

Construction of a Panel of Transgenic Mice Containing a Contiguous 2-Mb Set of YAC/P1 Clones from Human Chromosome 21q22.2

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Received December 2, 1994; accepted March 31, 1995

Libraries of the entire human genome, or regions of the genome, have been made in bacteria, yeast, and somatic cells. We have expanded this strategy using overlapping YACs and P1s from human 21q22.2 (the Down syndrome region) to create a panel of transgenic mice containing DNA that encompasses this region of the human genome. Together the members of the *in vivo* library, each with a unique transgene (four YACs and four P1s), contain approximately 2 Mb of contiguous DNA. The integrity, stable inheritance, and expression of a coding sequence for each member of the YAC panel are demonstrated, and the uses of the panel are described. © 1995 Academic Press, Inc.

INTRODUCTION

Down syndrome is caused by trisomy of chromosome 21 (Jacobs *et al.*, 1959; Lejeune *et al.*, 1959) and is associated with a range of defects in addition to mental retardation (Rahmani *et al.*, 1989; Korenberg *et al.*, 1990, 1992, 1994). These include immune deficiencies, increased susceptibility to leukemia, heart and gut defects, typical facies including brachycephaly, and the invariant development of Alzheimer disease-like neuropathology by the fourth decade. The Down syndrome region of human chromosome 21 has been shown to share extensive synteny with a segment of mouse chromosome 16 (Reeves and Miller, 1992; Doolittle *et al.*, 1993). Mice trisomic for this chromosome have been used as a model for Down syndrome and display features reminiscent of the syndrome (Gearhart *et al.*, 1986; Reeves *et al.*, 1986; Epstein *et al.*, 1990). However, the utility of these mice as a model is limited since they die *in utero*. This may be because mouse chromosome 16 contains extensive sequences in addition to that syntenic with human chromosome 21. Recently, an improved mouse model has been described

in which there is trisomy only for the region of mouse chromosome 16 that is conserved with human chromosome 21 (Davisson *et al.*, 1993). The partially trisomic chromosome 16 mice survive to adulthood and also display Down syndrome phenotypes. These studies suggest that individual genes may play a role in generating the distinct phenotypic features of Down syndrome, and it may thus be possible to identify the genes responsible for these features through the evaluation of animals that are transgenic for limited stretches of chromosome 21.

The region of mouse chromosome 16 syntenic to the Down syndrome region of human chromosome 21 is of additional interest since the murine mutation *weaver* has been mapped to this region, between the markers *Cbr* and *Pcp-4* (Mjaatvedt *et al.*, 1993). The *weaver* mutation is characterized by motor abnormalities (Lane, 1964) and a variety of central nervous system abnormalities, most prominently failure of cerebellar maturation (Rakic and Sidman, 1973a,b). *In vitro* (Gao *et al.*, 1992) and *in vivo* (Gao and Hatten, 1993) studies suggest that the presence of the wildtype *weaver* gene product should complement absence of the normal product. Due to the extensive synteny between the mouse and the human genetic maps in the Down syndrome region, it is likely that a human homologue of *weaver* is situated in this region of 21q22.2.

To investigate the Down syndrome region of human chromosome 21 and the syntenic region of mouse chromosome 16 containing the *weaver* gene, we have constructed a completely contiguous clone-based map using YACs and P1 phage that covers much of the region. This map was used to create an *in vivo* library of transgenic mice, each containing a component of the map. Individual members of the panel of transgenic lines contain large segments of human DNA, up to 670 kb, which are likely to include several distinct coding sequences. Stable inheritance of the intact human transgenic inserts and expression of known coding sequences contained within the inserts were shown to occur in the transgenic animals. This panel, which uses

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mice as a vector to propagate segments of human DNA, should be a valuable resource to understand the molecular basis of Down syndrome and to complement *in vivo* the *weaver* mutation as a step in the cloning of this gene.

MATERIALS AND METHODS

Development of a YAC and P1 clone-based map. General molecular biology techniques were performed as described (Sambrook *et al.*, 1989). The YAC clones 230E8, 745H11, 141G6, 152F7, and 285E6 were previously mapped to 21q22.2 by Chumakov *et al.* (1992) and later reported to be nonchimeric (Dufresne-Zacharia *et al.*, 1994). The YAC 285E6 was confirmed as being nonchimeric using fluorescence *in situ* hybridization to human metaphase cells (data not shown). The YAC was stable as judged by lack of deletion products on CHEF gel analysis. The P1 clones 1017, 1048, 1121, and 0073 were isolated from the DuPont library (Shepherd *et al.*, 1994) by filter hybridization using inter-*Alu* probes derived from YAC 745H11. The overlaps of these YACs and P1s were determined by PCR using previously mapped STSs and seven newly developed STSs (Fig. 1 and Table 1). The new STSs include six derived from cDNA sequences (21ES0302, 21ES0303, 21ES0305, 21ES0203, 21ES0291, and 21ES0123) and one derived from the Sp6 end of a P1 clone (1109S). The cDNA clones were isolated by hybrid selections from a fetal brain library (Clontech) using pools of chromosome 21 P1 clones (J.-F.C. and Y.Z., unpublished result) as genomic DNA probes. The hybrid selection procedure was described in detail previously (Cheng *et al.*, 1994). The isolated cDNAs were mapped by filter hybridization to both P1s and chromosome 21 YACs. The overlap between YACs 152F7 and 285E6 was demonstrated using a P1 phage that was isolated by virtue of the fact that both YACs hybridized to the phage. An STS was derived from the phage, and primers generated from the STS specifically amplified the correct fragment from both YACs using the PCR. The YAC 285E6 was shown to contain the human homolog of the mouse *Pop-4* (Ziai *et al.*, 1986; Nordquist *et al.*, 1988; Chen and Orr, 1990) by performing the PCR using primers that amplify the coding region of the mouse gene (Table 1). These primers also recognize the human PCP4 gene and produced the correct product from yeast DNA that contained the YAC 285E6, but not the other YACs from the contig.

Preparation and purification of YAC DNA. High-molecular-weight yeast DNA was prepared as described (Sambrook *et al.*, 1989) except that high-density yeast plugs were used for the purification of the YAC DNA. To make 13 ml of yeast plugs, 500 ml of saturated yeast culture was employed. YAC DNA was purified for microinjection as described (Schedl *et al.*, 1993) with some modifications. The yeast plugs were bisected with a razor blade and loaded onto a preparative well of a CHEF gel, and the DNA was separated. The outer segments of the preparative lane were stained with ethidium bromide and used to locate the YAC DNA. The relevant part of the gel was excised, and the agarose containing the YAC DNA was cut into 2.5-cm-long segments and embedded in a 4% Nusieve gel. The DNA was subjected to CHEF electrophoresis under conditions optimized for the size of the YAC. The usual conditions were an included angle of 90°, 4 V/cm gradient, switch time 50 s, and 10-h run time. After the YAC DNA had been concentrated in the 4% Nusieve, the agarose was equilibrated in 100 mM NaCl, 10 mM Tris-Cl, pH 7.5, 1 mM EDTA, pH 8.0, by incubation in 5 vol of the buffer for two 30-min incubations. The agarose was melted by incubation at 68°C for 12 min and digested using 4 μ l of 1 unit μ l⁻¹ β -agarase (New England Biolabs) per 100 mg of gel with incubation at 42°C for 2 to 3 h. Microdialysis employed a Millipore Minitan-S polysulfone membrane filter (100,000 NMWL) and was for 2 h to overnight against 100 mM NaCl, 10 mM Tris-Cl, pH 7.5, 0.1 mM EDTA, pH 8.0.

Purification of P1 phage DNA. P1 phage DNA was purified as described (Callow *et al.*, 1994) followed by a 2-h microdialysis step against 5 mM Tris-Cl, pH 7.5, 0.1 mM EDTA using Millipore VS

filters (pore size 0.025 μ m). For some preparations, the GeneClean (Bio 101) step was omitted. For other preparations both the GeneClean and microdialysis steps were omitted and replaced with five washes using a Millipore Ultrafree MC unit (30,000 NMWL). The washes used microdialysis buffer and each spin reduced the volume of the DNA twofold before restoration to the previous volume.

Creation of transgenic mice. Microinjection of DNA at a concentration of 1 ng μ l⁻¹ was into FVB zygotes and was performed as described (Hogan *et al.*, 1986). This concentration of DNA results in about one to five copies of YAC DNA being injected into the fertilized egg.

PCR screening of mice. Mouse tail DNA was prepared as described (Hogan *et al.*, 1986). Primers were chosen from DNA sequence using PRIMER 0.5 (Whitehead Institute). PCR reactions were performed in a 20- μ l volume, containing 1 μ l of 0.1 μ g μ l⁻¹ of mouse tail DNA, 2 μ l of 10 \times PCR buffer, 2 μ l of 2 mM dNTP, 1 μ l of each primer at 10 μ M, and 0.2 μ l of 5 unit μ l⁻¹ *Taq* polymerase (Boehringer-Mannheim). The 10 \times PCR buffer was 10 \times PCR buffer + Mg²⁺ (Boehringer-Mannheim; this is 100 mM Tris-Cl, pH 8.3, 15 mM MgCl₂, 500 mM KCl) for all primers except 10 \times TNK100 (100 mM Tris-Cl, pH 8.3, 50 mM NH₄Cl, 15 mM MgCl₂, 1 M KCl) was used for the 21ES0203 and 21ES0291 primers. The reactions were subjected to an initial denaturation step of 94°C for 3 min followed by cycles of 94°C for 30 s, 50 to 58°C (depending on the primer: 65°C for the D21S395 primers; 58°C for the left YAC arm, right YAC arm, CBR, 21ES0302, D21S395, 21ES0303, and 21ES0305 primers; 57°C for the 1109S, 21ES0302, 21ES0303, 21ES0305, and D21S267 Fwd and Rev primers; 53.6°C for the D21S167 primers; 50°C for the PCP4 primers, and 55°C for the others) for 30 s, 72°C for 1 min, except 2 min was used for the PCP4 primers. All reactions used 35 cycles except for those employing primers D21S395, which used 40 cycles. Primers AFM238wc3m and AFM238wc3a were used for screening mice for the STS D21S267, the primers D21S267 Fwd and Rev were used for RT-PCR analysis of this STS (see below). The reaction products were analyzed on 3% agarose gels.

Inter-*Alu* fingerprinting of YAC transgenic mice. Inter-*Alu* DNA fragments were amplified by PCR using 400 ng of genomic DNA purified from transgenic mice and 40 ng of yeast DNA containing YACs as templates. A 50- μ l PCR reaction mix contains 5 μ l 10 \times buffer (500 mM KCl, 100 mM Tris-Cl, pH 8.0, 15 mM MgCl₂, 0.1% (w/v) gelatin), 5 μ l of 2 mM dNTP, 0.2 μ l of 5 unit μ l⁻¹ AmpliTaq polymerase (Perkin Elmer Cetus), and 0.5 μ l of the pDJ34 primer at 10 μ M. Forty cycles of PCR were carried out with a temperature profile of 95°C for 30 s, 58°C for 30 s, and 72°C for 2 min. Twenty microliters of the amplified DNA from mouse and 10 μ l of the amplified DNA from yeast were run on an agarose gel and blotted to a nylon membrane.

Inter-*Alu* fragments were also generated from 100 pg of the gel-purified YAC DNA under the same PCR conditions described above. Three microliters of the amplified DNA was labeled with [α -³²P]dCTP in a random priming reaction (Feinberg and Vogelstein, 1983) and preincubated with 0.5 mg ml⁻¹ of human placenta DNA (Sigma) in 300 μ l of hybridization solution containing 6 \times SSC (0.9 M NaCl, 90 mM sodium citrate, pH 7.0), 5% (w/v) dextran sulfate, 1% NaDodSO₄ at 65°C for 3 h. All DNA filters were preincubated with 0.5 mg ml⁻¹ of placental DNA in 3 ml of prehybridization solution containing 6 \times SSC, 10 \times Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), and 1% NaDodSO₄ for 12 h at 65°C and then hybridized with ³²P-labeled probes in 3 ml of hybridization solution at 65°C for 12 h. Subsequently the filters were washed twice in 2 \times SSC, 1% NaDodSO₄ and twice in 0.1 \times SSC, 1% NaDodSO₄ at 65°C. All washes lasted 15 min. X-ray film (Kodak) was exposed to the filters for 2 h at -70°C.

RT-PCR. RNA was isolated from mouse tissues using RNA STAT-60 (Tel-Test "B," Inc.). RNA (2.5 μ g) was extracted with phenol/chloroform, ethanol precipitated, treated with RQ1 RNase-free DNase (Promega), extracted with phenol/chloroform, and ethanol precipitated. The RNA was treated with SuperscriptII RNase H⁻ reverse transcriptase (Gibco BRL) according to the manufacturer's

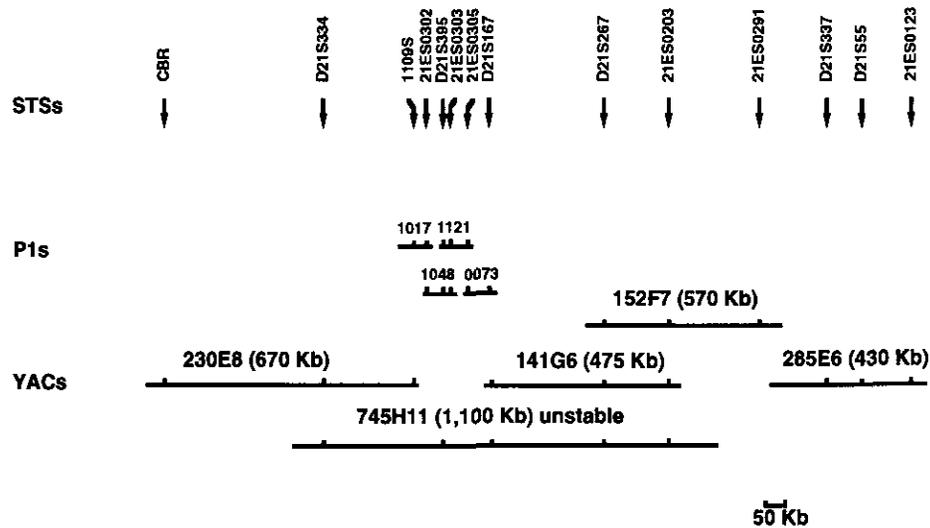


FIG. 1. Molecular map of the region between CBR and PCP4 in humans. All of the YACs shown were used for microinjection, except for 745H11, since this YAC was unstable. The P1 phage used for microinjection are shown, as are the STSs and ESTs used for the PCR screening of mouse pups. The human homolog of mouse *Pcp-4* (Ziai *et al.*, 1986; Nordquist *et al.*, 1988) maps to YAC 285E6.

instructions, using random hexamers as primers. One-tenth of the reverse transcription reaction was used in a PCR reaction under the conditions described above, except that 40 cycles were used for the 21ES0123 primers.

Determination of YAC transgene copy number. Southern blotting was used to determine the copy number of the YAC transgene for the full-length lines 230E8#55, 141G6#4, 152F7#12, and 285E6#67 (Table 6). Ten micrograms of YAC transgenic mouse DNA was digested to completion with *Bam*HI and Southern blotted. DNA corresponding to the YAC transgenes was identified using a probe made from the right YAC arm PCR product (Table 1), and DNA corresponding to a single-copy gene was detected using a probe made from the PCR product of primers that recognize the mouse embryonic α -globin genes (X) (Table 1). The intensity of positively hybridizing bands was compared using an ImageQuant phosphoimager (Molecular Dynamics) and the copy number of the YAC transgene determined by making corrections for the different lengths and specific activities of the probes. The YAC transgene copy number was determined for other full lines (Table 6) using a quantitative PCR assay. Human DNA and YAC transgenic DNA was serially diluted and the PCR performed using primers that recognize the STSs D21S334 for YAC 230E8, 21ES0203 for YACs 141G6 and 152F7, 21ES0123 for YAC 285E6. The human DNA acted as a comparison standard containing a single-copy gene. The reactions were analyzed on a 3% agarose gel and the products quantitated using the IS-1000 digital imaging system (Alpha Innotech Corp.). The range at which the amount of PCR product was linearly related to the target DNA concentration was determined and the copy number of the YAC transgene calculated by comparison with the results from the human DNA samples.

RESULTS

Molecular Map of the Down Syndrome/weaver Region

The results of the clone based map are shown in Fig. 1. This region encompasses much of the Down syndrome region at 21q22.2 (Rahmani *et al.*, 1989; Korenberg *et al.*, 1990, 1992, 1994) and includes the markers CBR and PCP4, between which mapping studies have indicated the *weaver* gene lies (Mjaatvedt *et al.*, 1993).

The physical map consists of a complete YAC contig and a P1 contig that extends over part of the YAC contig. There are five YACs and four P1s spanning approximately 2 Mb of DNA. Except for YAC 285E6, the other four YACs have been previously reported to be nonchimeric and nondeleted as suggested by the long-range restriction maps derived from both lymphocyte DNA and YACs (Dufresne-Zacharia *et al.*, 1994). This YAC contig completely covers the region between CBR and PCP4; however, YAC 745H11 is unstable, producing smaller fragments during growth (data not shown). This YAC was therefore not used in constructing transgenic mice. The omission of YAC 745H11 left a gap between YACs 230E8 and 141G6, which was filled using P1 clones.

In constructing a P1 clone map in the Down syndrome region, we used the inter-*Alu* fragments derived from YAC 745H11 to screen a human P1 library (see Materials and Methods). A subset of the isolated P1s form a contig that bridges the gap between YACs 230E8 and 141G6 (Fig. 1). The overlaps between P1s and YACs were detected by primers derived from six STSs, which include previously determined sequences (D21S395 and D21S167), newly isolated cDNAs (21ES0302, 21ES0303, and 21ES0305; J.-F. Cheng and Y. Zhu, unpublished results), and the sequence derived from the Sp6 end of the P1 clone 1109 (1109S). For example, the overlap between YAC 230E8 and P1 1017 was detected by 1109S, and the overlap between YAC 141G6 and P1 0073 was detected by D21S167. These primers together with other primers derived from known STSs (i.e., CBR, D21S334, D21S267, D21S337, and D21S55) and newly isolated cDNAs (i.e., 21ES0203, 21ES0291, and 21ES0123; see Table 1) were used in PCR assays to determine quickly the presence or absence of the cloned human DNA in transgenic mice (see below).

TABLE 1
PCR Primers

STS, EST, locus	Primer names	Primer sequence (5' to 3')	Product size (bp)	References
Left YAC arm	LA1	CCTGCTGCGCTTCGCTACTTGGAGC	222	Burke <i>et al.</i> (1987); K. Frazer, Berkeley pers. comm. (1994)
	LA2	GTCTTGCGCCTTAAACCAACTTGG		
Right YAC arm	RA1	CTTGAGATCGGGCGTTCGACTCGC	161	Burke <i>et al.</i> (1987); K. Frazer Berkeley, pers. comm. (1994)
	RA2	CCGCACCTGTGGCGCCGGTGATGC		
CBR	CARED-3'UA	GAGAAGAGAGTTGAACAGTG	235	Wermuth <i>et al.</i> (1988); Forrest <i>et al.</i> (1990, 1991); Avramopoulos <i>et al.</i> (1992)
	CARED-3'UB	CTCCTGCATCAGAGGAAATC		
D21S334	G51B07.1	AGTGATACCGTGCCTGGTC	277	Chumakov <i>et al.</i> (1992)
	G51B07.2	CCTAAATAAATGATGGTCCCTG		
1109S	1109S	TAATCGGCCCTTCTTGGACAG	217	This work
	1109S-2	TTTGCTTTGCATGGTTTTCA		
21ES0302	Fwd	ACAAGGGGTCATAGGCTGTG	336	This work
	Rev	TGACATCTTGGTTTGTGGGA		
21ES0303	Fwd	AAGCCAGGAGTGGAGACAGA	153	This work
	Rev	GTCTGTCACTTTCCCTTGTCC		
D21S395	E1-16-1	ATCCCAGCTCCTTGGCGACG	112	Chumakov <i>et al.</i> (1992); Ichikawa <i>et al.</i> (1993)
	E1-16-2	GCTGGGCGCTGCAGTTTACC		
21ES0305	Fwd	AAAGATCTCAACATGGAAAAATCC	166	This work
	Rev	AAACCTCTTTCCATTTCAGAAAGG		
D21S167	112	TCCTTCATGTACTCTGCA	170	Guo <i>et al.</i> (1990); Chumakov <i>et al.</i> (1992)
	113	TGCCCTGAAGCACATGTGT		
D21S267	AFM238wc3m	CCTCCAACCTGGGTGA	200	Chumakov <i>et al.</i> (1992); Weissenbach <i>et al.</i> (1992); Gyapay <i>et al.</i> (1994)
	AFM238wc3a	ATGGATCTGGATTCTATCTTC		
D21S267	Fwd	GCTATATCAAATCTTTCTTTGGAAAAC	225	This work
	Rev	TGTAACAAAATATTGGGTATTGTAATCTC		
21ES0203	Fwd3	CCCAGGTCCCTGGTCTCTTC	124	This work
	Rev2	GGGGGAAAATTTTCACGGAT		
21ES0291	Fwd	ACCTGGGGACTGTGTGTCTC	170	This work
	Rev	TCTCAGTCTTCGGGCACC		
D21S337	GA7E01.2	CCTTTCTGACCCCAACACAT	264	Chumakov <i>et al.</i> (1992)
	GA7E01.1	GAGAGCACAGTTTGTACACAGG		
PCP-4	PCP-4F	ATGAGTGAGAGACAAAGTGCC	189	Ziai <i>et al.</i> (1986); Nordquist <i>et al.</i> (1988); Chen and Orr (1990)
	PCP-4R	CTAGGACTGTGATCCTGCCTTTTT		
21ES0123	Fwd1	TTTCGCCTCAGCGTTACC	142	Cheng <i>et al.</i> (1994)
	Rev1	CTTGCCATGCACAACCTGC		
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH1	ACCACAGTCCATGCCATCAC	452	Arcari <i>et al.</i> (1984); Ercolani <i>et al.</i> (1988); Nasrin <i>et al.</i> (1990)
	G3PDH2	TCCACCACCTGTGTCTGTA		
Mouse embryonic α -hemoglobin gene (X)	X1	GATCATGACCGCCGTAGG	311	Leder <i>et al.</i> (1985); C. Paszty, Berkeley, pers. comm. (1994)
	X2	CATGAACCTGTCCAGGCTT		
Alu repeats	pDJ34	TGAGCCGAGATCGCGCCACTGCACTCCAGCCTGGG	Variable	Breukel <i>et al.</i> (1990)

Creation of a Panel of YAC/P1 Transgenic Mice

Pups resulting from zygote microinjection with YAC and P1 DNA were screened using the PCR, employing primers designed to detect sequence tagged sites (STSs) from the human and vector sequences. Table 1 shows the STSs and primers employed in the PCR screens of the pups. Tables 2 and 3 show the results of the screens, and these results are summarized in Table 4. For each YAC, 2 to 10 independent lines of transgenic mice harboring a full-length insert were created. In addition, for each YAC

clone between 8 and 13 lines of mice containing incomplete segments of the YAC inserts were created. The P1 phage DNA was injected as pools of two overlapping phage DNA mixed together in equal concentration, and the DNA was thus microinjected as three different pools. At least 1 line of mice was created for each P1 phage that bridged the gap in the YAC contig.

Assessment of Transgene Integrity and Copy Number

Several approaches have been used to assess transgene integrity including (1) inter-Alu PCR fin-

TABLE 2
PCR-Based STS Analysis of YAC Transgenic Mice

YAC 230E8					
Number of pups	Left YAC arm ^a	CBR	D21S334	Right YAC arm	
3 ^b	+	+	+	+	
1	-	+	+	+	
1	+	+	-	+	
3	+	-	+	-	
1	-	+	-	+	
1	-	+	-	-	
2	-	-	+	-	
51	-	-	-	-	

YAC 141G6					
Number of pups	Left YAC arm	D21S167	D21S267	21ES0203	Right YAC arm
10 ^b	+	+	+	+	+
2	+	+	+	-	+
3	+	-	+	+	-
1	+	-	-	+	-
2	-	-	+	+	-
2	+	-	-	-	-
1	-	+	-	-	-
1	-	-	+	-	-
48	-	-	-	-	-

YAC 152F7					
Number of pups	Left YAC arm	D21S267	21ES0203	21ES2091	Right YAC arm
2 ^b	+	+	+	+	+
1	+	+	-	+	+
1	-	+	+	-	+
1	+	-	-	+	-
1	-	+	+	-	-
1	-	-	+	-	+
1	-	+	-	-	-
1	-	-	+	-	-
1	-	-	-	-	+
82	-	-	-	-	-

YAC 285E6					
Number of pups	Left YAC arm	D21S337	21ES0123	Right YAC arm	
5 ^b	+	+	+	+	
1	+	+	+	-	
1	+	-	+	-	
1	+	-	-	+	
1	-	+	+	-	
1	-	+	-	+	
1	+	-	-	-	
1	-	+	-	-	
2	-	-	+	-	
1	-	-	-	+	
76	-	-	-	-	

^a +, presence of STS; -, absence of STS.

^b Mice that harbor full-length YACs.

gerprinting, (2) STS content mapping, (3) analysis of linkage and transmission frequencies of the YAC STSs, and (4) analysis of expressed sequences encoded by the YAC transgenes.

Inter-*Alu* PCR fingerprinting (Fig. 2) was used to

assess YAC integrity in transgenic mice containing a full-length YAC as judged by STS content mapping (see below). The exponential nature of the PCR resulted in some intensity differences of fingerprint bands between experiments where the yeast and mouse DNA were

TABLE 3
PCR-Based STS Analysis of P1 Transgenic Mice

P1 1017 + P1 1048			
Number of pups	1109S ^a	21ES0302	D21S395
1 ^b	+	+	+
1	+	+	-
2	-	+	-
43	-	-	-
P1 1048 + P1 1121			
	21ES0302	D21S395	21ES0305
1 ^b	+	+	+
2	+	-	-
1	-	+	-
2	-	-	+
11	-	-	-
P1 1121 + P1 0073			
	D21S395	21ES0305	D21S167
5 ^b	+	+	+
1	+	+	-
7	-	+	+
1	+	-	-
1	-	+	-
56	-	-	-

^a +, presence of STS; -, absence of STS.

^b Mice that harbor both P1 phage.

used. Nevertheless, every band present when the yeast DNA was used as a substrate was also present when the transgenic mouse DNA was used. The presence of a large number of bands in the YAC and transgenic samples supports a high degree of resolving power of this technique for the detection of rearrangements and deletions. The similarity of YAC and corresponding transgenic YAC fingerprints suggests that the YAC has not been rearranged during its integration into the mouse genome.

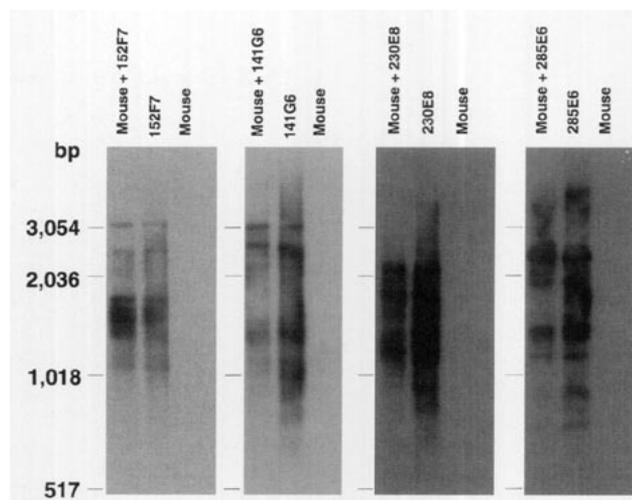


FIG. 2. Inter-*Alu* PCR fingerprinting of the full-length YAC transgenic mice. Each panel shows the fingerprint obtained from the transgenic mice and yeast containing the YAC. No fingerprint signal was obtained from nontransgenic littermates.

STS content mapping of transgenes utilized STSs that probed at three to four approximately equal intervals along the length of each of the injected YAC DNAs and that probed the injected P1 DNA between one and three times. PCR primers that recognize known human STSs and other STSs derived from DNA sequence analysis of the region were used (Table 1). The primers chosen for these studies gave PCR products of the predicted size only when human or YAC DNA was present as a substrate. Nontransgenic mouse DNA when used as target DNA failed to give PCR products of the correct size. Tables 2–4 show the results of PCR screens of mice transgenic for YAC and P1 DNA. Animals containing incomplete segments of the injected YACs did not show any obvious systematic pattern of retention of DNA fragments. For example, there were animals with fragments from both the left and the right ends of the YAC but that lacked central portions of the YAC. Several studies in which YAC transgenics have been characterized by STS content mapping have shown

TABLE 4
Summary of PCR Screening of Transgenic Mice

YAC	Number of pups screened	Number of transgenic pups	Percentage of transgenic pups	Number with full-length YAC	Percentage with full-length YAC
230E8	63	12	19	3	5
141G6	70	22	31	9	13
152F7	92	10	11	2	2
285E6	91	15	17	5	6
P1 pool	Number of pups screened	Number of transgenic pups	Percentage of transgenic pups	Number with two P1s	Percentage with two P1s
1017 + 1048	47	4	9	1	2
1048 + 1121	17	6	35	1	6
1121 + 0073	71	15	21	5	7

TABLE 5
Inheritance of STSs in Offspring of YAC Transgenic Mice

YAC	Line	Number of transgenic G1 offspring	Number of G1 offspring	Percentage of transgenic G1 offspring	Number of transgenic G2 offspring	Number of G2 offspring	Percentage of transgenic G2 offspring
230E8	42	0	3	0	—	—	—
	50	6	13	46	22	38	58
	55	1	14	7	—	—	—
141G6	2	1	18	6	—	—	—
	4	3	17	18	—	—	—
	21	0	5	0	—	—	—
	28	8	25	32	23	41	56
152F7	12	4	16	25	78	180	43
	57	3	16	19	19	35	54
285E6	38	1	6	17	—	—	—
	52	2	10	20	9	18	50
	67	11	15	73	38	82	46
	84	5	10	50	30	57	53

that transgenic animals with a full complement of STSs predominantly contain a full-length copy of the YAC (Jakobovits *et al.*, 1993; Lamb *et al.*, 1993; Peterson *et al.*, 1993; Strauss *et al.*, 1993; Frazer *et al.*, 1995). This assessment was based on characterization of the authentic protein product, the coding sequences of the gene being stretched out over a significant segment of the YAC.

A further method used to confirm transgene integrity was demonstration of a single linkage group of STSs in offspring of founder and G1 mice. For each YAC, at least two lines containing a full-length YAC have been shown to pass on all of the relevant STSs from the founder animals (G0) to the offspring (G1) in Mendelian fashion as one linkage group (Table 5). Similarly, at least one full-length line for each YAC has been shown to pass on all of the relevant STSs from the G1 generation to the next generation (G2) (Table 5). The mean of the transmission frequencies for the YACs for generations G1 to G2 was 48.5% (242/499), which is not significantly different from the expected Mendelian ratio of 50% ($\chi^2 = 0.22$, $df = 1$, $P < 0.7$). However, the mean transmission frequency for the YACs from the founder animals (G0) to the G1 generation was 26.8% (45/168), which is significantly different from the expected Mendelian ratio ($\chi^2 = 36.2$, $df = 1$, $P < 0.005$). The most likely explanation is that some of the founder animals are mosaics.

The expression of a gene (EST) on one each of the full-length YAC transgenes was examined to ascertain whether the gene was correctly expressed. For each of the four YACs correct expression was found (see below), which provides further supporting evidence that the transgene is intact.

Although the experiments described here would not detect small deletions in the transgenes, the multiple independent lines of transgenic animals provide a safeguard against such rearrangements, which in any case would be difficult to detect. It would be extremely un-

likely that the same deletion would occur independently in two lines of transgenic animals. Thus, evaluations of the biological properties of each transgenic YAC should use two or more independent lines. The integrity of the P1 phage in the transgenic mice was not assessed further. However, previous reports have shown that in the majority of cases there is no rearrangement of the transgenic DNA and there is correct expression of genes contained within the phage (Linton *et al.*, 1993; Callow *et al.*, 1994). The copy number of YAC transgenes was determined using Southern blotting and quantitative PCR. The results are shown in Table 6. The copy number of most lines was low, being about one to three copies per genome.

Expression of Transgenes

To investigate whether the human YAC DNA integrated into the mouse genome displayed expression of the resident genes, one transcription unit was examined for each YAC using reverse transcription-PCR (RT-PCR) to analyze expression in a transgenic mouse containing a full-length YAC. The results are shown in Fig. 3. The STS CBR is contained within the transcript of the gene

TABLE 6
Copy Number of YAC Transgenes

YAC	Line	Transgene copy number
230E8	50	1
	55	1
141G6	2	9
	4	1
	28	3
152F7	12	1
	57	2
285E6	52	3
	67	1

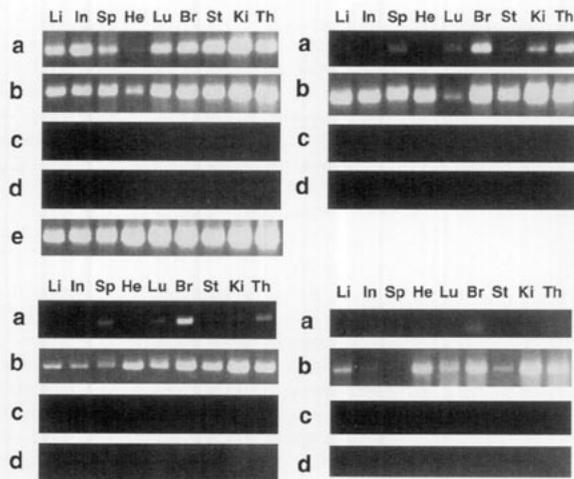


FIG. 3. RT-PCR analysis of expression of human genes contained within YACs integrated in the mouse genome. (Upper left) 230E8; (lower left) 141G6; (upper right) 152F7; (lower right) 283E6. *Tissues are abbreviated as follows: liver (Li), intestine (In), spleen (Sp), heart (He), lung (Lu), brain (Br), stomach (St), kidneys (Ki), and thymus (Th). (a) The expressed sequences are detected by primers that recognize the following STSs and ESTs: CBR, which is harbored within YAC 230E8 (upper left), D21S267, which is harbored within YACs 141G6 (lower left) and 152F7 (upper right), and 21ES0123, which is harbored within YAC 285E6 (lower right). (b) A control for equal loading of RNA in each lane of the gel is provided by primers that recognize the gene for the housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase (G3PDH). (c) For each YAC, a mock RT reaction omitting the RT was performed, showing that the signal was not due to DNA. (d) Similarly, for each set of primers, an RT-PCR reaction was performed on RNA from tissues obtained from a nontransgenic litter mate. The absence of PCR products showed that the primers specifically recognized only the human transcripts and not the endogenous mouse sequences. (e) The G3PDH primers were used in an RT-PCR reaction using RNA from tissues obtained from a nontransgenic litter mate, showing that intact RNA was obtained from this animal.

for human carbonyl reductase, which is considered to be a housekeeping gene (Forrest *et al.*, 1991; Wirth and Wermuth, 1992). The STS resides within YAC 230E8, and a line of mice containing this YAC expresses the carbonyl reductase gene fairly uniformly in all tissues examined. The STS D21S267 is contained within a transcription unit that has been shown to be expressed predominantly within the human brain (Cheng and Zhu, 1994). This STS is found within the YACs 141G6 and 152F7, and two lines of mice each containing one of these YACs express the gene containing this STS. The pattern of expression of the STS is almost identical in the two lines of mice that harbor the two different YACs, and furthermore expression of the human gene is particularly strong in the mouse brain. The expressed sequence tag (EST) 21ES0123 is found on YAC 285E6 and is known to be expressed in fetal human brain (Cheng *et al.*, 1994). The EST was found to be expressed in the brain of the transgenic mouse. Thus, for each of the transgenes analyzed here, the human gene appears to be correctly expressed when borne on a YAC vector and integrated into the mouse genome.

DISCUSSION

In this study we describe the creation of a series of independent lines of transgenic mice containing separate YAC and P1 phage DNAs that together cover approximately 2 Mb of contiguous human sequence from the Down syndrome region on chromosome 21. This panel of transpolygenic mice may be thought of as an *in vivo* library of this defined segment of the human genome that can be used for many purposes, including mapping and gene discovery. Results from our own laboratory (Frazer *et al.*, 1995) as well as from others (Choi *et al.*, 1993; Forget, 1993; Jakobovits *et al.*, 1993; Lamb *et al.*, 1993; Peterson *et al.*, 1993; Schedl *et al.*, 1993; Strauss *et al.*, 1993) show that for the vast majority of cases, YAC transgenes are correctly and efficiently expressed. This finding is corroborated by the results described in this report, which show, within the limited quantitation capabilities of RT-PCR, correct expression of human expressed sequence tagged sites on each of the YACs when integrated into the mouse genome. The advantage of propagating cloned DNA in a higher eukaryote, that is, a mouse, is that direct functional assays can now be used to extract biologically meaningful information from the expressed transgenic sequences.

The cloning of genes through their biological impact, including by *in vivo* complementation, has proved to be a successful strategy in both prokaryotes and the lower eukaryotes, although it has not been practicable for higher eukaryotes. With the recent development of techniques for cloning of large segments of DNA in YAC and P1 vectors and the successful creation of transgenic mice containing this DNA (Choi *et al.*, 1993; Jakobovits *et al.*, 1993; Lamb *et al.*, 1993; Peterson *et al.*, 1993; Schedl *et al.*, 1993; Strauss *et al.*, 1993) it is now feasible to cover targeted regions of the human genome in mice as demonstrated in the present study. Crossing members of the 21q22.2 *in vivo* panel with *wv/wv* mice may enable us to test the feasibility of moving from mapping information for the *weaver* mutation to localization of the cognate gene and definitively placing it on a YAC or P1 phage within the panel. In addition, the panel of mice described here will be of utility in the identification of individual genes whose increased dosage results in the distinct features of Down syndrome.

There was no apparent relationship between the lengths of the YACs and the proportion of animals that contained the full-length YAC. This suggests that it may be possible to obtain transgenic animals with full-length inserts using even larger YACs. Not only were transgenic animals containing the entire length of the YAC obtained in this study, but also animals that contained parts of the YACs fragmented in an apparently random fashion were created. This fragmentation is a powerful advantage of the approach described here. The mice harboring fragmented YACs will be an addi-

tional resource for the finer mapping of genes associated with identified phenotypes; the number of breakpoints obtained as a result of the microinjection process are far more numerous than could practically be obtained using breakpoints that occur as a result of meiotic recombination during classical genetic mapping.

Although the panel of mice described in this study covers nearly 2 Mb of sequence, this represents only about 0.07% of the human genome. It currently appears beyond the scope of the approach described here to make a complete panel of the human genome in mice. However, this goal might be more attainable if mixtures of purified DNA preparations from two or more different YACs could be used for microinjection, much as was described in this study using P1 phage. At 670 kb, the 230E8 YAC appears to be the largest stretch of DNA from which a mouse with an intact transgene has been made. It may be possible to cover larger segments of the human genome with even larger YACs. Nonetheless, the more limited approach described in this study is likely to provide valuable substrates for the analysis of other targeted segments of the human genome.

ACKNOWLEDGMENTS

D.J.S. is supported by a Research Fellowship from Trinity College, Cambridge, UK. J.F.C. and Y.Z. are supported by the United States Department of Energy. E.M.R. is an Established Investigator with the American Heart Association and is supported by the National Institutes of Health NHLBI Grant PPG HL18574. This study was performed at the Lawrence Berkeley Laboratory Human Genome Center through the United States Department of Energy under Contract DE-AC03-76SF00098, University of California, Berkeley. Additional support was provided by a Laboratory Director's Research and Development Fund.

Note added in proof. Recent experiments suggest that mouse *Pcp-4* hybridizes distal to YAC 285E6. To complete the panel of transgenic mice so that it contains the human homolog of the *weaver* gene, we are currently creating mice containing the YAC 750F7.

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