B66 The Effect of Primer Melting Temperature, Sequence, and Amplicon Length on PCR Inhibition

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After attending this presentation the attendee will be familiar with the impact of various parameters on PCR inhibition, and the basic mechanism of a selection of PCR inhibitors.

This presentation will impact the forensic community by providing information on PCR inhibition which can be utilized to improve analysis of DNA samples where PCR inhibitors are present.

The presence of source contaminants commingled with DNA template presents a challenge in forensic human identification. The effects of these compounds on the PCR reaction can vary from attenuation to complete inhibition. 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 purification step. Exogenous contaminants arise due to improperly controlled hygienic or laboratory conditions.

This project involves the utilization of real time PCR to study the mechanism of various PCR inhibitors, as well as the examination of the effect of amplicon length, primer melting temperature, and sequence on PCR inhibition. It is reasonable to assume that smaller amplicons would be less susceptible to inhibition due to the mechanism of primer extension by Taq polymerase. However, based on preliminary studies in our laboratory, inhibition mechanisms are more affected by the sequence than length. Since the presence of inhibitors can affect the amplification efficiency of any primer set, studies were initiated to examine a variety of parameters on the DNA typing process.

In this study, inhibitors which may be present in the sample itself were examined. These inhibitors can commingle with the DNA sample upon exposure to various environmental conditions. Although a wide range of PCR inhibitors have been reported, several common PCR inhibitors known to affect forensic samples were chosen for these studies: hematin, found in blood; indigo, a dye found in denim; melanin, a pigment found in skin and hair; humic acid, found in soil and other environmental samples; collagen, found in bone; calcium, another component of bone sample; tannic acid, a component of leaf litter; and others. The inhibitors were tested singly and in combinations that were likely to be present in forensic samples.

Experiments were conducted to determine the range of inhibitor concentration at which a consistent and significant change in signal was observed during rt-PCR (qPCR) analysis at a standard template concentration. The threshold inhibitor concentration was defined as the lowest concentration of inhibitor that shifted the signal to a higher Ct threshold. A range of concentrations for each inhibitor was then selected, and the inhibitors were added singularly and then were combined in different ratios to determine the effect of the mixtures.

Studies were also performed to further determine the effect of amplicon length sequence and primer melting temperature on the threshold inhibitor concentrations. For this study, a single locus comparison was made using primers with three different melting temperatures and three different amplicon lengths. After determining the normal uninhibited amplification efficiency for each primer pair, a range of concentrations of inhibitors was tested on each primer pair. Finally the level of inhibition for each primer pair was calculated and the results were compared between amplicon lengths and primer melting temperatures as well as among the different types of inhibitors.

DNA, PCR Inhibition, Rt-PCR

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