

Sixty New STSs (Sequence-Tagged Sites) of Human Chromosome 21

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Abstract

From human chromosome 21-specific libraries, 22 *Sfi*I linking clones and 38 P1 clones were isolated and regionally mapped on the chromosome. The terminal sequences of these clones were determined and pairs of PCR primers were generated which could specifically amplify the sequenced regions. These sequence-tagged sites (STSs) should be useful for constructing a high resolution map of human chromosome 21.

Key words: human chromosome restriction enzyme *Sfi*I; linking clone; P1 clone, high-resolution physical map; PCR

1. Introduction

Human chromosome 21 is the smallest chromosome (about 50 Mb long) in the human genome¹ and extensive efforts have been made to construct genetical and physical maps.^{2,3,4,5} A Contig of the yeast artificial chromosome⁶ and a complete *Not*I restriction map⁷ of the long arm of the chromosome have already been constructed. However, high resolution maps should be constructed for the detailed analysis of the chromosome. Sequence-tagged sites (STSs) have been shown to be a powerful tool for physical mapping,⁸ so that it is desirable to construct a high-resolution STS map of the chromosome. To accomplish this, we have attempted to generate many chromosome 21-specific STSs. In this paper, we report 22 STSs from chromosome 21-specific *Sfi*I linking clones, 27 STSs from the *APP* gene region, 9 STSs from the D21S8 region and 2 STSs from the D21S16 region.

2. Materials and Methods

2.1. Isolation of *Sfi*I linking clones

A *Sfi*I linking library of human chromosome 21 using the plasmid vector pTZ19R was constructed as described previously⁹ from a chromosome 21-enriched phage library (LA21NS002). Plasmid DNA from each clone was isolated using an automated plasmid isolation system (KURABO, Model PI 100), digested with *Hinc*II and *Sfi*I to eliminate overlapping and non-linking clones, and then

sequenced using an automated DNA sequencer (Pharmacia ALF) as described by Hattori et al.¹⁰

2.2. Isolation of P1 clones

P1 clones carrying the known STS markers were isolated from a chromosome 21-specific P1 library (manuscript in preparation) by polymerase chain reaction (PCR)-based screening. P1 DNA was prepared by the KURABO automated plasmid isolation system and purified with RNase A treatment and polyethyleneglycol-NaCl precipitation.¹¹ The terminal sequences were determined as described previously⁹ using a fluorescent sequencing primer for the P1 vector (p1FL-1 and P1FR-1, see Table 2) by an automated DNA sequencer (Pharmacia ALF).

2.3. PCR conditions

The PCR reaction was carried out using a Perkin-Elmer Cetus thermal cycler in 25 μ l of reaction mixture containing the buffer solution supplied from Promega, 50-100 ng of template genomic DNA or approximately 0.1 ng of cloned plasmid DNA, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 15 pmol of each primer and 1 unit of *Taq* DNA polymerase (Promega). Amplification was carried out by 30 cycles of denaturation (94°C for 1 min), annealing (57°C for 1 min except 65°C, 1 min for Sf-484) and extension (72°C for 1 min). Half of each PCR product was analyzed by electrophoresis on a 3% Nusieve/1% agarose gel.

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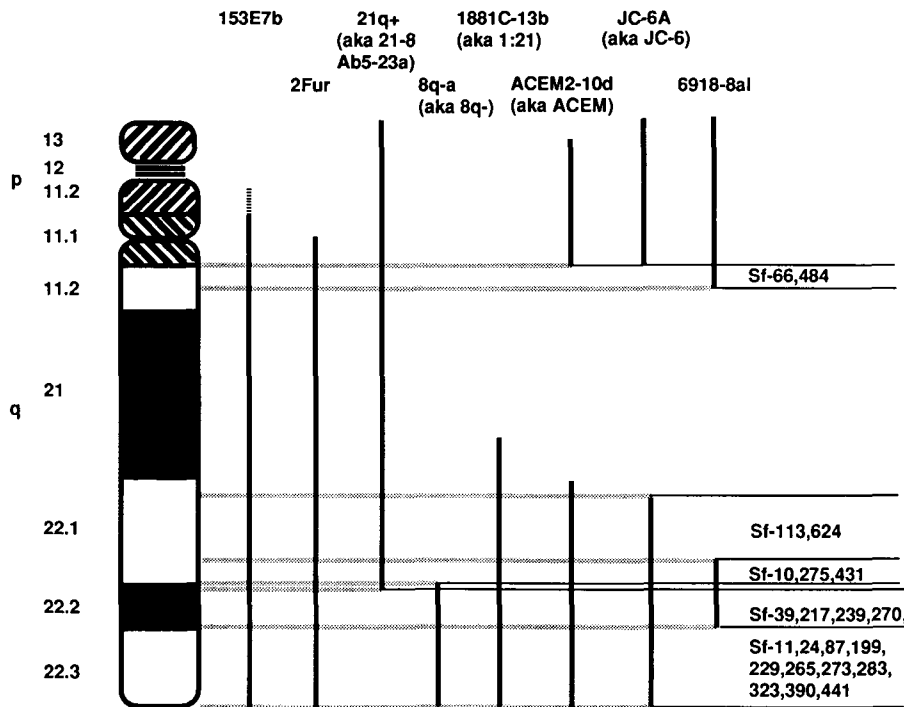


Figure 1. Regional assignment of *Sfi*I linking clones to the hybrid cell mapping panel. The solid lines indicate the portion of human chromosome 21 present in the indicated hybrid cell line. Shaded horizontal lines indicate relative positions of breakpoints on adjacent chromosomes. The cytogenetic region to which the linking clones are assigned by PCR or Southern hybridization is indicated at the right of the ideogram.

3. Results and Discussion

3.1. Sequence-tagged *Sfi*I sites

So-called rare-cutters' restriction sites are useful landmarks for large-scale physical mapping, but most of these sites contain a CpG dinucleotide sequence and are clustered in a so-called CpG island.¹² *Sfi*I is one of the rare-cutters but its recognition sequence does not contain the CpG dinucleotide, thus making *Sfi*I sites a useful marker for physical mapping. For these reasons, we attempted to isolate *Sfi*I linking clones and generate sequence-tagged *Sfi*I sites on chromosome 21. We previously constructed a *Sfi*I linking library from a flow-sorted chromosome 21-rich library.⁹ From this library, 198 clones were isolated and sequenced, and 72 independent clones were finally obtained. To select chromosome 21-specific clones, a set of oligonucleotide primers for each clone was synthesized, and PCR amplification was carried out using the primer. DNAs from human placenta, mouse liver and WAV17 (a human/mouse hybrid cell line containing human chromosome 21 as its sole human component) were used as templates. The cloned DNA was also used as a reference template. Among the 72 clones tested, the primers for 22 clones generated the expected size of products for both human and WAV17 DNAs but not for mouse DNA, indicating that they were chromosome 21-specific. Another

39 clones were found to be derived from human chromosomes but not from chromosome 21. The remaining 11 clones contained non-human DNA inserts. The PCR primer sequences, the length of the amplified products and the annealing temperatures for the chromosome 21-specific clones are listed in Table 1. Sf-273 and Sf-390 had two *Sfi*I sites. Among 22, 6 clones (Sf-113, 199, 239, 283, 390, 431) were Alu-positive and one clone (Sf-390) L1-positive. Digestion of *Sfi*I linking clones by several CpG enzymes (*Not*I, *Mlu*I, *Bss*HIII or *Sac*II) revealed that 14 of 22 *Sfi*I linking clones had no restriction site for the above CpG enzymes (Table 1). Thus a considerable number of *Sfi*I sites were thought to be out of CpG islands. The regional mapping of the *Sfi*I linking clones was performed by PCR using the template DNAs from the human/hamster (or mouse) hybrid cells containing various portion of human chromosome 21. The results are summarized in Fig. 1. The Sf-66 could not be mapped by PCR amplification for unknown reasons. Therefore, Sf-66 was mapped to 21q11.2 by Southern hybridization. It should be noted that the distribution of the *Sfi*I sites appeared not to be random. Eleven clones (50%) were mapped to the band 21q22.3, which represents approximately 20% of the long arm of the chromosome. The majority of them were mapped to R-band region.

Table 1. Sequence-tagged sites (STSs) specific for chromosome 21

Clone name	Insert size (kb)	Number of internal restriction sites				STS name	PCR primer sequences (5' to 3')		PCR product size (bp)
		Nt	Mi	Bs	Sc		Forward	Reverse	
Sf-10	4.7			6	1	Sf-10UV	CCCAAAGCAAGAGTAGATGG	AGGTGTGTGCGCTCAGATTTTC	90
Sf-11	3.0					Sf-11RV2	CTCTACTTAGTTGCCACTG	TTTGCTAGGCTTCCACACC	169
Sf-24	5.5		2	1		Sf-24UV	CATTCGCCCTTGATGGGC	TGTGCCCTGAGCATCAGG	106
Sf-39	4.5	2		3	1	Sf-39UV	TTGGGGATGATCAGGGGTG	GACGAGGCTGTTTTCGGAG	137
Sf-66	4.3					Sf-66RV	AGCTTAACCAAAATGTTGACTC	ATTTGCCAAGTTAATGGCTGC	113
Sf-87	0.9					Sf-87RV	ACTTTACCATGCAACTTAGGG	TCCTTTTTTTGGGCTCCTGCC	108
Sf-113	3.9					Sf-113UV	GCTTCCAGAATGGAAGTGG	TGAGCCTTGTCTGTGTATC	123
Sf-199	4.0	1				Sf-199UV2	TGCTTAACAGGCATCCAGGT	CTGCTTGTGTTACCTTCCATC	116
Sf-217	3.7					Sf-217UV	CTTGACGAGGGACAATATG	ATAAAGATGAGGCAGAGATGG	162
Sf-229	1.1					Sf-229RV	AGCTTACCACACATGAGGC	TGGCCAGTCTCTATGAGG	95
Sf-239	3.6					Sf-239RV	GCATAGAATAACAGCTCTGAC	ATGTGATTTTAGTACAGCTTCC	87
Sf-265	0.5					Sf-265UV	CTCAAAGGCAGTTGGCTG	CCTGGATCTGCTATCCTGC	110
Sf-270	2.4					Sf-270UV	TGTAGACAAAGTCACCAGGC	CTCCAGGATAATTCATGGGG	80
Sf-273*	3.9			1		Sf-273UV	GGTGCTTCTTTGATTCAGC	CITGGGAAGTGTGTGGCAC	122
Sf-275	0.7					Sf-275UV	AGGCAAAGAGTACTTTGGGC	GGTGTAGGGAAGGAGAAC	213
Sf-283	5.7	1	5			Sf-283UV2	TAGTATCCACATGCCGGAG	CCTGATCTCCAAGAGGATGC	165
Sf-323	3.2	1				Sf-323UV	AGCTTACCGGCTTCCCTTG	AGCTGGCCTGTCCCTCAATC	96
Sf-390*	7.1	1	1		7	Sf-390UV	CTGATAACTAGGTTCTTGTGTG	ATCCAGAGGCTGGGTCTG	120
Sf-431	1.3					Sf-431UV	TTTTGGTCCGGTGGAAAGTC	AAGCGACTGGTCAAGAGG	93
Sf-441	4.1					Sf-441RV	CAACAATTGAGGTGTCAAAGTC	TACCGTCTTCCAGTCTCAC	80
Sf-484	1.7					Sf-484RV2	TGCCCTCCTGTGGCTTGGAA	AAAGGCCACATGTACAATGC	97
Sf-624	1.9					Sf-624UV	GGCATGAATCTGGCATTCC	TTGACCTAAAGTGTATCCGCC	122

Nt: *NotI*, Mi: *MluI*, Bs: *BssHIII*, Sc: *SacII* * This clone had two *SfiI* sites.

Table 2. P1 clone-derived STSs

Clone	PCR primer sequences (5' to 3')		Length of PCR products (bp)	
Sequencing primer for P1				
P1FR-1	CGCTAATACGACTCACTATAGGGA			
P1FL-1	TTGGCCGTCGACATTTAGGTGACA			
Developed P1 STS				
APP region				
APPEX-887	ACAAGGGCAGCGTTTGAGG	GCAGGGATGTAACATCCATA	117	P
APPEX1	ATCCCACTCGCACAGCAGC	CAGGAGCAGTGCCAAACCG	73	P
APPEX3	CCCTGAAGTGCAGATCACC	AGCAGCGGTAGGGAATCAC	122	P
APPEX5	ACCAACTTGCATGACTACGG	TCTGCATAGTCTGTCTGC	170	P
APPEX6	AAGTAGTAGAAGTAGCAGAGG	TGGTGGTTCTCTCTGTGGC	120	P
APPEX4	TGGTGAAGTTTGTAAAGTGATGC	GTACCTCTTTGGCGACGGT	116	P
APPEX8	GTCCCAAAGTTTACTCAAGAC	TCTTCCCTTCCCTCAGGTG	88	P
APPEX9	TTCCTACAACAGCAGCCAGT	CTGGGACATTCTCTCTCGG	134	P
APPEX10	AGTCTGTATTCAAAGGATGAAC	TGATGTTTGGTAGGAAATGGG	167	P
APPEX11	TGGAATCTTTGGAACAGGAAG	CGAGGAGGAACAGCCTGC	142	P
APPEX12	ACAGCCTCGTCACGTGTTT	GATCTGAGCGGCTTTCTTGG	124	P
APPEX13	TTATGACACACCTTCCGTGTG	AACTTCATCCTTGAATCTCCTC	98	P
APPEX13A	TCCCCCATTGAATGGTCTTG	GTGGTACTACAGTAAGCACAG	122	P
APPEX14	AATCAGATGACGTCTTGGCC	GCTGGAGATCGTCCAGGC	144	P
APPEX16	AATACAGGTTCTGGGTTGAC	GACAAACAGTAGTGAAAGAG	147	P
APPEX17	AAAGGTGCAATCATTGGACTC	CCACTTGGAACATGCAGTC	149	P
T-1539R	TCATGGAAGTTTTTGCTCCC	CTAAGGTAAATACACTGCTCC	153	S
T-1539R	CATATAAAGCTTTTCCACCC	TGATCTCAGCTCTTTCCTGG	87	S
O-459R	TTAGGTTCCCCCCACATGG	AGCGATGTGAGGCACACTG	105	S
O-648R	CGGGAGGCATAGGTGCAG	TCATTCTCCGCTGAGAAACC	128	S
O-648L	AAGGCTTATCATCGGTAGCC	CCACTTGAGAGATAGTGAG	131	S
T-1559R	GGAAACCTGGCTGTTTCCC	CAGAATAGATGGGAGGGTTC	123	S
T-1559L	CAATATGCTGAGAATAAT	GATTAGCCAGGGCCATCC	112	S
O-491L	TTTTGCTGATAAGGTACCCAG	CAGGACTCCCAGGGACTC	174	S
T-1715L	CTCTCCCTTTTGACCTGC	TTTCTCCTACCTGAGTCCTG	75	S
T-364R/E6	AATTTAACGGTCCACATCTACG	GGATGGAAGAAGCTGTTTCAG	110	S
T-364L	GTTTGGCCAGGCTCATAGG	GCCCATGAACATGAGATAGC	104	S
<i>D21S8</i> region				
T-1850R	CCTAGTTGGTGGGTGATAGC	AACTAGGTGATGTAGTCTACC	117	S
T-1850L2	TTCAAAAGTTAAAACCTAGGGC	GGAAATTGTAACCCCTCATC	96	S
O-453R	GCAAACCACCATGGCACAC	AGGCACATCACGTATCAGG	175	S
O-453L2	GTATGTAGCACCTCCCACC	GCTTCTGGGGAGCCTCAG	107	S
O-576R	CAATACAAGGAGTTGGTTGAC	GTGCTTAGCGGAGATGGCT	95	S
O-672R2	ATGGCCAGTTAAGTTGACG	AGACCTATTTGTTGCAAGCAC	113	S
O-672R	GATGCAAAATGGACTGATGC	ATCATGTGTTTCTAAATCTGAG	82	S
O-11R2	GCCCAGCCCTCTAGTGCA	GACCAAAGAGGAAATAATGACC	78	S
O-11L	GATCATAGTGAGGACTGAAG	TATAAAGCATTTACCATGTGCC	81	S
<i>D21S16</i> region				
O-204R	GGAAAATCTGTACACTGAAAGC	TACAAACTATGATCGATCAGC	87	S
O-204L3	CCATAAACACAATCTTCAAGGC	CTATAAACACAGTAAACCAGC	81	S

P, the sequences were published (13). S, the sequences were determined in this work.

3.2. High-density STS maps of APP and D21S8 region

A contig of YAC clones covering the entire region of the chromosome 21 long arm has been constructed,⁶ but it is absolutely necessary to construct the contigs of P1, cosmid and/or lambda clones for the detailed analysis of the chromosome. Since small regional contigs may be

constructed independently by many groups using their own library systems, it is desirable to generate a high-density STS map which may allow us to link the contigs from different groups. For these reasons, we attempted to generate a high-density STS map of two adjacent loci, *APP* and *D21S8*. At first, the *APP* locus was taken as

- (A) *APP* locus
 (Cen) T364L - APPEX17 - APPEX16 - APPEX14 - T364R/E6 - APPEX13A - APPEX13 - APPEX12
 - O491L - APPEX11 - APPEX10 - T1715L - APPEX9 - APPEX8 - APPEX6 - T1559L - APPEX5 -
 APPEX4 - APPEX3 - O459R - O648L - T1559R - T1539R - O648R - APPEX1 - APPEX887 - T1539L -
 (Tel)
- (B) *D21S8* locus
 O672R2 - T1850R - O453R - D21S8 - O672L - O453L2 - O11L - T1850L2 - O11R2 - O576R -

Figure 2. Order of the STS in *APP* and *D21S8* loci. The STSs at the *APP* locus are ordered from centromeric side (Cen) to the telomeric side (Tel). The direction of STSs of the *D21S8* locus is unknown.

an example in which STSs can be easily generated at a high density from published sequence data. Twenty STSs were generated from each exon of the *APP* gene and the promoter region. Then eight independent P1 clones were isolated from a chromosome 21-specific P1 library by using those STSs, and the terminal sequences of their insert DNAs were determined. A set of PCR primers for each terminus were prepared, and PCR amplification by these primers enabled us to clarify the positional relation of the termini of each clone. The results are summarized in Fig. 2A and Table 2. We also attempted to construct an STS map of the *D21S8* locus which is adjacent to the *APP* locus. The STS for *D21S8* hit four P1 clones and STSs from the terminal sequences of those four clones hit another four overlapping clones. Furthermore an additional overlapping clone was obtained, and finally the relationship of these sequence-tagged sites was clarified (Fig. 2B, and Table 2). The density of STSs in these regions may be high enough to clarify the relations of P1 and cosmid contigs from different sources.

In conclusion, 60 new sequence-tagged sites were generated, which should be useful for high resolution mapping of human chromosome 21.

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