THE EFFECTS OF DNA SEQUENCE AND LENGTH ON INHIBITOR INTERACTIONS IN REAL TIME PCR AMPLIFICATION.

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The DNA left at crime scenes is not usually found in pristine conditions. The purpose of DNA extraction is to only extract the DNA from a sample, however this may not always be the case. Depending on where the sample came from, it is possible to co-extract other components along with the DNA, either from the sample itself or the environment, which have the capability to interfere with the PCR process and reduce the amount of DNA amplified or give a false estimation of the amount of DNA recovered. This can lead to incorrect DNA profiles, which can result in the guilty running free or an innocent taking their place. When the sample is already limited, this can be a major setback in the forensic analysis. The aim is to identify the most effective kinds of DNA sequences that are able to detect PCR inhibition in real-time PCR. This research examines the effect of the size and sequence of the sample being amplified with increasing amount of common known PCR inhibitors in relation to the amount of inhibitory effects seen using real-time PCR. Three novel DNA sequences with GC content ranging from 32 to 53% and embedded primer sequences giving amplicons of 80, 160 and 230bp allow us to simultaneously compare between sequence composition and size. These sequences are directly compared to the amplification and melt curves of Autosomal and Y DNA using the Plexor HY system. With this, we can determine the kinds of sequences that are most affected by different inhibitors and determine their mechanism. The results show that amplicon sequence has little to no impact on inhibitory effects, while the longer amplicons are strongly affected regardless of inhibitor type. Although the amplification of all samples show more inhibition as the size of the amplicon increased, it is possible for some more subtle changes in the melt curves to be seen with smaller amplicons of DNA-interacting inhibitors. From this we conclude that the current method of extrapolating the amount of inhibition based upon an amplicon of a smaller size than some of the final PCR products can be misleading. ⌘