International Congress Series 1288 (2006) 310–312

www.ics-elsevier.com

In-house validation of the PCR amplification kit «Mentype® Argus X-UL»

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Abstract. The forensic utilization of the Mentype® Argus X-UL PCR amplification kit, that contains 4 X-STRs plus the Amelogenin, was evaluated in this study. Preliminary tests showed that the diminution of PCR reaction volume to 12.5 μl increased the sensitivity of the kit. With these conditions, full genetic profiles were obtained with 100 pg DNA. Female/male DNA mixtures produced full profiles from the female or male minor contributor with respectively 2-fold and 5-fold excess of the major contributor. Intra- and inter-day reproducibility of allele sizing, stutter height and heterozygous balance were compatible with the forensic use of this kit. Allelic frequencies and other population data were generated by analyzing 100 persons of Swiss Caucasian origin. © 2005 Published by Elsevier B.V.

Keywords: STR; Paternity testing; Identification; X-chromosome

1. Introduction

Conventional paternity cases, for which samples from the mother, a putative father and one or several children are available, are easy to handle with autosomal short tandem repeat markers (STRs). However, these latter may reach their limits for the investigation of complex kinships or cases for which samples of key persons are not available (deficiency cases). Indeed, the more distant the relatives, the less chance they have to share autosomal alleles that are identical by descent. Excluding mutations, the same mitochondrial haplotype is shared by every members of a maternal lineage. Similarly, a unique Y-chromosome haplotype should be present in every men belonging to the same paternal

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0531-5131/ © 2005 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.08.042

lineage. These haploid markers may thus efficiently complement autosomal STRs to unravel paternity tests involving distant relatives. In some circumstances, X-chromosome STRs (X-STRs) may have similar features [1]. Therefore it could be useful to evaluate the forensic utilization of these X-markers.

The Mentype® Argus X-UL PCR amplification kit (BioType AG) has recently been commercialized. This kit contains 4 X-STRs: DXS8378, HPRTB, DXS7423 and DXS7132 plus the gender marker Amelogenin as an amplification control. In this study, 100 persons of Swiss Caucasian origin were analyzed in order to validate the forensic utilization of this kit and to determine some population data.

2. Material and methods

Blood or buccal swabs were obtained from 100 unrelated volunteers of Swiss Caucasian origin. DNA was extracted with the QIAamp DNA Mini Kit following the manufacturer's instructions. PCR amplifications were performed using the Mentype® Argus X-UL PCR amplification kit according to the manufacturer's protocols as well as with half PCR reaction's volume. Amplified DNA was analyzed on an ABI Prism 3100 with GeneMapper software from Applied Biosystems.

The detection threshold of the Biotype X-STR kit was determined by analyzing dilutions of the sample XX74 (from 1000 to 6.25 pg/μl DNA). DNA mixtures from the female XX74 and male XY1 reference samples from the kit were typed to determine the detection threshold of the minor fraction of the mixture. Concentration ratios tested were from 40 : 1 to 1 : 1. The intra-day genotyping precision was evaluated by analyzing 10 independent amplifications of the sample XX74 on the same run. This sample was further analyzed 14 times on different days to estimate the inter-days genotyping precision. The electropherogram's peak height was used to calculate the stutter percent and the heterozygous balance. Observed heterozygosities (Ho) were calculated as direct counts. Allele frequencies and expected heterozygosities (He) were computed with the FSTAT package [2]. Hardy–Weinberg equilibrium was tested for each locus by permuting alleles among individuals with the same program.

3. Results and discussion

A forensic evaluation of the Mentype® Argus X-UL PCR kit is presented in this study. Preliminary results showed that the diminution of PCR reaction volume from 25.0 to 12.5 μl allowed to enhance the sensitivity of the kit. With the smallest volume, allelic peaks were 2 to 5 times higher and full profiles were obtained with 100 pg DNA. Mixture analyses illustrated that full female profiles could be detected with an excess of 2 : 1 male DNA. This was not symmetrical since full male profiles could be detected with an excess of 5 : 1 female DNA. This reflects that, with equal DNA concentration, the number of copies of homozygous male alleles was twice that of heterozygous female alleles. Some alleles of the female or male minor profile could be sporadically detected even with an excess of 40 : 1 of the major contributor.

Standard deviations of allele sizes were comprised between 0.01 bp for the DXS7423 13-allele and 0.05 bp for the DXS8378 11-allele when replicate analyses of the XX74 reference sample were conducted on the same run. When this sample was analyzed on different days, standard deviations increased and ranged from 0.04 bp for the DXS8378 11-allele to 0.14 bp for the DXS7132 15-allele. Three standard deviations were below 0.5 bp, meaning that a genotyping precision of 1 bp could be

Table 1
Allele frequencies and other characteristics of the 4 X-STRs in the Swiss population

Locus	DXS8378	HPRTB	DXS7423	DXS7132	Samples considered
Allele 8	–	0.007	–	–	50 women and 50 men
Allele 9	0.033	0.007	–	–	
Allele 10	0.307	–	–	–	
Allele 11	0.340	0.147	–	0.020	
Allele 12	0.280	0.320	–	0.093	
Allele 13	0.033	0.280	0.087	0.293	
Allele 14	0.007	0.167	0.287	0.313	
Allele 15	–	0.067	0.360	0.200	
Allele 16	–	0.007	0.220	0.053	
Allele 17	–	–	0.047	0.020	
Allele 18	–	–	–	0.007	
Stutter size [%]	6.7 ± 2.5	9.5 ± 2.1	6.6 ± 1.6	9.8 ± 3.5	
Heterozygous balance [%]	89.8 ± 7.6	92.0 ± 7.3	90.8 ± 7.9	90.1 ± 8.6	50 women
Ho	0.700	0.820	0.700	0.680	
He	0.719	0.759	0.721	0.775	
P (H–W)	0.44	0.83	0.56	0.11	

achieved. The mean stutter size per locus was comprised between 6.6% and 9.8%, the heterozygous balance between 89.8% and 92.0% (Table 1). A maximal stutter value of 28.3% was observed at the locus DXS7132 and the lowest heterozygous balance observed was 70.4% at the locus DXS7423. These latter observations imply that care should be taken when interpreting DNA mixtures with the X-STRs kit tested here.

Allele frequencies and other population parameters given in Table 1 are very similar to those reported in the German population [3]. No deviations from H–W equilibrium were found within the sub-sample of 50 women (Table 1). The Mentype® Argus X-UL PCR amplification kit is therefore suitable for its forensic use in our laboratory.

Acknowledgements

The authors wish to thank the technicians of the laboratory and especially Alessandra Arlettaz who worked on this X-STRs project during her 6 months' probationary period at the institute.

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