A54 Development of Streptavidin-Biotin Binding of DNA Amplicons Methods for the Typing and Re-Typing of Forensically Relevant Short Tandem Repeats

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After attending this presentation, attendees will be informed of a method which would allow the re-amplification of a DNA target that has already undergone the Polymerase Chain Reaction (PCR) and is a now a constituent of the amplified work product. This will be accomplished by removing the amplicons and other PCR components such primers, dNTP's, etc, to sequester the original target DNA such that it is available for re-amplification with additional human identification chemistry. This would allow DNA crime laboratories and their analysts to genotype limited or exhaustive samples using a variety of kits/chemistries without the need for additional extraction or evidence processing.

This presentation will impact the forensic science community by demonstrating an approach that would allow for: (1) testing of both autosomal- and Y- STRs for limited sexual assault samples; (2) the ability of the analyst to re-amplify with mini-STR's or an enhanced amplification scheme (i.e., more Taq Polymerase, repair enzymes, more BSA) if it is realized that the DNA was degraded, damaged, and/or PCR inhibited: and, (3) the re-amplification of an exhaustive sample due to an amplification failure.

The first stage of development concentrates on maximizing PCR efficiency with primers that have been functionalized with biotin. In this study, the TPOX locus was used to test this optimization. Amplification of the TPOX locus was optimized by varying the concentrations of magnesium chloride ($MgCl_2$) and primers. The amplified products were then electrophoresed and stained with GelStar® Nucleic Acid Gel Stain (Lonza Inc, Walkersville, MD). The resulting bands were analyzed using ImageJ - a public domain open source image processing software.[1] The optimal concentrations were determined to be those used with Sample 3-5 (i.e. 2.25 mM $MgCl_2$ and 0.06 µM primers) (Figure 1).

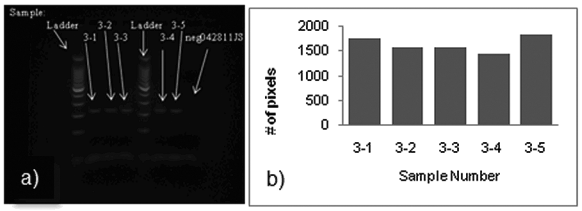

Figure 1: a) Agarose gel electrophoresis of the amplified product with non-biotinylated primer and varying concentrations of $MgCl_2$ and primer; b) Number of pixels (signifying signal intensity) of the amplified product analyzed with ImageJ.

Once optimized conditions for the amplification of TPOX were determined, it was of interest to confirm whether modification of the forward primer with biotin had a significant impact on amplification. As a result, new forward primers of the same sequence which were functionalized with biotin were used during amplification and the reagent concentrations once again varied. Agarose gel electrophoresis was performed as with the amplicons originating from the PCR using the non-biotinylated primer and the gel analyzed using ImageJ as previously described. In contrast to the aforementioned results, the optimal reagent concentrations were determined to be those used with Sample 4-4 (2 mM $MgCl_2$ and 0.04 µM primers) (Figure 2). This indicates that functionalization of the forward primer had an impact on the PCR, suggesting that optimization of the amplification for methods which use modified primers must be performed with the specific primer of interest.

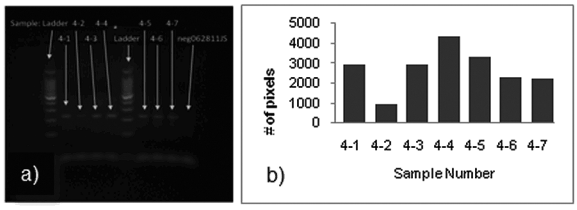

Figure 2: a) Agarose gel electrophoresis of the amplified product with biotinylated primer and varying concentrations of $MgCl_2$ and primer; b) Number of pixels (signifying signal intensity) of the amplified product analyzed with ImageJ.

Once the reaction is optimized, the clean-up procedure can be used on 1ng of DNA. First, the amplified DNA product is run on the 3130 Genetic Analyzer. Then the remaining sample is cleaned using post PCR purification to

remove salts and any unincorporated primers. The amplicons can then be removed from the sample using Streptavidin coated magnetic beads. More specifically, the beads bind to the biotin on the primer (now hybridized to the amplicon) leaving the original DNA in the supernatant for subsequent re-amplification.

Reference:

1. http://rsbweb.nih.gov/ij/. Accessed August 1, 2011.

Streptavidin, Biotin, PCR Optimization

 ** Presenting Author*