Chromosomal Region of the Cystic Fibrosis Gene in Yeast Artificial Chromosomes: A Model for Human Genome Mapping

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A general strategy for cloning and mapping large regions of human DNA with yeast artificial chromosomes (YAC's) is described. It relies on the use of the polymerase chain reaction to detect DNA landmarks called sequence-tagged sites (STS's) within YAC clones. The method was applied to the region of human chromosome 7 containing the cystic fibrosis (CF) gene. Thirty YAC clones from this region were analyzed, and a contig map that spans more than 1,500,000 base pairs was assembled. Individual YAC's as large as 790 kilobase pairs and containing the entire CF gene were constructed in vivo by meiotic recombination in yeast between pairs of overlapping YAC's.

THE MOST SERIOUS GAP IN CURRENT APPROACHES TO THE physical mapping of the human genome lies between "the cosmid and the centimorgan" (*1*). Cosmids, which are DNA clones propagated in *Escherichia coli*, provide convenient access to segments of human DNA up to 50 kilobase pairs (kb) in length (*2*). The centimorgan, which is a unit of genetic distance, represents the high end of the resolution achievable in linkage-mapping studies of human families (*3*); in typical regions of the human genome, 1 centimorgan (cM) corresponds to approximately 1 megabase pair (Mb). Typically, saturation cloning of a large chromosomal region produces a series of "contigs"—groups of overlapping clones that collectively span a particular part of the region. Individual contigs are often only 100 to 200 kb in length (*4*). Mapping of larger intervals requires the direct analysis of uncloned genomic DNA, a process that has been facilitated by the development of pulsed-field gel electrophoresis and the discovery of restriction enzymes that cleave human DNA infrequently (*5*).

The cloning of DNA segments of several hundred kilobase pairs into yeast artificial chromosomes (YAC's) provides a way to extend recombinant DNA analysis to megabase pair–sized regions of the human genome (*6–8*). We now describe an extensive test of the suitability of YAC cloning for isolating and analyzing large blocks of human DNA. In particular, we emphasize the use of sequence-tagged sites (STS's), a class of landmarks advocated as the natural "common language" of physical mapping projects (*9*).

Isolation of YAC clones from the cystic fibrosis region. An STS consists of a short, single-copy DNA sequence that can be detected by using the polymerase chain reaction (PCR) (*9*). A given region of human DNA can be mapped by determining the order of a series of STS's and measuring the distances between them (Fig. 1A). YAC-based "STS-content mapping" involves screening by PCR to detect the clones that contain particular STS's (*10, 11*) and then using the presence or absence of additional STS's to define the overlap relations among the YAC's (Fig. 1B).

The cystic fibrosis (CF) region of human chromosome 7 has been intensively studied. The CF locus was localized by genetic studies to the approximately 1.0-Mb region between the markers KM.19 and J3.11 (*12, 13*). Molecular techniques were then used to identify the gene itself, which has been designated the CF transmembrane conductance regulator (CFTR) gene (*14–16*) (Fig. 2).

A set of seven PCR assays (*17, 18*) that amplify STS's within and flanking the CFTR gene was used to screen our human-YAC library (*19*). We obtained 30 YAC clones (Fig. 3), the subsequent characterization of which included determination of YAC size by pulsed-field gel electrophoresis (*6, 8*), assessment of the presence or absence of 16 STS's (Figs. 3 and 4), and analysis with five DNA-DNA hybridization probes (*20*) (Fig. 3).

The data in Fig. 3 provided sufficient information to establish the relative order of all but two STS's (Fig. 5). Problems arose, however, when we attempted to estimate the spacings between the STS's by incorporating additional information, such as the sizes and restriction maps of the YAC's. For instance, analysis of the 725-kb yCF-1 indicated the presence of approximately 325 kb of DNA to the right of sCF4 that contained neither the expected STS's nor the nearby Sal I site. Extensive characterization of yCF-1, including the subcloning of the insert ends, indicated that only ~400 kb at the left end of this YAC contained DNA from the CF region, with the remaining DNA being derived from human chromosome 10 (*21*).

A total of six similar anomalies was found among the isolated YAC clones (indicated by the wavy lines in Fig. 5). It is likely that most or all of these artifacts represent events in which two or more unrelated segments of human DNA have been joined together during the cloning process. Such anomalous clones do not affect the ordering of STS's, but they do hinder efforts to define the physical spacing between STS's on the basis of YAC size.

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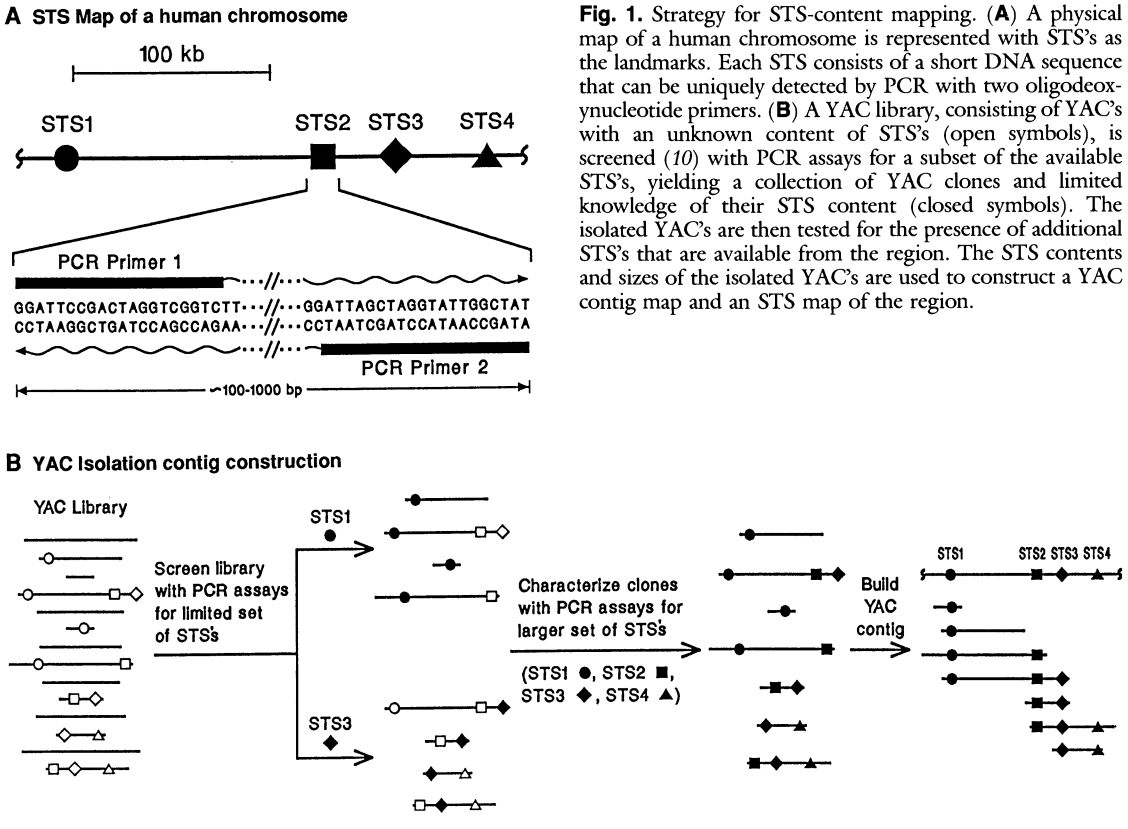


Fig. 1. Strategy for STS-content mapping. (**A**) A physical map of a human chromosome is represented with STS's as the landmarks. Each STS consists of a short DNA sequence that can be uniquely detected by PCR with two oligodeoxynucleotide primers. (**B**) A YAC library, consisting of YAC's with an unknown content of STS's (open symbols), is screened (*10*) with PCR assays for a subset of the available STS's, yielding a collection of YAC clones and limited knowledge of their STS content (closed symbols). The isolated YAC's are then tested for the presence of additional STS's that are available from the region. The STS contents and sizes of the isolated YAC's are used to construct a YAC contig map and an STS map of the region.

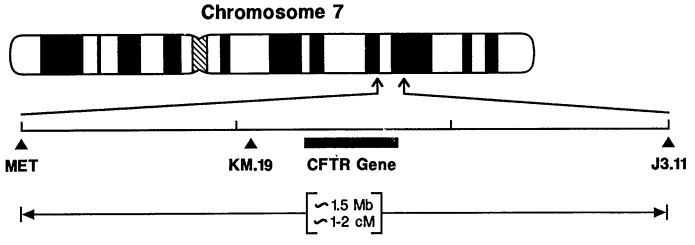


Fig. 2. The CF region of human chromosome 7q. The CFTR gene spans more than 250 kb within the interval defined by the genetic markers KM.19 and J3.11 (*14*).

Six YAC's (yCF-1, yCF-8, yCF-7, yCF-4, yCF-5, and yW30-5) that collectively span all the CFTR exons and a considerable amount of flanking DNA were extensively characterized. The localizations of restriction sites for Nru I and Sal I in these YAC's were used to fix the positions of several important STS's (for example, sL12, sCF4, sW305R, and sW305L) over the first 1 Mb of the contig map. Clones from the right portion of the contig map were aligned less precisely. As a whole, the map presented in Fig. 5 is in excellent agreement with previous results (*14*).

Construction of YAC-YAC recombinants by homologous recombination in yeast. Although several of the primary YAC isolates contained significant portions of the CFTR gene (for example, yCF-8, yCF-7, yCF-10, and yCF-4), none contained the genomic segment of more than 250 kb over which the exons are distributed. We considered homologous recombination between a pair of overlapping YAC's to be a promising route to a single YAC that would span the entire gene. Because the average ratio of physical to genetic distance in yeast is only 3 kb/cM (*22*), a high frequency of meiotic recombinants would be expected even if the overlap between two YAC's was as short as 100 kb. A representation of the recombination between yCF-8 and yCF-7 is shown in Fig. 6.

YAC Clone	sL12	sXV2C	sCS7	sKM19	sD9	sJ44	sCF2	sCF4	sCF10	sCF13	sCF19	sCF24	sW305R	sW30	sW305L	sJ311	W91	W46	W35	W32	J29
yXV2C-1		+																			
yKM19-3			+	+	+	+															
yKM19-2			+	+	+																
yD9-1					+	+															
yD9-2					+																
yD9-3					+																
yCF-1	+	+	+	+	+	+	+	+													
yCF-11					+	+	+														
yCF-8					+	+	+	+	+												
yCF-10					+	+	+	+	+	+	+										
yCF-3							+	+	+	+											
yCF-9							+	+	+	+											
yCF-12								+	+	+											
yCF-7								+	+	+	+	+									
yCF-4										+	+	+	+								
yCF-2											+	+									
yCF-6												+									
yCF-5												+	+								
yW30-5													+	+	+		+				
yW305R-2													+								
yW30-4														+	+		+				
yW30-3														+	+			+	+	+	
yW30-2														+	+			+	+	+	
yW30-1														+	+			+	+	+	
yW30-6														+	+						
yJ311-3														+	+	+		+	+	+	+
yJ311-2																+			+	+	
yJ311-5																+				+	
yJ311-1																+					+
yJ311-7																+					+

Fig. 3. Characteristics of YAC clones from the CF region. The 30 isolated YAC clones (designated with a "y" before their name) are listed along with the results of testing for the presence or absence of 16 STS's and five probe sequences. +, the YAC contains a particular STS or probe sequence.

Haploid yeast strains of opposite mating types containing each of the two parental YAC's (*23*) were mated to yield diploid strains containing both YAC's. The diploids were allowed to undergo meiosis and sporulation, and the spores in the resultant tetrads were separated by micromanipulation. Since both parental YAC's contained the same yeast genetic markers, we could only use physical assays (PCR-based assessment of STS content and size measurement by pulsed-field gel electrophoresis) to identify recombinants. YAC-containing spores were analyzed for the presence of sCF2 and sCF24. Detection of both STS's indicated the presence of either a yCF-8/7-R recombinant or two unrecombined parental YAC's in the same spore, possibilities that could be distinguished by pulsed-field gel electrophoresis.

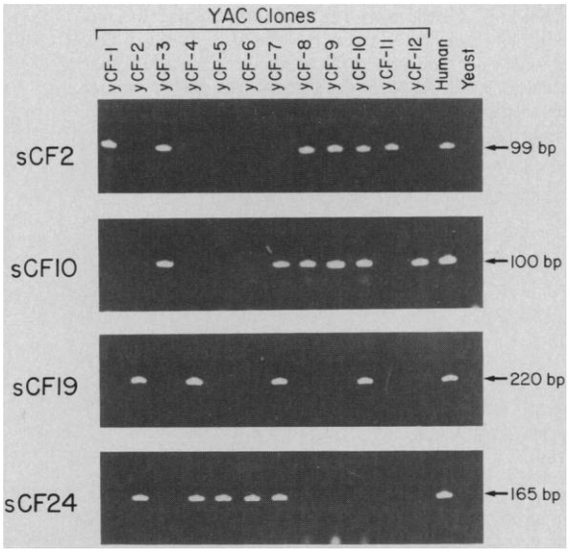


Fig. 4. Determination of STS content in YAC clones. The 12 YAC clones containing portions of the CFTR gene (yCF-1 through yCF-12) were tested for the presence of sCF2, sCF10, sCF19, and sCF24 by PCR assays carried out with cells from yeast colonies as the source of template DNA (*29*). Total human and yeast DNA (100 ng) were included as positive and negative controls, respectively. PCR products were separated on polyacrylamide gels and detected by staining with ethidium bromide (*10*). The migration positions and sizes of the PCR products are indicated.

For the yCF-8 × yCF-7 mating, we analyzed 36 spores. Two complete tetrads were found where two spores contained YAC's identical to the parental YAC's and two spores contained YAC's of the characteristics expected for the large and small recombinant products (Fig. 7). By the same general approach, we constructed three other YAC-YAC recombinants (*24*). Two products (yCF-1/8/7-R and yCF-1/7/5-R) resulted from the recombination of three YAC's in two successive steps. STS-content mapping and size assessment allowed the alignment of the four YAC-YAC recombinants within the YAC contig map (Fig. 5). The largest YAC-YAC recombinant constructed (yCF-1/7/5-R) was 790 kb, thereby containing roughly half of the DNA isolated from the CF region.

Recombination between two YAC's provides an efficient means of obtaining YAC's that are free of cocloned segments of DNA from outside the CF region. For example, yCF-1 contains a cocloned segment to the right of its chromosome 7 DNA, whereas yCF-8/7-R contains a cocloned segment to the left. Both cocloned blocks of DNA are eliminated during the construction of the recombinant yCF-1/8/7-R.

Characterization of the CFTR gene in the YAC-YAC recombinants. Because each of the three largest YAC-YAC recombinants contained the entire CFTR gene and appeared to be free of cocloned DNA, these clones were chosen for more detailed characterization

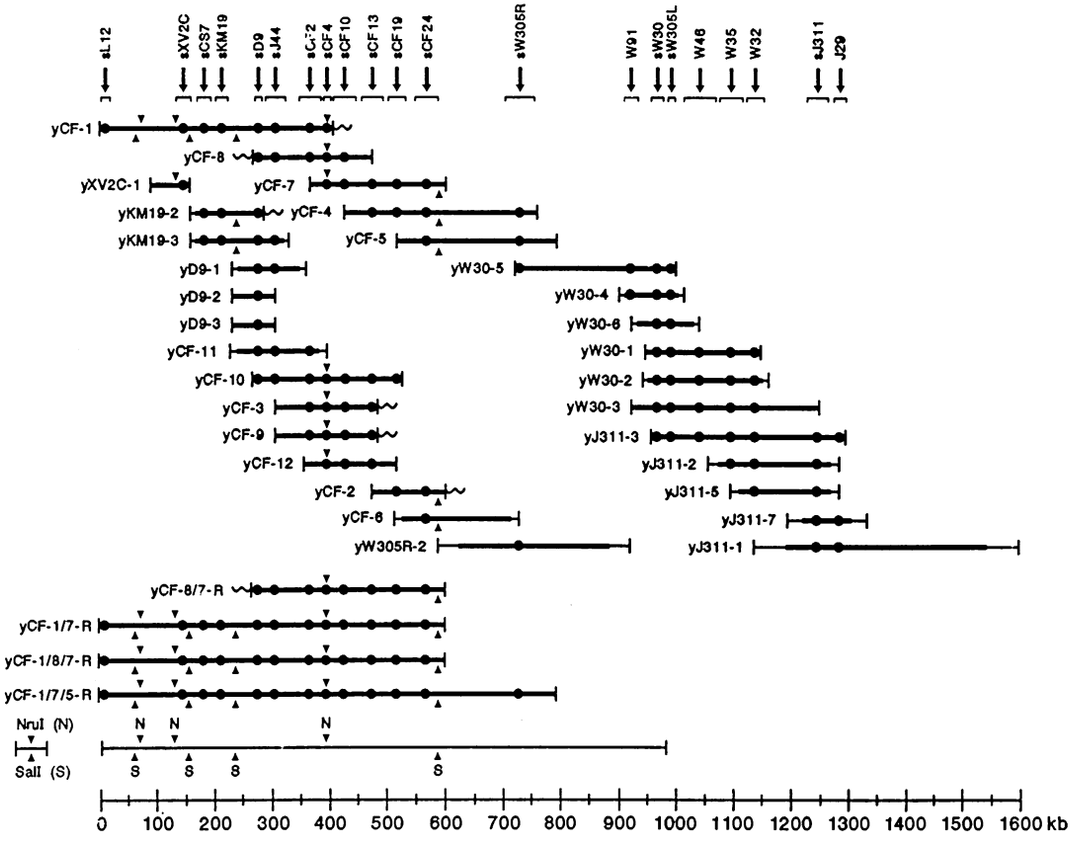


Fig. 5. Contig map of YAC clones from the CF region. The STS-based physical map of the CF region is based on the characterization of the 30 primary YAC isolates and four YAC-YAC recombinants. The indicated order of STS's was deduced from the data in Fig. 3, with the exception of sCS7 and sKM19, which were known from other studies, to map in the order indicated (*13*). Restriction mapping of the YAC's spanning the first 980 kb of the contig map revealed the location of several Nru I and Sal I sites, which are indicated as arrowheads above and below the depicted YAC's, respectively. The vertical lines indicate the farthest to the left or right that each YAC could extend on the basis of STS content, presence or absence of the indicated Nru I or Sal I sites, and YAC size. Some YAC's are represented with a combination of thick and thin horizontal lines, with the former representing the YAC length positioned within the center of the interval to which the YAC was mapped. The presence of cloned DNA known not to be derived from the CF region is denoted as a wavy line at the appropriate end of a YAC. The horizontal brackets below the STS or probe designations indicate the intervals within which the sequences were localized; the arrows and closed circles that symbolize these sites are arbitrarily placed at the center of the corresponding interval.

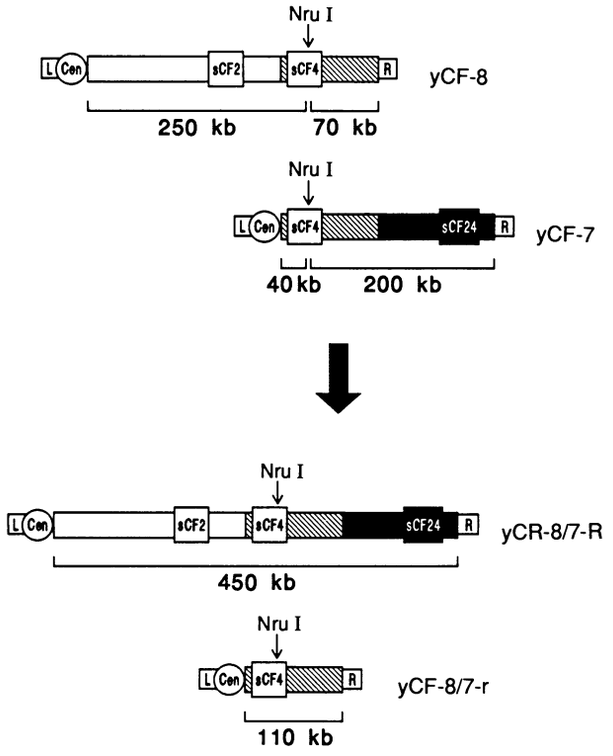


Fig. 6. Schematic model for the recombination of two YAC's, yCF-8 and yCF-7. Mapping of these YAC's with Nru I revealed the relative alignment of the two clones. The region common to both parental YAC's (hatched area) is approximately 110 kb in size and contains sCF4. The sizes and STS content of the regions distinctive for each YAC (open and closed areas in yCF-8 and yCF-7, respectively) were also determined. Homologous recombination between yCF-8 and yCF-7 within the common segment of human DNA would yield two products: a large YAC (yCF-8/7-R) containing the common and two distinctive regions, and a smaller YAC (yCF-8/7-r) consisting only of the 110-kb common region. Since the two parental YAC's are in the same orientation with respect to the vector arms, each of the reciprocal products obtained after recombination would be expected to contain a single centromere.

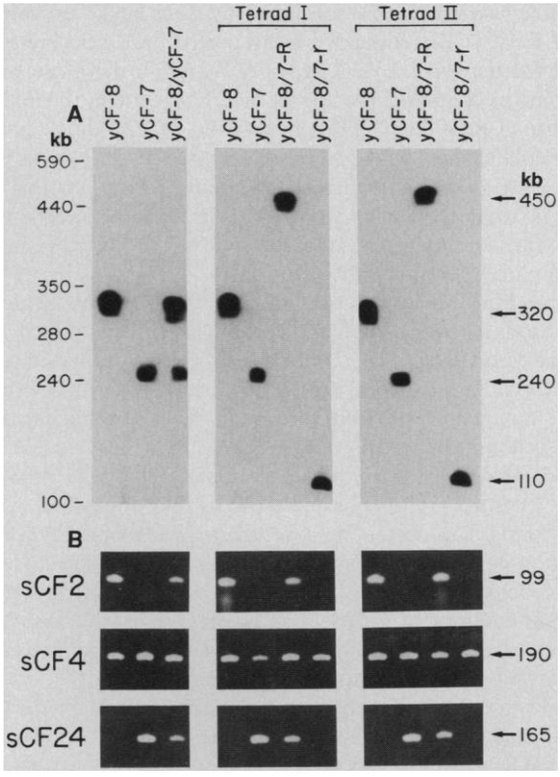
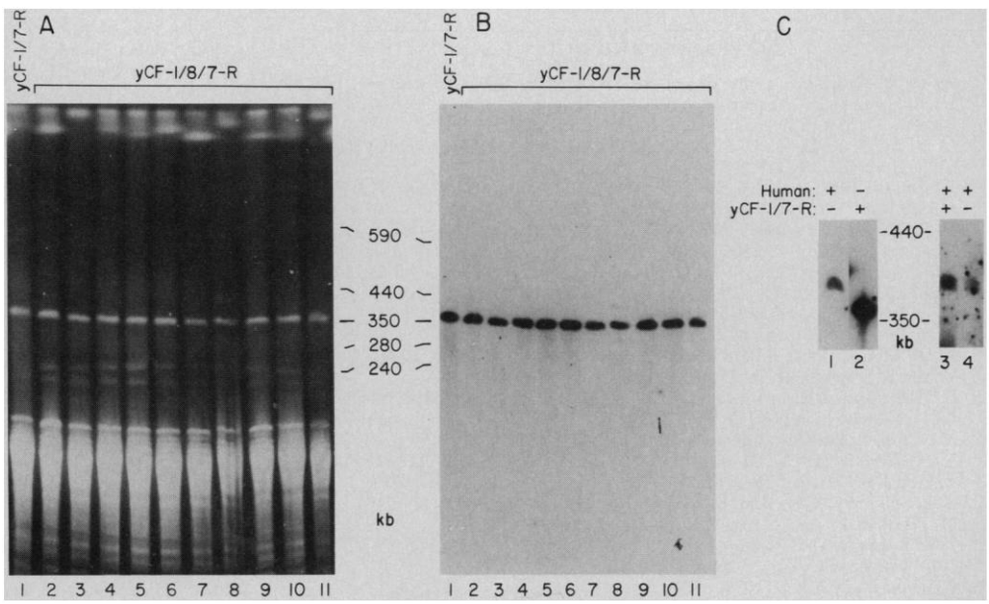


Fig. 7. Characterization of recombination products from the yCF-8 × yCF-7 mating. Yeast strains containing yCF-8 and yCF-7 were mated, and the resulting spores were analyzed, yielding two tetrads containing YAC's that appeared to be the products of reciprocal recombination. (**A**) Intact chromosomes from each of the parental YAC clones, the diploid strain yCF-8/yCF-7, and the indicated spores from each tetrad were separated by pulsed-field gel electrophoresis and analyzed by gel-transfer hybridization with ^{32}P-labeled total human DNA as the probe. For both tetrads, designated I and II, the parental YAC's, yCF-8 (320 kb) and yCF-7 (240 kb), as well as the recombinant products, yCF-8/7-R and yCF-8/7-r, were each found in a single spore. The two recombinant YAC's were of the predicted sizes (450 kb and 110 kb, respectively), as shown in Fig. 6. (**B**) Each of the yeast strains analyzed in (A) was tested for the presence of sCF2, sCF4, and sCF24 by PCR as described in the legend to Fig. 4.

Fig. 8. Characterization of YAC-YAC recombinants containing the intact CFTR gene. Sal I–digested DNA from yCF-1/7-R (lane 1 in A and B) or ten independent yCF-1/8/7-R isolates (lanes 2 to 11 in A and B) was separated by pulsed-field gel electrophoresis. The gel was (**A**) stained with ethidium bromide and (**B**) analyzed by gel-transfer hybridization with the use of the insert of pCFTR4.6 as the ^{32}P-labeled probe (*26*). (**C**) Sal I–digested total human DNA (lanes 1, 3, and 4) or yCF-1/7-R (lanes 2 and 3) was analyzed as in (B). The same amount of human DNA (approximately 5 μg) was subjected to electrophoresis in each case, whereas the amount of yeast DNA was approximately 1.0 μg in lane 2, 0.1 μg in lane 3, and 0 μg in lanes 1 and 4. Thus, the minor discrepancy between the apparent size of the Sal I fragment containing the CFTR gene in human DNA and in the three larger recombinant YAC's is an artifact associated with the variable amounts of DNA subjected to electrophoresis.



with three restriction enzymes. First, the three larger recombinants derived from yCF-1 contained the reported (*12*) Not I restriction site located between sXV2C and sCS7 (*25*). Furthermore, all four recombinants contained the appropriate Nru I site in the middle of the fourth exon of the CFTR gene (sCF4) (*14, 25*). Finally, two Sal I sites flanking the CFTR gene and separated by 380 kb have been reported on the basis of direct analysis of human genomic DNA (*14*). Electrophoretic analysis revealed that the CFTR gene resides on the same Sal I fragment in yCF-1/7-R, yCF-1/8/7-R, yCF-1/7/5-R, and human genomic DNA (Fig. 8).

As an additional test of authenticity, we sought to catalog the CFTR exons in the recombinant YAC's with cDNA-derived probes (*26*) that detect each of the 23 Eco RI fragments known to contain the gene's coding sequences (*15*). The identical 23 exon-hybridizing Eco RI fragments were detected in all four recombinants and in human genomic DNA (*25*).

Implications for human genome mapping. The STS-based strategy outlined in Fig. 1 has been successfully applied to assemble a YAC contig map containing more than 1.5 Mb of DNA from the CF region of human chromosome 7. Complete connectivity was achieved between the XV.2C, CS.7, and KM.19 cluster of genetic markers at the one end and J3.11 at the other. Even the best mapped portion of this approximately 1-Mb interval has not previously been assembled in continuous cloned coverage (*14*).

Two features of our study have direct implications for the global physical mapping of the human genome. First, if our experience with the CF region proves typical, the continuity achievable with YAC-based STS-content mapping appears adequate for the mapping of whole chromosomes, which average 130 Mb in size. It should be possible to determine the order and relative orientations of a set of 1- to 2-Mb contig maps with the aid of supplementary data acquired by linkage mapping, in situ hybridization, and somatic cell genetics. Second, the primary reliance on PCR detection of STS's, rather than on DNA-DNA hybridization or restriction-site mapping, will facilitate the comparison of maps constructed by different methods and provide universal access to the mapped DNA (*9*).

STS-content mapping shifts much of the experimental burden of genomic analysis to the process of defining an adequate set of STS's. In our study, most of the STS's either had already been defined during efforts to localize the CFTR gene or could be readily designed based on the cDNA sequence. In larger scale applications, a high proportion of the effort would be devoted to the generation of large numbers of STS's de novo.

REFERENCES AND NOTES

1. As far as we are aware, this phrase was first used by David Botstein in a talk summarizing the proceedings of an EMBO workshop on "Approaches to Genome Analysis," held 4 to 7 October 1985, in Heidelberg, FRG.
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17. The human-YAC library was screened (*10*) with the use of PCR assays that detected the following seven STS's: sXV2C, sKM19, sCF2, sCF10, sCF24, sW30, and sJ311. All STS's are designated with an "s" before their name.
18. All PCR assays were performed as described (*10*) for 30 to 35 cycles of 92°C for 1 minute; the indicated annealing temperature (see below) for 2 minutes, and 72°C for 2 minutes in a Perkin-Elmer Cetus (Norwalk, CT) thermal cycler. The annealing temperatures for each PCR assay were as follows: 52°C (sXV2C), 55°C (sKM19), 58°C (sL12, sCF2, sCF24, sW305R, and sW30), 60°C (sCS7, sD9, sJ44, sW305L, and sJ311), and 62°C (sCF4, sCF10, sCF13, and sCF19). The sequences of oligodeoxynucleotide primers and PCR product sizes for sXV2C [C. L. Rosenbloom *et al.*, *Nucleic Acids Res.* 17, 7117 (1989)], sCS7 [C. Williams *et al.*, *Lancet* **ii**, 103 (1988)], sKM19 [G. L. Feldman, R. Williamson, A. L. Beaudet, W. E. O'Brien, *ibid.*, p. 102], sD9 [A. Huth *et al.*, *Nucleic Acids Res.* 17, 7118 (1989)], and sJ311 [H. Northrup, C. Rosenbloom, W. E. O'Brien, A. L. Beaudet, *ibid.*, p. 1784] have been described. The sequences of the oligodeoxynucleotide primers used to amplify an 860-bp segment of sJ44 were 5′-CAATGTGATTGGTGAAACTA-3′ and 5′-CTTCTCCTCCTAGACACCTGCAT-3′ (P. Ray, personal communication), whereas the primers used to amplify a 420-bp segment of sW30 (5′-CCTGTATACTAGTAAAGGAGTGAG-3′ and 5′-TTTAATCCCTAAGGGCCTGGAGAC-3′) were derived from the reported DNA sequence (*27*). PCR assays that amplify portions of exons 2, 4, 10, 13, 19, and 24 of the CFTR gene were developed from the cDNA sequence (*15*), with the sequences of the oligodeoxynucleotide primers and sizes of the PCR products being as follows: sCF2 (5′-CTGGACCAGACCAATTTTGAGG-3′ and 5′-TTCAGATAGATTGTCAGCAG-3′; 99 bp), sCF4 (5′-GAAGTCACCAAAGCAGTACAG-3′ and 5′-GCTATTCTCATCTGCATTCC-3′; 190 bp), sCF10 (5′-CAGTTTTCCTGGATTATGCCTGG-3′ and 5′-GTTGGCATGCTTTGATGACGCTTC-3′; 100 bp), sCF13 (5′-CTGTGTCTGTAAACTGATGGC-3′ and 5′-TGGATGCTGTTGTCTTTCGG-3′; 605 bp), sCF19 (5′-GTTCATTGACATGCCAACAG-3′ and 5′-TCTGGCCAGGACTTATTGAG-3′; 220 bp), and sCF24 (5′-GAAGAGAACAAAGTGCGGCAG-3′ and 5′-CTGTCTCCTCTTTCAGAGCAG-3′; 165 bp). Three STS's were derived from sequences at the ends of YAC inserts. These sequences were obtained by subcloning total yeast DNA into the lambda vector EMBL3, isolating lambda clones containing the YAC vector-insert junctions, and sequencing the human DNA beginning at the cloning site (*21*). This procedure was used to define sL12 from yCF-1, as well as sW305R and sW305L from yW30-5. The sequences of the two oligodeoxynucleotide primers and sizes of the PCR products are as follows: sL12 (5′-AGCCAGGATAAAAACGGAGGTGGTC-3′ and 5′-GATTGTGGCTAATAACAACAACAAG-3′; 140 bp), sW305R (5′-CTTTAATCCTTTCCACTGGG-3′ and 5′-TGCAAGTCCCTTGCCCTTTC-3′; 225 bp), and sW305L (5′-CCACAACCAACTA-ATTTTGG-3′ and 5′-GCTTAGGATTCTTACCCTC-3′; 120 bp).
19. The construction and propagation of the human-YAC library used have been described (*8*). This library at present consists of approximately 70,000 clones with an average insert size of 250 to 300 kb, thereby covering the human genome with fivefold redundancy.
20. The DNA probes W91, W46, W35, W32, and J29 have been described [(*27*) and M. C. Iannuzzi *et al.*, *Am. J. Hum. Genet.* **44**, 695 (1989)].
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23. Because all primary YAC isolates were in the same host strain (AB1380), which is mating type MATa (*28*), the formation of diploid strains containing two YAC's required the prior step of introducing one of the two parental YAC's into a yeast strain of opposite mating type, MATα. To carry out this step, we crossed the yeast strain containing the YAC with the MATα strain AB1610 (*28*), isolated a MATa/MATα diploid containing the YAC, sporulated the diploid, and identified MATα spores that contained the YAC. These steps were facilitated by the use of a colony-PCR assay that distinguishes between the MATa, MATα, and MATa/MATα genotypes (*29*).
24. The four YAC-YAC recombinants (of the indicated sizes) were derived from the following pairs of parental YAC's (with the indicated amounts of homologous overlap): yCF-8/7-R (450 kb) from yCF-8 and yCF-7 (110 kb of overlap), yCF-1/7-R (600 kb) from yCF-1 and yCF-7 (40 to 50 kb of overlap), yCF-1/8/7-R (600 kb) from yCF-1 and yCF-8/7-R (150 to 200 kb of overlap), and yCF-1/7/5-R (790 kb) from yCF-1/7-R and yCF-5 (80 kb of overlap). The presence of cocloned DNA attached to yCF-1 and yCF-8/7-R precluded more precise estimates of the homologous overlaps with these YAC's. In the construction of the four recombinant YAC's, the numbers of independently derived isolates (and the total number of spores analyzed) were as follows: 2 (36), 1 (76), 11 (56), and 5 (64), respectively. The number of spores analyzed cannot be used to calculate recombination frequencies because spores were derived from complete and incomplete tetrads and, in some cases, the tetrads appeared to be derived from diploid cells that did not contain one copy of each parental YAC.
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26. pCFTR4.6 is a partial cDNA containing all the coding sequences of the CFTR gene except for the 3′ portions of exon 24 and a 57-bp deletion in exon 6 (M. L. Drumm and F. S. Collins, personal communication).
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