Human placental alkaline phosphatase PstI RFLP alleles are the result of a single base substitution

Robert C. Penhallow and Howard H. Sussman
Laboratory of Experimental Oncology,
Department of Pathology, Stanford University
School of Medicine, Stanford, CA 94305, USA

Source/Description: A 1.7 kb probe resulting from BamHI and KpnI digestion of a full length placental alkaline phosphatase (PLAP) cDNA was previously shown to detect a PstI RFLP of the PLAP gene (1). Specific oligonucleotide primers were used to (i) analyze the RFLP by polymerase chain reaction amplification of genomic DNA and (ii) sequence the alleles.

Polymorphism: DNA samples from individuals homozygous for the polymorphic alleles (PL28, A1/A1; PL1, A1/A2) were amplified by PCR. To confirm their genotype the resulting 404 bp products were digested with PstI and analyzed by Southern blot. PL28 DNA yields fragments 156 bp and 248 bp in size; PL1 DNA is uncut (Fig. A, Pst + lanes). (The 156 bp fragment is intronic in origin and is not recognized by the cDNA probe.) Direct sequencing of amplified PL1 and PL28 DNA (Fig. B) shows PL28 contains a native PstI site (5'-CTGCAG-3', allele 1) and PL1 to be mutated by an A for C substitution at the 4 position of the PstI site (5'-CTGAAG-3', allele 2). The remaining amplified sequences of the two alleles were identical. **Frequency:** Normal chromosomes of randomly chosen individuals: A1 = 0.82, A2 = 0.18, n = 46 (1).

Not Polymorphic For: Chromosome 2, q34-37 (2).

Mendelian Inheritance: Codominant Mendelian inheritance demonstrated in nine families (1).

Probe Availability: Not applicable.

Other Comments: The oligonucleotide sequences and conditions used for amplification are as follows: 5'-GTCTCCTCCTCTGTCAAATGGGAGT-3' and 5'-CCTGTTGGAGGGTTCAAGTCCGGT-3'. Genomic DNA (1 µg) was amplified in 100 µL with Taq polymerase (Perkin Elmer Cetus); 200 µM of dATP, dCTP, dGTP, TTP; 50 pmol of each primer (Operon Technologies); 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl₂. Samples were processed for 35 cycles consisting of 1 min at 94° (denaturing), 2 min at 55° (annealing) and 3 min at 72° (elongation). Amplified DNA from individuals homozygous for either allele was sequenced by dideoxynucleotide chain-termination.

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References: (1) Tsavaler, L., Penhallow, R.C. and Sussman, H.H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7680-7684; (2) Martin, D., Tucker, D.F., Gorman, P., Sheer, D., Spurr, N.K. and Trowsdale, J. (1987) *Ann. Hum. Genet.* **51**, 145-152.

Further characterization of SacI RFLPs at the Acid Alpha Glucosidase (GAA) locus

S. Tzall, F. Martiniuk and R. Hirschhorn
Department of Medicine, New York University
Medical Center, 550 First Avenue, New York,
NY 10016, USA

Source/Description: A 1.9 kb cDNA EcoRI segment subcloned into pUC 19. (GAA67; '2kb') (1) and subfragments indicated below.

Polymorphism: SacI detects two 2 allele polymorphisms: A: 5.5 kb (A1) and 5.3 kb (A2), A alleles also detected by a 0.2 kb SacI/EcoRI fragment, bp 3236 to 3406 (2); B: 3.8 kb (B1) and 3.4 kb (B2), B alleles also detected by a 0.65 kb SacI/NcoI subfragment, bp 1857 to 2520 (2).

Frequency: SacI A polymorphism studied in 34 unrelated Caucasians: gene frequency of 5.5 kb allele = 0.12 and of 5.3 kb allele = 0.88, heterozygote frequency = 0.23. SacI B polymorphism studied in 39 unrelated Caucasians: gene frequency of 3.8 kb allele = 0.05 and of 3.4 kb allele = 0.95, heterozygote frequency = 0.08. Frequencies of heterozygotes and homozygotes fit Hardy-Weinberg equilibrium.

Chromosomal Localization: 17q23.

Mendelian Inheritance: A polymorphism: codominant segregation in one 3-generation family (11 members) and two 2-generation families (6 members each). All families were monomorphic for the B polymorphism.

Probe Availability: Available from F. Martiniuk, NYU Medical Center.

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References: (1) Martiniuk *et al.* (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9641-9644; (2) Martiniuk *et al.* (1990) *DNA*, in press.