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Short Communication

YEAST ARTIFICIL CHROMOSOME (YAC) CLONES AND SEQUENCE TAGGED SITE (STS) MARKERS ANCHORED AT HUMAN CHROMOSOME 21

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Human chromosome 21 has been well-characterized genetically because it includes several potential genes involved in numerous inherited disorders, particularly Down syndrome (Scoggin and Patterson, 1982) and familial Alzheimer's disease (St. George-Hyslop *et al.*, 1987). Several known genes or restriction fragment length polymorphic (RFLP) DNA markers have been mapped regionally on chromosome 21 (Watkins *et al.*, 1985, 1987; Tanzi *et al.*, 1988). In order to clarify genome structure and to define molecular bases of these inherited disorders, great efforts to make a physical map have been made during the past decades.

Yeast artificial chromosome (YAC) vector developed recently provides a powerful tool for cloning several hundred kilobases of exogenous DNA in yeast cells and has allowed us to analyze a large region of human genome (Burke *et al.*, 1987; Hieter *et al.*, 1990; Imai and Olson, 1990a; Kai *et al.*, 1990; Yokoyama *et al.*, 1990).

We have constructed a YAC library from a human lymphoblastoid cell line, CGM-1 according to the Burke's method (Burke and Olson, 1990) with a slight modification (Imai and Olson, 1990a). The library contains 14,000 clones with an average insert DNA size of 360 kb and the total length of human insert DNA was estimated approximately to be two equivalents of human haploid genome.

We have chosen several known genes and RFLP markers localized in chromosome 21 in order to isolate YAC clones by using the polymerase chain reaction

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Table 1. The collection of YAC clones and STSs anchored at human chromosome 21.

Location	Symbol	PCR primer set	Anneal temp. (°C)	PCR product size (bp)	YAC (RGB No.)	Length (kb)
21q11.2	D21S13	5'-GGG AAG TCA CCT AAC ATA CAG CAG TGG ACT-3' 5'-GGG AAT CCA GAT AGC CTC ACT TGT CAC AAT TCT GTC-3'	65	264	HY21-121	640
21q21	D21S111	5'-GTA GTA GTG CCA CCC AGT TTC AGG C-3' 5'-CAG CTG CAT ACT TAT GTG AAG CC-3'	65	311	HY21-106	280
	APP	5'-CAA TAC AAG GAT ATC TGA GTG-3' 5'-GGA ATT CCT ACA ACA GCA TC-3'	55	177	HY21-109	400
	APP	5'-GGT GTT CTT TGC AGA AGA TGT GGG-3' 5'-ACA TGC AGT CAA GTT TAC CTA CC-3'	65	170	HY21-108	380
21q22.1	SOD1	5'-TGC GTC GTA GTC TCC TGC AG-3' 5'-GCC TTC TGC TCG AAA TTG ATG-3'	55	152	HY21-116	590
	SOD1	5'-CAT ATA GGC ATG TTG GAG ACT TGG-3' 5'-CTG GAT CTT AGA ATT CGC G-3'	60	219	HY21-107	180
	IFNAR	5'-GCT CAG ATT GGT CCT CCA GAA G-3' 5'-GCT TAA ACC ATC CAA AGC CC-3'	65	111	HY21-117	480
	GART	5'-GTA GCT GAA GAT GTG GAT GC-3' 5'-GGC TTC ATT CCT CTT TAA CC-3'	68	205	HY21-114	340
	GART	5'-GTA GCT GAA GAT GTG GAT GC-3' 5'-GGC TTC ATT CCT CTT TAA CC-3'	68	205	HY21-166	590
	D21S82	5'-CTG TTC TAG GAT AGC AAG TG-3' 5'-GAT AGT ACC TGA GAC TAG G-3'	65	153	HY21-118	440
	D21S17	5'-GGT TTC CTC ACA CTG ATA GC-3' 5'-TGG AAG TTG TCA CTG CAG-3'	65	153	HY21-141	590
21q22.2	HMG14	5'-GAT GCA TGC ACA TGA CCA TGG-3' 5'-CAG ACT TGG CTT CTT TCT CTC CTG C-3'	55 / 65	243	HY21-113	590
	D21S15	5'-CGT GCT TTC AAG CAG TCA C-3' 5'-GTG TTA AGA TCT GCA AGG CC-3'	65	188	HY21-142	280
	BCEI	5'-TTC AGA GAC GTG TAC AGT GG-3' 5'-GAC GTC GAT GGT ATT AGG ATA G-3'	65	148	HY21-111	280
21q22.3	D21S19	5'-CCA CAT TAG CAC AGG AAA TAT TGG TTG GTT-3' 5'-AAA CAC ATC TGT TTC ATG GTG TAA GTT ACT-3'	65	304	HY21-123	570

(PCR) with a pair of oligodeoxynucleotide primers designed from the nucleotide sequences of each DNA fragment (Green and Olson, 1990; Anand *et al.*, 1991). Five microliters of reaction mixture contains 50 ng of DNAs from YAC library, 5′- and 3′-end primers at a concentration of 25 μM in 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.01% gelatin (Sigma, #G-2500), dATP, dCTP, dGTP, and TTP at 200 μM each (TaKaRa, Kyoto), and 0.25 unit of recombinant *Taq* polymerase (Perkin Elmer Cetus) with 20 μl of mineral oil overlay for 35 cycles at 94°C for denaturation (1 min), at the indicated temperature (55–65°C) in Table 1 for annealing (2 min), and at 72°C for polymerization (2 min) in a Perkin Elmer Cetus automated thermal cycler.

The library was screened for positive YAC clones with a combination of PCR and filter hybridization. Eight pools of DNAs from five pooled DNA of master plate each, were screened by PCR by the methods of Green and Olson (1990) and Anand *et al.* (1991). In a second round of PCR screening, master pools of DNAs from the positive DNA pools were tested. A DNA pool of master plate was prepared from one filter representing a set of 384 YAC clones. Finally, positive YAC clones were detected by colony hybridization with the appropriate ^{32}P-labeled PCR product. Subsequent positive YAC clones were analyzed further by pulse-field gel electrophoresis and successive Southern blot hybridization to determine the size of insert DNA as described previously (Imai *et al.*, 1990b).

We have successively isolated fifteen YAC clones from the library by PCR-mediated screening and these results were summarized in Table 1. Twelve YAC clones were assigned to chromosome 21q22 and the others to 21q11 or 21q21. The total length of human DNA inserts was calculated about 6.6 Mb, which is equivalent to 16.5% that of the human chromosome 21. With these YAC clones anchoring at chromosome 21, we are now trying to isolate YAC linking clones to make a contig map of human chromosome 21 by means of "inverse-PCR" (Riley *et al.*, 1990) or "YAC-*Alu* PCR" (Breukel *et al.*, 1990; Cole *et al.*, 1991; Tashiro *et al.*, 1991).

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