Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1598–1602, March 1989
Genetics

Yeast artificial chromosomes with 200- to 800-kilobase inserts of human DNA containing HLA, V_κ, 5S, and Xq24-Xq28 sequences

(cell hybrid/cloning)

RANDALL D. LITTLE*, GIOVANNI PORTA*, GEORGES F. CARLE*, DAVID SCHLESSINGER*†, AND MICHELE D'URSO‡

*Departments of Microbiology and Immunology and Genetics, and Center for Genetics in Medicine, Washington University School of Medicine, Saint Louis, MO 63110; and ‡International Institute of Genetics and Biophysics, Via Marconi 10, Naples, Italy 80125

Communicated by Paul A. Marks, November 28, 1988

ABSTRACT **Sequences hybridizing to several human gene probes have been recovered as cloned inserts in yeast artificial chromosomes (YACs). Among 2300 YACs made from human leukocyte DNA (totaling about 0.1 genomic equivalent of human DNA) we have found two, 200 and 780 kilobases (kb), containing sequences of V_κI immunoglobulin (V = variable); one, 240 kb, with class I HLA; and 11, 200–800 kb, with 5S rRNA-encoding DNA (rDNA). Fifty human YACs from a hamster–human cell hybrid with only the Xq24-Xq28 portion of the X chromosome include one that contains two anonymous probe sequences, DX13 and St14, previously inferred by indirect means to lie within about 70 kb of one another in Xq28. The YACs specific for human DNA arise at a frequency equivalent to the fraction of cellular DNA that is human-specific. Furthermore, the human YACs, formed in a 280-fold excess of hamster DNA, do not hybridize to a hamster DNA probe, indicating that individual YACs do not contain a combination of human and hamster DNA. To confirm that sequences are not scrambled, the YACs containing V_κI or DX13 and St14 sequences were shown to produce restriction fragments identical in mobility to fragments detected by the same probes in total human DNA digested with the same enzymes. YACs may therefore provide large clones to bridge gene mapping at the chromosome level to molecular analyses of small fragments of genomic DNA.**

Restriction fragment length polymorphism (RFLP) analysis has abetted formal genetic analysis to produce chromosomal alignments of human genes with a resolving power of 1–10 million base pairs (Mbp) (1, 2). Most laboratories, however, work with small cloned segments of DNA up to 0.05 Mbp (3)—i.e., on a much smaller scale. Fitting together numbers of the small pieces into a more extensive map (by "walking" from one to another) is very difficult. Pulsed-field gel electrophoresis (PFGE), which can fractionate DNA molecules up to the Mbp range, has helped to elucidate some features of the organization of larger DNA fragments (4–6). Analyses on this scale are difficult, however, and even then the larger fragments have remained unavailable in cloned form.

In addition to helping in genome mapping, large cloned fragments of DNA would permit many studies of the expression of genes with a DNA complement too large to fit into the 50-kilobase (kb) maximum content of cosmid cloning vectors. There has therefore been considerable interest in possible ways to clone large segments of DNA.

One potential route is provided by the use of yeast artificial chromosomes (YACs) as cloning vectors (7, 8). The initial vectors reported have been shown to accept DNA inserts of up to 1 Mbp. This permits the formulation of a systematic approach to complex genome analysis, in which YACs could provide continuity between total organismal DNA and established cloning and sequencing technology. This would facilitate the isolation of complete genes and their alignment in proper context.

To apply YAC technology to the human genome, libraries must be organized and screened. For general goals, a library of YACs containing inserts of total human DNA is useful. But for many purposes, a delimited region of a chromosome is of interest, and specialized libraries are desired. One route to such libraries starts from a somatic cell hybrid in which the only human DNA is derived from the chromosomal region of interest. We have used one library constructed from total human DNA and another from a hybrid cell that contains the distal portion of the long arm of the X chromosome as its only human DNA content.

Initial screening efforts have started to test the quality of the libraries, asking (*i*) whether a YAC is a pure clone of one piece of DNA (or whether it might instead contain cocloned pieces of DNA from different chromosomal locations); and (*ii*) whether DNA is cloned into YACs at comparable frequencies from different portions of the genome. The results of screening with several probes indicate that YAC clones contain inserts of single fragments of human DNA; furthermore, test sequences are detected in specific clones at frequencies consistent with their representation in human DNA. In these cases, restriction fragment lengths in genomic DNA are preserved in YACs. In one of them, two RFLPs of the order of 70 kb apart in the genome have been isolated in a single cloned DNA fragment.

MATERIALS AND METHODS

Cells. The yeast strains used are described in Burke *et al.* (7) and include AB972 (for chromosomal size markers), AB1380 as a host for YAC transformation, and HY1 (7) as a control strain with a human YAC. Mammalian cells used include human–hamster cell hybrid X3000-11 (9), human fibroblast cell line WSI (American Type Culture Collection), Chinese hamster cell line CHO (American Type Culture Collection), and circulating human leukocytes from a normal donor.

DNAs. Cloned probes used for hybridizations include a V_κI probe (V = variable) (10), an HLA B7 probe (12), a 5S rRNA-encoding DNA (rDNA) probe (13), the St14-1 probe (DXS52) (14), and the DX13 probe (DXS15) (15), the latter two from Xq28. All probes were purified as restriction fragments and labeled using the random primer method of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: YAC, yeast artificial chromosome; Mbp, million base pairs; rDNA, rRNA-encoding DNA; PFGE, pulsed-field gel electrophoresis; RFLP, restriction fragment length polymorphism; V, variable.

†To whom reprint requests should be addressed.

Feinberg *et al.* (16). DNA from the cell line WSI was used as a human genomic probe; DNA from CHO cells was used as a hamster genomic probe. Genomic probes were nick-translated (3) for use as hybridization probes.

High molecular weight DNA was extracted from yeast and mammalian cells as described (5, 8). Yeast cells were embedded and lysed in agarose as described using the protocol of Carle and Olson (17). Ladders of λ DNA were prepared as reported (6); these and *Hin*dIII digests of λ DNA and *Hae* III digests of φX174 DNA were used as size markers in gel electrophoresis.

Gel Electrophoresis and Southern Analyses. High molecular weight DNA was digested with restriction enzymes and separated on conventional agarose gels as described (3). Yeast chromosomes were separated on a CHEF gel apparatus (6) using switching regimes described in each figure legend.

Unidirectional and bidirectional nitrocellulose blots and hybridizations were done as suggested by the manufacturer (Schleicher & Schuell). Blotting and hybridization conditions for nylon membranes (Sureblot, Oncor) were also as described by the manufacturer.

YAC Library Construction. The total human DNA YAC library was prepared by D. Burke and is described in detail elsewhere (8). Briefly, high molecular weight DNA was partially digested with *Eco*RI and ligated to the vector, pYAC4, to yield products with an average size of >200 kb. The X3000-11 YAC library was constructed in essentially the same manner. The resulting average size of artificial chromosomes was 150 kb.

Yeast Colony Screening. Yeast colony screening was done essentially as described in ref. 18 and an unpublished protocol kindly provided by R. Rothstein (Columbia University, New York), with some modifications. Transformants were picked from transformation plates and patched directly onto synthetic yeast medium (18) lacking uracil and tryptophan in arrays of 50 or 100 colonies and grown for 3–4 days at 30°C. These plates were either repatched or replicated onto a nitrocellulose disc (Schleicher & Schuell, BA85) on YPD medium (18). Colonies were grown overnight at 30°C. The nitrocellulose discs were placed on Whatman 3 filter discs (in square Petri plates) prewet with 3 ml of a solution of 1 M sorbitol/0.1 M sodium citrate, pH 7.0/60 mM EDTA/150 mM mercaptoethanol/1 mg of yeast lytic enzyme per ml (ICN). Plates were sealed in bags and incubated overnight at 37°C. To lyse the cells, the filters were successively treated with 1 ml each of the following solutions (placed as a pool on SaranWrap): 0.5 M NaOH for 7 min; 0.5 M Tris·HCl (pH 7.5), 2× SSC (SSC is 150 mM NaCl/15 mM sodium citrate) for 2 min; and twice with 2× SSC for 2 min each. The filters were air dried and baked for 2 hr. Filters were prehybridized at 65°C for 2 hr in 5× SSPE (3), 5× Denhardt's solution (3), 0.1% SDS, 0.05 mg of poly(A) per ml, and 0.05 mg of denatured salmon sperm DNA per ml. This solution was removed and replaced with fresh prewarmed solution. Denatured probe was added to 1–2 × 10^6 cpm/ml. Hybridization was overnight at 65°C; washes were typically 2× SSC/0.1% SDS twice for 15 min, 1× SSC/0.1% SDS twice for 30 min, and 0.2× SSC/0.1% SDS twice for 30 min, all at 65°C. Filters were dried and exposed to film.

RESULTS

Screening of 2300 YACs Containing Human DNA Inserts. The first requirement for a convenient analysis of the representation of sequences in a YAC library is an adequate screening technique. In general, we have found that positive signals can be obtained without significant background by an adaptation of hybridization of labeled probes to DNA from yeast transformants lysed on filters (see *Materials and Methods*). As earlier observed with λ phage (19), the presence of a human DNA insert in a colony of yeast can be confirmed by its hybridization to a probe of radiolabeled human DNA. In this case, the signal comes largely from highly repetitive sequences, the dispersion distance of which is about 10 kb, much smaller than a YAC. Greater than 95% of the transformants are conveniently detected in this way. The signal in some cases may result from hybridization to more than one repetitive element, since some clones detected by labeled total human DNA do not hybridize with an *Alu* (20) probe (data not shown). The presence of the insert in a YAC can be confirmed by Southern analysis (3) of chromosomes separated by pulsed-field gel electrophoresis (4–6).

The same screening technique can be extended to the use of gene-specific probes as illustrated in Fig. 1. First (Fig. 1*A*), a filter bearing DNA from 50 yeast transformants individually streaked out has been hybridized with a nick-translated probe of a class I HLA B7 DNA fragment (12). A single positive clone is clearly visible. This was the only positive clone found in the library of 2300 clones. Since the library contains

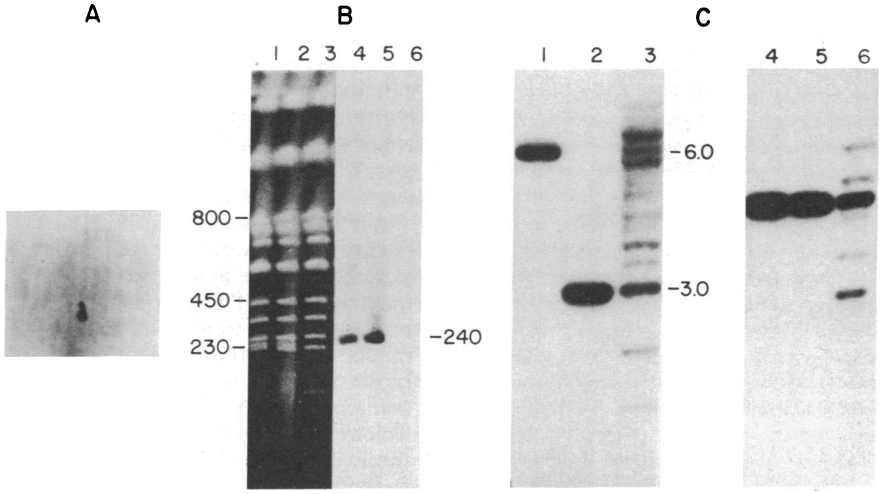

FIG. 1. YACs containing HLA and $V_\kappa I$ probe sequences. (*A*) One of 50 streaked YAC transformants hybridizes to a radioactive HLA type I probe. (*B*) PFGE (switching time, 60 sec for 23 hr) of chromosomes from the transformant containing the HLA probe sequence (lanes 1 and 2 and 4 and 5) and from a different YAC-containing strain (lanes 3 and 6). (*C*) DNA was extracted from two transformants containing a $V_\kappa I$ sequence (lanes 1 and 2 and 4 and 5) and from lymphocytes (lanes 3 and 6), digested with *Bgl* II (lanes 1–3) or *Bam*HI (lanes 4–6), and then electrophoresed in agarose gels, transferred to nitrocellulose membranes, and probed with labeled $V_\kappa I$ DNA. All sizes are in kb.

enough DNA to cover 10% of the human genome, and there are on the order of 20 copies of the probe sequence in total human DNA, one would expect on the order of one positive clone (21). Next (Fig. 1*B*), single colonies of yeast purified from the positive patch in Fig. 1*A* were grown in culture, immobilized, and lysed in agarose blocks (17). The chromosomes were then fractionated by pulsed-field gel electrophoresis (Fig. 1*B*, lanes 1 and 2). Ethidium bromide staining of the gel detects an extra chromosome of about 240 kb in the transformants compared to the DNA from another yeast strain containing a different small YAC (Fig. 1*B*, lane 3). Lanes 4–6 (Fig. 1*B*) show the corresponding autoradiogram obtained when the DNA in the gel was transferred to a nitrocellulose membrane and hybridized to the radioactive HLA probe DNA. As expected, the YAC in the transformants gave a signal absent from yeast DNA.

Comparable screening of the library with a V_κI immunoglobulin probe (10) produced two clones: one of 200 kb, another of 780 kb. In this case, the exact number of possible loci of genes and pseudogenes is unknown, but it can be estimated at about 100 (11). Thus, the finding of two positive clones in a library large enough to contain 10% of a genomic equivalent is consistent with the expectation for random cloning. The two YACs were analyzed further. To begin to compare the nature of their V_κ sequences to that in total DNA, DNA was extracted (8) from lymphocytes of the donor of the library and from each of the two YAC clones. The DNA preparations were digested to completion with either of two restriction enzymes (*Bgl* II and *Bam*HI) and fractionated by conventional agarose gel electrophoresis (ref. 3; Fig. 1*C*), and the DNA was transferred to nitrocellulose. The V_κI probe then detected a single characteristic fragment from each YAC after digestion with each enzyme. The probe detected a band of the same size in total human DNA in each case. This suggests that the YACs contain segments of DNA that retain the sequence of the probe and its immediate vicinity, since scrambling of the DNA would be expected to alter the relative positions of restriction sites in the neighborhood.

In additional screening experiments, a probe of 5S rDNA 200 base pairs (bp) long (13) detected 15 positive clones in the 2300 colonies, again with no background seen in any other clones. Fig. 2 shows the corresponding electrophoretic pattern of yeast chromosomes from minipreps of the 15 candidates and the confirmatory Southern hybridization of a single YAC in at least 11 of the 15 with the 5S probe. In preliminary work, 4 of these 15 candidates have been tested and confirmed by restriction mapping to contain single tracts of 5S sequence. Since 5S rDNA comprises about 0.1% of total human DNA, this frequency of positive signals is not surprising for a library with random representation of genomic DNA; but no detailed probability calculation is possible, since the 5S sequences are partly clustered and partly dispersed (13, 22).

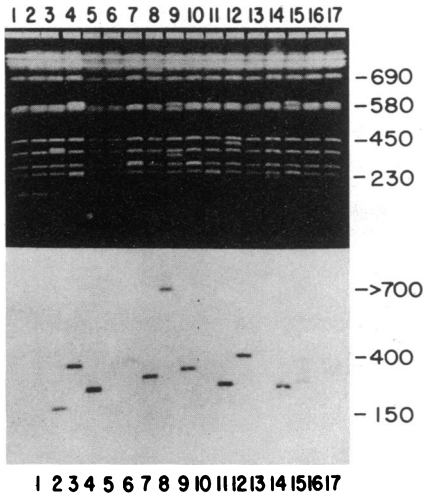

FIG. 2. PFGE analysis of 15 YACs that hybridize to human 5S rDNA. Each transformant was grown in liquid culture and its DNA was extracted and fractionated in a CHEF apparatus (switching time, 40 sec for 22 hr). (*Upper*) Ethidium bromide-stained gel showing the yeast chromosomes and candidate YAC chromosomes. (*Lower*) Autoradiogram of the DNA transferred to nitrocellulose and hybridized to nick-translated 5S rDNA. Control samples include HY1 (lane 1) and AB1380 (lane 17). Sizes are in kb.

Screening of an Xq24-Xq28 Library. One way to assess the usefulness of YAC clones as a mapping tool is to focus on a defined portion of the genome for which many probes are available. For this purpose, for example, a specialized library can be made from a somatic cell hybrid containing a portion of human DNA in a rodent background. We started from a human–hamster hybrid that is reported to contain Xq24-Xq28 as its total complement of human DNA (9). The hybrid cell was made by fusing Chinese hamster cells and human fibroblasts. As in other cases, human chromosomes are discarded during subsequent growth and treatments of the hybrid cells; the tip of the human X chromosome was retained by selection for a gene in Xq26 during the "reduction" of the hybrid.

The library was designed to have an average insert size of 150–200 kb; in that case, about 200 clones of human-specific DNA would be sufficient to include one equivalent of the DNA from that region. One advantage of this approach is that because of the biological prefractionation, all clones containing human DNA will presumptively contain DNA from the chromosomal region of interest. Furthermore, large numbers of probes from that region of chromosomal DNA are not required to find the initial bank of candidates for mapping purposes: total human DNA can be used as a probe.

YAC transformants were streaked out as in Fig. 1*A* and replica-plated onto membrane filters. Replicate filters were then hybridized with probes of nick-translated total human or total hamster DNA. As expected, most of the transformants hybridized to the hamster DNA. In contrast, a few transformants hybridized little or not at all with hamster DNA but hybridized strongly with the human DNA. Control experiments with the YAC library of total human DNA described above showed that, in agreement with earlier studies (19), >95% of the colonies containing human DNA inserts were easily and selectively recognized by the human DNA.

It is evident that human clones must be recovered over a background of large numbers of hamster YACs. Another disadvantage of this approach is that each transformant is initially embedded in agar, so that the work of isolating/repurifying and finding human-positive clones is greater than it would be with λ or other conventional vehicles. Nevertheless, it is possible to screen thousands of transformants per week.

Among the first 14,000 colonies screened, 50 human-positive clones were detected, or 1/250, the same percentage expected for the amount of human X chromosome DNA in the hybrid cell. This suggests that at least there is no relative bias against human DNA, and cloning may very well be random. Furthermore, in the same sample, the hamster DNA probe showed no hybridization with any of the clones containing human DNA. Fig. 3 includes a further analysis of 9 clones to demonstrate the specificity of the hybridization at the level of individual chromosomes. A CHEF gel analysis (6) is shown stained with ethidium bromide (Fig. 3 *Left*). The YACs are evident. The DNA in the gel was then transferred to two membranes in a sandwich blot, and the replicate filters were probed with the total human DNA probe (Fig. 3 *Center*) or hamster DNA (Fig. 3 *Right*). The seven candidates with human YAC chromosomes all showed hybridization only

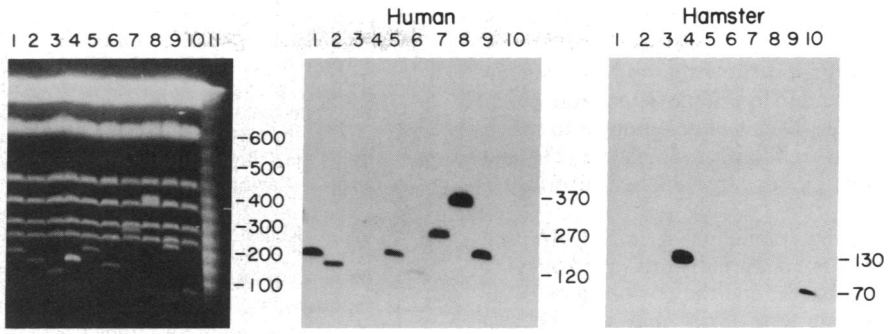

FIG. 3. PFGE of chromosomes from seven transformants containing human Xq24-Xq28 DNA sequences (lanes 1–3 and 5–9) and two transformants containing hamster DNA sequences (lanes 4 and 10). The switching regime was ramped from 25 sec to 50 sec for 20 hr. (*Left*) Gel stained with ethidium bromide. (*Center*) DNA transferred to nitrocellulose and probed with labeled total human DNA. (*Right*) Replicate membrane probed with total hamster DNA. Sizes are in kb.

with the human probe; only the two YACs containing hamster DNA showed a strong signal with hamster DNA as probe (Fig. 3 *Right*). The inserts vary in size up to 370 kb, with an average in the range of 150 kb.

These results indicate that there are no chimeras present—i.e., the clones seem to contain either human or hamster DNA. Thus, multiple cloning events during the formation of YACs may be rare, since such events in the presence of a 250-fold excess of hamster DNA would have been expected to yield clones with hamster DNA whenever human DNA was incorporated.

One clone, XY58, has been analyzed in some further detail. The DNA in transformed colonies and the isolated 150-kb YAC both hybridize to two RFLP probes, DX13 (15, 23) and St14 (14, 24). Other human YACs tested showed no hybridization to either probe. The inclusion of both probe sequences in a single YAC of 150 kb is consistent with PFGE analysis of restriction digests of human DNA (23). Those studies implied that the two segments of DNA are on the order of 70 kb apart in the genome.

Fig. 4 shows a Southern analysis of control DNA and XY58

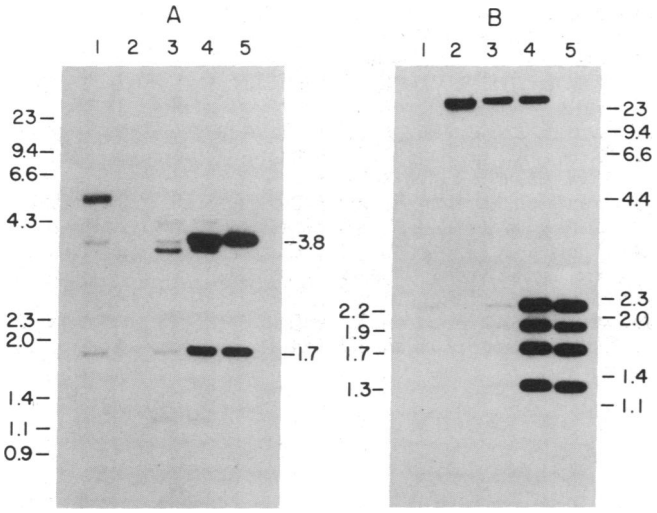

FIG. 4. XY58, a YAC containing St14 and DX13 segments of Xq28. (*A*) Total human DNA (lane 1), hamster DNA (lane 2), or hamster–human hybrid DNA (lane 3) was digested with *Taq* I, separated by agarose gel electrophoresis, transferred to a nylon membrane, and probed with St14 DNA. The comparable analysis was of YAC XY58 DNA, mixed with hamster–human hybird DNA (lane 4) or digested by itself (lane 5). (*B*) Electrophoretic analysis of DNAs digested with *Eco*RI. The order of samples was as in *A*. DNA was transferred to a nylon membrane and probed with radiolabeled DX13 DNA. Size markers (in kb) were *Hin*dIII-digested λ DNA and *Hae* III-digested φX174 DNA.

DNA probed with DX13 or St14 after digestion with restriction enzymes. Comparably digested total human DNA, DNA from the hamster–human hybrid cell, and the YAC show the same hybridization pattern for DX13: *Eco*RI fragments of 2.2, 1.9, 1.7, and 1.3 kb (Fig. 4*B*).

The pattern with St14 is more complex, as is expected for a locus that contains a number of sequences that hybridize to the probe (24). Fig. 4*A* (lanes 1–3) shows results with control DNAs digested with the enzyme *Taq* I. DNA from hamster cells (lane 2) shows only one weak signal, which is also seen in the hamster–human hybrid cell DNA (lane 3). In contrast, DNA from normal human lymphocytes (lane 1) shows a characteristic set of fragments that hybridize to the St14 probe. DNA from the hamster–human hybrid cell (lane 3) contains the "constant" 3.8-, 2.2-, and 1.7-kb bands seen in all human DNAs (14), but, as expected for human DNA from a different parental cell, several other bands (4.3, 3.6, and 1.0 kb) differ from those in the human DNA, presumably because of the presence of different polymorphic sites. XY58 DNA mixed with hybrid cell DNA (lane 4) or tested by itself (lane 5) clearly contains two of the constant DNA fragments seen in the hybrid DNA from which it was derived (3.8 and 1.7 kb; Fig. 4*A*, lanes 4 and 5). Thus, the DNA region containing the multiple sequences complementary to St14 is apparently only partially included in XY58 (see *Discussion*).

DISCUSSION

Single-copy human DNA probes give strong signals in screening assays of YACs with little or no interfering background (Fig. 1). This is because the complexity of yeast DNA (2×10^7 bp per genome) is two orders of magnitude less than that of human DNA. Thus, even with only a single copy of each YAC per cell, much less than high copy number conventional bacterial plasmids, the screening techniques and libraries are clearly adequate to yield YACs containing sequences that hybridize to specific probes. This permits one to begin more definitive testing of the potential of YACs in human genome analysis.

Since the human DNA clones occur in proportion to their DNA content in the somatic cell hybrid, cloning seems to be generally random—or in any case, bias in the cloning of Xq24-Xq28 human DNA is the same as that for total DNA. The frequencies with which clones containing probe sequences are found in the partial library of total human DNA are also not inconsistent with their representation in the total genome (though there is only limited information about the number of pseudogenes to be expected for each probe).

The failure to find chimeras of human and hamster DNA strongly supports the idea that a YAC tends to arise by the ligation of a single fragment of restricted DNA into the vector arms. This reduces the possibilities of any rearrangements or

recombination with extraneous DNA during the formation and recovery of clones.

The results with XY58 (Fig. 4) show that in this case two RFLP probes have been found to hybridize to the same YAC. Some St14 and DX13 sequences have been thought to reside within 70 kb of one another on the X chromosome (23), and the YAC system provides clones large enough to bridge a distance that great.

As in the case of V_κ, the fragments of total DNA that hybridize with probe DX13 are apparently preserved over the range of at least some kilobases, and the cocloning of DX13 and St14 suggests that long-term order may also be preserved. For St14, several standard fragments are absent, but the probe sequence is repeated a number of times over about 60 kb (14) and is highly polymorphic in a region that contains repetitive elements (24). Thus, the simplest possibility is that the human DNA insert in the YAC ends in the middle of the St14 region. Alternatively, it is still possible that DNA in the YAC insert has been partially rearranged in this region.

Detailed tests of the fidelity of such human YACs to genomic DNA are necessary, but there is already some additional relevant information. The initial report on the vectors used here (7) showed that the first YAC studied (HY1) contained a 120-kb *Sma* I fragment that was also detected in restriction digests of total human DNA. In our own further studies, YACs including the entire genes encoding factor IX and glucose 6-phosphate dehydrogenase have been shown to have all of the exon-containing restriction fragments expected after digestion with several enzymes (R.D.L., unpublished data). Other recent studies with YAC libraries from complex lower eukaryotic organisms also suggest that YACs consist of cloned representative DNA segments, each from a piece of contiguous DNA. For example, for *Caenorhabditis elegans*, YACs have been recovered that bridge a number of gaps in a map inferred from saturation cloning and analysis of cosmids (25). And in the case of *Drosophila melanogaster*, a number of YACs have been mapped by *in situ* hybridization to specific bands on polytene chromosomes (D. Garza and D. Hartl, personal communication).

These results show that libraries of total human DNA and of DNA from a selected portion of the genome can be organized by current protocols and can be analyzed for the representation and fidelity of clones of single and multicopy genes. The results increase the circumstantial evidence that YAC vectors may indeed provide a bridge between pedigree/pulsed-field gel mapping and conventional technology for subcloning and sequencing.

We are grateful to Dr. Terry Rabbits for the V_κI probe; to Dr. William Folk for the hamster 5S DNA probe; to Dr. Harry Orr for the HLA type I probe; to Dr. Morimasa Wada for the gel analysis in Fig. 4; to Dr. Tullio Labella for expertise and advice on handling hybrid cells; and to Antonio Caputi for help in organizing the Xq library. Dr. Maynard V. Olson provided many instructive discussions, and Dr. Lucio Luzzatto made many helpful suggestions. Funding for this project was provided by National Institutes of Health Grant GM10448 and grants from the James S. McDonnell Foundation and the Monsanto/Washington University Biomedical Agreement as well as auxiliary funds from Progetto Strategico "Genoma Umano" of Consiglio Nazionale delle Ricerche Italy.

1. Donis-Keller, H., Green, P., Helms, C., *et al.* (1987) *Cell* **51,** 319–337.
2. Drayna, D. & White, R. (1985) *Science* **230,** 753–758.
3. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
4. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **37,** 67–75.
5. Carle, G. F. & Olson, M. V. (1984) *Nucleic Acids Res.* **12,** 5647–5664.
6. Vollrath, D. & Davis, R. W. (1987) *Nucleic Acids Res.* **15,** 7865–7876.
7. Burke, D. T., Carle, G. F. & Olson, M. V. (1987) *Science* **236,** 806–812.
8. Burke, D. T. (1988) Dissertation (Washington Univ., Saint Louis, MO).
9. Nussbaum, R. L., Airhart, S. D. & Ledbetter, D. H. (1986) *Am. J. Med. Genet.* **23,** 457–466.
10. Bentley, D. L. & Rabbitts, T. H. (1981) *Cell* **24,** 613–623.
11. Straubinger, B., Thiebe, R., Pech, M. & Zachau, H. G. (1988) *Gene* **69,** 209–214.
12. Sood, A. K., Pereira, D. & Weissman, S. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 616–620.
13. Hart, R. P. & Folk, W. R. (1982) *J. Biol. Chem.* **257,** 11706–11711.
14. Oberle, I., Drayna, D., Camerino, G., White, R. & Mandel, J. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2824–2828.
15. Drayna, D., Davies, K. E., Hartley, D. A., Mandel, J. L., Camerino, G., Williamson, R. & White, R. L. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2836–2839.
16. Feinberg, A. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
17. Carle, G. F. & Olson, M. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3756–3760.
18. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Laboratory Course Manual for Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Murphy, P. O. & Ruddle, F. H. (1985) *Somatic Cell Mol. Genet.* **11,** 433–444.
20. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
21. Orr, H. T. & DeMars, R. (1983) *Nature (London)* **302,** 534–536.
22. Reddy, R., Henning, D., Rothblum, L. & Busch, H. (1986) *J. Biol. Chem.* **261,** 10618–10623.
23. Patterson, M., Kenwrick, S., Thibodeau, S., Faulk, K., Mattei, J.-F. & Davies, K. E. (1987) *Nucleic Acids Res.* **15,** 2639–2651.
24. Mandel, J. L., Arveiler, B., Camerino, G., Hanauer, A., Heileg, R., Koenig, M. & Oberle, I. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51,** 195–203.
25. Coulson, A., Waterson, R., Kiff, J., Sulston, J. & Kohara, Y. (1988) *Nature (London)* **335,** 184–186.