B3 Verification of STR Alleles by Alternative Primer Pairs Through a Singleplex PCR System

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The Singapore National DNA Database Laboratory uses the ABI IdentifilerTM kit for DNA profiling of the 13 CODIS loci. Routinely, researchers encounter allele ambiguities such as off-ladder range alleles, microvariant alleles, allele peak imbalances and tri-alleles among the different DNA loci typed. In order to determine whether these are true alleles or PCR artifacts, alternative primers of the questioned DNA locus are used to confirm the alleles typed. An allele is automatically assigned by its DNA locus specific Genotyper™ macro. Using the FGA locus as an example, the singleplex PCR of the questioned DNA locus approach is described here.

This presentation will impact the forensic community and/or humanity by demonstrating how the systematic singleplex PCR approach with automated allele assignment method will allow routine confirmation and resolution of STR allele ambiguities encountered during routine DNA profiling in the laboratory.

PCR artifacts such as stutter products and non-template nucleotide additions and other factors such as microvariants, allele dropout, allele imbalance and mutations can arise that may interfere with the clear interpretation and genotyping of the alleles present in the DNA template.

In the National DNA Database Laboratory, the Identifiler™ DNA typing kit is used in routine DNA profiling. The DNA is extracted and amplified from blood stained FTA™ collection cards of convicted offenders. Duplicate DNA typing is performed for each sample and results are compared for consistency and quality before uploading into the CODIS system. On some occasions, heterozygote allele peak imbalances, three banded allele patterns, off-ladder range alleles, microvariant alleles and spurious PCR artifacts are encountered. In order to resolve interpretation difficulties and allow genotyping of the correct STR alleles present in the DNA template, alternative fluorescent (6-FAM) labelled primer pairs using Promega® Powerplex™ 16 or mini STR primer (Butler *et al.* 2003) sequences of each DNA locus are obtained. For each DNA locus, amplifying the alleles obtained from either the diluted Identifiler™ or Powerplex™ 16 allelic ladders as template creates the allelic ladder. A Genotyper™ macro is then written for automated allele assignment based on the amplified ladders for each DNA locus. The questioned DNA samples are then amplified using the DNA locus-specific singleplex PCR primers. Alleles are assigned on the PCR products using its DNA locusspecific Genotyper™ macro. The identity of the PCR products is verified as either as PCR artifacts or as true allele amplified from the DNA templates.

An example of this approach using the FGA locus is discussed here. The Powerplex™ 16 primers for the FGA locus are used. Out of the 70 blood samples with allele peak ambiguities observed using the Identifiler™ DNA typing kit, 29 samples were confirmed to be off-ladder range alleles, 30 samples with peak imbalances were corrected and 10 tri-alleles were verified. The corrected imbalanced peak indicated point mutations at the primer binding position of the Identifiler™ FGA primers. Off-ladder range alleles were confirmed to be alleles of the FGA locus. The remaining sample with an imbalance peak typed using the Identifiler™ DNA typing kit is likely to be a tri-allele pattern as the imbalance peak was consistent with the singleplex PCR result. The tri-allele should consist of a homozygote allele peak with an imbalanced third allele.

In conclusion, this singleplex PCR approach with automated allele assignment allows routine confirmation and resolution of STR allele ambiguities encountered during routine DNA profiling in the laboratory.

STR, Peak Imbalance, PCR

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