Detection and Characterization of the DNA Inhibitory Agent Found in Carpet Backing

Carrie A. Thomas and Deanna M. Calabrese
Department of Forensic Science, Henry C. Lee College of Forensic Sciences and Criminal Justice

Abstract:

Deoxyribonucleic acid (DNA) analysis is a vital part of forensic science. It begins by the identification of a blood stain which contains DNA. Unique to each individual, DNA is the hereditary material that makes up the building blocks of a human being. The application of DNA to forensic science is vital in determining the aspects of a crime scene. Dried blood found at a crime scene is often found on carpet backing. This presents a problem to analysts. There is an inhibitory agent that prevents the amplification phase of DNA analysis from happening. The results come back as no DNA or any identifiable markers present on running a quantification test or profiling test. By determining the part of the carpet backing that has the inhibitory compound a technical note could be written on preparing a sample for analysis.

Introduction:

It is a well-known issue throughout the forensic science community that polymerase chain reaction (PCR), when performed on carpet backing, shows inhibition. PCR acts as a molecular copy machine. This process makes copies of DNA so that analysis can be performed on it. It is unknown what compound in carpet backing inhibits the PCR reaction from occurring. This in turn prevents any DNA from being analyzed. This causes an issue in the forensic science community because DNA analysis is vital in identifying the source of a biological fluid.

Carpet backing has three layers, including an adhesive layer. Prior research conducted shows that the tuffs of the carpet do not inhibit the PCR reaction from occurring [8]. The backing of carpet inhibits PCR as a synergistic effect of a combination of the layers. The inhibitor is bound to the molecule of DNA itself.

The research conducted was done to detect the presence of an inhibitor in specific layers of carpet and to characterize its effects.

When DNA is found at a crime scene it is often in the form of dried blood. Often, blood is found on carpet. Carpet backing contains an inhibitory agent that prevents the polymerase chain reaction process, which therefore enables DNA analysis. DNA analysis consists of five major parts: collection/identification, extraction, quantification, amplification and profiling. The polymerase chain reaction occurs during quantification and amplification of DNA. It is vital to forensics because it makes more copies of DNA. The polymerase chain reaction or PCR acts as a molecular photocopier to make exact copies of DNA. The unknown compound in the carpet backing prevents this "photocopying" from occurring.

Research done in various scientific fields has yielded similar results in attempts to perform

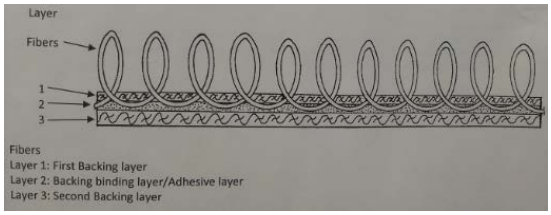


Figure 1

PCR on carpet samples containing known DNA samples. An example of this can be seen in research done by the Center for Environmental Studies at the University of Las Vegas. An experiment was performed to locate biological contamination on various flooring surfaces. DNA in all organisms is made of the same essential components; therefore the same methods of PCR can be used in the detection of other biological organisms. The research found significant differences in the ability to amplify DNA from carpeted flooring in comparison to other types of flooring.

The problems faced when amplifying DNA through PCR, in regards to testing on carpet, are due to an issue with the binding of taq polymerase. Taq polymerase is a thermal stabilizer which becomes activated at a specific temperature. The taq polymerase, upon activation, recognizes the DNA and begins to extend it, which allows for the amplification of new DNA strands. According to a technical note published in the Journal of Forensic Science, the inhibition of PCR is recognized as the result of a chemical compound present in the substance being analyzed. It is noted that at first testing, the DNA is unable to be amplified. After the addition of sodium hydroxide (NaOH) a chemical reaction occurred, which caused the inhibitor to become inactivated and the amplification of PCR to proceed. [8].

Prior research conducted in 2010 by Mallory Halliquist and Ryan Johnson revealed that the inhibitory compound was actually present in the carpet's backing layers and not in the fibers. Carpet backing consists of three layers: the first backing layer, a binding/adhesive layer, and a second backing layer. One or more of these layers prevents PCR from working properly.

The polymerase chain reaction's main function is to make more DNA from the template strands which are found in a blood sample. This is known as a process of amplification.

Extraction is a procedure that lyses or breaks open a cell's nucleus to release pure DNA. This technique was used to purify the DNA from our samples to test for the inhibition of PCR. We used two extraction techniques, one to 'screen' and the other to show inhibition of the initial extraction.

Quantification is a process that determines how much DNA is present in a sample. It is vital to determine how much DNA is present so that a ratio of DNA sample to water can be deduced in order to analyze it with the Promega PowerPlex® 16 HS Kit. If the sample has too much DNA then it will not read.

This kit processes the PCR products which are now called amplicons, after undergoing amplification. It compares the amplicons to allelic frequencies amongst a population. The kit amplifies nine genetic markers. These markers are referred to as Short Tandem Repeats (STRs). An STR is a repeating sequence in the DNA that is particular to a percentage in a specific population. These markers

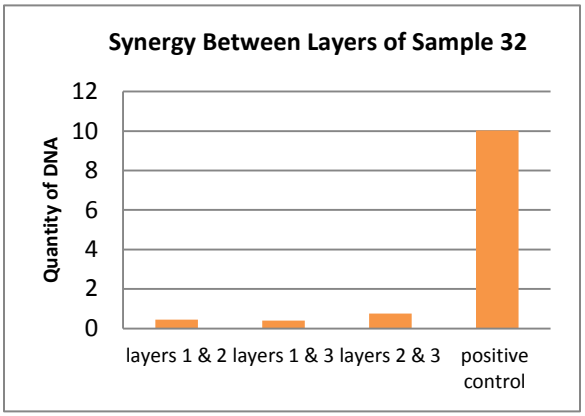


Chart 1: Quantity of DNA of combination of layers.

are each marked with a fluorescent dye individualizing each marker. It is then run through the GeneMapper® ID Software, which assesses the amount of fluorescent dye emitted by an STR and measures it as a peak.

Materials and Methods:

The carpet samples were dissected into the various layer combinations or single layers desired, and spotted with 50 µL of blood.

The samples were allowed to dry for a period of 24 hours and were then extracted following the protocol outlined in the QIAGEN® QIAamp® DNA Investigator manual for "Isolation of Total DNA from Paper and Similar Materials" and "Isolation of Total DNA from Small Volumes of Blood or Saliva."

The extracts were quantified using the Applied Biosystems 7500 Real-Time PCR Systems and Applied Biosystems™ ABI Quantifiler™ Kit.

The amount of human DNA present in each sample is determined and then amplified using Real-time PCR.

Further analysis of PCR inhibition was determined by use of the Promega PowerPlex® 16 HS Kit, which was visualized through use of the ABI GeneMapper® ID Software version 3.2.

Results and Discussion:

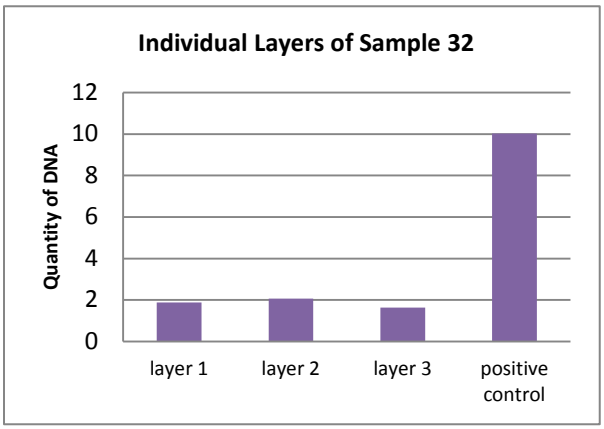


Chart 2: Quantity of DNA of single layers.

PCR inhibition found in carpet backing contains a synergistic effect, meaning it is affected by a combination of layers of carpet. This can be seen in chart 1; the combined layers show significantly more inhibition then the individual layers (Chart 2).

Similar results were seen with whole carpet, indicating that there is synergy between extracted layers of carpet. More inhibition was found to be present in samples containing all three backing layers then in combinations of any two layers. This can be compared to chart 1 which shows the individual layers having some inhibition, although not to the same extent as the whole carpet backing.

When multiple DNA aliquots from carpet sample 32 and various other carpet samples were analyzed, it was found that the overloaded samples, containing a significant amount of DNA, showed signs of inhibition. Figure 3 shows clear signs of inhibition; there are only two visible peaks in what should be a severely overloaded sample. Figures 2 and 3 both contain the same amount of DNA and should have similar peak heights. Figure 3, which is from just one layer of carpet, has much larger peak heights compared to those in figure 2, which shows that when a combination of carpet layers is involved, there is more inhibition than when a single layer is involved.

Samples analyzed containing less DNA were found to have larger and more distinguishable peak heights. When the concentrations of both the inhibitory compound and DNA were lowered a better profile is obtained. This can be seen in figures 4 and 5.

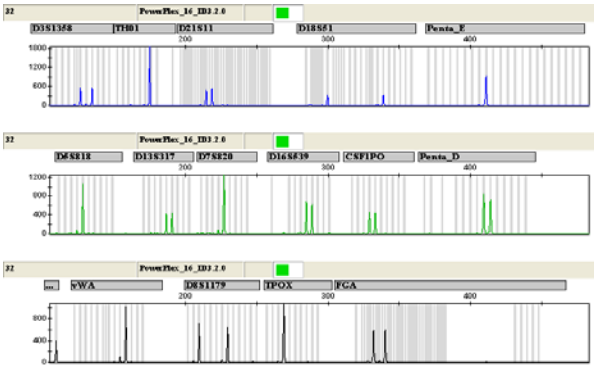


Figure 2

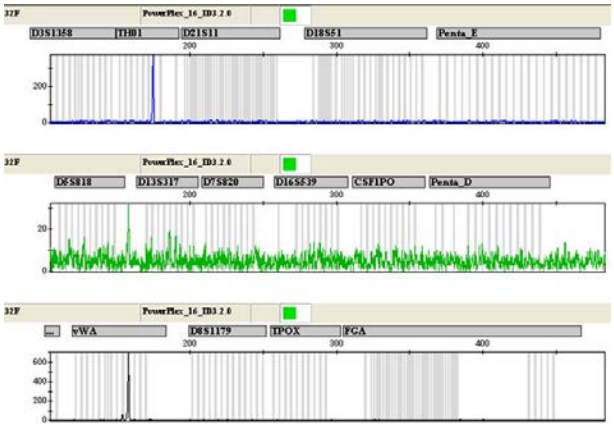


Figure 3

Because there is five times more DNA in the electropherogram of figure 4, this should be represented in the peak heights at each locus; which is not the case. Certain loci show smaller peak heights in the sample with more DNA than in the one with less. When the volume designated for analysis is increased the corresponding amount of inhibitor is also increased, causing the electropherogram to show significant signs of inhibition. Therefore the use of smaller or diluted aliquots of DNA can be used to develop a more complete and distinct profile.

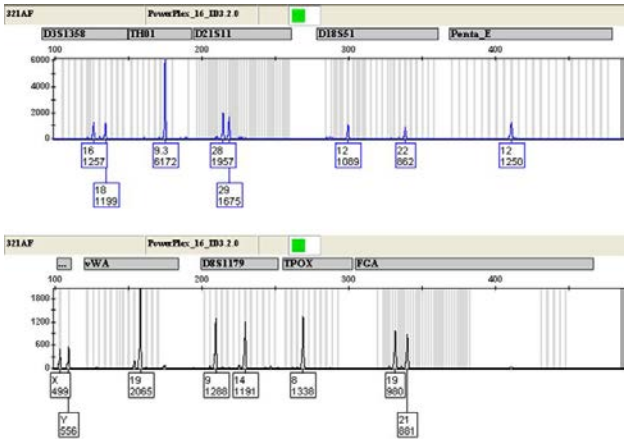


Figure 4

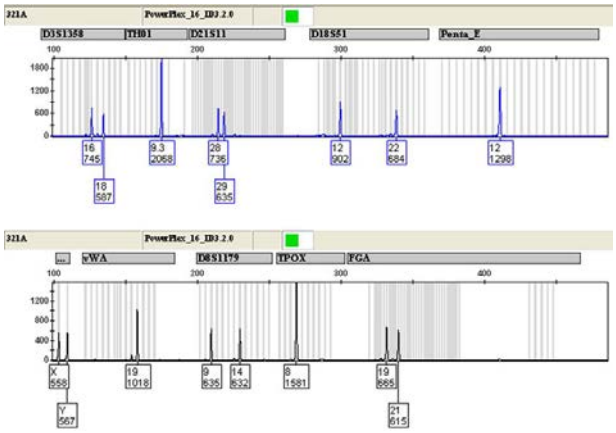


Figure 5

Undetectable or low quantities of DNA determined during quantification were amplified and injected. Those samples were found to have uninhibited electropherograms.

Conclusion:

Our results indicated that there is a synergy between the different layers of carpet backing, and that no single layer is solely responsible for PCR inhibition found in carpet backing. This can be seen in chart 1; the combined layers show significantly more inhibition then the individual layers (Chart 2).

The most successful method for analyzing DNA samples found on carpet samples, when use of fibers is unavailable, would be to remove at least one layer of the carpet. The most productive protocol would be to separate each individual backing layer of the carpet and extract the DNA separately for PCR amplification and analysis. Steps may also be taken to reduce the amount of inhibition found in electropheragrams by diluting the samples used for injection.

Acknowledgements:

We would like thank the Henry C. Lee College of Criminal Justice and Forensic Sciences for the provision of materials and instruments, the University of New Haven and the Associate Provost Office for the stipend to perform this research. We would also like to thank the SURF program for the opportunity to perform undergraduate research.

We would also like to thank Dr. Michael Adamowicz and the forensic science department for their dedication and guidance throughout the project.

References:

[1]Buttner, M. P., P. Cruz-Perez, and L. D. Stetzenbach. "Enhanced Detection of Surface-Associated Bacteria in Indoor Environments by Quantitative PCR." *Applied and Environmental Microbiology* 67.6 (2001): 2564-570. Print.

[2] Bourke, Michael T., Carol A. Scherczinger, Carll Ladd, and Henry C. Lee. "NaOH Treatment to Neutralize Inhibitors of Taq Polymerase." *Journal of Forensic Science* (1999): 1046-050. Web. 04 Mar. 2012.<http://libraryresources.cqu.edu.au/JFS/PDF/vol_44/iss_5/JFS4451046.pdf>.

[3] Nation Human Genome Institute. "Polymerase Chain Reaction (PCR)." Nation Institutes of Health. Web.27Feb.2012. <http://www.genome.gov/10000207>

[4] QIAGEN®. "Protocol: DNA Purification from Dried Blood Spots (QIAamp DNA Minit Kit)." *QIAamp® DNA Mini and Blood Mini Handbook.* Third Edition. April 2010. 43-44.

[5] QIAGEN®. "Protocol: Isolation of Total DNA from Small Volumes of Blood or Saliva." *QIAamp® DNA Investigator Handbook.* April 2010. 36-38.

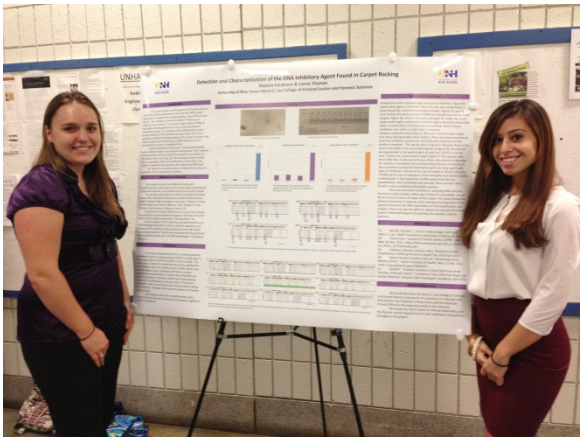
[6] Butler, John M. *Fundamentals of Forensic DNA Typing*. Amsterdam: Academic/Elsevier, 2010. 111-21. Print.

[7] *QIAGEN-QIAmp DNA Mini Kit*. Web. 03 Mar. 2012. <http://www.qiagen.com/products/genomicdnastabilizationpurification/qiaampsystem/qiaampdnaminikit.aspx#Tabs=t>.

Figure 4

[8] Hallquist, Mallroy. Johnson, Ryan. *Detection of DNA Amplification Inhibiting Factors in Carpet"* July 2010-April 2011

Biography:



Carrie Thomas (L) is currently a senior at the University of New Haven majoring in Forensic Science and Biology. She hopes to continue her education in the field of biology. This was Carrie's first experience with scientific research and she would like to continue exploring the different areas of biological research.

In her spare time Carrie participates in the Forensic Science and Chemistry Club, and is an avid reader and skier.

Deanna Calabrese (R) is currently a senior pursuing Bachelor degree in Forensic Science and Biology at the University of New Haven. She hopes to continue her education in the field of bioengineering. This was Deanna's first experience with scientific research and she enjoyed working in this field of biology.

In her spare time, Deanna is the Treasurer of the Forensic Science and Chemistry Club. She enjoys hiking, reading and ice skating.