INTRODUCTION

Over the past few years, large-scale DNA sequencing has resulted in determination of the entire sequences of bacteria, yeast, fruit fly, worm (Caenorhabditis elegans), mouse, and human genomes. Comparison of the genome sequences of different organisms or species will provide useful information about functionally important sequences that are conserved in evolution. For most genomes, an important breakthrough for building sequence-ready maps was the development of the large-insert bacterial artificial chromosome (BAC) cloning system and the construction of representative genomic BAC libraries [1,2]. These BACs provide a resource that bridges the mapping and sequencing of specific genes involved in the main cell mechanisms. Moreover, the sequence of many genes in turn will open the way for systematic studies of gene function. The traditional method of isolating a certain gene from a BAC library has some limitations. It is a very time-consuming process that involves the analysis of hundreds of thousands of random clones. Furthermore, the routine gene-cloning strategy cannot be applied to the study of a particular region with a variable structure. To isolate a specific region from the genome of individuals, a new library must be constructed each time.

A new approach called transformation-associated recombination (TAR) cloning has recently emerged, which allows entire genes and large chromosome regions to be specifically and accurately isolated from total genomic DNA [3,4]. This non-enzymatic procedure for gene cloning can be accomplished using the yeast Saccharomyces cerevisiae, an organism that has a high level of intermolecular recombination between homologous DNAs during transformation. Cotransformation

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of yeast spheroplasts by a TAR vector containing short specific sequences (“hooks”) that flank a desired region or a gene along with a total genomic DNA and subsequent recombination between the vector and genomic DNA results in selective isolation of a complete copy of the gene as a circular yeast artificial chromosome (YAC).

To generalize application of the TAR cloning technique, we have developed a new strategy for the re-isolation of any desired genomic fragment identified in a BAC library. The strategy is based on the use of a new shuttle pTARBAC1 vector (Fig. 1) containing a YAC cassette (a yeast-selectable marker and a centromeric region) for the construction of BAC libraries. BAC clones obtained with this vector can be easily converted into TAR vectors by endonuclease digestion that deletes most of the internal sequence in the insert but the unique flanking sequences, which can function as hooks in TAR cloning. We used two human pathogens, *Giardia lamblia* and *Trypanosoma brucei*, to construct libraries using a pTARBAC1 vector. We demonstrate here that large-insert clones generated in *Escherichia coli* can be transferred into yeast cells with a high efficiency and fidelity and vice versa.

We also show that protozoa BAC clones generated with the new vector can be used for direct re-isolation of specific genomic fragments by TAR cloning.

**RESULTS**

Selective Re-isolation of Large DNA Fragments from a Total Genome Using BAC Clones as “TARgeting” Vectors

The TAR-cloning-based strategy for re-isolation of any desired genomic fragment that has been already cloned as a BAC is described in Fig. 2. A prerequisite for the rescue of a specific chromosomal region by *in vivo* recombination in yeast is the presence of a TAR cloning cassette containing a yeast-selectable marker (**HIS3**) and a centromere (**CEN6**) in the vector used for a library construction. We constructed such an *S. cerevisiae/E. coli* shuttle cloning vector, pTARBAC1. Whereas the vector is able to propagate in *E. coli*, its ability to propagate in yeast cells obligatorily depends on the presence of a yeast origin of replication (autonomously replicating sequence, or ARS element) in the captured genomic insert. ARS-like elements represented by short AT-rich sequences

![FIG. 1. The pTARBAC1 vector. The hybrid BAC/YAC vector pTARBAC1 is shown, which differs from pBACE3.6 by the presence of a yeast centromere (CEN6) and a yeast-selectable marker (HIS3). Genomic inserts were cloned into EcoR1 sites for the RPCI-90 *G. lamblia* library and into *SacI* sites for the RPCI-102 *T. brucei* library.](http://www.idealibrary.com)

![FIG. 2. Re-isolation of large DNA fragments from a total genome using BAC clones as targeting vectors. Genomic fragments are cloned into a pTARBAC1 shuttle vector. The targeted wild-type chromosomal region (ABCD...XYZ) can be “pooled out” from the BAC/YAC library by screening of the library with specific markers. The ability of the BAC/YAC to replicate in yeast is provided by the ARS sequence(s) contained within part B–Y of the insert, which can function as a replication origin (ori) in yeast. After deletion, the ARS sequence(s) is deleted. This ARS-deficient “TARBAC” vector contains the yeast centromere CEN6, the yeast-selectable marker HIS3, and two arms (A and Z) homologous to a genomic sequence. After linearization, this TAR “rescue” vector is cotransformed with mutant genomic DNA (Abcd...xyz) into yeast spheroplasts. Recombination between the arms (A and Z) and genomic DNA leads to re-isolation of the region of interest as a circular YAC. Because pTARBAC1 contains a BAC cassette, the YAC isolate can be transferred into *E. coli* cells.](http://www.idealibrary.com)
are common in eukaryote genomes. For all analyzed genomes, the frequency of these sequences is approximately one ARS-like sequence per 20–40 kb genomic DNA [5]. Based on this estimate, most of DNA inserts bigger than 50 kb would contain at least one ARS element.

One or two such elements should present within part “B–Y” of the BAC insert in Fig. 2. This ARS element along with a CEN sequence present in the vector would provide stable propagation of the BAC clone in yeast cells as an additional yeast chromosome. The BAC clone can be easily converted into a TAR cloning vector for re-isolation of the original insert from genomic DNA by digestion of the BAC DNA with a specific restriction enzyme that does not cut the vector itself but removes most of the insert including the ARS-like sequence. The deleted BAC will remain the pTARBAC1 vector with two flanking genomic sequences (Fig. 2, A and Z). Cotransformation of such a construct along with genomic DNA into yeast spheroplasts and subsequent recombination would result in re-isolation of the genomic fragment as an autonomously replicating circular YAC. The YAC clone can be detected among transformants by PCR using a pair of diagnostic primers as described for isolation of genes by TAR cloning [4]. The presence of the BAC cassette allows transfer of the circular YAC back into E. coli for easy DNA isolation. Our strategy (Fig. 2) can be applied to most BAC/YAC clones containing an ARS-like element except those with an ARS near either end of the clone inserts.

Construction of Large-Insert BAC/YAC Libraries Using the pTARBAC1 Vector

We used the pTARBAC1 vector to construct representative BAC/YAC libraries for several species, including T. brucei (the RPCI-102 library) and G. lamblia (the RPCI-90 library). We estimated the size distribution and the ratio of non-recombinant clones from 160 randomly isolated clones of each library. We used digestion of a subset of clones from each group with NotI to determine the size of the insert (Figs. 3A, RPCI-102, and 3B, RPCI-90). We found an average insert size...
study on density of these sequences in protozoa genomes. Although these sequences are frequent in mammalian DNA, there has been no systematic cloning depends on the presence of an ARS-like element within this region [3]. Although these sequences are frequent in mammalian DNA, there has been no systematic cloning depends on the presence of an ARS-like element within this region [3]. Although these sequences are frequent in mammalian DNA, there has been no systematic cloning depends on the presence of an ARS-like element within this region [3].

Fidelity of BAC/YAC Transfer between Yeast and E. coli

Artifacts such as chimeras and deletions are frequent in large YACs generated by a standard method based on in vivo ligations [8–10]. Because the frequency of these artifacts is low in YACs generated by TAR method [4], they presumably result from recombinational repair of transforming DNA in yeast cells. Our strategy of re-isolating BAC inserts by in vivo recombination requires the double shuttling of inserts from BACs to YACs to BACs. Therefore, we investigated the fidelity of the insert DNA shuttling between yeast and E. coli. We transformed 20 randomly selected BACs from the RPCI-102 library into yeast spheroplasts. We isolated the relevant circular YACs propagated in yeast for ~ 20 generations from yeast transformants and shuttled them back to E. coli. For the transfer of YAC/BACs from yeast to E. coli, we used a modified protocol [11]. In a typical experiment with an ~ 150 kb YAC/BAC, 1 µl melted plug prepared by the modified method yielded 100–500 chloramphenicol-resistant colonies. We compared the final BACs (BACs shuttled from E. coli to yeast and back) to the original BACs using

Density of ARS-like Sequences in the Low-Complexity Genome of T. brucei

Direct isolation of the specific genomic region by TAR cloning depends on the presence of an ARS-like element within this region [3]. Although these sequences are frequent in mammalian DNA, there has been no systematic study on density of these sequences in protozoa genomes. We therefore analyzed individual BAC clones from the T. brucei library for the presence of ARS sequences. For this, we assessed DNA isolated from 40 randomly selected BACs for its ability to transform yeast spheroplasts with a high efficiency. Most (35 of 40) of the T. brucei BACs transformed yeast with a high efficiency, indicating the presence of at least one ARS element in the cloned genomic fragment. The efficiency of transformation for these clones was ~ 5 × 10^4 transformants per microgram BAC DNA. We obtained no or few (up to five) His^ sup + transformants with DNA from five other ARS-deficient BACs. Because the average size of the inserts was 107 kb, the predicted density of ARS elements in the T. brucei genome was approximately one ARS-like sequence per 120 kb. These data are different from the ARS-like element distribution in mammalian genomes, in which one ARS-like sequence is present per 20–40 kb [5]. However, our data are in agreement with a previous report that the density of genes in the African trypanosome genome is very high [6]. Because the ARS-like sequences are very AT-rich [7], they most likely are present only in the noncoding regions.

Re-isolation of T. brucei Specific Genomic Regions by TAR Cloning Using Individual BAC/YAC Clones

We used five ARS-containing BACs (clones Try 1–5) with insert sizes greater than 150 kb (150 kb, 152 kb, 172 kb, 170 kb, and 180 kb, respectively) to generate TAR cloning vectors. To obtain BAC/YAC rescue vectors for further TAR cloning experiments, we deleted the insert sequences in these BACs by treating the BACs with NotI, which lacks recognition sites in the pTARBAC1 vector. After being digested with NotI and re-circularized by ligation, the deleted BACs were transformed into E. coli. We purified plasmid DNA from bacterial cells and, before using it in TAR cloning experiments, we assessed its ARS activity by yeast spheroplast transformation. Typically, we obtained 1000–8000 transformants with 100 ng plasmid DNA with the plasmids containing an ARS-like sequence.

Based on the results of double digestion with NotI and EcoRI, the size of the remaining ends (“hooks”) of the deleted Try 1–5 BAC clones were 10.0 kb + 10.0 kb; 4.0 kb + 2.2 kb, 7.0 kb + 3.8 kb; 4.1 kb + 1.2 kb, and 4.1 kb + 3.2 kb, respec-
tively. Three BAC deletion derivatives (Try2, Try4, and Try5) lost ARS elements as a consequence of deletion, whereas the other two retained ARS elements. We used two ARS-deficient BAC derivatives, Try2 and Try4, for further TAR cloning experiments.

We transformed yeast spheroplasts with the linearized deleted BACs (Try2 or Try4) along with “gently prepared” high-molecular-weight T. brucei DNA. We used 5 μg genomic DNA, 1 μg linearized vector, and 2 × 10^8 yeast spheroplasts in each transformation experiment. Transformants were selected on synthetic medium lacking histidine. We obtained about 400 His⁺ transformants in five independent transformation experiments for Try2, and 120 transformants for Try4 in one single transformation. We randomly selected 20 individual His⁺ transformants from each group. We prepared YAC DNA (called TAR-YAC isolates here) from those clones using an alkaline lysis procedure and shuttled it back to E. coli to obtain the relevant TAR-BAC transformants. We failed to obtain relevant TAR-BAC transformants for 4 of 20 TAR-YACs from the Try2 group and 6 of 20 from the Try4 group. This could be explained by either the integration of the TAR vectors into one of yeast chromosomes or the large size of the inserts in YACs maintained in these clones. (BACs with inserts bigger than 300 kb transform E. coli poorly.) We excluded these clones from further analyses.

To demonstrate the rescue of the targeted chromosomal regions, we characterized all TAR-BACs by fingerprint analysis after complete digestion with NotI and EcoRI (Figs. 5A and 5B) and sequencing with BAC-specific primers. In the Try2 group, 3 of 16 TAR-BACs (clones 1, 5, and 13) had fingerprints identical to that of the original BAC (150 kb). For the Try4 group, only clone 12 was completely identical to the original BAC (180 kb).

FIG. 5. Fingerprints of the TAR-BACs for Try2 and Try4 groups. Analysis of 16 TAR-BAC transformants of the Try2 group (underlined) and 14 TAR-YAC isolates of the Try4 group. All TAR-BACs were digested with NotI (A) and EcoRI (B) for comparison with the original BAC clones (150 kb and 180 kb, respectively). O, original BAC clone; other lanes, individual TAR-BACs shuttled from different YAC isolates. For the Try2 group, three TAR-BACs (1, 5, and 13) are identical to the original BAC. For the Try4 group, one TAR-BAC (12) is identical to the original BAC.
used 9 primers to sequence the 2236-bp T7 arm in both directions and 14 primers for the 4058-bp SP6 arm (Fig. 6). The TAR-BACs identical to the original BAC clone should contain complete and non-rearranged targeting sequences. Clones 1, 5, and 13 had all priming sites from both arms and, therefore, retained both “hooks.” Also, the sequences obtained from the further downstream region of the EcoRI site were identical (data not shown). This confirms the fingerprinting results that all these TAR-BACs were identical to the original Try2 BAC clone. Clones 3, 6, 7, and 11 had all priming sites from one arm but only part of the another arm. Clones 2, 8, 9, 12, 14, and 15 had only fragments of arms. The other clones (4 and 10) had completely lost the SP6 arm. One clone presumably lost both arms, as none of the 23 primers worked.

Recent studies of the mechanism of TAR cloning have shown that when a targeting sequence is unique, nonhomologous end-joining is the main mechanism of generation of background clones (unpublished data). Deletion of the targeting sequences in the TAR-BAC clones indicates that these clones are a result of homologous recombination rather than nonhomologous end-joining.

TAR Cloning of the Specific Sir2 Locus from Total Genomic DNA

In T. brucei, Sir2 is a single-copy gene. We used five BACs (with insert sizes ranging from 50 kb to 160 kb) containing Sir2 to test our cloning approach. All five BACs contained the ARS element, based on their stable propagation in yeast cells. The BACs were deleted by digestion with EcoRI, religated, and then transformed into E. coli. Two of the deleted BACs were subsequently confirmed by yeast transformation to have lost the ARS element(s): clones 5J20 and 27M6. These two linearized deleted BACs were transformed into yeast spheroplasts along with T. brucei genomic DNA to re-isolate this genomic region. Ten randomly selected TAR-YAC clones from each group were shuttled to E. coli for further analysis. We assessed each of these colonies by PCR using a primer specific to the gene and also compared the TAR-BAC DNA with the original (nondeleted) BAC using EcoRI and NotI restriction fingerprinting (data not shown). For 5J20, 6 of 10 TAR-BAC isolates were identical to the original BAC. For clone 27M6, 3 of 10 TAR-BACs analyzed were identical to the original BAC. Based on these results, we conclude that a single-copy gene can be efficiently and accurately re-isolated from simple genomes by TAR cloning using a BAC derivative as a “TARgeting” vector.
**DISCUSSION**

Over the past few years, a new technique has emerged that allows the specific isolation of entire genes and regions directly from total genomic DNA [4,13]. This technique draws on a unique characteristic of yeast *S. cerevisiae*; that is, that recombination is highly efficient during transformation between homologous DNAs. Moreover, the genomic DNA of all eukaryotes contains multiple copies of short AT-rich sequences that can function as an origin of replication (ARS element) in yeast. For mammals, the frequency of such sequences is approximately one per 30 kb genomic DNA. These two features have allowed the development of a new method for direct gene isolation by *in vivo* recombination in yeast (TAR cloning) [3]. Homologous recombination between a TAR vector containing short 5′ and 3′ sequences flanking a gene of interest cotransformed with a total genomic DNA results in the rescue of the gene as a circular YAC. This circular YAC can be easily separated from linear yeast chromosomes for a further physical analysis. Alternatively, the YAC can be “retrofitted” to a BAC and transferred into *E. coli* cells [14].

Here we have demonstrated that a genomic BAC library constructed with a vector carrying a yeast-selectable marker and a *CEN* sequence can be used for re-isolation of DNA fragments from total genomic DNA by TAR cloning. Most of the BAC clones from such a library can be converted to a TAR cloning vector by removal of most of the internal sequence in its insert. Such a TAR vector may be used repeatedly for re-isolation of the same genomic region by *in vivo* recombination in yeast.

We successfully applied this strategy to the relatively small genome of *T. brucei*. Despite a high content of CG pairs in the genome (and therefore a lower density of potential ARS-like sequences), randomly selected genomic regions were re-isolated as circular YAC/BACs by TAR cloning with a high efficiency. Between 5 and 60% of yeast transformants containing YACs with a region of interest when a BAC lacking an internal sequence and genomic DNA were co-transformed into yeast spheroplasts. The genomic regions were isolated from the *T. brucei* genome with a high fidelity. Based on the results of fingerprint analysis and direct sequencing of BAC ends, there were no detectable DNA rearrangements in the YAC/BAC isolates. We found no rearrangements during transfer of the YAC/BACs between yeast and *E. coli*.

Given these results, we conclude that genomic regions of unicellular eukaryotes might be easily re-isolated by a TAR technique using BAC clones as a starting material. This new strategy of re-isolation of genomic fragments may be very useful for studying human pathogens. For example, African trypanosomes are able to express a large repertoire of different antigens during infection, which enables the parasite to avoid the immune response of its host. Cloning of different antigenic variations may help in producing a vaccine against the disease. In addition, sequence information may provide an important model for some general processes in eukaryotic differentiation.

Application of this strategy to the re-isolation of mammalian genomic fragments should be investigated. In contrast to protozoa genomes, mammalian genomes contain multiple repeats. Therefore, it is very likely that after the removal of the internal sequence from a BAC, the flanking ends would still contain some repeats. Such constructs would clone random DNA, as has been shown for TAR vectors containing *Alu* or LINE repeats [3]. In this case, to guarantee specificity of cloning, the “hooks” should be sequenced first and then only unique regions will remain for “TARgeting.”

**MATERIALS AND METHODS**

**Construction of the pTARBAC1 vector.** The *CEN6*-HIS3 YAC cassette was removed from pVC604 TAR cloning vector [4] by digestion with SpeI and cloned into the blunt-ended *NdeI* site in the pBAC-5.6 vector [15,16]. The resulting hybrid BAC/YAC vector, called pTARBAC1, contains a yeast centromere, *CEN6*, a yeast-selectable marker, *HIS3*, and the *F*-factor origin of replication (Fig. 1). Cloning of large genomic inserts and the selection of recombinant clones have been described [16,17]. Genomic inserts can be cloned into either *EcoRI* or *BamHI* sites and replace the “stuffing,” pUC-LINK. Thus, when *BamHI* sites are used for cloning, the *EcoRI* sites will be deleted from the vector, allowing generation of internal deletions in the BAC by treatment with *EcoRI*. Other sites available for the same purpose include *AatII*, *AvrII*, *BlnI*, *BsaI*, *SfiI*, and *XcmI*.

**Construction of large-insert BAC/YAC shuttle libraries.** A published procedure [17] was used to construct two BAC/YAC shuttle libraries for *T. brucei* (the RPCI-102 library) and *G. lamblia* (the RPCI-90 library). *T. brucei* 427-strain agarose-embedded DNA was partially digested with *Mbol* and cloned into the *BamHI* site of the pTARBAC1 vector. For the RPCI-90 library, *G. lamblia* agarose-embedded DNA was partially digested with a combination of *EcoRI* and *EcoR*I methylase and cloned into the pTARBAC1 vector at the *EcoRI* sites.

**Analysis of the large-insert BACs.** Single BAC colonies were grown overnight in 1.5 ml Luria-Bertani (LB) medium containing 20 μg/ml chloramphenicol. DNA was extracted using an automated plasmid isolation system (AutoGen 740; Integrated Separation Systems (Framingham, MA) or a manual extraction procedure as described before [17]. About 0.5 μg DNA was dissolved in 100 μl TE, pH 8.0. We used 1 μl DNA for PCR analysis. To estimate the size of the inserts, 5 μl DNA was digested with 0.1 U *NcoI* (New England Biolabs). The digestion was analyzed by field-inversion gel-electrophoresis as described before [17]. High-molecular-weight markers from New England Biolabs (Beverly, MA) were used to estimate the molecular weights of the resultant bands.

**Deletion of large-insert BACs from the RPCI-102 library to create TAR cloning vectors.** BAC DNA (~25 ng DNA) was digested with 1 U *EcoRI* (New England Biolabs) at 37°C for 3 h. After heat inactivation of the endonuclease at 65°C for 20 min, 0.5 mM ATP (final concentration; Sigma, St. Louis, MO) and T4 DNA ligase (Life Technologies, Grand Island, NY) were added and the reaction mix was incubated for another 2 h at room temperature. After ethanol precipitation, the DNA was dissolved in 20 μl TE, pH 8.0. Then, 2 μl of the sample was transformed into DH10B electro-competent cells (Life Technologies). A single colony of the transformants was inoculated in 500 ml LB culture and grown overnight at 37°C. The deleted BAC DNA was isolated using a Qagen kit according to the suggestions of the supplier. Before being used for TAR cloning, the deleted BACs were made linear by digestion with *EcoRI*, purified by phenol–chloroform extraction, and dissolved in double-distilled H2O at a final concentration of around 1.0 μg/ml.

**DNA probe and PCR analysis of Sir2.** A *T. brucei* Sir2-specific fragment (GenBank acc. no. AF102869) was labeled with [α-32P]dCTP by random-priming DNA synthesis [18] and used to screen the RPCI-102 *T. brucei* library. The posi-
tive BAC clones obtained from this screening were confirmed by PCR amplification with two gene-specific primers of *Try2*. Five BAC clones ranging from 50 to 160 kb were identified and used for further TAR cloning experiments. *Try2*-positive YACs were identified by PCR using diagnostic primers and the following conditions: 2.0 μl YAC DNA with a 0.5-μM concentration of each primer, 25 μM dNTPs, 1.5 mM MgCl₂, and 0.5 U Taq polymerase in a 25-μl volume. The DNA was first denatured at 96°C for 4 min, and amplification was then done at 94°C for 30 s, reannealing at 50°C for 30 s, and extension at 72°C for 30 s, for a total of 30 cycles. PCR products were separated by electrophoresis through 0.8% agarose gels and detected by ethidium bromide staining.

**Fingerprinting of BACs.** After Autogen preparation, 5 μl BAC DNA was digested with *Eco*RI and separated by gel electrophoresis with the BioRad Horizontal system. The fragments were separated in 1% Seakem agarose gel at 45 V and 14°C for 16 h, and were stained with ethidium bromide to view the digestion patterns.

**Sequencing of TAR-BAC isolates for the Try2 clone.** After *Eco*RI deletion and linearization, a deleted BAC with two arms of 2.2 kb and 4.1 kb was obtained for the clone Try2 and used as a TAR-cloning vector to rescue the same 150-kb Try2-containing genomic fragment from genomic DNA. We completely sequenced both arms and generated 23 primers for analysis of the TAR-BAC isolates (Fig. 6, positions and directions of primers).

**Preparation of chromosome-sized DNA in solid agarose plugs for TAR cloning.** About 4 × 10⁶ cells from the wild-type *T. brucei* 427 strain [19] were pelleted and resuspended in 3.0 ml TE (50 mM EDTA and 10 mM Tris, pH 7.5). This cell mixture was separated into 300-μl aliquots and incubated at 42°C. An equal volume of pre-warmed 1% agarose–ethylenediamine tetraacetic acid (low-melt-agarose) was added to each aliquot, mixed completely by vortexing, and poured to the Bio-Rad molds. Agarose plugs (75 μl) containing approximately 15 μg high-molecular-weight *T. brucei* DNA were prepared using a standard procedure [17].

**Yeast strain and transformation.** The highly transformable *S. cerevisiae* strain VL6-48 (MATa, his3-Δ1, trp1-Δ1, ura3-52, lys2-101, met14 c14) [4], from which HIS3 is deleted, was used for transformations. Spheroplasts that enable efficient transformation were prepared using a published protocol [4]. To assess the presence of an ARS sequence(s) in captured genomic inserts, approximately 10 ng DNA of the original large-insert BACs or internally deleted BACs was used for yeast transformation. For TAR cloning transformation, 1.0 μg DNA was added to the yeast-containing plugs (25 μl, containing about 5 μg genomic DNA) before the agarose plugs were melted and treated with agarase [3-4]. Yeast transformants were selected on plates containing synthetic complete medium lacking histidine.

**Preparation of circular YAC DNA from yeast transformants using an alkaline lysis procedure.** Single YAC colonies were grown 48 h in 6 ml synthetic standard selective medium without histidine. The DNA was extracted using an automated yeast genomic DNA isolation system (AutoGen 740; Integrated Separation Systems) with subsequent improvements. REAGENT 2 (sodium hydroxide and Sarkosyl) and REAGENT 3 (potassium acetate, phenol, and ethanol) from the standard plasmid DNA isolation system were used instead of REAGENT 2 (Tris HCl, EDTA, and Sarkosyl) and REAGENT 3 (potassium acetate and phenol) from the standard yeast extract. About 50 ng YAC DNA was obtained from 8.0 ml culture and dissolved in 100 μl TE, pH 8.0. To transfer YAC/BACs into bacteria, 2 μl DNA were used to transform DH10B electrocompetent cells (Life Technologies).

**REFERENCES**


Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession numbers AF255334 and AF255335.