

NEW STRATEGIES TO OVERCOMING PCR INHIBITION USING MUTANT TAQ POLYMERASES AND PCR ENHANCERS

Hillary Nguyen, Hanna Bennett, Phil Nhan, and Steven B. Lee.
Forensic Science Program. San Jose State University, San Jose CA 95192, USA

Since its introduction in the mid-1980s, the use of DNA, namely 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 Polymerase Chain Reaction (PCR). PCR has permitted the analysis of very low quantity, low quality DNA samples. However, crime scene samples 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 (1-6). 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 (7). A review on forensic implications of PCR inhibition was recently published (8).

Although the causes of PCR inhibition are still not fully known, three mechanisms have been proposed 1, 2, 7. 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 (7).

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 excrements, molecular pathologists studying preserved and mounted specimen, 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 (9-18).

In this study, a mutant Taq polymerase and two different PCR enhancers were tested for their ability to overcome inhibition: OmniTaq (DNA polymerase technologies, St. Louis, Missouri), PCR enhancer cocktail (DNA polymerase technologies, St. Louis, Missouri references 19 and 20) and PCRboost (Biomatrix, San Diego, CA reference 21-23).

OmniTaq 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 (19-20). PCR and STR boost are proprietary enhancers from Biomatrix Inc (21). Previous tests conducted in our laboratory have shown improved amplification using PCRboost from DNA samples containing indigo dye, hematin, humic acid, and phenol chloroform (21-23).

This project will explore the amplification enhancement of OmniTaq, PEC and PCR boost on low quantity and low quality DNA samples that contain varying amounts of inhibitors (eg humic acid and calcium). Enhancement will be evaluated on replicate 1, 0.5 and 0.25ng samples with and without inhibitors at different concentrations using qPCR (Plexor® HY, Promega, Madison WI) and STR multiplex typing (Identifiler, Identifiler plus, Identifiler Direct and Powerplex® 16HS).

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