Dinucleotide repeat polymorphism in the human angiotensinogen gene

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Source and Description of Clone: λ charon 4A M49A clone containing the last 3' exon of the human angiotensinogen gene (1). A 2.5 kb *Pst*I fragment of M49A was subcloned in Bluescript SK II vector and nucleotide sequences flanking the (CA)₁₅ repeat were determined (EMBL accession number: X62854). PCR primers were designed using the OLIGO program (2).

PCR Primers:

Ang5: 5'TAGATCTCTCAGCTATTACAAGG 3' (CA strand)
Ang6: 5'GTTTCAGAGAACTGACCTGTGG 3' (GT strand)

Polymorphism: Allelic fragments were resolved on 5% denaturing polyacrylamide gels. Length of PCR products and frequencies of alleles determined from 100 unrelated European caucasians were:

allele	length (nt)	freq.	allele	length	freq.
A1	133	.005	A7	121	0.40
A2	131	.02	A8	119	.065
A3	129	.03	A9	117	.13
A4	127	.03	A10	115	.035
A5	125	.09	A11	113	.025
A6	123	.17			

Heterozygosity 75%.

Chromosomal Localization: Angiotensinogen gene has been mapped to 1q42-q43 (3).

Mendelian Inheritance: Co-dominant Mendelian inheritance was observed in 85 informative families with 170 children.

PCR Conditions: PCR was performed in a total volume of 20 microliters with 200 ng of genomic DNA, 8 pmoles of each primer, 2 mM MgCl₂, 200 mM dNTP, 50 mM KCl, 5 mM Tris-HCl, pH 8.3, 0.01% gelatin, 0.5 U *Taq* polymerase (Boehringer). Primer Ang5 was 5' end labeled with ³²P γ -ATP with the resulting specific activity 0.1–0.05 μ Ci/pmol. Amplification was for 35 cycles with denaturation at 94°C 1 min, annealing at 55°C 1 min, extension 72°C 2 min. PCR products were heat denatured in 95% formamide, resolved on 5% sequencing gels, and autoradiographed on Amersham MP film.

References: 1) Gaillard, I., Clauser, E. and Corvol, P. (1989) *DNA* 8, 87–99. 2) Rychlik, W. and Rhoads, R.E. (1989) *Nucl. Acids Res.* 17, 8543–8551. 3) Isa, M.N., Boyd, E., Morrison, E., Harrap, S., Clauser, E. and Connor, J.M. (1990) *Genomics* 8, 598–600.

A microsatellite polymorphism at the D3S11 locus

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Source/Description: A human genomic library in EMBL3 was screened with a human repeat free fragment probe of E41 at locus D3S11 (1). Three steps of chromosome walking identified a clone, lambda E41–31, which is approximately 30 kb from the original probe. One *Hae*III fragment of lambda E41–31, subcloned in M13, hybridised to poly(dC-dA). Sequencing of this subclone identified a (GT)_n repeat which detects 7 alleles. The subclone was designated E41–31.13GT. Flanking sequences were used to design PCR primers to amplify the repeat sequence.

PCR Primers:

E41–31.13 1 5'-CAAACCTTTCCACAGTATCGTTC-3'
E41–31.13 2 5'-GTTTCCTTGAGAAGAATGGAGC-3'

Frequency: Estimated from 52 chromosomes of unrelated European Caucasians.

E41–31.13GT		
Allele	Size (nt)	Frequency
B1	147	0.14
B2	145	0.12
B3	143	0.23
B4	141	0.14
B5	139	0.17
B6	137	0.05
B7	135	0.15

Heterozygosity = 93% PIC = 0.88

Chromosomal Localisation: Localised to 3p14-p21 (1).

Mendelian Inheritance: Co-dominant segregation was observed in 6 two or three generation families.

PCR Conditions: PCR is performed in 12.5 μ l containing: 50 ng DNA, 12.5 pmoles of each primer, 1.0 mM MgCl₂, 10 mM Tris-Cl pH 8.3, 50 mM KCl, 25 μ M dATP and 200 μ M of other dNTPs, 1 unit *Taq* polymerase (Perkin-Elmer/Cetus), 0.01% gelatin and 0.5 μ l of ³⁵S-dATP at 500 Ci/mmol (Dupont). Amplification is for 35 cycles with denaturation at 94°C, annealing at 57°C and extension at 72°C, all for 1 minute each.

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Reference: 1) Dietzsch, E. *et al.* (1986) *Nucl. Acids Res.* 14, 8698.

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