

Alternative primers for DYS391 typing: advantages of their application to forensic genetics

Leonor Gusmão^{a,b}, Annabel González-Neira^a, Paula Sánchez-Diz^a,
María Victoria Lareu^{a,*}, Antonio Amorim^{b,c}, Angel Carracedo^a

^a*Institute of Legal Medicine, University of Santiago de Compostela, E-15705 Santiago de Compostela, Galicia, Spain*

^b*IPATIMUP, Instituto de Patologia e Imunologia Molecular, Universidade do Porto, Porto, Portugal*

^c*Faculdade de Ciências, Universidade do Porto, Porto, Portugal*

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Abstract

The amplification of the STR DYS391, using the primers described in the Genome Data Base (GDB: G00-365-251), shows not only an additional band to the Y-specific one in males with a size range of 26 bp less than those of DYS391 locus alleles, but also a polymorphic pattern in females in the same size range as the additional band observed in males. The DYS391 pattern in families reflects a Y-specific linked locus and also a polymorphic X locus with an X-linked pattern of inheritance. A first screening in the X homologous locus allowed the identification of five different alleles. Allele frequencies were explored in different population groups for both the Y locus and the homologous locus in the X chromosome showing a similar allele distribution pattern in the X and Y homologous loci. An alternative reverse primer was designed to amplify the Y-chromosome specific STR in order to improve the specificity and applicability of this system to forensic genetics. Comparative results of the amplification with the new and the previously described primers proved that with this new primer there is a substantial increase in the specificity of the amplification. Moreover, a smaller fragment is amplified with a size out of the range of the alleles of the other Y-STRs usually used in forensic applications, therefore simplifying its inclusion in multiplex systems. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: DYS391; Forensics; X-chromosome STR; Y-chromosome STR; X–Y homology

*Corresponding author. Tel.: +34-81-582-327; fax: +34-81-580-336.

E-mail address: apimllar@uscmail.usc.es (M.V. Lareu)

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1. Introduction

Although different kinds of polymorphism were described on the haploid male-specific portion of the human Y chromosome, STRs proved, until now, to be the most suitable markers in forensic genetics due to their diversity levels, relatively low mutation rates and technical simplicity [1–5]. Since the number of Y-STRs is limited, the presence of artefacts in the amplification does not necessarily imply discarding the use of a particular STR in forensics, but we should be aware of possible problems in interpretation. This is the case of DYS391 and DYS393 systems where additional amplification products are present in males and can be typed in females [6,7].

In this work, DNA samples from both males and females were used to amplify the STR DYS391 using the primers described in the Genome Data Base. Segregation studies were performed in paternity cases.

To ascertain the possible homology between the two loci (DYS391 and its X chromosome counterpart) these two STRs were analyzed in male and female samples from the three major human population groups (Caucasians, Africans and Asians).

The major application of the Y chromosome STRs in forensics is the detection of the male component in male–female mixtures. In this context, it is crucial to assure that Y STRs are really Y-specific and that female DNA is not co-amplified. For this reason, sequence analysis was carried out in both the DYS391 locus and in its X chromosome counterpart and new, alternative Y-specific primers were designed.

2. Material and methods

2.1. DNA samples

Population samples ($n=31$ – 132) were obtained from healthy unrelated individuals from three population groups, Caucasians (Northern Portugal), Asians (Macao) and Africans (Mozambique).

Genomic DNA was extracted as described by Valverde et al. [8]. The quantification was performed using fluorescence detection with DyNAQuant 200 (APB, Uppsala, Sweden).

Previously extracted and quantified DNA samples were used to prepare the male/female mixtures. The samples were diluted in order to obtain a DNA concentration of 5 ng/ μ l and then used to prepare the mixtures. From the whole final mixture (1:1–1:80), 2 μ l were used in a 25- μ l PCR reaction volume to amplify DYS391.

2.2. Amplification conditions

PCR amplification was performed using 5 ng of genomic DNA in a 25- μ l reaction volume comprising 1.5 mM MgCl₂, 1 U Taq DNA polymerase, 200 mM of each dNTPs and 0.25 μ M of each primer (Table 1). After a 95°C pre-incubation step for 2 min; 30 cycles: 94°C denaturation for 2 min, annealing at 55°C for 1 min and extension at 72°C for 1 min; then finally a 10-min extension at 65°C, in a Perkin-Elmer thermocycler.

Table 1
DYS391 primer sequences used in this work

Set 1 (in GDB)
Forward 5'-CTATTCATTCAATCATACACCCA-3'
Reverse 5'-GATTCTTTGTGGTGGGTCTG-3'
Set 2
Forward 5'-CTATTCATTCAATCATACACCCA-3'
Reverse 5'-CTGGGAATAAAATCTCCCTGGTTGC'-3
Set 3
Forward 5'-CTATTCATTCAATCATACACCCA-3'
Reverse 5'-CTGGGAATAAAATCTCCCTGGTTGCAAG'-3

2.3. Detection system

Fragment size determination of the PCR amplified products was performed using an ABI 377 automatic sequencer (Perkin-Elmer). After the amplification, 1 μ l of the PCR products was combined with the lane size standard TAMRA500, denaturated and loaded onto a 4.25% polyacrylamide denaturing gel. Genotyping was performed using the Genescan 2.1 Analysis software, with an allelic ladder obtained by the mixture of previously sequenced samples for the most common alleles. Allele nomenclature was made according to Kayser et al. [5].

2.4. Sequencing conditions

For sequence analysis, PCR amplified fragments were separated by electrophoresis, eluted from acrylamide gels according to the method described by Gusmão et al. [9], reamplified and purified with Microspin S-300 HR columns (Pharmacia). A dideoxy cycling sequencing reaction was carried out using ABI Rhodamine Dye terminator kit (PE). The products were purified by ethanol precipitation and run in a 6% denaturing gel on an ABI 377 sequencer. The results were analysed automatically using the Data Collection Software 377-18.

A total of 30 fragments from all the allelic classes found in our population study were sequenced in both the X and Y homologous loci..

3. Results and discussion

3.1. Population data and segregation analysis

Population data is shown in Table 2.

The three populations studied were found to be polymorphic for both X and Y-chromosome loci and in all of them the allele 10 was found to be the most frequent. A higher variation was found for Y-STR allele frequencies between different population groups than for the X-STR. The observed genotype distributions in female samples were in accordance with Hardy–Weinberg expectations.

Table 2
DYS391 and its X homologue allele frequencies

Allele	Population								
	North Portugal			Mozambique			Macau		
	Female X	Male X	Y	Female X	Male X	Y	Female X	Male X	Y
7	0.00	0.00	0.00	0.00	0.00	0.00	9.38	0.00	0.00
8	7.14	7.59	0.00	4.55	8.24	0.00	0.00	0.00	0.00
9	2.38	5.06	5.34	4.55	5.88	2.35	6.25	6.98	4.69
10	66.7	63.3	52.7	65.90	64.70	82.40	65.60	76.70	67.20
11	23.8	24.1	41.2	25.00	20.00	15.30	15.60	16.30	26.60
12	0.00	0.00	0.00	0.00	1.18	0.00	3.12	0.00	1.56
13	0.00	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00
<i>n</i>	42	79	131	44	85	85	32	43	64

The segregation results obtained in the study of father–mother–child trios (obtained from paternity cases with *W* values over 99.999%) were in accordance with what is expected for an X-linked way of inheritance.

3.2. Sequencing results

It is well known that human sex chromosomes have evolved from an ordinary pair of autosomes and the modern X–Y gene pairs are the remaining ‘fossils’ where extensive sequence identity between ancestral X and Y chromosome once existed [10].

The results obtained for the sequenced X and Y chromosome amplified fragments are those shown in Table 3.

For *DYS391*, identical sequences were observed in all the sequenced alleles except for the variable number of TCTA repeats and a base substitution (C/G) at position 87 upstream of the tandem repeat detected on the Y chromosome. For the X homologous locus only, a variation in the number of TCTA repeats was observed.

From these results and from the population data it may be concluded that both sequences in X and Y-chromosomes derived from the same homologous sequence where insertion/deletion and mutation events occurred during the evolution.

3.3. Amplification with primer set 1

When using the previously described primers to amplify *DYS391* (set 1) unspecific amplification occurred out of the range of the alleles for this locus. This unspecific amplification was not responsible for a mistype of the real alleles of this system. However, when male–female mixtures are typed, it is responsible for decreasing the amplification efficiency of the Y-specific alleles in favour of the unspecific amplification. When increasing the female component in the mixture, the amplification of the Y alleles below a certain concentration ratio is not efficient due to the preferential amplification of the major component (X alleles). For this reason, based on the results obtained by sequence analysis of both amplified products a new reverse primer was designed.

Table 3
Sequence results

Fwd primer-TA (TCTG)₃ (TCTA)_nTCTG (CCTA) TCTGCCTG (CCTA)₂TCCCTCTATGGCAATTG
 TA (TCTA)_nTCTG (CCTA)₂ CCTG TCTATGGCAGTTG

CTTGCAACCAGGGAGATTTTATTCCAGGAGATATTTGGCTATGTCTGCAACAATTTTTTTGGTTGTCACAAATGGG
TTTACAACCAAGGAGATTTTATTCCAGGAGATATTTGGCTATGTCTGCAACA TTTTTTGGTTGTCACAAATGGG

ATGAATGTTACTGGCATCTGGTGGGTGGAGCCAGAGATGCTGCTCAACACCCTACAGTGCACAAGA-Rev primer
 ATGAATGTTACTGGCATCTGGTGGGTGGAGCCGGAGATGCTGCTCAACACCCTACAGTGCACAAGGA

Sequence of the 146 bp fragment amplified with the primer set 2. The annealing regions of the forward and reverse primers are underlined.

5' - CTATTCATTCAATCATACAACCCAGTATACAAAGATTAGAAAGCCAGGTAATAGCAAGTTCTGG
 AGAAGATTCCAGGAAACAGGCTCATTCACTGCTGGAGCGCAGCCATTCTGGAGAGCAACC
AGGGAGATTTTATTCCAG - 3'

Sequence results of the larger fragment amplified with the primer set 2. This data corresponds to the sequence of a female DNA sample typed with 10 repeats when amplified with primer set 1. The annealing regions of the forward and reverse primers are underlined. The annealing region of the previously described reversed primer is underlined and in *italic*; in **bold** is marked the A→G transition differentiating Y and X chromosome.

5' - CTATTCCAATCATACAACCCATA(TCTA)₁₀TCTGCCTACCTACCTGTCTATGGCAGTTGTTTACAAC
 CAAGGAGATTTTATTCCAAGGAGATATTTGGCTATGTCTGGCAACATTTTTTTGGTTGTCACAAAT
 GGGATGAATGTTACTGGCATCTGGTGGGTGGAGCCCGGAGATGCTGCTCAACACCCTACAGTGC
 ACAGGACAGACCCACCACAG**GAA**TCTCCATCCAGCCTTAAATGTCCTAGTACTGAATCCAAG
 AAACCCTATCTTAGTATGAAAATGTTGGTCTATCTATACCTGCTTATCCATCCATCCATGTCTAT
 CTACCTAACCTATCCATCCATTCATTCTTATCTTACGTATCCATCTATTCATCCATCCATCCATC
 TATCCATCCATTTATCCATCCGATCTATCTGTCTATCTGTCCATGTCTATCTACCTAACCTATCCA
 TCCATTCACTTATCTTACGTATCCATCTATTCATCCATCCATCCATCTATCCATCCATTTATC
 CATCCGATCTATCTGTCTATCTGTCTGTCTACTCATCTATCTAGCTAGCTAGCTATGGCAGTTGT
 TTGCAACC AGGGAGATTTTATTCCAG - 3'

3.4. Amplification with primer set 2

The design of a new set of primers was undertaken in order to increase as much as possible the number of mismatches with the sequence of the non-Y-specific fragments.

To allow the inclusion of this STR in multiplexes, the primer was also designed bearing in mind the size range of the alleles in other Y-STRs. The fragments amplified with primer set 2 were in a size range between 136 and 156 bp and could be multiplexed with the Y-STRs more commonly used in forensics.

Using the primer set 2 it was possible to observe an inhibition of the amplification of the X-STR previously sequenced. However, with this primer set, two PCR fragments were amplified in addition to the DYS391 alleles:

1. A 146-bp fragment that overlaps with the allele 10 of DYS391 was amplified, both in

male and female samples. Sequence analysis was undertaken and the results are shown in Table 3

2. Larger alleles were amplified in a range of 642–692 bp. These fragments amplify in male as well as female samples and reflect the number of repeats in both X and Y-chromosome STRs previously detected by the primer set 1. From the sequence results it was possible to conclude that using these primers two fragments are amplified, since the annealing site of the reverse primer has been duplicated (Table 3). In male DNA samples, two different size PCR fragments are produced (the larger one comprising the smaller, and both fragments including the DYS391 STR locus). In the amplification of female DNA samples, since this new primer was designed to prevent amplification of the smaller fragment, amplification only occurs in the size range of 642–662 bp. Furthermore, sequence data also provided evidence for the differential amplification efficiency between X and Y chromosome amplified fragments: the transition A/G inside the annealing region of the set 1 reverse primer (Table 3).

3.5. Amplification with primer set 3

To avoid the amplification of the two additional fragments obtained in the amplification when using the primer set 2, a third reverse primer was designed by the addition of 3 bp on the 3' extreme of the primer set 2. The addition of these three base pairs was made in order to create a mismatch in the 3' end of the primer, with all the sequences except the one that corresponds to the DYS391 specific alleles (Table 3).

It was observed that the yield of PCR amplification product was greatly increased when the primer set 3 was used.

3.6. Forensic applications

To check the efficiency of the newly designed reverse primer, comparative studies of the amplification with primer sets 1 and 3 were undertaken. Primer set 2 was not used for this comparison, since this primer was not useful for forensic purposes.

To test the improvement of the Y-specificity with the new reverse primer, a comparative study of the amplification using male and female samples was undertaken. From the results (Fig. 1) it can be concluded that with primer set 3 there is no amplification of the X-chromosome locus. These two primer sets were also used to obtain comparative results of the amplification of different mixtures of male and female DNA (the results are shown in Fig. 2). Using the primer set 1, the amplification of the Y-specific locus starts to fail in mixtures where the male component is less than 1 to 40. On the other hand with primer set 3 we still have amplification in male–female mixtures of 1 to 80.

We have obtained good results in degraded samples (including bones) in which the first set of primers failed to amplify the DYS391 locus. Short STRs and amelogenin still produced reliable results in some of these cases. In all cases the new primer set 3 gave

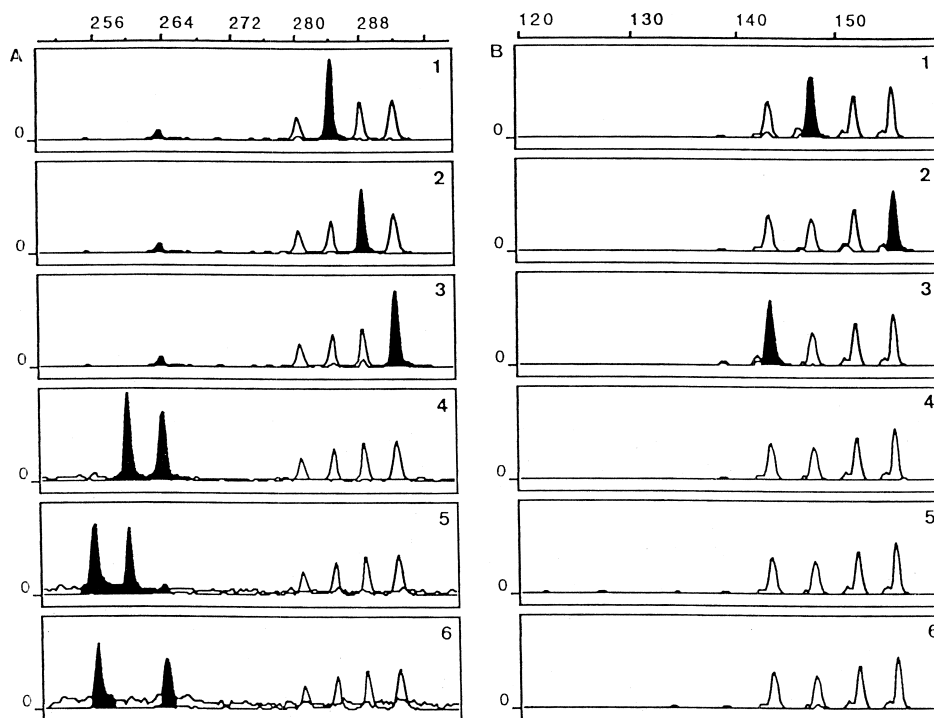


Fig. 1. DYS391 amplification in males (samples 1–3) and females (samples 4–6) using the primer set 1 (A) and primer set 3 (B).

good results and a reliable DYS391 typing was made, proving the better efficiency of this new set of primers over primer set 1.

In conclusion, when compared to the original primer set, the new reverse primer designed to amplify DYS391 (primer set 3) shows a greater suitability for forensic casework since it achieves (i) a higher Y-specificity and (ii) a reduction of the size of the PCR fragment. It also has the advantage that it can easily be included in multiplexes to be detected either in mono or polychromatic platforms, because the amplified fragments do not overlap in size with the other STRs often used in forensic routine.

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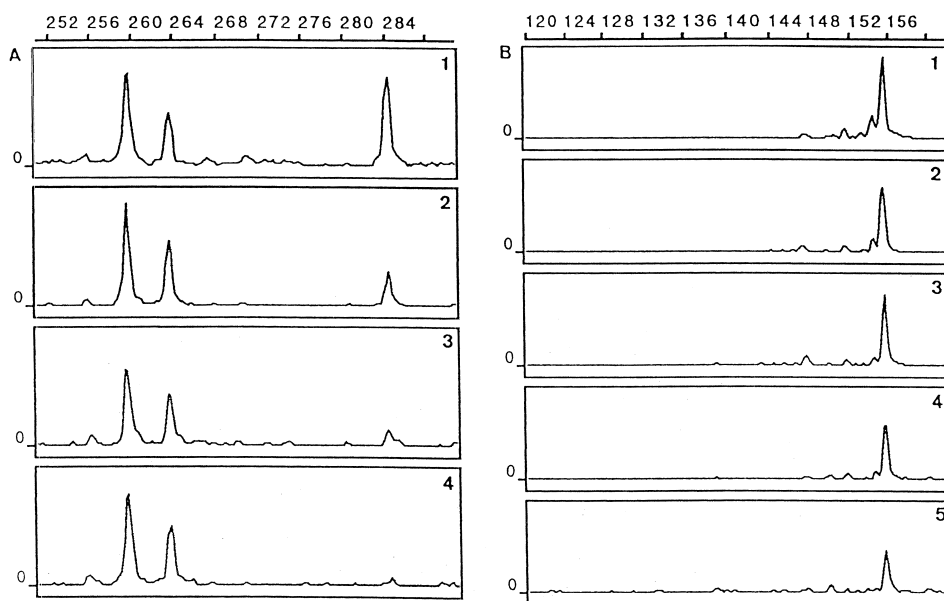


Fig. 2. DYS391 amplification in male–female mixtures at different proportions: male–female 1:5 (1), 1:10 (2), 1:20 (3), 1:40 (4) and 1:80 (5). Results with primer set 1 (A) and primer set 3 (B).

References

- [1] L. Roewer, J. Arnemann, N.K. Spurr, K.H. Grzeschik, J.T. Epplen, Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts, *Hum. Genet.* 89 (1992) 389–394.
- [2] P.A. Underhill, L. Jin, A.A. Lin, S.Q. Mehdi, T. Jenkins, D. Vollrath, R.W. Davis, L.L. Cavalli-Sforza, P.J. Oefner, Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography, *Genome Res.* 7 (1996) 996–1005.
- [3] M.A. Jobling, A. Pandya, C. Tyler-Smith, The Y chromosome in forensic analysis and paternity testing, *Int. J. Leg. Med.* 110 (1997) 118–124.
- [4] P. De Knijff, M. Kayser, A. Cagliá, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, L. Roewer, Chromosome Y microsatellites: population genetics and evolutionary aspects, *Int. J. Leg. Med.* 110 (1997) 134–149, Appendix 141–149.
- [5] M. Kayser, A. Cagliá, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, P. de Knijff, L. Roewer, Evaluation of Y-chromosomal STRs: a multicenter study, *Int. J. Leg. Med.* 110 (1997) 125–133, Appendix 141–149.
- [6] L. Gusmão, A. González-Neira, C. Pestoni, M. Brión, M.V. Lareu, A. Carracedo, Robustness of the Y STRs DYS19, DYS389I and II, DYS390 and DYS393: optimization of a PCR pentaplex. *Forensic Sci. Int.* 106 (1999) 163–172.
- [7] B.M. Dupuy, B. Olaisen, DYS393 and its X-chromosome counterpart. *For. Sci. Int.* (2000), in press.
- [8] E. Valverde, C. Cabrero, R. Cao, Population genetics of three VNTR polymorphisms in two different Spanish populations, *Int. J. Leg. Med.* 151 (1993) 251–256.

- [9] L. Gusmão, A. Amorim, M.J. Prata, L. Pereira, M.V. Lareu, A. Carracedo, Failed PCR amplifications of MBP-STR alleles due to a polymorphism in the primer annealing region, *Int. J. Leg. Med.* 108 (1996) 313–315.
- [10] B.T. Lahn, D.C. Page, Four evolutionary strata on the human X chromosome, *Science* 286 (1999) 964–967.