The SNPforID Assay as a Supplementary Method in Kinship and Trace Analysis

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Keywords

SNP · Kinship · Snapshot · Multiplex PCR ·
Paternity testing

Summary

Objective: Short tandem repeat (STR) analysis using commercial multiplex PCR kits is the method of choice for kinship testing and trace analysis. However, under certain circumstances (deficiency testing, mutations, minute DNA amounts), STRs alone may not suffice. **Methods:** We present a 50-plex single nucleotide polymorphism (SNP) assay based on the SNPs chosen by the SNPforID consortium as an additional method for paternity and for trace analysis. The new assay was applied to selected routine paternity and trace cases from our laboratory. **Results and Conclusions:** Our investigation shows that the new SNP multiplex assay is a valuable method to supplement STR analysis, and is a powerful means to solve complicated genetic analyses.

Schlüsselwörter

SNP · Vaterschaft · Snapshot · Multiplex PCR ·
Vaterschaftstestung

Zusammenfassung

Hintergrund: In der modernen Vaterschafts- und Spurenanalytik ist die Untersuchung sogenannter Short Tandem Repeats (STRs) heute das Mittel der Wahl. In bestimmten Fällen (Defizienzen, Mutationen in den STR-Systemen, sehr geringe DNA-Mengen) kann die STR-Analyse allein unter Umständen zur Klärung nicht ausreichen. **Methoden:** Wir stellen als zusätzliche Methode einen von uns auf Basis der vom SNPforID-Konsortium vorgeschlagenen Single Nucleotide Polymorphisms (SNPs) entwickelten, 50 SNPs umfassenden Assay vor. Dieser neue Multiplex-Assay wurde in verschiedenen Routinefällen angewandt. **Ergebnisse und Schlussfolgerungen:** Unsere Untersuchungen zeigen, dass die SNP-Analyse eine wertvolle Ergänzung der STR-Untersuchung darstellt und geeignet ist, zur Lösung komplizierter Spuren- und Vaterschaftsfälle eingesetzt zu werden.

Introduction

Beginning in the mid-1980s, DNA analysis revolutionized routine forensic work such as kinship or trace investigations [1, 2]. Shortly after genetic fingerprinting by restriction fragment length polymorphisms analysis (RFLP) was established, the polymerase chain reaction (PCR) was presented as a method for artificially amplifying specific DNA fragments [3]. With this method individual specific polymorphic short tandem repeats (STRs) could be detected. This enabled the analysis of even

minute amounts of biological material, such as DNA from old or degraded skeletal specimens [4–7], putrefied tissues [8, 9], burned remains [10, 11] or forensically relevant traces, e.g. single hairs [12] or swabs from touched objects [13, 14].

Today, STR analysis is also a powerful tool for kinship analysis [15, 16]. Modern multiplex PCR kits allowing the amplification of 15 or 16 STRs simultaneously in 1 reaction [17, 18] usually provide the genetic information necessary for resolving most questions frequently encountered in paternity and relationship cases. According to the German guidelines,

the investigation of paternity cases by STR analysis alone is allowed as long as 12 genetic markers or their products are investigated [19]. Consequently, commercially available STR typing kits have become the preferred tool in kinship analysis and relationship testing, making paternity analysis much faster and also cheaper. Time consuming RFLP testing on the other hand has disappeared from most routine labs, leaving most expert witnesses without a second method even though a second system is recommended by German guidelines [20].

The sole application of STR analysis in paternity testing can be problematic in various ways, and might lead to ambiguous results: i) due to a low probability of relationship or when an exclusion of fatherhood is suggested by just 1 or 2 STR loci, e.g. when (unknowingly) testing close relatives of the alleged father; ii) in cases where only 1 of the alleged parents is available for testing (so-called deficiency cases), and STR results provide insufficient information for an exclusion; iii) because of the relatively frequently occurring germ-line step mutations in STR loci (addition or the diminution of 1 or 2 repeats; mutation rate between 0 and 7×10^{-3} according to [21]) that cannot be distinguished from exclusions. Most laboratories supplement such cases with additional autosomal STR markers, alternative primer sets for STR systems that showed ambiguous results or, if applicable, Y chromosome STR typing. This can, however, lead to additional problems such as mutations in primer binding sites or additional ambiguous results, further hampering the resolution of these cases.

Single nucleotide polymorphisms (SNPs) have been extensively investigated in a forensic context in recent years. The use of SNPs in paternity testing has been described by Børsting et al. [22, 23]. SNPs rely on short amplicons (usually less than 100 bp), display a much lower mutation rate than STRs (approximately 10^{-8} [22]) and can be easily detected using the same laboratory equipment used for STR analysis. Since SNPs are much less polymorphic than STRs, the investigation of a high number of loci is necessary: approximately 4.2 SNPs with allele frequencies of 0.5 give the same power of exclusion as 1 STR locus [24], and 50 SNPs with allele frequencies of 0.2–0.8 result in the same likelihood ratio as 12 STRs [25].

To resolve complicated paternity cases that cannot be settled by STRs alone, we decided to implement the SNPforID assay comprising 52 autosomal SNPs [26] as a supplementary method in our routine forensic genetic lab. After establishing and optimizing the new assay, we tested the usefulness of SNP typing on several routine cases that could not always be conclusively solved by STR analysis alone.

Material and Methods

Samples

In the implementation of the method and the sensitivity study, the commercially available standards 9948 male DNA and 9947A female DNA

(Promega, Mannheim, Germany) were used. Both were serially diluted to 1 ng/μl–15 pg/μl. In addition, DNA extracted from blood samples donated voluntarily by lab personnel was investigated in the same way. To test the new assay for kinship analysis, we used DNA material from the routine collective of the Institute of Legal Medicine in Kiel. We only investigated paternity cases in which the participants had agreed to further scientific investigations according to the German law on genetic investigation (GenDG). 11 cases were chosen: 3 cases with high paternity indices for the alleged father, 3 cases with 1 mutation in at least 1 of the STR systems, and 5 cases in which the alleged father had been previously excluded through STR analysis. These exclusion cases were additionally simulated as motherless cases. To test the assay on routine trace analysis material, we also investigated highly degraded DNA samples from different cases (6 DNA samples from telogen hairs, DNA from putrefied tissues and buccal swabs from 3 different identification cases). All findings were compared with previously obtained STR-typing results using commercial multiplex-PCRs (AmpFISTRIdentifiler™ (Applied Biosystems, Carlsbad, CA, USA), Powerplex 16 HS, Powerplex ESX and ESI (all Promega)).

Methods of SNP Typing

Of the 52 SNPs analyzed previously with the SNPforID assay, 2 polymorphisms could not be satisfactorily detected in our lab. The other 50 SNPs were included in our 50-plex assay as shown in detail in table 1. All primer sequences were taken from the paper by Sanchez et al. [26], and analyzed using the PubMed SNP database (www.ncbi.nlm.nih.gov/pubmed). They were additionally checked for primer dimers, hairpin structures, etc., using the primer 3.2 software (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The HPLC-purified primers were ordered from Biomers, Germany. Amplified fragments were between 59 and 115 bp long.

Multiplex PCR

The amplification of all 50 SNPs was performed in 2 Multiplex PCRs comprising 21 and 29 primer pairs, respectively. A Qiagen® Multiplex PCR kit (Qiagen, Hilden, Germany) was used in a total volume of 25 μl for each PCR, and contained 1 μl DNA extract (1 ng DNA content in paternity cases, pure DNA extract from minute trace samples without prior DNA quantification, and various DNA concentrations for sensitivity experiments). Amplification was conducted with the following PCR program: denaturation at 95 °C for 15 min followed by 35 cycles of 94 °C for 30 s, 60 °C for 90 s and 72 °C for 60 s, and a final extension step with 60 °C for 30 min in a GeneAmp® PCR system 9700 (Applied Biosystems).

Detection of PCR Products on Polyacrylamide Gels

To confirm PCR success we preferred to use the highly discriminative and more sensitive polyacrylamide gels (PAG) with subsequent silver staining rather than agarose gel electrophoresis. 5 μl of each PCR product was separated on a PAG and detected using modified silver staining [27].

Single-Base Extension

PCR products were purified using ExoSAP-IT (Affymetrix) according to the manufacturer's protocol. Single-base extension (SBE) was done using the ABI PRISM® SNaPshot™ kit (Applied Biosystems) with 1 μl of the respective PCR product in 2 separate reactions, analogous to the above-described PCRs, in a total volume of 8 μl. The fragments were amplified in the following SBE program: 30 cycles at 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s in the same PCR machine. SBE products were purified using FastApp Thermosensitive Alkaline Phosphatase (Fermentas) according to the manufacturer's protocol.

Table 1. SNPs investigated in this study. Chromosome locations, database numbers, alleles and frequency data as given by Sanchez et al. [26] are shown. Also given are the concentrations for every SNP in the first PCR; Standard 52-plex: concentrations according to Sanchez et al. [26]; Optimized 50-plex: concentrations showing the best results in our study

SNP	Ch.	NCBI rs No.	TSC no.	Allele	America frequency	Asia frequency	Europe frequency	Standard 52-plex, $\mu\text{mol/l}$	Optimized 50-plex, $\mu\text{mol/l}$
1	1	Rs1490413	TSC0724193	A/G	0.59/0.41	0.46/0.54	0.47/0.53	0.06	0.13
2	2	Rs876724	TSC0208870	C/T	0.72/0.28	0.46/0.54	0.69/0.31	0.02	0.10
3	3	Rs1357617	TSC0496080	A/T	0.85/0.15	0.80/0.20	0.65/0.35	0.02	0.06
4	4	Rs2046361	TSC1065282	A/T	0.65/0.35	0.70/0.30	0.80/0.20	0.03	0.15
5	5	Rs717302	TSC0039610	A/G	0.92/0.08	0.83/0.17	0.45/0.55	0.04	0.10
6	6	Rs1029047	TSC0253802	A/T	0.61/0.39	0.27/0.73	0.37/0.63	0.06	0.20
7	7	Rs917118	TSC0229630	C/T	0.10/0.90	0.50/0.50	0.50/0.51	0.03	N
8	8	Rs763869	TSC0065968	C/T	0.48/0.52	no data	0.56/0.44	0.09	0.10
9	9	Rs1015250	TSC0097236	C/G	0.55/0.45	0.40/0.60	0.15/0.85	0.03	0.06
10	10	Rs735155	TSC0027519	A/G	0.38/0.62	0.82/0.18	0.41/0.59	0.05	0.13
11	11	Rs901398	TSC0177752	C/T	0.39/0.61	0.29/0.71	0.34/0.66	0.02	0.10
12	12	Rs2107612	TSC1108144	A/G	0.64/0.36	0.75/0.25	0.70/0.30	0.05	0.08
13	13	Rs1886510	TSC0904551	C/T	0.85/0.15	no data	0.50/0.50	0.01	0.08
14	14	Rs1454361	TSC0684657	A/T	0.57/0.43	0.62/0.38	0.47/0.53	0.02	0.03
15	15	Rs2016276	TSC0326920	A/G	0.91/0.09	0.59/0.41	0.79/0.21	0.17	0.40
16	16	Rs729172	TSC0028090	A/C	0.23/0.77	0.06/0.94	0.38/0.62	0.02	0.08
17	17	Rs740910	TSC0105771	A/G	0.92/0.08	0.92/0.09	0.57/0.43	0.08	0.10
18	18	Rs1493232	TSC0729796	A/C	0.44/0.56	0.39/0.61	0.56/0.44	0.07	0.10
19	19	Rs719366	TSC0044147	C/T	0.24/0.76	0.15/0.85	0.38/0.62	0.06	0.10
20	20	Rs1031825	TSC0334834	A/C	0.60/0.40	0.41/0.59	0.30/0.70	0.03	0.10
21	21	Rs722098	TSC0050288	A/G	0.17/0.83	0.50/0.50	0.86/0.14	0.12	0.10
22	22	Rs733164	TSC0023085	A/G	0.26/0.74	0.16/0.84	0.34/0.66	0.02	0.05
23	10	Rs826472	TSC0557086	C/T	0.70/0.30	0.66/0.34	0.44/0.56	0.04	0.08
24	21	Rs2831700	no data	A/G	0.42/0.58	0.50/0.50	0.62/0.38	0.04	0.08
25	14	Rs873196	TSC0202434	C/T	0.30/0.70	0.19/0.81	0.37/0.63	0.03	0.08
26	16	Rs1382387	TSC0544547	G/T	0.55/0.45	0.40/0.60	0.25/0.75	0.02	0.08
27	12	Rs2111980	TSC1113476	A/G	0.69/0.31	0.60/0.40	0.56/0.44	0.01	0.05
28	8	Rs2056277	TSC1082757	C/T	0.70/0.30	0.75/0.25	0.55/0.45	0.03	0.10
29	18	Rs1024116	TSC0247167	A/G	0.37/0.63	0.12/0.88	0.50/0.50	0.03	0.08
30	6	Rs727811	TSC0062764	A/C	0.30/0.70	0.66/0.34	0.61/0.39	0.03	0.10
31	1	Rs1413212	TSC0607362	A/G	0.61/0.39	0.47/0.53	0.41/0.59	0.05	0.10
32	17	Rs938283	TSC0357388	C/T	0.09/0.91	0.17/0.83	0.30/0.70	0.03	0.05
33	4	Rs1979255	TSC0925231	C/G	0.38/0.62	0.33/0.67	0.35/0.65	0.03	0.08
34	9	Rs1463729	TSC0377760	A/G	0.79/0.21	0.48/0.52	0.56/0.44	0.04	0.10
35	11	Rs2076848	TSC0022275	A/T	0.38/0.62	0.58/0.42	0.49/0.51	0.04	0.05
36	3	Rs1355366	TSC0491662	A/G	0.40/0.60	0.95/0.05	0.70/0.30	0.05	0.10
37	2	Rs907100	TSC0186810	C/G	0.74/0.26	no data	0.48/0.52	0.04	0.08
38	13	Rs354439	TSC0700528	A/T	0.50/0.50	0.58/0.42	0.64/0.36	0.05	0.08
39	22	Rs2040411	TSC1056845	A/G	0.75/0.25	0.08/0.92	0.71/0.29	0.03	0.05
40	7	Rs737681	TSC0033074	C/T	0.56/0.44	0.80/0.20	0.58/0.42	0.03	0.10
41	21	Rs2830795	no data	A/G	0.88/0.12	0.45/0.55	0.67/0.33	0.02	0.08
42	5	rs251934	TSC0220872	C/T	0.26/0.74	0.16/0.84	0.42/0.58	0.01	0.05
43	21	rs914165	TSC0197658	A/G	0.66/0.34	0.27/0.73	0.49/0.51	0.07	0.05
44	1	rs10495407	no data	A/G	0.11/0.89	0.42/0.58	0.29/0.71	0.05	0.10
45	9	rs1360288	TSC0501229	C/T	0.70/0.30	0.50/0.50	0.50/0.50	0.04	0.05
46	10	rs964681	TSC0270699	C/T	0.37/0.63	0.23/0.77	0.41/0.59	0.03	0.12
47	20	rs1005533	TSC0082071	A/G	0.55/0.45	0.60/0.40	0.45/0.55	0.03	0.05
48	15	rs8037429	no data	C/T	0.58/0.42	0.42/0.58	0.62/0.38	0.03	N
49	1	rs891700	TSC0162577	A/G	0.53/0.47	0.53/0.47	0.57/0.43	0.04	0.10
50	13	rs1335873	TSC0829150	A/T	0.91/0.09	0.28/0.72	0.36/0.64	0.03	0.08
51	22	rs1028528	TSC0253071	A/G	0.35/0.65	0.65/0.35	0.74/0.26	0.02	0.04
52	15	rs1528460	TSC0798410	C/T	0.65/0.35	0.36/0.64	0.29/0.71	0.06	0.10

No data = No information given in the mentioned paper; SNP = single nucleotide polymorphism; Ch. = chromosome, NCBI = National Center for Biotechnology Information; rs = reference SNP; TSC = the SNP Consortium; A = adenine; C = cytosine; G = guanine; T = thymine; N = this primer was removed from our assay.

Fig. 1. Checking of amplification success prior to single-base extension (SBE). Aliquots of 5 µl of each PCR product from 6 different samples were separated on a PAG and detected using a modified silver staining. Left: Results from the 21-plex; right: the 29-plex amplicons. NTC = No template control, L = molecular weight marker (GeneRuler™ Low range DNA ladder, Fermentas GmbH, Germany).

Fragment Analysis

Detection of SNP products was done on an ABI PRISM™ 3130 Genetic Analyzer (Applied Biosystems) using 2 µl purified SNaPshot product in 11.7 µl HD formamide and 0.3 µl LIZ120 standard (all Applied Biosystems). The data were processed using the GeneMapper™ ID Software version 3.2.

Biostatistical Calculations and Evaluations

Probabilities of fatherhood and paternity indices were calculated according to Essen-Möller with a self-constructed software tool (PAnalyzer vers. 2.1) using European frequencies obtained from [26]. Calculation of genotype frequencies was made using the same frequencies and again a self-designed program (SNP-Ivers1.2). In this study all samples were analyzed using European frequencies; however, if the origin of the investigated persons was not European, Asian or American data could also be used and are part of our program.

Results and Discussion

Sensitivity and Establishment of the Presented Assay

Review of the published primer sequences uncovered 2 faulty primers: rs2076848 (SNP 35) and rs914165 (SNP 43) showed a wrong nucleotide sequence. The corrected sequences generated the expected fragments. 2 SNPs had to be removed from the assay: SNP 7 (rs917118) and SNP 48 (rs8037429) were not included in the multiplex PCR because they led to C artifacts that hampered the evaluation of other SNPs. To obtain a satisfactory detection threshold, the remaining 50 PCR products were generated in 2 different multiplex reactions (21- and 29-plex). Figure 1 shows a PAG of different samples inserted into the 2 multiplex reactions.

In our approach the PCR conditions presented in [26] did not lead to a reliable and sensitive amplification. Most primer concentrations had to be increased (see table 1 for details). The application of the Qiagen Multiplex-PCR kit gave more robust and sensitive results compared to the ImmoMix™ (Bioline, Luckenwalde, Germany) and to a self-made PCR mix

using Immolase (Bioline; data not shown). This necessary procedure is in accordance with studies from other groups such as that of Børsting and colleagues, who also reduced the number of SNPs from 52 to 49 and modified specific PCR conditions [23].

Fragment analysis conditions were also optimized to achieve signals between 1,000 and 6,000 relative fluorescence units (rfu). The use of 2 µl SEB products led to strong and very clear signals. The chosen analysis conditions led to a detection threshold down to 500 pg in dilution experiments using the 9947a female DNA (Promega), while 100 pg still allowed the detection of 36 out of 50 SNPs (72%), and 25 pg template DNA still led to 19 detectable SNPs (38% success rate); see figure 2 for exemplarily results of a dilution experiment with the 29-plex.

Surprisingly, comparing the 2 SNP multiplex assays, we found that the 29-plex was more sensitive than the 21-plex. With < 500 pg template DNA, significantly lower number of successfully amplified SNPs were found in the 21-plex. Using only 25 pg template DNA, approximately half of the 29 SNPs were still detectable, whereas only 5 SNPs were detected in the 21-plex (23%). Keeping the multiplex assay separated in 2 different multiplex PCRs might be useful in cases in which template DNA is limited. In routine work it may be an option to use the 29-plex PCR first and then, depending on the outcome, subject the sample material to the less-sensitive 21-plex.

Comparison of Routine Trace Analysis and Identification Cases

After optimization, our SNP assay was used to investigate routine samples from various forensic cases. We initially chose 3 simple identification cases (severely putrefied bodies) that could not be solved by STR analysis. DNA was extracted from different soft tissues as previously described by our working group [9], and inserted into the assay. SNP detection worked very well even with DNA of low quality and quantity. As noted above, the 29-plex seemed to be more robust and reliable than the 21 SNP multiplex. In each of the 3 cases, ap-

Fig. 2. Sensitivity of the established 29-plex. Electropherogram after separation of specific reaction products on an ABI3130. Different DNA concentrations were subjected to the described 29-plex. The figure shows that with as little as 25 pg template DNA some SNPs are still detectable.

Fig. 3. Genetic analysis of single hairs using STR and SNP typing. Amplification success in percent of successfully typed markers after analyzing 17 STRs or 50 SNPs. Every analysis was done using 1 µl pure DNA extract as template.

plication of our assay led to an adequate phenotype frequency that was comparable to the STR analysis results using the highly sensitive Powerplex ESX kit [18].

DNA extracted from 6 telogen hairs was then investigated and compared in the same way. Hairs with telogenic roots represent the majority of trace hairs collected in connection with criminal cases [12], and contain only little, if any, nuclear DNA [27]. SNP detection was successful in all 6 samples, while STR analysis gave no reproducible results in 1 of 6 cases. Furthermore, SNP typing led to higher phenotype frequencies than STR analysis. Again, the 29-plex gave a better amplification success than the 21-plex. We achieved a much higher amplification rate for our assay compared with STR typing using the Powerplex ESX17; whereas STR analysis yielded a maximum

of 4 successfully amplified loci, our SNP-plex generated between 21 and 42 SNPs (see fig. 3 for a summary of the results). In conclusion, SNP typing provides a promising new tool for the analysis of minute and/or highly degraded DNA samples, and allows not only mitochondrial [28], but also autosomal investigation of degraded hair samples.

Comparison of Different Paternity Cases

We applied our assay in selected paternity investigations. In all cases, SNP-typing results were compared to those of STR analysis (amplification of 17 STRs using the Powerplex 16 HS and AmpFISTRIdentifiler multiplex PCR kits).

Table 2. Results for paternity calculation using 17 STRs versus 50 SNPs. Values for combined paternity index and probability of paternity calculated using a self-developed software program

Case	Remarks	Combined paternity index (PI)		Probability of paternity (W), %	
		17 STRs	50 SNPs	17 STRs	50 SNPs
1	no mutations	1.53×10^8	2.14×10^7	>99.999999	99.999959
3	no mutations	7.32×10^7	1.41×10^5	>99.999999	99.9992925
3	no mutations	5.13×10^7	2.35×10^6	99.999998	99.9999575
4	2-step paternal mutation in D2S1338	2.22×10^3	1.10×10^6	99.955068	99.999909
5	1-step paternal mutation in FGA	8.71×10^4	1.65×10^8	99.998851	99.9999994
6	two 1-step mutations, VWA and Penta D	3.22×10^1	6.32×10^8	96.990151	99.9999998

Trio Cases

In all 3 regular trio cases (cases 1–3 in table 2) involving mother, child and putative father, STR analysis gave higher combined paternity indices (PI) and probabilities of paternity (W) than SNP typing. However, SNP analysis alone was always sufficient to issue the highest verbal statement in Germany ('paternity practically proven') with W values above 99.9% (see table 2 for more detailed information). Our results support the findings of Børsting et al. [22] who calculated PIs between 489 and 6.31×10^8 with an average of 1.32×10^6 after investigating 72 Danish trio cases with 52 SNPs.

Mutations

STR analysis of 3 trio cases with mutations in different STR loci (cases 4–6 in table 2) gave lower combined PIs than investigation of the samples with our multiplex SNP assay. These results demonstrate one of the main advantages of SNP typing: the low mutation rate of approximately 10^{-8} . STR analysis on the other hand can lead to false paternal or maternal exclusions due to a higher mutation rate and consecutive gain or loss of a limited number of tandem repeats, possibly as a result of slippage of the DNA replication complex during DNA synthesis [29]. Most common mutations using STR typing are false paternal or maternal exclusions, in which an obligate gene differs only slightly in its size from its presumed predecessor in an otherwise confirmed parent. This is unproblematic in cases with at least 3 mismatches that, according to guidelines, lead to the exclusion of the alleged father. In cases with less than 3 mismatches, the overall mutation rate of the investigated loci that ranges between 5×10^{-4} and 7×10^{-3} [21, 30] can be used to calculate paternity probabilities. In this study, all 3 cases showed no mismatches after SNP typing. Thus, the additional investigation of SNPs always led to much higher and more reliable paternity probabilities. In 1 case with 2 mutations after STR analysis (case 6 in table 2), SNP typing was necessary to obtain the required W value of at least 99.9%.

Exclusions of Fatherhood

Five trio paternity cases were chosen in which the alleged father was excluded via STR analysis. Investigation of 17 STRs resulted in 3–13 exclusions, while SNP typing ($n = 50$) led to an average of 7.2 exclusions (6–8). The results are in accordance with the calculations by Krawczak [24], who estimated that approximately 4.2 SNPs with allelic frequencies of 0.5 each give the same power of exclusion as 1 STR locus. We did not distinguish between genetic inconsistency and opposite homozygosity despite possible silent alleles; the latter is a strong indicator against paternity. In accordance with our results, Børsting et al. [22] found an average of 10 mismatches in their study.

Deficiency Cases

The above-mentioned 5 exclusion cases were additionally calculated as (simulated) deficiencies, i.e. without consideration of the mother. Analysis of 17 STRs resulted in 2–8 exclusions between child and alleged father. In 1 of the 5 cases the exclusion of fatherhood was not reliably possible. The supplementary application of our SNP assay, however, led to 3–8 additional excluding markers, reliably solving all cases. Recently, it was shown that in complicated cases (deficiencies, mutations/genetic inconsistencies) SNP typing was not only supportive, but even more informative than the analysis of 15 STRs in the 6 investigated cases [31].

Conclusion

Our study shows that the implementation of the SNP multiplex assay as a second method is a useful tool to increase the informative value of kinship and trace analyses, and that our assay comprising 50 SNPforID markers can be easily used as a standard method to confirm and complement STR results. The application of SNPs will frequently be suitable to differentiate between mere mutations and real exclusions in STR mismatch cases. Furthermore, our SNP assay will increase the reliability of results in deficiency cases. Under usual circumstances, the investigation of a large number of STRs provides

a reliable paternity testing even without consideration of the mother. However, in cases with related alleged fathers (e.g. biological father and his full brother), false inclusions can occur [32]. Even an unrelated man might be falsely included [33]. In summary, the application of SNPs to supplement genetic analysis can be recommended, especially as no additional machinery is needed.

Disclosure Statement

The authors declare that there are no conflicts of interest regarding this publication.

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