A148 Comparison of Different Amplification Reagents for Alleviating Inhibitory Effects of Indigo Dye in PCR

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After attending this presentation, attendees will have learned about the effectiveness of a commercially available PCR enhancing reagent in comparison to 3X Taq polymerase/BSA in alleviating inhibitory effects of indigo dye during PCR.

This presentation will impact the forensic science community by presenting data to confirm the effectiveness of different reagents in combating inhibitors such as indigo dye during PCR. This knowledge is important because forensic scientists can better select which reagents to use in their future PCR studies based on the outcome of this experiment.

PCR is an indispensable tool in forensic science studies. All who use PCR are likely to be impacted by inhibitors at some time, but the wide range of forensic sample types and variety of sampling conditions encountered renders forensic DNA samples particularly susceptible to inhibitors. There are several approaches that have been utilized to address PCR inhibitors including simply diluting the sample. This approach may not always be effective depending on the concentration of the inhibitor and the template concentration. Another method recently developed involves removing inhibitors from DNA samples using an automated instrument. This method appears promising, but the backlogs at crime labs, and the expense and time of purifying each sample before PCR may make this method impractical for all cases.

Another method of addressing PCR inhibitors is to find reagents that will alleviate the inhibiting effects during the PCR process. Knowing which reagents to select to accomplish this goal has multiple advantages. First, this may reduce sample consumption as the number of re- amplifications of inhibited samples may be reduced. Second, it may also increase productivity by reducing the need for analysis and review of replicate samples. Third, it will also likely reduce costs.

A number of studies claim that the use of a variety of reagents can enhance PCR performance by improving sensitivity and specificity during amplification of genomic DNA templates. The goal of this study is to evaluate the effectiveness of different reagents in alleviating the inhibitory effects of indigo dye (known inhibitor) from denim, which is a frequently encountered evidentiary substrate from blue jeans.

Control 9947a DNA will be first quantified using qPCR and spiked with different amounts of Indigo dye to mimic samples that contain co- extracted indigo dye. In addition, DNA extracted from blood delivered on blue jeans will also be utilized. Comparisons of three reagents will be performed on control DNA spiked with Indigo Dye and DNA extracted from blood on denim at different concentrations: (1) A PCR enhancing reagent (commercially available); (2) tripling the amount of DNA polymerase with additional bovine serum albumin; and, 3) a combination of both 1 and 2. After PCR, capillary electrophoresis will be used to separate the amplicons and computer analysis will be used to evaluate the results in terms of degree of amplification (eg peak heights and peak height ratios) and the number of alleles recovered. It is hypothesized that the combination of both approaches will be most effective in alleviating inhibitory effects of indigo dye.

The long term goal of this study is to investigate the possible mechanisms of indigo dye inhibition on PCR. Overcoming inhibition with different reagent strategies may provide useful information to this end. There are several possible mechanisms of inhibition. First, indigo dye molecules may bind to DNA and inhibit the amplification process. Second, it is possible that indigo dye interferes with primer extension by the DNA polymerase. If such is the case, PCR cannot take place properly. Finally, in order to utilize indigo dye for staining denim, it is must be reduced to the negatively charged leucoindigo (soluble form) and when in solution (as it may be in co-extracted DNA samples), may interfere with the cofactors (eg Mg++) necessary for successful PCR.

Other experiments with varying amounts of DNA template, indigo dye, and additional Mg2+ will also be performed. Knowing the indigo dye inhibitory mechanisms is important because further research can be made to enhance reagents aimed to alleviate indigo effects on PCR, thus making this tool more accurate and effective in forensic identification. **PCR, Inhibition, Indigo Dye**

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