A84 Multiplex Amplification of Deletion/Insertion Polymorphisms: A New Kit on the Block

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The goal of this presentation is to detect deletion/insertion polymorphisms from evidence samples which have been subjected to various adverse environmental conditions. Nuclear DNA extracted from these compromised body fluids can often be degraded and low in amount. Extracted DNA samples may also contain substances that inhibit polymerase chain reaction (PCR) used for the amplification of genomic DNA. Short tandem repeats (STRs), due to their high discriminatory power, is currently the method of choice for human identification. However, detection of STR loci requires large fragments of nuclear DNA, and commercially available kits used to detect STR loci from forensic samples do not always yield complete profile from degraded or inhibited samples. This research discusses a combination of the extraction methods and short deletion/ insertion polymorphisms (DIPS or Indels) which can help analysts acquire additional information from body fluids different from that obtained with only the STR amplification kits.

This presentation will impact the forensic science community by introducing deletion and insertion polymorphisms that can be derived from degraded and inhibited evidence samples. Currently, forensic community uses STR profile from genomic DNA for identity. Addition of the deletion and insertion polymorphisms along with the sex determining locus, Amelogenin, would greatly enhance the capabilities of the scientists and help them obtain more genetic information from compromised samples.

Forensic scientists are constantly looking for ways to obtain complete DNA profiles from less than ideal biological samples. Some of the procedures to achieve this are to use different types of extraction techniques and more sensitive amplification kits. Saliva samples deposited on cigarette butts easily become less than ideal biological samples due to the environment where cigarette butts are often discarded during the commission of a crime. Temperature, humidity, and soil are only some of the factors which can hasten the process of degradation. Amplification of these types of samples may show allelic dropouts, peak imbalance, and low intensity peaks.

Tar and nicotine, some of the components of cigarettes themselves, can act as inhibitors. The concentrations of these ingredients can also adversely affect the amplification process. In addition, saliva samples deposited on cigarette butts that are exposed to detrimental environmental conditions may yield lower than optimal amounts of DNA, and the DNA in these extracts may be degraded and/or contain inhibitors.

One of the ways to improve DNA amplification when faced with degraded and inhibited samples is to change the size of the desired amplicons. The Investigator DIPplex kit takes advantage of short amplicons which are more likely to be amplified in degraded DNA samples. The short amplicons in this kit makes the DIPplex kit an ideal vehicle for analyzing degraded forensic samples such as body fluids deposited on cigarettes and then exposed to various adverse environmental conditions.

DNA from cigarettes butts, exposed to unfavorable environmental conditions, was extracted with various techniques and subjected to amplification using commercially available kits for detection of short tandem repeat polymorphisms. The same extracts were also amplified using the DIPplex kit in order to detect deletions/insertions in the genomic DNA.

Although the STR polymorphism and deletion/insertion polymorphisms cannot be directly compared, an assessment of the profiles can be easily made. It is concluded that the DIPplex kit is a highly sensitive and useful tool when amplifying degraded DNA samples. This assay can yield more identifying information from degraded samples than using only STR amplification. A combination of STR amplification and the deletion/insertion polymorphisms would give the forensic analysts more capabilities to analyze compromised samples.

STR, Insertion/Deletion, Polymorphisms

