B97 Mitochondrial Coding and D-Loop Analysis Using Pyrosequencing

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This presentation will describe a fast analysis tool for mtDNA analysis including extended information retrieved by analysis of coding regions in the mitochondrial genome.

In forensic analysis, mitochondrial DNA is often used when the evidence material contains limited amounts of nuclear DNA. We have developed a novel, rapid and easy to use typing system for analysis of mitochondrial DNA based on the Pyrosequencing technology. This is a rapid, non-electrophoretic, single-tube sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light. The pyrosequencing system was first developed for analysis of the highly polymorphic mitochondrial D-loop and a database of D-loop sequences from 200 randomly selected blood donors has been compiled. However, a more discriminatory mitochondrial DNA analysis would be preferable, especially in cases where none or few differences in comparison to the Cambridge reference sequence are found between different individuals. Therefore, the system has been extended to also include analysis of short fragments in the coding region.

The coding region fragments were chosen, based on the diversity determined in 26 Swedish as well as 52 European whole mitochondrial sequenced individuals, and cover the most informative polymorphic sites throughout the entire mitochondrial genome. A database over coding region variation in 150 individuals has been compiled for the 19 different coding region fragments that were designed and optimised for pyrosequencing analysis. After the initial database analysis samples with none or one difference from the Cambridge reference sequence in the Dloop were studied in more detail. In order to achieve a better discrimination than D-loop analysis alone between these individuals, 8 of the 19 coding sequence fragments were chosen as a part of the complete typing system. The fully developed typing system, for an optimal discrimination, consists of 18 PCR fragments and 24 pyrosequencing reactions: 8 D-loop reactions, 8 haplogroup determining reactions and 8 reactions analysing additional SNPs for a possibility to distinguish between individuals identical in the D-loop sequence.

The pyrosequencing D-loop typing system has been evaluated in analysis of 50 previously sequenced forensic casework evidence materials. All pyrosequencing results were identical to the Sanger sequences with an average read length of 45-100 nucleotides. The coding fragment analysis has shown a capability to reduce the number of not distinguishable D-loop analysed samples (due to only one or no differences) by as much as 80%. Moreover, coding region analysis has been used successfully in a forensic case from 1952 to exclude a suspect exhibiting only one D-loop difference in comparison with the evidence material. In the future, this system, for analysis of the most informative parts of the whole mitochondrial genome, will be more informative and faster, than the D-loop sequence analysis used routinely today.

mtDNA, Pyrosequencing, Coding mtDNA

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