The most common way to successfully type forensic DNA samples is Short Tandem Repeat (STR) analysis, which uses PCR. However, this process is beset by the presence of PCR inhibitors that do not allow for the full amplification of DNA. The PCR inhibitors can be present as a side effect of the PCR inhibitors. Therefore, when analyzing biological samples found on carpet, the analysis must be performed after some additional work is done to remove the PCR inhibitors before the DNA can be extracted.

Chelating is the binding of specific metal ions using a chelating agent. A common chelating agent used in molecular biology is EthyleneDiamineTetraAcetic Acid (EDTA). Metal ions will combine with proteins in order to block with EDTA. These metal ions are responsible for the activity of DNAse, an enzyme that breaks the phosphodiester bonds of DNA, that cleaving. Removing these metal ions in order to stop DNAse from working is a major requirement in order to use PCR inhibitors. EDTA can sometimes remove the samples because magnets are no longer available. Magnesium is a metal ion, which is bound to the molecules and become heat-resistant. This, therefore, inhibits the effect of DNAse and prevents the amplification of the DNA. For this reason, residual EDTA from the de-chelating procedure needs to be removed prior to PCR amplification.

Materials and Methods:

Over 3/4s of carpet samples were typed with the most common kit being the 5 µL of DNA extract from the 1.5 M NaCl and nuclease. Dna was added to each sample and the samples were then run through one of four possible carpet cleaners: Carburex, Carpromise, Recon, or deionized water. Samples were run through according to recommendations on the bottles or until the blood stains were no longer visible, and then added to dry overnight. The samples were then cut into sections which would allow for the most DNA to be extracted from each. The samples were then cut off of each of the sections and the DNA was then extracted, purified, and concentrated using the QIAamp DNA Blood Kit (Qiagen). Further analysis of DNA was performed using the QIAamp DNA Blood Kit (Qiagen). A 400 µL reaction was run in each concentration and the DNA was then added to each sample and the samples were then run through one of four possible carpet cleaners: Carburex, Carpromise, Recon, or deionized water. Samples were run through according to recommendations on the bottles or until the blood stains were no longer visible, and then added to dry overnight. The samples were then cut into sections which would allow for the most DNA to be extracted from each. The samples were then cut off of each of the sections and the DNA was then extracted, purified, and concentrated using the QIAamp DNA Blood Kit (Qiagen).

The protocol was then followed. A 1.5 M NaCl and nuclease were used to prepare the DNA for PCR.

The PCR was then followed by the addition of the DNA to the PCR mixture and the samples were then run through the PCR machine. The DNA was then analyzed and the concentrations were visualized through use of the ABI GeneMapper® 10 software version 3.2.1.

In the next step of the protocol, a de-chelating procedure similar to that found in “Comparison of Two Methods for Isolating DNA from Humid Material for STR Analysis” [19] was followed. Carpet was cut into sections of 10 cm² and the DNA was then removed. DNA was then added to each sample and the samples were then run through the PCR machine. The DNA was then analyzed and the concentrations were visualized through use of the ABI GeneMapper® 10 software version 3.2.1.

The nucleotides were then electrophoresed on a 3.0% agarose gel in TAE buffer. The gel was then stained with ethidium bromide and viewed under UV light. The samples were then run through the electrophoreser. In addition to different kits for the 1.5 M NaCl, samples were then run through procedures or using the same procedure as previously described.

This procedure was repeated exactly using only 30 µL aliquots of DNA in 50 µL was determined to be sufficient. After the results of the 1.5 M NaCl, a third procedure was also performed, but was altered slightly. In the third trial, 3 µL aliquots of DNA were spotted on the slides. This was confirmed using a 1.5 M NaCl solution. After the results of the 3 µL trial, a fourth procedure was also performed, but was altered slightly. In the third trial, 3 µL aliquots of DNA were spotted on the slides. This was confirmed using a 1.5 M NaCl solution. After the results of the 3 µL trial, a fourth procedure was also performed, but was altered slightly. The samples were then run through the electrophoreser. In addition to different kits for the 1.5 M NaCl, samples were then run through procedures or using the same procedure as previously described.