DEVELOPING AN UNDERSTAND OF PCR INHIBITION USING REAL TIME PCR AND MELT CURVE ANALYSIS

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PCR inhibition is a common problem in forensic analysis that can result in numerous problems in STR amplification including peak imbalance, allele loss, and problems in data interpretation. Inhibitory effects can result in the loss of larger alleles, mimicking DNA degradation or the effects can be quite different, producing sequence specific allele dropout. In general inhibitory substances affect PCR amplification in one of two ways. Either replication is blocked through effects on the polymerase, or the inhibitor binds DNA template, reducing its available concentration. Certain inhibitors can do both.

The goal of this presentation is to demonstrate ways to better interpret real time PCR data in order to identify inhibitory mechanisms. In doing this we can assist the analyst in data interpretation and ultimately suggest best practices for cleanup and purification of samples. In the project we have utilized the Plexor® HY real time PCR quantification system in combination with the Powerplex® HS STR amplifications to examine the effects of a wide variety of PCR inhibitors. By comparing PCR amplification plot and effects on melt curves with allele loss we are able to ascertain and classify PCR inhibitors by their mechanism. These effects are further clarified through the use of curve fitting algorithms to model the various effects of the inhibitors on the amplification process. In our results, substances affecting DNA template tend to affect Ct and produce melt curve shifts while those affecting the polymerase generate an altered amplification curve. All inhibitors produce dropout of larger alleles, but those inhibitors binding DNA tend to produce more complex and sequence specific patterns.