

# A Forensic Validation Study on the Effectiveness of the Streck Philisa<sup>®</sup> High-Speed Thermocycler

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## Abstract

While DNA analysis can be extremely helpful, it is not a quick process. It can take more than 3 hours to complete the PCR amplification of forensic samples. Streck, a company that sells clinical laboratory products, has created the Streck Philisa<sup>®</sup> Thermocycler, which claims to be a high-speed thermocycler, and would complete PCR in about 30 minutes. The purpose of this project is to perform a validation study showing that the Streck Philisa<sup>®</sup> high-speed thermocycler provides the same quality results in less time than a regular thermocycler on forensically relevant samples. Various DNA sample types were collected. The DNA was extracted, quantified, amplified, separated, and analyzed using standard forensic DNA procedures and kits, with the exception of the thermocycler. Amplification was carried out using the Streck Philisa<sup>®</sup> high-speed thermocycler. Initial results showed that the instrument did in fact work, however results were not always optimal. Electropherograms of the analyzed DNA samples exhibited increased stutter peaks and PCR inhibition. These problems were especially evident in those samples with smaller quantities of DNA like hair and nail clippings. Decreasing the volume of master mix and the amount of DNA from 25  $\mu$ L of master mix plus approximately 1 ng of DNA to a 12.5  $\mu$ L sample and approximately 0.65 ng of DNA greatly enhanced the quality of the data. The addition of Bovine Serum Albumin (BSA) to the amplification mix improved results by helping with allele dropout and by reducing the amount of stutter.

## Introduction

Deoxyribonucleic acid (DNA) is a vital piece of evidence used in forensic science. It's very important to analyze biological samples found at crime scenes in order to make identifications, linkages, and to aid in the reconstruction of the crime. Since DNA is often found in small quantities or poor quality, the polymerase chain reaction (PCR) is used to make exact copies of a specific portion of the DNA. The products of PCR are known as amplicons. PCR is carried out using a thermocycler. A thermocycler is an instrument that heats and cools the DNA sample in order to synthesize the DNA amplicons in conjunction with an enzyme called DNA polymerase.

PCR has been a successful and useful method used to make millions of copies of DNA, which are then used for analysis and profiling. DNA profiling utilizes STR's, or short tandem repeats. Most of our DNA is identical to each other's. There are regions of a person's DNA sequence that do differ from person to person. These variations in the DNA sequences between individuals are known as polymorphisms. Certain sequences with high degrees of polymorphism are extremely useful in the analysis of DNA. One class of polymorphisms used for individualization are known as short tandem repeats. These are very short pieces of DNA, around 2-5 base pairs long that are repeated in tandem numerous times. STR's are helpful because each person has a unique combination of the different number of copies of the specific repeated sections of the DNA [1]. In this project, the Promega PowerPlex<sup>®</sup> 16 HS amplification kit was used. This is a multiplex STR system that amplifies 16 loci, which refers to the location of a specific DNA sequence on a chromosome. The kit also included the primers necessary to replicate the appropriate parts of the DNA that are going to be amplified during the PCR process.

PCR occurs in 3 steps; denature, annealing, and extension. The first step, denaturation, refers to heating up the DNA in order to break down the hydrogen bonds which

hold the DNA double helix together. This step allows the two strands to separate, creating single stranded DNA. The second step, annealing, refers to the process that allows the various STR primers to bind to their complimentary sequence on the template strand of DNA. The third step, extension, is when an enzyme known as *Taq* polymerase extends the primers by adding nucleotides and using the target DNA strand as a template [2]. The resulting strands created are called amplicons. The PCR process can be repeated numerous times, which allows for millions of copies of DNA to be made [3].

It typically takes around 3 hours for a regular thermocycler to perform the amplification of the STR markers used in forensic DNA analysis. In a forensic laboratory setting, where hundreds of DNA samples need to be analyzed and used for DNA typing, 3 hours is a long time to wait to analyze the amplicons. This could potentially contribute to backlog situations. The Streck Philisa<sup>®</sup> high-speed thermocycler has the potential to cut down the amount of time it takes to perform PCR from 3 hours to just 30 minutes [4]. This would allow forensic scientists to analyze more DNA samples in a lesser amount of time, making the lab more efficient.

## Materials and Methods

Buccal swabs, liquid blood, hair samples, and nail clippings were obtained from human participants with written, informed consent after UNH IRB approval. Once collected, the DNA from samples of each type was extracted and purified. DNA from buccal swabs and blood was extracted using the QIAGEN<sup>®</sup> QIAamp<sup>®</sup> DNA Mini Kit while DNA from hair and nail clippings was extracted using the QIAGEN<sup>®</sup> QIAamp<sup>®</sup> DNA Investigator Kit. The following protocols were used for the extraction of the respective DNA sample types; "Buccal Swab Spin Protocol" [5], "Isolation of Total DNA from Nail Clippings and Hair"

[6], and “Blood and Body Fluid Spin Protocol” [7]. These protocols were followed as per the kit manuals.

Quantification of the extracted DNA was performed using the Applied Biosystems™ (AB) Quantifiler® Kit and the AB 7500 Real-Time PCR System. DNA amplification was carried out using the Promega PowerPlex® 16 HS kit and performed on the Streck Philisa® High Speed Thermocycler. Either SpeedSTAR™ HS DNA Polymerase or PhilisaFAST™ DNA Polymerase was included in the master mix. For comparison purposes, amplification was also performed with an AB 9700 thermocycler. Separation and detection of the individual alleles was performed on an AB 3130xl PRISM sequencing instrument. Resulting data was analyzed and individual genotypes of the DNA samples were visualized using the AB GeneMapper® ID Software version 3.2.1.

SpeedSTAR™ HS DNA Polymerase Parameters		PhilisaFAST™ DNA Polymerase Parameters	
96°C for 60 seconds		96°C for 60 seconds	
10 Cycles of the Following:			
94°C	10 seconds	94°C	10 seconds
60°C	10 seconds	60°C	10 seconds
70°C	20 seconds	70°C	25 seconds
22 cycles of the Following:			
90°C	10 seconds	90°C	10 seconds
60°C	10 seconds	60°C	10 seconds
70°C	20 seconds	70°C	25 seconds
60°C for 180 seconds		60°C for 180 seconds	
Total Run Time: approximately 28 minutes		Total Run Time: approximately 31 minutes	

Figure 1: The Streck Philisa® High Speed Thermocycler was used with the parameters above depending on the type of polymerase used.

## Results and Discussion

Initial results showed that the Streck Philisa® high-speed thermocycler did not amplify various DNA sample types as well as the AB 9700 regular thermocycler did. Figure 2a shows an electropherogram of a DNA profile recovered from nail clippings amplified with the Streck Philisa® high-speed thermocycler using a 25 µL sample volume and SpeedSTAR™ HS DNA polymerase. The electropherogram clearly shows that amplification was not successful; no allelic peaks were evident.

Electropherograms of the data showed that DNA amplified using the Philisa® thermocycler exhibited higher stutter peaks and increased inhibition. PCR inhibition was evident in samples from hair and nail clippings. In order to enhance the quality of results, alterations were made to both the master mix used for amplification as well as to the PCR parameters on the instrument. Figure 2b shows an electropherogram of a DNA profile recovered from the same nail clippings pictured in Figure 2a. The sample was amplified with the Streck Philisa® high-speed thermocycler using a 12.5 µL sample volume which included 1 µL of 2 µg/µL BSA solution and the PhilisaFAST™ DNA polymerase. The BSA solution was added in order to obtain a final BSA concentration of 0.16 mg/mL. It can be seen that the additions of BSA and the PhilisaFAST™ DNA polymerase, as well as the decrease in total volume, and increase in PCR extension time from 20 to 25 seconds produced a complete profile with no evidence of inhibition. Lower stutter peaks were also observed using this combination.

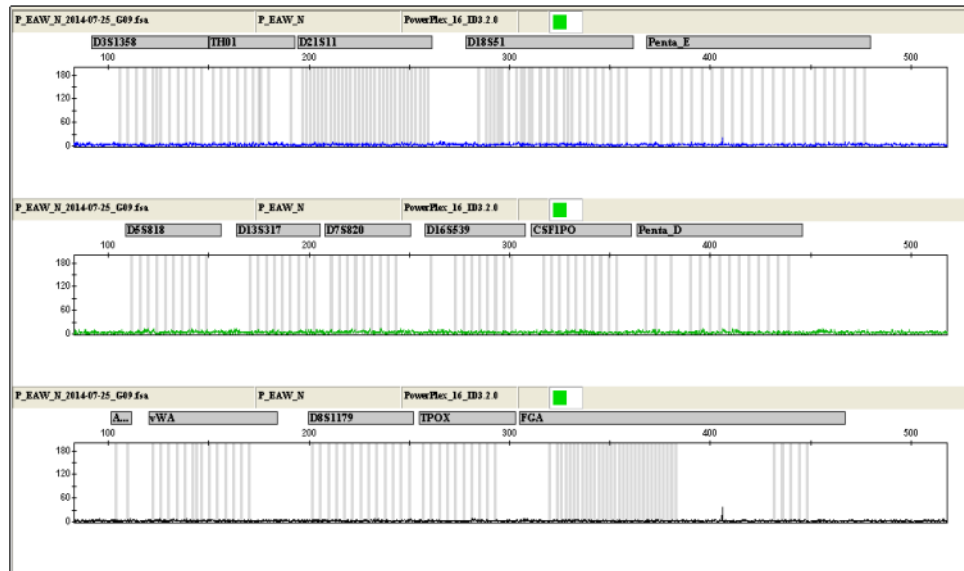
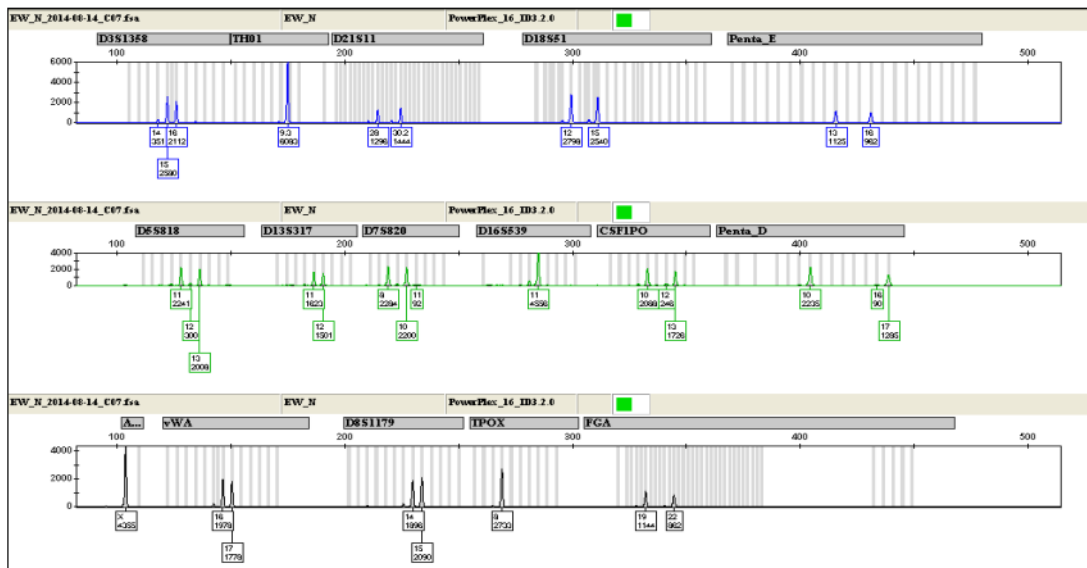


Figure 2a: Electropherogram of a DNA profile recovered from nail clippings amplified with the Streck Philisa high-speed thermocycler using a 25 µL amplification volume and Speed STARTM HS DNA polymerase. It can be seen that amplification was not successful.



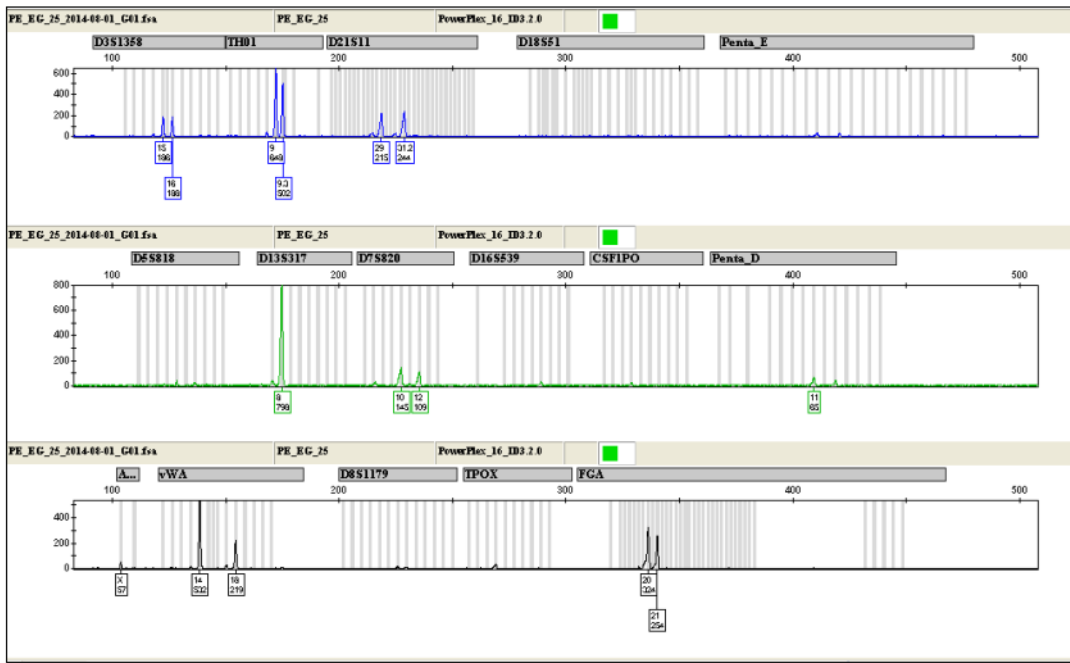
**Figure 2b.** Electropherogram of a DNA profile recovered from same nail clippings pictured in Figure 2a. The sample was amplified with the Streck Philisa<sup>®</sup> high-speed thermocycler using a 12.5  $\mu$ L amplification volume which included 1  $\mu$ L of 2  $\mu$ g/ $\mu$ L BSA solution and PhilisaFAST<sup>™</sup> DNA polymerase. It can be seen that the reduced volume, new enzyme, and the addition of BSA allowed for a complete profile.

PCR inhibition was one of the problems seen in those sample extracted from fingernails and hair. “PCR inhibitors generally exert their effects through direct interaction with DNA or interference with thermostable DNA polymerases. Direct binding of agents to single-stranded or double-stranded DNA can prevent amplification” [8]. Inhibition was causing DNA samples from hair and nail clippings to experience allele dropout of larger loci, like Penta E and Penta D. The data in Figure 3 shows the number of alleles per locus present for the Penta E and Penta D loci in two different DNA samples recovered from hair clippings. When samples were amplified using the AB 9700 thermocycler, allele dropout did not occur. But when using the Philisa<sup>®</sup> thermocycler, Penta E and Penta D in both of the samples experienced dropout and were not amplified. It was found that by decreasing the total volume of the amplification mix from 25  $\mu$ L to 12.5  $\mu$ L, adding 1  $\mu$ L of 2  $\mu$ g/ $\mu$ L BSA solution to the total volume, and increasing extension time of the PCR cycle from 20 to 25 seconds the inhibition was decreased. Under these modified amplification conditions allele dropout no longer occurred.

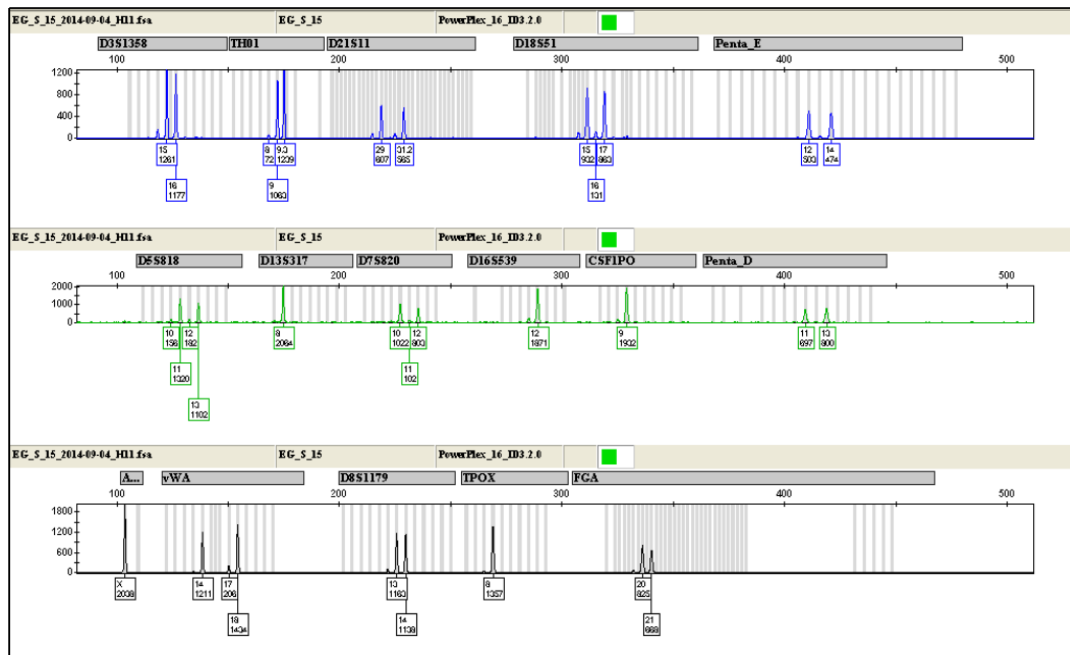
Figure 4a shows an electropherogram of DNA from a buccal swab using an amplification volume of 25  $\mu$ L and the PhilisaFAST<sup>™</sup> DNA polymerase. As clearly seen in the electropherogram, an incomplete DNA profile was obtained. Figure 4b on the other hand shows an electropherogram of the DNA from the same buccal swab DNA extract used in Figure 4a, except the amplification volume in this experiment was decreased to 15  $\mu$ L using 1  $\mu$ L of 2  $\mu$ g/ $\mu$ L BSA solution for a final BSA concentration of 0.13 mg/mL, and the same PhilisaFAST<sup>™</sup> DNA Polymerase as used before. This electropherogram shows a complete DNA profile.

		<i>Number of Alleles per Locus for Penta E and Penta D Loci</i>		
		AB 9700	Initial Philisa <sup>®</sup>	Philisa <sup>®</sup>
		Thermocycler	Thermocycler	Thermocycler
		Amplification	Amplification	Amplification
			(25 $\mu$ L with	(12.5 $\mu$ L with
			SpeedSTAR <sup>™</sup> HS	PhilisaFAST <sup>™</sup> DNA
			DNA Polymerase)	Polymerase, 0.16 mg/mL
				BSA, 25 second
				extension)
Hair	Penta E	2	0	2
Sample 1	Penta D	2	0	2
Hair	Penta E	2	0	2
Sample 2	Penta D	2	0	2

**Figure 3.** Data table which shows the number of alleles per locus present for the Penta E and Penta D Loci for two DNA samples recovered from hair. Data is shown by the type of thermocycler used, along with volume and type of DNA polymerase used. It can be seen that while initial amplification of the samples using the Streck Philisa<sup>®</sup> high-speed thermocycler resulted in the dropout of alleles for the Penta E and Penta D loci, the reduced volume along with PhilisaFAST<sup>™</sup> DNA Polymerase and addition of BSA resulted in full profiles.



**Figure 4a.** Electropherogram of DNA profile recovered from a buccal swab amplified with the Streck Philisa<sup>®</sup> high-speed thermocycler using a 25 µL amplification volume and PhilisaFAST<sup>™</sup> DNA Polymerase. It can be seen that amplification was not successful and an incomplete profile was obtained.



**Figure 4b.** Electropherogram of DNA profile recovered from the same buccal swab pictured in Figure 4a. The sample was amplified with the Streck Philisa<sup>®</sup> high-speed thermocycler using a 15 µL amplification volume which included 1 µL of 2 µg/µL BSA solution and PhilisaFAST<sup>™</sup> DNA Polymerase. It can be seen that the reduced volume and addition of BSA allowed for a complete profile.

Higher stutter peaks were another issue seen in the data collected when using the Streck Philisa<sup>®</sup> high-speed thermocycler for amplification. While it was not expected, the addition of BSA to the sample actually helped with decreasing stutter peaks. Decreasing the amount of DNA amplified from approximately 1 ng to 0.65 ng also decreased the abundance of higher stutter peaks.

In order to further investigate the role of BSA in reducing the amount of inhibition and high stutter peaks present in samples amplified with the Streck Philisa<sup>®</sup> high-speed thermocycler, experiments were set up to test the effectiveness of various BSA concentrations. Five BSA solutions of different concentrations were made. The BSA solutions created were of the following concentrations: 1.5 µg/µL, 1.75 µg/µL, 2 µg/µL, 2.25 µg/µL, and 2.5 µg/µL. Using DNA from the same hair sample, 1 µL of each of the differing BSA concentration solutions was added to a separate master mix for amplification of each specific BSA concentration. The final amplification master mixes had concentrations of 0.12 mg/mL, 0.14 mg/mL, 0.16 mg/mL, 0.18 mg/mL, and 0.20 mg/mL respectively. Each of the five samples were amplified using the Philisa<sup>®</sup> high-speed thermocycler, and then injected using capillary electrophoresis to separate and visualize alleles. Based on the resulting electropherograms, it was determined that the 2 µg/µL BSA solution, resulting in a concentration of 0.16 mg/mL was the most effective in decreasing inhibition from PCR inhibitors and reducing high stutter peaks associated with the amplification of samples using the Streck Philisa<sup>®</sup> high-speed thermocycler (Figure 2b).

## Conclusions

Initial results showed that the Streck Philisa<sup>®</sup> high-speed thermocycler was able to carry out amplification, just not with the same quality of results as a standard thermocycler would. Electropherograms of the analyzed DNA samples exhibited increased stutter peaks and PCR inhibition. Samples with lower DNA concentrations, like hair and nail clippings, experienced dropout of larger alleles. The quality of results were greatly enhanced through the addition of BSA to the master mix, by increasing extension time for PCR from 20 to 25 seconds, and by decreasing the amplification volume and the amount of input DNA from 25 µL of master mix plus approximately 1 ng of DNA to a 12.5 µL volume and approximately 0.65 ng of DNA.

This project will be continued throughout the 2014-2015 school year in order to continue to refine the quality of data obtained from the use of the Streck Philisa<sup>®</sup> high-speed thermocycler for amplification. The possibility of decreasing PCR amplification time from 3 hours using the AB 9700 thermocycler to just 30 minutes with the Streck Philisa<sup>®</sup> high-speed thermocycler makes the point of this work clear.

## References

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## **Biography**

Christina Martins is currently a senior at the University of New Haven, double majoring in Forensic Science and Biology. She is currently a member of the Biology Club, Alpha Lambda Delta First Year Honor's Society, Students Making an Impact on their Living Environment (S.M.I.L.E.) Community Service Club, and is serving as the Executive Assistant for the Regenerative Medicine Club. In her spare time, she enjoys participating in Rec Sports, reading, and watching hockey. Christina has a passion for both science and research, and after graduation plans to attend graduate school to pursue a Ph.D. in microbiology.

