B140 Detection of STR and SNP Markers on the Y Chromosome Using the Pyrosequencing Technology

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After attending this presentation, the attendees will learn about the principles of the Pyrosequencing technology and the possibility to detect variations found on the Y-chromosome by using this flexible and rapid method.This typing system is based on wellknown Y-SNPs and Y-STRs that can be used in analysis of DNA found in degraded forensic or ancient samples.

This typing system is based on a new technology which will improve the analysis of short DNA fragments that are found in ancient samples and in forensic casework materials. This technology detects variations found in the genome and is very reliable and roubust.

Analysis of DNA sequence variation on the Y-chromosome is a useful tool in forensic casework analysis, especially in cases of sexual assault where mixtures of female and male DNA are found. Moreover, analysis of Y-chromosome markers is valuable in studies of paternal relationships. The routinely used Y-STR assay, which is based on determination of size after fragment separation, is reliable and robust but will require analysis of large fragments if the assay is multiplexed in order to analyze multiple markers simultaneously. To be able to analyze ancient DNA and forensic casework samples that have been exposed to severe environments resulting in degradation, amplification of short fragments is often necessary. We have developed a system based on the pyrosequencing technology (SQA analysis), which allow sequence analysis of short stretches of DNA to detect SNP and STR variants found on the Y-chromosome.

Pyrosequencing is a non-electrophoretic, single-tube sequencingby-synthesis method, which is rapid in comparison to many other technologies used for SNP and STR analysis. The pyrosequencing assay is flexible, easy to perform and will reveal the sequence of the repeats in the STR rather than the length. Since the actual sequence is determined in this assay additional information such as the nature of a mutational event can be achieved. Moreover, the PCR fragments can be designed to be very short, covering only a few bases outside the actual repeat unit, to ensure a highly sensitive assay.

In this study, we have designed a typing system based on wellknown Y-STR and Y-SNP markers to be analyzed by pyrosequencing. A major advantage with commonly used markers is that allele frequency information from many different populations is available. A first set of STR markers, DYS 392, DYS389-II, DYS438 and DYS 390 has been evaluated. The PCR products for these markers yield fragments between 80 and 227 nucleotides. The repeat lengths for a number of male samples were easily interpreted in the pyrosequencing analysis and all products

were male specific. A second set of markers, DYS19, DYS391, DYS389-I and DYS393 is undergoing further development for pyrosequence analysis. The system also consists of 19 previously reported SNP markers on the Y-chromosome, which are selected to be highly informative in Scandinavians. The primers for each fragment were designed to be as short as possible, resulting in PCR products between 48 and 96 nucleotides. Specificity of the primers was first confirmed by evaluation in singleplex PCR reactions, followed by analysis of the primers in multiplex PCR reactions to save valuable material.

In addition to the multiplexed PCR, time and reagents can be saved by multiplex pyrosequencing analysis. A first combination of the STR markers DYS392 and DYS438 have been analyzed simultaneously and the repeat length of both markers can easily be inferred in the pyrogram. The SNP markers have also been developed for performance of multiplex pyrosequencing. The SNP positions studied so far in these multiplexes were successfully interpreted and scored for the different polymorphisms.

In this study we have analyzed variations found on the Y-chromosome using the pyrosequencing technique. The use of short fragments containing Y-SNP and Y-STR markers in this system will improve the possibility to amplify and analyze degraded DNA in casework analysis or ancient DNA studies. When fully developed analysis of teeth from remains in a family grave, which were found 1915 in the church of Varnhem, will be performed. These are the disputed remains of the founder of Stockholm, Birger Jarl (1205-1266) and his son. Analysis of Y-chromosome SNP and STR variation might support a paternal relationship between the two individuals in this case.

Y-STR, Y-SNP, Pyrosequencing Technology

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