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Sequence-Tagged Sites (STSs) Spanning 4p16.3 and the Huntington Disease Candidate Region

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The generation of sequence-tagged sites (STSs) has been proposed as a unifying approach to correlating the disparate results generated by genetic and various physical techniques being used to map the human genome. We have developed an STS map to complement the existing physical and genetic maps of 4p16.3, the region containing the Huntington disease gene. A total of 18 STSs span over 4 Mb of 4p16.3, with an average spacing of about 250 kb. Eleven of the STSs are located within the primary candidate *HD* region of 2.5 Mb between *D4S126* and *D4S168*. The availability of STSs makes the corresponding loci accessible to the general community without the need for distribution of cloned DNA. These STSs should also provide the means to isolate yeast artificial chromosome clones spanning the *HD* candidate region.

INTRODUCTION

The maturing of technologies for manipulating, cloning, and sequencing DNA has made the mapping and sequencing of the entire human genome a feasible goal. Several different approaches are being employed to generate physical maps of human chromosomes, including long-range restriction mapping by pulsed-field gel electrophoresis, ordering of isolated clones by use of irradiation-reduced hybrid lines or fluorescence detection-based *in situ* hybridization, and isolation of overlapping clone sets (or "contigs"), particularly using cosmids or yeast artificial chromosomes. The completion of the genome map requires that information from each of the different mapping procedures can be readily compared and related to the genetic linkage map being constructed in parallel. It has been proposed that the use of sequence-tagged sites (STSs), for which specific primer pairs yield a characteristic polymerase chain reaction product as common signposts on the maps, would fulfill the requirement of a "common language" for comparison of the different maps (Olson *et al.*, 1989).

The p16.3 cytogenetic band of chromosome 4 is one of the more extensively mapped regions of the human genome as a result of the search for the defect causing Huntington's disease (Gusella, 1991). The HD gene has been confined to a segment of 4p16.3 between *D4S10* and the telomere (Gilliam *et al.*, 1987a). A long-range restriction map of this region includes only one gap and spans 4.5 Mb from *D4S10* to the telomere (Bućan *et al.*, 1990; Bates *et al.*, 1991). Irradiation-reduced hybrids have also been applied to mapping 4p16.3 (Cox *et al.*, 1989; Doucette-Stamm *et al.*, 1991). Numerous phage and cosmid clones have been mapped to the region and several have yielded DNA polymorphisms, permitting construction of a detailed genetic map (MacDonald *et al.*, 1989a; Youngman *et al.*, 1989; Allitto *et al.*, 1991a). To assist in comparing the evolving physical maps being generated with different cell lines and techniques and to lay the foundation for isolating overlapping YAC clones for the *HD* candidate region, we have generated a set of 18 STSs spanning 4p16.3 at an average spacing of 250 kb.

MATERIALS AND METHODS

Origin of cell lines and clones. HHW416, a human–hamster hybrid containing only human chromosome 4, and HHW693, containing only human 4pter–4p15.1 and 5p15.1–5cen as part of a naturally occurring t(4;5) chromosome with 5q deleted, have been reported previously (Wasmuth *et al.*, 1986). Clones used for generation of primer sets have been used successfully in Southern blot experiments, and their positions on genetic and physical maps have been described previously and are listed in Table 1. STSs have been named by adding * followed by a sequential identification number to the locus symbol.

DNA sequencing and preparation of oligonucleotides. DNA sequence was obtained by the procedure of Sanger *et al.* (1977). Primers were synthesized using a Biosearch Cyclone DNA synthesizer or an Applied Biosystems DNA synthesizer or were purchased from Genosys (Houston).

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TABLE 1

Description of 4p16.3 Sequence-Tagged Sites

STS	Clone	PCR primer sequences	PCR product size (bp)	PCR reaction mix	PCR program
*D4S10**1	G8	5′ GCACCTGGATCTCGGGCTTCTGG 3′ 5′ AGAATGGGCTGCGGGAACGGGAG 3′	192	A	1
*D4S10**2	I5	5′ CGGGCTGGGTTCCAGCAAGG 3′ 5′ CATATGTTGAAGTCCTGCCG 3′	213	B	2
*D4S126**1	p309	5′ GCATGTACACTGTGAAGCCATAGTA 3′ 5′ TTGCCCTGGCTTCACTGTGCCTCAA 3′	184	B	3
*D4S125**1	pYNZ32	5′ AGGTGGTTCTGCCCTGAGAGCCTTT 3′ 5′ CAGCTGAGGAGGTGCCTCTGCTCCT 3′	159	B	4
*D4S180**1	L19ps11	5′ CTGGTTCTCAAGAGTGTCTC 3′ 5′ CATAATGTTGGTGGCTGCTG 3′	103	A	1
*D4S95**1	pBS674-VR	5′ GAGTCTACCGGTGCCAAAAGGAAG 3′ 5′ TGGCCTCTCCAGATGGAATGTGCTC 3′	1066	A	3
*D4S182**1	pY12Eco2.3	5′ GCCTTGGGGCAGGGGCCGGTGAGTA 3′ 5′ TCTATGAATTTCAAGGTGGCCATCT 3′	213	B	3
*D4S181**1	L14ps7	5′ AGGGCCAGTGAGCCTTCAAGCCTTA 3′ 5′ AATAAAGTTGTCATGGCCACGTCAT 3′	141	B	3
*D4S43**1	C39Kpn4.5	5′ GTACCCTTGAAGGGATATATTCCTT 3′ 5′ TCTAATGACTAGTGATGTTGGTCAT 3′	243	B	3
*D4S43**2	LCD450	5′ GACTGGTTGTTTGAGGGCGTTG 3′ 5′ TCCTTGACTCTGCTTCAGC 3′	378	A	1
*D4S166**1	L6-H10	5′ GGCCTGATCTGAGGTTGTGGAAACG 3′ 5′ CTGTACCCAGGAGCATTCTCTCCC 3′	895	A	1
*FGFR3**1	pBS385H-A	5′ AAAGGTAGCAGTCCAGGC 3′ 5′ TGTGTGTCCCAGGCAG 3′	202	A	1
*D4S168**1	E4ps2	5′ TCCCAGGACTCTTCTGGGCACAGGG 3′ 5′ CACCAGGGGCTCTCAGGACCT 3′	118	A	1
*D4S115**1	p252.3	5′ ATCAAGTCGAGGGACCTGGGCT 3′ 5′ CAGACAGCAGAGTCCACGGACAG 3′	164	A	1
*IDUA**1	p157.9	5′ GGATCCCTCTGGCATCTC 3′ 5′ TACTATCATTACCCATCC 3′	230	B	3
*D4S232**1	I14ps18	5′ TGCACCCACAACAGCAGGTGGCGAT 3′ 5′ CATCCGGCGTCTCTCTGGTAATG 3′	175	A	1
*D4S90**1	D5	5′ GTCCAGAGGAAGATGTGTAGGGAC 3′ 5′ CTACCACACCAGATCGACTAAGC 3′	599	A	1
*D4S142**1	88-18	5′ CTGGGGAGGCAAAGCCAGACCATAT 3′ 5′ GATAGTAGTTCCCACGAGATTCCTC 3′	267	B	3

Polymerase chain reaction. The polymerase chain reaction and cycling conditions for each primer set are listed in Table 1 as buffer mixture A or B and programs 1–4. Mixture A consists of 67 m*M* Tris–HCl, pH 8.8; 6.7 m*M* $MgCl_2$; 16.6 m*M* ammonium sulfate; 10 m*M* β-mercaptoethanol; 10% DMSO; and 1.25 m*M* dNTPs. Mixture B is 10 m*M* Tris–HCl, pH 8.3; 1.5 m*M* $MgCl_2$; 50 m*M* KCl; 0.01% gelatin; and 200 μ*M* dNTPs. Both sets of reactions included 10 ng/μl of each primer, 0.02 unit/μl *Taq* DNA polymerase (Perkin–Elmer/Cetus), and 10 ng/μl genomic DNA and were carried out using one of the following programs designated in Table 1:

(1) 2′@94°C,30x(1′@94°C,1′@60°C,1′@72°C);
(2) 2′@94°C,30x(1′@94°C,1′@55°C,1′@72°C);
(3) 2′@94°C,30x(1′@94°C,1′@57°C,3′@72°C);
(4) 2′@94°C,30x(1′@94°C,1′@65°C,3′@72°C).

RESULTS

Generation of STSs

Individual clones from a variety of sources have been positioned previously on the long-range restriction map of 4p16.3 (Bućan *et al.*, 1990; Whaley *et al.*, 1991; Bates *et al.*, 1991; Lin *et al.*, 1991). The list of those selected for sequence analysis to provide a source of STSs is presented in Table 1, along with the primer pair, amplification conditions, and predicted size of the amplification product for each resulting STS.

Each of the STS reactions was initially tested using DNA from HHW416, a human × hamster hybrid line containing only human chromosome 4, or from HHW693, a human × hamster hybrid line containing 4p15.1–4pter as part of a translocation chromosome and DNA from tsH1, the hamster parent (Wasmuth *et al.*, 1986). Since 5p15.1–5cen is the only other human DNA present in HHW693, the chromosome 4 origin of each PCR product could be confirmed using hybrids containing either chromosome 4 (HHW416) or chromosome 5 (HHW599) as the only human DNA. Subsequently, the STS reactions were demonstrated also to yield the expected fragment as the primary product in amplifying human genomic DNA from a variety of sources, such as lymphoblastoid cell lines, placenta, or other tissues, and as the only product from the clone used to generate the corresponding STS.

Figure 1 shows typical examples of PCR amplification products from genomic DNA for several of the STSs

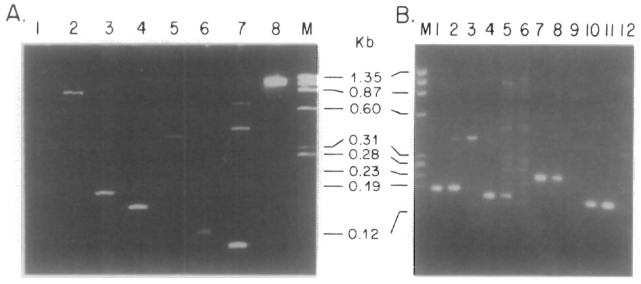

FIG. 1. Representative 4p16.3 STS amplification products: Ethidium bromide-stained gels containing representative PCR products from the STSs listed in Table 1. (**A**) A 6% polyacrylamide gel containing the following STS PCR products from HHW693 DNA: lane **1**, no DNA control; lane **2**, *D4S166**1; lane **3**, *D4S10**1; lane **4**, *D4S115**1; lane **5**, *D4S43**2; lane **6**, *D4S168**1; lane **7**, *D4S180**1; lane **8**, *D4S95**1. (**B**) A 1.2% agarose gel containing the products of PCR reactions for *D4S126**1 (lanes **1–3**), *D4S125**1, (lanes **4–6**), *D4S182**1 (lanes **7–9**), and *D4S181**1 (lanes **10–12**) using human DNA (lanes **1, 4, 7, 10**), HHW416 DNA (lanes **2, 5, 9, 11**), and hamster DNA (lanes **3, 6, 9, 12**). In **A** and **B**, lane **M** contains *Hae*III-digested ϕX174 DNA.

reported here. In each case, the major PCR product closely matches the predicted size. Occasionally, a fragment was also amplified from hamster DNA but was readily distinguishable from the human product based on its size. For example, the ~450-bp product from the *D4S126**1 STS reaction seen in both the HHW416 and hamster lanes is due to amplification of hamster DNA (Fig. 1B, lanes 2 and 3), as are the ~450-bp and ~700-bp products in the *D4S180**1 STS amplification (Fig. 1A, lane 7) and the numerous light bands in the *D4S125**1 amplification (Fig. 1B, lanes 5 and 6).

Location of STSs

The location and corresponding locus symbol for the 18 STSs and for eight polymorphisms for which PCR assays are available are given in Fig. 2. Two STSs are given for the extended *D4S10* locus, to which Huntington's disease was first linked (Gusella *et al.*, 1983). *D4S10**1 was based on a sequence reported by Stapleton (1988) to cover polymorphic *Hin*dIII site 2 and a polymorphic *Hae*III site detected by phage clone G8. Base changes at positions 66 and 152 of the 193-bp PCR product are predicted to be the basis for the *Hin*dIII and *Hae*III RFLPs, respectively, which could now be assayed by digestion of the PCR product with these enzymes. *D4S10**2 maps 14 kb distal to *D4S10**1, adjacent to but not spanning a second polymorphic *Hin*dIII site, referred to as *Hin*dIII 1. The latter can be assayed by PCR using a primer set reported by McIntosh *et al.* (1989) to produce a 93-bp product crossing this site.

*D4S126**1 was generated using the sequence of a random subclone from cosmid BJ14 (Allitto *et al.*, 1991a). This segment falls within a region of increased recombination between *D4S10* and *D4S125* and lies near the centromeric limit of the candidate region for *HD*. *D4S125**1 was derived from the sequence adjacent to a variable number of tandem repeats (VNTR) polymorphism (Nakamura *et al.*, 1988). The VNTR itself can be assayed by PCR of a much larger product (1400–2200 bp) using primers designed by Richards *et al.* (1991). *D4S180**1 is located in cosmid L19 at the *D4180* locus, distal to the region of increased recombination (Lin *et al.*, 1991).

*D4S95**1 was designed to span a large single-copy sequence adjacent to the VNTR at this locus (Wasmuth *et al.*, 1988). The resulting PCR product overlaps extensively with the single-copy probe, pBS674D, used in Southern blot analysis of the VNTR. It does not overlap, however, with the 990 to 1600-bp PCR product of the primer set reported by Allitto *et al.* (1991b) used for PCR assay of the VNTR. *D4S95**1 lies within the region of 4p16.3 that displays linkage disequilibrium with *HD* (MacDonald *et al.*, 1991a; Snell *et al.*, 1989; Theilmann *et al.*, 1989).

*D4S182**1 and *D4S181**1 were derived from subclones of cosmids Y12 and L14, respectively (Lin *et al.*, 1991). *D4S182**1 is adjacent to a VNTR polymorphism that has yet to be sequenced. *D4S181**1 maps within the 250-kb *Not*I fragment distal to *D4S182*, but has not yet been localized more precisely. This will require the more detailed restriction map of this segment that will be generated from YAC cloning of the region.

Two STSs are reported for the extended locus *D4S43*, which spans 170 kb. The more centromeric *D4S43**1 was generated from the sequence adjacent to the VNTR polymorphism detected by probe pKP1.65 (MacDonald *et al.*, 1989b). We have recently developed a PCR assay for this polymorphism that produces products ranging from 184 to 478 bp in size (Horn *et al.*, 1991). Primers for *D4S43**2 were generated from the sequence of the anonymous cDNA, LCD2, reported in Gilliam *et al.* (1987b). This STS is located near the telomeric end of *D4S43*, approximately 85 kb distal to *D4S43**1. Although we have not sequenced the PCR product, the correspondence to the predicted size suggests that it probably contains little or no intron sequence, but rather derives from a single exon.

*D4S166**1 is located in a clone that spans the distal breakpoint of an interstitial deletion chromosome that splits 4p16.3 into proximal and distal portions and has been used in regional somatic cell hybrid mapping panels (Whaley *et al.*, 1991). *FGFR3**1 corresponds to the sequence of the single-copy probe pBS385H-A, which formerly defined the *D4S99* locus (Smith *et al.*, 1988). This STS sequence has recently been shown to form part of the 3′ untranslated region of the mRNA transcript of a fibroblast growth factor receptor gene. Consequently, this locus has been renamed *FGFR3* (Thompson *et al.*, 1991).

*D4S168**1 was generated from a single-copy subclone of cosmid E4, the cloned segment closest to the centromeric side of the remaining gap in the long-range restriction map of 4p16.3 (Whaley *et al.*, 1991). *D4S115**1 was derived from single-copy sequence immediately adjacent to another VNTR polymorphism (MacDonald *et al.*, 1989b). We are currently optimizing a PCR assay for this VNTR to complement those developed for the more

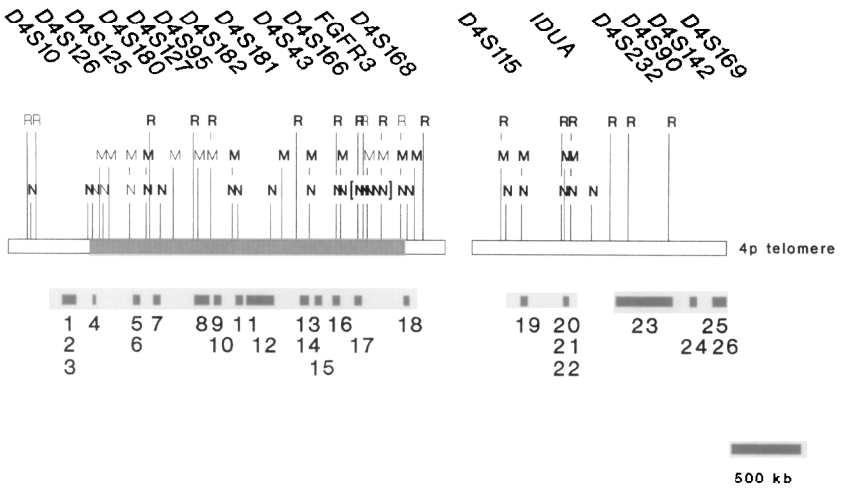

FIG. 2. Physical map locations of 4p16.3 STS and polymorphism PCR assays. The locations of 18 new STSs (listed in Table 1), two site polymorphisms, two dinucleotide repeat polymorphisms, and four VNTR polymorphisms for which PCR assays have been established are shown below the long-range restriction map of 4p16.3 with a black line to indicate the smallest interval to which each has been localized. The corresponding locus numbers are given above the map. The proximal and distal portions of the map are separated by a gap of unknown size, where fragments have not yet been visualized. The map of the proximal region is drawn from Bates *et al.* (1991), Lin *et al.* (1991), and Whaley *et al.* (1991), and the map of the distal region is drawn from Whaley *et al.* (1991). Sites (N, *Not*I; M, *Mlu*I; R, *Nru*I) prone to partial digestion are shown in lighter typeface. The Huntington disease gene candidate region (Bates *et al.*, 1991) is shown as the cross-hatched portion of the map. Square brackets around the *Not*I sites at *FGFR3* denote the presence of additional sites that have not all been mapped precisely. The PCR assays shown in the figure are: **1,** *D4S10**1; **2,** *D4S10**2; **3,** *D4S10* *Hin*dIII polymorphism assay (McIntosh *et al.*, 1989); **4,** *D4S126**1; **5,** *D4S125**1; **6,** *D4S125* VNTR assay (Richards *et al.*, 1991); **7,** *D4S180**1; **8,** *D4S127* dinucleotide repeat assay (Taylor *et al.*, 1991); **9,** *D4S95**1; **10,** *D4S95* VNTR assay (Allitto *et al.*, 1991b); **11,** *D4S182**1; **12,** *D4S181**1; **13,** *D4S43**1; **14,** *D4S43* VNTR assay (Horn *et al.*, 1991); **15,** *D4S43**2; **16,** *D4S166**1; **17,** *FGFR3**1; **18,** *D4S168**1; **19,** *D4S115**1; **20,** *IDUA**1; **21,** *IDUA* *Kpn*I polymorphism assay (Scott *et al.*, 1991a); **22,** *IDUA* VNTR assay (Scott *et al.*, 1991b); **23,** *D4S232**1; **24,** *D4S90**1; **25,** *D4S142**1; and **26,** *D4S169* dinucleotide repeat assay (Pritchard *et al.*, 1991).

centromeric loci (Allitto *et al.*, in preparation). Together, *D4S168**1 and *D4S115**1 may permit the isolation of YAC clones that will span the gap in the physical map, which has not been bridged by other techniques.

*IDUA**1 lies within the *D4S111* locus, which has recently been renamed *IDUA*, encoding α-L-iduronidase, the disease gene in Hurler syndrome (MacDonald *et al.*, 1991b). Two other segments can be amplified from this locus, one crossing a polymorphic *Kpn*I site (Scott *et al.*, 1991a) and a second spanning a VNTR polymorphism (Scott *et al.*, 1991b). *D4S232**1 is from cosmid I14, which lies within a 400-kb segment of the telomeric *Not*I fragment of 4p (Whaley *et al.*, 1991), but has not been assigned more precisely due to differences in methylation patterns affecting the *Nru*I sites in pulsed-field gel mapping of this region. It is probably located close to *PDEB*, encoding the β polypeptide of cGMP phosphodiesterase, the human homologue of the mouse *rd* gene (Weber *et al.*, 1991; Bowes *et al.*, 1990).

*D4S90**1 was generated from the single-copy probe D5, which detects RFLP with *Hinc*II and *Stu*I (Youngman *et al.*, 1989). This locus is currently the most telomeric marker that has been formally placed on the linkage map of the chromosome. The PCR product crosses both a *Hinc*II and a *Stu*I site within D5, but we have not determined whether either of these represents a polymorphic site. *D4S142**1 was derived from 88-18, a single-copy probe detecting an *Msp*I RFLP and located at the proximal end of a YAC clone spanning the 100 kb that includes the telomere of 4p (Bates *et al.*, 1990). It is located proximal to the recently reported *D4S169* locus, for which a simple-sequence repeat can be assayed by PCR (Pritchard *et al.*, 1991).

DISCUSSION

The average spacing between our new STSs from *D4S10* to the telomere is about 250 kb, with the density being highest in the *HD* candidate region. The most notable segments without STSs are the 450 kb between *D4S180* and *D4S95* and the gaps of indeterminate size between *IDUA*, *D4S232*, and *D4S90*. The first of these gaps contains a dinucleotide repeat polymorphism at *D4S127* for which a PCR reaction is available (Taylor *et al.*, 1992). Filling the gaps between *IDUA*, *D4S232*, and *D4S90* will then require a more specific positioning of *D4S232*. Also unrepresented is the region between *D4S168* and *D4S115* for which pulsed-field fragments have yet to be visualized. It is unlikely that this unmapped gap is large, given the number of random clones already isolated from 4p16.3, and it may be possible to cross it by isolating YAC clones using the *D4S168* and *D4S115* primer sets.

Taken together with the single-site, dinucleotide repeat, and VNTR polymorphisms for which PCR reactions have been worked out, the 18 STSs reported here bring to 26 the number of PCR assays that could be used to screen for YACs in 4p16.3. The 11 new STSs between and including *D4S126* and *D4S168* should prove sufficient to isolate YAC clones spanning most or all of the *HD* candidate region (Bates *et al.*, 1991). Moreover, the density of STSs in the segment can be increased if

needed by developing additional primer sets from the ends of YACs as they are isolated. We have already isolated YAC clones using the *D4S95* and *D4S166* STS assays and are pursuing the goal of cloning the *HD* candidate region and the rest of 4p16.3 in its entirety. Of more immediate impact, the development of these 18 STSs throughout 4p16.3 makes the corresponding loci and their surrounding regions readily accessible to the entire research community without the cost or difficulty of distributing cloned DNAs.

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