

## Genetics and Molecular Biology



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### REFERÊNCIA

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## Impact of a chromosome X STR Decaplex in deficiency paternity cases

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### Abstract

Deficiency paternity cases, characterized by the absence of the alleged father, are a challenge for forensic genetics. Here we present four cases with a female child and a deceased alleged father in which the analysis of a set of 21 or 22 autosomal STRs (AS STRs) produced results within a range of doubt when genotyping relatives of the alleged father. Aiming to increase the Paternity Index (PI) and obtain more reliable results, a set of 10 X-linked STR markers, developed by the Spanish and Portuguese Group of the International Society for Forensic Genetics (ISFG), was then added. Statistical analysis substantially shifted the results towards the alleged fatherhood in all four cases, with more dramatic changes when the supposed half-sister and respective mother were the relatives tested.

**Keywords:** forensic genetics, ChrX STR Decaplex, X-linked markers, deficiency paternity case, Paternity Index.

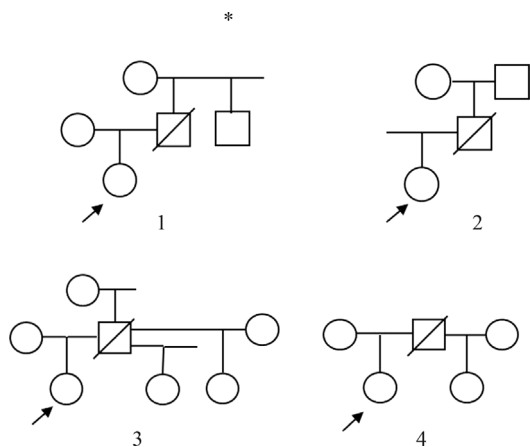
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In the laboratory of the Brazilian Federal District Civil Police, about 11% of civil paternity investigation cases are characterized by “deficiency”, *i.e.* the alleged father is absent. In such cases, close relatives make up for this absence by providing material for analysis, with the disadvantage that the Combined Paternity Index (PI) obtained is invariably lower than the index obtained if the alleged father is available for analysis. Furthermore, in some cases the exclusive use of autosomal STR markers may be insufficient to obtain PI values above an acceptable limit.

The introduction of X chromosome short tandem repeats (ChrX STR) in forensic genetics has a recent history (Szibor *et al.*, 2003; Shin *et al.*, 2005; Szibor, 2007). Markers located on this chromosome have a particular inheritance pattern: women are dizygous and men are hemizygous, with the latter receiving their single X from the mother. This makes these markers particularly suited in deficiency paternity cases. ChrX STRs can, however, only be used in paternity case disputes involving daughters, as there is no allele inherited by descent in a father-son relationship. As ChrX STRs are located on a single chromo-

some, investigators must take the proper precautions when using these markers for genotyping and need to take into consideration genetic linkage and possible linkage disequilibrium amongst them (Edelmann *et al.*, 2004, Gomes *et al.*, 2011; Pinto *et al.*, 2011).

Here we present the use of this class of markers in four paternity deficiency cases, all including a female child and a deceased alleged father who was not analyzed. The number of subjects genotyped in each case was variable. Figure 1 displays the pedigrees of the families along with the probands that were identified by arrows. All individuals shown in the pedigrees were tested for autosomal (AS) and X STRs except for the putative uncle in family 1 and the putative grandfather in family 2, who were both genotyped only for AS STRs. All cases were previously analyzed for either 21 or 22 AS STRs using the AMPFISTR Identifier® (Applied Biosystems, CA, USA), Powerplex® 16 System, GenePrint FFFL Multiplex kit and PowerPlex® ES Monoplex System SE33 (Promega Corp., Madison, WI, USA) amplification systems. The results of the four cases failed to achieve PI values equal to or greater than 10,000, this being the cut off point set according to the laboratory of the Brazilian Federal District Civil Police internal protocol, considering for the alternative hypothesis an unrelated person (Table 1). Genotypes and frequencies of the allele for AS STRs transmitted by the deceased father are presented in



**Figure 1** - Pedigrees of the four deficiency paternity cases.

Supplementary material Table S1, and the formulae used to calculate PIs, considering the profile configurations depicted with AS STRs in the four cases, are presented in Tables S2-S5).

These four cases were then analyzed with the X-Decaplex system, composed of 10 ChrX STRs: DXS8378, DXS9898, DXS7133, GATA172D05, GATA31E08, DXS7423, DXS6809, DXS7132, DXS9902 and DXS6789 (Gusmão *et al.*, 2009). These STRs are located in three of the four linkage groups described by Szibor *et al.* (2003) for the X chromosome.

Briefly, the X STR laboratory analysis protocol was as follows: DNA was obtained from blood samples and/or oral swab employing the rapid NaOH extraction method (Richards *et al.*, 1993). Genomic DNA (1 ng) was amplified in a 10 µL total volume of PCR reaction mix in a GeneAmp 9600 thermocycler (Perkin-Elmer). The products were loaded into a 3130xl Genetic Analyzer (Applied Biosystems, CA, USA). Data was collected by DataCollection v3.0 software and alleles were assigned using GeneMapper v3.2.

The statistical parameters analyzed were the Paternity Index (PI) and Probability of Paternity (PP), with the assumption of an *a priori* probability of 0.5 for the latter. Genotypes and frequencies of the allele for X-linked STRs transmitted by the deceased father are shown in Table S6. The formulae used when the putative grandmother was the genotyped relative (cases 1 and 2) can be seen in Table 2.

When a putative half-sister was genotyped to reconstruct the putative father’s haplotype (cases 3 and 4), statistical analyses were performed according to the formulae proposed by Ayres and Powley (2005). The formulae were modified in order to exclude sub-structure parameters (Table 3). In case 4, the haplotype of the alleged father could not be reconstructed at three markers (DXS8378, DXS7423 and DXS6789), where the alleged half-sister and her mother shared the same heterozygous genotype. For each of these three markers, the formula  $f_a/f_a+f_b$  was used to es-

**Table 2** - Formulae used to calculate PI considering the profile configurations depicted with ChrX STRs, in cases 1 and 2.

Mother	Daughter	Putative grandmother	Formulas
AB	AB	AB	$1/(a+b)$
AB	AA	AB	$1/2a$
AA	AB	AB	$1/2b$
AA	AA	AB	$1/2a$
AB	BC	AC or CD	$1/2c$
AC	CC	BC	$1/2c$
AC	AC	BC	$1/[2(b+c)]$
AA	AB	BB	$1/b$
Absent	AB	BC	$1/4b$
Absent	AB	BB	$1/2b$
Absent	BB	BC	$1/2b$

**Table 3** - Formulae to calculate PI considering the profile configurations depicted with ChrX STRs, in cases 3 and 4.

Mother	Daughter	Putative half-sister	Formulas
AA	AA	A	$1/a$
AA	AB	B	$1/b$
AB	AA	A	$1/a$
AB	AB	A	$1/(a+b)$
AB	AC	C	$1/c$
AA	AA	A or B	$(a/a+b)/a$
AB	AA	A or B	$(a/a+b)/a$
AB	AC	B or C	$(a/a+b)/a$

**Table 1** - Combined Paternity Index (PI) and Probability of Paternity (PP) for autosomal STRs (AS STRs), X chromosome STRs (ChrX STRs) and a combination of AS/ChrX STRs.

	AS STR		ChrX STR		AS STR + ChrX STR	
	PI	PP	PI	PP	PI	PP
Case 01	0.0054	0.537	776	99.87	4.19	80.74
Case 02	10.01	90.92	5.31	83.70	51.35	98.09
Case 03	55.83	98.24	164 063.94	99.9994	9 159 128.01	99.999989
Case 04	975.49	99.90	1 527 368.04	99.99993	1 489 932 249.34	99.99999993

timate the probability of the paternal obligatory allele, also identified in the allelic profile of the daughter, as being part of the haplotype of the alleged father. In this formula,  $f_a$  denotes the frequency of occurrence of the paternal obligatory allele identified in both the genotype of the daughter and the genotype of the putative half-sister, and  $f_b$  denotes the other allele, identified in the profile of the latter. The value obtained was used as the probability of the alleged father has forwarded the paternal mandatory allele for the daughter, in the hypothesis of prosecution (HP) for each marker. The allelic frequencies used were the weighted average frequencies of four Brazilian populations obtained from Gusmão *et al.*, 2009. A Microsoft Office 2007 Excel Worksheet (Microsoft Corporation) was used to perform the calculations of PI and PP for both classes of markers. In case 3, the genotypes of the putative grandmother and the one motherless alleged half-sister were used only to identify the paternal allele of the putative deceased father in a locus where the other biological half-sister and her mother shared the same heterozygous genotype.

A first result was that we observed no inconsistencies by genotyping the four cases with the X-Decaplex system. Table 1 shows the statistical evaluation of the four cases for each class of marker separately, and for both classes together. In cases 1 and 2, the paternity index obtained by adding the ChrX-STRs data (4.19 for case 1 and 51.35 for case 2) still remained substantially lower than the cut off point according to the laboratory of the Brazilian Federal District Civil Police internal protocol. It should be pointed out that in case 1, the AS STRs produced a PI of only 0.0054 and in case 2, despite an initial PI of 10.01, the mother of the tested female was not available for analysis.

In case 3, the PI based on 21 autosomal markers reached 7,000. In order to raise the PI to 10,000, the respective samples were amplified for the autosomal marker SE33. An inconsistency in a repeat unit was, however, found between the putative father's rebuilt genotype and that of the tested child. The inconsistency was interpreted as a mutation, this moving the PI to 56. By incorporating additional information from X-Decaplex it was possible to achieve a final PI close to 9,000,000, consistent with paternity. The most dramatic change occurred in case 4, where a PI of less than 1,000 achieved by genotyping 21 autosomal STRs rose to a final PI close to 1,500,000,000 after joining the PI of the 10 ChrX markers.

Due to inheritance patterns, STRs located on chromosome X are well suited to evaluate cases of deficiency paternity. In these scenarios, the product rule depends on the absence of a linkage disequilibrium between the loci tested in the population of interest (Gusmão *et al.*, 2009). In a joint study carried out by the Spanish and Portuguese ISFG Working Group (ISFG), no linkage disequilibrium was observed between the loci of the X-Decaplex system in 15 Ibero-American populations, four of which were from Brazil (Gusmão *et al.*, 2009). Additionally, no linkage dis-

equilibrium was observed in the Brazilian Federal District population, which includes the tested individuals (Trindade-Filho, unpublished data).

If we assume that the individuals analyzed are inserted within a population in linkage equilibrium, then the 10 loci of the X Decaplex system could be considered in the statistical evaluation for all four cases. In cases 3 and 4, the linkage equilibrium alone allowed the authors to use the product rule to estimate the frequency of occurrence of the reconstructed haplotype of the alleged father.

For cases 1 and 2, the estimate of the frequency of occurrence of the reconstructed haplotype of the alleged father is more complex. When ChrX markers are investigated in a deficiency paternity case, the putative grandmother plays the same role as the putative father when autosomal STRs are used, and the statistical evaluation should be made under the same principle if two STRs are closely linked. Here we refer to the principle that says: if two markers do not segregate independently, the product rule can get undermined. This is the picture in cases 1 and 2, as the 10 X markers are located in three of the four linkage groups above mentioned. Nevertheless, we followed the guidance of Gill *et al.* (2012) who argue that if one assumes a linkage equilibrium at the population level, the effects of linkage in pedigrees spanning a few generations in forensic caseworks has no effect at all, unless at least one individual is involved in at least two transmissions of genetic material, either as a parent or a child, and that individual is a double heterozygote at the loci in question. And this are not the issues in our pedigrees.

Our results are in agreement with the literature and demonstrate the usefulness of ChrX STRs in selected cases of deficiency paternity (Edelmann *et al.*, 2004; Aquino *et al.*, 2009), especially if biological daughters of the alleged father and their mothers are available for reconstructing his haplotype. If the profile of the alleged father can be reconstructed, it should be emphasized that the haplotype from ChrX markers is more informative than the genotype from autosomal markers (Szibor *et al.*, 2003), considering the same allele frequencies and degree of polymorphism among the loci tested.

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## Supplementary Material

The following online material is available for this article:

Table S1 - Genotypes for AS-STRs and frequencies of the paternal obligatory alleles (APO) identified in the profile of the daughter

Table S2 - Formulae to calculate PI considering the profile configurations depicted with AS STRs, in case 1.

Table S3 - Formulae to calculate PI considering the profile configurations depicted with AS STRs, in case 2.

Table S4 - Formulae to calculate PI considering the profile configurations depicted with AS STRs, in case 3.

Table S5 - Formulae to calculate PI considering the profile configurations depicted with AS STRs, in case 4.

Table S6 - Genotypes for X-linked-STRs and frequencies of the paternal obligatory alleles (APO) identified in the profile of the daughter.

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**Table S1: Genotypes and frequencies of the allele for AS-STRs transmitted by the deceased father.**

Case 1	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	Penta E	Penta D	LPL	F13B	FESFPS	F13A01
Mother	12-13	28-29	10-12	12-12	18-18	6-6	11-12	12-13	19-23	14-15	16-18	11-11	13-15	9-13	22-27	5-11	10-10	9-12	9-10	10-11	6-7
Daughter	11-12	28-28	10-10	11-12	14-18	6-8	11-11	12-13	23-24	14-15	15-18	9-11	13-15	9-12	25-27	7-11	10-14	9-10	10-10	10-11	6-6
Putative Grandmother	13-14	28-29	9-10	10-12	14-15	7-9.3	11-12	11-11	19-20	13-15	15-16	9-10	14-18	10-12	21-25	10-13	13-14	11-12	6-10	11-12	7-14
Putative Uncle	13-14	29-30	9-10	9-12	15-15	6-9.3	9-11	11-11	16-19	13-14	15-16	9-12	17-18	12-12	20-25	5-13	10-13	11-12	10-10	12-12	3.2-14
FAF	0,060	0,144	0,310	0,279	0,082	0,146	0,282	0,246 0,139	0,081	0,286 0,150	0,133	0,125	0,098 0,174	0,335	0,099	0,096	0,055	0,428	0,317	0,234 0,415	0,194

Case 2	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	Penta E	Penta D	LPL	F13B	FESFPS	F13A01
Daughter	13-14	29-30.2	10-12	10-12	13-16	7-9.3	11-12	11-11	19-19	15-15	17-17	8-8	13-17	11-12	24-25	7-14	9-13	10-12	9-10	11-12	3.2-5
Putative Grandmother	13-14	28-29	11-12	11-13	14-16	9-9	8-12	11-11	18-24	12-13.2	14-17	8-8	17-17	11-12	24-24	5-12	10-13	10-12	8-9	8-12	3.2-7
Putative Grandfather	12-14	32.2-34	9-11	12-12	14-15	7-9.3	9-13	10-11	17-19	14-15	15-18	8-8	17-18	11-12	19-22	7-12	10-12	9-12	6-10	11-13	5-6
FAF	0,265 0,256	0,241	0,150	0,316	0,283	0,279 0,21	0,323	0,312	0,118	0,150	0,247	0,460	0,173	0,336 0,335	0,156	0,096	0,1675	0,428 0,227	0,273 0,317	0,415 0,213	0,132 0,259

Case 3	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	Penta E	Penta D	LPL	F13B	FESFPS	F13A01	SE33
Mother	13-14	32.2-32.2	10-12	11-12	14-17	6-6	12-13	10-11	17-20	12-13.2	14-16	8-11	12-14	12-13	21-23	8-12	9-12	10-10	9-10	12-12	6-7	17-23.2
Daughter	11-13	29-32.2	11-12	12-12	14-16	6-7	11-12	10-11	17-23	12-13	14-16	8-11	12-20	12-13	21-22	8-17	12-16	10-10	9-9	12-12	7-7	17-18
Putative Grandmother	12-14	28-28	8-11	11-12	15-16	7-9	11-11	9-13	17-23	14-14.2	17-18	8-8	15-20	13-14	23-24	12-13	11-11	10-10	9-10	12-12	6-7	14-18
Putative Half-sister 1	14-16	28-30	11-12	12-12	15-16	7-7	11-11	9-13	18-23	13-15.2	18-19	8-11	14-20	12-13	20-22	12-17	11-13	10-11	10-10	12-12	6-6	15-17
Putative Half-sister 2	12-14	29-29	12-12	11-11	14-15	7-9.3	11-12	10-11	17-22	13-14.2	16-17	8-11	15-17	12-12	22-26	13-15	8-11	9-10	9-9	10-12	6-6	14-18
Mother of Putative Half-sister 2	12-13	29-31.2	10-12	11-12	14-16	9-9.3	9-12	10-12	17-18	14-14.2	17-17	11-11	14-17	11-12	24-26	15-15	8-12	9-10	9-10	10-11	6-7	17-18
RPPF	14-?	28-29	12-(8 or 11)	11-12	15-?	7-?	11-?	11-(9 or 13)	22-23	13-(14 or 14.2)	16-18	8-?	15-(14 or 20)	12-(13 or 14)	22-(23 or 24)	13-(12 or 17)	11-?	10-?	9-10	12-?	6-?	14-15 or 17
FAF	0,060	0,241	0,232	0,316	0,283	0,279	0,282	0,117 0,312	0,099	0,234	0,087 0,257	0,460 0,247	0,033	0,335 0,177	0,164	0,055	0,030	0,428	0,273	0,213	0,250	NA

Case 4	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	Penta E	Penta D	LPL	F13B	FESFPS	F13A01
Mother	12-13	30-31	9-9	12-12	18-18	6-9.3	11-13	9-13	18-22	12.2-14	16-16	11-11	12-16	10-12	21-24	12-19	10-12	10-12	6-6	11-12	5-16
Daughter	13-14	30-31	9-11	10-12	15-18	9.3-9.3	12-13	12-13	18-22	12.2-14	14-16	8-11	16-17	12-13	21-23	9-19	10-12	10-12	6-6	10-11	5-6
Putative Half-sister	11-13	28-30.2	10-11	9-10	15-15	7-9.3	8-12	12-12	17-18	12.2-13.2	16-17	8-11	12-19	11-13	23-26	10-16	5-10	10-11	6-10	10-11	3.2-7
Mother of Putative Half-sister	13-13	28-30	10-10	9-13	15-15	6-7	8-11	11-12	17-25	13-13.2	14-17	8-11	12-12	13-13	21-26	10-14	5-9	10-11	9-10	11-11	3.2-7
RPPF	11-?	30.2-?	11-?	10-?	15-?	9.3-?	12-?	12-?	18-?	12.2-?	16-?	(8 or 11)-	19-?	11-?	23-?	16-?	10-?	10 or 11-?	6-?	10-?	3,2 or 7-?
FADF	0,256	0,206 0,084	0,232	0,255	0,314	0,212	0,323	0,246	0,058 0,094	0,030 0,286	0,087	0,460 f11=0,247	0,173	0,177	0,151	0,030	0,155 0,193	0,428 0,227	0,178	0,234	0,194

RPPF – reconstructed profile of putative father

FAF- frequencies of allele (or possible alleles) transmitted by the father

Table S2 - Formulas to calculate PI considering the profile configurations depicted with AS STRs, in case 1.

Mother	Daughter	Putative Grandmother	Putative Uncle	Formulas
AA	AB	AC	AD	
AB	BC	AD	AD	$(c/4)/c$
AB	BC	AD or DE	AE	
AB	AA	AB	BC	
AB	AA	AC	AC	$((1+a)/4)/a$
AB	AA	AB	AC	
AA	AB	BC	AC or CC	
AA	AB	BC	BD	$((1+b)/4)/b$
AB	BC	AC	AC	
AB	BC	CD	CE	$((1+c)/4)/c$
AA	AB	CD	AD	$(b/4)/b$
AB	AB	CC	CC	
AB	AB	CD	DE	$((a+b)/4)/(a+b)$
AB	AB	BC	AC	$((2+a+b)/4)/(a+b)$
AB	AC	CD	CC	
AB	BB	BC	BB	$((2+c)/4)/c$
AB	AB	BC	CC	$((1+a+b)/4)/(a+b)$
AB	AA	BC	CD	$(a/4)/a$

Table S3 - Formulas to calculate PI considering the profile configurations depicted with AS STRs, in case 2.

Daughter	Putative Grandmother	Putative Grandfather	Formulas
AB	AB	BC	$(a+2b)/8ab$
AB	AC	CD or DE	$1/8a$
AB	BB	CD	$1/4b$
AB	BC	CD or DE	$1/8b$
AB	AB	CC	$(a+b)/8ab$
AB	BC	AD or BD	
AA	AA	AB	$3/4a$
AA	BC	AD	$1/4a$
AA	AA	AA	$1/a$
AB	BB	BC	$3/8b$
AB	AB	AB	$(a+b)/4ab$



Table S4 - Formulas to calculate PI considering the profile configurations depicted with AS STRs, in case 3.

Mother	Daughter	Putative Grandmother	Putative Half-sister 1	Putative Half-sister 2	Mother of Putative Half-sister 2	Formulas
AB	AC	BD	BE	BD	AD	
AB	BC	CD	CD	BD	BC	$c/2c$
AB	BC	DD	DE	DF	BF	
AA	AB	CC	CD	BB	BE	
AB	BB	AB	BB	AA	AB	$1/2b$
AB	BB	AB	AA	BB	AB	
AB	BC	CD	BC	BB	AB	$(1/2)/2c$
AA	AB	BC	BB	BD	CD	$(1+b)/2b$
AB	BC	CC	CC	BC	BD	$(1+c)/2c$
AB	AB	CD	CD	AB	AE	$1/2ab$
AB	AB	CD	DE	BC	CC	
AB	BC	BC	CD	BE	BD	
AB	BC	DE	FG	EF	DE	$1/2c$
AB	BC	AD	CE	CF	DF	
AB	AB	AA	AB	AB	BB	$(1+a+b)/((2(a+b)))$
AB	BC	CD	AC	DE	AE	$((c/(a+c))/2c$
AB	AB	BC	AB	AA	AD	$1,5/2ab$
AB	BC	AD	AC	DE	EE	$((c/(a+c))/2c$
AA	AA	AA	AB	AC	AC	$(1+a)/2a$
AA	AA	AA	AA	AB	BC	
AB	BB	AB	AA	AA	AB	$b/2b$

Table S5 - Formulas to calculate PI considering the profile configurations depicted with AS STRs, in case 4.

Mother	Daughter	Putative Half-sister	Mother of Putative Half-sister	Formulas
AB	BC	BD	BB	
AB	BC	AD	AA	
AB	BC	CD	CC	$c/2c$
AB	BC	DE	DF	
AB	AB	CD	AC	$(a+b)/2(a+b)$
AA	AB	BC	CC or CD	
AA	AB	BB	BB	$(1+b)/2b$
AB	BB	BC	AC	
AB	BC	CD	AD or BD	
AB	BC	CC	CD	$(1+c)/2c$
AB	BC	BC	BB	
AB	AB	AC	CD	$(1+a+b)/2(a+b)$
AA	AB	AC	BC	$b/2b$
AA	AB	AB	AB	$((b/(a+b))+b)/2b$
AB	AB	AC	AC	$((a/a+c)+(a+b))/2(a+b)$
AA	AA	AB	BC	$(1+a)/2a$

**Table S6: Genotypes and frequencies of the allele for X-linked STRs transmitted by the deceased father.**

Case 1	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DSX7423	DXS6809	DXS7132	DXS9902	DXS6789
Mother	11-12	12-13	9-9	13-13	9-10	14-15	30-33	13-14	11-12	20-20
Daughter	11-12	13-13	9-10	13-13	6-10	14-14	30-33	14-14	11-12	20-21
Putative Grandmother	11-12	12-13	9-10	11-13	6-11	13-14	32-33	14-16.3	11-13	21-21
FADF	0,322 0,266	0,235	0,162	0,244	0,147	0,322	0,039 0,302	0,329	0,360 0,351	0,244
Case 2	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DSX7423	DXS6809	DXS7132	DXS9902	DXS6789
Daughter	11-12	8.3-13	9-11	9-13	8-11	14-14	32-35	11-14	11-13	15-20
Putative Grandmother	10-12	12-13	9-9	12-13	8-8	14-15	32-34	13-14	11-11	20-23
FADF	0,266	0,235	0,453	0,244	0,166	0,322	0,169	0,329	0,360	0,402
Case 3	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DSX7423	DXS6809	DXS7132	DXS9902	DXS6789
Mother	10-12	8.3-13	10-11	11-12	9-12	14-15	33-33	15-16	12-13	21-22
Daughter	10-12	12-13	11-11	11-12	8-12	15-15	33-34	15-15	12-13	21-21
Putative Grandmother	10-11	12-12	11-11	11-12	8-10	14-15	34-35	15-15	12-13	21-21
Putative Half sister 1	10-12	12-13	11-12	11-12	8-10	14-15	30-34	15-15	12-14	21-26
Putative Half sister 2	10-11	12-14	9-11	9-12	8-11	14-15	33-34	15-15	12-13	20-21
Mother of Half sister 2	11-11	11-14	9-11	9-13	6-11	13-14	32-33	15-15	11-13	20-22
RAPF	10	12	11	12	8	15	34	15	12	21
FADF	0,366 0,266	0,305	0,323	0,254	0,166	0,395	0,195	0,221	0,351 0,208	0,244
Case 4	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DSX7423	DXS6809	DXS7132	DXS9902	DXS6789
Mother	12-12	13-13	9-11	12-12	11-12	14-15	33-36	12-15	11-12	21-23
Daughter	12-12	13-13	9-11	12-15	11-12	15-15	33-36	12-17.3	11-13	22-23
Putative Half sister	10-12	13-13	9-9	9-15	11-11	14-15	33-34	16-17.3	12-13	21-22
Mother of Half sister	10-12	8.3-13	9-10	9-11	10-11	14-15	34-37	15-16	12-12.1	21-22
RAPF	10 or 12	13	9	15	11	14 or 15	33	17.3	13	21 or 22
FADF	0,266	0,235	0,453 0,323	0,030	0,225 0,101	0,395	0,302 0,030	0,030	0,208	0,130

RAPF – reconstructed allele of putative father

FAF- frequencies of allele transmitted by the father