A STUDY PCR INHIBITION MECHANISMS IN STR GENOTYPING BY QUANTITATIVE PCR

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It is well know that a variety of substance when coextracted with DNA from biological samples may result in PCR inhibition. This phenomena results in poor amplification, allele dropout and sometimes complete loss of signal. We have been examining the effects of various known PCR inhibitors including collagen, humic acid, tannic acid, indigo and calcium on the PCR. We have determined minimum concentrations for these inhibitors and have probed their effects on multiplex STR amplification.

We have also investigated the application of quantitative PCR for the determination of the mechanism by which these various inhibitors affect the PCR reaction. Through careful experimental design we can probe the effect of amplicon length, sequence and primer binding. The use of Syber Green with quantitative PCR also allows us to probe PCR take off cycle, reaction efficiency, and DNA melt curves as further clues to the action of inhibitors. Our overall results indicate that there are a variety of different mechanisms by which these compounds affect DNA amplification. These include sequence specific binding to template, Taq inhibition, and sequestration of reactants. The presentation will conclude with some general recommendations on how to recognize and treat these effects.