A High-Resolution Physical Map of Human Chromosome 21p Using Yeast Artificial Chromosomes

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The short arm of human chromosome 21 (21p) contains many different types of repetitive sequences and is highly homologous to the short arms of other acrocentric chromosomes. Owing to its repetitive nature and the lack of chromosome 21p-specific molecular markers, most physical maps of chromosome 21 exclude this region. We constructed a physical map of chromosome 21p using sequence tagged site (STS) content mapping of yeast artificial chromosomes (YACs). To this end, 39 STSs located on the short arm or near the centromere of chromosome 21 were constructed, including four polymorphic simple tandem repeats (STRs) and two expressed sequence tags (ESTs). Thirty YACs were selected from the St. Louis YAC library, the chromosome 21-enriched ICRF YAC library, and the CEPH YAC and megaYAC libraries. These were assembled in a YAC contig map ranging from the centromere to the rDNA gene cluster at 21p12. The total size of the region covered by YACs is estimated between 2.9 and 5 Mb. The integrity of the YAC contig was confirmed by restriction enzyme fingerprinting and fluorescence in situ hybridization (FISH). One gap with an estimated size of 400 kb remained near the telomeric end of the contig. This YAC contig map of the short arm of human chromosome 21 constitutes a basic framework for further structural and functional studies of chromosome 21p.

Human chromosome 21 is the smallest of the five acrocentric chromosomes, the short arm of which (21p) is mainly composed of tandemly arranged or interspersed arrays of different types of repetitive satellite sequences (Choo et al. 1988; Greig and Willard 1992; Vissel and Choo 1992). They are located mainly, but not exclusively, on the short arms of the acrocentric chromosomes. Therefore, chromosome 21p shares extensive homology with the short arms of the other acrocentric chromosomes. The highest homology is shared with chromosome 13p, because, mapping of markers using somatic cell hybrids and macrorestriction mapping has not identified differences between chromosomes 21p and 13p (Jorgensen et al. 1987; Van Camp et al. 1992). The highly repetitive nature of chromosome 21p and its extensive homology with other acrocentric chromosomes have hampered the search for chromosome 21p-specific markers and the construction of genetic and physical maps of this region. Most maps of chromosome 21 span the entire long arm (21q) (Chumakov et al. 1992; Nizetic et al. 1994; Gardiner et al. 1995; Korenberg et al. 1995), whereas only a few of them extend a short distance into the p-arm (Nizetic et al. 1994; Doering et al. 1995).

More than 90 genes are known on chromosome 21q (Antonarakis 1998), whereas only the ribosomal gene cluster (rDNA) was localized to chromosome 21p (Henderson et al. 1972). It is believed that the short arm of chromosome 21 and that of the other acrocentric chromosomes is composed almost exclusively of satellite sequences and that they are devoid of genes. However, the absence of genes might also reflect lack of detailed investigation of these genomic regions.

Trisomy of chromosome 21 results in Down syndrome (DS), the most common mental retardation–dysmorphology disorder in humans. Ninety-five per-

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cent of the DS cases have three copies of chromosome 21 due to a nondisjunction event that occurred in meiosis I or meiosis II (Epstein 1989). The remaining 5% of DS patients have a trisomy of chromosome 21 as a result of a Robertsonian translocation, mostly involving one of the other acrocentric chromosomes (Antonarakis 1993). The parental origin of the extra copy can be determined accurately using highly informative chromosome 21-specific polymorphic markers (Lamb et al. 1997). However, because no chromosome 21p or centromere-specific polymorphic markers are available, the study of the meiotic nondisjunction is based on data obtained from chromosome 21q markers only (Antonarakis et al. 1992). The availability of chromosome 21p-specific polymorphic markers would highly increase the informativness of such studies.

We describe the development of PCR-based markers located on chromosome 21p including nonpolymorphic STSs, simple tandem repeat (STR) markers and expressed sequence tags (ESTs). Furthermore, we used these markers to construct a yeast artificial chromosome (YAC) contig map of chromosome 21p ranging from the centromere to the rDNA gene cluster at 21p12. The integrity of the YAC contig map was validated by fluorescence in situ hybridization (FISH) analyses and restriction enzyme fingerprinting. The size of the contig was estimated to span a region of 2.9–5 Mb. One gap with an estimated size of 400 kb remained near the telomeric end of the contig. This YAC contig map of chromosome 21p is a valuable resource for the identification of chromosome 21p-specific polymorphic markers and expressed sequences, a research area that has not yet been explored.

RESULTS

Development and Characterization of STSs

The inserts of 12 plasmid clones mapped previously to the pericentromere or the short arm of chromosome 21 were sequenced from both sides using vector-based sequencing primers (Table 1). Homology to known sequences in the public databases was analyzed using the BLASTN algorithm (Altschul et al. 1990). As expected, pC_{HB} and pA_2 were 98%–100% homologous to the spacer and 28S coding sequence of the rDNA gene cluster, respectively, whereas Xba21 was 98% homologous to an α-satellite sequence. D21S190 and D21S191 were highly homologous to chromosome 21 genomic sequences, whereas the other sequence homologies were unrelated to chromosome 21 (Table 1). The sequences of three clones did not identify homologous sequences in the public databases.

The obtained sequences were used to develop PCR primer pairs. Also, primer pairs were developed from two repetitive α-satellite sequences, *D21Z4* and *D21Z3*

Table 1. Sequence Analysis of chromosome 21p Clones

			Sequence		Homology				
Locus	Clone	Location	Size (bp)	Acc. No.	Sequence	Location	Acc. No.	Region	p- value
D21S190	pVC1.21c	21q11.1	258	U04826	Human PAC LLNLP704G1150Q13	21	AJ006996	88469-88717	9.5e-77
	Xba21	21cen	160	AF145203	Human α-satellite DNA clone pSX21		X14303	1206-1359	1.3e-55
D21S5	pPW235D	21p11.2	5'-338	U76303	-				
			3'-294	AF152020	-				
D21S187	pVC10a	21p13	5'-226	U03253	-				
			3'-189	U03253	-				
D21S1276	pLSB77	21p11.2	5'-102	AF152019	-				
			3'-237	U03240	Human CMT1A gene, repeat unit	17p12-p11.2	L44121	1238-1434	5.9e-45
D21S188	pVC1.12c	21p13	291	U03246	EST yd48d11.r1 containing KER repeats		T83238	77-232	4.7e-82
D21S191	pVC1.23c	21p13	396	U03248	Human genomic DNA	21q11.1	AP000024	19258-19466	8.1e-60
								20177-20409	1.0e-71
D21S1277	pAW14a	21p12	5'-140	AF152018	EST aa90e03.s1		AA456946	11-140	2.2e-28
					EST yt69a11.s1		R93354	249-369	2.0e-25
					Human genomic clone bWXD759	X	AC004074	28233-28361	4.4e-24
					Human BAC clone GR331P03		AC002464	56033-56157	5.2e-24
					EST zh48e01.s1		W91984	4-105	2.8e-23
					Human PAC clone 323B6	X	Z83841	47419-47547	9.8e-23
					EST ye29g10.r1		R94184	1-78	1.0e-20
			3'-356	U03234	Human PAC clone pDJ181p7	15q11-q13	AC006596	17982-18126	2.7e-70
					Human BAC clone GR331P03		AC002464	54168-54436	3.3e-47
					Human genomic clone bWXD759	X	AC004074	29916-30190	2.1e-42
					Human PAC clone 323B6	X	Z83841	49257-49411	6.9e-40
	3C6	21p	78		Human PAC clone DJ0988L12	7q11.23-q21.1	AC004454	85462-85532	1.1e-11
D21S1278	pAW32a	21p11.2	5'-491	U03252	-				
			3'-230	AF152017	-				
rDNA	pC_{HB}	21p12	5'-139	U76302	Human rDNA intergenic spacer sequence		X68195	4915-4985	7.1e-37
			3'-115	U76301	Human rDNA intergenic spacer sequence		X68195	5228-5334	2.6e-39
rDNA	pA_2	21p12	273	AF152922	Human 28S ribosomal RNA gene		M11167	4144-4416	1.2e-98

Six insert sequences were incomplete because the 5′ and 3′ sequences did not overlap. Homology searches were performed using the BLAST algorithm (Altschul et al. 1990). (Acc. No.) GenBank accession no.

(Vissel et al. 1992), one expressed sequence, hmc01a06 (Chen et al. 1996, 1997), and four $(CA)_n$-repeat-containing clones ABM–C61, ABM–C62D, ABM–C78 and *D21S411* (Bosch et al. 1996; A. Bosch and X. Estivill, unpubl.) (Table 2). First the markers were analyzed on a monochromosomal somatic-cell hybrid mapping panel, indicating that all markers were located on chromosome 21 as well as on chromosome 13. In addition, most of them also amplified sequences on other acrocentric chromosomes and one or more nonacrocentric chromosomes (Table 2). PCR amplification of an extended-mapping panel of partial chromosome 21 somatic cell hybrids localized the markers in four clusters separated by the 21p breakpoints of the cell hybrids (Table 3A). In addition to the novel markers, *D21S286*, *D21S381*, *D21Z1*, *D21S275* (Chumakov et al. 1992), and *D21S237*, (Watkins et al. 1985) were regionally localized to the pericentromeric region of chromosome 21. Xba21 did not amplify the somatic cell hybrid DNA, and pA_2 was positive on mouse and hamster DNA; therefore, it could not be mapped unambiguously.

Selection and Characterization of YACs

The St. Louis and CEPH human YAC libraries were screened by PCR or hybridization with Xba21, *D21S187*, *D21S1276*, *D21S5*, *D21S188*, *D21S191*, *D21S1277*, *D21S1278*, pC_{HB}, and pA_2, resulting in the identification of 14 YACs. Furthermore, we obtained 22 YACs from the chromosome 21-enriched ICRF YAC library and six CEPH YACs mapped previously to the centromere or short arm of chromosome 21 by PCR, hybridization, or FISH (Chumakov et al. 1992; Nizetic et al. 1994). PCR amplification of the selected YACs identified 42 YACs that were positive for the markers with which they were selected. The selected YACs were

Table 2. PCR Primer Sequences and Chromosomal Assignments of Chromosome 21p Markers

Locus	Clone	Type	Primer sequences	Size (bp)	Chromosomal assignment
D21S190	pVC1.21c	STS	CTGACAAGGAATTAGTAACC TACACCTATTTGCCATTTGC	133	13, 18, 21
—	ABM-C61	STR	CCTAGCTTTCTGTCAGCAGT CAACATGATTCCACATAATGTA	450	13, 14, 15, 21, 22
—	hmc01a06	EST	TGGATGTCACTCTCATCCTTG TCGAAGAAGAACATCCATGAG	122	13, 22, 21, Y
—	ABM-C62D	STR	CCACTGTTGTACAAATCAACTA TCTAAGAATACCTGTAGGCATA	143	13, 15, 21, Y
—	Xba21	STS	CAATCCCGTTTCCCACG CTTGTTTGTGATGTGTGCCC	133	N.D.
D21Z4	pTRA-4	STS	ATTTGGAGGCCTTTGTGGCTATGG GGCCTCAAAACGCTCCAAGTATCC	287	13, 14, 21
D21Z3	pTRA-1	STS	TGAGCCATTTGAGGCCTACTGTG TGTCTCCAAACTGCTCAATCAGAA	138	13, 14, 21
—	ABM-C78	STR	CCTCTTCTATGCCAGTCTTAA CAGCTCTAAAGTAAGCTTGGA	109	9, 13, 14, 15, 20, 21, 22
D21S5	pPW235D	STS	CCAAAGGTTACTGCAGTCTC GGGGGTCATATAAAGGAAAC	123	13, 14, 21
D21S187	pVC10a	STS	TACATGCAAATGACCAAGAG ATCAGCAATGCCTTCTAAGT	400	4, 9, 13, 14, 15, 21, 22
D21S1276	pLSB77	STS	GGCCTGATGTCTGCCTTAGAT GCCATAGGTGAGCAACAGGA	108	1, 3, 9, 13, 14, 15, 20, 21, 22, Y
D21S188	pVC1.12c	STR	TTCTGTGTCATCTGAACTGG ACGCACATTGAATACTGAGG	184	9, 13, 14, 15, 20, 21, 22, Y
D21S191	pVC1.23c	STS	TGATTGGTATGACTTTGTTCCCAG AGATGTCCACCCAGTGCTGC	179	3, 4, 13, 14, 15, 21, 22
D21S411	ABM-C74	STR	GAACCATTATAAGTTGACCATC ACATGTTAACATGCTATATCTGT	196–206	3, 4, 13, 14, 15, 21, 22
D21S1277	pAW14a	STS	AACTAAGGGCTCTAAAGCAT AATGGGAGCACAAGTTCTAA	300	3, 4, 9, 13, 14, 15, 21, 22, Y
—	3C6	STS	CAAGAGGGCACTGAACAG GCACTCTTAGCTTTGCCA	59	1, 4, 13, 14, 15, 21, 22, Y
D21S1278	pAW32a	STS	TCATACAGAGTATAACACCAGGAC GTCTTATTGTGATAGGCTTGC	266	1, 10, 13, 14, 15, 20, 21, 22, Y
rDNA	pC_{HB}	STS	CGAGGTGACTCTCGGTTTGC TTTCGCAAGCAGGCATTTG	370	13, 14, 15, 21, 22
rDNA	pA_2	EST	TATGAACGCTTGGCCGC CGGTAACGCAGTGTCTAAGGC	204	N.D.

(N.D.) Not determined.

Table 3. Somatic Cell Hybrid Mapping on Chromosome 21p

Locus		Clone	WAV17	AHVI-17	JC-6A	ACEM2-10d	153-E7b	R2-10W	2FUr1	Region
A	*D21S190*	PVC1.21c	+	+	+	+	+	+	+	21q11.1
	D21S286	G51E07	+	+	+	+	+	+	+	21q11.1
	D21S381	E341	+	+	+	+	+	+	−	21cen
	D21Z1	L1.26	+	+	+	+	+	+	−	21cen
	—	ABM-C61	+	+	+	+	+	+	−	21cen
	D21S275	G51G19	+	+	+	+	+	+	−	21cen
	D21S237	PPW265D	+	+	+	+	+	+	−	21cen
	—	Hmc01a06	+	+	+	−	+	+	−	21cen
	—	ABM-C62D	+	+	+	−	+	+	−	21cen
	D21Z4	PTRA-4	+	+	+	+	+	+	−	21cen
	D21Z3	PTRA-1	+	+	+	+	+	+	−	21cen
	—	ABM-C78	+	+	+	+	+	−	−	21cen
	D21S5	PPW235D	+	+	+	+	+	−	−	21p11.1
	D21S187	PVC10a	+	+	+	+	+	−	−	21p11.1
	D21S1276	PLSB77	+	+	+	+	+	−	−	21p11.1
	D21S188	PVC1.12c	+	+	+	+	−	−	−	21p11.1
	D21S191	PVC1.23c	+	+	+	+	−	−	−	21p11.2–p12
	D21S411	ABM-C74	+	+	+	+	−	−	−	21p11.2–p12
	D21S1277	PAW14a	+	+	+	+	−	−	−	21p11.2–p12
	—	3C6	+	+	+	+	−	−	−	21p11.2–p12
	D21S1278	PAW32a	+	+	+	+	−	−	−	21p11.2–p12
	—	pC_{HB}	+	+	+	+	−	−	−	21p11.2–p12
B		1F8R	+	+	+	+	+	+	−	21cen
		5A12R	+	+	+	+	−	−	−	21p11.2–p12
		891B10L	+	+	+	−	+	+	−	21cen
		831B6R	+	+	+	−	+	+	−	21cen
		2C9L	+	+	+	−	+	+	−	21cen
		829D2R	+	+	+	+	+	−	−	21p11.1
		837F5L	+	+	+	+	+	−	−	21p11.1
		2C9R	+	+	+	+	+	−	−	21p11.1
		891B10R	+	+	+	+	+	−	−	21p11.1
		4E9R	+	+	+	+	−	−	−	21p11.2–p12
		2E4L	+	+	+	+	−	−	−	21p11.2–p12
		4E9L	+	+	+	+	−	−	−	21p11.2–p12
		1B8L	+	+	+	+	−	−	−	21p11.2–p12
		2C2R	+	+	+	+	−	−	−	21p11.2–p12
		5B7L	+	+	+	+	+	+	−	21cen
		2E4R	+	+	+	+	−	−	−	21p11.2–p12

(A) STSs, STRs, and ESTs derived from chromosome 21p or centromeric clones. **(B)** STSs derived from 21p YAC terminal fragments. Names ending with L and R indicate, respectively, left and right YAC end STSs. (+ or −) Positive or negative PCR amplification.

reexamined for their marker specificity by PCR amplification, and true positive YACs were used in the YAC content mapping. Sizes of the YACs were estimated by PFGE and ranged from 100 to 1600 kb (Table 4).

YAC Contig Construction

STS content mapping of the 42 selected YACs was performed with the 24 chromosome 21p markers (Table 4). All markers were positive on one or more YACs, except ABM–C62D, which was negative for all YACs. Again, Xba21 did not amplify YAC DNA and was excluded from further STS content mapping. Together, 26 YACs were identified that contained multiple markers and were assembled into a YAC contig map based on their marker content (Shimizu et al. 1995; Wang et al. 1995, 1997; Korenberg et al. 1997). The STS content of each YAC was verified using DNA of five different single YAC colonies. For four YACs—4C7, 781G5, 1B8, and 5B7—the STS content of the clones was not identical, possibly because of internal deletions of the YACs. For these YACs, we considered STSs that amplified four of the five clones. The YAC contig showed two gaps, one between hmc01a06/*D21S237* and *D21Z3* and one between *D21S1278* and pC_{HB}. Next, we isolated 34 end fragments of 15 YACs from the preliminary YAC contig map and 4 YACs containing one single STS. Their DNA sequence was determined and the homology to sequences in public databases was analyzed. The left ends of 831B6, 1F8, and 3A8 were 94%–98% homologous to the repetitive α-satellite sequence α21-I, whereas the left ends of 5A12 and 2C2 were 78%–84% homologous to a satellite III sequence. The left and right ends of A24F7 were identical to the 18S coding sequence and the intergenic spacer se-

Table 4. STS Content Mapping of Chromosome 21p YACs

Screenings	YAC addresses	Library	Size (kb)	pA2	pCHB	2E4R	5B7T	D21S1278	4C2R	3C6	1B8T	4E9T	D21S1277	D21S1411	3E4T	D21S191	D21S188	4E9R	D21S1276	2C9R	D21S187	D21S15	8378SL	ABM-C78	8290ZR	D21Z3	2C9L	D21Z4	831B6R	EST hmc01a06	891B10L	D21S237	3A8R	5A12R	D21S275	ABM-C61	1F8R	D21Z1A	D21S381	D21S264	D21S190
pA2	A184D4	S	100	+																																				
pCHB	A24F7	S	100	+	+																																				
2E4R	814A11	C	1600	+	+	+																																			
	2E4	I	750	+	+	+	+																																		
	5B7	I	530	+	+	+	+	+																																	
D21S1278	B169D10	S	120	+	+	+	+	+	+																																
D21S1278	B194A6	S	100	+	+	+	+	+	+	+																															
	2C2	I	230	+	+	+	+	+	+	+	+																														
	4E12	I	220	+	+	+	+	+	+	+	+	+																													
	4E9	I	220	+	+	+	+	+	+	+	+	+	+																												
	2H5	I	750	+	+	+	+	+	+	+	+	+	+	+																											
	5B9	I	280	+	+	+	+	+	+	+	+	+	+	+	+																										
	1B8	I	800	+	+	+	+	+	+	+	+	+	+	+	+	+																									
D21S1277	B97B12	S	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+																								
D21S191	B117H3	S	210	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																							
	3G8	I	440	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																						
D21S188	F9B6	S	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																					
D21S188	B8C6	S	270	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																				
D21S5	965D4	C	1480	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																			
D21S5	976D5	C	1300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																		
D21S5	837F5	C	520	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																	
	1B2	I	800	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																
D21S187	B35E11	S	480	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+															
	4B2	I	700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+														
D21S1276	B46E11	S	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+													
	2C9	I	700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+												
ABM-C78	891B10	C	1000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
ABM-C78	482H9	C	280	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+										
ABM-C78	829D2	C	710	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
ABM-C78	89D10	C	360	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
	831B6	C	1400																																						
831B6R	906C1	C	1600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Xba21	G32G11	S	620	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
	3A8	I	350	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	5A12	I	420	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
	1F8	I	400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	4C7	I	440	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	781G5	C	1125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	4E7	I	600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	4C1	I	550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	52H5	C	350																																						
	857B10	C	480	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	317D9	C	380	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	770B3	C	1600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1A2	I	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1H2	I	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1F3	I	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2E9	I	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Column heads 5–42 show the markers used in the STS content mapping. (Colume 1) Markers used in the YAC library screening. (Colume 3) (I) ICRF YAC library; (C) CEPH YAC library; (S) St. Louis YAC library. (Shaded areas) positive PCR amplifications; (–) negative PCR amplification. (n.d.) Not determined.

quence of the rDNA gene cluster, respectively, confirming that this YAC is located within the rDNA gene cluster. Furthermore, a 74-bp region of the right end of 1F8 was 80% homologous to a cDNA sequence of the human protein kinase C-like gene (*PRKC-L*), related to the protein kinase C gene family (Bacher et al. 1991). No other end sequences showed any homology to known sequences.

Twenty-seven STSs were developed from the nonrepetitive YAC end sequences, and their chromosomal location was determined by PCR analysis of the monochromosomal somatic cell hybrid mapping panel (Table 5). Fourteen STSs were positive on chromosome 21, as well as on other acrocentric and nonacrocentric chromosomes, and were regionally localized to chromosome 21p using the extended partial chromosome 21 somatic cell hybrid panel (Table 3B). Their location was consistent with the position of the corresponding YACs in the contig map, except for 5B7L and 5A12R. YAC 5B7 is located in 21p11.2–21p12, whereas its left end mapped to the centromeric region. Conversely, 5A12R localized to 21p11.2–12, whereas the YAC mapped in the 21cen region. 3A8R did not PCR amplify the somatic cell hybrids and could not be localized. All chromosome 21p YAC-end STSs were included in the YAC content mapping. However, this did not result in the closure of the two gaps in the YAC contig. Therefore, STSs 2E4R, ABM–C78, 831B6R, and ABM–C62D flanking the gaps were used to screen the CEPH YAC and megaYAC libraries. Six additional YACs were selected, four positive with ABM–C78, one with 831B6R, and one with 2E4R (Table 4). STS content mapping of these YACs resulted in closing the gap between hmc01a06/*D21S237* and *D21Z3* by YAC 891B10, as it was positive for markers at both sides of the gap. Next, both ends of 891B10, the right end of 829D2, and the left end of 89D10 were isolated and sequenced. The left end of 89D10 was 88% homologous to the repetitive satellite sequence α21-II. The other three sequences did not identify homologous sequences in the public databases and were used to develop STSs. All three STSs were mapped to chromosome 21 and other chromosomes using the monochromosomal cell hybrid mapping panel (Table 5). The regional location was determined by PCR amplification of the extended partial chromosome 21 somatic cell hybrid panel and was consistent with the location of the YACs in the contig (Table 3B). Inclusion of the additional STSs confirmed the closure of the gap between hmc01a06/*D21S237* and *D21Z3*. Unexpectedly, 891B10R was present only in 891B10, but not in overlapping YACs.

Together, the STS-content mapping resulted in a contiguous YAC map ranging from *D21S190* to *D21S1278* (Fig. 1). The contig comprises 30 YACs that contain multiple markers. Of four YACs, both ends were isolated and mapped to chromosome 21 and of five additional YACs, one end was localized to chromosome 21, while the other end represented a chromosome 21-repetitive sequence. Of nine YACs, one or both ends did not map to chromosome 21 (Table 5). The YAC-contig map contains 39 markers including 17 nonpolymorphic STSs, four polymorphic STRs, and two ESTs. STR marker ABM–C62D, which was localized by somatic cell hybrid mapping in a region covered by YACs, was absent from all YACs localized in that region.

YAC Contig Validation

Seven overlapping YACs—831B6, 891B10, 2C9, 4B2, 3G8, 4E9, and 2E4—representing the complete region covered by the YAC contig map were analyzed using *Hin*dIII–*Bam*HI fingerprinting using an *Alu* repeat as hybridization probe (Fig. 2). At least six bands of the same size were observed for each pair of YACs with overlapping marker contents. The total size of the commonly shared fragments ranged between 19- and 66 kb, that is, between 14.5% and 81.5% (average 54.5%) of the summed sizes of *Alu*-positive bands. Also, these seven YACs and the centromeric YAC 1F8 were used as hybridization probes in FISH analyses of metaphase chromosomes. Ten metaphase spreads of each YAC were examined. Hybridization with 4E9 resulted in chromosome 21p-specific signals using standard procedures (Fig. 3A). For the other YACs, extra signal amplification was needed. Of these, 831B6 and 1F8 specifically recognized the short arm of chromosome 21. For the remaining five YACs, the background signal was high despite the use of 50-fold excess of human *Cot-1* DNA. However, in addition to signals on chromosome 21p, other signals with significantly stronger intensity than the background were detected on other acrocentric chromosomes (data not shown).

Gap Size Estimation

The markers pC_{HB} and 2E4R, flanking the remaining gap were used as hybridization probes to screen the chromosome 21 cosmid library LL21NC02 (HGMP Resource Center, UK). Eight cosmids were selected with pC_{HB} and two with 2E4R. The cosmid insert sizes were estimated between 38 and 48 kb by PFGE analysis of *Sfi*I digested cosmid DNA. Cosmids 89b9 (pC_{HB}) and 15f5 (2E4R) were hybridized in situ on released DNA fibers (Fig. 3B). The gap size, calculated based on the length of cosmid signals and the distance between them averaged over 10 signal pairs, was estimated at 400 kb.

STR Analysis

Five STR markers containing $(CA)_n$-repeat sequences (*D21S188*, *D21S411*, ABM–C78, ABM–C62D, and ABM–C61) were mapped to chromosome 21p, al-

Table 5. YAC End STSs and Their Chromosomal Locations

	Name	Primer sequences	Size (bp)	Chromosomal assignment
A	89B10L	TGAAACTGTTTTTGGCAGGTAGG GTAGGATGGGCAGAAGTGGTCAG	306	4, 7, 13, 21, 22, X, Y
	891B10R	TTATTTTGCCGNGCTTCAAGACCT GAGAGCCAGTGTCCTTATACAAAG	96	4, 13, 21
	2C9L	ACGCCTGCTGTGTTCTCTTGGACT TGATGGATCGGTGAATTTGCTCTG	168	13, 21
	2C9R	AATGAGGTCAAATGAACAATACAA TCAGCAGTCAGAAGAAAAGGAA	150	4, 9, 13, 14, 21
	4E9L	TGATGAATGAGGTGACTGTG AACTTTACGTTTCCCTTTCC	102	3, 4, 13, 15, 21, 22
	4E9R	AATCTCCATGGTGTTTGAAC ATCCAGAATTTGTAGGGCTT	183	3, 4, 13, 14, 15, 21, 22, Y
	2E4L	TTTGTGTTTTAATGCCTTCT TGCTACAAACTTGAGACTTT	156	13, 14, 15, 21, 22
	2E4R	TGACACTTAAATGAGCTGCT TTCAAATTTCTGAAATCCAA	100	13, 14, 21
B	1F8R	TCTTGGCCCTAGTCTGGTCCACTC AGAGCCAGGGTGGGTCCTCTGT	181	13, 14, 15, 21, 22, Y
	5A12R	CGTAGCAAGTATGTTTTCAAAGAA AGCCACCAAGACCCACCTTA	51	4, 13, 14, 15, 21, 22
	3A8R	ACTTAATAATTTGCCCACCAT AACCAAAGAAATGTAGATTTACC	58	4, 7, 13, 14, 15, 21, 22, Y
	831B6R	CTTTGTGAGCGCGGCAGACT TAGAGAACGTGGACGGATTATTTT	145	13, 21, Y
	2C2R	ACGGTTTACGTTTCCCTTTCCTAA TGGTGCATTATTGATGAATGAGGT	111	13, 14, 15, 21, 22
C	829D2R	AAAGCACGTTGAAAGGTCATAC ACAGCCTTTACAGTGTTGAAGTA	243	9, 13, 15, 20, 21
	5B7L	CATGCGTGCCCAGCTACACTTGA ACATGGGCACGCATCTTT(C/T)TGC	60	4, 13, 14, 15, 21, 22, Y
D	4C7L	TGTCTGGGAGTGCTAGAAGT ATGCCTCCAGGTTTCTCTAT	94	20
	4C7R	CCTCTATAGGACAGCAGGAA CAGACAGCATTCTAGGTGCT	70	4
	G32G11L	ATGTTTTGAGGGGTGGATTGTTTA TCTGTTGCCTGGTGACTGGAC	247	5, 11, 22
	G32G11R	CAGTTGAAATGGTTGTATGA ATCCAGTTGAGTTTATCCA	100	20
	B35E11L	CCAGGATATGAAATGTCAGCT ATCTCGATTTTTATAGCTCAACTC	110	5, 18
	1B2L	AGGGACTGAAATAAAATGAAAC CAATGTGCAAAAATAAACTGA	88	14
	1B2R	ACTCAATTACCTTTTTCCTCCATC TTCAGAAAATTTGATTTGAGGAGA	112	9
	837F5L	CTTCAATGCCCAGATACAGATGAA CCCCGCCGACTTTATTTAGCT	93	9, 13, 14, 15, 21, 22, Y
	837F5R	TTGAACAAGGAAGAAATCCAAAAC CAAGTCCTGGGCTTCCCTTTAC	100	X
	965D4L	TAAATTTGAAGTTGTTGCCTAATG TCCTTAATGGATTTCTGATGGA	163	14
	965D4R	CTGCTTTCAGGGATGGGCTTAA AAAAGATGATTCCCAGTCCCACAT	100	4, 22, Y
	F9B6R	GTGGGCCTTAATGCAATGATTTC TTGGCATCCCCTGGCTTGTAT	129	1
	3G8L	CATCATAAATAAACAAAAACATTAGA CATGCAAGCTCCATTTAGAG	128	11
	1B8L	AAGGCTTCTTGGAAAGGAACT TTGTGATTGGTATGGGAGAATC	93	1, 3, 4, 13, 14, 21, 22
	1B8R	AGAAATAAAACTNGCANGGG GTATGANTTCACCCACATCT	100	3

YAC addresses followed by L and R indicate *left* and *right* YAC end STSs, respectively.
(A) STSs of YACs with both ends mapping to chromosome 21. **(B)** YACs with one end mapping to chromosome 21 and one end homologous to satellite repeat sequences. **(C)** YACs with one end isolated and mapped to chromosome 21. **(D)** YACs with one or two ends not mapping to chromosome 21.

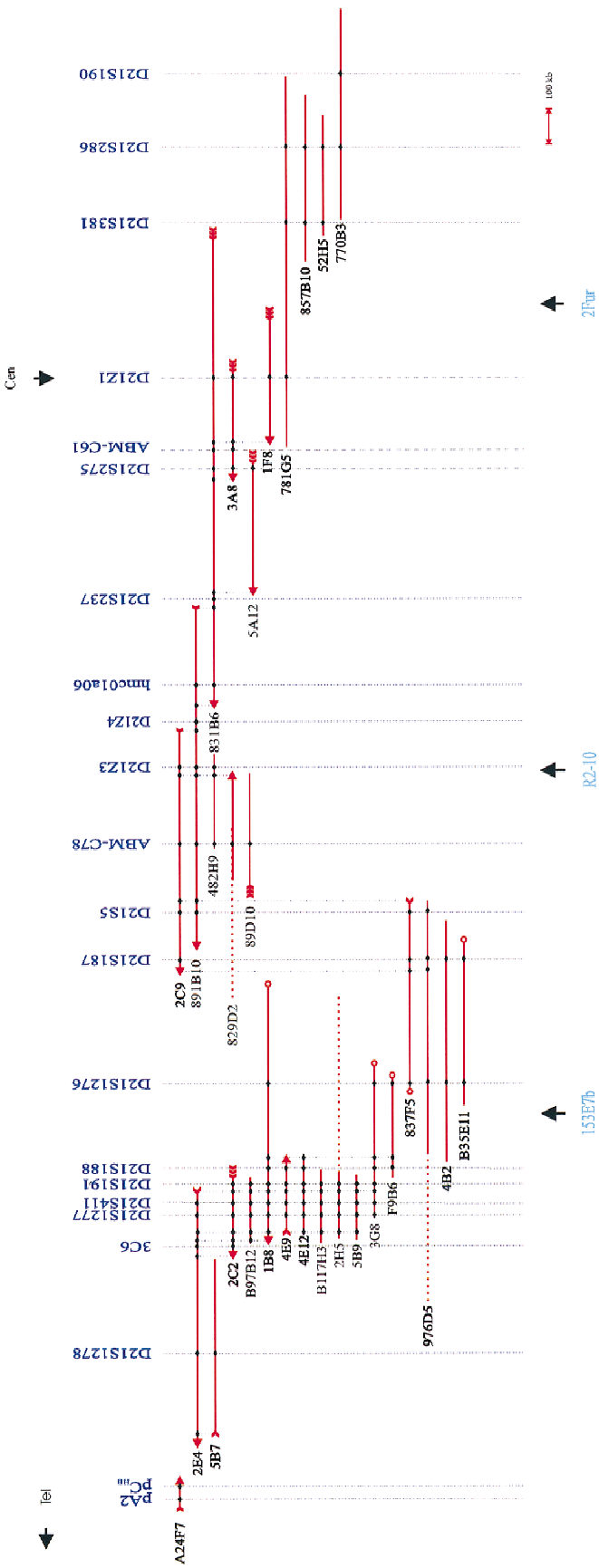

Figure 1 Chromosome 21p YAC contig map. The orientation of the YAC contig is based on the regional location of *D21Z1* and pC_{HB}. Horizontal lines represent YACs. Dotted lines indicate possible chimerical regions of the YACs. (Single arrowheads) Chromosome 21 YAC left (tails of arrows) and right (heads of arrows) ends; (triple tails of arrows) YAC end fragments homologous to α-satellite or satellite III repetitive sequences; (open circles) nonchromosome 21 YAC ends.

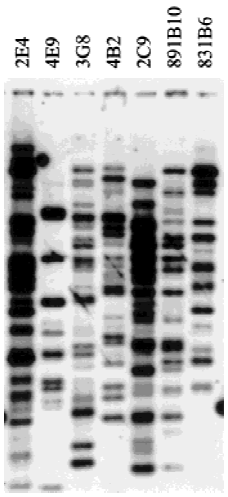

Figure 2 *Hin*dIII–*Bam*HI fingerprint analysis of YACs.

though none of them was chromosome 21-specific (Table 3). When the STRs were PCR amplified on genomic DNA of unrelated individuals and analyzed, a complex pattern of constant and variable bands emerged. *D21S188* showed 16 different alleles with frequencies ranging from 1% to 97% and 1 constant allele in 39 unrelated individuals. Analysis of *D21S188* in CEPH families 1333, 1334, and 1347 demonstrated Mendelian inheritance of each allele (Fig. 4). Comparison of different alleles with the patterns obtained using somatic cell hybrids WAV17, GB3, HDm-5, WegrothB3, HorlI, and WegrothD2, each containing a single acrocentric chromosome, did not allow for the identification of chromosome 21-specific alleles.

DISCUSSION

We describe the construction of an STS-based YAC-contig map of chromosome 21p ranging from the centromere (*D21Z1*) to the rDNA gene cluster at 21p12 (pC_{HB}). Because chromosome 21p markers were lacking, we first developed 19 novel STSs from plasmid clones localized previously to chromosome 21p. Analysis of the markers on a monochromosomal somatic cell hybrid mapping panel indicated that in addition to chromosome 21, all markers amplified sequences of chromosome 13, substantiating previous observations that among the acrocentric chromosomes, 13p and 21p share the highest degree of homology. Previous homology studies related to repetitive satellite sequences (Jorgensen et al. 1987; Van Camp et al. 1992), whereas our data extend the observation of this homology to nonrepetitive sequences. In addition to chromosome 21 and 13, all markers amplified sequences of other acrocentric chromosomes. Furthermore, 59% of the markers amplified nonacrocentric chromosomes.

By using somatic cell hybrids containing different regions of chromosome 21, we were able to sublocalize the markers on chromosome 21p without the interference of the other human chromosomes. *D21S187*, which by hybridization on DNA of chromosome 21 somatic cell hybrids showed two bands corresponding to locations in 21p11.2 and 21p13, respectively (Van Camp et al. 1990), was assigned by PCR to the centromeric locus only. This might be explained if the PCR primer pair of *D21S187* is locus specific and amplifies only the 21p11.2 sequence. Alternatively, if sequence homology is high between both loci, PCR amplification might result in fragments of similar or identical size that cannot be separated by agarose gel electrophoresis. *D21S188* and *D21S191*, both localized to 21p13 by hybridization (Van Camp et al. 1990), were mapped to 21p11.2–p12 by PCR, as they were positive on the somatic cell hybrid ACEM2-10d (Table 3). It is possible that the plasmid clones map to the 21p13 region but have weak homology to a more centromeric region that is recognized only by PCR. However, the ambiguities between hybridization and PCR in both cases involve the same somatic cell hybrid ACEM2-10d containing a complex 21;21 translocation chromosome (Van Keuren et al. 1989), which might indicate that ACEM2-10d is highly unstable, as reported previously (Graw et al. 1995).

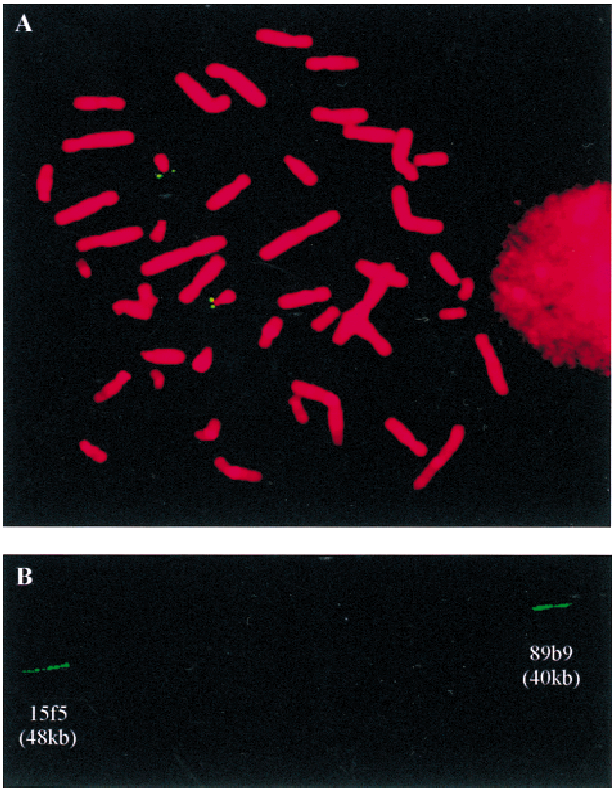

Figure 3 FISH analyses. (*A*) Metaphase FISH analysis of YAC 4E9. (*B*) Fiber FISH analysis of cosmid probes 15f5 and 89b9 to estimate the gap size between *D21S1278* and pC_{HB}.

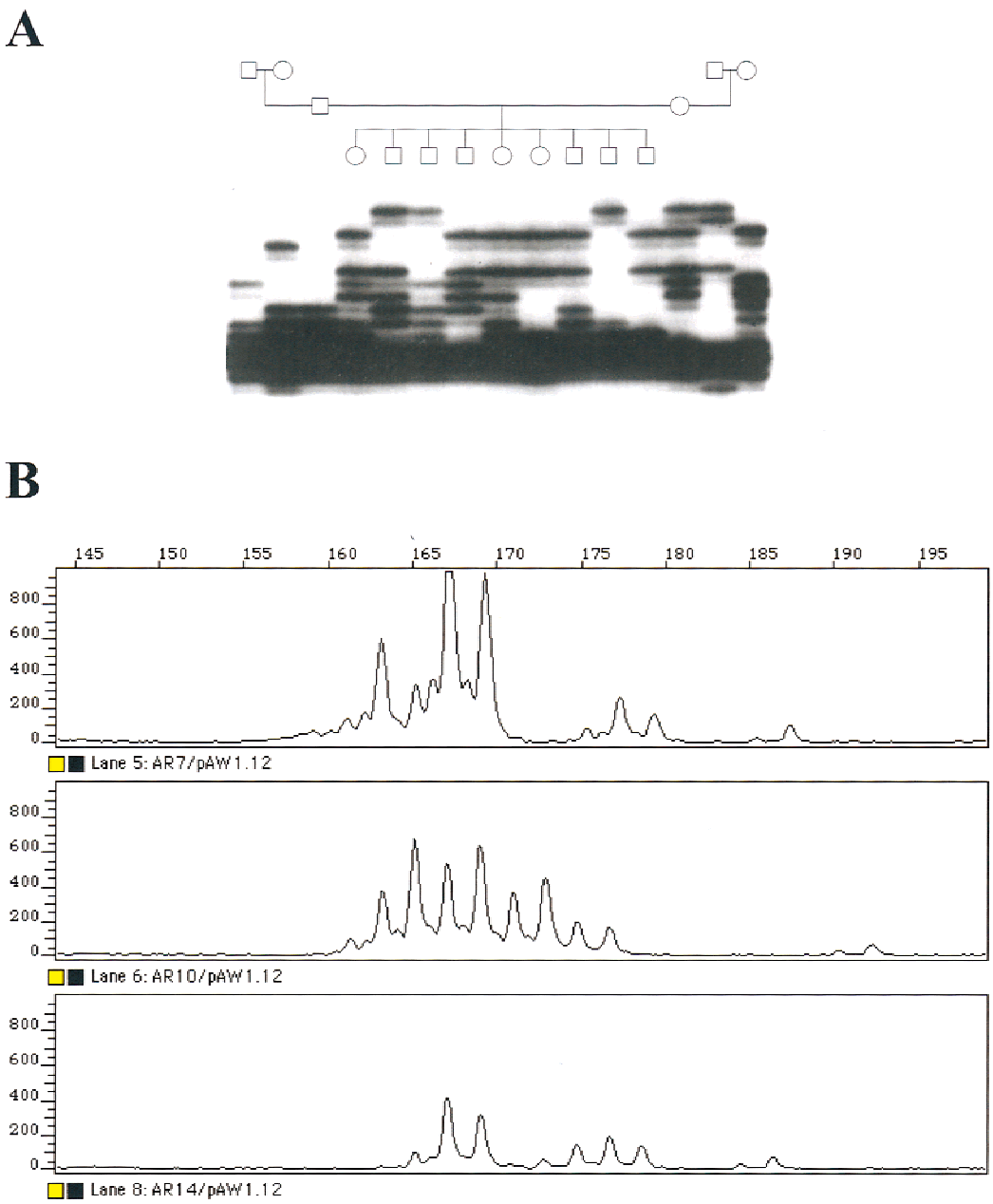

Figure 4 Analysis of $(CA)_n$ repeat *D21S188*. (*A*) Segregation analysis of *D21S188* in CEPH pedigree 1347 using a radiolabeled PCR primer and autoradiography. (*B*) Fluorescent analysis of *D21S188* in three at random individuals.

Using the newly developed markers, YACs were selected from the St. Louis and CEPH YAC libraries, and additional YACs from the chromosome 21-enriched ICRF YAC library were obtained. The YACs were assembled in a contig based on their STS content. The chimerism of the YACs was determined by sequence homology analysis and somatic cell hybrid mapping of YAC ends (Table 5). Ten YACs were localized to chromosome 21p, as both ends either mapped to chromosome 21p or were homologous to repetitive sequences present in chromosome 21p. Five YACs were considered chimerical, because one end fragment did not map to chromosome 21p. Four YACs with both ends not mapping to chromosome 21 were excluded from the YAC contig. Inclusion of the YAC ends in the contig mapping and selection of six additional CEPH megaYACs resulted in a map that was contiguous from the centromere to the rDNA genes at 21p12, with the exception of a region between pC_{HB} and *D21S1278*, where a gap remained. The contig consists of 30 multiple-locus YACs and 39 markers including 32 nonpolymorphic STSs, 4 polymorphic STRs, and 2 ESTs.

The pericentromeric region spanned by the YAC contig map partly overlaps with the published 21q YAC contig maps (Chumakov et al. 1992; Gardiner et al. 1995; Korenberg et al. 1995). The locations of YACs 831B6, 781G5, and 52H5 in our contig map are compatible with these maps. In the hybridization-based physical map of chromosome 21 reported by Nizetic et al. (1994), *D21S5*, *D21S187*, *D21S1276*, *D21S188*, *D21S191*, *D21S1277*, and *D21S1278* were localized in the 21p region. The order of these markers and YACs are the same as in our map; however, the orientation is reversed. In our YAC contig map (Fig. 2), *D21S1278* is located at 21p11.2–p12, at the telomeric end of the YAC contig, whereas *D21S5* is located in the centromeric region of the contig. The location of these STSs was determined by both PCR amplification of the chromosome 21 somatic cell hybrids and STS-content mapping of the YACs. 2E4 and 5B7 contain *D21S1278* and were mapped in the 21p11.2–p12 region; however, 2E4 was also positive with the STSs *D21S275* and ABM–C61 located in the pericentromeric region of chromosome 21. Furthermore, the left end of 5B7 was localized to the pericentromere. 5A12 was mapped to the pericentromeric region, whereas its right end localized to 21p11.2–p12. Together, these data suggest the presence of homologous sequences at two loci on chromosome 21p, which may explain why 2E4 overlapped with 5A12 by hybridization, resulting in the localization of *D21S1278* toward the centromere on chromosome 21 in the hybridization-based YAC map (Nizetic et al. 1994). From centromere to telomere, the order of repetitive satellite sequences in this map is α21-I (*D21Z1*, 831B6L, 1F8L, and 3A8L), satellite III (5A12L), α21-II (*D21Z4*, *D21Z3*, 89DD10R), and satellite III (2C2L), which is consistent with the results reported by Shimizu et al. (1995), Trowell et al. (1993), and Ikeno et al. (1994).

The minimal physical size of the region covered by YACs was estimated at 2.9 Mb, based on the insert lengths of nonoverlapping and nonchimerical YACs 831B6, 2C9, 4E9, 5B7, and A24F7. The maximum size was estimated at 5 Mb, based on the sizes of overlapping YACs 831B6, 891B10, 2C9, 4B2, F9B6, 4E9, 2E4, and A24F7. The size of the gap was estimated at 400 kb by fiber FISH using cosmids selected with YAC ends flanking the gap. The size of the region between the centromere and the rDNA cluster at 21p12 estimated from our YAC contig is significantly smaller than that derived from the PFGE map of the satellite repeat sequences on chromosome 21p (Doering et al. 1995; Korenberg et al. 1997). Possibly, this is due to the large variability of the size of the short arm and the centromere of chromosome 21. The exact size could not yet be determined accurately (Antonarakis 1998). Also, it is possible that a reasonable part of chromosome 21p is located distal to rDNA, a region not covered by our YAC contig map. Another explanation for the discordance in size estimations is that instability of long repetitive sequences in YACs leads to internal deletions, an observation that has been reported (Doering et al. 1996) and that might result in a YAC contig map that is shorter than the genomic size of the region. Two STSs (ABM–C62D and 891B10R) that localized to the pericentromere and 21p11, respectively, by somatic cell hybrid mapping were absent from YACs covering that region. These results suggest that deletions or other kinds of rearrangements might have occurred in some YACs of the contig map. However, when the STS content of each YAC was performed on five individual clones, only four YACs showed different STS contents.

All YACs in the contig were initially selected by chromosome 21p markers that were also present on at least one other chromosome and therefore might not be derived from chromosome 21. We investigated the chromosomal origin of seven YACs (831B6, 891B10, 2C9, 4B2, 3G8, 4E9, and 2E4) covering the region mapped by the contig using metaphase FISH. YAC 1F8 was analyzed by FISH because 74 bp of its right end was 80% homologous to *PRKC-L*, a member of the protein kinase C gene family. Three YACs—831B6, 1F8, and 4E9—specifically recognized chromosome 21p, indicating that they are derived from chromosome 21. In addition, 4E9 gave clear hybridization results without excessive signal amplification, suggesting that a major fraction of this YAC contains single- or low-copy sequences. The other four YACs recognized loci on other acrocentric chromosomes, in addition to chormosome 21p. In each case, the chromosome 21 signal was the strongest, or of equal intensity to the other signals. No YAC could be excluded from chromosome 21p. Based on the results of the FISH analysis and the sequence analysis of YAC end fragments, the YAC contig can be considered representative of chromosome 21p. Also, five of the seven YACs were selected from the ICRF library, which is an enriched chromosome 21 YAC library. This further supports the belief that the YACs most probably are derived from chromosome 21p.

We identified five chromosome 21p STR markers of the $(CA)_n$-repeat type and located four of them in the YAC contig map. We have analyzed these STR markers for their locus specificity by somatic cell hybrid mapping; however, none of them was chromosome 21p specific. All five STRs amplify multiple loci in the human genome and thus show a complex pattern of variable and constant bands. PCR amplification of somatic cell hybrids containing single human acrocentric chromosomes could not identify chromosome-specific alleles. *D21S188* was the most polymorphic showing Mendelian inheritance and it is a useful marker for DNA fingerprint analyses. We used this marker successfully to confirm the identity of EBV-transformed cell lines versus genomic DNA of many

samples in a fast and reliable manner. When used in multiplex analyses with other highly polymorphic STRs, *D21S188* can be applied for paternity testing and forensic applications investigation of the genetic relation between different samples.

Finally, it is interesting to note that hmc01a06, which is derived from an exon-trapping experiment using chromosome 21 cosmids (Chen et al. 1996), mapped to the pericentromeric 21p region in our YAC contig map (Fig. 2). Also, pAW14a (*D21S1277*) and 1F8R are partly homologous to several cDNA clones and a CpG-rich region of a genomic clone. These results suggest genes and/or pseudogenes might be located on chromosome 21p.

In conclusion, we have constructed a high-resolution physical map spanning a region of 2.9–5 Mb of the centromere and the short arm of chromosome 21, one of the acrocentric chromosomes. The current YAC contig map can be used to identify chromosome 21p-specific polymorphic markers. Such markers will be useful for nondisjunction studies in DS but have not yet been identified. Also, the YACs of this contig can be used as starting material for structural and functional studies of chromosome 21p, and the investigation of the existence of chromosome 21p genes, a study that has not yet been performed.

METHODS

Chromosome 21 Plasmid Clones

Nine plasmid clones localized previously to chromosome 21p or the pericentromeric region of 21q by Southern blot hybridization were selected. *D21S190* (pVC1.21c), *D21S5* (pPW235D), *D21S187* (pVC10a), *D21S1276* (pLSB77), *D21S188* (pVC1.12c), and *D21S191* (pVC1.23c) are subclones from phages derived from chromosome 21-specific phage libraries (Watkins et al. 1985; Van Camp et al. 1990; Stuyver et al. 1991). Additionally, pA_2 and pC_{HB} are clones of the 28S coding and spacer region of the rDNA gene cluster, respectively (Sylvester et al. 1986). Finally, Xba21 contains an *Xba*I fragment of the chromosome 21 somatic cell hybrid 153E9A that was selected with an alphoid DNA probe (Thompson et al. 1989).

Two additional plasmid clones were identified by screening the chromosome 21 phage library LL21NL01 using total human DNA or Blur8 containing human *Alu* repetitive DNA (Rubin et al. 1980). Somatic cell hybrids WAV17 (Graw et al. 1995), Hdm-15 (Lugo et al. 1987), and AHVI-17 (Cox and Epstein 1985) were used to select for chromosome 21 phage clones. Two clones, F14 and F32, were characterized in detail, and low-copy fragments were subcloned in plasmid pUC18. pAW14a (*D21S1277*) contains a 2.1-kb *Eco*RI fragment and pAW32a (*D21S1278*) a 0.8-kb *Eco*RI–*Sst*I fragment. Additionally, one plasmid clone, 3C6, was generated using microdissection of banded human metaphase chromosome 21p and microcloning (Bennett et al. 1995).

Finally, clones containing a $(CA)_n$-repeat were isolated from the flow-sorted chromosome 21 phage library LA21NS01 by hybridization using a $(GT)_{10}$ oligonucleotide probe. Four clones, *D21S411*, ABM–C78 (GenBank accession no. AF187011), ABM–C62D (GenBank accession no. AF187010), and ABM–C61, were localized to chromosome 21p by Southern blot hybridization of a somatic cell hybrid mapping panel as described elsewhere (Bosch et al. 1996; A. Bosch and X. Estivill, unpubl.). Clone pVC1.12c (Van Camp et al. 1990) was shown to contain a $(CA)_n$ repeat by hybridization with a $(CA)_n$ probe (Sigma-Aldrich, St. Louis, MO).

Development and Characterization of STSs

The DNA sequence of plasmid clones was determined using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequences were analyzed on an automated DNA sequencer model 373A (Applied Biosystems), and PCR primer pairs were designed. Of the sequences of *D21S411*, ABM–C78, ABM–C62D, and ABM–C61, PCR-primer pairs flanking the $(CA)_n$ repeat were selected. One EST, hmc01a06, was derived from the sequence of an exon-trapped product that mapped to the pericentromere of chromosome 21 (Chen et al. 1996, 1997). Additionally, the sequence of two α-satellite repeat sequences, *D21Z3* and *D21Z4* (Vissel et al. 1992), were used to develop PCR primer pairs. Finally, five additional STSs: [*D21Z1* (L1.26), *D21S275* (G51G10), *D21S237* (pPW265D), *D21S381* (E341), and *D21S286* (G51E07)] were selected from the Genome Database (GDB) based on their location in the centromere of chromosome 21 (Warburton et al. 1991; Chumakov et al. 1992; Tanzi et al. 1992).

Human genomic DNA and DNA from somatic cell hybrids were PCR amplified in a total volume of 25 µl containing 50–100 ng of template DNA, 10 pmoles of each primer, 1.5–2.0 mM $MgCl_2$, 200 µM dNTPs, and one unit of *Taq* DNA polymerase (GIBCO BRL, Gaitherburg, MD) using a DNA thermal cycler 480 (Perkin-Elmer-Cetus, Norwalk, CT). The first denaturing step was at 94°C for 1.5 min, followed by 30–40 amplification cycles consisting of a denaturing step at 94°C for 1 min, annealing at the empirically defined optimal temperature for 1.5 min and extension at 72°C for 1.5 min. A final extension step was performed at 72°C for 4 min. The PCR products were separated on a 1.5% agarose gel and visualized on a UV transilluminator after ethidium bromide staining. First, a monochromosomal somatic cell hybrid mapping panel (BIOS Laboratories, New Haven, CT) was used to establish the chromosomal assignment of the STSs. Next, the STSs were analyzed using an extended mapping panel of human chromosome 21 somatic cell hybrids, composed of WAV17, JC-6A, ACEM2-10d, 153-E7b, R2-10W, 2Fur1 (Graw et al. 1995), and AHVI-17 (Cox and Epstein 1985), each containing different regions of human chromosome 21.

Selection and Characterization of YACs

The St. Louis YAC library (Brownstein et al. 1989) was screened by PCR using STSs Xba21, *D21S76*, *D21S187*, *D21S188*, *D21S191*, *D21S1277*, *D21S1278*, pC_{HB}, and pA_2 in the context of the International Chromosome 21 Joint YAC-Screening Effort (JYSE) at the Eleanor Roosevelt Institute. The CEPH YAC and megaYAC libraries (Chumakov et al. 1992) were screened by both PCR amplification and hybridization using markers ABM–C78 and *D21S5*. In addition, YACs of the ICRF chromosome 21-enriched YAC library and CEPH library that were selected previously and mapped to the centromere or the p-arm of chromosome 21 (Chumakov et al. 1992; Nizetic et al. 1994) were included.

Single YAC colonies were grown at 30°C for 48 hr in 20 ml of selective AHC medium (6.7 grams/liter yeast nitrogen base without amino acids, 10 grams/liter casein hydrolysate, 10 mg/liter adenine and 2 grams/liter glucose) in the absence of tryptophan and uracil. Total YAC DNA and agarose plugs were prepared as described elsewhere (Cruts et al. 1995). To determine the size of the YACs, the YAC and yeast chromosomes were separated by PFGE using the CHEF Mapper XA apparatus (Bio-Rad) and the chromosomes were visualized by ethidium bromide (0.1 µg/ml) staining. Alternatively, the DNA was transferred to Hybond-N+ membranes (Amersham), and the YAC chromosomes were identified by hybridization with ^{32}P-labeled human genomic DNA. The sizes of the YAC chromosomes were estimated using the chromosomes of *Saccharomyces cerevisiae* strain YP148 as size marker.

YAC Contig Mapping

The STS content of the YACs was determined by using standard PCR amplifications. YAC vectorette PCR (Riley et al. 1990) and a linker-mediated technique (Cruts et al. 1995) were performed to isolate YAC end fragments. Alternatively, we used inverse PCR (Ochman et al. 1988; Triglia et al. 1988) with the following modifications: An amount of 0.2 µg of YAC DNA was digested with 10 units of *Rsa*I and *Ssp*I in One-Phor-All (OPA) buffer (Pharmacia, Upsalla, Sweden) and circulated by self-ligation using 10 units of T4 DNA ligase in a total volume of 200 µl by incubation overnight at room temperature. A second restriction-enzyme digestion was performed using *Fsp*I for the YAC left end (centromeric side) and *Sma*I for the YAC right end (URA3 side). The primer pairs 1207 (5′-AGCCAAGTTGGTTTAAGGCGCAAGGACT-3′) and 161 (5′-CGATGCTGTCGGAATGGACGATATC-3′), complementary to the YAC left vector arm, and 1208 (5′-TCGAACG-CCCGATCTCAAGATTACG-3′) and 162 (5′-GCATGTCTC-CATTCACTTCCCAGAC-3′), complementary to the YAC right vector arm, were used to amplify both ends of the human insert fragments. The PCR products were separated by agarose gel electrophoresis, excised from the gel, purified, and PCR amplified again using the same primers. The YAC end fragments were directly sequenced using the same primers as in the PCR amplification. Alternatively, the PCR products were subcloned into plasmid vector pUC18 or pGEM-T (Promega, Madison, WI) and sequenced using vector primers. PCR primer pairs were developed and PCR was performed on human genomic DNA and DNA from somatic cell hybrids and YACs.

Restriction Enzyme Fingerprinting of YACs

About 3 µg of YAC DNA embedded in agarose plugs was digested with 5 units of *Hin*dIII and *Bam*HI at 37°C for 6 hr. The reactions were terminated by heat inactivation or the restriction enzyme at 65°C for 10 min. DNA fragments were separated on a 0.7% agarose gel at 2 V/cm for 20 hr. The DNA was transferred to Hybond-N+ membranes by Southern blotting, and hybridization was performed using *Alu* clone pPD39 (Batzer et al. 1994) as probe.

FISH Analysis

Metaphase chromosomes were prepared from human lymphoblast cell lines according to standard procedures. Free DNA fibers were achieved from nuclei fixed to silanized microscope slides. Briefly, slides were submerged in 55 ml of lysis buffer (0.5% SDS, 50 mM EDTA, 200 mM Tris at pH 7.4) for 10 min in an upright position. Fifty milliliters of absolute ethanol was added dropwise to the lysis solution, and the slides were kept in this solution for 10 min. Finally, the slides were fixed for 30 min in 70% ethanol at room temperature and air-dried. FISH was performed as described (Pinkel et al. 1986; Tissir et al. 1995) using hybridization probes that were biotinylated with the Biotin-Nick Translation kit (Boehringer Mannheim). Briefly, the hybridization mixture contained 50% formamide and 10% dextran sulfate in 2× SSC. Repetitive sequences were suppressed with 50-fold excess of human *Cot-1* DNA. After overnight incubation at 37°C, the slides were washed three times at 45°C in 50% formamide and 2× SSC and three times in 0.1× SSC. Probes were detected with avidin–FITC (Vector Laboratories), amplified with biotinylated goat anti-avidin (Vector Laboratories) and a second layer of avidin-FITC. Fluorescent signals were analyzed on an Axioskop microscope (Zeiss, Germany). Images were captured using a CCD camera (Applied Imaging, UK). The length of probe signals and the gap were measured from digitized images with the CytoVision software (Applied Imaging).

STR Analysis

The polymorphic STRs *D21S188*, *D21S411*, ABM–C78, ABM–C62D, and ABM–C61 (Bosch et al. 1996; A. Bosch and X. Estivill, unpubl.) were detected using PCR on human genomic DNA of CEPH families 1333, 1334, 1347, or 7 unrelated individuals, and a set of somatic cell hybrids containing acrocentric chromosomes. GB3 contains chromosome 13 (Scheffer et al. 1986), HDm-5 (Lugo et al. 1987) and WegrothB3 (Geurts van Kessel et al. 1983) chromosome 14, HorlI chromosome 15 (Heisterkamp et al. 1982), and Wegroth D2 chromosome 22 (Geurts van Kessel et al. 1983). The PCR reaction was carried out in a total volume of 10 µl containing ~100 ng template DNA, 0.1 unit of *Taq* DNA polymerase, 5 pmoles of each primer, one of which was γ-^{32}P end-labeled, 100 µM dNTP, and 1.0 mM $MgCl_2$. PCR amplification was carried out as described above. Aliquots of the PCR products were denatured at 94°C for 5 min and separated on a 6% denaturing polyacrylamide gel containing 8 M urea at 55 W for 4.5 hr. The polymorphic alleles were visualized by overnight autoradiography.

Alternatively, *D21S188* was PCR amplified using one fluorescently labeled primer and separated using an automated DNA sequencer ABI 373 (Applied Biosystems). The fragments were analyzed using the GeneScan 672 software (Applied Biosystems).

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