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Yeast artificial chromosomes spanning 8 megabases and 10–15 centimorgans of human cytogenetic band Xq26

(cloning/DNA/genetic map/physical map/genome)

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ABSTRACT **A successful test is reported to generate long-range contiguous coverage of DNA from a human cytogenetic band in overlapping yeast artificial chromosomes (YACs). Seed YACs in band Xq26 were recovered from a targeted library of clones from Xq24–q28 with 14 probes, including probes for the hypoxanthine guanine phosphoribosyltransferase- and coagulation factor IX-encoding genes and nine probes used in linkage mapping. Neighboring YACs were then identified by 25 "walking" steps with end-clones, and the content of 71 probes in cognate YACs was verified by further hybridization analyses. The resultant contig extends across 8 million base pairs, including most of band Xq26, with an order of markers consistent with linkage data. YAC-based mapping, thus, permits steps toward a fully integrated physical and genetic map and is probably adequate to sustain most of the human genome project.**

The Human Genome Initiative and similar projects aim to map and sequence complex genomes. This goal requires the generation of very long-range continuity in cloned DNA. Such coverage may be achievable by overlapping yeast artificial chromosomes (YACs; ref. 1), which are 100 to >1000 kilobases (kb) long (2).

Libraries of YACs for *Caenorhabditis elegans* (3), *Drosophila* (4), and human DNA (5, 6) have been organized, and prior mapping with such YACs has produced contigs of up to 2 megabases (Mb) (7–10). Significant questions remained, however: for mammalian genomes, could a large proportion of the genomic DNA be cloned in YACs, or would frequent "holes" be unavoidable? Also, many YACs bring together noncontiguous cocloned segments of chromosomes. Would cocloning occur at a frequency that confounds construction of accurate physical maps in the multi-megabase range?

These concerns are dispelled here for a region that covers 0.25% of the genome in overlapping YACs. Starting from a collection of YACs specific for Xq24–q28 (11–13), a contig of 8 Mb has been assembled that includes most of band Xq26. This scale is sufficient to permit an attempt to compare and unify the genetic and physical maps encompassing several disease-producing genes in this region.

MATERIALS AND METHODS

Construction and Screening of YAC Library. The library was targeted to bands Xq24–q28 (11–13) by isolating YACs containing human DNA from a somatic cell hybrid, X3000.11 (14), which contains only that portion of the human genome. The library now includes 820 clones corresponding to about three genomic equivalents. Five additional YACs for this contig were obtained from a total human library (6), including two clones, telomeric to probe a329R, which cover the only segment not found in the Xq24–q28 collection. YACs are named in accord with standard recommendations, with the prefix y for YAC, W for Washington University in St. Louis, XD for the laboratory of origin, and an accession number (XY837 in ref. 13, for example, is yWXD837 here).

Hybridization Probes Used to Organize the YAC Contig. All probes were oligolabeled and hybridized to DNA in a matrix array of lysed YAC clones on a nylon filter, as in refs. 12 and 13.

Probes from Other Laboratories. Probes for loci were as in Human Gene Mapping 10.5 (HGM10.5) (15, 16) and were named accordingly, including 07-03 (locus DXS79, from F. H. Ruddle, Yale University), pDSK1 (*HPRT*, from C. T. Caskey, Baylor University), St1 [locus DXS86, from J. L. Mandel, Institut National de la Santé et de la Recherche Médicale (INSERM), Strasbourg], 36B-2 (locus DXS10, from American Type Culture Collection), plambda2.7 (locus DXS177, from B. N. White, Queen's University, Kingston), c11 (locus DXS144E, from J. L. Mandel), pX58c (locus DXS99, from B. N. White), pG44 (locus DXS64, from L. M. Kunkel, Harvard Medical School), cX44.1 (locus DXS155, from G. J. B. van Ommen, Rijksuniversiteit, Leiden, The Netherlands), p52A (locus DXS51, from American Type Culture Collection), cX38.1 (locus DXS102, from G. J. B. van Ommen), pTG397 [coagulation factor IX (F9)-encoding gene from J. L. Mandel], pHT.1 (*MCF2*, from D. Birnbaum, INSERM, Marseille), and E2 (locus DXS403, from B. R. Jordan, Centre National de la Recherche Scientifique, Marseille). Probes prE and pr9 were obtained from G. G. Brownlee, Oxford University.

End-Clones and *Alu* PCR Fragments. End-clones in Table 1 are named as p(plasmid) followed by the YAC (yWXD) number and designation of origin from the DNA nearest the left (L) or right (R) vector arm. For end-cloning, the YAC was digested with the first enzyme, and the terminal fragment was cloned into Bluescript (Stratagene). Probes for screening were excised with the combination of enzymes shown in Table 1. *Alu* PCR products, named with the prefix "a" in the text, were generated from a YAC as in ref. 17 with one of four *Alu* consensus primers A–D. Primers were used singly for *Alu–Alu* fragments to confirm clone overlaps (Fig. 1) or with primers from the left (LS2) or right (RA2) pYAC4 vector sequences to generate *Alu*-vector PCR end fragments (Table 1) (D. Freije and D.S., unpublished work; details available on request).

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Abbreviations: YAC, yeast artificial chromosome; HPRT, hypoxanthine guanine phosphoribosyltransferase; Mb, megabase(s); cM, centimorgan(s); F9, coagulation factor IX.
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Southern Blot Analysis. Southern analysis was done as in ref. 18, with DNA probes (50 ng) prehybridized 1 hr at 68°C with 500 ng of sonicated human DNA.

RESULTS

The region chosen for study includes the completely sequenced *HPRT* (19) and the gene encoding F9 (20), lesions in which, respectively, cause Lesch–Nyhan syndrome and hemophilia B. A relatively large number of anonymous probes, some of which identify restriction fragment length polymorphisms (21), have also been assigned to band Xq26, thus making this a model region in which to seed and extend pilot contigs.

Assembly of Contigs. Fig. 1 shows a schematic of the contig. Each YAC is shown by a line proportional to its size, as determined by pulsed-field gel electrophoresis (22). The contig covers 0.25% of the human genome and contains 94 YACs, including 11% of the YACs in the Xq24–q28 library.

Assembly of the contigs was initiated from the collection of YACs specific for bands Xq24–q28 by localizing a number of clones to Xq26–27, either by *in situ* hybridization (23) or by identifying YACs containing probes previously assigned to the Xq26–27 region (see refs. 11–13).

The apparent coverage of Xq26 DNA in the YAC library, assessed by screening with probes, is encouraging. The YACs contain about three equivalents of the estimated 50-Mb content of Xq24–Xqter. Screening of the library has continued to yield an average of three YACs for each probe with a distribution indistinguishable from the expectation of Poisson statistics for random cloning (refs. 11–13).

Starting from this set of initial clones, overlapping or neighboring YACs were identified by "walking." In one approach, a radioactively labeled YAC was itself used as a hybridization probe against the total collection to find overlapping clones (12). In another, fragments were isolated from the ends of YAC inserts and used as probes against the library. This approach involved one of two methods: (*i*) Small fragments [up to 6 kilobases (kb)] spanning the junction between the YAC vector and insert sequences were directly subcloned into a plasmid (Table 1, Method *i*). (*ii*) A second method used a modification of the *Alu*-vector PCR (ref. 17; *Materials and Methods* and Table 1, Method *ii*). To clarify the organization of clones in specific regions of the contigs, additional probes from internal sites of particular YACs were obtained by using *Alu–Alu* PCR (ref. 17; indicated by "a" and YAC of origin in Fig. 1).

Verification of Contigs. Extensive coverage across a region is no guarantee that YACs retain the structure of the human genome at higher resolution. Quality is traditionally assessed by the internal consistency of the structure of overlapping clones and by the fidelity of the clones to uncloned human DNA. In one type of test, a set of YACs from a contig are digested with site-specific restriction enzymes, and the fragments are fractionated by gel electrophoresis. A particular probe found in at least one of those YACs can then be used as a probe against the panel of YAC DNAs in a Southern analysis. In this way, one can check that DNA fragments containing the probe are present only in the YACs that putatively overlap in that region, that it has the same size in all of them, and that the size is the same as that observed in total human DNA that has been comparably digested.

As expected for a set of overlapping YACs with internally consistent overlaps, a probe for locus DXS64, probe prE, and a probe for locus DXS102 (Fig. 2 *A–C*) are seen to detect DNA fragments of the same size in all the overlapping YACs and *only* in the overlapping YACs. The same size fragments are seen in control human DNA (cell line CGM1; ref. 6) and hybrid cell line X3000 (14), supplemented, in some cases, in total DNA by additional species from other chromosomal sites. (One fragment hybridizing the probe for locus DXS64 in YAC yWXD760 is out of line because the clone insert ends within the probe sequence.) Similar tests have now been applied to a total of 20 probes and end-clones, and the results are completely consistent in all cases.

Verification by Southern analysis is not trivial because such experiments in several regions of Xq24–q28 have shown that certain probes hybridize to restriction fragments arising from more than one location. Fig. 2*D* shows one of four examples of false positives. YAC yWXD820 was positive in hybridization with probes from two disparate locations on the q arm of the X chromosome, the probe for p813L from band Xq28 (data not shown) and end-clone p636L probe in this region. However, two *Taq* I fragments were detected by the probe for p636L in total human DNA (cell line CGM1) and in DNA from the X3000.11 hybrid. One of the *Taq* I fragments was seen in YACs yWXD636 and yWXD503; yWXD820 contained the other and likely arises from band Xq28 but contains a sequence that cross-hybridizes with DNA from band Xq26.

Colony hybridization tests also missed two positive clones. For example, p491R as a probe hybridized to YACs yWXD491, yWXD476, and yWXD829 but did not hybridize with yWXD792, which seemingly overlapped them. How-

FIG. 1. Xq26 contig. Hybridization probes and genetic loci are placed on the map at the locations indicated above the contig. YACs are drawn to the indicated scale of Mb, with the YAC accession numbers and probes for specific loci (DXS), plasmid end-clones (p), and *Alu–Alu* and

Table 1. Hybridization probes used to organize YAC contigs

	Method *i*	Method *ii*		Size,
End-clone	Probe for screening	Probe	Primers	kb
p229L	1.7-kb *Hin*dIII/*Eag* I	a457R	RA2+*Alu*C	0.6
p229R	1.2-kb *Hin*dIII/*Sna*BI	a325L	LS2+*Alu*A	2.0
p342L	0.3-kb *Pst* I/*Eag* I	a893R	RA2+*Alu*B	0.5
p342R	1.0-kb *Pst* I/*Sna*BI	a382R	RA2+*Alu*A	0.7
p639R	0.4-kb *Pst* I/*Sna*BI	a963L	LS2+*Alu*D	1.0
p529R	4.0-kb *Hin*dIII/*Sna*BI/ *Eco*RI	a962R	RA2+*Alu*A	0.5
p529L	1.8-kb *Hin*dIII/*Eag* I	a329R	RA2+*Alu*C	0.9
p476L	2.7-kb *Pst* I/*Eag* I	a962L	LS2+*Alu*A	2.0
p491R	2.4-kb *Pst* I/*Sna*BI	a390L	LS2+*Alu*D	0.6
p476R	0.9-kb *Pst* I/*Sna*BI	a840R	RA2+*Alu*A	0.8
p491L	1.0-kb *Pst* I/*Eag* I	a840L	LS2+*Alu*B	0.5
p446R	0.8-kb *Pst* I/*Sma* I/ *Hin*dIII	a371R	RA2+*Alu*B	0.6
pA32G5L	0.6-kb *Xba* I/*Eco*RI	a522L	LS2+*Alu*B	3.0
p311L	0.6-kb *Hin*dIII/*Eag* I	a515R	RA2+*Alu*A	0.8
p258L	5.5-kb *Hin*dIII/*Eag* I	a6L	LS2+*Alu*D	0.6
p662L	0.7-kb *Hin*dIII/*Eco*RI			
p843L	1.0-kb *Hin*dIII/*Eco*RI			
p636L	1.4-kb *Pst* I/*Eag* I			

End-clones and *Alu*–vector PCR products (a probes) were made and named as indicated in text. For example, p229L was a *Hin*dIII fragment cloned from the human insert sequence nearest the left (L) pYAC4 vector arm of yWXD 229; and the probe for screening was excised with *Hin*dIII and *Eag* I. a457R was an *Alu*-vector product from the insert sequence nearest the R vector arm of yWXD457. LS2 and RA2, left and right pYAC4 vector sequences, respectively.

ever, Southern hybridization showed yWXD792 to be positive as well.

The Problem of Cocloning. Among the discrepancies that could be resolved by Southern analysis with a number of probes are those arising from YACs that contain inserts derived from two nonadjacent fragments of DNA. This limitation of YAC cloning, like similar problems with cosmid and λ cloning, can be overcome by analyzing enough clones with enough probes.

Because the YACs used here are made from a somatic cell hybrid cell line containing a 300-fold excess of hamster over human DNA (14), most YACs that contain a substantial amount of cocloned DNA are easily spotted by their hybridization to radiolabeled hamster DNA. Of the 94 YACs in the current contigs, 17, or ≈20%, have been demonstrated to contain cocloned DNA (wavy lines at the termini of YACs in Fig. 1). Fourteen YACs had hybridized to hamster DNA. In addition, two of 35 end-clones tested also hybridized to hamster DNA but not to other YACs or to human DNA; these YACs contained only a small amount of cocloned hamster DNA.

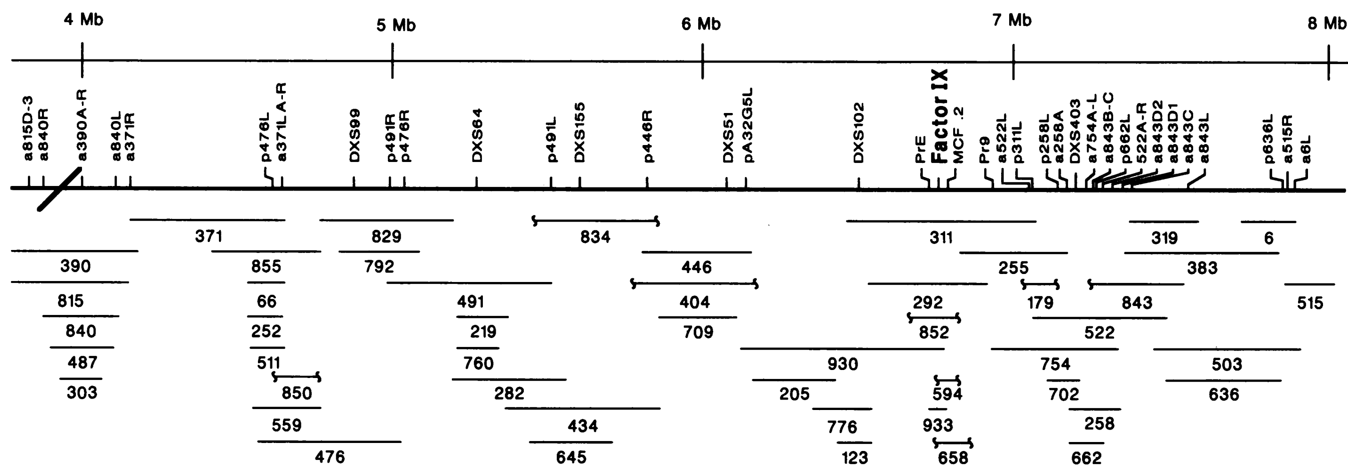
One clone was more difficult to analyze. Probes for p662L and p522R both hybridized to YAC yWXD843, and consistent with the contig structure (Fig. 1), the probe for p843L then hybridized to yWXD319, yWXD383, yWXD636, and yWXD503. The probe for p843R, however, from the other end of the clone, hybridized only to itself and to a clone, yWXD237, that contained none of the other probes in the immediate vicinity. Furthermore, yWXD843 hybridized to a probe for DXS180, a locus in Xq28. The situation was clarified by screening with five *Alu–Alu* probes made from yWXD843: two probes linked the clone to more centromeric YACs, whereas three probes linked it to more telomeric ones (Fig. 1). The portion of the clone that overlaps YAC yWXD237 and locus DXS180 is absent from the other clones in the contig that yield a self-consistent map, and the YAC DNA, therefore, probably arose from a human–human DNA cocloning event.

DISCUSSION

There are regions that are unstable or unclonable in YACs (2); but it seems likely that as in band Xq26, contigs can be assembled relatively easily in other genomic regions as large as a cytogenetic band. For the 50 Mb of Xq24–q28, for example, the library of YACs used contains three genomic equivalents with an average size of 250 kb. From statistical considerations, one would expect to achieve 32 contigs, with an average of 42 clones covering 2 Mb (ref. 24; walking with end-clones is the limiting case of clones that overlap on the basis of a very small extent of DNA in common). It is encouraging that in unpublished work, in accord with expectation, these and other contigs covering >90% of Xq26–q28 have reached an average size in the megabase range (25).

Quality of the YAC Map. As indicated in Figs. 1 and 2, the short-range structure of the genome around the probes tested is generally conserved in this YAC contig, as in other recent reports of contigs of 1 Mb or longer (for example, refs. 8–10). The verification has been extended to longer-range structure in three ways:

(*i*) The extent and distances within the contig were refined with pulsed-field gel mapping with *Not* I, *Nru* I, and *Mlu* I, and overlapping YACs showed completely self-consistent patterns of restriction sites (unpublished data). The sizes of restriction fragments generated by the rare cutters were also consistent with the map.



Alu–vector products (a) as in text and Table 1. An "s," or wavy line, indicates a cocloning event; where the location of material cocloned from outside the region is known, the s is only at that end.

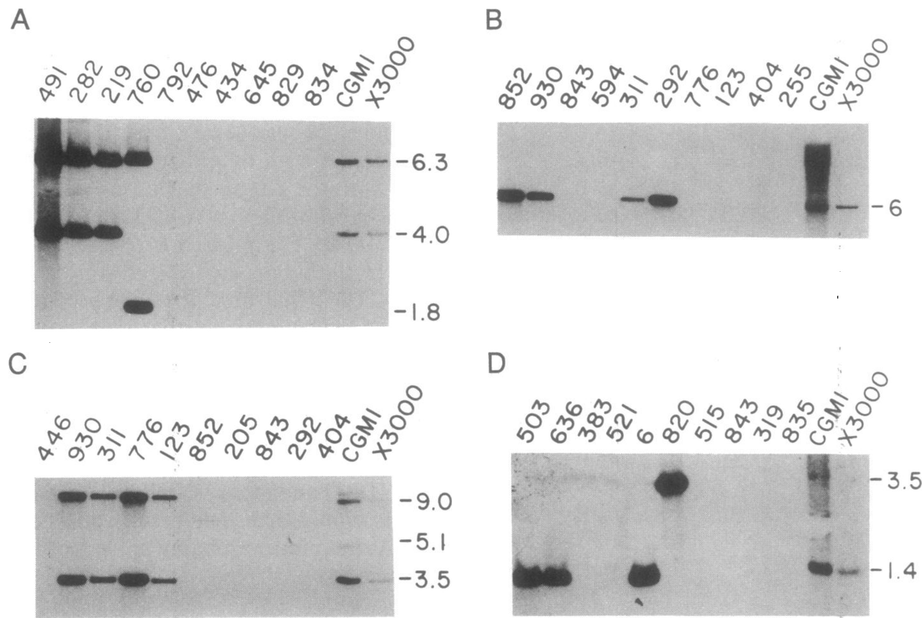


FIG. 2. Verification of probe content in overlapping YACs by Southern blot analysis. YACs are named by their accession numbers, as in Fig. 1. The probe for locus DXS64 (*A*), probe prE (*B*), the probe for locus DXS102 (*C*), and probe p636L (*D*) are listed in text. Probes were hybridized to DNAs from the indicated YAC clones (0.5 μg each), from the hamster–human hybrid cell line X3000.11 (10 μg), and from a human lymphoblast line CGM1 (10 μg); hybridized bands were digested with *Taq* I, electrophoresed in a 1% agarose gel, and blotted onto a Sure Blot (Oncor, Gaithersburg, MD) membrane. Sizes of bands are indicated in kb.

(*ii*) In two cases where the analysis of clones from the q arm of chromosome X has been pushed to the level of gene expression, copies of *G6PD* (26) and *HPRT* (27) cloned into YACs have both produced active enzyme.

(*iii*) Perhaps the most encouraging indication of long-range validity of the map is agreement of the order of physical and genetic linkage probes.

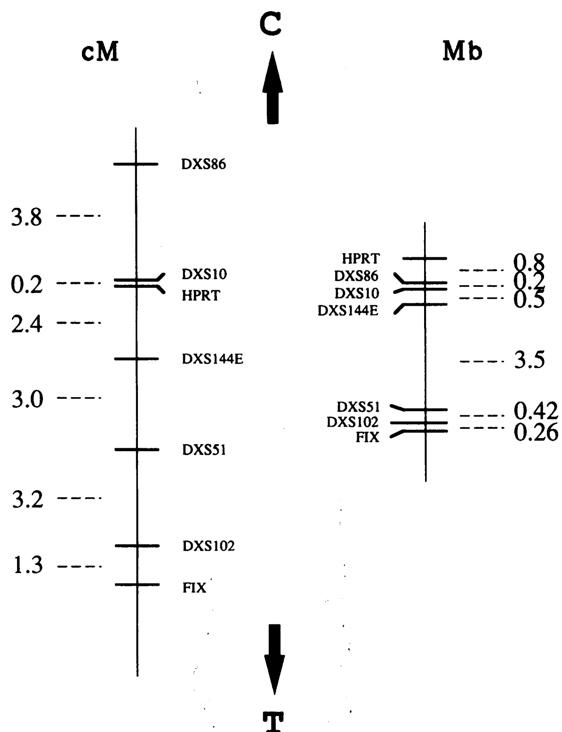


FIG. 3. Comparative schematic of part of the physical and genetic map from the gene encoding F9 (FIX) to the region around *HPRT*. Physical distances are from Fig. 1; genetic linkage distances are from the summary genetic map in ref. 15 (p. 502). C, centromere; T, telomere.

Comparison of Physical and Genetic Maps. One way to align and orient contigs along the chromosome is provided by linkage probes that have been used to make genetic maps. Many of these have been placed in a relatively unambiguous order (15, 16). The results thus far provide a starting point for comparing and merging a significant portion of the recombination map and the physical map.

The probe order and the summary linkage map from Human Gene Mapping 10 (15) are compared for this region in Fig. 3. The order and orientation of the contig with respect to the centromere agree; the one major exception is the discrepant order and relative distances of the probes for locus DXS10, locus DXS86, and *HPRT*. More recent data, however, brings the linkage map into agreement. Reilly *et al.* (28) find that, in agreement with the contig, the probe for *HPRT* is nearer the centromere, and the probes for loci DXS10 and DXS86 show no recombination and lie in close proximity (on the same *Bss*HII restriction fragment).

The recombination distance between the two most distant probes used in the analysis (for *HPRT* and F9-encoding gene) is 10–15 centimorgans (cM; 1 cM is thought to correspond roughly to 1 Mb); but the reported recombination distances between pairs of restriction fragment length polymorphisms in the contig vary from 2 to 8 cM/Mb. For example, probes for locus DXS99 and F9-encoding gene are suggested to be ≈2 Mb and, from two-point mapping (15, 16), 4 cM apart. More extreme, DXS51 and F9 DNA, only 800 kb distant, are 8 cM apart.

The confidence limits of current genetic linkage estimates are, unfortunately, too broad to sustain such strict comparisons (15, 16). Nevertheless, the estimates indicate how genetic and physical data are likely to interact in the future. The current physical map provides the material to determine whether any hot or cold spots for recombination occur in this region and to specify such sites to the level of sequence. In conjunction with further genetic data, the contig map should also abet conventional approaches to localize genes, including genes for disease states. For example, YACs in the contig contain the oncogene *MCF2* (29) in intact genomic form. And from McKusick's compilation (30), the contig may already include genes involved in Börjeson–Forssman–Lehman syn-

drome, X chromosome-linked hypoparathyroidism, albinism–deafness, and Lowe syndrome.

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