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SHORT COMMUNICATION

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DYS19 and amelogenin in artificial blood stains with defined amounts of male and female cells

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Abstract The sensitivity of the DYS19 and the amelogenin STR systems for amplifying Y-specific fragments was assayed using artificial bloodstains with varying amounts of male and female (non-template) DNA in different ratios. The study confirmed the high sensitivity of both systems in detecting male-specific PCR fragments in stains containing 10–25 template molecules even in the presence of large amounts of female DNA in the mixture by silver-stain detection. However, blood mixtures which contain less than 10% male cells could be reliably typed only when at least 100 template molecules were present in the artificial bloodstain, due to increasing amounts of hemoglobin from the female blood which is a PCR inhibitor.

Key words DYS19 · Amelogenin · Artificial bloodstains · STR system · Sensitivity

Introduction

The commonly used and well characterized Y-chomosomal marker DYS19 [1, 2] exhibits a repeat nucleotide sequence with the tetranucleotide structure $(GATA)_3$ GGTA $(GATA)_{10–14}$. The size of amplified fragments ranges from 186 bp (13 GATA repeats) to 202 bp (17 GATA repeats) in Caucasian populations. Due to its chomosomal location only male individuals posesses alleles. The amelogenin system [3, 4] provides an X-chromosome (106 bp) and a Y-chromosome specific fragment (112 bp). Therefore with both STR systems Y-specific and therefore male DNA can be detected, but only with DYS19 can information on a specific male be obtained.

The aim of present study was to examine the overall sensitivity of both STR systems and the capability of detecting Y-specific fragments in mixtures with stain material (blood) originating from male and female individuals. Therefore these STRs were tested using artificial blood stains spotted onto filter paper. Male and female DNA was present as a mixture defined by the number of male and female white blood cells in the stain. This application represents practical casework where male cells to be analysed in a male/female mixture are not spermatozoa, which otherwise could be separated by differential lysis [5, 6]. It provides information about the sensitivity of the STR system by using template molecules per examined stain and not per PCR sample and therefore confirms also the quality and efficiency of the DNA extraction.

In addition, population data for 100 unrelated male individuals from the Frankfurt/M. area (Germany) were generated.

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Materials and methods

Blood stain preparation

A blood cell count was performed for a male and a female donor and the blood samples were mixed resulting in a defined ratio of male to female white blood cells, i.e. 100, 75, 50, 25, 10, 5, 2 and 1% and no male cells in the mixture. These samples were diluted with 0.9% NaCl solution to concentrations of 5–1000 cells per µl and 2–10 µl was spotted onto filter paper (Whatman 3 MM) to produce artificial bloodstains with 10000, 5000, 2000, 1000, 500, 100, 50, 20 and 10 cells in total. So each blood stain contained a defined amount of template molecules (i.e. the amount of Y-chromosomes) together with a defined amount of non-template molecules per stain.

DNA extraction, amplification and detection of the PCR products

Each blood stain was completely cut out from the filter paper and DNA was extracted using standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation [7]. The DNA from each blood stain was dissolved in 20 µl distilled water.

DYS19 and amelogenin PCR was carried out using 50% of the DNA of each sample (10 µl), i.e. half the number of cells per blood stain was analysed in one PCR-reaction. For amplification the following conditions were used: 10 mM Tris HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM of each dNTP, 160 µg/ml BSA, 0.2 U Amplitaq polymerase, pH 8.3, in a volume of 25 µl. A Perkin Elmer

2400 Thermocycler was used applying 30 s at 94 °C, 30 cycles with 30 s for denaturation at 94 °C, 30 s annealing at 60 °C (amelogenin) or 56 °C (DYS19) and 30 s extension at 72 °C followed by a final extension step of 7 min at 72 °C. Primers were applied as described elsewhere [1, 3] at a concentration of 10 pmol of each primer per reaction. The PCR products were separated on 6.5% native polyacrylamide gels and detected after silver staining. Each experiment was repeated three times.

Preliminary experiments (data not shown) were performed to find out the area of the detection limit, so the analysis of stains with clear positive or clear negative results was not necessary.

Population study using DYS19

DNA samples from 100 unrelated Caucasian male individuals were amplified as described above and typed by comparison with an allelic ladder to obtain population data.

Results and discussion

The present investigation demonstrates that the sensitivity of DYS 19 and the amelogenin system is high enough to amplify about 10 template molecules even in the presence of a 100-fold excess of non-template (female) DNA (Tables 1 and 2). Both PCR-systems exhibited the same high sensitivity. However, the reliability of an unambiguous typing is reduced in mixtures containing only 10% or less male DNA. For exact typing of these samples a minimum of about 100–250 template molecules was found to be necessary. However, a related study presented by Prinz et al. [8] which examined the detection of alleles of four Y specific STRs in a multiplex system showed a detection limit of 50 pg male DNA without female DNA and a detection limit of 400 pg male DNA within 800 ng female DNA. Assuming a DNA content of ca. 6.6 pg DNA per diploid cell, 50 pg DNA corresponds to an amount present in 7–8 cells. In this study the sensitivity of both STRs was also found to be in this order of magnitude (10–20 cells) indicating an efficient DNA extraction procedure. When analysing mixtures, Prinz et al. [8] demonstrated that an amount of DNA representing about 60 male cells can be detected in a mixture where the amount of female DNA represents about 12000 cells. However, in the present study blood stains instead of isolated DNA were used. Therefore the DNA extracted was not as pure as the DNA used by Prinz et al. [8]. With increasing amounts of female DNA the amount of hemoglobin contamination per sample can also increase, which is known to be an inhibitor of the PCR process [9].

The aim of present study was to confirm the applicability of DYS19 and amelogenin on stains of male/female mixed origin and not on mixed DNA samples. As indicated mixed samples with negative results for a supposed male component should be interpreted carefully with these STRs in practical casework, particularly when the ratio of male/female cells in the stain is unknown. Considering their sensitivity, the DYS19 and the amelogenin systems are equally applicable in forensic casework when male DNA has to be detected in a mixture of male and female DNA. It should be taken into account that in samples containing very degraded DNA, the sensitivity of DYS19 may be more affected than the amelogenin system, be-

Table 1 Typing results using the DYS19 system

Number of total cells in PCR	Percentage of male cells								
	100%	75%	50%	25%	10%	5%	2%	1%	0
5	–	–	–	–					
10	0	0	0	–					
25	+	+	+	0					
50	+	+	+	0					
250	+	+	+	+	+	0	–	–	–
500					0	0	0	0	–
1000					0	0	0	0	–
2500					+	+	0	0	–
5000					+	+	+	0	–

– = negative results in all three experiments, + = positive results in all three experiments, 0 = positive result in only one or two out of three experiments

Table 2 Typing results using the amelogenin system

Number of total cells in PCR	Percentage of male cells								
	100%	75%	50%	25%	10%	5%	2%	1%	0
5	–	–	–	–					
10	0	0	0	–					
25	+	+	0	–					
50	+	+	+	+					
250	+	+	+	+	0	–	–	–	–
500					0	–	–	–	–
1000					+	0	0	–	–
2500					+	+	0	–	–
5000					+	+	+	0	–

– = negative results in all three experiments, + = Y-specific fragment detected in all three experiments, 0 = detection of the Y-specific fragment only one or two out of three experiments

Table 3 Allele frequencies in a sample of 100 unrelated males

Allele	Frequency
13 (186 bp)	0.12
14 (190 bp)	0.44
15 (194 bp)	0.22
16 (198 bp)	0.14
17 (202 bp)	0.09

cause of the larger fragments generated (about 200 bp in DYS19 vs. about 100 bp in the amelogenin system). In addition, the X-chromosome specific fragment of the amelogenin system can be considered as an internal control of the failure of the PCR in the individual reaction. On the other hand, a positive DYS19 signal does not only indicate "male" but also gives individual-specific fragments.

The population study revealed five alleles with frequencies similar to other Caucasian populations (Table 3) [1, 10, 11].

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