A171 Detection of Deletion/Insertion Polymorphisms From Challenged Samples

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After attending this presentation, attendees will learn an alternate method of detecting polymorphism from environmentally insulted evidence samples.

This presentation will impact the forensic science community by allowing the scientist to generate insertion/deletion polymorphism profiles from samples where complete Short Tandem Repeat (STR) DNA profiles may not be possible to obtain.

Analysis of Short Tandem Repeats (STRs) is currently the most commonly used method for human identification. However, DNA extracted from evidence samples exposed to environmental insults does not always yield complete STR profiles. Light, humidity, elevated temperatures, and bacterial or fungal contaminants all degrade DNA, which in turn can lead to the loss of genetic information. Also, the efficiency of the PCR amplification process is reduced when inhibitors such as salts, heme in blood, indigo dye found in denim, phenolic compounds, melanin found in skin and hair, humic acid from soil, and collagen and calcium in bone are present in the extracted DNA. Degradation and inhibition can lead to loss of signal, peak imbalance, and allelic dropout with current STR technology. In situations where DNA is highly degraded, the molecule becomes fragmented and the chances of obtaining complete profiles are reduced. Typically, the larger amplicons are the first to fall below the detection limit. This problem has prompted research in the area of extraction and amplification methods to obtain complete DNA profiles from these types of compromised samples.

The Investigator DIPplex® Kit from Qiagen combines 30 insertions and deletions (InDels) markers as well as Amelogenin in a single PCR reaction. These unlinked markers are distributed across 19 chromosomes. One significant advantage of this kit is that stutter peaks commonly seen as artifacts of STR analysis do not appear in DIPplex® profiles. The PCR products generated by the primers are no larger than 150 base pairs. Due to the small amplicon size, the assay is highly sensitive and the manufacturer claims it can create a full DNA profile from as little as 63pg of DNA.

The current study focuses on the detection of insertion/deletion polymorphisms from challenged samples using the Investigator DIPplex® kit from Qiagen. Unlike the PCR amplification kits currently available in the forensic community that amplify 15 or more STR loci, the DIPplex® kit allows for multiplex amplification of 30 bi-allelic areas of known InDels plus the Amelogenin locus. This PCR amplification kit uses reduced amplicon sizes (maximum of 150bp), similar to SNPs, improving the amplification of degraded samples. The combination of current STR analysis procedures and small amplicon sizes makes InDels suitable for pristine as well as degraded DNA evidence samples.

This research included analyzing different types of body fluids from humans, some of which have also been subjected to environmental insults. Body fluids such as saliva, blood, semen, and nasal secretions, etc. from male and female human donors were analyzed to determine if they yield consistent profiles from the same donor. Another goal of this research was to assess the DIPplex® kit's capacity for samples that closely resemble forensic casework evidence. For the purpose of validating the kit, samples from various animals were also included to determine if the kit is species specific.

The experiments indicated that complete DIPplex® profiles can be obtained from degraded samples which yielded either partial or no STR profiles. These samples included washed bloodstains deposited on various types of fabrics, such as denim.

Reducing the primer concentration as well as the total reaction volume gave results comparable to profiles obtained when following the manufacturer's recommended protocol. Increasing the cycle number from the recommended protocol also improved the yield of profiles from DNA extracts which were degraded and below optimum quantity.

Direct amplification with 1.2mm blood and saliva punches from various substrates yielded complete profiles when using less than the recommended cycle number.

DNA extracted from several animals yielded either no profile or profiles dissimilar to humans. The results indicate that analysis of bi-allelic insertion/deletion polymorphisms can be useful in supplementing data obtained with STR profiles.

InDels, DNA Polymorphism, STR

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