ENHANCEMENT STRATEGIES FOR OVERCOMING PCR INHIBITORS

Clarissa Trogdon, Phil Nhan, Steven Lee
San Jose State University, Forensic Science Program, Justice Studies Development,
San Jose, CA 95192-0050

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 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 a novel reagent, known as STRboost (Biomatrica, Inc.), that was 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.

This project will examine different amplification strategies with and without STRboost on varying amounts of inhibited DNA samples spiked with indigo dye, and phenol chloroform.

Replicate samples of control DNA at 0.62 ng spiked with varying amounts of inhibitors will be prepared. Real-Time PCR (qPCR) with the Quantifiler Kit (Applied Biosysems) will be used to quantify the samples and evaluate the level of inhibition via monitoring the IPC values(17). PCR amplification will be performed using the Identifiler, Identifiler Plus Kit (Applied Biosystems) and/or the Powerplex 16 HS Kits (19) as per the manufacturers recommendations. The Identifiler protocol will also be modified with and without 2x Taq and BSA, as well as with and without STRboost. The products will be separated and detected using capillary electrophoresis (CE) on the ABI 310 or the ABI 3130 Genetic Analyzer (Applied Biosystems). Analysis of the data will be performed using GeneMapperID from Applied Biosystems, which assesses both quantitative and qualitative data of all samples.

Results indicate that when STRboost is used with the Identifiler Kit, peak height and heterozygous peak balance is improved. Results on samples spiked with inhibitors using the additional amplification strategies will be presented.

References:

1. Al-Soud, W.A. & Radstrom, P. (2001). Purification and characterization of PCR-inhibitory components in blood cells. *Journal of Clinical Microbiology, 39*(2), 485-493

2. Barbisin, M., Fang, R., O'Shea, C.E., Calandro, L.M., Furtado, M.R., & Shewale, J.G. (2009) Developmental validation of the Quantifiler Duo DNA Quantification Kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. *Journal of Forensic Science, 54*(2), 305-319. doi: 10.1111/j.1556-4029.2008.00951.x
3. Bessetti, J. (2007). PCR inhibition. Profiles in DNA. Promega Corporation. www.promega.com
4. Brevnov, M.G., Pawar, H.S., Mundt, J., Calandro, L.M., Furtado, M.R., & Shewale, J.G. (2009) Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. *Journal of Forensic Science, 54*(3), 599-607. doi: 10.1111/j.1556-4029.2009.01013.x
5. Broemeling, D.J., Pel, J., Gunn, D.C., Mai, L., Thompson, J.D., Poon, H., & Marziali, A. (2008). An instrument for automated purification of nucleic acids from contaminated forensic samples. *National Institute of Health, 13*(1), 40-48
6. Jiang, J., Alderisio, K.A., Singh, A., & Xiao, L. (2005). Development of procedures for direct extraction of cryptosporidium DNA from water concentrates and for relief of PCR inhibitors. *Applied and Environmental Microbiology, 71*(3), 1135-1141. doi:10.1128/AEM.71.3.1135–1141.2005
7. Kemp, B.M., Monroe, C. & Smith, D.G. (2006). Repeat silica extraction: A simple technique for the removal of PCR inhibitors from DNA extractions. *Journal of Archaeological Science, 33*, 1680-1689
8. Kermekchiev, M.B., Kirilova, L.I., Vail, E.E. & Barnes, W.M. (2009). Mutants of taq DNA polymerase resistant to PCR inhibitors allow DNA amplification from whole blood and crude soil samples. *Nucleic Acids Research, 37*(5), 1-14. doi:10.1093/nar/gkn1055
9. Kontanis, E.J. & Reed, F.A. (2006). Evaluation of real-time PCR amplification efficiencies to detect PCR inhibitors. *Journal of Forensic Science, 51*(4), 795-804. doi:10.1111/j.1556-4029.2006.00182.x
10. Kreader, C.A. (1996). Relief of amplification inhibition in PCR with bovine serum albumin or T4 Gene 32 protein. *Applied and Environmental Microbiology, 62*(3), 1102-1106
11. Lantz, P.G., Matsson, M., Wadstrom, T., & Radstrom, P. (1997). Removal of PCR inhibitors from human faecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. *Journal of Microbiological Methods, 28* (3), 159-167
12. Larkin, A. & Harbison, S.A. (1999). An improved method for STR analysis of bloodstained denim. *International Journal of Legal Medicine, 112,* 388-390
13. Mulero, J.J., Chang, C.W., Lagace, R.E., Wang, D.Y., Bas, J.L., McMahon, T.P., & Hennessy, L.K. (2008) Development and validation of the AmpF*l*STR MiniFiler PCR Amplification Kit: A miniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. *Journal of Forensic Science, 53*(4), 838-852. doi: 10.1111/j.1556-4029.2008.00760.x
14. Opel, K.L., Chung, D., & McCord, B.R. (2010). A study of PCR inhibition mechanisms using real time PCR. *Journal of Forensic Science, 55*(1), 25-33. doi: 10.1111/j.1556-4029.2009.01245.x
15. Stark, P.C., Mullen, K.I., Banton, K., Russotti, R., Soran, D. & Kuske, C.R. (2000). Pre-PCR DNA quantitation of soil and sediment samples: Method development and instrument design. *Soil Biology & Biochemistry, 32,* 1101-1110
16. Wilson, I.G. (1997). Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology, 63*(10), 3741-3751
17. www.appliedbiosystems.com
18. www.biomatrica.com
19. www.promega.com