**INTRODUCTION**

The purpose of this work is to develop a method which would allow for the amplification of a DNA target which has already been amplified and is now a constituent of the amplified `work product`. This will be accomplished by removing the amplicons and other PCR components such as primers, dNTP's, etc. to sequester the original target DNA such that it is available for re-amplification with additional human identification chemistries. This would allow DNA crime laboratories to genotype limited or exhaustive samples allowing for the amplification of a DNA target which has already been amplified.

**METHOD**

**Experiment 1:**

**PCR Optimization**

Agarose Gel Electrophoresis

ImageJ Analysis

**PCR Optimization With Biotinylated Primers**

**Figure 1.** A) Image of gel after amplification with biotinylated primers (Samples 1 to 6) and the same samples after cleaning with Streptavidin coated magnetic beads and re-amplification (Samples 1-1 to 1-4). B) The graph is a representation of the results generated by ImageJ. C) Table of DNA recovered based on 1ng original input.

**Experiment 2:**

**Amplification With Biotinylated primers**

Streptavidin-Biotin clean-up of amplified `work-product`

Centrifugal Filtration of amplified `work-product`

**Re-Amplification of target**

Agarose Gel Electrophoresis

gPCR

ImageJ Analysis

Capillary Electrophoresis

GeneMarker® HID Analysis

**RESULTS**

**Figure 2.** A) Image of gel after amplification with biotinylated primers (Samples 1 to 6) and the same samples after cleaning with Streptavidin coated magnetic beads and re-amplification (Samples 1-1 to 1-4). B) The graph is a representation of the results generated by ImageJ. C) Table of DNA recovered based on 1ng original input.

**METHOD DEVELOPMENT**

**Figure 3.** RT-PCR amplification plot. IPC from Quantification Negative and the IPC from Sample 1 and (c) Sample 2 after cleaning with Streptavidin coated magnetic beads, indicating significant inhibition of amplification.

**Figure 4.** A) Image of gel after amplification with biotinylated primers (Samples 11-1 to 11-4) and the same samples after cleaning with Streptavidin coated magnetic beads and re-amplification (Samples 11-1 to 11-4). B) The graph is a representation of the results generated by ImageJ. C) Table of DNA recovered based on 1ng original input.

**Figure 5.** RT-PCR amplification plot. IPC from Quantification Negative and the IPC from Sample 3 and replicate Sample 4 after cleaning with Streptavidin coated magnetic beads and centrifugal filters, indicating an additional post-PCR clean up is required to negate inhibition.

**CONCLUSIONS**

A novel method which allows for re-amplification of DNA which has already been amplified and is now a constituent of the amplified `work product` was developed. This method is expected to be utilized with, and provide additional processing options for exhaustive samples.

- that may benefit from multiple amplifications - such as traditional- and Y-STR - and have traditionally been deemed of sufficient quantity/quality for only one amplification (i.e. DNA yields < 1 ng).
- where there was a technical issue with the amplification (i.e. contamination of negative control or thermal cycler deficiency).

The optimal PCR component concentrations for non-biotinylated primers were not the same for the biotinylated primers, suggesting optimization of amplification conditions with biotinylated primers is required prior to use (Figure 1).

Figures 2 and 3 show that without centrifugal filtration, PCR inhibition is prevalent. Hence additional processing, following Streptavidin-Biotin binding, is necessary. Centrifugal filters may be used in this capacity to remove remaining excess primers, salts, and additional inhibitors that may be present in the supernatant.

Figure 4 shows that when the TPOX locus was amplified, signal at ~230 bases was observed. After method clean-up the DSS818 locus (~130 bases) was amplified and run on the agarose gel. These lanes showed no TPOX bands suggesting that the original genomic DNA remains in the supernatant for future DNA processing.

Further, there was no indication of remaining TPOX amplicons from the original amplification demonstrating that clean-up was successful and that the Streptavidin-Biotin post-PCR clean-up process was sufficient and ensured amplicons were not present to complicate down-stream amplifications. However, Figure 4c indicates there is significant loss of genomic DNA incurred during the clean-up process. As a result, re-amplification may be able to be accomplished only a finite number of times.

Future studies will include evaluating the number of times this method can be applied to a sample and identification of the LOD (the original mass required for re-amplification success).