



A66 New Strategies to Overcoming PCR Inhibition Using Mutant Taq Polymerases and PCR Enhancers

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After attending this presentation, attendees will gain an understanding of how inhibitors found in DNA extracts such as phenol-chloroform, humic acid, collagen, and calcium affect Polymerase Chain Reaction (PCR) amplification, and whether mutant Taq DNA polymerases and PCR enhancers will aid in overcoming the inhibition to recover a DNA profile.

This presentation will impact the forensic science community by providing potential strategies to overcome PCR inhibition that may be found in low template, low quality DNA samples such as samples resulting from degraded skeletal remains. Selection of amplification strategies and downstream methods, such as the inclusion of PCR enhancers and/or new mutant Taq polymerases, will likely improve typing on crude, inhibited samples encountered in missing person, mass disaster, and cold cases.

Since its introduction in the mid-1980s, the use of DNA profiling in forensic science has revolutionized the justice system on a worldwide scale. DNA profiling is comprised of multiple steps and procedures including DNA extraction, quantification, and amplification using PCR. PCR has permitted the analysis of very low quantity, low quality DNA samples. Crime scene samples, however, are often found in very poor condition and are often mixed with extraneous materials that may co-extract with the DNA.

There are several known, commonly encountered inhibitors to PCR: calcium, collagen; humic acid; hematin; melanin; indigo dye; detergents; and, phenol-chloroform used in DNA extractions. These inhibitors may interfere with the cell lysis or capture of components necessary for DNA extraction by causing DNA degradation and/or inhibiting DNA polymerase amplification of target DNA. A review on forensic implications of PCR inhibition was recently published.

Although the causes of PCR inhibition are still not fully known, three mechanisms have been proposed. These include: (1) binding of inhibitor to Taq polymerase; (2) blocking of amplification sites due to inhibitor-template binding; and, (3) decreasing processivity due to interaction of the inhibitor with Mg^{2+} cofactors or other components of PCR.

Detection and overcoming PCR inhibition are critical challenges faced by forensic molecular biologists and many others such as microbiologists studying soil samples, molecular evolutionary biologists studying ancient remains and preserved samples, molecular ecologists studying animal excrement, molecular pathologists studying preserved and mounted specimens, and molecular archaeologists and anthropologists studying ancient human remains. Commonly utilized methods for overcoming inhibition in the forensic DNA community include: (1) diluting the samples (thereby also diluting inhibitors in the sample); (2) additional cleaning of the sample by purification; (3) including additional DNA polymerase and Bovine Serum Albumin; (4) utilizing STR multiplexes that are inhibitor resistant such as Minifiler, Identifiler Plus, and Powerplex 16HS; and, (5) adding PCR enhancers.

In this study, a mutant Taq polymerase and two different PCR enhancers were tested for their ability to overcome inhibition: Omnitag (DNA Polymerase Technologies); PCR enhancer cocktail (DNA Polymerase Technologies references 19 and 20); and, PCRboost (Biomatrica).

Omni tag is an inhibitor-resistant Taq polymerase mutant and PCR enhancer cocktail (PEC) consists of a mixture of nonionic detergent, L-caritine, D-(+) trehalose, and heparin. PCR and STR boost are proprietary enhancers from Biomatrica, Inc. Previous tests have shown improved amplification using PCRboost from DNA samples containing indigo dye, hematin, humic acid, and phenol chloroform.

This presentation explores the amplification enhancement of Omnitag, PEC, and PCR boost on low quantity and low quality DNA samples that contain varying amounts of inhibitors (Phoh, humic acid, collagen, and calcium). Enhancement were evaluated on replicate 1, 0.5, and 0.25 ng samples with and without inhibitors at different concentrations using qPCR (Plexor HY) and STR multiplex typing (Identifiler, Identifiler plus, Identifiler Direct, and Powerplex 16HS).

PCR Inhibition, Mutant Taq, PCR Enhancement