B32 The Development of Polymorphic Combined DNA Index System (CODIS) Short Tandem Repeat (STR) Primers for Unbalanced DNA Mixture Analyses

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After attending this presentation, attendees will understand the advantages of using polymorphic STR primers for unbalanced DNA mixture analyses.

This presentation will impact the forensic science community by detailing the development of polymorphic CODIS STR primers and how these primers can be utilized to detect a minor contributor in a DNA mixture.

Currently, there are several methods available to help analysts assess mixed DNA profiles, including comparison of relative peak heights of STRs, Y-chromosomal Short Tandem Repeat (Y-STR) analysis, or the use of expert software systems; however, these methods have several limitations. For instance, it is often difficult to detect minor contributors in extremely unbalanced mixtures due to Polymerase Chain Reaction (PCR) amplification bias, Y-STR haplotypes being shared by paternal relatives, and the question of whether expert software system analysis violates defendants' rights (e.g., the Confrontation Clause).[1-3]

In the research presented, an alternative method for analyzing DNA mixtures that exploits polymorphic PCR primer sites was explored. Combining a Single Nucleotide Polymorphism (SNP) or an Insertion/Deletion (INDEL) polymorphism with an STR can create a tool suitable for analyzing mixtures when one component is in much lower quantity than the other. In this case, a conserved primer flanks one side of the STR, while the other primer is designed to anneal to a specific SNP or indel allele on the opposite side of the STR. This allows an analyst to specifically target one DNA component in a mixture depending on the polymorphism present. For example, if a major contributor is homozygous for a deletion at one locus, the primer set designed for the insertion would be used to target the minor contributor. Specifically targeting SNP or INDEL alleles absent in the major contributor overcomes the PCR amplification bias that otherwise occurs.[4]

Polymorphic primer sites near the expanded set of CODIS STR loci were identified using the University of California, Santa Cruz database genome browser and allele-specific primer pairs were designed using Primer3 web version 4.0.0.[5-7] PCR conditions were optimized for each primer set by varying cycle number, primer concentration, and annealing temperature using a thermal gradient thermocycler. DNA collected from volunteers was then isolated from buccal swabs, quantified, and amplified in singleplex reactions. When amplification was successful with just one allele-specific primer set for a given locus, the individual was classified as homozygous; successful amplification with both allele-specific primer sets for the same locus was classified as heterozygous. Results were confirmed by sequencing the SNP or INDEL locus.

After the DNAs were quantified and genotyped, mixtures at ratios ranging from 1:10 to 1:1000 were created. DNA from an individual homozygous for an SNP or INDEL associated with a marker was used as the major contributor with input quantities ranging from 1ng to 100ng, and DNA from an individual heterozygous or homozygous for the other SNP or INDEL allele was used as the minor contributor with input quantities as low as 20pg. Using this method resulted in detection of minor contributor alleles via gel electrophoresis at DNA mixture ratios of up to 1:1000. Primers were then labeled with fluorescent dyes for fragment analysis via capillary electrophoresis. Mixtures genotyped with the labeled primers were consistent with known minor contributor alleles.

In conclusion, the availability of polymorphic CODIS STR primers provides a valuable tool for forensic biologists. Successful amplification of the minor contributor will allow the STR genotype to be compared to a reference sample or database, and established allele frequencies for the STR loci can be used to calculate random match probabilities. The development of polymorphic primes will allow analysts to deconvolute and easily apply statistics to unbalanced DNA mixtures that may otherwise be uninformative.

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