

Construction And Calibration Of Allelic Ladders For The PCR-Based Systems D8S320 and AR

P. Huber¹, W. Schmidt² and J. Holtz¹

¹Inst. of Forensic Medicine, University of Bonn, Germany

²IHF, Hamburg, Germany

Introduction

Amplified fragments of STRs and AmpFlips were analyzed after electrophoretic separation and staining with ethidium bromide or silver. Exact allele determination requires comparison with allelic ladders. We construct such ladders for the tetrameric STR D8S320 and the trimeric X-linked polymorphism in the Androgen receptor gene (AR). Calibration of the ladders was performed by comparing the results of conventional separation on PAGE and subsequent silver staining with those of the ABI Sequencer in connection with the GeneScan Software 672.

Based on these ladders we conducted population studies for both systems.

Material and Methods

Systems:

D8S320 (8q); Primers: Riley et al., 1993

AR (Xq 11-12); Primers: Sleddens et al., 1992

PCR amplification conditions:

Hot start: 5 min 94 °C, 5 min 80 °C; denaturation: 1 min 94 °C, annealing: 45 s 60 °C, extension: 45 s 72 °C, cycles: 13, denaturation: 1 min 94 °C, annealing: 45 s 62 °C, extension: 45 s 72 °C (3 s elongation each cycle), cycles: 17, final extension: 10 min 72 °C

Electrophoretic separation:

native, vertical PAA gels: AR (8% T), D8S320 (7% T); detection method: silver staining

Construction and calibration of the allelic ladders:

1-3 µl of the selected PCR products were pooled and diluted 10⁶-fold with water. 20 µl of the dilution were reamplified in a 100 µl reaction volume using the described conditions above.

For calibration the same dilutions were amplified with dye labelled primers (D8S320: labelled with „Fam“, AR: labelled with „Rox“) under the same conditions except the cycle number (D8S320, total cycle number: 25; AR, total cycle number: 32).

Fragment size was determined by running on the ABI Sequencer 373 A with an internal standard (GenScan 350, labelled with „Tamra“) using the GenScan Software 672.

Results

Allelic ladders

The AR - allelic ladder contains the alleles 16-32 (Fig. 1). The fragment sizes range from 190 bp to 238 bp (Fig. 3). The determined sizes are 1 bp bigger than the sizes calculated according to the sequence published by Lubahn et al. (1989).

The D8S320 - allelic ladder includes 5 alleles (in bp): 394, 398, 402, 410, 418 (Fig. 2+4).

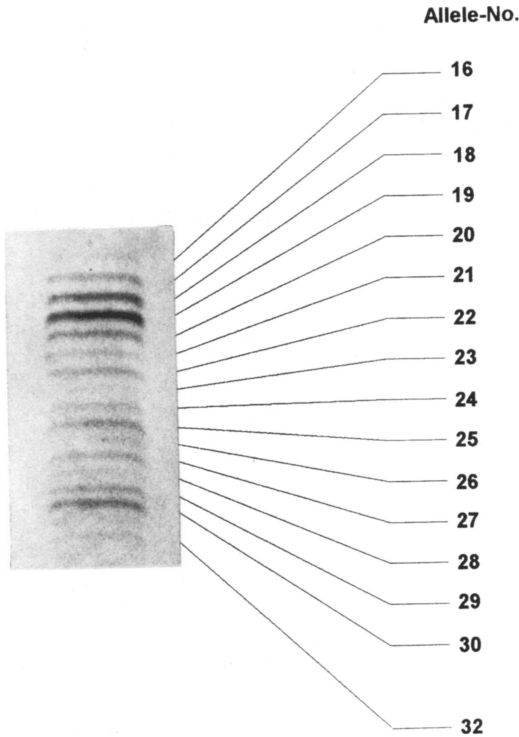


Fig. 1 AR - allelic ladder
The ladder contains the alleles between 16 and 32.
(nomenclature according to Edwards et al., 1992)

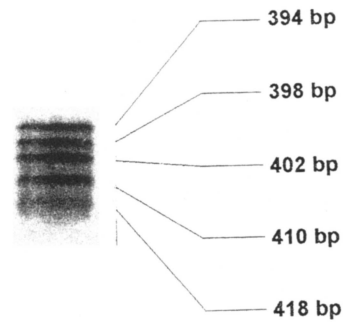


Fig. 2 D8S320 - allelic ladder
The ladder includes 5 alleles

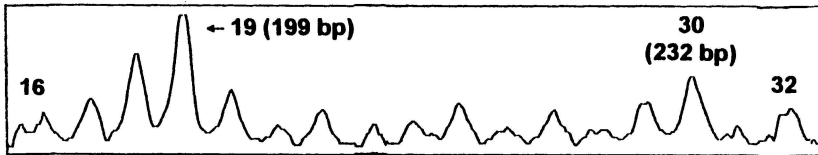


Fig. 3 AR - allelic ladder: fragment size determination
The fragment sizes were determined using the ABI sequencer 373 A and the GeneScan software 672.
The determined sizes are 1 bp bigger than the calculated sizes according to the sequence published by Lubahn et al. (1989).

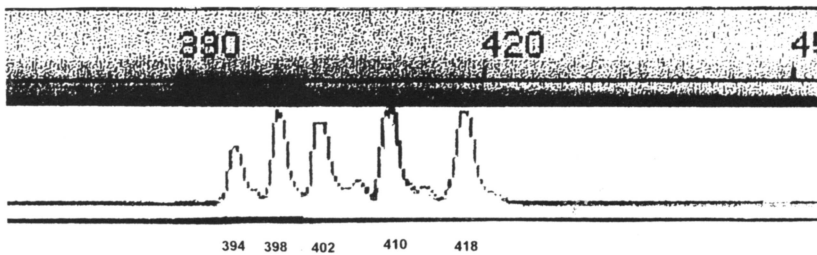


Fig. 4 D8S320 - allelic ladder: fragment size determination
The length (in bp) of the 5 alleles in the ladder are: 394, 398, 402, 410, 418

Population studies

The studies were conducted on samples of unrelated individuals selected from the north-west German population (area of Bonn); AR: n=153 (female: 77, male: 76), D8S320: n=128

Table 1: AR allele distribution (n=230)

Table 2: D8S320 allele distribution (n=256)

allele-no ¹⁾	% ,female (n = 154)	% ,male (n = 76)	%, pooled (n = 230)	allele (bp)	%
16	1,3	1,3	1,3	386	0,4
17	9,1	5,3	7,8	390	0
18	7,1	6,6	7,0	394	3,1
19	14,9	17,1	15,2	398	14,5
20	17,5	19,7	18,3	402	14,8
21	6,5	11,8	8,3	406	19,1
22	9,7	7,9	9,1	410	13,7
23	5,8	11,8	7,8	414	16,4
24	8,4	4	7	418	17,6
25	5,8	5,3	5,7	422	0,4
26	3,9	0	2,6		
27	3,3	4	3,5		
28	3,3	1,3	2,6		
29	2	1,3	1,7		
30	1,3	1,3	1,7		
31	0	0	0		
32		1,3	0,4		

1) nomenclature according to Edwards et al., 1992

Hardy-Weinberg equilibrium and forensic efficiency data

Table 3

Locus	Hardy-Weinberg equilibrium	power of exclusion	discrimination power	heterozygosity [%]
AR	no significant deviation ($p > 0,05$) (5-allele model; $\chi^2 = 17,91$; $df = 14$; $0,2 < p < 0,3$)	0,81 ²⁾	0,98 ²⁾	77,9 ²⁾
D8S320	no significant deviation ($p > 0,05$) (7-allele model; $\chi^2 = 34,94$; $df = 27$; $0,1 < p < 0,2$)	0,70	0,95	73,4

2) for female individuals

References

- Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992): Genetic variation at five trimeric and tetrameric repeat loci in four human population groups. *Genomics* 12: 241-253
- Lubahn DB, Brown TR, Simental, JA, Higgs, HN, Migeon CJ, Wilson EM, French SF (1989): Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. *Proc Natl Acad Sci USA* 86: 9534-9538
- Riley R, Nelson L, Lu J, Robertson M, Ballard L, Connolly J, Ward K (1993): Tetranucleotide polymorphism at the D8S320 locus. *Hum Mol Genet* 2: 1512
- Sladdens HFBM, Oostra BA, Brinkmann AO, Trapman J (1992): Trinucleotide repeat polymorphism in the androgen receptor gene (AR). *Nucleic Acids Res* 20: 1427