Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4256–4260, June 1990
Genetics

Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents

(preparative pulsed-field electrophoresis/physical mapping)

HANS M. ALBERTSEN*, HADI ABDERRAHIM, HOWARD M. CANN, JEAN DAUSSET, DENIS LE PASLIER, AND DANIEL COHEN

Centre d'Étude du Polymorphisme Humain (CEPH) 27, rue Juliette Dodu, F-75010 Paris, France

Contributed by Jean Dausset, March 22, 1990

ABSTRACT **Prior to constructing a library of yeast artificial chromosomes (YACs) containing very large human DNA fragments, we performed a series of preliminary experiments aimed at developing a suitable protocol. We found an inverse relationship between YAC insert size and transformation efficiency. Evidence of occasional rearrangement within YAC inserts was found resulting in clonally stable internal deletions or clonally unstable size variations. A protocol was developed for preparative electrophoretic enrichment of high molecular mass human DNA fragments from partial restriction digests and ligation with the YAC vector in agarose. A YAC library has been constructed from large fragments of DNA from an Epstein–Barr virus-transformed human lymphoblastoid cell line. The library presently contains 50,000 clones, 95% of which are greater than 250 kilobase pairs in size. The mean YAC size of the library, calculated from 132 randomly isolated clones, is 430 kilobase pairs. The library thus contains the equivalent of approximately seven haploid human genomes.**

To physically map genomes of complex organisms a number of techniques have been developed. These techniques include pulsed-field gel electrophoresis (1, 2), chromosome jumping (3), and cloning of large DNA fragments in yeast artificial chromosomes (YACs) (4, 5). Physical mapping, isolation of large genes plus all regulatory sites, and description of long-range genomic organization will depend on isolating and analyzing fragments of several hundred kilobase pairs of contiguous DNA. Intuitively appealing, the YAC system must be rigorously examined to understand cloning efficiency as a function of size and the extent to which the clones faithfully represent their genomic origin. Until recently (6), all YAC experiments had been carried out with DNA purified and manipulated in solution. In these early experiments one major problem has been small mean size of the YACs. This is probably due to extended handling of DNA in solution. As each manipulation of high molecular mass DNA in solution is potentially damaging, a protocol that minimizes damage is desirable. Further, it is known that strand damage initiates particular repair mechanisms in *Saccharomyces cerevisiae* and, consequently, rearrangements might be expected in the transformed YAC molecule (7). YACs that are not consistent with expected restriction enzyme patterns have been observed (8, 9), indicating the necessity of knowing the frequency of YAC rearrangements and the nature of the mechanisms involved. We performed a series of experiments designed to provide information that was incorporated into a protocol for constructing a YAC genomic library. These experiments and the resulting library are described in this report.

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.

MATERIALS AND METHODS

Chemicals and lyticase were obtained from Sigma and growth media were from Difco. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs, bacterial alkaline phosphatase was from IBI, and proteinase K was from Boehringer Mannheim. Agarase was obtained from Calbiochem, Zymolyase was from Seikagaku Kogyo (Tokyo), and Novozyme 234 was from Novo Biolabs (Copenhagen). Agarose (SeaKem and SeaPlaque; low melting point) was obtained from FMC. Biodyne membranes were purchased from Pall.

Yeast Strains. Yeast strains tested for transformation were *S. cerevisiae* AB1380 (α *ura3-52 can1-100 lys2-1 trp1 ura3 his5* ψ^+), BOY45 (α *his4 leu2-13,112 ura3 trp1 GAL:HO rad1::LEU2*), M1615 (α *ilv1-22 trp arg4-16 ade2 ura3-52*), SHY-3 (**a** *ste VC9 ura3-52 trp1-289 leu2-3 leu2-112 his3-Δ1 ade1-101 can1-100*), SX4-6A (**a** *ura3 trp1-289 ade2-1 his3-532 inos$^-$ can^R*), STX170-1A (α *ade2-1 his5-2 lys1-1 trp5-48 can1-100 ura3-1 leu1 gal1* ψ^+ *SUQ5*), W839-1C (α *ade2-1 can1-100* or *can1-100,x his3-11,15 leu2-3,112 trp1-1 ura3-1 rad1::LEU2+ rad52-8::TRP1+*), Y90 (**a** *ura3-52 lys2-801 ade2-101 trp1-901*), Y93 (**a** *ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200*), and YNN281 (**a** *trp1-Δ his3-Δ200 ura3-52 lys2-801 ade2-1 gal1b*).

Media. All yeast cultures were grown on either complete medium (YPD) or selective medium lacking uracil or uracil and tryptophan. These media have been described by Sherman *et al.* (10).

Transformation Experiments. Transformation was carried out according to Burgers and Percival (11) with the following modifications. Spheroplasts were prepared with Zymolyase-T20 (stock preparation containing 1 unit/μl). Four samples of 1×10^9 yeast cells were incubated with various amounts of Zymolyase-20T (5–40 units) for 15 min. The spheroplasts were washed once in 1 M sorbitol and then placed in YPD containing 1 M sorbitol for 30 min. The cells were resuspended in STC buffer (11), and the described protocol was followed. Spheroplast preparations chosen for transformation experiments were those which, under phase-contrast optics, showed less than 2% lysed spheroplasts in 1 M sorbitol and 100% lysis after H_2O was added to the microscope slide. The ligated and size-fractionated DNA (10 μl per Petri dish) was used for transformation. Usually 50 Petri dishes were prepared in a transformation experiment. For "retransformation" experiments, yeast cells containing a YAC of known size were cultured in medium lacking uracil. Cell concentration was determined and agarose plugs containing 2×10^9 cells per ml were prepared. After purification of the chromosomes and agarase treatment, 10 μl (equal to 2

Abbreviations: YAC, yeast artificial chromosome; CHEF, contour-clamped homogeneous electrophoresis.

*To whom reprint requests should be addressed.

$\times 10^7$ YAC molecules) of the sample was used to transform AB1380 cells as described (11).

Analytical Gel Electrophoresis. Analytical pulsed-field gel electrophoresis was run on a contour-clamped homogeneous electrophoresis (CHEF) (12) apparatus (Bio-Rad) using SeaKem agarose in 0.5× TBE (1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). Analytical separations were performed at 10°C and 200 V for 20 hr and with 50-sec switch times gradually increasing to 90 sec. Analytical field-inversion electrophoresis (2) with 1% SeaKem gels in 0.5× TBE was used to determine YAC size. Electrophoretic conditions were 14°C and 5.5 V/cm for 20 hr; the switch time was gradually increased from 9 sec to 60 sec, with a switch ratio of 3:1. Restriction digests for fingerprint analysis were separated on 0.6% SeaKem agarose in 0.5× TBE, for 20 hr at 5.5 V/cm.

Fingerprint Analysis of YAC Chromosomes. Purified YACs in agarose plugs were equilibrated with an appropriate restriction enzyme buffer prior to digestion. Complete digests were performed in 1 vol of restriction buffer per plug with enzyme added to 5 units/μg of DNA. Restriction digests of YACs were separated using unidirectional gel electrophoresis, transferred to Biodyne nylon membranes (Pall), and hybridized with total human DNA.

Radioactive Labeling and Hybridization. Probes were labeled by the random-primer method (13), and filters were hybridized and rinsed as described (5).

Protocol for Preparing Human YAC Library. *Purification of yeast and human DNA.* Human DNA was purified from Epstein–Barr virus-transformed human male lymphoblastoid cell line as described (12). Plugs (120 μl) for preparative electrophoresis contained 40 μg. Large batch preparations of yeast chromosomes were prepared as described (14). Minipreps of yeast chromosomes were prepared from 2 ml of YPD cultures that had grown to saturation. The cells were resuspended in 240 μl of 0.5% SeaPlaque agarose and plugs were prepared. The plugs were incubated for 3 hr at 37°C in SCEM (11) in the presence of 10 units of Zymolyase per ml. Then the plugs were incubated overnight in NDS (12) with proteinase K (0.5 mg/ml) at 50°C and rinsed several times in 50 vol of TE (10 mM Tris·HCl/5 mM EDTA, pH 7.5).

Vector preparation. The vector pYAC4 was used to construct YACs (5). Prior to ligation the vector was linearized with *Bam*HI and the two restriction sites were dephosphorylated with bacterial alkaline phosphatase. After phenol extraction, the cloning site of the linearized vector was cleaved with *Eco*RI. The *Eco*RI site was not dephosphorylated.

Partial restriction digests. Human DNA in agarose plugs was partially digested with *Eco*RI as described by Albertsen *et al.* (15).

Preparative CHEF I. The partially digested human DNA was size-fractionated using a CHEF (12) apparatus (Bio-Rad). The gel was 1% SeaPlaque low-melting-point agarose in 0.5× TBE. Preparative electrophoresis was performed at 10°C and 200 V for 18 hr, with a 15-sec switch time.

Ligation in agarose. Human DNA was recovered from a preparative CHEF gel in an agarose slice (1 ml). After equilibration with ligation buffer for 1 hr, a molar excess of vector was added to the insert DNA (40:1) and the agarose slice was briefly melted at 68°C. When the molten agarose had cooled to 37°C, 10 μl of T4 DNA ligase (400,000 units/ml) was added to the agarose in 1 ml of fresh ligation buffer. The ligation reaction mixture was incubated overnight at room temperature.

Preparative CHEF II. The ligation products were size-fractionated as described above.

Agarase treatment of agarose plugs. DNA used for transformation was liberated from the protective agarose matrix by agarase. SeaPlaque agarose plugs were equilibrated with 30 mM NaCl, melted at 68°C and transferred to 37°C, and agarase (40 units/ml) was added. The sample was incubated for 2 hr (16).

Transformation. After agarase treatment, the DNA was immediately used for transformation as described above. Colonies appeared after 4 days of incubation and were tested for the desired genotype before adding them to the library.

Storage of YAC clones. Clones are stored in YPD medium containing 20% (vol/vol) glycerol at −80°C in 96-well microtiter plates in triplicate.

RESULTS

Various factors influencing YAC transformation efficiency, size, and stability were analyzed. The preparation of spheroplasts is crucial for transformation efficiency. Of three commercially available enzymes used to prepare spheroplasts, Novozyme 234, Lyticase, and Zymolyase-T20, the last gave better preparations as judged by morphology and transformation efficiency. We also screened 10 yeast strains as hosts for YAC transformation. We found no advantage in using strains other than AB1380. Accordingly, we used this strain for the experiments described here.

Agarase treatment of agarose slices containing DNA of desired size is important for our transformation protocol. Accordingly, agarase treatment should not degrade the transforming DNA. We tested the effect of agarase on a preparation of yeast chromosomes and found no evidence of degradation (data not shown).

The following experiment was performed to validate the conditions used for preparative electrophoresis to separate DNA fragments in the desired size range. Yeast chromosomes were separated in one dimension using preparative conditions. A slice from this electrophoretic gel, containing the separated chromosomes, was recovered and subjected to electrophoresis in the second dimension under analytical conditions allowing separation of fragments of greater than 1300 kilobase pairs (kb). Fig. 1 shows yeast chromosomes

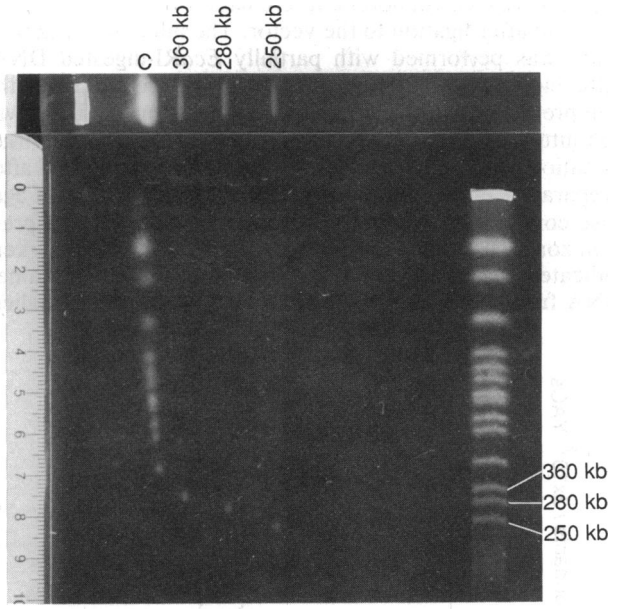

FIG. 1. Yeast chromosomes from strain STX454.1B[a] (Yeast Genetic Stock Center) separated in two dimensions using preparative and analytical conditions. A slice from the preparative gel was cut out, and the chromosomes were separated in a second dimension under analytical conditions ("L"-shaped display, left part of gel). Marker yeast chromosomes separated using analytical conditions are shown on the right. The sizes (360, 280, and 250 kb) of the smallest yeast chromosomes are shown. Region C is the compression zone.

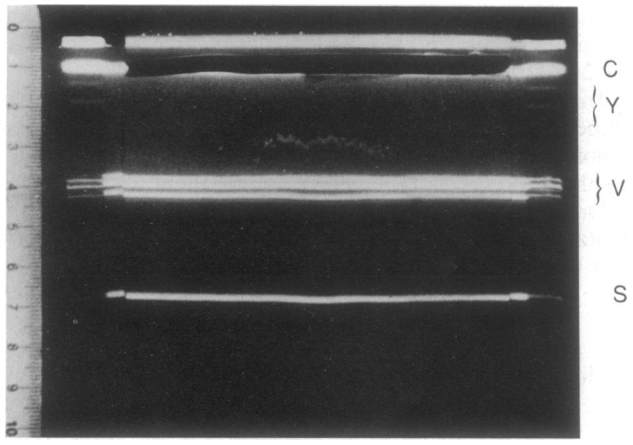

FIG. 2. After ligation of vector to *Eco*RI partially digested human DNA, the ligation products were size-fractionated using preparative CHEF. Yeast chromosome markers are included in both sides of the gel to guide the recovery of the DNA in the compression zone. The lanes with the yeast chromosomes plus a small part of the gel containing ligated human DNA were excised from the gel, stained with ethidium bromide, and replaced. The location of the compression zone (the heavily stained band at the 1-cm marker) could thus be determined, and the corresponding part of the unstained gel was excised and stored in TE. To confirm recovery of the desired fraction, the remainder of the preparative gel was stained as shown here. Region C is the compression zone. Region Y is the smallest yeast chromosomes. Region V contains the three possible products of ligated vector arms. Region S is the spacer fragment released from the vector by *Bam*HI. Contamination of the yeast marker lanes with ligation products is seen in regions V and S.

separated in two dimensions. With the preparative conditions used, chromosomes larger than 300 kb comigrated in a compression zone with limited mobility and resolution. The smaller chromosomes migrated ahead of the compression zone and their resolution was size dependent.

To obtain high molecular size DNA for transformation, preparative electrophoretic fractionation was performed before and after ligation to the vector. The initial size fractionation was performed with partially *Eco*RI-digested DNA. After electrophoresis an agarose slice was recovered from the compression zone. After ligation to the vector, the DNA was submitted to a second preparative electrophoretic size fractionation. Fig. 2 shows an ethidium bromide-stained gel after preparative electrophoresis of ligated DNA. A slice of agarose containing the ligated human DNA from the compression zone was removed prior to staining. The yeast markers indicated that the fraction isolated from the gel contained DNA from 300 kb to approximately 1500 kb. The smallest band in the gel represented the fragment between the two telomeres released by digestion with *Bam*HI. The next three higher bands represented all possible ligation products of the two vector arms (left/left, right/right, and left/right). Thus this gel provides information about molecular size of the human DNA fraction isolated and the efficiency of vector ligation.

Retransformation experiments involved purification of the chromosomes of a yeast strain harboring a YAC of known size and transforming the DNA into AB1380. To study the relationship between YAC size and transformation efficiency the following experiment was performed. Five YACs of known size, 180 kb, 490 kb, 600 kb, 700 kb, and 1050 kb, were transformed into AB1380 cells. Fig. 3 indicates that there is an inverse relationship between YAC size and the transformation efficiency. For this experiment, transformation efficiency varied from 2000 (1050-kb YAC) to 330,000 (180-kb YAC) transformant colonies per μg of DNA. To test the hypothesis that polyamines improve YAC transformation efficiency, the same experiment was carried out with transformation buffer containing spermidine at 0.7 mM and spermine at 0.3 mM. There is no apparent effect of polyamines added to the transformation buffer.

After transformation, occasionally, YACs smaller than the input YAC have been recovered. The integrity of these clones was suggested by their growth on selective medium, lacking uracil and tryptophan, indicating the presence of both telomeric regions. These clones showed no subsequent size variation on further growth in culture. DNA from these YAC clones was digested with various restriction enzymes and fingerprints were compared to that of the transforming YAC. Fig. 4 *A–D* shows an analytical CHEF gel, the corresponding autoradiogram of five YAC clones after retransformation with a 1050-kb YAC, the *Pvu* II restriction digests stained with ethidium bromide, and the fingerprint patterns of these five clones, respectively. Fingerprints generated with *Pvu* II and *Xba* I showed that the clone in the center lane was identical to that of the original transforming YAC of 1050 kb in size. The four other clones varied from 250 kb to 420 kb (Fig. 4*B*). Comparison of the fingerprints in Fig. 4*D* shows that some restriction fragments of the 1050-kb YAC were missing from the smaller clones. Each of the remaining restriction fragments of the smaller clones was found in the fingerprint of the 1050-kb YAC. The results of the fingerprint analysis were consistent with an internal deletion accounting for the reduced size of the smaller clones. A possible novel fragment (i.e., not found in the 1050-kb clone) was observed in Fig. 4*D*, lane 1 (indicated by an arrow). This could represent a junction fragment from a rearranged YAC insert.

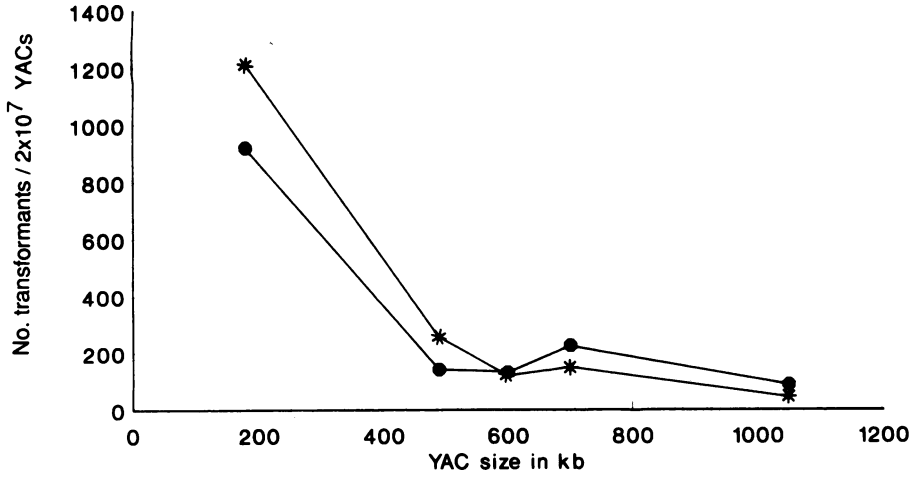

FIG. 3. Retransformation experiment involving five YACs ranging in size from 180 kb to 1050 kb. *, Experiment performed without polyamines; ●, experiment performed with polyamines (0.7 mM spermidine/0.3 mM spermine) added to the transformation buffer. The *x* axis indicates molecular size of the transforming YACs. The *y* axis indicates the number of transformants per 2×10^7 YAC molecules.

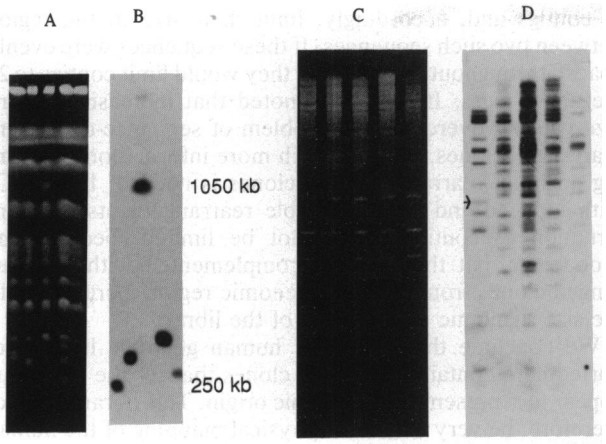

FIG. 4. Fingerprint analysis of five YACs obtained after retransformation with a 1050-kb YAC. The fingerprint of the 1050-kb YAC in the center lane of each panel is identical to that of the transforming YAC. An ethidium bromide-stained CHEF gel of the five clones is shown in *A*. The corresponding autoradiogram is shown in *B*. The five clones were digested with *Pvu* II and the electrophoretic pattern, developed with ethidium bromide, is shown in *C*. The corresponding autoradiogram after hybridization with human DNA is shown in *D*. The order of the five clones is the same in each panel. The arrow in *D* indicates a novel fragment (not present in the central lane) that might represent the junction fragment in the rearranged clone.

Clonally unstable YACs represent another type of rare rearrangement that we have observed in 2 clones among 132 YAC clones tested for size. One of these clones is shown in Fig. 5*A*. A complex pattern of at least four bands is seen, suggesting intraclonal size variation. This clone was streaked out on selective medium, and secondary clones were recovered and their sizes were determined (Fig. 5*B*). Six of the secondary clones also show at least two bands (clones 3, 5, 7, 8, 9, and 10). The other clones (lanes 1, 2, 6, and 11) each show a single band, suggesting that they are stable; however, they differ in size.

The YAC library was constructed with techniques used for the experiments just reported. Techniques especially useful for preparing the library were enrichment of very high molecular mass human DNA fragments on preparative CHEF gels (before and after ligation) and ligation of vector and human DNA in low-melting-point agarose. The library contains 50,000 YAC clones that were stored in YPD medium

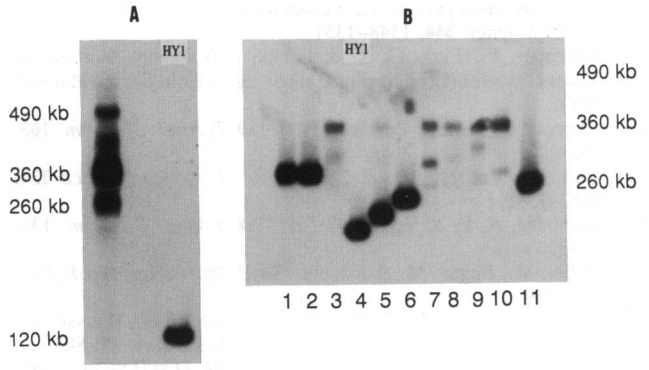

FIG. 5. (*A*) DNA from a single colony was separated by CHEF in parallel with YAC clone HY1 of 120 kb (gift from D. Burke, Department of Genetics, Washington University School of Medicine, Saint Louis). (*B*) Cells from the original colony were streaked out on selective medium. Chromosomes were purified from the resulting new single colonies and separated by CHEF. The Southern blot was hybridized with human DNA. Secondary sequence unstable clones show decreased intensity and are observed in lanes 3, 5, 7, 8, 9, and 10.

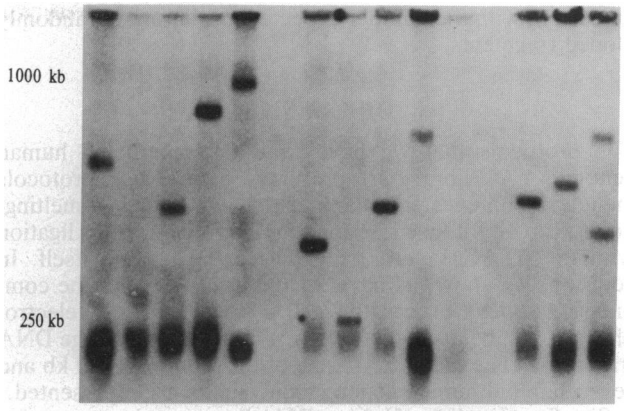

FIG. 6. Randomly chosen clones from each transformation were analyzed by pulsed-field gel electrophoresis and transferred to a filter. An autoradiogram of the filter hybridized with total human DNA is shown here. The size distribution observed is consistent with the expected size distribution of YACs obtained using preparative CHEF for enrichment of large DNA fragments.

in 20% glycerol at −80°C. The number of clones obtained varied among the transformations, the range being 200–10,000.

A sample of five clones were routinely chosen at random after transformation to determine molecular size of the insert. Chosen clones were hybridized with human DNA after analytical field-inversion gel electrophoresis and transferred to a membrane (Fig. 6). The size distribution of 132 YAC clones is shown in Fig. 7. The mean size of clones in the YAC library is 430 kb, as calculated from the mean size of selected clones weighted by the total number of transformants from each transformation.

Five YAC clones from the library, ranging in size from 180 kb to 1050 kb, have been used as probes for fluorescent *in situ* hybridization. Each of the five YACs was unambiguously assigned to a particular region of a different human chromosome (T. Kievits and G.J.B. van Ommen, personal communication). This suggests that single fragments have been cloned in the YACs analyzed.

Clones from a portion of the library have been identified by colony hybridization with probes for HLA class I sequences, *HLA-DRA*, and a subtelomeric interspersed repeat sequence (STIR) (17). The number of clones obtained was in each case

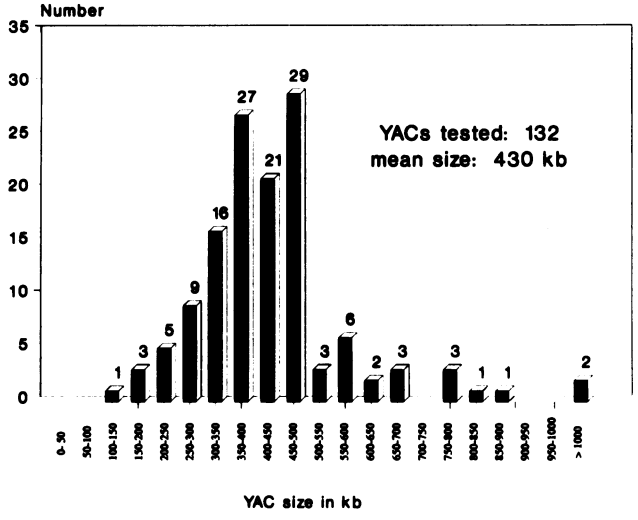

FIG. 7. Size distribution of 132 randomly chosen YAC clones. The calculated mean size of 430 kb is not directly reflected by this figure (see text).

consistent with the number expected in a library of randomly cloned fragments.

DISCUSSION

The protocols that we have used to construct a human genomic YAC library are presented here. These protocols include techniques for manipulating DNA in low-melting-point agarose for size fractionation, before and after ligation with the pYAC4 vector, and during the ligation itself. In addition, we show, as have Anand *et al.* (6), that the compression zone of preparative gels by pulsed-field gel electrophoresis can be used effectively for separation of large DNA fragments. The mean YAC size of this library is 430 kb and seven haploid human genome equivalents are represented.

Size fractionation of human DNA fragments in preparative agarose gels under the conditions reported here permits us to isolate large human DNA fragments ranging from 300 kb to approximately 1500 kb. The lower threshold, 300 kb, is relatively sharp, minimizing contamination with fragments of lower molecular size. This threshold was chosen to take advantage of favorable transformation efficiencies associated with the lower range of the selected size fraction (see Fig. 3) and still provides considerably larger inserts than those of cosmids. It is plausible that YACs of 250 kb to 300 kb observed in the library reflect reduced separation efficiencies of heavily charged preparative CHEF gels, predisposing to comigration of fragments smaller than expected in the compression zone.

An experimental technique, important for generating the observations presented here, involved the transformation of AB1380 host cells with DNA from a transformed yeast clone carrying a YAC of a given size. This technique permitted us to demonstrate and analyze clonally stable rearrangements in YACs. These rearranged YACs appeared to be smaller than the transforming YAC used in the experiment. As chromosomes larger than the original YAC have never been observed in these experiments and as no change in size of the natural yeast chromosomes has been observed, this type of rearrangement is likely to occur intrachromosomally. Fingerprints of rearranged YACs indicate a loss of genetic material. Our hypothesis is that the rearrangements are related to nicks in the YAC DNA induced by manipulation and that internal deletions are generated during repair of these nicks prior to the first mitosis after transformation. With careful manipulation of the YAC DNA, the frequency of these induced rearrangements should be minimized. We estimate the frequency of stable, induced rearrangements in the YAC library to be no greater than 5%. The basis for this estimate is the weighted distribution of clones tested for size.

The clonally unstable rearrangements, estimated to occur in less than 2% of the YAC library, may well represent a sequence-dependent phenomenon, driving recombination.

The significance of the two types of observed rearrangements on physical mapping with YACs should be considered. The frequency of clonally unstable rearrangements in this library, with a mean YAC size of 430 kb, is estimated to be approximately 2% (note that this frequency is dependent on insert size). Assuming that particular sequences account for the unstable rearrangements, our observations suggest that about 140 of these sequences are found in the human genome. Any YAC clone carrying one or more of these sequences is likely to be rearranged and not representative of its genomic region of origin. These clones are unacceptable for inclusion in contigs and, accordingly, limit their size to the region between two such sequences. If these sequences were evenly spaced throughout the genome, they would limit contigs to 20 megabase-pairs. It should be noted that increasing library size will not overcome the problem of sequence-dependent rearranged clones. Clearly much more information concerning unstably rearranged YAC clones is needed. For YACs with induced and clonally stable rearrangements the construction of contigs should not be limited, because the probability that they will be complemented with nonrearranged clones from the same genomic region increases with the size (genomic equivalents) of the library.

We conclude that the YAC human genomic library reported here contains large size clones that for the most part appear to represent their genomic origin. This library should, therefore, be very useful for physical mapping of the human genome.

We acknowledge G. Carle for advice and discussions and D. Burke and M. Olson for the YAC vectors and the yeast strain AB1380. We especially thank J. Weissenbach for his invaluable support and encouragement, and M. S. Cazelle, M. P. Pankowiak, J. L. Sambucy, and C. Soravito for skilled technical assistance. We also thank R. Rothstein, B. Dujon, B. Ozenberger, and the Carlsberg Research Laboratory for the generous gift of the yeast strains included in this study. H.M.A. is supported by a fellowship from the Danish Medical Research Council, H.A. is supported by a fellowship from the Société de Secours des Amis des Sciences, and H.M.C. is supported by the Institut National de la Santé et de la Recherche Médicale (INSERM). This work was supported in part by National Institutes of Health Grant 1R01 GM41387-01 and the Association Française contre les Myopathies.

1. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **37**, 67–75.
2. Carle, G. F., Frank, M. & Olson, M. V. (1986) *Science* **232**, 65–68.
3. Poustka, A., Pohl, T. M., Barlow, D. P., Frischauf, A.-M. & Lehrach, A. (1987) *Nature (London)* **325**, 353–355.
4. Murray, W. M. & Szostak, J. W. (1983) *Nature (London)* **305**, 189–193.
5. Burke, D. T., Carle, G. F. & Olson, M. V. (1987) *Science* **236**, 806–812.
6. Anand, R., Villasante, A. & Tyler-Smith, C. (1989) *Nucleic Acids Res.* **17**, 3425–3432.
7. Kunz, B. A. & Haynes, R. H. (1981) *Annu. Rev. Genet.* **15**, 57–89.
8. Little, R. D., Porta, G., Carle, G. F., Schlessinger, D. & D'Urso (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1598–1602.
9. Brownstein, B. H., Silverman, G. A., Little, R. D., Burke, D. T., Korsmeyer, S. J., Schlessinger, D. & Olson, M. V. (1989) *Science* **244**, 1348–1351.
10. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
11. Burgers, P. M. J. & Percival, K. J. (1987) *Anal. Biochem.* **163**, 391–397.
12. Chu, G., Vollrath, D. & Davies, R. W. (1986) *Science* **234**, 1582–1585.
13. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
14. Bellis, M., Pages, M. & Roizes, G. (1988) *Nucleic Acids Res.* **16**, 6749.
15. Albertsen, H. M., Le Paslier, D., Abderrahim, H., Dausset, J., Cann, H. & Cohen, D. (1989) *Nucleic Acids Res.* **17**, 808.
16. Michiels, F., Burmeister, M. & Lehrach, H. (1987) *Science* **236**, 1305–1308.
17. Rouyer, F., de la Chapelle, A., Andersson, M. & Weissenbach, J. (1990) *EMBO J.* **9**, 505–514.