

A new primer set in a SRY gene for sex identification

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Abstract. Determination of sex using the amelogenin sex test has been routinely performed in forensic casework, prenatal diagnosis and DNA data basing. However, the test does not appear to be entirely reliable. We designed a new pair of primers that target a section of the SRY gene resulting in 96-bp male-specific PCR fragment that can be coamplified with multiplex STR kits which assures unambiguous and quick gender identification. The applicability and specificity of the primers were confirmed with DNA from *DAM* sample. © 2005 Published by Elsevier B.V.

Keywords: Sex determination; Amelogenin; *DAM*; SRY; Multiplex STR kit

1. Introduction

Sex determination not only can be an important piece of information in various forensic investigations, especially in sexual assault cases, but can also be useful in prenatal diagnosis of foetus with a known family history of genetic disorder affecting only male children. Although different PCR-based methods are known to identify a sample as originating from a male or a female, the only sex test included in commercially available human identification PCR kits for gender determination is based on the amelogenin sex test described by Sullivan et al. [1]. The test is quick and effective, however, new studies showed that it is not always reliable. The frequency of the deletion of Y copy of the amelogenin gene has been reported that occurs between 8% and 0.018% depending on the population [2–7] and “deleted-amelogenin males” (designed as *DAMs*) would have been identified as women. Considering the consequences of the result obtained using only an amelogenin marker, some authors have already reported the use of new genetic markers lying on the sex-determination region Y (SRY) on the Y chromosome [2,4,5,7–9]. In all cases, the accuracy of sex determination has been tested by an additional PCR reaction

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using only the primers for SRY or the combination of primers for SRY and amelogenin. But this procedure is labourious and time consuming. To overcome the drawback, we investigated possibilities to design a new pair of primers so that the size of the SRY amplification product as well as PCR conditions would suit the amplification reactions of commercially multiplex STR kits. We designed a new primers set which after amplification yielded a 96-bp product. In this study, a newly designed SRY marker was analysed for determination of sex as a part of multiplex STR kits regarding its sensitivity and specificity by testing with low amount of male DNA and with high amount of female DNA, respectively. In the last stage, the applicability of SRY marker was tested with DNA from deleted-amelogenin male.

2. Materials and methods

At first, we tested DNA from 9947A and 9948 cell-lines declared as female and male DNA, then from three samples of which two were randomly female and male, while the last one was a deleted-amelogenin male from our previous study [7]. Buccal epithelial cells were collected from persons using cotton swabs. DNA from these samples was isolated according to Walsh et al. [10]. The amplification of a male-specific SRY marker situated in the Y chromosome sex determination region was amplified using newly designed forward primer SRY-150F (5'AGC AGT CAG GGA GGC AGA TCA 3') labelled with 6-Fam, while the reversed primer was SRY-245R as described by Lo et al. [8]. The newly designed SRY primers flank a 96-bp region. A singleplex amplification was carried out in a 25- μ l total volume containing 1 U AmpliTaq Gold Polymerase (Applied Biosystems), 0.25 μ M (0.5 μ M, 0.75 and 1.25 μ M) of each primer and 2.5 μ l 10 \times Gold STR buffer (AB). Concentration of DNA varies from 0.25 ng to 10 ng. PCR reaction was performed on each of the templates. Samples were amplified through 28 cycles comprising 1 min at 94 $^{\circ}$ C, 45 s at 60 $^{\circ}$ C (or 45 s at 59 $^{\circ}$ C) and 1 min at 72 $^{\circ}$ C following initial denaturation at 95 $^{\circ}$ C for 11 min and final incubation at 72 $^{\circ}$ C for 60 min. In the next stage of analysis, a new primer pair binding to the SRY gene was coamplified with the commercial multiplex PCR kits AmpFISTR SGM Plus and PowerPlex 16 according to the technical manuals. Separation and detection of the alleles were carried out using capillary electrophoresis on an ABI 310.

3. Results and discussion

In our previous study, we showed that the individual, although a phenotypically normal male, was genotyped as female using either the AmpFISTR Plus or PowerPlex 16 kits according to the deletion of Y chromosome-specific amelogenin [7]. The male nature of the individual has been identified after additional PCR reactions using primers for different Y-specific markers (e.g. SRY and STR). The SRY marker we used was described by Lo et al. [8] and yielded 137-bp PCR product that overlaps in size with some alleles from STR loci used in multiplex kits, so additional PCR reaction for determination of gender must be performed. We investigated the sequences between those forward and reverse primers of SRY marker described by Lo et al. [8] for possible primer that produces smaller PCR fragment. These sequences were analysed with respect to coamplification with the STR primers in different commercial multiplex kits. We decided to use the same reverse primer SRY-245R as in our previous study while for forward primer we choose the sequences 96-bp upstream from it. At first, different amplification conditions and primers concentrations were tested

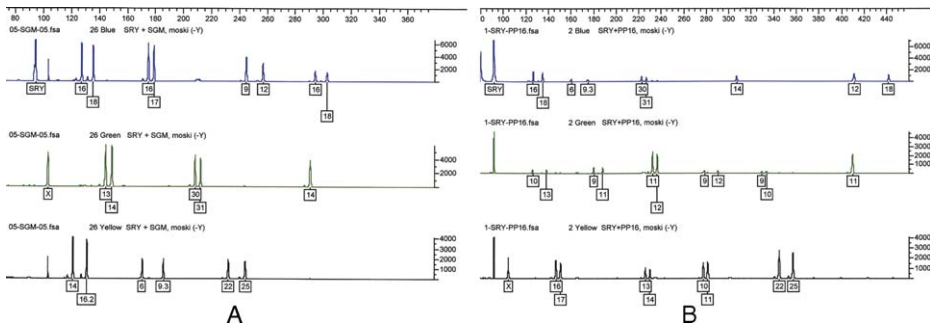


Fig. 1. Panel A shows electropherogram of sample from deleted-amelogenin male coamplified with SRY marker and AmpFISTR SGM Plus kit, while panel B shows electropherogram of sample from deleted-amelogenin male coamplified with SRY marker and PowerPlex kit. It can be clearly seen that the amelogenin Y-specific PCR product is lacking, while a peak ~92 bp identified as the 96-bp SRY amplification product is seen in both panels.

using DNA isolated from 9947A and 9948 cell-lines. We tested the amplification at two annealing temperature: 60 °C and 59 °C while both are used in commercial multiplex STR kits. We found no difference in amplification between them. In the male sample, the amplification resulted in only one band peak with apparent size ~92 bp, putatively identified as the 96-bp SRY amplification product. We did not observe any reproducible peaks over minimum threshold in the female sample even with high quantity of female DNA at the concentration of 0.6 μ M for forward primer and 1.25 μ M for reversed primer. We obtained clear peak even with 0.25 ng of template. After the optimisation of amplification for SRY marker, we succeeded to coamplify the new designed SRY marker with STR loci in AmpFISTR SGM Plus as well as in PowerPlex multiplex kits without any artefact using male DNAs, but it was absent from females. Finally, the male phenotype of the individual who was genotyped as female by lacking the amelogenin Y-specific PCR product was confirmed by the amplification of SRY marker using both multiplex STR kits, respectively (Fig. 1).

References

- [1] K.M. Sullivan, et al., A rapid and quantitative DNA sex test: fluorescence-based PCR analysis X–Y homologous gene amelogenin, *BioTechniques* 15 (1993) 636–641.
- [2] F.R. Santos, A. Pandya, C. Tyler-Smith, Reliability of DNA-based sex tests, *Nat. Genet.* 18 (1998) 103.
- [3] P.E. Roffey, C.I. Eckhoff, J.L. Kuhl, A rare mutation in the amelogenin gene and its potential investigation ramifications, *J. Forensic Sci.* 45 (5) (2000) 1016–1019.
- [4] K. Thangaraj, A.G. Reddy, L. Singh, Is the amelogenin reliable for gender identification in forensic casework and prenatal diagnosis? *Int. J. Leg. Med.* 116 (2002) 121–123.
- [5] M. Steinlechner, et al., Rare failures in the amelogenin sex test, *Int. J. Leg. Med.* 116 (2002) 117–120.
- [6] A. Michael, P. Brauner, Erroneous gender identification by amelogenin sex test, *J. Forensic Sci.* 49 (2) (2004) 258–259.
- [7] K. Drobníč, Unusual failure of gender determination using amelogenin test, Poster Presented at Third European–American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, 2003 Sept 1–5, p. 36, Zagreb, Croatia.
- [8] Y.M. Lo, et al., Quantitative analysis of fetal DNA in maternal plasma and serum: implication for noninvasive prenatal diagnosis, *Am. J. Hum. Genet.* 62 (1998) 768–777.
- [9] B. McKeown, J. Sickley, A. Riordan, Gender assignment by PCR of the SRY gene: an improvement on amelogenin, *Prog. Forensic Genet.* 8 (2000) 433–435.
- [10] P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, *BioTechniques* 10 (1991) 506–513.