

NOVEL INHIBITION-RESISTANT TAQ MUTANT ENZYMES AND PCR ENHANCERS DESIGNED FOR FORENSICS

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Major problem with PCR-based forensic tests of samples containing blood and soil are false negative results and low sensitivity caused by inhibitory substances. The effect of the main PCR inhibitors in such samples, hemoglobin and humic acid, 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 these pre-PCR steps we have recently engineered mutants of Taq polymerase (OmniTaq and Omni Klentaq) highly resistant to blood and soil inhibitors (Kermekchiev, M. et al., *Nucleic Acids Res.*, 2009 Apr; 37(5):e40. Epub). 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 showing that the mutant enzymes, combined with PEC, can amplify human targets, including STR markers from crude samples containing whole blood, soil, or combination of both, where plain Taq and AmpliTaq Gold fail to perform. Identical results are shown with dry blood spots, directly subjected to amplification without DNA extraction. The new enzymes also generate STR profiles from swab specimens without any DNA purification steps prior to amplification.

We also illustrate that OmniTaq and Omni Klentaq in the presence of PEC can outperform AmpliTaq Gold in amplifying nuclease treated partially hydrolyzed DNA. With damaged DNA, extracted from a forty year aged hair shaft, the novel enzymes can produce a STR profile with some spurious alleles, using the PowerPlex 16 kit, while no STR profile is produced with the AmpliTaq Gold enzyme. Development of Taq mutants / PEC combinations optimized for overcoming inhibition in other than blood and soil specimens is underway. The novel enzyme-enhancer systems could eliminate, in many cases, the need to purify DNA prior to PCR and speed-up, lower the cost and increase the efficiency of forensic DNA testing.

The work on this project is funded by an ongoing NIH Grant Award 2008-DN-BX-K299.