A sequence tagged site (STS) detects EcoRI polymorphisms in the human acidic fibroblast growth factor gene

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The DNA sequence of the human acidic fibroblast growth factor (aFGF) gene has been reported (1–3) and the gene has been localized to chromosome 5q 31.3–33.2 (4). We have combined this information in a sequence tagged site (STS) (5), designated FGFA.7/5q31–33 for inclusion in the human genome map. Using the polymerase chain reaction (PCR) described below, a fragment of 324 bp was amplified from as little as 12.5 ng of human genomic DNA (Fig. 1A). The fragment contains part of the DNA sequence from one of the upstream, untranslated exons of the human aFGF gene (unpublished results).

The two primers used in the PCR are 32 and 30 nucleotides in length, respectively. The sequence of the forward primer is from nucleotides 1 to 32 and the reverse primer is from nucleotides 324 to 295 (see below). The reactions were carried out in a final volume of 100 μ l containing from 12.5 ng to 1 μ g of genomic DNA, 25 pmol of each oligonucleotide, 200 μ M dNTP, 10 μ l of 10 $\times$ reaction buffer [100 mM Tris-HCl (pH 8.3 at 25°C), 500 mM KCl, 15 mM MgCl₂, 0.01% (w/v) gelatin]. The reaction mixture was heated at 95°C for 5 minutes and then allowed to cool to 72°C prior to adding 2.5 units of Taq polymerase (Perkin-Elmer Cetus). After adding Taq polymerase, the denaturation step was carried out at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 2 minutes for a total of 40 cycles. The reaction mixture was then extended at 72°C for 15 minutes and subjected to agarose gel electrophoresis. A single band of 324 bp was detected corresponding to the expected STS (Fig. 1A, lanes 2–7). Restriction enzyme digestions (data not shown) and sequencing (see below) were used to confirm that the correct product was amplified. The anticipated sequence (Accession No. X59612) of the PCR product is as follows:

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ATCCCCAAGGCTAGGAGGCAACCTACTAAGAGGTGGGTATGGTGTGTTTTCAC 60
TCAGTCTCTTCATCGGGTTTCTCTGAGCTCCATTCATACAGAAAGGAGCAGGAGAGA 120
CGAGCAAGTGGATCCAGAGCCTTCGCTCCAGGGGAATCAGGGGATCGGCTCTTTTCT 180
GGAGGAGACCTCCTCTCTGATGGTGAATGGGAATCCTCTCTCTCGAGCAGCCTGCT 240
GCAGCTGTCTGGTAGAGAGTGTGGCATTGCAAGAGCTGTCTCTCGCCAGAAAGAAA 300
GCACCCAGAGCCAGGCAAGAG 324
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The amplified fragment was digested with PstI and the 229 bp fragment was cloned into the SmaI and PstI sites of pBluescript vector KS (+). The resultant plasmid was designated p1.A (Δ PstI). The cloned DNA was sequenced and shown to be identical to the genomic DNA isolated from a chromosome 5 specific library (unpublished results). Genomic Southern blotting and hybridization was carried out using the cloned DNA as a probe. Three EcoRI fragments of 3.2 kbp, 2.4 kbp and 1.0 kbp were detected (Fig. 1B). According to Heitz *et al.* (6), EcoRI polymorphisms occur in this region as follows: allele 1 (2.4 kbp):

82.9%, allele 2 (3.2 kbp): 11.6%, and allele 3 (1.0 kbp): 5.5%. To demonstrate the direct utility of this STS to retrieve clones from genomic DNA libraries, we have used PCR with the two primers to isolate six YAC clones containing the human aFGF gene (unpublished results).

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Figure 1A. Analysis of PCR products following electrophoresis on a 3% NuSieve and 1% Seakem agarose gel and ethidium bromide staining. Lane M contains 0.25 μ g of ϕ X174 DNA digested with HaeIII. Lane 1 contains 10 μ l of amplified DNA using control template and control primers supplied by Perkin-Elmer Cetus. Lanes 2 to 7 contain 10 μ l of amplified DNA using 1 μ g, 500 ng, 100 ng, 50 ng, 25 ng and 12.5 ng of human genomic DNA isolated from placenta as a template, respectively. A single band of 324 bp is visible in lanes 2–7 corresponding to the expected STS. **1B.** Southern blotting analysis of EcoRI polymorphisms. Genomic DNA was isolated from an acute nonlymphocytic leukemia patient (#SN109 as described in ref. 7) (Lane 1) and two normal individuals (Lanes 2 and 3). The DNA was digested with EcoRI, electrophoresed on an agarose gel and hybridized to the XbaI-PstI fragment derived from p1.A(Δ PstI). Arrows indicate three alleles with sizes of 3.2 kbp, 2.4 kbp and 1.0 kbp, respectively.