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Comparison of paternity indices based on typing of 15 STRs, 7 VNTRs and 52 SNPs in 50 Danish mother–child–father trios

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Abstract. Fifty Danish paternity cases from the year 2004 were selected based on the results obtained with the AmpF/STR® Identifiler® PCR amplification kit. In all cases, the calculated paternity index (PI) was higher than 10,000, and there was not observed any genetic inconsistency between mother and child, or between father and child. DNA from the selected trios was used to type 7 VNTRs (D2S44, D5S43, D5S110, D7S21, D7S22, D12S11 and D16S309) with the RFLP technique and 52 SNPs using a PCR multiplex with 52 PCR primer pairs and two SBE multiplexes with 23 and 29 SBE primers, respectively. PIs were calculated based on each set of loci (STRs, VNTRs and SNPs). On average, the typical PI obtained from analyses of 15 STRs was 4 times higher than the typical PI obtained from analyses of 7 VNTRs, and 17 times higher than the typical PI obtained from analyses of 52 SNPs. However, in only 3 trios analyzed for SNPs and 1 trio analyzed for VNTRs, PI < 10,000. No genetic inconsistency was detected between mother and child or between father and child in the SNP-analyses. In contrast, one father–child and one mother–child genetic inconsistency were observed in the VNTR-analyses.

Keywords: Paternity case work; Short tandem repeat; Variable number tandem repeat; Single nucleotide polymorphism

1. Introduction

At the Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, 15 STRs are analyzed in all paternity cases. If PI < 10,000 or one or two

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genetic inconsistencies are detected between the tested man and the child, or between mother and child, additional testing of 7 VNTR loci is performed. VNTRs are analyzed by endonuclease digestion and Southern blot techniques that are both time-consuming and require large amounts of sample material compared to PCR based methods. The purpose of this work was to investigate whether the 52-SNP-plex developed by the SNP*for*ID consortium [1] would be a suitable replacement for the 7 VNTRs in paternity case works.

2. Materials and methods

Fifty Danish paternity cases from 2004 were selected using the following criteria: (1) PI > 10,000 based on the results from the STR-analysis, and (2) no genetic inconsistency in the STR-analysis. The work presented here was approved by the Danish Ethical Committee (KF-01-037/03). DNA was purified from blood by chelex extraction or by using the FlexiGene DNA kit (Qiagen).

Fifteen autosomal STR markers were typed using the AmpF*l*STR® Identifiler® PCR amplification kit (Applied Biosystems) and the PCR products were analyzed in an ABI Prisms® 3100 Genetic Analyzer (Applied Biosystems).

For VNTR-analysis, DNA was digested with 1 μl 40 U/μl *Hin*fI (Roche) and quantified using Hoechst 33258 (Polysciences Inc.). A total of 1 μg digested DNA was separated in 1% 20 cm agarose gel by electrophoresis in 1× TBE buffer for at least 16 hours. The NICE DNA Sizing Ladder (Orchid) was used as size marker. Standard conditions for Southern blotting and hybridisation were applied [2]. Hybridisation was performed using CDP-coupled single locus DNA probes (Cellmark Diagnostics). After wash, the nylon membranes were incubated for 10 min at room temperature with CDP-star-substrate as recommended by the manufacturer (Cellmark Diagnostics). X-ray films were analyzed using a digitizing pad and DNAVIEW 22.17.

The 52-SNP-plex PCR and single base extension (SBE) reactions were performed as described [1] and the SBE products were analyzed in an ABI Prisms® 3100 Genetic Analyzer.

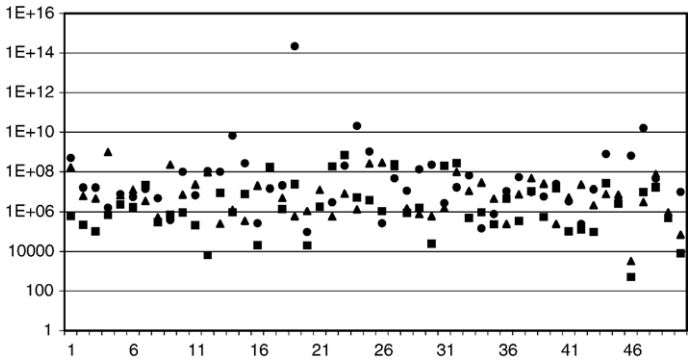

Fig. 1. PIs calculated in 50 Danish trios based on the analysis of 15 STRs (circles), 7 VNTRs (triangles), and 52 SNPs (squares).

Table 1
Estimated number of hours used for analysis

	Hours of hands-on labour and analysis of data		Hours of incubation and instrument runs	
	Per batch	Per sample	Per batch	Per sample
STR	5.75	0.07	8.25	0.09
VNTR	19	0.32	147	2.45
SNP	9	0.10	9	0.10

One batch of samples consisted of: STR: 88 samples, VNTR: 60 samples, SNP: 94 samples.

Paternity indices (PI) were calculated using DNAVIEW 27.21 [3] and in-house allele frequency databases.

3. Results

The PIs obtained from the STR-, VNTR-and SNP-analyses of the 50 trios are shown in Fig. 1. No genetic inconsistency was detected in the SNP-analyses. In contrast, one father–child and one mother–child genetic inconsistency were observed in the VNTR-analyses.

In Table 1 an overview of the estimated time used to perform the laboratory work and the data analysis for each set of loci are shown.

4. Discussion

Genetic inconsistencies in true mother–child–father trios were expected to occur with relatively high frequency (paternal; 15 STRs: 0.026, 7 VNTRs: 0.019; maternal; 15 STRs: 0.006, 7 VNTRs: 0.008) because of meiotic mutations occurring with high frequency in tandem repeats, and the two genetic inconsistencies observed in the VNTR-analyses were in accordance with the expected number. SNP loci mutate much less frequently, and therefore SNPs were considered ideal for additional testing in paternity cases where genetic inconsistencies were detected in the standard STR-analysis. The typical PI obtained by analyzing 52 SNPs was satisfactory even though PI < 10,000 in 3 out of 50 trios because, in practical casework, this index will always be multiplied with that obtained from the analysis of the 15 STRs.

Based on the data in Table 1, we estimated that duplicate analyses of 150 samples for 15 STRs, 7 VNTR, and 52 SNPs required 6, 50 and 8 workdays, respectively. Furthermore, estimations of the laboratory expenses, not including wages, for analysis of each set of loci showed that VNTR-analysis was approximately 8 times more costly than the STR- and SNP-analyses. Thus, in addition to being a more suitable marker set for additional testing in paternity cases, SNP-analysis is also both faster and cheaper.

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