B55 Analysis of Nuclear STR Markers Using Pyrosequencing Technology

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This presentation will present a new rapid, reliable and robust technology for DNA identification in forensics.

DNA analyses in forensic investigations have made a great contribution to the possibility to convict a perpetrator or free an innocent suspect. With the help of rapid developments in molecular technology new methods are becoming faster and more sensitive to allow effective and accurate DNA analyses to aid criminal investigations.

Forensic analysis has traditionally been performed using nuclear STRs (short tandem repeats) due to the high number of alleles at each locus. The genetic diversity of STRs makes these markers highly discriminating and suitable for individual identification in forensic investigations. DNA in forensic samples as well as in ancient DNA, however, is often subjected to a harsh environment of degrading agents, which might affect the ability to amplify longer fragments of nuclear DNA. Therefore, we selected loci harbouring the shortest repeat units and designed a PCR assay to amplify short fragments between 70-200 base pairs. Another advantage is that the STRs were selected from the STRBase web site, which represents markers that have been extensively investigated for their usage in forensic applications with well-known allele frequencies from different populations. The use of these markers can thereby easily be transformed to new technology platforms remaining their large informative property.

Although fragment analysis commonly used for nuclear DNA analysis is well established and reliable, it also requires larger fragment sizes that may be difficult to amplify on materials of poor quality and limited amounts. In this study we have used Pyrosequencing to analyse 11 different STRs (CSF1PO, TH01, TPOX, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539 and Penta D). The Pyrosequencing technology is based on an indirect luminescent detection of organic pyrophosphate (PPi) released from each dNTP upon DNA chain elongation. The four nucleotides are added stepwise and following base incorporation, PPi is released and used as a substrate for ATP sulfurase, which results in formation of ATP. The enzyme Luciferase uses ATP to convert luciferin to its oxi-derative, which creates a light output detectable by a CCD camera. Prior to each cyclic addition of nucleotides, the excess of dNTPs is degraded by apyrase. The results are shown in a pyrogram where each peak height is proportional to the number of incorporated nucleotides.

This new technology provides a faster turnaround time of the analysis and is highly robust. The post-PCR handling takes about 2 hours and represents a flexible platform for different types of analysis such as; mutation detection, sequencing of short fragments, SNP and STR analysis. The pyrosequencing method also generates a more easily interpreted and reliable scoring of simple and complex repeats due to the higher resolution obtained by actual sequence analysis of the repeat and absence of common gel artefacts like stutter bands. As the peak heights are proportional to the incorporated nucleotides, the decrease in signal intensity by half and the specific pattern that arises after the end of the repeat makes it possible to resolve different alleles of a heterozygous genotype.

In this study we describe the first system for analysis of STRs using Pyrosequencing. Eleven STR loci were analyzed among 100 Swedish individuals to generate allele frequencies for the Swedish population. The system has also been tested for analysis on several forensic materials from previously analyzed forensic case works.

DNA, STR, Pyrosequencing

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