SENSITIVE ANALYSIS OF STR AND SNP MARKERS USING THE PYROSEQUENCING TECHNOLOGY

DNA from casework samples and ancient materials are in many cases degraded and difficult to analyze. Amplification of mtDNA or short nuclear fragments is often necessary for a successful analysis of degraded samples. We have developed several sensitive, rapid, flexible and easy-to-use SNP and STR typing systems based on the Pyrosequencing technology.

This is a non-electrophoretic, single-tube sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light. For severely degraded DNA samples a mtDNA analysis system has been developed. Two PCR fragments covering the HVI and HVII regions in the d-loop are analyzed rapidly in eight pyrosequencing reactions. In addition, 16 fragments in the coding region of the mitochondrial genome are used for additional discrimination. Each fragment covers multiple polymorphic SNPs and the average read length in the pyrosequencing reactions is 45-75 nucleotides.

When nuclear DNA can be isolated from degraded or limited samples, SNP analysis is likely to be most successful as the amplicons can be designed very short. A system for analysis of 19 previously reported SNP markers on the Y-chromosome has been developed. The PCR products are between 48 and 96 base pairs and the most informative markers have been optimized to allow analysis in triplex PCR and pyrosequencing reactions. If a nuclear DNA analysis is permitted on degraded or limited samples, obviously a STR analysis will be preferred due to the large number of alleles at each locus.

As a complement to the routinely used STR assays, pyrosequencing-based systems of short PCR fragments covering only a few bases outside the actual repeat unit, have been developed. Eleven widely used autosomal STR loci harboring short repeat units have been analyzed using pyrosequencing. The PCR fragments are spanning between 70-200 base pairs. 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. Furthermore, eight commonly used Y-STR markers have been analyzed in fragments between 80 bp and 227 bp. In order to save valuable material multiplex PCR and pyrosequencing reactions are under development. Since the actual sequence is determined rather than the repeat length in this assay there is a possibility to achieve additional information such as the nature of a mutational event. We have analyzed genetic variation in both the nuclear and mitochondrial genome using the pyrosequencing technique.

Pyrosequencing is a robust and flexible system that can handle SNP analysis, STR analysis or sequencing of short stretches of DNA with two hours post-PCR handling. The use of short fragments containing SNP or STR markers will improve the possibility to amplify and analyze degraded DNA in casework analysis or ancient DNA studies. The developed systems have been evaluated for the allele frequencies in the Swedish population and tested on several forensic materials from non-probative cases as well as on medieval bone specimens.