NOVEL TAQ MUTANT ENZYMES AND PCR ENHANCERS DESIGNED FOR FORENSICS:
GENOTYPING WITHOUT DNA EXTRACTION

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Major problems with PCR-based forensic tests of samples containing blood, soil, or other inhibitory substances are false negative results and low sensitivity caused by such inhibitors. The effect of the PCR inhibitors is primarily associated with inactivation of Taq DNA polymerase. Therefore, various protocols and DNA extraction procedures are being used to purify DNA prior to PCR. These extra steps add to cost, are time-consuming, may not completely remove inhibitors, or may lead to losses of target DNA. As a novel alternative to these pre-PCR steps we have engineered mutants of Taq polymerase (OmniTaq and CesiumTaq) highly resistant to PCR inhibitors and possessing a built-in hot-start feature. We also developed novel PCR enhancer cocktails (PECs) which further improve the performance of the mutant enzymes in crude samples, and increase the specificity and sensitivity of DNA detection.

We present data on direct STR genotyping of human DNA from a variety of crude samples relevant to the forensic practice, including blood, soil, humic acid, semen, bile, indigo dye, tannins, cigarette butts, aged buccal swabs, soda can swabs, showing that our genetically engineered enzymes OmniTaq and CesiumTaq, supplemented with the PEC enhancers, can outperform two top commercial kits available today, AmpFlSTR Identifiler-Plus and PowerPlex-16 HS, generating complete allele profiles with 17 crude samples tested while skipping the DNA extraction. In comparison, out of the 17 crude samples, the PowerPlex 16HS kit generated 12 full and 5 partial profiles, and the Identifiler-Plus kit, with 14 of these 17 samples attempted, generated 6 full profiles, 2 partial profiles, and no profiles were generated in the other 6 samples. The mutant enzymes generate full STR profiles with blood on FTA cards, no matter if the blood was untreated or treated with the common anticoagulants EDTA, heparin, or citrate.

The tests described above were performed by combining our enzyme/enhancer/buffer master mixes with the PowerPlex-16HS primers, using the same cycling conditions, so these master mixes can be easily implemented in the forensic practice without any significant changes in the STR typing protocol.

Our novel inhibition-resistant enzyme/enhancer systems could eliminate, in many cases, the need to purify DNA prior to PCR, and reduce the cost, time and false negatives in forensic DNA testing.

Acknowledgments:
We thank Promega Corp. and Bode Technology for kindly providing the PowerPlex-16 HS kit lacking the enzyme, and various forensic specimens, respectively, which made this evaluation possible.
This work was supported by Grant # DN-BX-K299 from The National Institute of Justice.