International Congress Series 1239 (2003) 481–485

A nomenclature for YCA II which is compatible with the ISFG guidelines for Y-STR analysis

Ulrike Schmidt [a,*], Sabine Lutz [a], Lutz Roewer [b]

[a] *Institut für Rechtsmedizin, Albert-Ludwigs-Universität, Albertstr. 9, 79104, Freiburg, Germany*
[b] *Institut für Rechtsmedizin, Humboldt-Universität, Berlin, Germany*

Abstract

In the present study, nine selected whole blood samples containing different alleles of YCA II were analyzed. PCR products were cloned, allelic and stutter inserts were separated and allelic inserts were sequenced. The numeric alleles 1–8 contained 17–24 CA/TG repeat units, respectively. An additional short allele (SA) containing 11 repeat units was also sequenced. Based on our results we propose a repeat-based nomenclature of the locus YCA II and the revision of the nomenclature in the Y STR Haplotype Reference Database (YHRD, available at http://ystr.charite.de).

Keywords: YCA II; Nomenclature; Cloning; YHRD; Extended haplotype

1. Introduction

YCA II is a highly informative dimeric Y-chromosomal short tandem repeat (STR) [1,2] located at the Yq region [3]. Combined with DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385 (= "minimal haplotype"), it builds the so-called "extended haplotype" as it was defined for the online Y STR Haplotype Reference Database (YHRD, available at http://ystr.charite.de). By additional typing of YCA II, 20% more of the European Y chromosomes can be distinguished than by the minimal haplotype alone.

YHRD uses the numeric nomenclature published by Kayser et al. [2]. So far, there is no agreeable repeat-based nomenclature for YCA II. This is probably due to the dinucleotide repeat structure PCR which typically produces a relatively high amount of stutter products 2

* Corresponding author. Tel.: +49-761-203-6869; fax: +49-761-203-6858.
E-mail address: ulischmi@ukl.uni-freiburg.de (U. Schmidt).

0531-5131/03
PII: S0531-5131(02)00480-6

($n-2$) or even 4 ($n-4$) bases shorter than the corresponding main allele peak. As a consequence, sequence analysis of the purified PCR product leads to inhomogenous results.

The aim of this study was to provide consistent sequence information about available alleles of YCA II in order to establish a repeat-based nomenclature for this locus.

2. Materials and methods

DNA was extracted from nine selected whole blood samples containing the numeric YCA II alleles 1–8 [2] and an additional short allele (SA) that was detected in fragment length analysis being approximately 136 bp long (Table 1). Initially, the samples had been typed during an ongoing population study. Designation of numeric alleles was according to defined standards supplied by L. Roewer.

2.1. PCR

Primers used for PCR were the ones described by Mathias et al. [1]. Cycling conditions (PTC-200, MJ Research) were according to Kayser et al. [2] but the annealing temperature was adjusted: 47 °C for four cycles, 46 °C for four cycles and 45 °C for 21 cycles. Results of amplification were checked on a 2.0% agarose gel (TBE) with ethidium bromide.

2.2. Cloning

PCR products (Fig. 1a) were ligated into the cloning vector pCR®2.1-TOPO using the TOPO TA Cloning® kit (Invitrogen, USA) and 20 colonies per sample were picked (sample number 3:18 colonies). Plasmids were isolated from incubated bacterial cultures and purified utilizing the Plasmid Mini® kit (Qiagen, Hilden, Germany). Plasmid DNA was roughly quantified on a 0.8% agarose gel (TBE) with ethidium bromide against defined DNA standards. From each clone picked a PCR was performed (Fig. 1c) using a fluorescence labelled forward primer (5′ FAM–TAT ATT AAA TAG AAG TAG TGA 3′). Fragments were separated and detected on an ABI 373A DNA sequencer.

Table 1
Composition of examined blood samples

Sample number	Numeric YCA II alleles
1	1–1
2	3–3
3	4–4
4	5–5
5	6–6
6	7–7
7	3–8
8	2–4
9	SA[a]-5

[a] SA: short allele; PCR fragment with a length of ~ 136 bp.

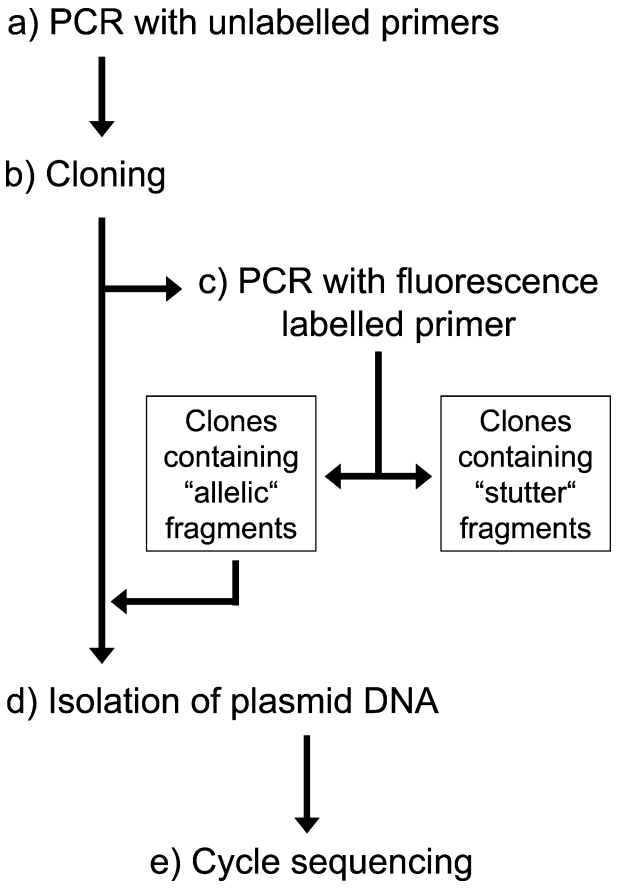

Fig. 1. Examination of the samples (flow chart).

2.3. Cycle sequencing of plasmid DNA

Plasmid DNA was sequenced using ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Weiterstadt, Germany) and M13 forward and reverse primers, respectively (binding sites on pCR®2.1-TOPO vector). Cycle sequencing conditions: 8 μl Ready Reaction Mix, 10 pmol primer and ~ 100 ng plasmid DNA in a 20 μl reaction volume, primer annealing temperature was 48 °C for 15 s (PTC-200, MJ Research). Electrophoresis and detection of fluorescence labelled chain termination products were performed on an ABI 373A DNA sequencer.

3. Results and discussion

A homogenous template for the sequencing reaction was received by cloning of PCR products. Performing a PCR with the fluorescence labelled primer and the DNA from the

Table 2
Results of sequence analysis and proposed repeat-based nomenclature

Numeric allele[a]	Number of repeat units	Length of PCR fragment (bp)	Sequenced fragment length (bp)	Allele designation (this study)
–	...[b]	...[b]	...[b]	...[b]
–	11	± 136	134	11
–	...[b]	...[b]	...[b]	...[b]
1	17	± 148	146	17
2	18	± 150	148	18
3	19	± 152	150	19
4	20	± 154	152	20
5	21	± 156	154	21
6	22	± 158	156	22
7	23	± 160	158	23
8	24	± 162	160	24
–	...[b]	...[b]	...[b]	...[b]

[a] According to Kayser et al. [2].
[b] Among the samples that have been analyzed in our laboratory (n = 300), only the alleles mentioned in this table have been detected.

picked bacteria colonies (see Fig. 1c), the length of the ligated original PCR fragments (see Fig. 1a) could be detected even before isolation and sequencing of the plasmid DNA. Thus, the ligated "allelic" and "stutter" PCR products could be determined and separated.

Plasmid DNA of the clones that contained "allelic" PCR fragments and exhibited a distinct single signal was used for cycle sequencing. The results of fragment length analysis and of the sequence analysis are given in Table 2.

The differences in length between the PCR fragments as detected by fragment length analysis and the sequenced fragments is due to the addition of a single adenosine by *Taq* DNA polymerase [4], and to the influences of the fluorochromes used as dye labels and the physical conditions of electrophoretic separation (current, gel temperature, etc.) as well.

The nucleotide sequence of YCA II as derived from our data is shown in Fig. 2. Priming sites as given by Mathias et al. [1] are underlined. Fragments containing a single CA (TG) repeat unit would be 114 bp long. Numeric allele 3 contains 19 repeat units and is 150 bp long (sequenced fragment length, see Table 2).

According to the ISFG guidelines for Y-chromosome STR analysis [5] nomenclature of alleles should follow the number of complete repeats. Numeric alleles 1–8 of YCA II and an additional short allele containing 11 repeat units were analyzed. Sequencing results have been consistent without exception. As a consequence, we want to propose a repeat-based nomenclature for YCA II (Table 2). In this way, additional YCA II alleles that have

```
  1 TATCGATGTA   ATGTTATATT   AAAAAATG(CA)n GCTAACCTTA

 41 TGGTGAAAGA   CTGACAGATT   TTACTCTAAG   AGCAAAAACA

 81 AGGATGCCTG   TTTTCACTAC   TTCTATTTAA   TATA
```

Fig. 2. Sequence of YCA II as found in this study.

not yet been detected can easily be inserted into the nomenclature. Since a repeat-based nomenclature is a prerequisite for further forensic application of this locus, databasing and evaluation, the revision of the nomenclature in the YHRD should be discussed.

Acknowledgements

This work has been supported in part by the Wissenschaftliche Gesellschaft in Freiburg. The authors wish to thank Mrs. Nicola Meier and Mrs. Tanja Schmitt for expert technical assistance.

References

[1] N. Mathias, M. Bayes, C. Tyler-Smith, Highly informative compound haplotypes for the human Y-chromosome, Hum. Mol. Genet. 3 (1994) 115–123.
[2] M. Kayser, A. Caglià, D. Corach, et al., Evaluation of Y-chromosomal STRs: a multicenter study, Int. J. Legal Med. 110 (1997) 125–133.
[3] M. Kayser, Y-chromosomale DNA-Polymorphismen: Charakterisierung, Populationsgenetik, Anwendung in molekularer Anthropologie und forensischer Individualisierung. Dissertation, Humboldt-Universität Berlin, 1998.
[4] J.M. Clark, Novel non-templated nucleotide addition reactions catalyzed by prokaryotic and eukaryotic DNA polymerases, Nucleic Acids Res. 16 (1988) 9677–9686.
[5] P. Gill, C. Brenner, B. Brinkmann, et al., DNA commission of the international society of forensic genetics: recommendations on forensic analysis using Y-chromosome STRs, Int. J. Legal Med. 114 (2001) 305–309.