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Low-frequency chimeric yeast artificial chromosome libraries from flow-sorted human chromosomes 16 and 21

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ABSTRACT **Construction of chromosome-specific yeast artificial chromosome (YAC) libraries from sorted chromosomes was undertaken (*i*) to eliminate drawbacks associated with first-generation total genomic YAC libraries, such as the high frequency of chimeric YACs, and (*ii*) to provide an alternative method for generating chromosome-specific YAC libraries in addition to isolating such collections from a total genomic library. Chromosome-specific YAC libraries highly enriched for human chromosomes 16 and 21 were constructed. By maximizing the percentage of fragments with two ligatable ends and performing yeast transformations with less than saturating amounts of DNA in the presence of carrier DNA, YAC libraries with a low percentage of chimeric clones were obtained. The smaller number of YAC clones in these chromosome-specific libraries reduces the effort involved in PCR-based screening and allows hybridization methods to be a manageable screening approach.**

It has been reported that flow-sorted chromosomes can be a source of DNA for generating chromosome-specific yeast artificial chromosomes (YACs) (1). This suggested the possibility of using such an enriched DNA source to construct entire libraries representing a given chromosome in YACs. Chromosome-specific libraries constructed from flow-sorted chromosomal DNA in both λ and cosmid vector systems have been described (2, 3) and have proven to be valuable resources for gene isolation and physical mapping (4). Several chromosome-specific libraries have been constructed in YAC vectors utilizing genomic DNA from somatic cell hybrids (1, 5, 6). The ability to use flow-sorted chromosomal DNA to produce chromosome-specific YAC libraries results in a smaller number of total clones, highly enriched for the chromosome of interest, compared to using genomic DNA. Reducing the number of clones minimizes the effort involved in utilizing the library for physical mapping and gene isolation by allowing more rapid screening with both PCR and hybridization-based methods. The nanogram quantities of high-quality DNA isolated from flow-sorted chromosomes, when used to construct YAC libraries in the presence of carrier DNA, results in a few percent chimeric YACs compared to chimera frequencies in the 50% range found in total human genomic YAC libraries (7). We report here on the use of flow-sorted chromosomal DNA, from human chromosomes 16 and 21, to construct complete digest YAC libraries using restriction endonucleases that have infrequent recognition sites.

MATERIALS AND METHODS

Chromosome Sorting and Library Construction. Human chromosomes 16 and 21 were isolated from monochromosomal somatic cell hybrids CY18 (4) and WAV-17 (8), respectively, by bivariate fluorescence-activated flow sorting (2, 9); 5–10 × 10^6 chromosomes (0.5–1.0 μg of DNA) were collected in tubes coated with 400 μl of 1% low-melting-point agarose (LMA) and recovered by centrifugation at 1500 × *g* for at least 1 hr. LMA plugs containing chromosomes were deproteinized overnight at 50°C in ESP (0.5 M EDTA, pH 8.0/1% sodium lauryl sarkosyl/2 mg of proteinase K per ml) and then washed in ET (10 mM Tris·HCl, pH 8/50 mM EDTA) containing 0.01% phenylmethylsulfonyl fluoride for 1 hr followed by five or six washes in TE (10 mM Tris·HCl, pH 8/1 mM EDTA) before digestion with either *Cla* I or *Eag* I or double digestion with *Not* I and *Nhe* I (Boehringer Mannheim and New England Biolabs). Digestions were performed overnight at 37°C by adding 200 μl of the appropriate 1× buffer and enzyme to the washed plug. The plug, containing digested DNA at 1.0–2.0 ng/μl, was washed in three changes of 50 mM NaCl and melted at 65°C. Ligations were performed by mixing 1.0 μg of YAC vector arms pJS97 and pJS98 (10) into the molten plug at 65°C and cooling to 37°C before adding ligation buffer and ligase (50 μl of 10× buffer and 5 μl of ligase; 2 × 10^6 units/ml; New England Biolabs). The ligation mixtures were allowed to cool to room temperature and then incubated at 16°C overnight. Ligations were size selected on 0.8% LMA pulsed-field gels using a 4-sec pulse at 150 V for 14–20 hr. Focused ligation products were cut out of the gel, washed in three changes of 50 mM NaCl, and melted at 65°C. The molten size-selected ligation, at a concentration of ≤1.0–2.0 ng/μl, was either transformed directly into spheroplasts or first diluted with an equal volume of 50 mM NaCl and then with an equal volume of 2× STC (2 M sorbitol/20 mM Tris·HCl/20 mM $CaCl_2$, pH 7.5) to help minimize resolidification of the LMA. Fifty microliters of molten LMA or 100 μl of molten, diluted LMA was transformed into 500 μl of YPH250 spheroplasts with 25–50 μg of sheared carrier DNA (herring sperm or *Escherichia coli*) and 1× polyamines (11, 12). Spheroplasts of YPH250 were made and transformed as described (11) and the transformation efficiency was >1 × 10^6 colony-forming units per μg of pJS97 plasmid DNA. Transformants were picked and transferred to 96-well microtiter plates.

Characterization and Screening. Average YAC size was estimated by pulsed-field gel electrophoresis of chromosome-sized DNA from YAC strains, transfer of the gel to a nylon membrane, and hybridization to human Cot1 DNA (BRL). Inter-*Alu* PCR products for mapping of YACs in somatic cell hybrids were generated (13) by using genomic DNA from human, rodent, and hybrid cell lines (1) and from yeast plugs containing chromosome-sized DNA made as described (11). Electrophoresis, transfer, and hybridization were performed as described (1) with minor modifications. Probes were labeled with a Prime-It kit (Stratagene) and preannealed when necessary with 50 μg of human Cot1 DNA (BRL).

Fluorescence *in Situ* Hybridization (FISH). Yeast genomic DNA was prepared as described (11) and ≈500 ng was biotin

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Abbreviations: YAC, yeast artificial chromosome; LMA, low-melting-point agarose; FISH, fluorescence *in situ* hybridization.

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labeled (BRL) with 3x polymerase. Fifty nanograms of biotinylated DNA, 1.0 μg of salmon sperm DNA, 2.0 μg of human Cot1 DNA (BRL), and 3.0 μg of yeast DNA were hybridized to human metaphase chromosomes in a vol of 50 μl under a coverslip (22 × 50 mm) as described (14). After hybridization at 37°C for ≥16 hr, slides were washed and stained as described (14) with minor modifications.

Colony Hybridization. Standard density colony grids were stamped manually from 96-well microtiter plates onto nylon membranes and grown 3–4 days at 30°C on selective medium lacking uracil and tryptophan. The filters were placed on Whatman 3M paper soaked with SPEM (1.0 M sorbitol/10 mM sodium phosphate, pH 7.5/10 mM EDTA/30 mM 2-mercaptoethanol) containing 1 mg of 20T zymolyase (ICN) per ml and incubated at 37°C overnight. Filters were then denatured, neutralized, hybridized, and washed as described (1). High-density grids (2 × 2, 4 × 2, and 3 × 2) were stamped on nylon membranes by a Biomek 1000 robot (Beckman) with a high-density gridding tool and were prepared for hybridization as described above. DNA pools for PCR screening of the libraries were made as chromosome-sized DNA in LMA and diluted 1:10 or 1:20 before screening. The pooling scheme consists of three levels: (*i*) large pools composed of all clones in each complete digest library, (*ii*) small plate pools consisting of each microtiter plate within a complete digest library, (*iii*) row and column pools of microtiter plates.

RESULTS

Transformation efficiencies were 500–2400 colony-forming units per μg of DNA sorted. Sort purities of human chromosomes 16 and 21 ranged from 85% to 95% as determined by FISH of an aliquot of the sorted chromosomes to human Cot1 DNA. Since both human chromosomes were sorted from monochromosomal somatic cell hybrids, any contamination during sorting is likely to be from rodent chromosomes and therefore does not complicate characterization of the human YACs. After transformation, recombinants (colonies containing an artificial chromosome) were estimated to be between 75% and 90% by colony color assay (15). There were 560 and 2500 transformants obtained for chromosomes 16 and 21, respectively. After adjusting for nonrecombinants (vector background) and sort purity, there were estimated to be 450 chromosome 16 YAC strains (all *Cla* I clones) and 1700 chromosome 21 YAC strains (≈300 *Cla* I clones, ≈500 *Eag* I clones, and ≈900 *Not* I/*Nhe* I clones), which had an average YAC size of 200 kb (Fig. 1). Since the YAC clones were constructed by using restriction endonucleases with recognition sites that are not randomly distributed, it is more accurate to refer to numbers of clones than chromosome equivalents in the library. YACs were constructed by complete digestion of flow-sorted chromosomal DNA rather than partial digestion due to the difficulty in controlling and reproducing partial digestion of small and varying amounts of DNA in the sorted samples. Since a single total digest library would not result in complete representation of the chromosome, three different total digests were performed for chromosome 21 DNA to attempt to maximize representation of the original chromosome in the combined libraries.

Location of YACs on the chromosome of origin was confirmed by mapping human YACs in somatic cell hybrids or by FISH to human metaphase chromosomes (Table 1). Somatic cell hybrid mapping of YACs was done with YAC end clones or inter-*Alu* PCR products (Fig. 2). Of 24 human chromosome 21 YACs mapped using hybrids, all localized on human chromosome 21, as would be expected since the YACs were generated from DNA that was flow sorted from a monochromosomal hybrid. FISH analysis of chromosome 21 YACs has also confirmed that the human YACs obtained from flow-sorted chromosome 21 DNA do localize to chromosome 21. Fig. 3 is a representative example of 1 of 85 YACs that were analyzed by FISH, showing a strong hybridization signal on both chromosome 21 homologues, which places this YAC on the distal long arm of chromosome 21. A strong, discrete signal on both chromosome 21 homologues was observed in the large majority of metaphase spreads and a weak, sporadic signal was not reproducible. All 85 of the human YACs analyzed by FISH mapped on chromosome 21.

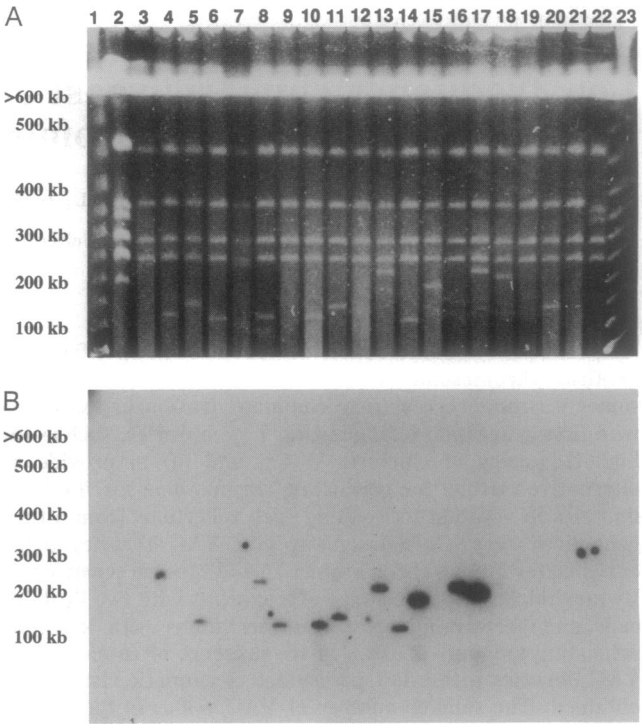

FIG. 1. Electrophoretic karyotype and Southern blot of yeast strains containing YACs. (*A*) Ethidium bromide-stained CHEF (Bio-Rad) gel. Lanes: 1 and 23, λ ladder; 2, positive control yeast strain containing a human YAC; 3–21, random yeast strains resulting from transformation with human chromosome 21 DNA ligated to YAC vectors; 22, negative control yeast strain containing a mouse YAC. (*B*) Southern blot of *A* probed with human Cot1 DNA. Human YACs comprise 58% (11/19), mouse YACs (hybridization not shown) comprise 16% (3/19), and nonrecombinants comprise 26% (5/19) of this sample, supporting the estimate that ≈60% of the transformants contain human YACs given ≈75% recombinants and a chromosome sort purity of ≈85%.

Somatic cell hybrid mapping of YACs was primarily used to confirm localization on chromosome 21, although the results—namely, that the YAC ends or PCR products localize in the same general region of the chromosome—were also consistent with a low frequency of chimeric clones. FISH was the primary method used to estimate the percentage of chimeric YACs (Table 1). Since the YACs were constructed from flow-sorted chromosomal DNA, most chimeric YACs will involve human fragments from different regions of the same chromosome. Therefore, the ability to observe two discrete FISH signals on chromosome 21 was tested in mixing experiments. In one experiment, two YACs, previously mapped by FISH to proximal and distal regions of chromosome 21q, were hybridized together to the same metaphase spreads, and two discrete signals on chromosome 21q were observed (data not shown). In another experiment, two YACs known to contain overlapping sequences were hybridized to the same metaphase spreads, and discrete signals could not be distinguished. Therefore, while some chimeric YACs may not be identified by FISH, most chimeras should be detectable by this method since, by chance,

Table 1. Localization of human YACs to chromosomes 16 and 21

Method	Chromosome	Hybridization probe	Number localized	Chimera frequency
Somatic cell hybrid mapping panel	21	YAC end clones	4 (8 end clones)	0%
		YAC inter-*Alu* PCR products	20	0%
FISH	21	Whole YAC (yeast genomic DNA)	85	1–8%
	16		38	5–10%

Examples of mapping in somatic cell hybrids and by FISH are shown in Figs. 2 and 3, respectively.

most chimeric YACs will contain two fragments from distinctly different locations on the chromosome. Of the 109 YACs characterized, 102 were not chimeric (24/24 nonchimeric YACs mapped in somatic cell hybrids and 78/85 nonchimeric YACs mapped by FISH). Seven of the YACs analyzed by FISH resulted in two signals on chromosome 21 as follows: (*i*) 3 of these YACs hybridized to the centromere region and short arm of chromosome 21 and the short arms of all the acrocentric chromosomes. (*ii*) 3 other YACs hybridized to 21q22, the short arm of chromosome 21, and the short arms of all the acrocentric chromosomes. One of these 3 YACs that maps to 21q22 is known to contain a low-abundance repeat. In addition, chromosome walks from both ends of this YAC have identified overlapping YACs that have been localized to 21q22 by a single, discrete FISH signal. (*iii*) One final YAC hybridizes to the centromere region of chromosome 21 in the majority of metaphases and also the proximal long arm of chromosome 21 in ≈10% of metaphases. The two signals observed as a result of FISH of these 7 YACs are possibly due to the presence of a repetitive

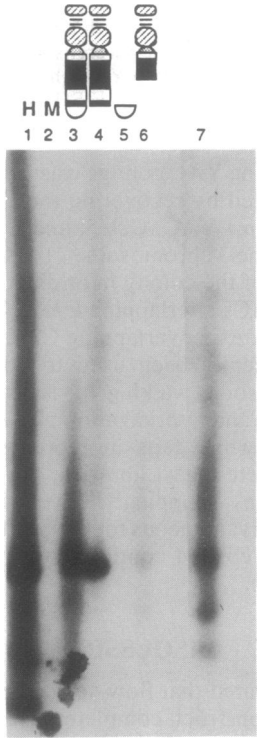

FIG. 2. Somatic cell hybrid mapping panel. Southern blot of inter-*Alu* PCR products of genomic DNA from cell lines hybridized with inter-*Alu* PCR products of a chromosome 21 YAC. Lanes: 1, human; 2, mouse; 3, WAV-17; 4–6, somatic cell hybrids containing the portion of chromosome 21 indicated (1). Lane 7, longer exposure of lane 6. Filter was hybridized with YAC inter-*Alu* PCR products that were preannealed with human Cot1 DNA, which reduces hybridization from the repetitive portion of the sequence but does not eliminate it, resulting in some background hybridization in the lanes. The inter-*Alu* PCR products of this YAC are human (lane 1) and map on human chromosome 21 (lane 3). The regional location of the YAC is determined by the pattern of hybridization in the remaining lanes. Hybridization in lanes 4 and 6 and the absence of hybridization in lane 5 localize the inter-*Alu* PCR products of this YAC to a region of chromosome 21 present in common in the hybrids represented in lanes 4 and 6, which corresponds to region 21pter–21q21. The banding pattern and intensity in the lanes that are hybridizing to the inter-*Alu* PCR products of the YAC are not always identical. Although there are several explanations, this is probably because the amplification of specific chromosome 21 sequences from a given cell line will be affected by the homologue that is present and the genetic background competing for the primer sequences. Therefore, all the chromosome 21-specific PCR products may not be amplified from all the cell lines. While this hybridization pattern is not definitive evidence against chimerism, since the YAC could contain two fragments of noncontiguous DNA from the region 21pter–21q21, it is consistent with the YAC being nonchimeric. A clear chimeric YAC, for example, would result in hybridization in lanes 1, 3, 4, and 5 or in lanes 1, 3, 5, and 6.

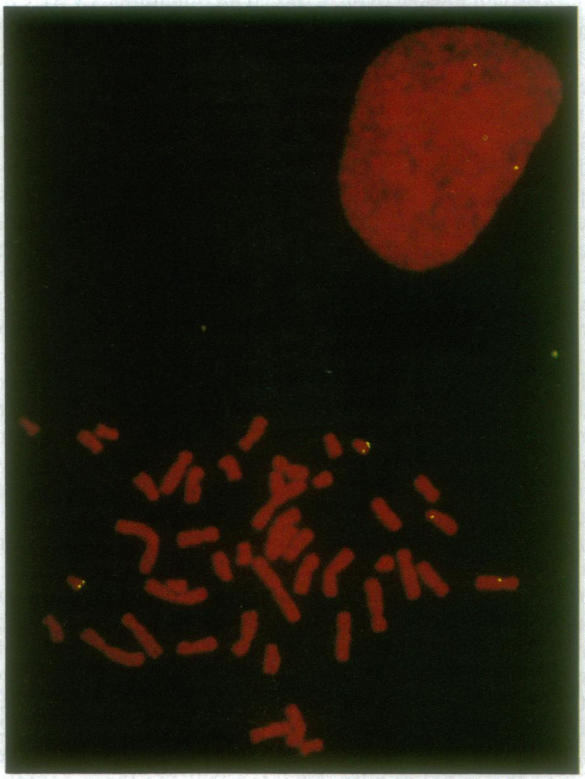

FIG. 3. FISH of human chromosome 21 YAC E9F7 to metaphase human chromosomes. Genomic DNA was isolated from yeast strains containing human YACs, labeled with biotin, and hybridized to human metaphase chromosomes. One of 85 YACs tested is shown and maps to a single location on the distal q arm of human chromosome 21. A strong, discrete hybridization signal on both homologues of chromosome 21 was seen in the large majority of metaphase spreads and background signal was not reproducible in different metaphase spreads. Any human–human chimeric YACs in the flow-sorted chromosome 21 library would be composed of two chromosome 21 DNA fragments. Therefore, a chimeric YAC would be expected to result in two discrete signals on chromosome 21. The ability to observe two FISH signals on chromosome 21 was confirmed by hybridizing two previously characterized YACs together (data not shown). Of the 85 YACs characterized by FISH, 78 resulted in single, discrete hybridization signals on chromosome 21, which is consistent with them being nonchimeric.

sequence, since cross-hybridization to the short arms of acrocentric chromosomes is involved in six of the seven results and 1 of those 6 YACs is known to contain a low-abundance repeat. In this scenario, there is no other explanation for only 1 of the 7 YACs that results in two FISH signals, which would suggest a chimera frequency ranging from 0.9% (1/109), if the 24 YACs mapped in somatic cell hybrids are taken into account, to 1.2% (1/85) if the analysis is limited to YACs analyzed by FISH. Alternatively, the two FISH signals could be the result of these 7 YACs being chimeric clones, which would indicate the chimera frequency to be between 6.4% (7/109) and 8.2% (7/85) in the chromosome 21 libraries.

Similar results have been obtained for the chromosome 16 library, as described below (Norman Doggett, personal communication). Of 38 YACs analyzed by FISH, 15 analyses resulted in two signals on chromosome 16. Ten of these were determined to be due to the presence of 2 YACs. Subsequent isolation of those YACs into individual strains by characterizing single colonies from the original strain indicated that the original strain was a mixture of 2 different YAC colonies. Additional analysis of the clones in the chromosome 16 library by pulsed-field gel electrophoresis has revealed the frequency of double-picked clones (mixture of 2 YAC strains) in the arrayed library to be ≈10%. The remaining 5 YACs that resulted in two FISH signals were checked against additional data for the presence of a low-abundance repeat, since it has been shown that up to 10% of chromosome 16 is composed of repetitive DNA sequences and that of chromosome 16 cosmids analyzed by FISH 29% result in two FISH signals and 75–90% of those are due to the presence of repetitive sequences on the cosmids. Of these 5 YACs, 1 contains a low-abundance repeat and 2 do not, based on hybridization to chromosome 16 cosmids. There is no information available about the presence of repetitive sequences in the other 2 YACs. Therefore, the frequency of chimeric clones in the chromosome 16 YAC library is estimated to be between 5% and 10% (2–4/38).

The representation of the chromosome 21 libraries was addressed by FISH, PCR screening, and colony hybridization screening. Initial FISH results indicated all three chromosome 21 libraries contained YACs, which were distributed along the chromosome (Fig. 4). However, other results suggest that the *Eag* I and *Not* I/*Nhe* I YACs may cluster in some regions of the chromosome, such as the centromere, since 34 positives, compared to an expected number of 5, were identified when colony grids were hybridized with end clones from a centromeric *Eag* I YAC. While it is possible that these 34 YACs were identified due to the presence of a low-abundance repeat, 1 of the YACs has also been localized to the centromere region of chromosome 21 by FISH. PCR and hybridization screening have identified YACs for 72% (21/29) of the sequences we have used to screen the libraries.

Since the initial results indicated that the three complete digest YAC libraries of chromosome 21 might represent nonidentical regions of the chromosome, the ability to use

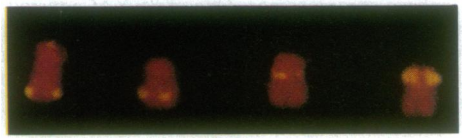

FIG. 4. Analysis of the distribution of chromosome 21 YACs by FISH. Genomic DNA from at least 20 yeast strains containing human YACs from each complete digest library were hybridized by FISH to human metaphase chromosomes. Each library contained YACs that hybridized to various locations on chromosome 21. The locations of 4 chromosome 21 YACs, demonstrating distribution along the chromosome, are shown.

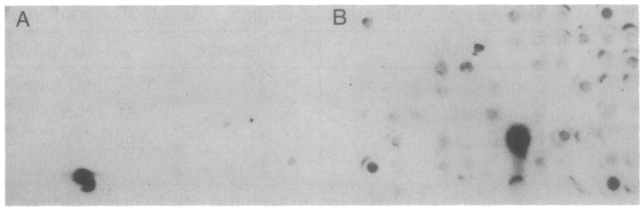

FIG. 5. Identification of overlapping YACs by hybridization of YAC end fragments to YAC colony grids. (*A* and *B*) Colony grids of part of the chromosome 21 YAC libraries probed with YAC end fragments. Both end fragments of YAC C2H5 were recovered by plasmid rescue. End fragments were then isolated from the plasmid, labeled by random priming, preannealed with human Cot1 DNA, and hybridized to colony grids from all three YAC libraries. (*A*) Colony grid representing YACs C2A1 to C2H12. (*B*) Colony grid representing YACs E9A1 to E9H12. Probes (both YAC ends from YAC C2H5) identify the YAC from which they were isolated, C2H5 (*A*), and an overlapping YAC, E9F7 (*B*). Both YACs have been localized to the same region of chromosome 21 by FISH and somatic cell hybrid mapping, which is consistent with these results.

them for constructing YAC contigs was tested. Chromosome walking was initiated by recovering the end fragments of a chromosome 21 *Cla* I YAC, C2H5, and hybridizing them to gridded arrays of the 3 chromosome 21 YAC libraries. Fig. 5 shows the results of the colony hybridization and identification of 1 of the YACs overlapping C2H5, which is an *Eag* I YAC, E9F7. The YACs overlapping C2H5 that were identified have subsequently been used to complete additional rounds of chromosome walking by repeating the cycle of YAC end recovery and hybridization of YAC ends to colony grids. Therefore, while gaps in representation are to be expected in complete digest libraries, the ability to identify overlapping YACs by "hopping" between different complete digest libraries is advantageous for physical map construction and for closure of cosmid contig maps, such as for human chromosome 16 (4).

DISCUSSION

We have demonstrated that flow-sorted chromosomal DNA can be used to construct complete digest YAC libraries, highly enriched for a specific chromosome, containing a low percentage of chimeric clones. Chimeric YACs in total human genomic libraries have been suggested to result from either coligation events or homologous recombination events between repetitive sequences on a fragmented piece of DNA and repetitive sequences on another fragment, or an intact YAC, present in the same cell (16). Most YAC cloning schemes include a large molar excess of vector in the ligation reaction to maximize the ligation of YAC vectors to all insert ends and reduce the ligation of inserts to each other (coligation). The ligations described here were performed with a low concentration of insert DNA as well as with a molar excess of vector DNA to attempt to minimize coligation events. Homologous recombination as a source of chimeric YACs can also be minimized by reducing both the percentage of fragments with "broken" or nonligatable ends and the frequency of cotransformation with multiple segments of human DNA. We have attempted to minimize DNA manipulation and any consequent shearing by performing all steps in the presence of LMA (11). To maximize the percentage of fragments with two ligatable ends, the starting DNA is >2 Mb and the restriction endonucleases (with the exception of *Eag* I) generate average-sized fragments of ≈200 kb. Despite these efforts, some percentage of the fragments will contain a broken end. The role of these fragments in chimera formation can be reduced by minimizing cotransformation. One way to achieve this is to include carrier DNA during trans-

formation, reducing the amount of human DNA to a small percentage of the total DNA being transformed, consequently reducing the likelihood that two human segments of DNA will cotransform the same cell. Indirect support of this theory is evidenced by the lower frequency of human–human chimeric YACs in libraries generated from somatic cell hybrid DNA (17) than in YAC libraries generated from human genomic DNA without carrier DNA (7, 16). In the YAC library created from a somatic cell hybrid, the percentage of human DNA is <1.0% of the total DNA and the excess of rodent DNA can be viewed essentially as having acted as carrier DNA during the transformation.

The low frequency of chimeric YACs obtained in the flow-sorted YAC libraries was likely due to several factors, then, including (*i*) ligating the insert DNA in dilute concentrations (≤1.0–2.0 ng/μl) with molar excess of vector, (*ii*) maximizing the percentage of fragments with two ligatable ends, (*iii*) performing yeast transformations with less than saturating amounts of DNA (18), and (*iv*) including carrier DNA during transformation. The frequency of chimeric YACs in the chromosome 21 library ranges from 1% to 8% and from 5% to 10% in the chromosome 16 library. These frequencies should be considered estimates, since some YACs resulting in two FISH signals may contain low-abundance repetitive sequences rather than being chimeric clones, and some chimeric YACs may not be detected due to the limitations of both FISH analysis and somatic cell hybrid mapping. For example, it is possible that a chimeric fragment that is <10–15% of a 200-kb YAC may escape detection by *in situ* hybridization. It is also possible that a chimeric YAC may contain two fragments that map close enough together on chromosome 21 that they are indistinguishable by FISH and appear to be nonchimeric by somatic cell hybrid mapping. However, given these limitations, the results suggest that the frequency of chimeric YACs probably does not exceed a few percent and is likely to be substantially lower than the 50% range reported for many total human genomic YAC libraries. This low frequency of chimeras is characteristic of all four independently constructed YAC libraries reported here, suggesting that it is a reproducible feature of this method for constructing YAC libraries and should be applicable to other sources of DNA as well as to DNA from flow-sorted chromosomes.

The average size of the YACs in both the chromosome 16 and 21 libraries is ≈200 kb. This is the expected average size after double restriction with *Not* I and *Nhe* I, near the expected average size after restriction with *Cla* I, and less than the expected average size after restriction with *Eag* I based on the range of fragment sizes generated in each digest. The average YAC size obtained in the *Eag* I library has likely been affected by the bimodal distribution of *Eag* I fragment sizes and a single size selection step after ligation. The larger size class of *Eag* I fragments probably transform inefficiently compared to the smaller *Eag* I fragments, and the smaller fragments were not thoroughly removed since only a single size selection was performed.

Screening of total human genomic YAC libraries can be accomplished through PCR and hybridization-based methods, although PCR may be more efficient because of the large number of filters required for hybridization screening, the effort involved in their preparation, and their limited ability to be reused (19). Screening of chromosome-specific YAC libraries by PCR methods is simplified, compared to screening of genomic YAC libraries, due to a reduction in number of DNA pools and PCRs required, with the extent of the reduction dependent on the pooling scheme. In addition, the relatively small number of YAC clones in the chromosome-specific library allows the option of hybridization screening to be more feasible since the number of filters required is significantly reduced. For example, the 1700 chromosome 21 YACs can be represented on two or three high-density filters the size of a microtiter plate or on 3.5 filters (8 × 12 cm) of standard density. The availability of the hybridization-based screening option eliminates the need to generate and test PCR primers for every probe utilized in library screening.

While complete digest libraries might be expected to underrepresent some regions of a chromosome, they also offer several advantages over total human genomic YAC libraries, including enrichment for a particular chromosome and a low frequency of chimeric clones. These flow-sorted YAC libraries, in conjunction with other chromosome-specific subsets of YACs such as from the large insert human genomic YAC library constructed by Centre d'Etude du Polymorphisme Humain (20), are valuable resources for the closure of the physical map of chromosome 16 and for increased resolution of the physical map of chromosome 21.

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