

Analysis of the short tandem repeat polymorphism D21S11 in German Caucasians

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INTRODUCTION

Short tandem repeat loci (STR) are polymorphic markers that can be used for human identification in forensic or paternity casework. We have investigated the polymorphism of the STR locus D21S11 in a German population sample.

SYSTEMS AND LOCUS: D21S11 (21q21)

POPULATION AND SAMPLE SIZE: Hesse (Germany)
N: 77

METHODS

Primersequences were chosen according to Sharma and Litt (1992). The 5' primer was labelled with HEX (6-carboxy-2',4',7',4,7-Hexachlorofluorescein, Perkin Elmer - ABD).

PCR amplification conditions: 95 C - 5 min; 28 cycles 95 C - 30 sec , 54 C 40 sec, 72 C 30 sec; 72 C 5 min. (Perkin Elmer 9600).

PCR reaction conditions: 10-100ng DNA, 5pmol 5'and 3' primer, 200 µmol dNTP's, 0,5 Units Taq DNA polymerase (Appligene) and the corresponding buffer (Appligene) in a final volume of 50 µl.

Electrophoretic methods: 6% polyacrylamide denaturing gel electrophoresis. The gels were run for 8h at constant power (30W) 1200V and 28mA on an ABD automated DNA sequencer 373A. Typing was performed by comparison with an ROX (6-carboxy-X-rhodamine) labelled internal standard generated from the vector pGL-2-Basic (Promega) using the Southern local method for fragment size assignment (GENESCAN software, Perkin Elmer - ABD).

Sequence analysis: Individual alleles were sequenced using the solid-phase sequence strategy. Analysis of sequence reactions was conducted on an ABD 373A automated DNA sequencer using the SEQUENCE NAVIGATOR software (Perkin Elmer - ABD).

Statistical analysis: The polymorphic information content (PIC) was calculated using the formula of Botstein (1980). The discrimination index (DI) and the matching probability (pM) were calculated by the method of Jones (1972). The sample gene diversity (geneD) (frequency of heterozygotes expected under Hardy Weinberg equilibrium was calculated as described by Kimpton (1993).

RESULTS

Observed genotypes

Gen.	Obs. (N)	Gen.	Obs.(N)	Gen.	Obs.(N)	Gen.	Obs.(N)
57 - 63	1	61 - 70	1	63 - 72	1	66 - 68	1
59 - 61	1	61 - 72	1	64 - 68	1	66 - 70	2
59 - 63	1	63 - 63	4	65 - 65	6	67 - 68	2
61 - 61	2	63 - 65	5	65 - 66	4	68 - 70	2
61 - 63	5	63 - 66	2	65 - 67	6	70 - 70	1
61 - 65	4	63 - 67	2	65 - 68	3	70 - 72	1
61 - 67	1	63 - 68	3	65 - 70	7		
61 - 68	3	63 - 70	3	66 - 66	1		

Allele frequencies

Allele (bp)	Frequency	Allele (bp)	Frequency	Allele (bp)	Frequency
57 (209)	0,007	64 (223)	0,007	68 (231)	0,097
59 (213)	0,013	65 (225)	0,266	70 (235)	0,117
61 (217)	0,13	66 (227)	0,071	72 (239)	0,026
63 (221)	0,201	67 (229)	0,071	73 (241)	0,02

COMMENTS

STR locus	HR	gene D	PIC	DI	pM	mean exclusion change
D21S11	0,818	0,838	0,819	0,99	0,012	68,12

The observed genotype distribution follows the Hardy Weinberg equilibrium (Chi-square value 0,368; df 65; $p > 0,99$). The sequence analysis of alleles at the D21S11 locus reveals a tetranucleotide repeat structure that contains a hexanucleotide repeat in alleles with even repeat numbers leading to size differences of 2 base pairs. Allele assignment was done as proposed by Urquhart (1994) counting each tetra- or hexanucleotide repeat as two or three dinucleotide repeats respectively. Locus D21S11 can be easily analysed by PCR amplification. Using an internal size standard and fluorescence labelled primers proper allele assignment of allele fragments differing by only 2 bp could be performed. Computer generated band sizes of the different fragments observed in our population sample exhibited a maximum band size range of 0,3 bp (corresponding to $\pm 0,15$ bp either side of the mean value).

REFERENCES

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