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SHORT COMMUNICATION

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New reference allelic ladders to improve allelic designation in a multiplex STR system

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Abstract This paper reports the composition of a new reference allelic ladder mixture for use with a multiplex DNA profiling system consisting of six short tandem repeat loci. The loci included in this mixture are HUMTH01, D21S11, D18S51, D8S1179, HUMVWAF31/A, HUMFIBRA/FGA and an amelogenin sex test. Sequence analysis of individual ladder alleles was carried out and allelic designations made in accordance with the recommendations of the International Society of Forensic Haemogenetics (1992; 1994). A series of rare alleles which increase the range of alleles previously reported were identified. By including some of the rare alleles into the ladder marker system, we have significantly improved the ability to identify new alleles in unknown samples.

Key words STR · Sequence · Databases · Allelic ladders · Allelic designation

Introduction

There are a number of DNA profiling systems based on short tandem repeat (STR) loci which have been evaluated for forensic use. They may be based on single locus (singleplex) analysis (Hagelberg et al. 1991; Alonso et al. 1993; Wiegand et al. 1993; Möller and Brinkmann 1994; Möller et al. 1995) or simultaneous analysis of multiple loci (multiplex) (Edwards et al. 1991, 1992; Lygo et al. 1994; Kimpton et al. 1994; Hochmeister et al. 1995; Pestoni et al. 1995). A multiplex system is currently employed in the UK by the Forensic Science Service for routine casework and intelligence database applications. This system has been termed the second generation multiplex (SGM) and is based on the co-amplification of six STR loci (HUMTH01, D21S11, D18S51, D851179, HUMVWAF31/A and HUMFIBRA/FGA) and an amelogenin sex test (Urquhart et al. 1995; Oldroyd et al. 1995; Sparkes et al. 1996a, b). Alleles in unknown samples are designated by comparison with ladder markers (Gill et al. 1995) which consist of alleles derived from actual samples. This paper reports the construction of a new reference allelic ladder marker mixture or cocktail for use with the SGM multiplex system.

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Materials and methods

The general strategy for ladder construction was firstly to isolate individual STR alleles from samples. The alleles were then sequenced according to methods described in Barber et al. (1996) and Barber and Parkin (1996). The labelled purified alleles were then mixed together to form a balanced ladder. In some cases. for example the amelogenin 'ladder', where both alleles from a sample were required for ladder construction, the genomic DNA was amplified using labelled primers and added directly to the ladder. However, these alleles were also purified and sequenced separately. After the allelic ladder cocktail had been prepared, it was validated on both Applied Biosystems (ABD) 373A and 377 automated sequencers using Genescan and Genotyper software.

Purification of single alleles and sequencing

Genomic DNA was extracted from buccal swabs or bloodstains using the chelex procedure described by Walsh et al. (1991). The recovered DNA was quantified by dot blot hybridisation to a higher primate specific probe (Waye et al. 1989; Walsh et al. 1992). Each sample was amplified using conditions shown in Appendix I. The sequences of the primers have been described previously (Urquhart et al. 1995; Oldroyd et al. 1995). Individual ladder alleles were isolated and sequence analysis was carried out. The consensus sequences reported in this paper are based on data obtained from both DNA strands of each allele.

Preparation of ladder cocktail

Single alleles were analysed on an ABD 377 automated sequencer, the products of each locus were diluted, mixed together, reanalysed and balanced to produce a single ladder for each locus with even peak heights [c. 1000 arbitrary units (AU)]. Each ladder was individually re-amplified to increase the signal strength (to 1000–5000AU) and to increase the volume of stock ladder. Finally the single ladders were mixed together to form a 'cocktail' in proportions so that all peak heights were balanced (between 100 and 1000AU).

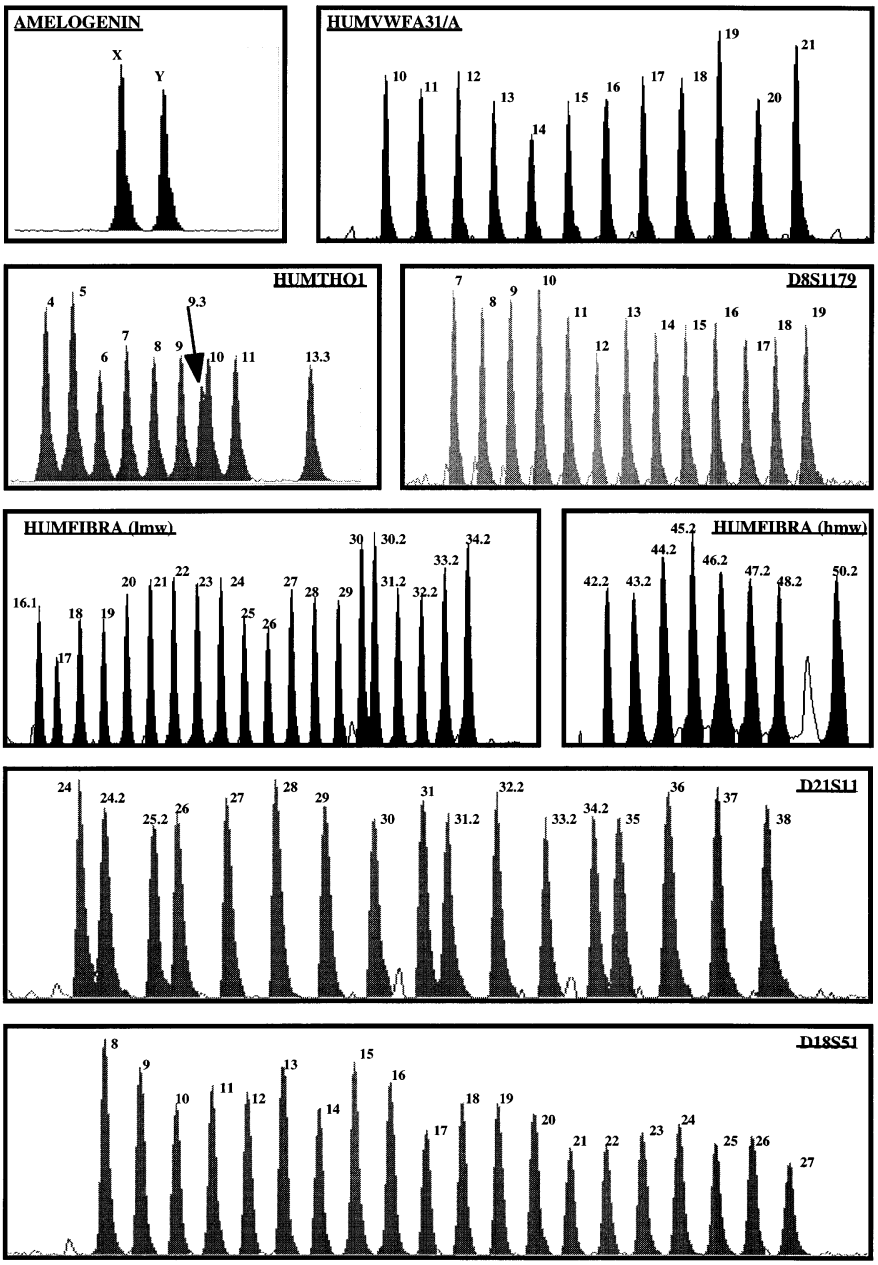

Fig. 1 Electrophoretograms of the allelic ladders. Each allele is labelled using the standard nomenclature. In HUMFIBRA, there is a stutter band which is coincident with the position expected of allele 49.2. This band is artefactual and will not be used for designation purposes

Results and discussion

The nomenclature used follows the recommendations of the DNA commission of the ISFH (1992; 1994) where the number of complete tandem repeats observed is designated by digit(s). The nomenclature of loci discussed in this paper is described by Gill et al. (1996). However, the nomenclature of alleles at the D21S11 locus follow the Möller et al. 1994 notation (Gill et al. 1997). Allele sizes determined using the SGM profiling system are larger by 1 base pair than those determined by sequencing methods (Appendix II). This results from the ability of DNA polymerase from *Thermus aquaticus* to catalyse a non-template mediated addition of a deoxyribonucleotide to the 3′ hydroxyl of PCR products. This has been termed the ‘n+1’ product and has been shown to occur with relatively high efficiency (Clarke 1988). Consequently the PCR conditions for the SGM have been optimised to favour the ‘n+1’, product in order to eliminate the formation of double peaks (Kimpton et al. 1993, 1994; Lygo et al. 1994). The sizes we report have been determined from sequence data and therefore refer to the ‘n’ peak. Some sequence data has already been published (Brinkmann et al. 1996; Barber et al. 1996; Barber and Parkin 1996; Puers et al. 1993; Möller et al. 1994).

Composition of the ladder mixes

Eight separate ladders have been prepared (Fig. 1). The allelic sequences and sizes of alleles contained in each ladder are shown in Appendices II and III. The range of each

Table 1 The ladder range for each locus defined by the extreme low molecular weight and high molecular weight alleles (using the standard nomenclature). The allelic ladder range previously described by Gill et al. (1996) is shown in parentheses

Locus	Low MW allele	High MW allele
HUMVWFA31/A	10 (13)	21 (21)
HUMTH01	4 (5)	13.3 (11)
D8S1179	7 (8)	19 (17)
HUMFIBRA/FGA	16.1 (17)	50.2 (46.2)
D21S11	24 (24.2)	38 (36)
D18S51	8 (10)	27 (22)

allelic ladder and the range previously described by Gill et al. (1996) is shown in Table 1.

Advantages of the extended ladder

The new ladder significantly extends the range of the current ladder markers, assisting in the identification of rare alleles, particularly those outside the previously known range. The improved range of identification for each locus is given in Table 1. All of the alleles in the ladder markers have been sequenced and designated in accordance with the recommendations of the ISFH DNA Commission (1992; 1994), hence the size of each marker is known definitively (Appendix II). Frequencies of rare alleles found in populations will be published elsewhere. The sequence of HUMTH01 allele 13.3 is particularly interesting. It shows homology with 9.3 since the partial .3 is found in the fourth repeat from the 5′ end. In addition, the second repeat from the 3′ end has an interesting T→C transition at the third base.

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APPENDIX I

Cycling conditions used for PCR amplification

D18	95° C for 60 s	D21	94° C for 30 s
	60° C for 30 s		58° C for 60 s
	72° C for 60 s		72° C for 30
Method: 28 cycles + 72° C for 10 min. Then hold at 4° C		Method: 26 cycles + 72° C for 10 min. Then hold at 4° C	
D8	94° C for 30 s	TH01 and	94° C for 45
	60° C for 60 s	VWA	60° C for 60 s
	72° C for 60 s		72° C for 60 s
Method: 30 cycles + 72° C for 10 min. Then hold at 4° C		Method: 28 cycles + 72° C for 10 min. Then hold at 4° C	
FGA	93° C for 60 s	Amelo	93° C for 30 s
	60° C for 60 s		58° C for 75 s
	72° C for 60 s		72° C for 15 s
Method: 30 cycles + 72° C for 10 min. Then hold at 4° C		Method: 30 cycles + 72° C for 10 min. Then hold at 4° C	

APPENDIX II

Allele designations and their sizes (including flanking sequence) for SGM loci. These sizes are derived from sequence data and therefore refer to the n peak

Locus	Allelic designation	Size (bp)	Locus	Allelic designation	Size (bp)	Locus	Allelic designation	Size (bp)	Locus	Allelic designation	Size (bp)
THOI	4	150	D8	7	157	D18	8	266	FGA	16.1	173
	5	154		8	161		9	270	(LMW)	17	176
	6	158		9	165		10	274		18	180
	7	162		10	169		11	278		19	184
	8	166		11	173		12	282		20	188
	9	170		12	177		13	286		21	192
	9.3	173		13	181		14	290		22	196
	10	174		14	185		15	294		23	200
	11	178		15	189		16	298		24	204
	13.3	189		16	193		17	302		25	208
				17	197		18	306		26	212
D21	24	203		18	201		19	310		27	216
	24.2	205		19	205		20	314		28	220
	25.2	209					21	318		29	224
	26	211	VWA	10	122		22	322		30	228
	27	215		11	126		23	326		30.2	230
	28	219		12	130		24	330		31.2	234
	29	223		13	134		25	334		32.2	238
	30	227		14	138		26	338		33.2	242
	31	231		15	142		27	342		34.2	246
	31.2	233		16	146						
	32.2	237		17	150	AMELO	X	106	FGA	42.2	278
	33.2	241		18	154		Y	112	(HMW)	42.3	282
	34.2	245		19	158					44.2	286
	35	247		20	162					45.2	290
	36	251		21	166					46.2	294
	37	255								48.2	302
	38									47.2	298
		259								50.2	310

APPENDIX III

Sequences of HUMTHO1 alleles in the ladder

*4	(AATG)4
5	(AATG)5
6	(AATG)6
7	(AATG)7
8	(AATG)8
9	(AATG)9
9.3	(AATG)6 ATG (AATG)3
10	(AATG)10
11	(AATG)11
*13.3	(AATG) AACG (AATG)8 ATG (AATG)3

* alleles 4 and 13.3 are novel (remaining alleles have been reported and sequenced by Puers et al. 1993)

Sequences of D21S11 alleles in the ladder

*24	(TCTA)4 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)6
*24.2	(TCTA)5 (TCTG)6 (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)9
*25.2	(TCTA)5 (TCTG)6 (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)10
26	(TCTA)4 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCA TA (TCTA)8
*27	(TCTA)5 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)9
**28	(TCTA)4 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)10
29	(TCTA)4 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)11
*30	(TCTA)6 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)11
30.2	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)10 TA TCTA
**31	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)12
31.2	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)11 TA TCTA
32	(TCTA)6 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)13
32.2	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)12 TA TCTA
33.2	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)13 TA TCTA
34.2	(TCTA)5 (TCTG)6 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)14 TA TCTA
**35	(TCTA)10 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)12
*36	(TCTA)11 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)12
*37	(TCTA)11 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)13
*38	(TCTA)13 (TCTG)5 (TCTA)3 TA (TCTA)3 TCA (TCTA)2 TCCATA (TCTA)12

* alleles 24, 24.2, 25.2, 27, 30, 36, 37 and 38 are novel; ** alleles 28, 31 and 35 were reported by Brinkmann et al. (1996). Remaining alleles have been reported and sequenced by Möller et al. (1994)

D18S51 sequences in the ladder

*8	(AGAA)8
9	(AGAA)9
10	(AGAA)10
11	(AGAA)11
12	(AGAA)12
13	(AGAA)13
14	(AGAA)14
15	(AGAA)15
16	(AGAA)16
17	(AGAA)17
18	(AGAA)18
19	(AGAA)19
20	(AGAA)20
21	(AGAA)21
22	(AGAA)22
23	(AGAA)23
24	(AGAA)24
25	(AGAA)25
26	(AGAA)26
27	(AGAA)27

* allele 8 is novel (remaining alleles have been reported and sequenced by Barber and Parkin 1996)

D8S1179 sequences in the ladder

*7	(TCTA)7
8	(TCTA)8
9	(TCTA)9
10	(TCTA)10
11	(TCTA)11
12	(TCTA)12
13	TCTA TCTG (TCTA)11
14	(TCTA)2 TCTG (TCTA)11
15	(TCTA)2 TCTG (TCTA)12
16	(TCTA)2 TCTG (TCTA)13
17	(TCTA)2 (TCTG)2 (TCTA)13
18	(TCTA)2 TCTG (TCTA)15
*19	(TCTA)2 (TCTG)2 (TCTA)15

* alleles 7 and 19 are novel (remaining alleles have been reported and sequenced by Barber and Parkin 1996)

HUMVWAF31/A sequences in the ladder

*10	TCTA TCTG TCTA (TCTG)4 (TCTA)3
*12	TCTA (TCTG)4 (TCTA)7
*13	(TCTA)2 (TCTG)4 (TCTA)3 TCCA (TCTA)3
14	TCTA TCTG TCTA (TCTG)4 (TCTA)3 TCCA (TCTA)3
15	TCTA (TCTG)4 (TCTA)10
16	TCTA (TCTG)4 (TCTA)11
16	TCTA (TCTG)3 (TCTA)12
17	TCTA (TCTG)4 (TCTA)12
18	TCTA (TCTG)4 (TCTA)13
19	TCTA (TCTG)4 (TCTA)14
20	TCTA (TCTG)4 (TCTA)15
21	TCTA (TCTG)4 (TCTA)16

* alleles 10, 12 and 13 are novel (remaining alleles have been reported and sequenced by Möller et al. 1994)

HUMFIBRA(FGA) sequences in the ladder

*16.1	(TTTC)3 TTTT TTCT (CTTT)5 T (CTTT)3 CTCC (TTCC)2
17	(TTTC)3 TTTT TTCT (CTTT)9 CTCC (TTCC)2
18	(TTTC)3 TTTT TTCT (CTTT)10 CTCC (TTCC)2
19	(TTTC)3 TTTT TTCT (CTTT)11 CTCC (TTCC)2
20	(TTTC)3 TTTT TTCT (CTTT)12 CTCC (TTCC)2
21	(TTTC)3 TTTT TTCT (CTTT)13 CTCC (TTCC)2
22	(TTTC)3 TTTT TTCT (CTTT)14 CTCC (TTCC)2
23	(TTTC)3 TTTT TTCT (CTTT)15 CTCC (TTCC)2
24	(TTTC)3 TTTT TTCT (CTTT)16 CTCC (TTCC)2
25	(TTTC)3 TTTT TTCT (CTTT)17 CTCC (TTCC)2

26	(TTTC)3 TTTT TTCT (CTTT)18 CTCC (TTCC)2
**27	(TTTC)3 TTTT TTCT (CTTT)13 CCTT (CTTT)5 CTCC (TTCC)2
28	(TTTC)3 TTTT TTCT (CTTT)20 CTCC (TTCC)2
29	(TTTC)3 TTTT TTCT (CTTT)15 CCTT (CTTT)5 CTCC (TTCC)2
*30	(TTTC)3 TTTT TTCT (CTTT)16 CCTT (CTTT)5 CTCC (TTCC)2
30.2	(TTTC)4 TTTT TT (CTTT)1 (CTTC)3 (CTTT)3 CTCC (TTCC)4
*31.2	(TTTC)4 TTTT TT (CTTT)15 (CTTC)3 (CTTT)3 CTCC (TTCC)4
*32.2	(TTTC)4 TTTT TT (CTTT)16 (CTTC)3 (CTTT)3 TCC (TTCC)4
*33.2	(TTTC)4 TTTT TT (CTTT)17 (CTTC)3 (CTTT)3 CTCC (TTCC)4
34.2	(TTTC)4 TTTT TT (CTTT)18 (CTTC)3 (CTTT)3 CTCC (TTCC)4
*42.2	(TTTC)4 TTTT TT (CTTT)8 (CTGT)4 (CTTT)13 (CTTC)4 (CTTT)3 CTCC (TTCC)4
*43.2	(TTTC)4 TTTT TT (CTTT)8 (CTGT)5 (CTTT)13 (CTTC)4 (CTTT)3 CTCC (TTCC)4
*44.2	(TTTC)4 TTTT TT (CTTT)11 (CTGT)3 (CTTT)14 (CTTC)3 (CTTT)3 CTCC (TTCC)4
*45.2	(TTTC)4 TTTT TT (CTTT)10 (CTGT)5 (CTTT)13 (CTTC)4 (CTTT)3 CTCC (TTCC)4
46.2	(TTTC)4 TTTT TT (CTTT)12 (CTTC)5 (CTTT)13 (CTCC)3 (CTTT)3 CTCC (TTCC)4
*47.2	(TTTC)4 TTTT TT (CTTT)12 (CTGT)5 (CTTT)14 CTTC)3 (CTTT)3 CTCC (TTCC)4
*48.2	(TTTC)4 TTTT TT (CTTT)14 (CTGT)3 (CTTT)14 (CTTC)4 (CTTT)3 CTCC (TTCC)4
50.2	(TTTC)4 TTTT TT (CTTT)14 (CTGT)4 (CTTT)15 (CTTC)4 (CTTT)3 CTCC (TTCC)4

** The sequence of allele 27 previously reported by Barber et al. (1996) is not the same as the sequence of allele 27 which has been included in this ladder

* Alleles 16.1, 27, 30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 47.2, 48.2 and 50.2 are novel (remaining alleles have been reported and sequenced by Barber et al. 1996)