Enhancement Strategies for Overcoming PCR Inhibitors

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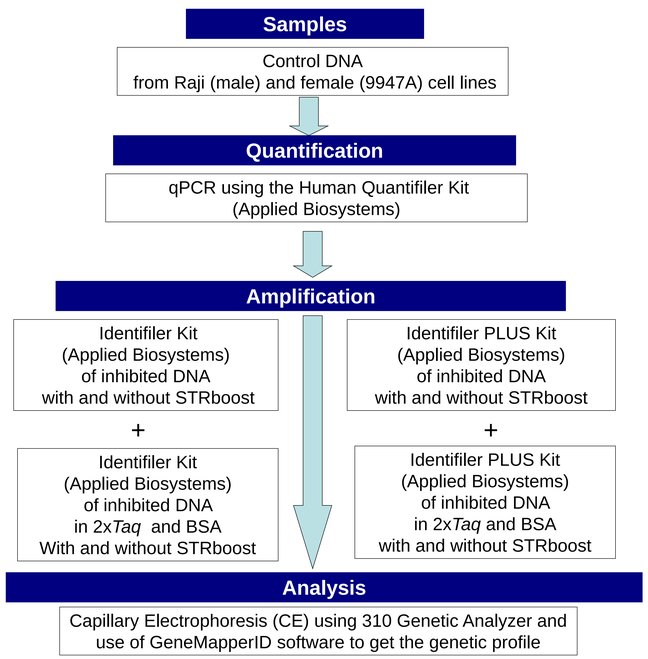
Abstract

The use of forensic DNA to solve crimes is well established. Forensic evidence samples are often degraded or contain environmental contaminants, which interfere with PCR amplification (3, 10, 14, 16). There are several known inhibitors to PCR: calcium, collagen, humic acid, hematin, melanin, indigo dye, and phenol-chloroform (4, 14). 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 (16). Three possible mechanisms of inhibition are (1) binding of the inhibitor to the polymerase, (2) interaction of the inhibitor with the DNA, and (3) interaction with the polymerase during primer extension (14). To overcome the effect of PCR inhibitors, purification from inhibitors prior to DNA extraction, removal of inhibitors during or after DNA extraction, or relief or suppression of the effect of PCR inhibitors when performing PCR can be utilized (6). Inhibitors may cause loss of signal, peak imbalance, and/or allelic dropout (14). There are several methods that are being utilized to overcome inhibition during PCR amplification, such as adding more *Taq* and BSA (10); as well as using kits designed to overcome inhibition, such as Identifiler Plus (Applied Biosystems) and PowerPlex 16 HS (Promega).

A new method to enhance amplification includes the use of novel reagents, know as PCRboost and STRboost (Biomatrica, Inc.), that have been reported to improve PCR performance five-fold or more on challenging and difficult to amplify samples (18). ***This project will explore the amplification enhancement of STRboost on low quantity and low quality DNA samples that are spiked with inhibitors.*** Specifically, this project will focus on samples spiked with indigo dye and phenol chloroform. Amplification with Identifiler and Identifiler PLUS kits (Applied Biosystems) of low quantity DNA samples with and without STRboost in the presence of varying amounts of inhibitors and the use of additional *Taq* polymerase and BSA were performed. Amplification utilizing kits not already designed to overcome inhibition of inhibited, low quantity DNA samples were improved using STRboost.

Methods



Results

Identifiler Kit amplification of PhOH-CHCl$_3$ spiked DNA with and without PCRboost

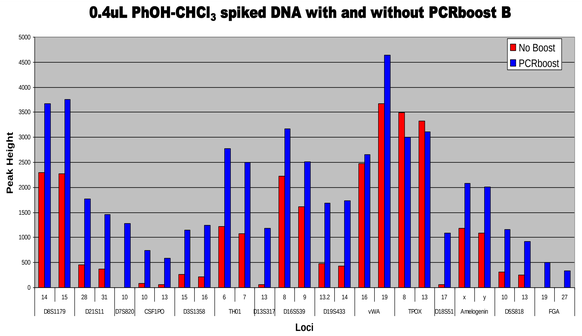


Figure 1. Amplification results of 0.62ng DNA spiked with varying amounts (0.4uL, 0.8uL, and 1.2uL) of Phenol/Chloroform/Isoamyl Alcolhol 25:24:1 (PhOH-CHCl$_3$) with and without PCRboost B using Identifiler Kit. At 0.4uL, PCRboost recovered 3 alleles at 2 loci, and nearly 4-fold enhancement was obtained for some alleles (D21S11, D3S135, D19S433, D5S818).

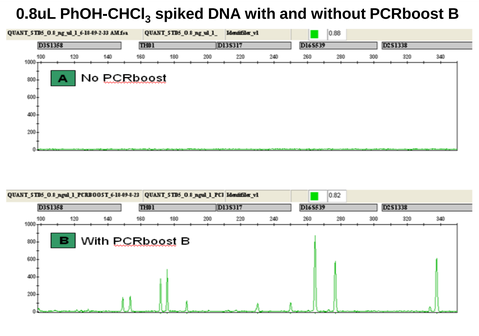


Figure 2. Electropherogram of 0.62ng with 0.8ul PhOH-CHCl$_3$ without PCR boost (A) and with PCRboost (B), a full profile was recovered. Note: additional peaks are due to spectral overlap/pull up.

Identifiler PLUS Kit amplification of PhOH-CHCl3 spiked DNA with and without STRboost

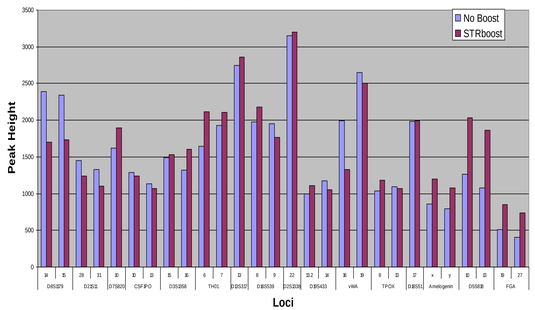


Figure 3. Amplification results of 0.62ng DNA spiked with varying amounts (0.6uL, 0.9uL, and 1.2uL) of PhOH-CHCl3 with and without STRboost using the Identifiler PLUS Kit. At 1.2uL, STRboost enhanced some alleles at 9 loci, some alleles at nearly 2-fold (D5S818 and FGA). Note: At 1.2uL using Identifiler Kit, no alleles were recovered above the 50rfu threshold even with PCRboost.

Identifiler Kit amplification of Indigo Dye spiked samples with and without PCRboost and/or "Superjuice"

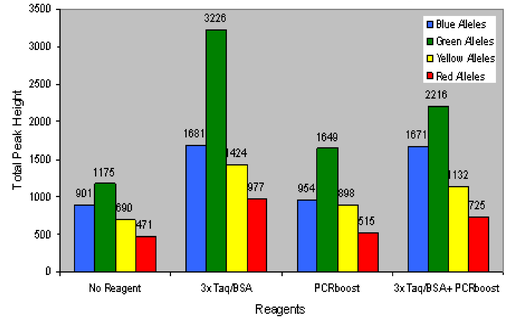


Figure 4. Amplification results of 0.0625 ng DNA spiked with 0.7mM of indigo dye enhanced with and without 3xTaq/BSA ("superjuice") and/or PCRboost using the Identifiler Kit. Results indicate a combination of both enhancers improved amplification but not as well as with superjuice alone.

Identifiler PLUS Kit amplification of Indigo Dye spiked samples with and without STRboost

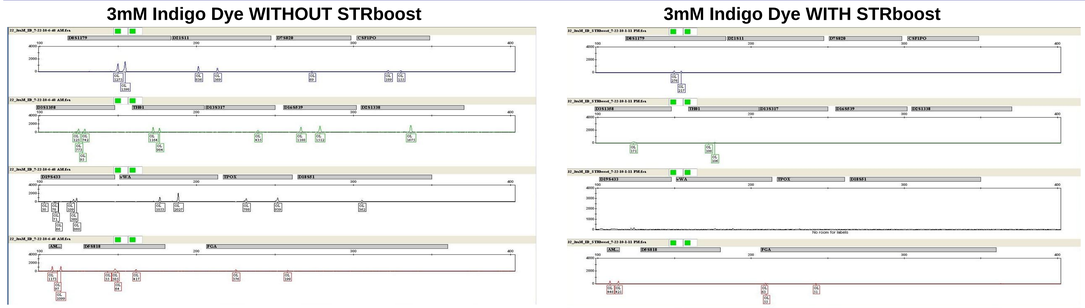


Figure 5. Amplification results of 0.62ng DNA spiked with 3mM Indigo Dye using the Identifiler PLUS Kit with and without STRboost. At 3mM, a combination of Identifiler PLUS additives and STRboost apparently inhibits amplification.

Conclusion

- Enhancement and recovery was achieved using STRboost and other amplification enhancers on low quality and low quantity inhibited samples using the Identifiler Kit.
  - Enhancement of amplification of 0.4uL PhOH-CHCl$_3$ inhibited DNA was also achieved (Figure 1).
  - A full profile was recovered from 0.8uL PhOH-CHCl$_3$ spiked DNA after the addition of PCRboost (Figure 2).
- PCRboost and STRboost amplified and enhanced the peak heights of PCR products from inhibited samples however, the enhancement was not as significant on control, uninhibited samples.
- Amplification kits (Identifiler PLUS) designed to overcome inhibition did not show significant improvement with additional enhancers, such as STRboost. In fact the amplification appeared to decrease with STRboost (Figure 3 and Figure 5).
  - Using the traditional Identifiler Kit with a combination of "Superjuice" (3xTaq/BSA) and PCRboost, did show enhancement; however, not as well as Superjuice alone, indicating that too many amplification enhancers may have an inhibitory effect (Figure 3, Figure 4, and Figure 5).
- PCRboost appears to enhance the amplification of loci differentially suggesting that the enhancement may be sequence and locus dependant.

Future Steps

- Evaluate different amplification strategies by modifying the Identifiler and Identifiler PLUS kit (Applied Biosystems) and PowerPlex 16 HS (Promega) protocols to include other amplification enhancing strategies with and without STRboost.
- Conduct further research and testing to understand why STRboost may have a greater affinity for certain loci.
- Evaluate results on mixtures and non-probative DNA samples with and without STRboost.
- Evaluate DNA stored in polymers and different storage buffers and tubes (e.g. DNA stored in Samplematrix and teflon tubes) with and without PCR boost to evaluate any synergistic effects of prior storage buffers and polymers on STRboost enhancement.
- Expand collaborations to include additional labs, sample types and amplification targets (e.g. Other inhibitors, mtDNA).

Acknowledgements

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