurred.

Unparalleled Data Quality

In addition to addressing the requirements outlined by ENFSI/EDNAP for additional loci and performance on specific challenging sample types, the NGM™ Kit has been developed according to Applied Biosystems stringent performance standards to provide gold standard data quality. Optimized as part of an integrated reagent, instrumentation, and software system, the NGM™ Kit offers maximum performance and efficiency on industry-standard capillary electrophoresis systems. A 1 ng input DNA recommendation maximizes heterozygote peak height balance within each profile. This allows laboratories to take full advantage of expert system analysis of database samples while increasing the level of confidence with which casework samples can be interpreted. Analysis of casework samples has been further enhanced through improvements

in Applied Biosystems primer manufacturing techniques, which minimize the presence of dye-related artifacts that can interfere with data interpretation (Figure 6).

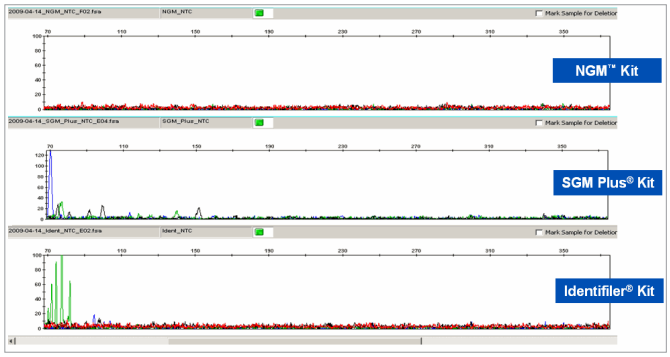

Figure 6: A comparison of negative control reactions for three different AmpF*ℓ*STR® Kits. The improved primer manufacturing process introduced for the NGM™ Kit results in significantly cleaner baselines than for previous AmpF*ℓ*STR® Kits.

Ease-of-Use

Increases in sample numbers have prompted many laboratories to invest in automated systems for the processing of both database and casework samples. To ensure compatibility with both manual and automated systems the NGM™ Kit will be available in two package sizes, the standard 200 reaction kit and a larger 1000 reaction kit. The larger package will include larger bottles to facilitate use on liquid handling platforms. The enzyme is now contained within the master mix, simplifying reaction set-up compared to the SGM Plus® and Identifiler® Kits, and cycling times have been shortened by approximately one hour, allowing laboratories to achieve better quality results more quickly.

Compatibility With Existing Databases

The importance of the inclusion of additional loci into new multiplexes has been eloquently discussed in the original publications on the subject. However, any new multiplex development must also take into consideration the volume of data already generated using existing kits and stored on national databases, which will be compared to any new data generated. Within Europe alone, over 6 million DNA profiles from both suspects and convicted offenders are stored on national databases, and much of this data has been generated using existing AmpF*ℓ*STR® Kits such as SGM Plus®, Identifiler® and SEfiler™ Plus. To facilitate comparisons between this valuable repository of data and profiles generated using the new chemistry, the NGM™ Kit uses identical primer sequences for all the STR loci common to other AmpF*ℓ*STR® Kits (Figure 7), allowing complete concordance. Primer sequences for the five new loci have been completely reengineered from published data to optimize both performance on forensic samples and position the loci within the new multiplex. The inclusion of these new loci has been achieved without compromising the performance of the existing SGM Plus® loci.

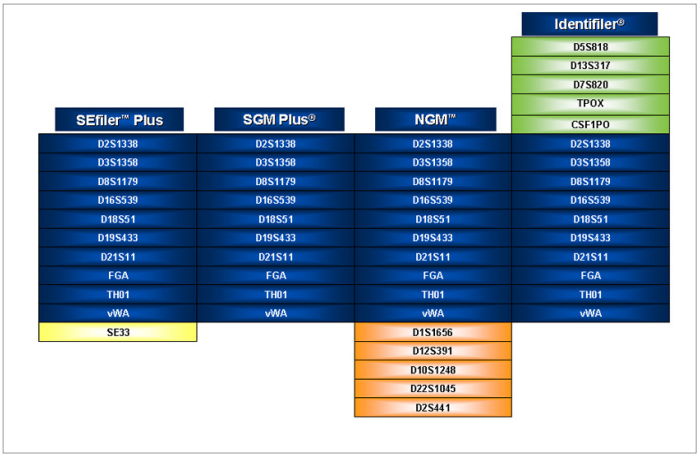

Figure 7: Comparison of common loci between the NGM™ Kit and existing AmpF*ℓ*STR® Kits. Loci in the blue boxes utilize identical primer sequences, maximizing concordance between results generated with different kits.

The Power of Collaboration

The capabilities of the new AmpF*ℓ*STR® NGM™ PCR Amplification Kit are a powerful testament to the benefits of collaboration. Since the initial publication of the recommendations on new loci in 2005, Applied Biosystems® has worked closely with laboratories within ENSFI and EDNAP to help ensure delivery of a chemistry with the capability to not only meet, but exceed their requirements. Since the establishment of a prototype system in 2007, our test sites have participated in three rounds of testing, and influenced the development process by providing essential feedback on the performance of the chemistry. This close relationship with operational forensic laboratories has enabled more thorough assessments of the chemistry at each stage of the process, on a much wider range of samples than would have been possible otherwise.