B67 SNPs by MIPS - SNP Typing Using Molecular Inversion Probes (MIPS)

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The goal of this presentation is to discuss a method to improve the multiplexing capacity for forensic SNP analysis. Attendees will be presented with a convenient method based on molecular inversion probes.

This presentation will impact the forensic science community by presenting a convenient method based on molecular inversion probes.

Background and purpose: Routine forensic DNA analysis is commonly performed using STR markers. However, when the DNA in the samples is fragmented, the routine analysis might fail due to the requirement for amplification of quite large fragments in the PCR. SNP based DNA typing, on the other hand, has the potential to be highly sensitive as the PCR products can be kept very short. Therefore, SNP testing holds promise for future analysis of degraded and compromised samples. However, SNP typing has a limitation in the multiplexing capacity of multiple targets in the PCR step. In this study the possibility of using molecular inversions probes (MIPS or Padlock probes) for forensic SNP typing was investigated. MIPS are linear oligonucleotides, 70-100 nt in length, with end-segments that are complementary to a target sequence. The allele specific nucleotide is located at the 3' end of the probe and hybridization to target sequence leads to circularisation of the probe by DNA ligase. Using two allele-specific MIPS for each locus provides a robust distinction between SNP variants. After hybridisation and ligation to perfectly matched targets the probe is cut and universal PCR-primers can be used to amplify and analyse a large number of targets in parallel.

Methods: In this initial study a total 20 SNPs were selected from http://www.cstl.nist.gov/div831/strbase/SNP.htm. At first, SNP detection was done by Pyrosequencing, but the assay will be transferred to an array platform in the future for easy and fast SNP detection.

Results and conclusions: SNP detection by Pyrosequencing gave correct genotyping. However, further optimization of the protocol is required as well as sensitivity tests. The high multiplexing capacity of MIPS make them a suitable for large scale SNP genotyping, but for use on forensic materials further evaluation is required.

DNA Analysis, Multiplax Analysis, Molecular Inversion Probes

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