SDPD Forensic Science Section – Forensic Biology Unit

Validation of the Yfiler® PCR Amplification Kit from Life Technologies Using a 3500 Genetic Analyzer

Baseline (signal-to-noise) - Analytical Threshold Study

Purpose

The goal of this study is to empirically determine the peak amplitude threshold (i.e. detection threshold) that allows for reliable interpretation of Yfiler® PCR Amplification Kit data. An analytical threshold defines the minimum height requirement at (and above) which detected peaks can be reliably distinguished from background noise. Because the analytical threshold is based upon a distribution of noise values, it is expected that occasional, non-reproducible noise peaks may be detected above the analytical threshold. An analytical threshold should be sufficiently high to filter out noise peaks. On the other hand, usage of an exceedingly high analytical threshold increases the risk of allelic data loss which is of potential exclusionary value. This study aimed to determine an ideal Analytical Threshold for the Yfiler® kit on 3500 Genetic Analyzers.

Materials and Methods

An amplification blank and DNA-containing samples were amplified using titrating amounts of input DNA and cycling parameters recommended by the manufacturer. The recommended parameters were: 10uL of TE for the blank, 0.5ng of DNA for amplification and 30 cycles with the Yfiler® kit. The amount of DNA amplified ranged from 0.7-0.04ng. The amplification product from the TE blank and the DNA-containing samples were injected for capillary electrophoresis on one 3500 instrument (3500B) according to the manufacturer's recommended parameters. Sixty-one DNA containing samples and one TE blank were used to analyze the analytical threshold. A detection threshold of 1 RFU was applied to all samples and blanks during GeneMapper ID-X (v1.4) analysis. All known allele peaks, PCR artifacts (i.e. stutter peaks and minus-A peaks), and peaks that spectrally overlapped with known peaks were removed prior to the assessment. The data was evaluated (from 60-460 bps) to determine the average baseline peak heights as well as the standard deviation of baseline peaks for each dye channel. Additionally, the RFU of the highest peak was determined for each dye channel that could not be identified as a DNA related peak or spectral artifact.

Results and Discussion

The baseline study evaluated the signal-to-noise ratio for the Applied Biosystems Yfiler® kit on the 3500 Genetic Analyzer. The instrumental noise (baseline) was examined from amplified TE blanks as well as from amplified single source samples (allele peaks, PCR artifacts, kit-specific

artifacts, and peaks that spectrally overlapped with known peaks were removed). A summary of the average, standard deviation, and maximum peak heights can be seen in Figure 1.

Amplified Samples (DNA peaks Removed)			
Channel	**Average**	**StDev**	**Max PH**
Blue	**4.99**	**3.41**	**57**
Green	**8.13**	**4.41**	**57**
Yellow	**15.00**	**5.70**	**59**
Red	**21.69**	**6.62**	**59**

Figure 1 – Peak Heights from samples amplified with Yfiler® and injected on 3500 Genetic analyzers

Average noise peak height and max peak height were similar between the blue and green channels and the yellow and red channels, respectively. Occasionally, noise peaks over ten standard deviations above the average were observed. In many of the amplified samples, four peaks of approximately 100 to 1200 RFU (between 60.0 and 90.0 bp, and at 114bp) were reproducibly observed in the green channel. A wide peak of 45-60 RFU (between 90 and 94bp) was reproducibly visible in the yellow channel. These were considered to be kit-specific artifacts and were excluded from baseline calculations.

The peak heights for the detection range of each dye channel were evaluated and separated according to size. The majority of the noise peak heights were below 10 RFU for the blue and green channels, below 20 RFU for the yellow channel, and below 30 for the red channel, as shown in Figure 2.

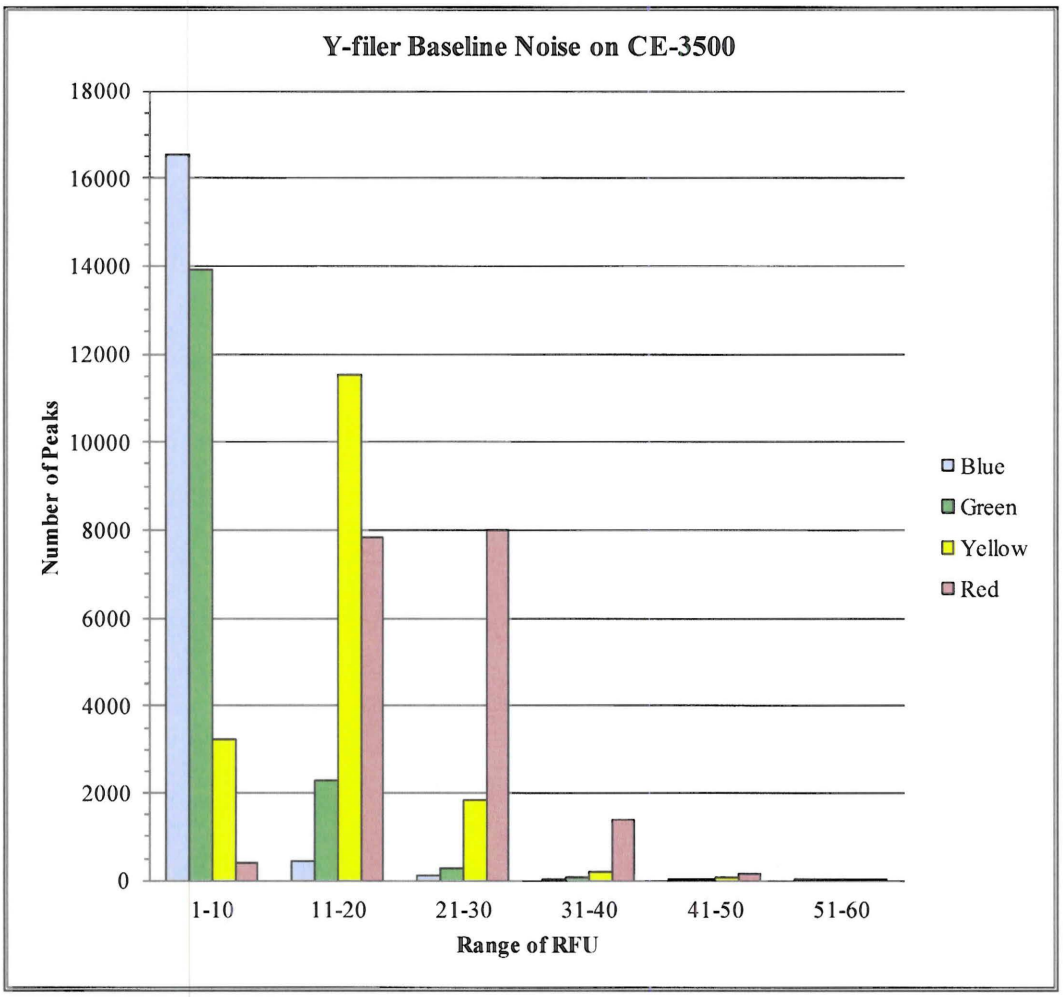

Figure 2 – Distribution graph evaluating the range of noise peaks

There are multiple ways of calculating an empirical analytical threshold from baseline data. Analytical thresholds are generally calculated by examining the baseline data and calculating the mean and standard deviation, then determining the threshold based on two or three times the standard deviation, or ten times the standard deviation. SWGDAM has previously given an example for determining the analytical threshold using two times the intensity difference between the highest peak in the baseline and the lowest trough (1). GeneMapper ID-X data generally omits negative baseline data (i.e. data below zero) in its collection of data. In order to compensate for this, one option in determining the analytical threshold would be to use a calculation of two or three times the highest peak in the baseline. Figure 3 shows three possible methods to help guide the analytical threshold value.

Method	Channel	Analytical Threshold (RFU)
Mean + 10 standard deviations	Blue	39
	Green	52
	Yellow	72
	Red	88
2X the highest baseline peak	Blue	114
	Green	114
	Yellow	118
	Red	118
3X the highest baseline peak	Blue	171
	Green	171
	Yellow	177
	Red	177

Figure 3 – Multiple methods to determine the analytical threshold

Conclusions

The data from the "Baseline Study" suggests that a peak amplitude threshold of 120 RFU is an appropriate analytical threshold for DNA casework.

References

Michael D. Coble, PhD. "Design and Execution of Validation Studies for Establishing DNA Mixture Interpretation Procedures". NFSTC DNA Mixture Interpretation Workshop. Largo, FL (March 15-17, 2011).

Validation Analysts

David Cornacchia, Criminalist

Shawn Montpetit
DNA Technical Manager

FB Supervisor Review

FB Supervisor Review

10/7/15

QA Review