A Forensic Validation Study on the Effectiveness of the Streck Philisa® 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® 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® 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® high-speed thermocycler.

Initial results showed that the instrument did in fact work, but not as well as a regular thermocycler. Electropherograms of the analyzed DNA samples exhibited increased stutter peaks and PCR inhibition. These problems were especially evident in those samples with smaller amounts of DNA like hair and nail clippings. Decreasing the volume of master mix and the amount of DNA from 25 µL of master mix plus approximately 1 nanogram of DNA to a 12.5 µL sample and approximately 0.65 nanograms 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 try and 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, known as amplicons. PCR is carried out using an instrument known as 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.

The Polymerase Chain Reaction (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. These polymorphisms are known as short tandem repeats, which are very short pieces of DNA, around 2-5 base pairs long that are repeated numerous times. STR's are helpful because each person has a different number of copies of the specific repeat section of the DNA [1]. In this project, the Promega PowerPlex 16 HS amplification kit, a multiplex STR system that amplifies 16 loci, which refers to the location of a specific DNA sequence on a chromosome, was used. The kit also includes 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 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 step 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 is activated by the temperature of the environment, and 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 carry out various cycles of PCR on the DNA samples. In a forensic lab 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, and could potentially contribute to backlog situations. The Streck Philisa® 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® QIAamp® DNA Mini Kit while DNA from hair and nail clippings was extracted using the QIAGEN® QIAamp® 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 Philisa*FAST*™ 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.

The Streck Philisa® High Speed Thermocycler was set with the following parameters:

SpeedSTAR™ HS DNA Polymerase Parameters		**Philisa*FAST*™ DNA Polymerase Parameters**	
96°C for 60 seconds		96°C for 60 seconds	
10 Cycles of the Following:		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:		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	

Results

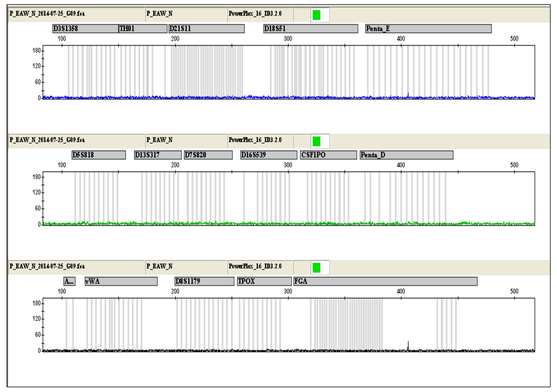

Figure 1a. Electropherogram of DNA profile recovered from nail clippings amplified with the Streck Philisa® high-speed thermocycler using a 25 µL sample and SpeedSTAR™ HS DNA Polymerase. It can be seen that amplification was not successful.

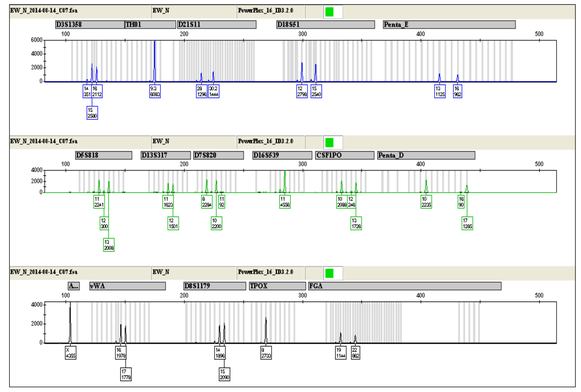

Figure 1b. Electropherogram of DNA profile recovered from same nail clippings pictured in Figure 1a. The sample was amplified with the Streck Philisa® high-speed thermocycler using a 12.5 µL sample which included 1 µL of 2 µg/µL BSA solution and Philisa*FAST*™ DNA Polymerase. It can be seen that the reduced volume, new enzyme, and addition of BSA allowed for a complete profile.

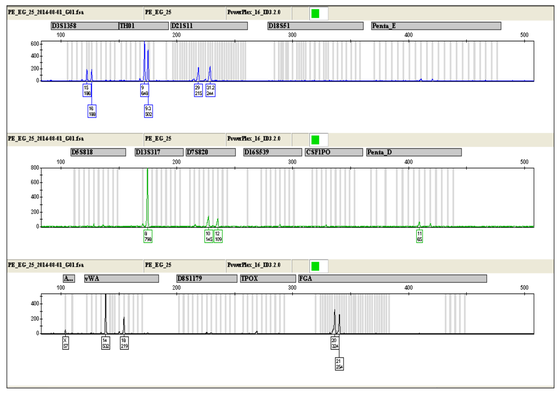

Figure 2a. Electropherogram of DNA profile recovered from a buccal swab amplified with the Streck Philisa® high-speed thermocycler using a 25 µL sample and Philisa*FAST*™ DNA Polymerase. It can be seen that amplification was not successful and an incomplete profile was obtained.

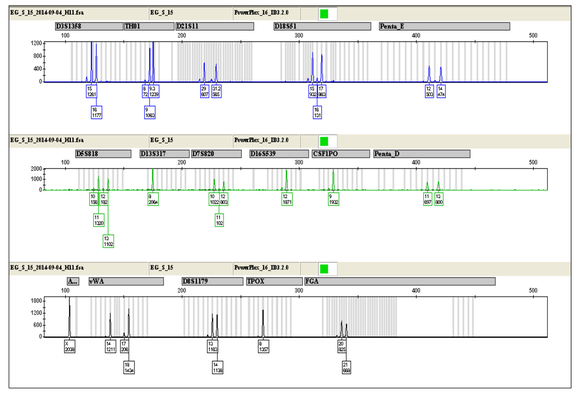

Figure 2b. Electropherogram of DNA profile recovered from the same buccal swab pictured in Figure 2a. The sample was amplified with the Streck Philisa® high-speed thermocycler using a 15 µL sample which included 1 µL of 2 µg/µL BSA solution and Philisa*FAST*™ DNA Polymerase. It can be seen that the reduced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

Number of Alleles per Locus for Penta E and Penta D Loci

		AB 9700 Thermocycler Amplification	**Initial Philisa® Thermocycler Amplification** (25 µL with SpeedSTAR™ HS DNA Polymerase)	**Philisa® Thermocycler Amplification** (12.5 µl with Philisa*FAST*™ DNA Polymerase, 1 µL of 2 µg/µL BSA solution, 25 second extension time)
Hair Sample 1	Penta E	2	0	2
	Penta D	2	0	2
Hair Sample 2	Penta E	2	0	2
	Penta D	2	0	2

Figure 3. Data table which shows the number of alleles per locus present for Penta E and Penta D Loci for 2 DNA samples recovered from hair clippings based on 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 the Streck Philisa® high-speed thermocycler resulted in the dropout of alleles for Penta E and Penta D loci, the reduced volume along with Philisa*FAST*™ DNA Polymerase and addition of BSA resulted in the reappearance of alleles once lost during amplification.

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 1a shows an electropherogram of a DNA profile recovered from nail clippings amplified with the Streck Philisa® high-speed thermocycler using a 25 µL sample and SpeedSTAR™ HS DNA Polymerase. The electropherogram clearly shows that amplification was not successful, and did not amplify at all. Electropherograms of the data showed that DNA amplified using the Philisa® thermocycler exhibited higher stutter peaks and increased inhibition. PCR Inhibition was especially evident in those samples with smaller amounts of DNA like hair and nail clippings. In order to try and enhance the quality of results, many changes were made to both the master mix used for amplification as well as with the actual PCR parameters of the instrument. Figure 1b shows an electropherogram of a DNA profile recovered from the same nail clippings pictured in Figure 1a. The sample was amplified with the Streck Philisa® high-speed thermocycler using a 12.5 µL sample which included 1 µL of 2 µg/µL BSA solution and Philisa*FAST*™ DNA Polymerase. The 1 µL of 2 µg/µL BSA solution was added in order to obtain a final BSA concentration of 0.16 mg/mL. It can be seen that due to the additions of BSA and the Philisa*FAST*™ 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 and lower stutter peaks.

PCR inhibition was one of the problems seen in those sample types with smaller amounts of DNA. "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 table in Figure 3 shows the number of alleles per locus present for Penta E and Penta D Loci for 2 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® 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 sample from 25 µL to 12.5 µL, adding 1 µL of 2 µg/µL BSA solution to the total volume, and increasing extension time of the PCR cycle from 20 to 25 seconds decreased the inhibition to a point where allele dropout no longer occurred. Figure 2a shows an electropherogram of DNA from a buccal swab using a volume of 25 µL and the Philisa*FAST*™ DNA Polymerase. As clearly seen in the electropherogram, an incomplete DNA profile was obtained and clear inhibition is evident. Figure 2b on the other hand shows an electropherogram of the DNA from the same buccal swab in Figure 2a, except the volume in this case was decreased to 15 µL using 1 µL of 2 µg/µL BSA solution for a final BSA concentration of 0.13 mg/mL, and the same Philisa*FAST*™ DNA Polymerase as used before. This electropherogram shows a complete DNA profile with no evidence of inhibition.

Higher stutter peaks were another issue seen in the data collected when using the Streck Philisa® 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 used from approximately 1 nanogram to 0.65 nanograms also decreased the abundance of higher stutter peaks.

Conclusions

Initial results showed that the Streck Philisa® high-speed thermocycler was able to carry out amplification, just not with the same quality results as a regular 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 amount of sample and amount of DNA from 25 µL of master mix plus approximately 1 nanogram of DNA to a 12.5 µL sample and approximately 0.65 nanograms 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® 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® high-speed thermocycler makes the point of this work clear.

References

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