SIMULTANEOUS PURIFICATION AND QUANTITATION OF DNA FROM DATABASE BLOOD AND BUCCAL SWAB SAMPLES

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Note: Figures can be found at end of document.

ABSTRACT

The purification process involves the use of magnetic particles that capture the DNA released from samples placed in a lysis buffer. The use of magnetic particles allows the flexibility of manual or robotic manipulation based on magnetic separation, filtration or centrifugation. This purification process automatically quantitates the DNA eliminating the necessity to quantitate the DNA in a separate step.

We have first applied this technique to the purification of DNA from liquid blood. Normalized peak heights of amplified alleles purified from a 7.5 fold range of blood (4 to 30µl) only varied between 0.5 and 1.2. Analysis of the purified DNA with PicoGreen® showed a similar narrow concentration range. Results using this technique for the purification and quantitation of DNA from buccal swabs also will be discussed.

INTRODUCTION

The analysis of DNA for identification purposes is a multi-step process, the ultimate success being dependent on the success of each step. Sample collection and DNA purification are critical aspects of this process since successful and reliable amplification of short tandem repeat (STR) loci require a specific range of input DNA (1, 2). Too low a DNA concentration results in allele dropout and allele imbalances due to stochastic effects (3). Too high a DNA concentration results in locus imbalance and artifact bands (4). Casework samples are quite variable in quantity, quality and levels of contamination and will not be discussed further. Samples for databasing and suspect profiling, while providing uncontaminated high quality DNA never the less can vary considerably in DNA concentration. The amount of DNA obtained from buccal swabs vary considerably depending on technique, swab type, recovery of cells and individual variations. DNA obtained from blood can vary significantly due to immune stimulation or suppression, which is common in the criminal population. This variation is compounded in bloodstains where variations in spotting techniques and wicking cause significant variations within the bloodstain.

These variations cause obvious problems when using FTA™or S&S 903 bloodstain punches directly in amplification reactions. In addition, the high capacity of FTA™ paper requires modification to the amplification cycle number to accommodate for the excess DNA being placed in the reaction (4). However, even purified DNA has presented problems due to inconsistencies in DNA quantitation systems. These problems in DNA purification and quantitation have resulted in difficulties in analyzing the amplified DNA and contribute to a significant failure rate.

To alleviate some of the DNA purification and quantitation problems, we are in the process of developing a system that purifies DNA for database purposes from liquid blood, bloodstains and buccal swabs and in the process quantitates the DNA so that a constant quantity can be used in amplification reactions.

MATERIALS AND METHODS

Materials

Blood was collected in 7 ml Vacutainer® tubes containing EDTA, stored at 4°C, and used within 3 weeks unless otherwise indicated. Amplification reactions were performed using Promega's *GenePrint*® PowerPlex™1.1 System with Gold ST★R buffer (Promega) and AmpliTaq Gold™DNA polymerase from Perkin-Elmer.

DNA Isolation from liquid blood

Liquid blood was incubated in 100µl of a lysis solution and a special magnetic resin for 5 minutes. The magnetic resin containing its capacity of DNA was separated on a magnetic stand from the lysis solution. The resin was then washed 3 times and allowed to air dry for 10 minutes. Water was added to the resin and the tubes incubated for 5 minutes at 60°C. The resulting DNA solution was then recovered after magnetically separating the resin. The whole procedure takes approximately 30 to 60 minutes depending on the number of samples.

DNA isolation from bloodstains and cotton buccal swabs

Bloodstains on FTA™and S&S 903 papers, cut to various sizes with a razor blade or 1.2 or 2.0mm Harris micro-punches (Life Technologies, Inc) and cotton buccal swabs were heated in 250µl of lysis solution for 30 minutes. The solid support and solution were placed in a spin basket without filter and centrifuged 2 minutes. This removes the DNA from the solid support. The magnetic resin was then added to the solution and DNA was isolated using the liquid blood procedure described above.

DNA analysis

The amount of DNA was determined by first determining the concentration of DNA using a PicoGreen® dsDNA quantitation kit (Molecular Probes). For functionality analysis as well as confirming quantitative data, 1µl of eluted DNA solution regardless of original sample size was amplified in a GenePrint® PowerPlex™ 1.1 reaction. Data analysis was performed on a Hitachi FMBIO® II with StaRCall™ software. Peak heights were determined for each locus and normalized.

THEORY OF QUANTITATION

The most efficient method of purifying DNA is to use a DNA binding resin. Although this technique does not provide as clean a DNA as organic purification methods, the DNA is sufficiently pure for amplification reactions, is less time consuming and can be automated. In many cases it is desirable to isolate all of the DNA. As resins have a maximum capacity for binding DNA, an excess of resin must be used. Adding twice the sample size will result in purifying twice the amount of DNA. The small box in Figure 1 demonstrates this concept. For database samples where sample size, quality and contamination are not an issue, not all of the DNA has to be isolated. By using a set amount of resin and an excess of DNA, a set amount of DNA will always be isolated. Adding twice the sample size only results in a small increase in the amount of DNA isolated. The large box in Figure 1 demonstrates this concept. Using this technique a set amount of DNA can be isolated from samples containing large differences in the amount of DNA as long as the DNA is in excess of the capacity for the resin to bind DNA.

ISOLATION AND QUANTITATION OF DNA FROM BLOOD

DNA was isolated from 6µl to 25µl of liquid blood using two different amounts of magnetic resin to determine the capacity of the resin and to assess how closely the experimental data fit theory. Figure 2 shows the total DNA recovered compared to the sample size using 500µg and 700µg of magnetic resin. Using 500µg of resin, the amount of DNA recovered varied between 60 and 100 ng and followed a typical saturation curve. Between 8µl and 25µl the recovered DNA was between 80 and 100ng. The 700µg

curve showed a similar saturation profile but had a higher saturation level and was not as close to saturation as the lower amount of resin. Even in this case the variation was only between 75 and 150ng with the midrange sample sizes giving 110 to 120ng of DNA. These results indicate that this magnetic resin was suitable for isolating a relatively uniform amount of DNA from a range of sample sizes. Defined amounts of DNA can be selected by using experimentally determined amounts of resin in a defined sample size range.

To determine the functionality and confirm the quantity of DNA isolated with the magnetic particles, we used 1µl of DNA solution from each of the samples isolated with 700 µg of resin in an amplification reaction. The samples were analyzed for the ability of the StaRCall™software to correctly identify each of 15 alleles from 8 STR loci. In addition, the variations in signal intensity at each allele were determined. Figure 3 shows that the peak intensities were very uniform for original blood sample sizes between 10 and 25µl. The 6µl blood sample had peak heights about half this intensity but were still easily analyzed by the StaRCall™ software. This is consistent with the PicoGreen® dsDNA quantitation assays that showed that the smallest samples had about half the amount of DNA.

ISOLATION OF DNA FROM OLD BLOOD

For efficient processing of database samples, samples must be accumulated and processed in batches. To test whether this system could efficiently isolate DNA from blood stored at 4°C for extended periods of time, we purified DNA from blood stored from 0 to 132 days. Figure 4 demonstrates that the same amount of DNA was isolated regardless of storage time. Up to one month the amplified alleles had similar standard deviations in peak height. However, after 132 days of storage the DNA appeared to be degraded resulting in decreased peak heights for the larger alleles and increased peak heights for the smaller alleles. Again, the StaRCall™software was able to correctly identify all of the alleles in every sample.

ISOLATION AND QUANTITATION OF DNA FROM BLOODSTAINS

A common way to store blood samples for databasing is to prepare bloodstains on either S&S 903 or FTA™ paper. These papers allow for long term storage and can be used directly in amplification reactions. Unfortunately, this storage method creates some problems. The DNA is not easily removed from the paper, preventing accurate DNA quantitation. The support, especially FTA™, has such a high capacity for DNA that even 1 mm punches require adjustments in the amplification protocols. Finally, the blood does not distribute evenly in the stain compounding the variations in white cells between individuals to give quite variable results.

To eliminate these problems, we have developed a simple 40 minute extraction protocol that includes incubation of the bloodstains in a lysis buffer followed by centrifugation in a spin basket to remove the DNA from the paper support. The resulting solution can then be processed as if it were liquid blood. Using this procedure we first analyzed bloodstains on S&S 903 paper. Figure 5 shows the results of processing stains containing 5µl to 50µl of blood. Normalized peak heights for the 15 observed alleles were almost identical between samples when 1 µl of DNA solution was amplified. Thus, a ten fold difference in input sample size resulted in identical amplification results.

We next examined $3mm^2$ to $57mm^2$ bloodstains on FTA™paper. Figure 6 shows the result of amplifying 1µl of the resulting DNA solution from each bloodstain. The smaller size bloodstains gave consistent peak heights while the larger stains had about a 20% reduction in peak height. This reduction may be the result of residual inhibitors that carried over from the blood or FTA paper. In any case, the StaRCall™ software correctly identified each of the 15 observed alleles in all of the samples.

ISOLATION AND QUANTITATION OF DNA FROM BUCCAL SWABS

Buccal swabs are an attractive alternative to bloodstains for collecting samples for database purposes. This collection procedure is noninvasive and less prone to contain infectious agents such as HIV and Hepatitis. Unfortunately, swabs are more difficult to process than blood or bloodstains and contain quite

variable amounts of DNA. To examine whether our extraction procedure would work on swabs, we processed samples from 12 different individuals. The extraction and purification procedure was identical to bloodstain processing. Preliminary results indicated that a Taq DNA polymerase inhibitor was present to various degrees in these samples. The results for a given individual were quite reproducible with some individuals having consistently high levels of inhibitor. Our more recent results indicate that we have been able to eliminate this inhibitor and are currently obtaining consistent results between individuals.

CONCLUSION

DNA analysis is a multi-step process, its success being dependent on the success of each step. While reliable commercial kits are available for the amplification of STR loci, they require a relatively narrow range of DNA concentration to produce accurate results. Unfortunately, reliable quantitation of DNA remains a problem. We have addressed this issue by using a purification process that isolates a set amount of DNA. This process is less susceptible to influences that affect quantitation results and eliminates a separate quantitation step altogether.

The system being developed is quite scalable. The amount of resin used determines the maximum amount of DNA that is isolated. However, the general range of sample size must also be determined to ensure that the DNA is in sufficient excess to saturate the resin. The system begins to generate erratic results if very large excesses of DNA are used. In these cases the large amount of DNA traps the resin and variable amounts of resin are lost during washing.

The current system is designed for single tube extractions and takes approximately 1 hour for 24 samples. However, a 96 well format is being developed for blood as well as bloodstains and buccal swabs. Preliminary experiments have shown the utility of using this method for the reliable isolation of a set amount of DNA for database analysis.

Because casework samples require human specific quantitation, this system can not be used to quantitate the DNA. However, using an excess of resin results in a very efficient method of purifying DNA from these samples. Preliminary results have been able to isolate DNA from bloodstains on S&S 903 paper containing less than 1µl of blood. These results show the flexibility of this system. One standard procedure can be used for a variety of sample types. Only the amount of reagents needs to be adjusted depending on sample size and whether quantitation or maximum yield is desired.

ACKNOWLEDGEMENTS

We would like to extend our thanks to the members of the Genetic Identity Research and Development group at Promega Corporation for their contributions of buccal swabs.

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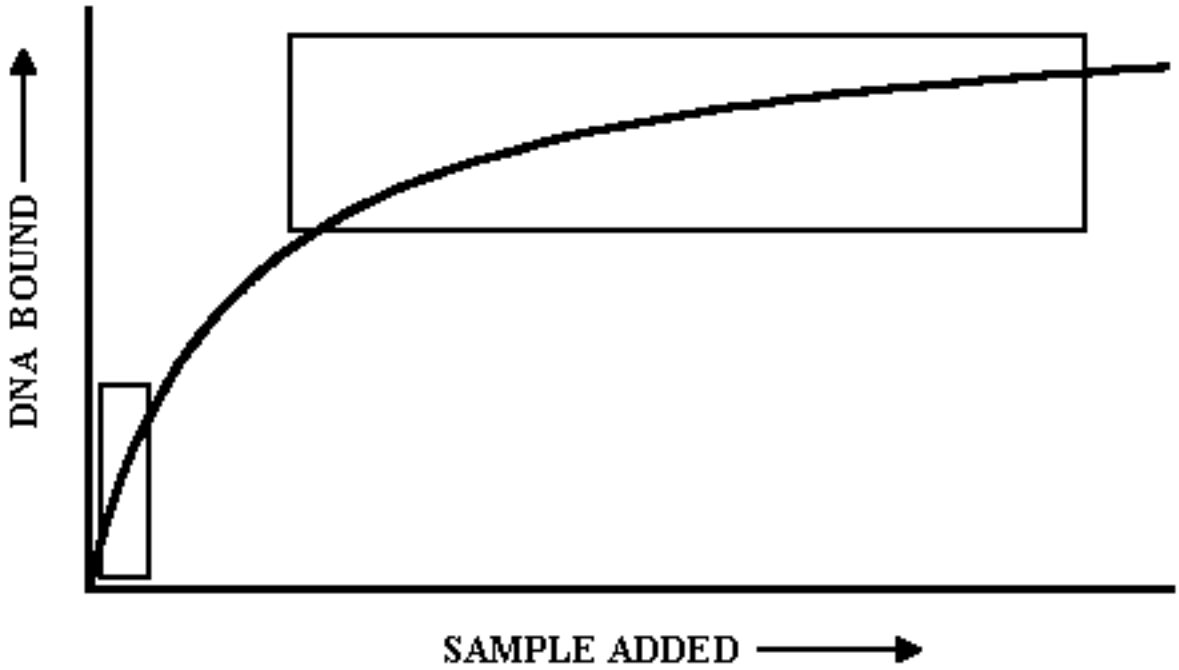

Figure 1. Theory behind quantitation during DNA purification. Resins have defined capacities for binding DNA. At high resin to DNA ratios, indicated by the lower left box, the amount of bound DNA is linearly related to the amount of sample. This ratio is used for high yield capture of DNA. At high DNA to resin ratios, indicated by the large upper box, the amount of bound DNA is nearly independent of sample size. This ratio is used to isolate a uniform amount of DNA regardless of sample size.

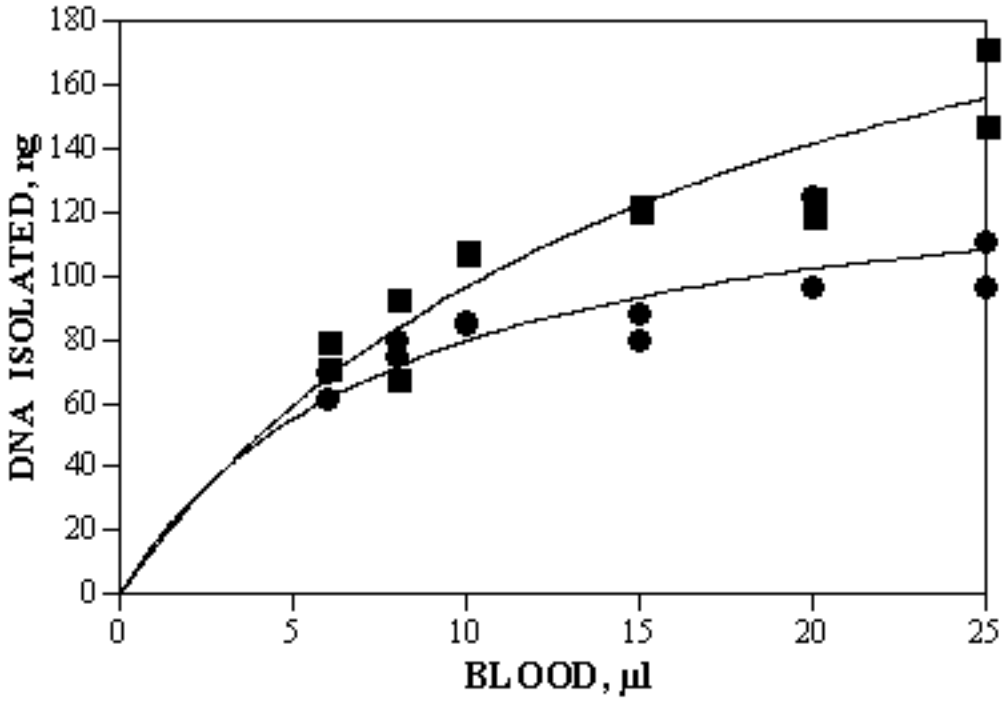

Figure 2. Isolation of DNA from various amounts of blood. DNA was isolated from various amounts of blood using either (●) 500 or (■) 700µg of resin. The total amount of DNA isolated was determined using a PicoGreen® dsDNA quantitation kit.

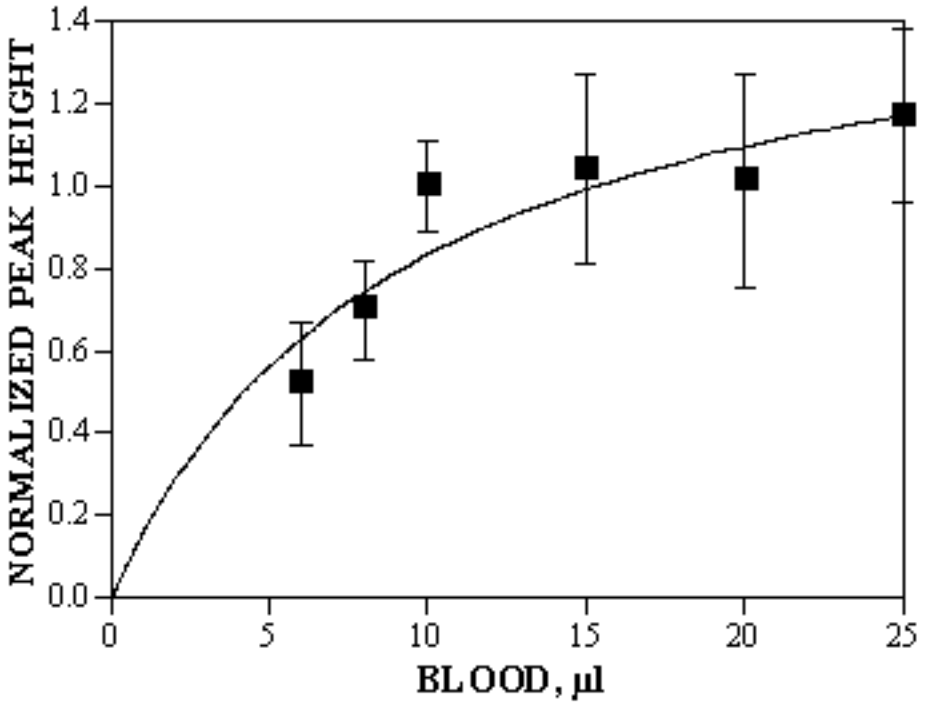

Figure 3. Analysis of DNA amplified from 1µl aliquots. One-µl aliquots from the DNA isolated using 700µg of resin in Figure 2 were amplified using the ***GenePrint***® PowerPlex™ 1.1 system. Peak heights for each of the 15 observed alleles were normalized against the 10µl blood sample. The error bars show standard deviations for each original sample size. These results are consistent with the amount of DNA determined using PicoGreen®.

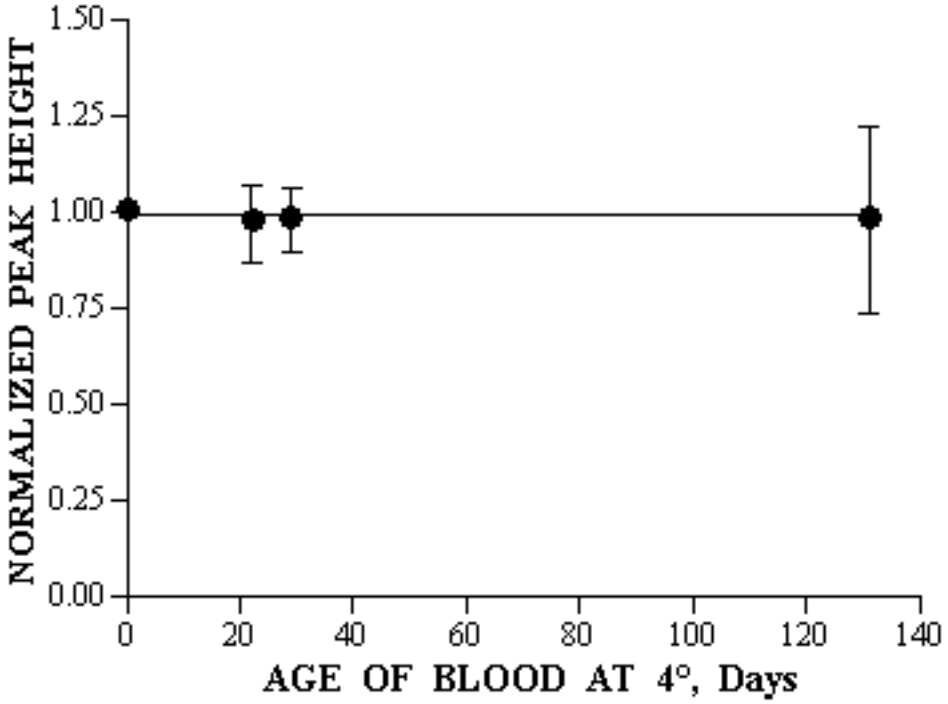

Figure 4. Isolation of DNA from blood stored at 4°C for extended time periods. Blood stored for various periods of time at 4°C were used to isolate DNA using the standard protocol and analyzed using Promega's *GenePrint*® PowerPlex™ 1.1 system**.**

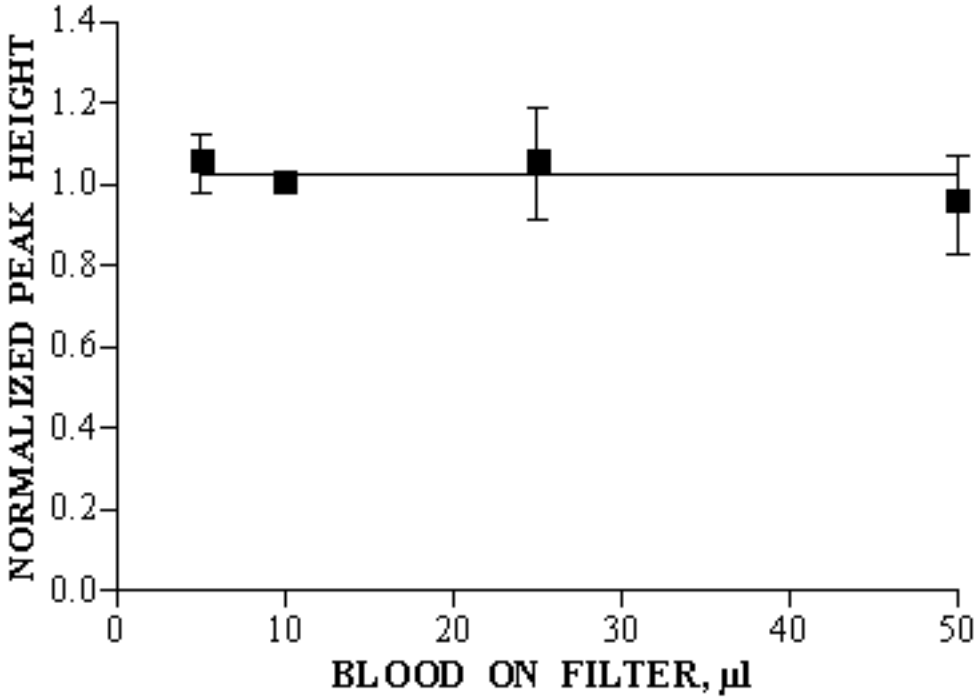

Figure 5. Isolation of DNA from S&S 903 paper. Two-month-old bloodstains on S&S 903 paper containing 5 to 50µl of blood were extracted and processed as described in Materials and Methods. The peak heights from 1µl amplification reactions at each of the observed 15 alleles were normalized to the peak heights observed for the 10µl sample. Error bars indicate the standard deviations observed for the 15 alleles at each original sample size.

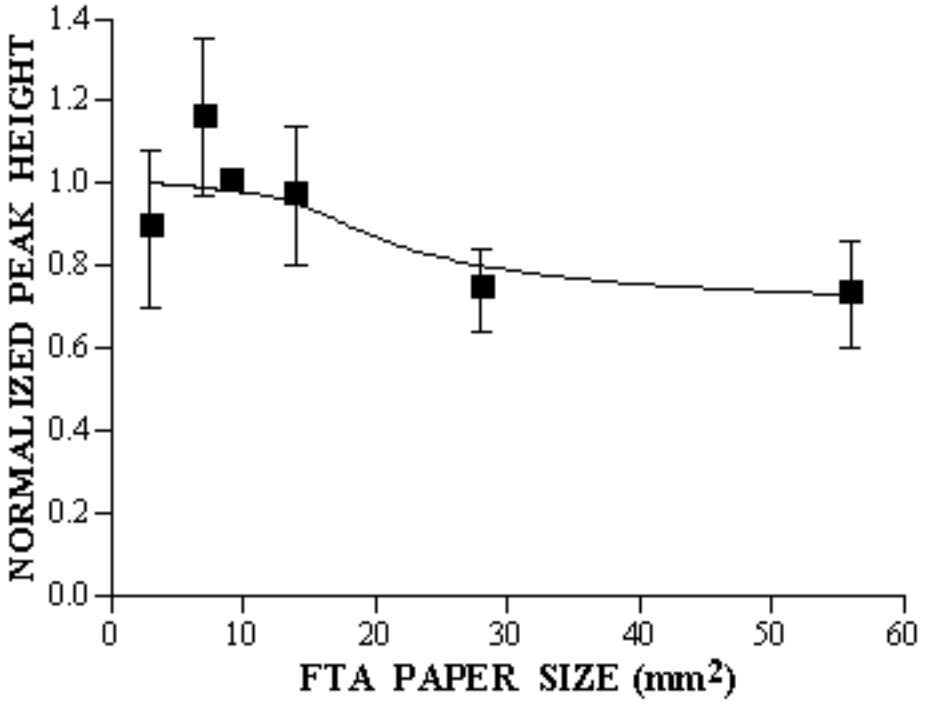

Figure 6. Isolation of DNA from FTA paper. 3mm^2 (2mm diameter Harris punch) to 57mm^2 bloodstains on FTA paper were extracted and processed as described in Materials and Methods. The peak heights from 1µl amplification reactions at each of the observed 15 alleles were normalized to the peak heights observed for the 9mm^2 sample. Error bars indicate the standard deviations observed for the 15 alleles at each original sample size.