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Validation of the Mentype® Argus X-UL kit

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Abstract. The Argus X-UL kit is a commercial multiplex system which contains Amelogenin for gender determination as well as four uncoupled X-chromosomal STR markers (DXS8378, HPRTB, DXS7423 and DXS7132). In this study, we present the results of some forensic validation studies including the following aspects: sensitivity, analysis of female/male mixtures, validation of our protocol consisting of blood on FTA cards and amplification in a small PCR reaction volume (10 ul). The use of these markers in a deficiency paternity case will also be shown. © 2006 Elsevier B.V. All rights reserved.

Keywords: X chromosome STR; Validation; Deficiency paternity testing

1. Introduction

With the aim of using X-chromosomal polymorphic markers in Swiss crime cases (female DNA on a male background) and particularly in kinship testing, a validation study of the Mentype® Argus X-UL kit was performed. The Argus X-UL kit is a commercial multiplex system which contains Amelogenin for gender determination as well as four uncoupled X-chromosomal STR markers (DXS8378, HPRTB, DXS7423 and DXS7132). In this study, we present the results of some forensic validation studies including the following aspects: sensitivity, analysis of female/male, female/female and male/male mixtures, validation of our protocol from blood on FTA cards and amplification in a small PCR reaction volume (10 μl). The use of these markers in a deficiency paternity case will also be shown.

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2. Materials and methods

PCR amplification was performed using the Mentype® Argus X-UL kit according to the instruction manual. The amplified products were detected using an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

3. Results and discussion

Robust and reproducible amplification results were obtained for the 4 loci (+amelogenin) for DNA template input of as low as 20–50 pg (30 cycles).

Several mixtures studies showed that the minor component could be detected at proportions up to 1:10. This is particularly important when female DNA traces are investigated against a male background, for instance female traces under fingernail scrappings of a man.

Our paternity cases are processed from blood on FTA cards. We wished to perform PCR reactions at low volumes (total reaction volume of 10 μl) of our blood punchouts using the Argus X-UL kit and obtain the same results in terms of quality and reproducibility that we would expect at the recommended volumes. The preparation of the FTA punchout 1.2 mm (disc) was done according to indications of the Whatman® protocol. Because of the small PCR volume and the quantity of DNA present on the disc it is important to reduce the number of PCR cycles. The number of PCR cycles was varied from 24 to 30 in order to determine the adequate number of PCR cycles. The best results were obtained for 26 cycles, with peak heights in the range of 1000–3000 rfu.

As an example of the application of X-STRs, a deficiency paternity case is provided. A deceased alleged father is believed to be the biological father of a child (daughter, D1). Available for testing are the mother (M1) and her daughter (D1) and a second mother (M2) and her daughter (D2), for which the paternity of the deceased man is not in question (Fig. 1).

The X-haplotypes of the biological fathers (F1 and F2) of daughter 1 (D1) and daughter 2 (D2) can be determined after comparing the daughters X haplotype with their mothers X haplotype. The X haplotypes of the biological fathers of D1 and D2 are different (Table 1).

One can therefore exclude with certainty that the two children have the same biological father. Under the hypothesis that the deceased man is the biological father of D2 one can therefore exclude him as being the father of D1. The limitation of X-STR markers in paternity testing is that only female children can be tested.

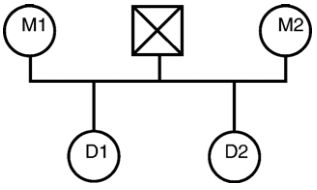

Fig. 1. Deficiency paternity case.

Table 1
X haplotypes of M1, D1, M2, D2 and F1 (father of D1) and F2 (father of D2)

	Amelogenin	DXS8378	HPRTB	DXS7423	DXS7132
M1	XX	10,11	10,12	15,16	13
D1	XX	10,12	10,11	16	13,14
F1	XY	12	11	16	14
M2	XX	11	13	14,15	14
D2	XX	10,11	13	14	14,15
F2	XY	10	13	14	15

This case clearly demonstrates that X-STR markers have the potential to solve some paternity cases, which cannot be solved by using autosomal markers.