J Hum Genet (2004) 49:169–171
DOI 10.1007/s10038-003-0123-2

SHORT COMMUNICATION

Koji Fujii · Kazumasa Sekiguchi · Kenshi Shimizu
Kentaro Kasai

A new sequenced allelic ladder marker for *D1S80* typing

Received: 24 October 2003 / Accepted: 11 December 2003 / Published online: 26 February 2004

Abstract A sequenced allelic ladder marker that contains 32 alleles consisting of alleles 13–44 was developed for *D1S80* (*MCT118*) typing. Each allele, after amplification with D1S80 primers, was cloned to the pT7Blue T-Vector. The plasmid DNAs of these clones were mixed and amplified with D1S80 primers to construct the allelic ladder marker. All the alleles prepared in this study were sequenced in both directions, using two types of forward primers and one reverse primer. The sequencing results revealed that all the alleles in our allelic ladder marker had the correct length corresponding to the allele number. This marker can be used for more effectively typing *D1S80*.

Keywords Forensic · DNA typing · Variable number of tandem repeat · *D1S80* · Allelic ladder marker

The nucleotide sequence data reported are available in the DDBJ database under the accession numbers AB121699–AB121730

K. Fujii (✉) · K. Kasai
Fourth Biological Section,
National Research Institute of Police Science,
6-3-1 Kashiwanoha, Kashiwa,
Chiba 277-0882, Japan
E-mail: fujii@nrips.go.jp
Tel.: +81-4-71358001
Fax: +81-4-71339153

K. Sekiguchi
Third Biological Section,
National Research Institute of Police Science,
6-3-1 Kashiwanoha, Kashiwa,
Chiba 277-0882, Japan

K. Shimizu
Forensic Science Laboratory,
Miyazaki Prefectural Police Headquarters,
1-8-28 Asahi, Miyazaki 880-8509, Japan

Introduction

D1S80 (*pMCT118*, GenBank accession number D28507) is a variable number of tandem repeat locus located on chromosome 1p35-36 with a repeat unit of 16 bp (Nakamura et al. 1988; Kasai et al. 1990). The *D1S80* locus is very useful for human identification because of its high polymorphism, especially in the Japanese population (Rand et al. 1992; Sugiyama et al. 1993; TWGFDM 1995). Baechtel et al. (1993) described an allelic ladder marker that consisted of alleles 16–37 and 41 of the locus, but they were not sequenced. Applied Biosystems (Foster City, Calif., USA) supplied the AmpliFLP D1S80 Allelic Ladder consisting of alleles 14 and 16–41 of the locus, but they only sequenced alleles 14, 16, 18, and 24 in the allelic ladder marker. In addition, Applied Biosystems stopped supplying the AmpliFLP D1S80 Allelic Ladder in 2003. When using the AmpliFLP D1S80 Allelic Ladder, we were not able to type alleles larger than allele 41. However, the alleles larger than allele 41 have been observed with a frequency of about 1% in the Japanese population (TWGFDM 1995). In order to type them, an allelic ladder marker that contains some alleles larger than the allele 41 is required. Here, we constructed a new allelic ladder marker consisting of alleles 13–44 of the *D1S80* locus and confirmed that all the alleles had the correct length corresponding to the allele number by sequencing.

Materials and methods

Blood samples were collected from 23 Japanese individuals that were heterozygous at the *D1S80* locus. Appropriate informed consent was obtained from each individual. DNA samples were purified from their blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA samples were amplified with D1S80 primers (Kasai et al. 1990), and each allele was separated on a 2% agarose gel or by denaturing high-performance liquid chromatography using a WAVE system (Transgenomic, Omaha, Neb., USA). Each separated *D1S80* allele was

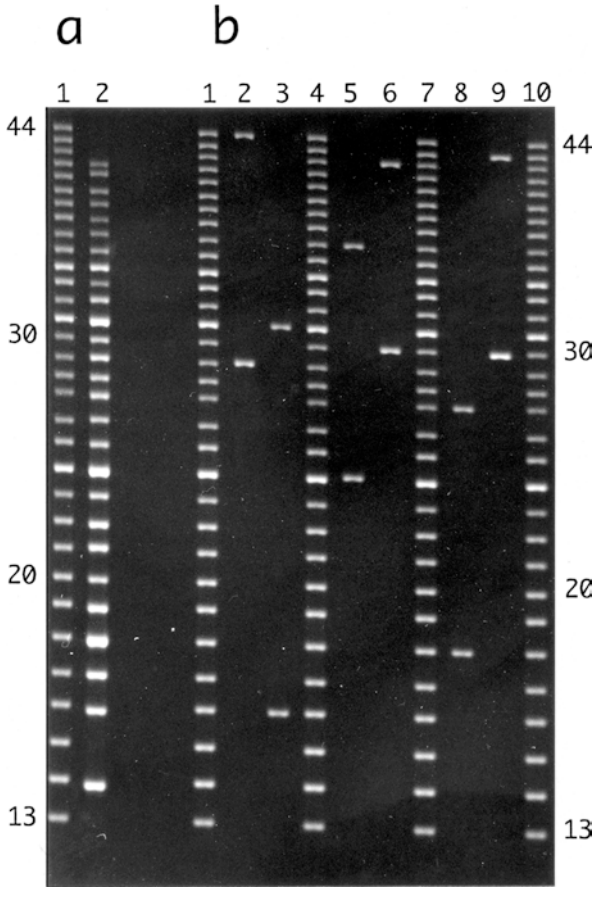

Fig. 1a, b A new sequenced allelic ladder marker for *D1S80* typing. **a** *Lane 1* A new allelic ladder marker consisting of alleles 13–44, *lane 2* AmpliFLP D1S80 Allelic Ladder (Applied Biosystems) consisting of alleles 14 and 16–41. **b** *Lanes 1, 4, 7, 10* A new allelic ladder marker, *lanes 2, 3, 5, 6, 8, 9* PCR products of blood samples amplified with D1S80 primers (*lanes 2, 29–44*; *lanes 3, 16–31*; *lanes 5, 24–36*; *lanes 6, 30–42*; *lanes 8, 18–27*; *lanes 9, 30–43*)

cloned to the pT7Blue T-Vector (Novagen, Madison, Wis., USA).

The plasmid DNAs containing the alleles 13–44 of the *D1S80* locus were mixed and amplified with D1S80 primers to construct an allelic ladder marker. The PCR was performed using 0.14–1.2 fg of each plasmid DNA in a 100-µl reaction volume with a TaKaRa PCR Thermal Cycler Dice (Takara Bio, Otsu, Japan). The PCR reaction mixture contained 1× Ex *Taq* buffer (2 mM Mg^{2+} Plus, Takara Bio), 0.2 mM dNTPs, 0.05 U/µl TaKaRa Ex *Taq* HS (Takara Bio), and 1.4 µM of each of the D1S80 primers. The thermal cycle was performed at 95°C for 1 min, followed by 27 cycles at 94°C for 1 min, 65°C for 1 min, and 70°C for 1 min. A final extension was performed at 70°C for 10 min.

All the plasmids containing the alleles 13–44 were sequenced. Four hundred nanograms of the plasmid DNA was sequenced with the T7 promoter primer, with the M13-21 primer, and with the Forward2 primer (5′-CAGCC CAAGG AAGAC AGACC-3′) reported by Watanabe and Shimizu (2002). The sequence reaction with the T7 promoter primer was performed at 95°C for 5 min, followed by 25 cycles at 95°C for 30 s, 46°C for 10 s, and 60°C for 4 min. The sequence reaction with M13-21 was performed at 95°C for 5 min, followed by 25 cycles at 95°C for 30 s, and 60°C for 4 min. The sequence reaction mixture of the T7 promoter primer and of the M13-21 primer contained 5% dimethyl sulfoxide and 5% glycerol. The sequencing reaction with the Forward2 primer was performed with the method described by Watanabe and Shimizu (2002).

Results and discussion

An allelic ladder marker consisting of the alleles 13–44 for use in *D1S80* typing was constructed (Fig. 1). The forward sequences were obtained from all the plasmids with the T7 promoter primer and the Forward2 primer, and the reverse sequences were obtained with the M13-21 primer. From the sequence data of the alleles 13–44, 13 types of repeat units were obtained, and the repeat units have been reported previously (Fig. 2, Duncan et al. 1997; Arakura et al. 1998; Liu et al. 1999; Watanabe and Shimizu 2002). The repeat units 2–13 consisted of 16 bp, and their sequences were designated as RMRRA CCACH RGVAA G. The first four repeats and the last three repeats were conserved expect for the allele 21. The allele 21 appears to contain a nucleotide substitution in the 19th repeat. However, we do not know whether the nucleotide substitution occurred in the PCR amplification before the cloning or was derived from an individual who provided the allele. All the alleles used in this study contained the same 5′ and 3′ franking region sequences that were similar to the sequence reported by Kasai et al. (1990). The lengths of all the alleles in this study were calculated using the following formula reported by Sekiguchi et al. (1993): 116 bp (5′ franking region) + 14 bp (first repeat) + 16(n−1) bp (second repeat through last repeat) + 32 bp (3′ franking region) = 146 + 16n bp, where n is the allele number. The sequencing result revealed that all the alleles in our allelic ladder marker had the correct length corresponding to the allele number, and we think that this marker can be used for more effectively typing *D1S80*. Takara Bio now supplies the TaKaRa D1S80 Allelic Ladder using the method decribed in this study.

```
   Repeat number  1  *  *  *  5  *  *  *  *  10 *  *  *  *  15 *  *  *  *  20 *  *  *  *  25 *  *  *  *  30 *  *  *  *  35 *  *  *  *  40 *  *  *  44
allele 44   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10-10-10- 9 - 9 - 8 - 9 - 9 - 9 -13-10-10-10- 9 -10-10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 43   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10-10-10- 9 - 9 - 8 - 9 - 9 -13-10-10-10- 9 -10-10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 42   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 -10-10-10-10- 9 - 9 - 8 - 9 - 9 -13-10-10-10- 9 -10-10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 41   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10-10- 9 - 9 - 8 - 9 - 9 -13-10-10-10- 9 -10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 40   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10-10- 9 - 9 - 8 - 9 - 9 -13-10-10-10- 9 -10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 39   5'FR - 1 - 2 - 3 - 4 - 5 - 6 - 3 - 7 - 8 - 9 -10- 9 - 9 - 8 - 9 - 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 - 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 38   5'FR - 1 - 2 - 3 - 4 - 5 - 6 - 3 - 7 - 8 - 9 -10- 9 - 9 - 8 - 9 - 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 37   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 - 8 - 9 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 -11- 9 - 9 -11- 9 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 36   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 - 8 - 9 - 8 - 9 - 9 - 8 - 9 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 35   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 - 8 - 9 - 9 - 8 - 9 - 9 - 8 - 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 34   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 - 8 - 9 - 9 - 8 - 9 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 33   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 -10-10- 9 - 9 - 8 - 9 - 9 - 9 - 8 - 9 -11- 9 -11- 9 - 9 -12- 7 -3'FR
allele 32   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 - 8 - 9 - 8 - 9 - 9 - 9 - 9 -10- 9 -10- 9 -10- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 31   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 6 - 3 - 7 - 8 - 9 -10-10- 9 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 30   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 -10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 29   5'FR - 1 - 2 - 3 - 4 - 5 - 6 - 3 - 7 - 8 - 9 -10- 9 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 28   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 27   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 8 - 8 - 9 - 9 -10- 9 -10- 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 26   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 8 - 9 -10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 25   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 -11- 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 24   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 23   5'FR - 1 - 2 - 3 - 4 - 5 - 6 - 3 - 7 - 8 - 9 - 9 -10- 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 22   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 8 - 9 -10-10- 9 - 9 - 8 - 9 -11- 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 21   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10-10- 9 - 9 - 8 - 9 -11- 9 -13-12- 7 -3'FR
allele 20   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 8 - 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 19   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 7 - 9 -10- 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 18   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 8 - 8 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 17   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 8 - 8 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 16   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 8 - 8 - 9 - 9 - 8 - 9 - 9 -12- 7 -3'FR
allele 15   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 9 - 9 - 8 - 9 -11- 9 - 9 -12- 7 -3'FR
allele 14   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 8 - 8 - 9 - 9 - 9 -12- 7 -3'FR
allele 13   5'FR - 1 - 2 - 3 - 4 - 4 - 5 - 3 - 8 - 8 - 9 - 9 -12- 7 -3'FR

  The sequences of the repeat units (1-13) and the 5' and 3' franking regions.
     1:TCAGC CCAAG GAAG        2:ACAGA CCACA GGCAA G     3:GAGGA CCACC GGAAA G     4:GAAGA CCACC GGAAA G     5:GAAGA CCACA GGCAA G
     6:GAGGA CCACA GGCAA G     7:GAAGA CCACC GGCAA G     8:GAGGA CCACC GGCAA G     9:GAGGA CCACC AGGAA G    10:GAGGA CCACC AGCAA G
    11:GAGAA CCACC AGGAA G    12:GAGGA CCACT GGCAA G    13:GAGGA CCACC GGGAA G
   5'FR:GAAAC TGGCC TCCAA ACACT GCCCG  CCGTC  CACGG  CCGGC  CGGTC  CTGCG  TGTGA  ATGAC  TCAGG  AGCGT  ATTCC  CCACG  CGCCA  GCACT
        GCATT CAGAT AAGCG CTGGC TCAGT G
   3'FR:CCTGC AAGGG GCACG TGCAT CTCCA ACAAG AC
```

Fig. 2 Sequence structures of alleles 13–44 of the *D1S80* locus

References

Arakura A, Liu C, Ota M, Fukushima H (1998) Subtyping and characterization of *D1S80* alleles in a Japanese population using PCR-RFLP. Int J Legal Med 111:183–187

Baechtel FS, Smerick JB, Presley KW, Budowle B (1993) Multigenerational amplification of a reference ladder for alleles at locus *D1S80*. J Forensic Sci 38:1176–1182

Duncan GT, Balamurugan K, Budowle B, Smerick J, Tracy ML (1997) Microvariation at the human *D1S80* locus. Int J Legal Med 110:150–154

Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (*pMCT118*) by the polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci 35:1196–1200

Liu C, Arakura A, Takayanagi K, Asamura H, Ota M, Fukushima H (1999) *D1S80* subtyping by PCR-RFLP: new nomenclature and further characterization. Leg Med 1:210–216

Nakamura Y, Carlson M, Krapcho K, White R (1988) Isolation and mapping of a polymorphic DNA sequence (*pMCT118*) on chromosome 1p [*D1S80*]. Nucl Acids Res 16:9364

Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data of 4 AMPFLPs. Int J Legal Med 104:329–333

Sekiguchi K, Sakai I, Mizuno N, Yoshida K, Kasai K, Sato H, Seta S (1993) DNA sequence analysis of PCR-amplified products of *MCT118* locus in Japanese DNA samples (in Japanese). Rep Nat Res Inst Police Sci 46:172–176

Sugiyama E, Honda K, Katsuyama Y, Uchiyama S, Tsuchikane A, Ota M, Fukushima H (1993) Allele frequency distribution of the *D1S80* (*pMCT118*) locus polymorphism in the Japanese population by the polymerase chain reaction. Int J Legal Med 106:111–114

TWGFDM (Technical working group for forensic DNA typing method) (1995) Reassessment on frequency distribution of *MCT118* and *HLADQα* alleles and genotypes in Japanese population along with the comparison with other population data (in Japanese). Rep Nat Res Inst Police Sci 48:171–185

Watanabe G, Shimizu K (2002) DNA sequence analysis of long PCR amplified products at the *D1S80* locus. Leg Med 4:37–39