B115 The Effect of PCR Inhibitors on the Amplification of Low Concentrations of Template DNA Using Reduced-Size STR Primer Sets

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After attending this presentation, attendees will be familiar with the affect of single and mixed PCR inhibitors on the amplification of low concentrations of human template DNA, as well as the effect of amplicon length on PCR inhibition.

This presentation will impact the forensic community and/or humanity by providing information on how various inhibitors affect DNA amplification at different template concentrations. This information may affect how amplification of low concentration samples will be approached in the future with respect to dealing with inhibitors.

The presence of source contaminants commingled with DNA tem- plate presents a challenge in forensic human identification. The effects of these compounds on the PCR reaction can vary from attenuation to com- plete inhibition of the amplification reaction. PCR inhibitors can be endogenous or exogenous to the reaction. Endogenous contaminants usually originate from insufficiently purified DNA template, and the inhibitor is co-extracted with the target DNA during the extraction or purifi- cation step. Exogenous contaminants arise due to improperly controlled hygienic or laboratory conditions.

This project covers the effect of inhibitors on the reduced size STR Miniplex primer sets at different template concentrations. The Miniplex primer set produces smaller amplicons, and increases the probability of obtaining a usable profile from degraded DNA. The size of the alleles in the Miniplex kits range from 60-284 base pairs. Since the presence of inhibitors can affect the amplification efficiency of any primer set, studies were initiated to determine if the decreased amplicon size would affect the level of inhibition or the concentration at which it occurs.

For this study, inhibitors which may be present in the sample itself were examined. These inhibitors can commingle with the DNA sample upon exposure to different environmental conditions. Although a wide range of PCR inhibitors have been reported, six common PCR inhibitors known to affect forensic samples were chosen for these studies: 1) hematin, found in blood; 2) indigo, a dye found in denim; 3) melanin, a pigment found in skin and hair; 4) humic acid, found in soil and other environmental samples; 5) collagen, found in bone; and 6) calcium, another component of bone samples. The inhibitors were tested singularly and in combinations which are likely to be present in forensic samples.

Five concentrations of each inhibitor were tested with each Miniplex kit to determine the threshold inhibitor concentration (TIC) at high template concentration (500 pg/25 µL for Big Mini and 250 pg/ 25 uL for Mini 2 and Mini 4). The TIC was defined as the lowest concentration of inhibitor that removed the signal from at least one locus in three replicate measurements. This threshold inhibitor concentration and slope of the curves obtained for different inhibitor concentrations were then compared for each inhibitor and each Miniplex. Initial results show a wide variation in threshold concen- tration and that their effect appears to be independent of amplicon length. In addition, different loci show variations in threshold levels for inhibition.

Studies were also performed to determine the effect of lower template concentrations on the threshold inhibitor concentration. Three concentra- tions of inhibitors were tested on different levels of DNA template for each of the Miniplex primer sets (500, 250, and 125 pg/25 µL for Big Mini, 250, 125 and 63 pg/25 µL for Mini 2 and Mini 4). The level of inhibition for each locus and each concentration was calculated and the results were compared. The rate of inhibition for each individual locus was also determined. Finally, combinations of inhibitors which could be found in forensic samples were studied. These were used on different template concentrations and the level of inhibition was calculated and compared between sets and loci.

PCR Inhibition, DNA Template Concentration, Miniplexes

 ** Presenting Author*