Short Communication

SEQUENCE TAGGED SITES OF MICROCLONES
OBTAINED BY MICRODISSECTION OF A
HUMAN CHROMOSOMAL REGION 11q23
AND ISOLATION OF YEAST ARTIFICIAL
CHROMOSOMES

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Summary A human chromosomal region 11q23-specific DNA library has been constructed by means of microdissection-microcloning method with polymerase chain reaction (PCR) technique (Seki *et al.*, Genomics 16: 1993). DNA sequences were determined for 25 microclones that contained approximately 300–500 bp insert and gave a unique (single copy) signal in Southern blot analysis. The sequence tagged site (STS) was designed and appropriate condition for PCR was determined for each unique microclone. Twelve STSs were established and used for PCR-screening of human genomic libraries constructed with yeast artificial chromosome (YAC). Thirteen YAC clones have been isolated from eight STSs. These chromosomal region-specific STSs and YAC clones will be useful in the positional cloning of disease-related genes localized to the q23 region of chromosome 11.

Key Words 11q23, DNA library, microdissection, STS, YAC

The 11q23 region of human chromosome 11 contains several genes responsible for genetic diseases, such as ataxia-telangiectasia (Gatti *et al.*, 1988; Sanal *et al.*, 1990; Richard *et al.*, 1993), tuberous sclerosis (Smith *et al.*, 1990), the cancer-break-points involved in a variety of chromosomal rearrangements (Trent *et al.*, 1989; Zieman-Van der Poel *et al.*, 1991), rare and common fragile sites (Sutherland and Ledbetter, 1989), and Epstein-Barr virus modification sites (Sutherland *et al.*, 1988;

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Seki *et al.*, 1992). To identify genes responsible for such genetic diseases and DNA sequences involved in chromosomal instability, a fine physical mapping of human chromosome 11 is an essential step. In an attempt, we have constructed a high-resolution cytogenetic map with 168 cosmid markers, including 70 RFLPs for human chromosome 11 and more than 40 cosmid clones were mapped in the 11q23 region (Hori *et al.*, 1992). Furthermore, a human genomic DNA library specific for the 11q23 region has been constructed by using a microdissection-microcloning procedure with polymerase chain reaction (PCR) method (Seki *et al.*, 1993). These cosmid markers and microclones have proven to be useful for further detailed genetic and physical mapping. The generation of chromosomal region-specific sequence tagged sites (STSs) would be highly valuable for isolating large genomic DNA fragments harbored in such as P1 phage vector and yeast artificial chromosome (YAC) and subsequently for the construction of contig maps with them.

The chromosome 11q23 region-specific microclones were obtained as pUC119 recombinants by microdissection-microcloning method as described previously (Seki *et al.*, 1993). Template plasmid DNA was prepared by an alkaline lysis procedure for DNA sequencing. DNA sequencing reaction was carried out using fluorescence dye-labeled M13 forward or reverse primers according to Pharmacia's autocycle sequencing protocol. DNA sequences for 25 microclones that contained approximately 300–500 bp insert and exhibited a unique (single-copy) signal in Southern blot analysis were determined by Pharmacia A.L.F. sequencer. The proper STS primers were designed by computer-aided analysis with GENETYX software (SDC, Japan). The primers were synthesized by using Milligen Cyclone-plus DNA synthesizer.

The predicted STS primers were tested by PCR amplification in 10 μ l reaction volume containing 10 mM Tris-HCl (pH 8.8), 2.5 mM MgCl₂, 150 μ M each dNTP, 200 ng DNA, 2 μ M each primer, and 0.2 unit of Taq DNA polymerase (Kurabo, Japan). Initial PCR conditions were denaturation at 95°C for 2 min followed by 32 cycles of 30 sec at 95°C, annealing for 1 min at the predicted annealing temperature for each primer set, and 1 min at 72°C. The annealing temperature has been tested for amplification using human genomic DNA as a template. An optimal temperature was determined for the amplification of the following test DNA samples: original microclone DNA, total human DNA, DNA from a hybrid cell line (J1-44) containing only a human chromosome 11 in CHO cell background (see Seki *et al.*, 1993). DNA samples from a somatic cell hybrid (TFX543) retaining the fragile X chromosome but not chromosome 11, mouse (NIH3T3) cells and yeast cells were also included as negative controls.

Table 1 summarizes chromosome 11q23-specific STS primer sets, sizes for PCR products, and PCR conditions that produced amplification products only for the templates from human genomic DNA and J1-44 DNA. Figure 1a shows representative results of PCR-assay on three STSs. Each STS gave a specific PCR

Table 1. Chromosome 11q23 region-specific STSs and YAC clones isolated.

No. Microclone	Primer sequence	STS size (bp)	PCR condition		YAC clone	Size (kb)
			Tan (°C) ^a	Mg (mM)		
1. pMD11C-7	5'-ACTGAGACCGTGAGAGTAGAG-3'	265	62	2.5	A2411-D11	360
	5'-CCACACCAGGCCATACCTTGA-3'				D005-F5 *	530
					B019-D4	200
2. pMD11C-13	5'-GTGAGACATGGATGCCACTTT-3'	190	62	2.5	A2302-B10	500
	5'-CTTCTCCATGGAGACTCCAG-3'				CE520-B12 *	500
3. pMD11C-23	5'-ACTCTTGAAAGCTTTTCTGACITC-3'	156	55	2.5	A2302-B10	500
	5'-CATATAGTGTATAAATAAGGTGAGC-3'				CE520-B12	500
4. pMD11C-31	5'-TCTGTAAATATGATTATGCA-3'	201	58	2.5	A2218-C3 *	560
	5'-TCACTAACICATTTGTAGGTACTC-3'					
5. pMD11C-104	5'-GGCCTGTGCCATCACACAGAC-3'	302	63	2.5	B018-D8	800
	5'-GGCCAGTAAATCAACCTTCTCAC-3'				341-D11	180
6. pMD11C-106	5'-GGTGATCCGTGATGGGCTG-3'	144	65	2.5	C3202-H6 *	270
	5'-CAGACAGTGAGAGGCTTCTTTG-3'					
7. pMD11C-107	5'-CCTTGGTATCACACTGTCIGAAC-3'	170	66	2.5	A1904-B1	520
	5'-AAGTCTCTCTGCAGCCCTCACAG-3'				A2207-F9	500, 300
8. pMD11C-115	5'-TTGCCAAGATACAGACTTAGTC-3'	229	61	2.5	432-H2 *	190
	5'-CAGAGAGCTAGATTGGGTC-3'				474-E7 *	330
9. pMD11C-5	5'-GCAGAGTTGGTTTCTGAGAGG-3'	285	62	2.5		
	5'-ACTGTAGTTGGGIGGTTATTATGGAC-3'					
10. pMD11C-32	5'-CAGGCAGGATAACCCAAAGTCC-3'	157	68	2.5		
	5'-CATCCATCTTGGAGGCCAC-3'					
11. pMD11C-111	5'-GTAATCCAGGCAAGAGATGATGG-3'	183	65	2.5		
	5'-CTAGCTCCACATCTCAGCAG-3'					
12. pMD11C-113	5'-AATGATTAGTCTTTGTGTGG-3'	213	57	2.5		
	5'-CAGAGTATTTTGCAGAAG-3'					

^a Tan (°C) is annealing temperature

* No chimerism was detected by FISH

product only for their original microclone DNA, total human DNA and J1-44 DNA, but not TFX543 and NIH3T3. The sizes of PCR products were identical to those expected from the nucleotide sequence data.

Using STS primers designed to amplify the DNA from 11q23-specific human genomic region, we screened two YAC libraries; a CEPH YAC library that consists of 52,000 YAC clones (mean insert size, 400 kb) and a YAC library (originally prepared and provided by Dr. T. Imai, Cancer Institute, Japan) of 15,000 clones (mean insert size, 360 kb). The human YAC library DNA were pooled to a 3D-system which consists of rows, columns, and layers so that a single clone can be identified from over 67,000 clones as few as 123 PCR reactions. Figure 1b shows an example of PCR-assay of five candidate YAC clones isolated by a STS from

Fig. 1. PCR assays of STS primers (a) and YAC clones isolated (b). a, PCR primers corresponding to sequence in the microclones pMD11C-7, -13 and -23 were used to amplify five test DNAs. b, PCR-assay of five YAC clones isolated in 3D-YAC screening system by using a STS primer set of pMD11C-23.

the microclone of pMD11C-23. Only two YAC clones, A2302-B10 and CE520-B12, were found to contain DNA sequence identified by the STS. We have isolated 13 YAC clones by using eight STS primers designed from chromosome 11q23-region specific microclones (Table 1). The human genomic DNA insert in YAC clones was tested by pulsed-field gel electrophoresis and Southern blot analysis by using *Alu* sequence as a probe (data not shown). All YAC clones contained a single human DNA segment and the estimated insert sizes ranged from 180 to 800 kb, except one case of 2007-F9 that carried two human YACs estimated at 500 and 300 kb. As shown in Table 1, two STSs of pMD11C-13 and pMD11C-23 identified the same two YAC clones. A preliminary restriction site fingerprinting test with *NotI*, *MulI*, *SfiI*, and *BssHII* on these two YAC clones indicated that two STSs were localized within 100 kb (data not shown). A preliminary examination on the chimerism of YAC's was done by fluorescence *in situ* hybridization (FISH) method. No chimerism was detected in six YAC clones tested so far (see Table 1).

Establishment of STSs from DNA markers that have been mapped to a specific region on a chromosome would be useful for a common language in the human genome analysis and will facilitate the characterization of radiation hybrids (Richard *et al.*, 1993; Gillett *et al.*, 1993) and the isolation of YAC clones (Foote *et al.*, 1992; Qin *et al.*, 1993) to contribute towards the construction of a large-range physical contig map of a specific subregion of the human genome. Recently Smith *et al.* (1993) have reported 370 STSs that cover entire chromosome 11 uniformly with an average spacing of 340 kb. Miwa *et al.* (1993) also reported 50 STSs established from cosmid markers which have been localized to the particular region of chromosome 11 by means of mapping based on a deletion panel of somatic cell hybrids (Tanigami *et al.*, 1992) and by fluorescence *in situ* hybridization (FISH) (Hori *et al.*, 1992). The construction of an integrated map of YAC clones with the published STSs, radiation hybrids, cosmid clones and linking clones (Sanford *et al.*, 1993; Hermanson *et al.*, 1992) will provide a highly efficient approach to the construction of a 11q23-region specific contig map. The integrated mapping and ordering of the YAC clones is currently under way by using pulse-field gel electrophoresis and multi-color FISH on prometaphase chromosomes and extended chromatin.

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