# SDPD FORENSIC SCIENCE SECTION

## Forensic Biology Section

### AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Summary

The SDPD Forensic Science Section currently employs Promega's PowerPlex Y kit for Y-STR analysis. Applied Biosystems has also released a kit for the analysis of Y-STRs: the AmpF*l*STR Yfiler PCR Amplification Kit. The Yfiler kit amplifies all of the loci present in the PowerPlex Y kit as well as five additional Y-STR loci, providing increased discrimination potential of Yfiler over the PowerPlex Y kit. Because Yfiler uses the same five dye chemistry as other AmpF*l*STR kits currently employed by the SDPD Forensic Science Section (Identifiler, Minifiler, etc), both autosomal STRs and Y-STRs can be electrophoresed in the same run, unlike PowerPlex Y. Electrophoresis of samples amplified using PowerPlex Y is currently only performed using the 310 Genetic Analyzer. This validation study examined the AmpFlSTR Yfiler Kit using both the 310 and 3130 Genetic Analyzers.

The sensitivity of Yfiler was examined using both the 310 and 3130 electrophoresis platforms. Full profiles were generally obtained using 0.2ng of male template. The data obtained support the manufacturer's suggested range of template (0.5-1.0ng of male DNA) and 0.75ng of male template DNA is recommended as a target for casework sample amplification. This study recommended a threshold of 100 RFU for the interpretation of casework samples.

Two person mixtures of male DNA at varying ratios were amplified using Yfiler. In DNA mixtures where greater than 80% of the DNA is from a single contributor, the DNA profile from the major contributor could be easily determined. In DNA mixtures where greater than 90% of the DNA is from a single contributor, DNA types from the minor contributor can drop out of a profile or be indistinguishable from stutter or other artifacts. This study recommended that the DNA profile from the minor contributor to some DNA mixtures should be determined with caution. Male DNA was amplified in the presence of varying amounts of female DNA using Yfiler. The presence of female DNA did not have any significant effect on Yfiler analysis.

In-house samples and samples from the NIST Standard Reference Material 2395 were amplified using Yfiler and PowerPlex Y to verify concordance. All samples gave concordant results for the loci common to both kits. Although direct comparison of Yfiler and PowerPlex Y data can be performed, this study recommended that samples for comparison be analyzed using the same primer set, if feasible.

Simulated casework samples were successfully amplified using Yfiler. Based on the results of these validation experiments, Yfiler has been shown to be a reliable tool and should be adopted for casework analysis.

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# SDPD FORENSIC SCIENCE SECTION

## AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Study

### SIMULATED CASEWORK STUDY

## INTRODUCTION:

The purpose of this study was to test simulated casework samples using the AmpF*l*STR Yfiler Amplification kit. Samples were selected to evaluate the ability of Yfiler to amplify degraded and/or inhibited samples. These samples were also amplified using Identifiler for comparison purposes (data not shown).

## MATERIAL AND METHODS:

**Samples**:

Previously extracted and quantitated DNA samples from a noncriminal missing person case and from a recent proficiency test were used for this experiment. DNA from additional simulated casework samples were extracted for testing, including: a swab of a steering wheel, a swab of a cell phone, a body swab of an area with moisturizing lotion, a cigar and a swab with cola flavored soda present. All but one of the samples was expected to have sufficient male DNA present based on previous quantitation or DNA typing results.

**Amplification**:

Approximately 1.0ng of male template DNA was amplified for each sample using the thermal cycling conditions recommended by the manufacturer. Previously prepared TE buffer was used to prepare all dilutions and to supplement amplification reactions to a final volume of 25ul.

**Capillary Electrophoresis and Data Analysis:**

Samples were prepared for electrophoresis on the 3130 Genetic Analyzer as described by the manufacturer in the section of the AmpF*l*STR Yfiler User's Manual Chapter 4 entitled "3100/3100-*Avant* Electrophoresis Setup". Analysis of data was performed using GeneMapper ID Software following the SDPD Forensic Biology Technical Manual using a detection threshold of 50RFU for the blue, green, yellow and red dyes.

**RESULTS:**

At least partial profiles were obtained from all samples. Partial profiles were only obtained from samples that were known to have degraded DNA or inhibitors present and from a sample with very little male DNA present. Samples that showed mixtures with Yfiler also showed mixtures with Identifiler. Samples prepared from male DNA with known Y-STR types gave the expected DNA results.

Other than in a sample with numerous off-scale peaks, artifacts were either below the 100 RFU threshold or below the filter percentages recommended for casework.

**CONCLUSIONS:**

It is recommend that Yfiler be approved for casework analysis.

**REFERENCES:**

AmpF*l*STR Yfiler User's Manual
SDPD Forensic Biology Technical Manual

# SDPD FORENSIC SCIENCE SECTION

## AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Study

### SENSITIVITY STUDY

**INTRODUCTION:**

The purpose of this study was to examine the sensitivity of the AmpF*l*STR Yfiler Amplification Kit and determine a minimum quantity of male DNA required to produce a full Y-STR DNA profile, and in addition, establish a general range of DNA concentrations that ensure the reliability and integrity of DNA typing results when using the AmpF*l*STR Yfiler Amplification Kit in conjunction with 310 and 3130 Genetic Analyzers.

**MATERIAL AND METHODS:**

**Samples**:

Two previously extracted and quantitated male DNA samples (DC and EP), together with the AmpF*l*STR Control DNA 007, were used in this study.

**Amplification**:

Approximately 0.1ng, 0.2ng, 0.5ng, 0.75ng, 1.0ng, 1.25ng, 1.5ng, 2.0ng and 4.0ng of DC and EP DNA and 0.1ng, 0.2ng, 0.5ng, 0.75ng and 1.0ng of AmpF*l*STR Control DNA 007 were amplified using the thermal cycling conditions recommended by the manufacturer. Previously prepared TE buffer was used to prepare all dilutions and to supplement amplification reactions to a final volume of 25ul.

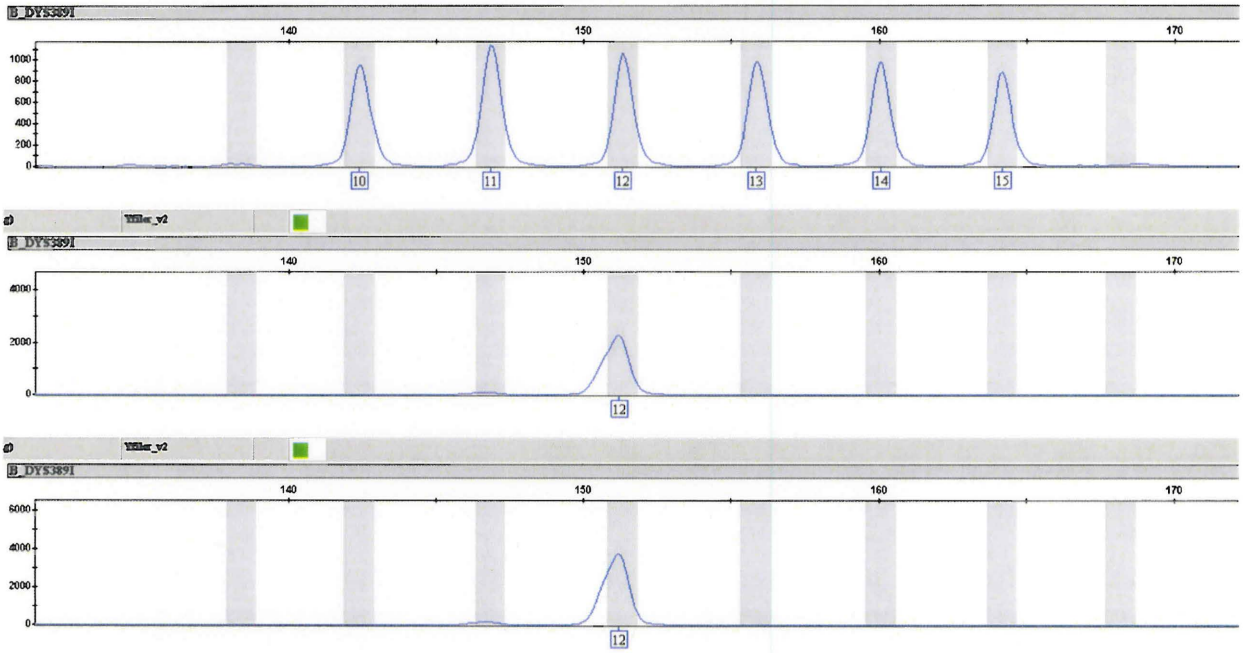
**Capillary Electrophoresis and Data Analysis:**

Samples were prepared for electrophoresis on the 310 Genetic Analyzer as described by the manufacturer. Samples were prepared for electrophoresis on the 3130 Genetic Analyzer as described by the manufacturer for "3100/3100-*Avant* Electrophoresis Setup". Analysis of data was performed using GeneMapper ID Software following the SDPD Technical Manual using a detection threshold of 50RFU for the blue, green, yellow and red dyes.

**RESULTS:**

Data collected using the 310 Genetic Analyzer generally showed greater peak heights for the same sample than data collected on the 3130 Genetic Analyzer. Sensitivity differences between the 310 and 3130 platforms have been observed previously. In the 3130 validation, peak heights were approximately forty percent higher on the 3130 than on the 310. Sensitivity differences have also been observed when using different capillaries and/or arrays on the same instrument.

Previous analysis showed significantly reduced peak heights at DYS389I relative to the other loci in blue (Initial Evaluation). In this study, the peak heights at DYS389I were not significantly reduced, although they did show broadening and/or shouldering of the peaks. Two examples in comparison to the allelic ladder are shown below.




Full profiles >50 RFU were obtained from all samples in this study regardless of the input template amount or electrophoresis platform. Each sample amplified with 0.1ng of template (DC, EP and 007) gave one allele with a peak height below 75 RFU and an additional allele with a peak height 100 RFU or below using the 3130 Genetic Analyzer. All samples run on the 310 Genetic Analyzer had peak heights greater than 100 RFU.

Peak heights greater than 500 RFU were observed for samples DC and EP for all input template amounts greater than 0.5ng, regardless of instrument platform. Peak heights greater than 500 RFU were observed for AmpF*l*STR Control DNA 007 for all input template amounts greater than 0.75ng, regardless of instrument platform. When amplifying 0.5ng of 007 Control DNA, all peak heights were greater than 500 RFU using the 310 Genetic Analyzer; however using the 3130, numerous peaks were below 500 RFU.

Off-scale data were obtained from DC and EP amplified with 4.0ng of template, regardless of platform; however off-scale data were obtained from both of these samples amplified with as little as 1.5ng using the 310 Genetic Analyzer.

Called stutter peaks were observed in samples amplified with as little as 0.5ng of template using the 3130 Genetic Analyzer (DC and EP) and as little as 0.1ng using the 310 Genetic Analyzer (EP). N+3bp artifacts were typically present at the trinucleotide repeat locus DYS392 and n-2bp artifacts were typically present at DYS19 in samples amplified with more than 0.5ng. Only one sample amplified with less than 1.0ng of DNA gave an n+3bp artifact greater than 100 RFU at DYS392 (EP, 0.5ng, 310, 106 RFU). No n+3bp or n+4bp artifacts were observed above 100 RFU at loci other than DYS392 unless 2.0ng or more was amplified.

Numerous additional artifacts were observed, typically in samples amplified with more than 1.0ng of template. At DYS19, n+2bp artifacts were observed. One n+8bp artifact was observed at DYS456 (DC, 1.5ng, 310, 72 RFU). Matrix effects (pullup and raised baseline) were common, especially when peak heights exceeded 3000 RFU. Pullup and raised baseline artifacts are dependent on the matrix or spectral. Less of these artifacts may be present if a new matrix or spectral is created for the genetic analyzers.

**CONCLUSIONS:**

This study demonstrates that 0.2ng is generally sufficient to produce full profiles greater than 100 RFU. Because off-scale data were obtained with as little as 1.5ng of template DNA and artifacts were more common in samples amplified using greater than 1ng of template, this study concurs with the manufacturer's suggested range of 0.5-1.0ng of male template DNA.

Because most artifacts observed in this study were below 100 RFU for samples amplified with 1.0ng or less template DNA, and because all samples amplified within the range of 0.5-1.0ng of male DNA gave full profiles above 100 RFU, this threshold is recommended for interpretation of casework samples.

Based on this study, we recommend that 0.75ng of male DNA be targeted using the AmpF*l*STR Yfiler amplification kit for casework.

**REFERENCES:**

AmpF*l*STR Yfiler User's Manual
SDPD Forensic Biology Technical Manual

# SDPD FORENSIC SCIENCE SECTION

## AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Study

### Male:Male DNA Mixture Study

## INTRODUCTION:

Mixtures of DNA from more than one person are frequently encountered in casework samples, and interpretation of mixed DNA profiles is an important aspect of forensic DNA analysis. The purpose of this study was to examine the ability of the AmpF*l*STR® Yfiler™ PCR Amplification Kit to amplify mixtures of two male DNA samples together in varying ratios.

## MATERIAL AND METHODS:

**Samples**:

DNA was extracted and quantitated from four in-house male samples (JB, LD, JS and WMD) for use in this study. The samples were diluted to 0.075ng/µl using previously prepared TE buffer. Two combinations of these samples were selected for amplification (JB/LD and JS/WMD). These two combinations were chosen to limit the number of loci where each sample shared alleles. Male:male mixtures were created with the following ratios: 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and1:20 using previously diluted samples.

**Amplification**:

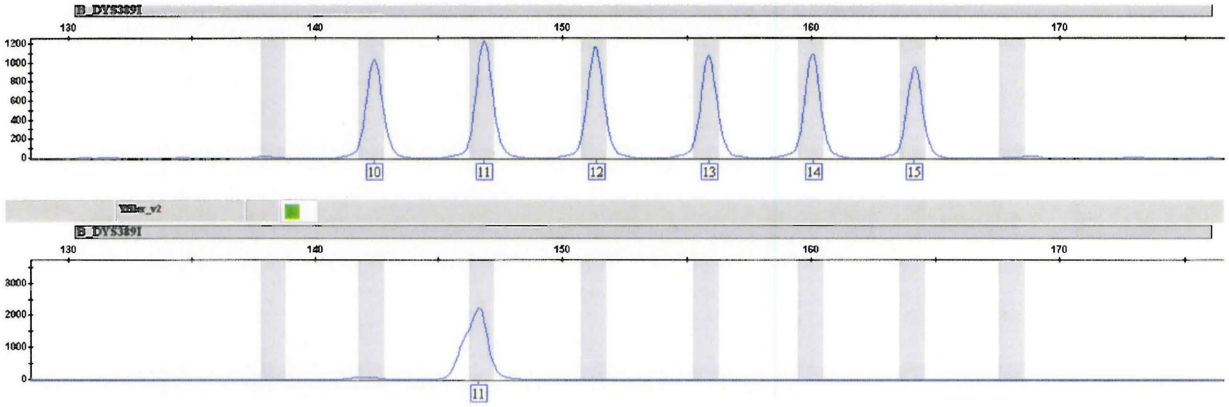
Based on the conclusions of the sensitivity study, approximately 0.75ng of total male DNA was targeted for each sample and mixture. Each of the four unmixed male samples as well as 10µl from each mixture ratio was amplified using the AmpF*l*STR Yfiler PCR Amplification Kit and the thermal cycling conditions recommended by the manufacturer.

**Capillary Electrophoresis and Data Analysis:**

Samples were prepared for electrophoresis on the 3130 Genetic Analyzer as described by the manufacturer in the section of the AmpF*l*STR Yfiler User's Manual Chapter 4 entitled "3100/3100-*Avant* Electrophoresis Setup". Analysis of data was performed using GeneMapper ID Software following the SDPD Forensic Biology Technical Manual using a detection threshold of 50RFU for the blue, green, yellow and red dyes.

**RESULTS:**

Previous analysis showed significantly reduced peak heights at DYS389I relative to the other loci in blue (Initial Evaluation). In this study, the peak heights at DYS389I were not significantly reduced, although they did show broadening and/or shouldering of the peaks. An example (JB) in comparison to the allelic ladder is shown below.



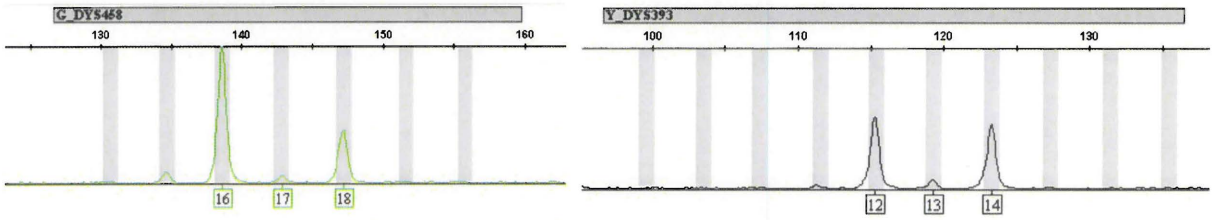

In mixed DNA samples with ratios of the two DNA contributors at 1:5, 1:2, 1:1, 2:1 and 5:1, all alleles from both contributors were detected above 100 RFU. In mixtures with ratios of the two contributors at 1:20, 1:10, 1:5, 5:1, 10:1 and 20:1, the heights of the alleles from the major contributor were significantly greater than the heights of alleles from the minor contributor. In general, peak heights from each contributor to the DNA mixtures were consistent with the expected ratios.

Three of the four mixed DNA samples with ratios of the two contributors at 1:10 (or 10:1) showed at last one allele from the minor contributor with a peak height below 100 RFU. The fourth sample (10 JB: 1 LD) had an allele at DYS456 that was not labeled by the GeneMapper software because it was below the stutter filter cutoff of 13.21% (417 RFU, 13.18%). In these mixtures, the alleles from the major contributor were detected well above the 100 RFU threshold.

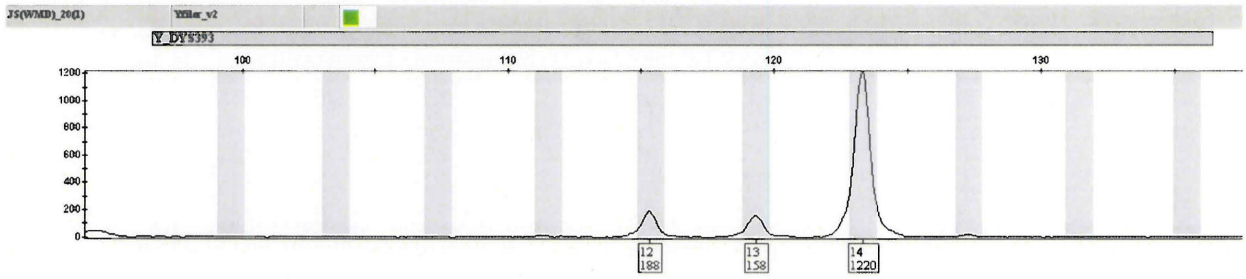
Each mixed DNA sample with a ratio of the two contributors at 1:20 (or 20:1) showed multiple alleles from the minor contributor that were either undetected or had a peak height less than 100 RFU. One sample (20 JS: 1 WMD) had five undetected alleles, three alleles with a peak height less than 100 RFU and two alleles that were not labeled because they were below the stutter cutoff. The alleles from the major contributor in these mixtures were detected well above the 100 RFU threshold.

Previous studies have shown that artifacts are common in samples amplified with Yfiler, especially at DYS19 and DYS392. In this study, the heights of these artifacts were similar to the height of alleles for the minor contributor in mixtures with ratios of the two contributors at 1:10 and 1:20 (or 10:1 and 20:1).

In mixtures where the alleles from the two contributors were separated by two repeat units, it was common to have an elevated stutter peak between the two alleles. This effect was most obviously observed in samples where peak heights of the alleles from each contributor were comparable. Two examples from the same sample are shown below.




One mixed sample [JS(WMD)_20(1)] showed significant peak height differences between alleles from the two contributors as expected. At DYS393, although the 12 allele is only 188RFU, the elevation of the stutter peak for the 14 allele is sufficient for this peak to be called as a 13 allele. Note that the stutter cutoff value for DYS393 is 12.58% and the 13 allele for this sample is 12.95% of the height of the 14 allele.




**CONCLUSIONS:**

In general, DNA mixtures should be interpreted with greater caution than single source samples. As in autosomal STRs, DNA mixtures with two contributors at ratios of 1:1, 1:2 and 2:1 may not allow for simple determination of which allele originated from which contributor to the mixture.

Because the alleles from the major contributors in mixtures with ratios of 1:20, 1:10, 1:5, 5:1, 10:1 and 20:1were detected at heights well in excess of the heights of the alleles from the minor contributor, it should be possible in many circumstances to determine the major contributor's DNA types. As the ratio of major to minor contributor increases (10:1, 20:1, etc), confident determination of the minor contributor's DNA types may become hampered by additional stutter peaks and artifacts as well as the loss of alleles due to dropout or filtering by the software; however it may be possible to obtain a partial DNA profile with confidence.

When a peak is called at the stutter position between two alleles in a mixed sample when the alleles are separated by two repeat units, the analyst should take into account that this called allele may be due to elevated stutter and not from a third (minor) contributor to the mixture. Analysts should be cautioned that this effect may result even if the smaller of the two alleles is below 200RFU.

The results obtained from mixed DNA samples using AmpF*l*STR Yfiler were generally consistent with past experience using other STR based analysis systems, and demonstrated that AmpF*l*STR Yfiler is suitable for analysis of male DNA mixtures in casework samples.

**REFERENCES:**

AmpF*l*STR Yfiler User's Manual
SDPD Forensic Biology Technical Manual

# SDPD FORENSIC SCIENCE SECTION

## AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Study

### Evaluation of Amplification Artifacts

**INTRODUCTION:**

The SDPD Forensic Science Section currently employs Promega's PowerPlex Y kit for Y-STR analysis. N-4bp stutter and other amplification artifacts are commonly detected using the PowerPlex Y system. The presence of these amplification artifacts can complicate interpretation of STR profiles, particularly from mixed samples. The purpose of this study was to evaluate the level of n-4bp stutter and other amplification artifacts using the Yfiler kit and to determine if the manufacturer's filter values should be adjusted.

**MATERIAL AND METHODS:**

Data were obtained from the previous studies of the Yfiler validation (Initial Evaluation, Sensitivity, Male:Female DNA Mixture Study, the Concordance Study and additional samples analyzed in conjunction with these studies). The samples in this study include multiple amplifications of twenty-five different male samples (nineteen in-house samples, five NIST samples and the 007 DNA Control provided in the Yfiler kit). Male:Male DNA mixtures and samples with off-scale data were not included in this study. Samples were evaluated to ensure that spikes or pullup were not present in stutter and other known artifact positions.

**RESULTS:**

Stutter peaks were not detected for all loci in all samples, particularly samples with less than 0.75ng of input DNA. All but four loci had more than eighty stutter peaks in this study. As expected, loci with relatively low stutter percentages (DYS438, DYS448, etc.) had fewer stutter peaks in this study than those with higher stutter percentages (DYS392, DYS456, etc.). No locus had all alleles in the allelic ladder represented and several loci had all or nearly all stutter peaks spread over only three alleles.

For each locus, all stutter values were plotted versus the allele for the corresponding stutter peak and these plots are attached. One outlier was removed from the data (NIST Sample B, DYS385, 17 allele, 25.68% stutter).

The average calculated stutter for the loci ranged from a low of 2.90% at DYS438 to 11.57% at DYS389II. The average stutter values for each locus are listed in an attached table and the values were below the manufacturer's default stutter filter for each locus. All but three loci had at least one sample with stutter above the stutter filter value, leading to called stutter peaks. In general, larger alleles at a given locus had higher stutter than smaller alleles and in almost all cases of called stutter peaks, the stutter was associated with one of the larger alleles at the locus.

Most samples had n-2bp artifacts at DYS19, the only locus to show this type of artifact. The ratio of the height of the n-2bp peak to the height of the main allele at DYS19 was also plotted. On average, the heights of these artifacts were 7.84% of height of the allelic peak.

Six samples also had n+2bp artifacts at DYS19. The heights of these artifacts averaged 1.76% of the height of the allelic peak.

The majority of samples showed n+3bp artifacts at the trinucleotide repeat DYS392. The ratio of the height of the n+3bp peak to the height of the main allele at DYS392 was plotted. On average, the heights of these artifacts were 5.40% of the height of the allelic peak.

Unlike previous observations with PowerPlex Y, no samples amplified with Yfiler showed n-9bp artifacts at DYS385.

**CONCLUSIONS:**

A few loci showed much lower average stutter than would be expected based on the manufacturer's default stutter filter value. This study only included DNA from twenty-five males, and not all alleles were represented in the study. It is likely that the stutter evaluation by the manufacturer included samples from a larger number of males, representing a wider range of alleles. The average stutter at DYS389I was less than half of the default stutter value for this locus. At this locus, multiple samples displayed lower peak heights relative to the other loci in blue and/or shouldering of the peak. These may have contributed to the low average stutter percent observed for this locus.

We recommend that the default stutter filter values be adopted for casework. Due to the fact that stutter generally increases as allele size increases, and because only a limited number of alleles were represented at several loci in this study, it is recommend that the stutter plots for each locus be employed to assess whether called peaks in stutter positions for relatively large alleles may be due to stutter alone or due to a second, minor contributor.

The current version of GeneMapper ID software does not allow for filtering of the n-2bp or n+2bp artifacts at DYS19 or the n+3bp artifacts at DYS392 observed in this study. We recommend that the data generated in this study be available to analysts to evaluate these artifacts for casework analysis. Genescan software previously employed by the SDPD Forensic Science Section allowed for filtering of the n-2bp artifacts at DYS19 and the n+3bp artifacts at DYS392, but not the n+2bp artifacts at DYS19. The manufacturer's default filter thresholds for Genescan are 10.21% for the n-2bp artifacts at DYS19 and 7.9% for the n+3bp artifacts at DYS392. We recommend that if future versions of GeneMapper ID allow for filtering of these artifacts, these values be adopted.

**REFERENCES:**

AmpF*l*STR Yfiler User's Manual
SDPD Forensic Biology Technical Manual

# SDPD FORENSIC SCIENCE SECTION

## AmpF*l*STR® Yfiler™ PCR Amplification Kit Validation Study

### Concordance Study

**INTRODUCTION:**

The SDPD Forensic Science Section currently employs Promega's PowerPlex Y kit for Y-STR analysis. The AmpF*l*STR Yfiler PCR Amplification Kit amplifies all of the Y-STR loci present in the PowerPlex Y kit as well as five additional Y-STR loci. The purpose of this study was to verify that the results obtained using the Yfiler kit are concordant with those obtained using the PowerPlex Y kit. In addition, this study verifies that the results obtained using the Yfiler kit for analysis of NIST traceable standards match the known results published by NIST.

**MATERIAL AND METHODS:**

**Samples**:

DNA from nineteen in-house male samples was previously amplified using the AmpF*l*STR Yfiler PCR Amplification Kit (see Initial Evaluation study). The same extracted and quantitated male DNA samples were used in this study. Samples A, B, C, D and E from the NIST Standard Reference Material 2395 were also used in this study.

**Amplification**:

Approximately 1ng of each in-house male DNA sample was targeted for amplification using the PowerPlex Y kit following the SDPD Forensic Biology Technical Manual. Approximately 0.75ng of each NIST Standard Reference Material sample was targeted for amplification using the Yfiler kit.

**Capillary Electrophoresis and Data Analysis:**

Samples amplified using Yfiler were prepared for electrophoresis on the 3130 Genetic Analyzer as described by the manufacturer in the section of the AmpF*l*STR Yfiler User's Manual Chapter 4 entitled "3100/3100-*Avant* Electrophoresis Setup". Samples amplified using PowerPlex Y were prepared for electrophoresis on the 310 Genetic Analyzer following the SDPD Forensic Biology Technical Manual

Analysis of data was performed using GeneMapper ID Software following the SDPD Forensic Biology Technical Manual. Yfiler data was analyzed using a detection threshold of 50 RFU for the blue, green, yellow and red dyes. PowerPlex Y data was analyzed using a detection threshold of 50 RFU for the blue, green and yellow dyes and a detection threshold of 200 RFU for the red dye.

**RESULTS:**

All in-house male DNA samples had concordant results between PowerPlex Y and Yfiler for all loci common to the two kits. Results for all tested NIST Standard Reference Material samples matched the results for the fifteen Yfiler loci listed in the Certificate of Analysis.

See attached summary tables

**CONCLUSIONS:**

It is common for STR kits from different manufacturers to use a different primer set for the same locus. Due to the use of different primer sets it is possible to have rare mutational events detected using one manufacturer's kit and not another. If possible, samples for comparison should be analyzed using the same primer sets. In circumstances where this is not feasible (previous analysis consumed a sample, a sample was analyzed by an outside laboratory using a different primer set, etc.), this study shows that comparison of Yfiler and PowerPlex Y STR data should be acceptable in most circumstances.

**REFERENCES:**

AmpF*l*STR Yfiler User's Manual
SDPD Forensic Biology Technical Manual
National Institute of Standards and Technology – Certificate of Analysis – Standard Reference Material 2395 – Human Y-Chromosome DNA Profiling Standard