SHORT COMMUNICATION

ISOLATION OF A cDNA-DERIVED EXPRESSED-SEQUENCE-TAG CLONE ON CHROMOSOME 15

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INTRODUCTION

Complementary DNA clones isolated from human brain cDNA libraries have been partially DNA sequenced to generate expressed sequence tags (EST) (Adams *et al.*, 1992). ESTs have been essential for construction of transcript maps across large chromosome regions and for identification of genes responsible for inherited diseases and cancers. A procedure for rapid chromosomal assignment of EST has been developed (Polymeropoulos *et al.*, 1992). Pairs of oligonucleotide primers for each ESTs were used to amplify by PCR DNA template human-rodent somatic cell hybrid chromosomal panels. They were able to localize 46 brain EST to chromosomes. One EST (EST00165) was mapped to chromosome 15, the chromosome involved in Angelman syndrome (AS), a complex disorder characterized by severe mental retardation and puppet-like upper-limb movements, which is caused by deletions of proximal 15q (Knoll *et al.*, 1989). In our study, a genomic clone for this EST has been isolated and used to determine whether it maps to the AS chromosome region.

MATERIALS AND METHODS

Isolation of phage clone

Preparation of plating cells (*Escherichia coli* LE392), phage titring, and phage plating were done as previously described (Sambrook *et al.*, 1989). The human genomic library in lambda EMBL3 (Clontech) was screened by PCR of phage lysates using a strategy described by Bloem and Yu (1990).

PCR Reaction

The PCR was carried out in 50 µl reaction volumes containing 10 µl of phage lysate or DNA preparation, 20 pmol of each primer, 50 mM KCl, 10 mM Tris pH 8.3, 1.0 mM MgCl₂, 0.001% gelatin, 100 µM dNTPs and 0.75 units of *Taq* polymerase (AmpliTaq,

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Figure 1a. The EST PCR amplification of a human genomic lambda library. Lane (1) amplification of the 105-bp product from a positive clone; (2) pBR322-*MspI* DNA size marker; (3, 4) negative clones; (5) a negative control - water; and (6) a positive control - total genomic DNA.

Perkin-Elmer Cetus). After an initial denaturation (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 2 min), amplification was carried out with 29 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 1 min). This was followed by a final cycle of denaturation (94°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 10 min). The primers were synthesized based on the EST00165 sequence (Polymeropoulos *et al.*, 1992), EST00165-L: 5'AGGATGACCTGAGTGAGCTG3' and EST00165-R: 5'CCATGGCAGCAAGGAAGTCT3'. The expected PCR product size was 105 bp. Total human DNA (50 ng) was used as template for positive control, and water for negative control. Ten µl samples were electrophoresed in 6 % PAGE gels in 0.5xTBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) for 30 min at 200 volts. The DNA-size marker used was pBR322-*MspI* (New England Biolabs). The gels were stained with ethidium bromide and the DNAs were visualized by illumination with short-wave UV.

Cloning of phage DNA

The preparation and purification of the phage clone, and the isolation of the phage DNA were done as previously described (Sambrook *et al.*, 1989). The phage DNA was digested with *Bam*HI and *Sal*I (New England Biolabs). The 5.3-kb fragment was confirmed to contain the EST sequence by PCR. It was ligated to a plasmid vector, pBluescript (Stratagene), and then transformed into competent *Escherichia coli* XL1-Blue cells (Stratagene). The transformants containing the recombinant DNA were identified by the blue/white plate assay, and then by the EST PCR.

Figure 1b. The restriction digestion of the EST phage clone. Lane (1) λ -HindIII DNA size marker; (2) the EST lambda clone digested with *Sall*; (3) the EST lambda clone digested with *Bam*HI; and (4) the EST lambda clone digested with *Sall* and *Bam*HI.

Figure 1c. The cloning of the EST 5.3-kb fragment into pBS vector. Lane (1) the pBS clone of the 5.3-kb fragment digested with *Sall* and *Bam*HI; (2) the EST lambda clone digested with *Sall* and *Bam*HI; and (3) the pBS vector digested with *Sall* and *Bam*HI.

Southern hybridization

The radioactive labelling, suppression of repeated sequences and Southern hybridization were done as previously described (Driscoll and Migeon, 1990). The zoo blot was prepared as previously described (Monaco *et al.*, 1986). The YAC clones (Kuwano *et al.*, 1992) used were from the CEPH YAC library: 273A2 (450 kb); St. Louis YAC library: B230E3 (250 kb), A229A2 (250 kb) and A203G1 (400 kb); and the ICI YAC library: 6GD4 (165 kb). The YACs were not chimeric, except A203G1.

RESULTS AND DISCUSSION

The titre of a human genomic library in lambda EMBL3 was 1.5×10^6 . It was plated at 50,000 plaque forming units (pfu) per plate on thirty 150-mm plates. The phage lysate was prepared from each plate and was used in PCR amplification with the EST primers. From the 50 phage-lysate preparations screened only one produced an amplification of the appropriately-sized product of 105 bp (Figure 1a). Others produced no PCR amplification. The titre of the positive lysate was 5×10^9 pfu/ml. The lysate was subsequently plated at 10,000, then 1,000 and then 100 pfu/plate. At each stage of plating,

appropriate numbers of plates were used to provide three-times coverage of the number of independent phages. At 100 pfu/plate, individual plaques were picked and tested separately. A positive plaque was identified. This was plated-out once more and its progeny plaques were tested. All were positive.

The DNA from the positive phage was prepared and digested with restriction endonucleases *Bam*HI and *Sal*I. These enzymes released the human insert from the lambda-vector arms, as well as, internally digesting the insert (Figure 1b). The digested DNA fragments were well-separated on an agarose gel and each DNA fragment was tested for amplification with the EST PCR. Only the 5.3-kb fragment gave the 105-bp PCR product. The same-sized PCR product was obtained from both cDNA and genomic DNA, therefore there may be no intron sequences between these two primer sites. The 5.3-kb fragment was cloned into pBluescript digested with *Bam*HI and *Sal*I (Figure 1c). It hybridized to a Southern blot of human genomic DNA suppressed for repeated sequences. The probe also hybridized to cow and rat genomic DNAs on a zoo blot, indicating the presence of unique sequences and evolutionary conservation. The clone was tested to determine whether it maps to our area of interest, the Angelman syndrome chromosome region. However, it did not hybridize to YAC clones, 273A2, B230E3, 6GD4, A229A2 and A203G1, which cover this region. Nonetheless, the clone hybridized exclusively to a mouse-human somatic cell line containing human chromosome 15. Moreover, with subsequent chromosomal sublocation, the EST can serve as an anchor point on human genome map to facilitate the ordering of large genomic clones and also as an initiation point for continuous sequencing of large genomic fragments.

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