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

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Abstract

From human chromosome 21-specific libraries, 22 SfiI 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 SsiI; 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 genome and extensive efforts have been made to construct genetic and physical maps. A contig of the yeast artificial chromosome and a complete NotI restriction map 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, 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 SfiI 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 SfiI linking clones

A SfiI linking library of human chromosome 21 using the plasmid vector pTZ19R was constructed as described previously from a chromosome 21-enriched phage library (LA21NS002). Plasmid DNA from each clone was isolated using an automated DNA sequencer (Pharmacia ALF) as described by Hattori et al.

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 polyethylene glycol-NaCl precipitation. The terminal sequences were determined as described previously 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 μl 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 Sfi-484) and extension (72°C for 1 min). Half of each PCR product was analyzed by electrophoresis on a 3% Nusieve/1% agarose gel.
3. Results and Discussion

3.1. Sequence-tagged SfiI 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 SfiI is one of the rare-cutters but its recognition sequence does not contain the CpG dinucleotide, thus making SfiI sites a useful marker for physical mapping. For these reasons, we attempted to isolate SfiI linking clones and generate sequence-tagged SfiI sites on chromosome 21. We previously constructed a SfiI 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 SfiI sites. Among 22, 6 clones (Sf-113, 199, 239, 283, 390, 431) were Alu-positive and one clone (Sf-390) L1-positive. Digestion of SfiI linking clones by several CpG enzymes (NotI, MluI, BstIII or SacI) revealed that 14 of 22 SfiI linking clones had no restriction site for the above CpG enzymes (Table 1). Thus a considerable number of SfiI sites were thought to be out of CpG islands. The regional mapping of the SfiI 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 SfiI 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) |
|------------|----------------|--------------------------------|----------|---------------------------------|----------------------|
| SF-10      | 4.7            | 6 1                             | SF-10UV  | CCCAAAGCAAGACTAGATGG            | AGGTGTCTGTCGATTTCC   | 90                   |
| SF-11      | 3.0            |                                  | SF-11RV  | CTTCTACTTACGGTCGCTGCGCTGAC      | TTTGCTAGGCTGCTACAACC | 169                  |
| SF-24      | 5.5            | 2 1                             | SF-24UV  | CATTCCCCCTTGTTGGGG            | TGTGCCTCTGAGCATCACG   | 106                  |
| SF-39      | 4.5            | 2 1                             | SF-39UV  | TTGGGAGATGACTGGGTTGG            | GACGAGGTGTGTGTTGAG    | 137                  |
| SF-66      | 4.3            |                                  | SF-66RV  | AGCTTAACAAAAATGGTGACTCT         | ATTTGCAAGTTAATGGCTGCA | 113                  |
| SF-87      | 0.9            |                                  | SF-87RV  | ACTTACCAACTGCACTAGGG            | TCTCTTTTTTTTTGCTTCGCC | 108                  |
| SF-111     | 3.9            |                                  | SF-111UV | CTGTTACAGAATGGAAGG             | TCGACCTCTGCTGCTAGTAC | 123                  |
| SF-199     | 4.0            | 1 1                             | SF-199UV | TGCTTGAACAGCATCCCCCTA           | CTTGCTTGTGTTACCTCCATC | 116                  |
| SF-217     | 3.7            |                                  | SF-217UV | CTGTCGAGCGAGGCAATATAG          | ATAAAGATAGGCAGAGATGG | 162                  |
| SF-229     | 1.1            |                                  | SF-229RV | AGCTTAACACAGCTACGAGCC          | TGCCAGCTCTCTACATGGC   | 95                   |
| SF-239     | 3.6            |                                  | SF-239RV | GCATAAGAATACAGCTGCTAC           | ATTTGATTTTTAGTACGCTCC | 87                   |
| SF-265     | 0.5            |                                  | SF-265UV | CTCAAAAGCGAGTGGGTG             | CTCTGGATCTGCTATCTGCC | 110                  |
| SF-270     | 2.4            |                                  | SF-270UV | TGCTAGACAAATGGTGACCC            | CTCCAGGATAATTGCTGGG  | 80                   |
| SF-273*    | 3.9            | 1 1                             | SF-273UV | GGTGCTCTTTGATGGGCTGCG         | CTAGTTGGAAGTGTGTGGCC | 122                  |
| SF-275     | 0.7            |                                  | SF-275UV | AGCCAAAGAGATCTTGGG             | CTGACCTAGGAAGGAGGAC   | 213                  |
| SF-283     | 5.7            | 1 5 5                           | SF-283UV | TTATGATCCACACGGCCGGG           | CTCTGACCTCAAAGAGGATGC | 165                  |
| SF-323     | 3.2            | 1 1                             | SF-323UV | AGCTTACGCGCTTGGCTGTC           | ATGCCAGGGTCGGTGCTGC  | 120                  |
| SF-390*    | 7.1            | 1 1 1                           | SF-390UV | CTGATAACTAGGCTTCTTGGG          | ATCCAGAGGTCTGGTGTCTG | 96                   |
| SF-431     | 1.3            |                                  | SF-431UV | TTTTGCTCGCTGGGTAAGGCTC         | ATGCTTGGCAGCTGGTGGCC | 93                   |
| SF-441     | 4.1            |                                  | SF-441RV | CAACTAAGGGTTGGTCAAGTC         | TACGCTTCTTCAGCTCTAC  | 80                   |
| SF-484     | 1.7            |                                  | SF-484RV | TGCCCTCTTGGTGGTGGGG           | AAGGCGCAATGTTAATCGAGC | 97                   |
| SF-624     | 1.9            |                                  | SF-624UV | GGCATGAATCTGCTGAGCCA          | TTGACCTAAGTGATCGC   | 122                  |

Nt: *NruI, Ml: *MluI, Bs: *BstHI, Sc: *SacII * This clone had two SfiI sites.
### Table 2. PI clone-derived STSs

| Clone   | PCR primer sequences (5' to 3') | Length of PCR products (bp) |
|---------|---------------------------------|----------------------------|
| P1FR-1  | GCAGGATGATAAACATCCCAAAACGGAG   | 117                        | P |
| P1FL-1  | CAGGAGCTACCATCAGCCCAAGGAGAGAG | 73                         | P |
| Developed PI STS |                              |                            |  |
| APP region |                                 |                            |  |
| APPEX-887 | ACAAGGGCCAGCGTTTGGAGG         | 122                        | P |
| APPEX1  | TGGGCGTGACATTTAGGGCAAAAAAGAGG | 167                        | P |
| APPEX2  | ACTCTCATCACTCTGAGGGTACAGAG    | 170                        | P |
| APPEX3  | TGGGTGTTTCTCTCTGAGGGTACAGAGG | 122                        | P |
| APPEX4  | TTCCTCTTCTCTGAGGGTACAGAG    | 116                        | P |
| APPEX5  | CGAGGATGATAAACATCCCAAAACGGAG | 88                         | P |
| APPEX6  | CAGGAGCTACCATCAGCCCAAGGAGAG | 134                        | P |
| APPEX7  | GCAGGAGCTACCATCAGCCCAAGGAGAG | 167                        | P |
| APPEX8  | CAGGAGCTACCATCAGCCCAAGGAGAG | 170                        | P |
| APPEX9  | ACTCTCATCACTCTGAGGGTACAGAG   | 122                        | P |
| APPEX10 | CGAGGATGATAAACATCCCAAAACGGAG | 88                         | P |
| APPEX11 | CAGGAGCTACCATCAGCCCAAGGAGAG | 134                        | P |
| APPEX12 | TGGGTGTTTCTCTCTGAGGGTACAGAG | 116                        | P |
| APPEX13 | CGAGGATGATAAACATCCCAAAACGGAG | 88                         | P |
| APPEX14 | ACTCTCATCACTCTGAGGGTACAGAG | 134                        | P |
| APPEX15 | CGAGGATGATAAACATCCCAAAACGGAG | 167                        | P |
| APPEX16 | CAGGAGCTACCATCAGCCCAAGGAGAG | 170                        | P |
| APPEX17 | ACTCTCATCACTCTGAGGGTACAGAG | 122                        | P |

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, but it is absolutely necessary to construct the contigs of PI, cosmids 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...
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 PI 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 PI regions 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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