A83 Who Needs Gold?

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The goal of this presentation is to explore the capabilities of various polymerases to overcome inhibition and generate short tandem repeat (STR) profiles from degraded, low template DNA samples encountered in forensic cases. Nuclear DNA extracted from evidence, which has been exposed to adverse environment conditions, may be degraded and the amount obtained may be lower than necessary for obtaining complete STR profiles. This research investigates a combination of extraction methods, various enzymes, and amplification protocols which can overcome the difficulties associated with amplification of low amounts of degraded DNA which may also contain inhibitors.

This presentation will impact the forensic science community by offering other options regarding enzymes used in polymerase chain reaction (PCR). Use of these enzymes and optimal amplification conditions can yield high quality human DNA profiles from low amounts of degraded DNA which may also contain inhibitors.

Forensic scientists are constantly looking for ways to attain complete human DNA profiles from less than ideal biological samples, particularly from low template DNA. DNA extracted from items of evidence which have been subjected to adverse environmental conditions can be degraded or inhibited in a way that could negatively impact the PCR reactions. Scientists in the past have changed amplification conditions in order to increase PCR efficiency which is crucial to the success of DNA analysis.

Cigarette butts retrieved from crime scenes are sometimes less than ideal forensic evidence. Temperature, humidity, and soil are some of the factors which can cause degradation of biological fluid deposited on cigarette butts. Tar and nicotine, a few of the components of cigarettes, can also act as inhibitors, as can humic acid in soil. The concentrations of these ingredients can adversely affect the amplification process. In addition, saliva deposited on cigarette butts that have been exposed to detrimental environmental conditions may yield lower than optimal amounts of DNA. DNA extracted from these items is also often degraded and contains inhibitors. Amplification of low template, degraded DNA can result in allelic dropouts, peak imbalance, and low intensity peaks.

This research explores the use of various polymerases during the amplification process of low template, degraded DNA samples which may also contain inhibitors. Although AmpliTaq Gold® is the polymerase of choice for obtaining human DNA STR profiles from forensic samples; there are other enzymes available in the scientific community. This study uses various polymerases and optimal amplification conditions to increase the efficiency and specificity of the PCR reaction, which in turn can yield better quality DNA profiles from low amounts of degraded or inhibited evidence samples.

The first part of this study focuses on amplification with polymerases other than the commonly used AmpliTaq Gold® polymerase to determine if other enzymes can yield similar or better STR profiles from an optimal amount of DNA which is neither degraded nor contains possible inhibitors. The second part of the study is conducted with DNA extracted from environmentally insulted cigarettes butts. This includes depositing known amounts of saliva from various donors on cigarette butts, exposing them to a range of temperatures, submerging the cigarette butts in water and burying them in soil. These conditions are carried out over varying lengths of time. Various extraction procedures are used and the amplification conditions, including the amount of enzymes, are optimized to obtain high quality STR profiles.

Complete DNA profiles were obtained using 0.1ng of uncompromised DNA and AmpliTaq Gold®, Ex Taq™, and Diamond Taq™ polymerases. Although all alleles were present, some of the alleles were below threshold parameters when 0.1ng of DNA was amplified with DFS Taq polymerase. Attempts to amplify 1.0ng or lower amounts of uncompromised DNA with Titanium® Taq and PrimeSTAR® HS DNA polymerases yielded either partial profiles or no profiles. Amplification parameters for these reactions were as recommended by the manufacturer of the kit used in this research.

While some of the compromised cigarette butts yielded full STR profiles, most extracts gave partial profiles when the recommended parameters of the amplification kit and the AmpliTaq Gold®, Ex Taq™, and Diamond Taq™ polymerases were used. When extraction procedures and amplification parameters, including the enzyme concentration, were optimized, more of the compromised DNA extracts yielded complete STR profiles.

This study indicates that by employing polymerases not commonly used in analyzing forensic evidence, analysts could potentially produce human STR profiles from low template, degraded DNA which may also contain inhibitors.

Polymerase, Inhibition, Degradation

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