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ANALYSIS OF PATERNITY CASES WITH A SINGLE EXCLUSION IN A GENETIC MARKER USING PRECISION ID GLOBALFILER™ NGS STR PANEL v2

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INTRODUCTION

Paternity test results can sometimes evidence incompatibilities in the allelic transmission from parents to children, when the expected alleles that the child should have received in a genetic marker do not match to any of the identified alleles in the alleged father and/or biological mother. This exclusion may result from a genetic mutation or other factors as null or silent alleles [1–3].
Capillary electrophoresis (CE) is the traditional method used in forensic genetics to analyze STRs (Short Tandem Repeats). Although the number of genetic markers studied is already high, it is not possible to know the exact allele number variation due to the lack of sequence data and, when necessary, it is important to consider the study of other genetic markers to investigate the existence of more exclusions. The application of Next-Generation Sequencing (NGS) technology may provide additional information, since it allows to detect and sequence simultaneously SNPs (Single Nucleotide Polymorphisms) present in the flanking regions and also distinguish isometric alleles with the same length but different sequences, that were misinterpreted as homozygous. By identifying repeat motif variation within STRs based on their sequence and not length as in the CE, NGS may be very useful to infer the origin of the genetic mutation and to increase the discrimination power [1–5].
The Precision ID GlobalFiler™ NGS STR Panel v2 is composed by 35 markers, including the same 20 autosomal STRs found in the GlobalFiler™ PCR Amplification Kit used in the CE, along with Y chromosome markers, amelogenin sex markers and additional multiallelic STRs markers [6–7].
The aim of this study was to verify if the NGS methodology using Precision ID GlobalFiler™ NGS STR Panel v2 provides valuable information in paternity cases with a single exclusion in a genetic marker and also to identify the parental origin of a mutant allele.

MATERIALS AND METHODS

A total of 45 reference samples (buccal swabs and blood stains), previously amplified with the GlobalFiler™ PCR Amplification Kit and sequenced by CE on the 3500 Genetic Analyzer, were selected from 15 paternity trio cases with a single exclusion, reported after GeneMapper ID-X Software analysis.
As recommended in the user guide, all samples were extracted with the PrepFiler Express™ Kit followed by purification on the AutoMate Express™ System. The extracted samples were quantified with the Quantifiler™ Trio Kit on the 7500 Real-time PCR System and, according to the quantification results, the samples were diluted to 67 pg/µL with nuclease-free water to prepare 15 µL of each diluted sample. Library preparation was automatically prepared with the Precision ID GlobalFiler™ NGS STR Panel v2 using Precision ID DL8 Kit on the Ion Chef™ System. Next, libraries were quantified with the Ion Library TaqMan™ Quantitation Kit on the 7500 Real-time PCR System. All barcoded libraries were diluted to 50 pM and then combined in a super-pool to ensure equal contribution in the sequencing run. Template preparation was performed on the Ion Chef™ System using the Ion S5™ Precision ID Chef & Sequencing Kit and the loaded Ion 530™ chips were sequenced on the Ion S5™ System, as described by manufacturer.
Finally, the results were analyzed with the Converge™ Software and then compared to the results obtained with CE.

RESULTS

NGS results were in concordance with those obtained by CE for all 15 paternity trios (Table 1). For the analysis, it was considered that the allele that the child has in common with the biological parent always comes from maternal origin, as it is the paternity that is being investigated.
Trios 1 to 4 revealed an exclusion at the FGA marker, in which there was a gain or loss of a CTTT repeat in father-child transmission.
Regarding trios 5 and 6, the exclusion was observed in the vWA marker with a mutation resulting from the gain of a TCTA repeat at the final sequence.
In trios 7 and 8, the exclusion occurred at the D12S391 marker. In trio 7, the father's allele 23 mutated to 24 with the gain of an AGAT repeat. For trio 8, the genotype of the child is the same as the mother, which does not allow to distinguish which allele was mutated from the father to the child and there may have been a gain or loss of an AGAT repeat in the initial sequence.
In trio 9, the mutation at the D2S1338 marker was associated with the loss of a TTCC repeat, while for trio 10 with an exclusion at the D7S820 marker, the mutation was the result of the loss of a GATA repeat.
Trio 11 was a particular case, since considering that one of the alleles 13 at the D19S433 marker was necessarily inherited from the mother, there are two possibilities of father-child transmission in which the gain or loss of an AAGG repeat may have occurred.
For trio 12, a mutation was observed in mother-child transmission at the D2S441 marker, in which the child received allele 11 from the father and the allele 12 from the mother mutated to 11 with the loss of a TCTA repeat.

Table 1. Summary of NGS results for 15 paternity trios with a single exclusion. Legend: F – father; M – mother; C – Child.

Trio		Allele 1	NGS Sequence Allele 1	Allele 2	NGS Sequence Allele 2
1 FGA	F	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2	25	[TTTC]3 TTTT TTCT [CTTT]17 CTCC [TTCC]2
	M	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2	27	[TTTC]3 TTTT TTCT [CTTT]19 CTCC [TTCC]2
	C	22	[TTTC]3 TTTT TTCT [CTTT]14 CTCC [TTCC]2	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2
2 FGA	F	20	[TTTC]3 TTTT TTCT [CTTT]12 CTCC [TTCC]2	26	[TTTC]3 TTTT TTCT [CTTT]18 CTCC [TTCC]2
	M	22	[TTTC]3 TTTT TTCT [CTTT]14 CTCC [TTCC]2	24	[TTTC]3 TTTT TTCT [CTTT]16 CTCC [TTCC]2
	C	22	[TTTC]3 TTTT TTCT [CTTT]14 CTCC [TTCC]2	27	[TTTC]3 TTTT TTCT [CTTT]19 CTCC [TTCC]2
3 FGA	F	21	[TTTC]3 TTTT TTCT [CTTT]13 CTCC [TTCC]2	24	[TTTC]3 TTTT TTCT [CTTT]16 CTCC [TTCC]2
	M	21	[TTTC]3 TTTT TTCT [CTTT]13 CTCC [TTCC]2	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2
	C	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2	23	[TTTC]3 TTTT TTCT [CTTT]15 CTCC [TTCC]2
4 FGA	F	19	[TTTC]3 TTTT TTCT [CTTT]11 CTCC [TTCC]2	25	[TTTC]3 TTTT TTCT [CTTT]17 CTCC [TTCC]2
	M	22	[TTTC]3 TTTT TTCT [CTTT]14 CTCC [TTCC]2	23.2	[TTTC]3 TTTT TT[CTTT]16 CTCC [TTCC]2
	C	20	[TTTC]3 TTTT TTCT [CTTT]12 CTCC [TTCC]2	22	[TTTC]3 TTTT TTCT [CTTT]14 CTCC [TTCC]2
5 vWA	F	18	[TCTA]1 [TCTG]4 [TCTA]13	18	[TCTA]1 [TCTG]4 [TCTA]13
	M	17	[TCTA]1 [TCTG]4 [TCTA]12	18	[TCTA]1 [TCTG]4 [TCTA]13
	C	17	[TCTA]1 [TCTG]4 [TCTA]12	19	[TCTA]1 [TCTG]4 [TCTA]14
6 vWA	F	15	[TCTA]1 [TCTG]4 [TCTA]10	18	[TCTA]1 [TCTG]4 [TCTA]13
	M	17	[TCTA]1 [TCTG]4 [TCTA]12	19	[TCTA]1 [TCTG]4 [TCTA]14
	C	19	[TCTA]1 [TCTG]4 [TCTA]14	19	[TCTA]1 [TCTG]4 [TCTA]14
7 D12S391	F	18	[AGAT]11 [AGAC]6 [AGAT]1	23	[AGAT]14 [AGAC]9
	M	18	[AGAT]11 [AGAC]6 [AGAT]1	19	[AGAT]12 [AGAC]6 [AGAT]1
	C	19	[AGAT]12 [AGAC]6 [AGAT]1	24	[AGAT]15 [AGAC]9
8 D12S391	F	17	[AGAT]10 [AGAC]6 [AGAT]1	21	[AGAT]14 [AGAC]6 [AGAT]1
	M	18	[AGAT]11 [AGAC]6 [AGAT]1	20	[AGAT]13 [AGAC]6 [AGAT]1
	C	18	[AGAT]11 [AGAC]6 [AGAT]1	20	[AGAT]13 [AGAC]6 [AGAT]1
9 D2S1338	F	23	[TGCC]7 [TTCC]13 GTCC[TTCC]2	26	[TGCC]8 [TTCC]15 GTCC[TTCC]2
	M	17	[TGCC]5 [TTCC]12	25	[TGCC]7 [TTCC]15 GTCC[TTCC]2
	C	17	[TGCC]5 [TTCC]12	25	[TGCC]8 [TTCC]14 GTCC[TTCC]2
10 D7S820	F	10	[GATA]10	13	[GATA]13
	M	11	[GATA]11	11	[GATA]11
	C	11	[GATA]11	12	[GATA]12
11 D19S433	F	12	AAGGTAGG [AAGG]10	14	AAGGTAGG [AAGG]12
	M	13	AAGGTAGG [AAGG]11	15	AAGGTAGG [AAGG]13
	C	13	AAGGTAGG [AAGG]11	13	AAGGTAGG [AAGG]11
12 D2S441	F	11	[TCTA]11	16	[TCTA]13 TTTA[TCTA]2
	M	12	[TCTA]12 rs74640515 (G/A)	12	[TCTA]12 rs74640515 (G/A)
	C	11	[TCTA]11	11	[TCTA]11
13 D5S818	F	12	[AGAT]12 rs25768 (A/G)	12	[AGAT]12 rs25768 (A/G)
	M	11	[AGAT]11 rs25768 (A/G)	12	[AGAT]12 rs25768 (A/G)
	C	11	[AGAT]11 rs25768 (A/G)	13	[AGAT]13 rs25768 (A/G)
14 D5S818	F	12	[AGAT]12 rs25768 (A/G)	12	[AGAT]12 rs25768 (A/G)
	M	12	[AGAT]12 rs25768 (A/G)	12	[AGAT]12 rs25768 (A/G)
	C	12	[AGAT]12 rs25768 (A/G)	13	[AGAT]13 rs25768 (A/G)
15 CSF1PO	F	10	[AGAT]10	11	[AGAT]11
	M	12	[AGAT]12	12	[AGAT]12
	C	8	[AGAT]8	12	[AGAT]12

Trios 13 and 14 showed the same flanking SNP rs25768 (A/G) at the D5S818 marker, with the father's allele 12 being mutated to 13 with the gain of an AGAT repeat.
In the case of trio 15 with an exclusion at the CSF1PO marker, a silent or null allele was found, since the allele 8 was not detected in any of the parents in both the CE and NGS methodologies.

DISCUSSION AND CONCLUSION

NGS technology allowed to obtain a concordant result with those by CE system, showing the reliability of both methods. However, NGS provides additional information about the STR repeat motif sequence, which could increase the discrimination power of several genetic markers. This methodology combined with the use of the Precision ID GlobalFiler™ NGS STR Panel v2 demonstrated to be useful to clarify the single exclusion, since it was possible to confirm the origin of the mutation by interpreting the sequence of the alleles for the majority of paternity trios. For the trio case with the silent or null allele, the discrepancy in allele inheritance may be due to a primer binding site mutation rather than a repeat mutation, since it was not detected in both parents or in any of the methodologies. Also, the presence of 9 additional multi-allelic STRs in the NGS panel compared to the traditional CE method may be an important tool in paternity cases with more than one allelic transmission mismatch. In a future research, it will be important to include the allele frequencies of these additional markers to calculate the paternity index, in order to improve the statistical values. Therefore, the Precision ID GlobalFiler™ NGS STR Panel v2 shows to be a powerful method for kinship analyses and typing reference samples through a sequence-based analysis.

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