

D12S391: A HIGHLY USEFUL STR FOR FORENSIC PURPOSES

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SYSTEM: CHLC. GATA 11 HO8.
LOCUS: D12S391
POPULATION: GALICIA (NW SPAIN)
SAMPLE SIZE: N= 130

METHODS**PRIMERS:**

FORWARD PRIMER: 5' AACAGGATCAATGGATGCAT 3'

REVERSE PRIMER: * 5' TGGCTTTTAGACCTGGACTG 3'
 (* primer 5' end labelled with fluorescent dye)

AMPLIFICATION CONDITIONS*PCR Singleplex reaction*

5-10 ng of genomic DNA in a 50 μ M reaction volume.
 10mM TRIS-HCL (pH8.3), 50mM Kcl, 0.01% Gelatin, 1.5mM MgCl₂, 200 μ M each dNTP,
 0.25 μ M each primer and 1.25 U AmpliTaq DNA Polymerase (Cetus, Emeryville, Calif).

PCR Triplex reaction

Identical buffer, dNTPs and enzyme concentration.

Primers concentration:

HUMCD4: 0.10 μ M each primer

HUMTHO1: 0.15 μ M each primer

D12S391: 0.25 μ M each primer

PCR CYCLING (Individual and triplex reaction)

94°C - 45 sec.

60°C - 60 sec. 30 CYCLES

72°C - 60 sec.

SEQUENCING

Sequencing from templates derived from PCR products were carried out using the fmol TM DNA Sequencing System Sample (Promega).

ALLELIC LADDER

It was obtained after combination of 12 sequenced alleles (Fig. 1).

DETECTION SYSTEM

A.L.F. DNA Sequencer (Pharmacia)

Analysis of fragment: 6% Polyacrylamide denaturing gels.

Electrophoresis conditions: 1600V, 42mA, 45W at 40°C for 4 h.

Sequencing: 6% Polyacrylamide denaturing gels.

Electrophoresis conditions: 1600V, 38mA, 45W at 50°C for 5 h.

RESULTS

POPULATION STUDY

12 alleles ranging from 210 to 254 bp were found.

Allelic designation of the STR D12S391 was made according the number of repeats.

Observed genotypes of D12S391 in the Galician population.

Gen.	Obs.	Gen.	Obs.	Gen.	Obs.	Gen.	Obs.
15-15	1	17-17	1	18-21	6	20-21	4
15-17	2	17-18	2	18-22	9	20-22	3
15-18	1	17-19	1	18-23	4	20-23	1
15-19	3	17-20	1	18-24	5	21-21	2
15-20	1	17-21	2	18-25	1	21-22	5
15-21	1	17-22	5	19-20	5	21-23	2
15-23	1	17-23	4	19-21	1	22-22	1
16-17	1	17-24	1	19-22	6	22-23	2
16-18	2	17-26	1	19-23	5	22-24	4
16-20	1	18-18	6	19-24	1	23-24	2
16-21	1	18-19	11	19-25	1	23-25	1
16-25	1	18-20	6	20-20	2	24-26	1

Allele frequencies of D12S391 in the Galician population.

Allele	Frequency	allele	frequency	allele	frequency	allele	frequency
15	0.0420	18	0.2252	21	0.0992	24	0.0534
16	0.0229	19	0.1298	22	0.1374	25	0.0153
17	0.0840	20	0.0992	23	0.0840	26	0.0076

The system is in Hardy-Weinberg equilibrium (Exact test: $p = 0.54132$).

Heterozygosity: 90.08%

Discrimination power: 98.04%

SEQUENCE STRUCTURE

The 12 alleles showed a common repeat structure with a core sequence:

(AGAT)₈₋₁₆(AGAC)₆₋₁₀(AGAT)₀₋₁

TRIPLEX

Triplex amplification of the HUMCD4, HUMTH01 and D12S391 has proved that it gives good results.

CONCLUSION

The characteristics of the system, including the high polymorphism, normal electrophoretic behaviour, easy amplification and easy multiplexing makes this STR considerably interesting for forensic purposes

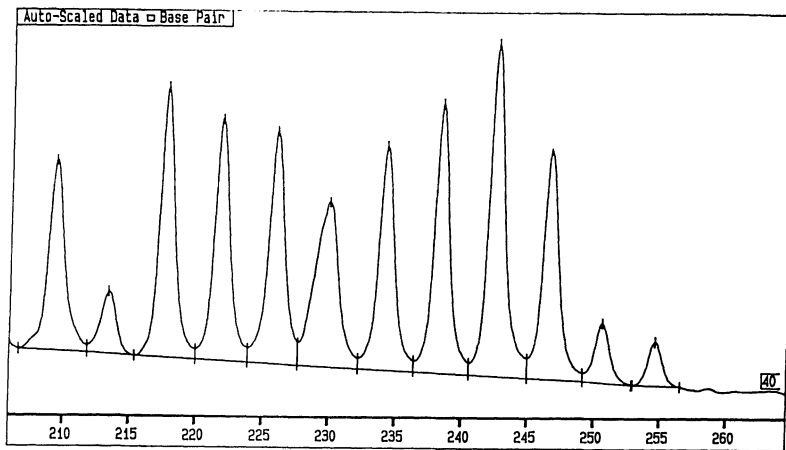


Figure 1. D12S391 allelic ladder. The allelic ladder was obtained after combination of 12 sequenced alleles.

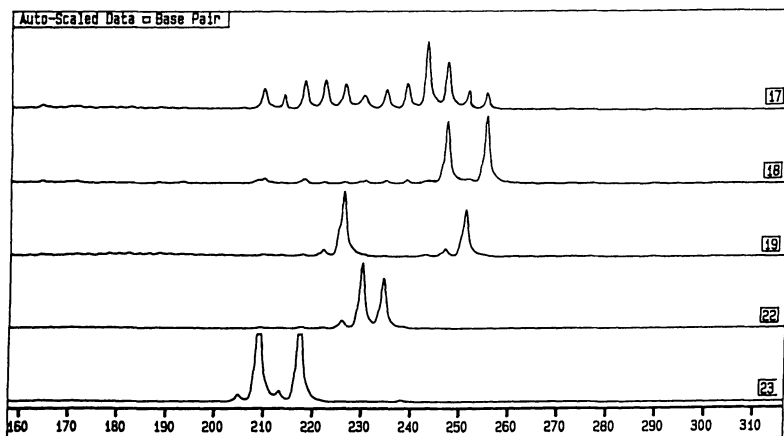


Figure 2. Electropherogram from the STR D12S391 from a 6% denaturing polyacrylamide gel. Lane 17 (allelic ladder), lane 18 (24-26), lane 19 (19-25), lane 22 (20-21) and lane 23 (15-17).