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STR sequence variants revealed by Pyrosequencing technology

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Abstract. Pyrosequencing is a fast, real-time, non-electrophoretic sequencing-by-synthesis method that can be used to genotype short tandem repeat (STRs) markers. In this study, a total of 18 Y-chromosome and autosomal STRs have been successfully analyzed using Pyrosequencing technology. Several sequence variants were detected, demonstrating that additional information besides the fragment length can be provided in a forensic DNA investigation by these assays.

Keywords: STR; Pyrosequencing; Sequence variant

1. Introduction

Short tandem repeats (STRs) are routinely used in forensic DNA analysis due to the high variability at each locus. Analysis of multiple loci results in a high discrimination power and thereby provides a very useful tool for individual identification [1]. The forensic community have evaluated and validated core sets of STR markers for autosomal as well as Y chromosome analysis and there are several commercial kits available [2–5]. However, in order to permit electrophoretic length separation of multiple markers, these kits require some PCR fragments of larger size. This can create difficulties in analysis of materials that are degraded or in limited amounts.

Pyrosequencing is a fast, non-electrophoretic, sequencing-by-synthesis method, based on a cascade of enzymatic reactions to monitor DNA synthesis. Nucleotide incorporation results in release of pyrophosphate (PPi) and production of detectable light. The produced light is proportional to the number of incorporated nucleotides and shown as peaks in a

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pyrogram [6,7]. The dispensation of nucleotides can either be cyclic or directed, i.e. pre-programmed according to a known sequence. In autosomal STR analysis, the two alleles in a genotype can be distinguished by a directed dispensation order and the use of a termination–recognition base (TRB). The TRB is usually the first occurring downstream base that is not a part of the repeat unit. Termination of the shortest allele in heterozygous genotypes will result in a reduction of the signal by half, while no signal reduction is seen in homozygous genotypes. In Y-chromosome STR (Y-STR) analysis, the first occurring nucleotide in the downstream target sequence that is not dispensed will terminate the reaction and result in a unique Pyrosequencing pattern allowing determination of the repeat length.

In this study, we have developed a system based on the Pyrosequencing technology to analyse autosomal- and Y-STRs in short PCR fragments to facilitate degraded DNA analysis. As the actual sequence is determined in addition to the fragment length, sequence variants within or near the repeat structure can not only yield additional information about a marker but also increase the discrimination capacity in a forensic DNA analysis.

2. Material and methods

Ten autosomal STR markers (CSF1PO, THO1, TPOX, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539 and Penta E) and eight Y-STR markers (DYS19, DYS389 I–II, DYS390, DYS391, DYS392, DYS393 and DYS438) were analyzed in control samples (Swedish blood donors) using Pyrosequencing. PCR primer pairs generating fragment lengths ranging between 66 and 233 bp, were designed using the Primer Express Software (Applied Biosystems, Foster City, CA, US). For conformation of autosomal STR genotypes, electrophoretic size separation using fluorescently labelled PCR primers was performed. All detected sequence variants were confirmed by Sanger sequencing.

3. Results

3.1. Autosomal STR sequence variants

Allelic variants were detected in four autosomal STRs and four Y-STRs using the Pyrosequencing technology. At the THO1 locus the incomplete allele 9.3 was detected in 61 genotypes of which 19 were homozygous for the variant. At the D5S818 locus a four base pair deletion, involving a CTCT motif next to the first ATCT repeat unit was observed in 45/114 genotypes (Fig. 1). A C/T SNP was observed at the 9th nucleotide upstream of the repeat at the D7S820 locus, (***C/***

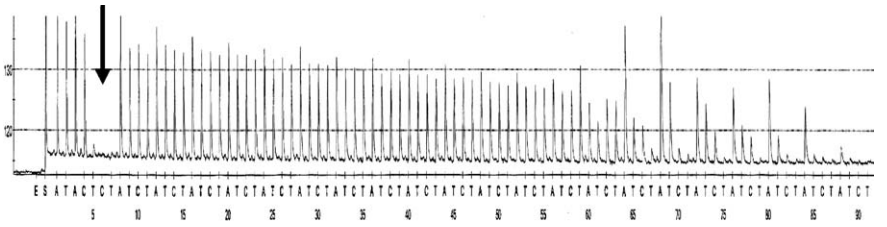

Fig. 1. Pyrosequencing results of a homozygous sample for the four base pair deletion at the D5S818 locus (ATCT).

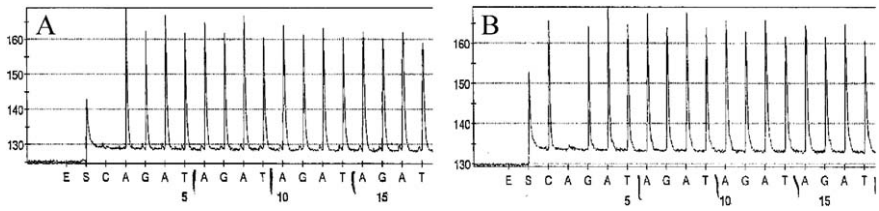

Fig. 2. Pyrograms showing the A/C SNP found at the DYS393 locus. A) Demonstrates an A at the SNP position (wild type). B) The pyrogram shows the C that is converting the repeat to CGAT(AGAT)$_n$.

TGAACTAAC[GATA]$_n$), in 9/114 genotypes. At the D8S1179 locus a variant allele, due to a G/C SNP, resulted in the altered repeat structure (TCTA)(TCTG)(TGTA)(TCTA)$_{11}$, in 4/114 genotypes.

3.2. Y-STR sequence variants

At the Y chromosome marker DYS391 locus a G/A SNP was observed one nucleotide upstream of the repeat, resulting in TCTG instead of TCTA. A G/A SNP was also observed at the DYS390 locus one nucleotide upstream of the repeat. At both these loci only one individual (out of 70) displayed the variation. In 10/70 individuals an A/C SNP was observed at the DYS393 locus in the first repeat unit, converting the repeat structure from (AGAT) to (CGAT). This variant was only seen in individuals with a total of 13 repeats (Fig. 2). Finally, at the DYS389II locus an A/G SNP resulted in (TCTG)$_6$ instead of (TCTG)$_{4–5}$ in one individual.

4. Discussion

In this study, we have investigated the use of the Pyrosequencing technology for STR analysis as a complement to the routine used fragment analysis. 18 STR markers have been analysed successfully and several sequence variants were detected. Most variants will not be detected by fragment analysis, demonstrating the possibility to increase the resolution using this technology. Thus, Pyrosequencing is a useful tool for rapid compilation of population databases, for detection of new or known allelic variants as well as for forensic analysis of a small set of STR markers with short amplicon lengths in degraded samples.

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