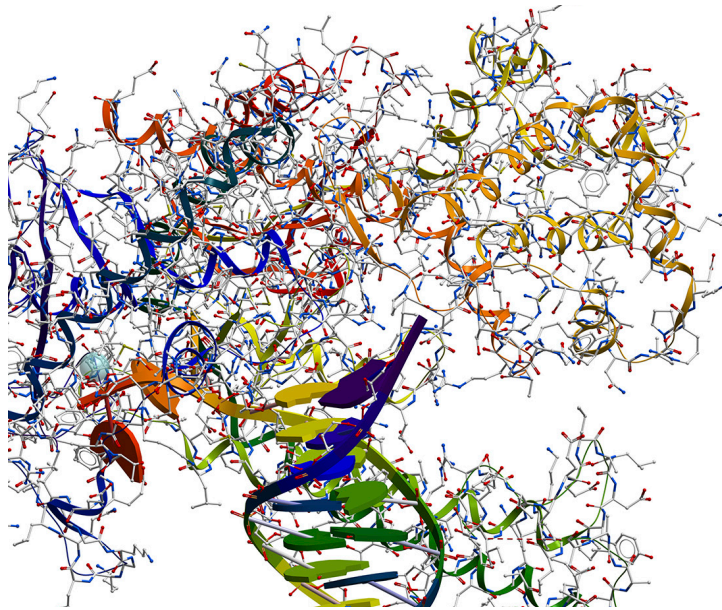




NIJ and DNA Polymerase Technologies

Addressing a Key Challenge for PCR-Based Forensic Tests

Moving Knowledge from Research to Impact



Synopsis of Problem and Solution

Polymerase Chain Reaction (PCR) inhibitors are present in blood, soil, and reagents, and represent a key challenge for PCR-based forensic tests, resulting in false negatives and poor sensitivity of results. Partial Short Tandem Repeat (STR) profiles obtained in the presence of PCR inhibitors appear similar to degraded DNA profiles, which is a major concern for downstream analysis and reporting results obtained from forensic samples. Thermofisher's AmpliTaq Gold®, a DNA polymerase enzyme that is widely used in forensic DNA testing, is sensitive to PCR inhibitors and can completely inhibit a PCR reaction in the presence of less than 0.1% whole blood. Therefore, current DNA typing protocols must be adapted to achieve a high degree of purity of the evidentiary sample. In general, DNA extraction procedures can be time-consuming or labor-intensive, can cause DNA loss, and may fail to completely remove common inhibitors. Additionally, multiple sample manipulations may increase the risk of cross-contamination. This NIJ funded research examined the effects of PCR inhibitors on the DNA polymerase enzyme from alternative *Thermus aquaticus* (Taq) mutants and explored an approach to eliminate the need to extract and purify DNA prior to PCR. Removing this step decreases the required time, lowers the cost, and increases the efficiency of forensic DNA typing.

"DNA Polymerase Technologies has done impressive work engineering Taq mutants and in determining the proper combination of PCR enhancer cocktails to assess STR profiles in challenging samples."

Robert Bever, Ph.D. Laboratory Director
Mitotyping Technologies, A Division of American
International Biotechnology

Benefits

The NIJ grants contributed to the development of novel Taq mutants:

- ▶ The combination of Taq mutants, optimized buffers, and PCR enhancer cocktails (PEC) enables the removal of the DNA extraction step usually required for amplifying DNA.
- ▶ The technology enables PCR in 40% blood (treated with anticoagulants), plasma, and serum.
- ▶ The technology is able to overcome other PCR inhibitors including urine, tannins, indigo dyes, melanin, feces/bile salts, sperm, soil/humic acid, plant extract, ethanol, GITC, milk, cheese, chocolate, seafood, meat, and water.

The Future

Currently available kits may contain robust DNA polymerases with enhancers to quell inhibition; however, these kits have only been shown to be reliable on buccal samples. DNA Polymerase Technologies PCR mixes and mutant taqs demonstrate the ability to directly amplify DNA obtained from challenging samples gathered from the field, including whole blood. A solution that is able to do this without a DNA extraction step would greatly simplify the identification process and remove the critiques currently used in the court room that eventually cause some DNA evidence to be dismissed.

NIJ-Funded Research

With NIJ grant support, DNA Polymerase Technologies developed novel, genetically-engineered mutants of Taq DNA polymerase that are highly resistant to Polymerase Chain Reaction (PCR) inhibitors. Additionally, DNA Polymerase Technologies optimized buffers and PECs to be compatible with the novel Taq polymerases. Protocols for STR genotyping of crude samples were designed to be compatible with the primers and cycling conditions of both PowerPlex® 16 HS (Promega) and AmpFLSTR® Identifiler® Plus (ThermoFisher), the two most commonly used kits in forensic DNA labs. The master mix provided with the kits were replaced with DNA Polymerase Technologies' master mix containing the alternative Taq mutants, along with an optimized buffer and PEC additions. In comparative tests of direct STR typing with challenging crude samples, the company's master mix outperformed comparable mixes from name brand suppliers.

Bringing Research to Practice

DNA Polymerase Technologies is working with InnoGenomics Technologies (www.innogenomics.com), a biotechnology company developing innovative genetic testing solutions that solve crimes and save lives. InnoGenomics has developed alternative DNA markers for identification as well as for diagnosis of diseases such as cancer and cardiovascular disease. The company's goal is to develop diagnostic tests that don't require extraction of DNA from blood or hair. The Taq mutants and PEC cocktails supplied by DNA Polymerase Technologies is enabling InnoGenomics to amplify cell-free DNA fragments that are in the blood or on hair (not DNA from the follicle) and to improve the sensitivity of their assays.



More Information

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