OVERCOMING PCR INHIBITION USING MUTANT TAQ POLYMERASES

Dominique N. Cooper[1, 2], Zachary Goecker[2], Jesse Ramirez[2], Ryan Yee[2] and Steven B. Lee[2]
[1]Jackson State University
[2]San Jose State University Forensic Science, Justice Studies

PCR-based forensic tests may have false-negative results or low sensitivity caused by powerful PCR inhibitors found in biological samples[1][7][9]. In some cases for amplification of DNA to be successful, it is necessary that purification of target DNA be completed before PCR. In an attempt to overcome PCR inhibition, enhance DNA amplification, and minimize the various time and labor consuming pre-PCR procedures[3][7], the purpose of this study is to evaluate the effects of the inhibitor resistant mutant of Taq DNA polymerase, OmniTaq (DNA Polymerase Technologies St. Louis, Missouri), on amplifying DNA samples containing inhibitors. The hypothesis is that the use of inhibitor resistant Taq polymerases will result in enhanced amplification on inhibitor spiked samples.

Biological samples were obtained from volunteers, and DNA was extracted using a standard organic phase extraction method and then quantified using agarose gel electrophoresis and quantitative PCR (qPCR) using the Plexor HY kit (Promega Madison WI). The DNA samples were quantified using a serial dilution of known DNA standards. In a separate experiment, a DNA standard was quantified in the presence of OmniTaq polymerase. Previous studies using a 2ng standard DNA sample with OmniTaq suggested that the mutant Taq appeared to inhibit PCR[10]. The use of OmniTaq with the 2ng standard indicated that, contrary to prior results, it did not inhibit PCR and appeared to enhance amplification.
The inhibitors evaluated for this study are Hematin (1mg/ml) and Phenol:Chloroform:Isoamyl Alcohol (24:24:1 Fisher Scientific Cat. # BP153-400). Standards were spiked with increasing amounts of inhibitors [Hematin (1mg/ml): 0.1ul, 0.3ul, 0.4ul, 0.6ul and Phenol Chloroform [0.1ul, 0.2ul, 0.4ul, 0.8ul] and then amplified with and without OmniTaq using 0ul, 1.0ul, 2.0ul, 3.0ul, or 4.0ul.

OmniTaq (4.0ul) partially overcame inhibition on Phenol Chloroform (0.2ul) and Hematin (0.1ul) spiked DNA partially supporting our hypothesis. Although inhibition was alleviated, the OmniTaq concentrations utilized did not fully overcome inhibition.
Future planned experiments will include repeating qPCR experiments with higher concentrations of OmniTaq, evaluating the effect of OmniTaq on STR amplification of inhibitor spiked samples, and evaluating OmniTaq PCR amplfication on DNA spiked with additional inhibitors (Humic acid and Melanin titrations).
Acknowledgements: This research was supported by an NSF-REU grant #1262832 to Dr. Julio Soto and Dr. Miri VanHoven in Biology at SJSU as co-PIs and to Dr. Steven Lee in Forensic Science at SJSU as research mentor.

The authors would like to thank DNA Polymerase Technologies for donating the Omnitaq utilized in this study.

Alaeddini, Reza. "Forensic implications of PCR inhibition—a review." Forensic Science International: Genetics 6.3 (2012): 297-305.

Bickley J., Short J. K., McDowell G., Parkes H. C. (1996). Polymerase chain reaction (PCR) detection of listeria monocytogenes in diluted milk and reversal of PCR inhibition caused by calcium ions. Lett. Appl. Microbiol; 22: 153–158.

Kermekchiev M., Kirilova L., Vail E., Barnes W. (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.

Kermekchiev, M. (2011). Taq Mutants Engineered for Forensics

Larkin, A. & Harbison, S.A. (1999). An improved method for STR analysis of bloodstained denim. International Journal of Legal Medicine; 112: 388-390.

Moreira D.(1998). Efficient removal of PCR inhibitors using agarose-embedded DNA preparations. Nucleic Acids Res;26: 3309–3310.

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 Wiedbrauk, D. L., J. C. Werner, and A. M. Drevon. 1995. Inhibition of PCR by aqueous and vitreous fluids. J. Clin. Microbiol. 33:2643–2646.

Zhang, Z., Kermekchiev, M., & Barnes, W. (2010). Direct DNA amplification form crude clinical samples using a PCR enhancer cocktail and novel mutants of taq . Journal of Molecular Diagnostics; 12(2): 152-161.

Hillary Nguyen, Hanna Bennett, Phil Nhan, Steven B. Lee (2012) New Strategies for Overcoming PCR Inhibition Using Mutant Taq Polymerases and PCR Enhancers. Poster presented at 2012 ISHI - http://www.promega.com/~/media/files/resources/conference%20proceedings/ishi%2023/poster%20abstracts/51%20poster.pdf?la=en