JOURNAL OF BACTERIOLOGY, Oct. 1979, p. 73-82
0021-9193/79/10-0073/10$02.00/0

Vol. 140, No. 1

Chimeric Plasmids for Cloning of Deoxyribonucleic Acid Sequences in *Saccharomyces cerevisiae*

R. K. STORMS,* J. B. McNEIL, P. S. KHANDEKAR, G. AN, J. PARKER,† AND J. D. FRIESEN

Department of Biology, York University, Downsview, Ontario, Canada M3J 1P3

Received for publication 9 March 1979

Two sets of plasmids, each carrying a *Saccharomyces cerevisiae* gene and a portion or all of the yeast 2-μm circle linked to the *Escherichia coli* plasmid pBR322, have been constructed. One of these sets contains a *Bam*HI fragment of *S. cerevisiae* deoxyribonucleic acid that includes the yeast *his3* gene, whereas the other set contains a *Bam*HI fragment of *S. cerevisiae* that includes the yeast *leu2* gene. All plasmids transform *S. cerevisiae* and *E. coli* with a high frequency, possess unique restriction endonuclease sites, and are retrievable from both host organisms. Plasmids carrying the 2.4-megadalton *Eco*RI fragment of the 2-μm circle transform yeast with 2- to 10-fold greater frequency than those carrying the 1.5-megadalton *Eco*RI fragment of the 2-μm circle. Restriction endonuclease analysis of plasmids retrieved from *S. cerevisiae* transformed with plasmids carrying the 2.4-megadalton *Eco*RI fragment showed that in 13 of 96 cases the original plasmid has acquired an additional copy of the 2-μm circle. These altered plasmids appear to have arisen by means of an interplasmid recombination event while in *S. cerevisiae*. A clone bank of *S. cerevisiae* genes based upon one of these composite plasmids has been constructed. By using this bank and selecting directly in *S. cerevisiae*, the *ura3*, *tyr1*, and *met2* genes have been cloned.

The discovery by Hinnen et al. (9) that the yeast *Saccharomyces cerevisiae* can be transformed with ColE1 plasmid DNA carrying the *S. cerevisiae leu2* gene has opened an entirely new approach to the genetics of yeasts. It has been shown that the frequency of *S. cerevisiae* transformation depends upon the choice of yeast DNA fragment. For example, a ColE1 vector carrying the *S. cerevisiae leu2* or *his3* gene transforms *S. cerevisiae* with a frequency of 10^{-7} per viable cell (9). A vector with its own yeast replicon, which can be provided, for example, by the chromosomal region surrounding the *trp1* gene (19) or by the self-replicating, genetically cryptic 2-μm yeast plasmid (2), yields transformation frequencies of 10^{-5} to 10^{-3} per viable cell. Those vectors that transform *S. cerevisiae* with a high frequency were found to exist autonomously (2), whereas those that transform with a low frequency were found only integrated in the chromosome (9).

These observations suggest the possibility of developing an *S. cerevisiae*-based cloning system in which one can select specific eucaryotic genes in a eucaryotic host. To be useful, such a gene cloning vehicle should be capable of transforming both *S. cerevisiae* and *Escherichia coli* with high frequencies and should be retrievable from both organisms. These properties would enable one to detect the expression of eucaryotic DNA sequences in yeasts, yet permit propagation of the cloned genes in *E. coli*, where high yields of plasmid DNA are readily obtained. A further desirable characteristic of the cloning vehicle is the presence of unique restriction endonuclease sites to enable the cloning of foreign DNA. Finally, the vehicle should carry genetic marker(s) that allow selection in both *S. cerevisiae* and *E. coli*.

We describe here two sets of composite plasmids which fulfill these criteria.

MATERIALS AND METHODS

Strains. The *E. coli* plasmids used were pBR313 (3) and pBR322 (4) from H. Boyer. The *E. coli* strains used were JF1125 *leuB recB recC hsdR hsdM lop-11 lig*$^+$ from the collection of R. Davis, JF1161 *hsdR lac gal metB* from the collection of W. Arber, and JF1184 *hisB436* from the collection of P. Hartman. *S. cerevisiae* strains used were the wild-type S288C and LL20 (α *leu2-3 leu2-112 his3-11 his3-15*), the latter from G. R. Fink. The spontaneous reversion frequency to leucine or histidine prototrophy in strain LL20 is less than 10^{-10}. The source of the *S. cerevisiae* 2-μm circle was a plasmid 82-6B, given us by J. Donelson.

Media. The complex medium used for the growth of *E. coli* was L-broth (10), and that for *S. cerevisiae* was YEPD (1% yeast extract, 2% peptone [Difco], 2%

† Present address: Department of Microbiology, Southern Illinois University, Carbondale, IL 62901.

glucose). The defined medium used for growth of *E. coli* was M9 plus 0.2% glucose (1), and that for *S. cerevisiae* was YNB plus 2% glucose, containing 0.67% yeast nitrogen base without amino acids (Difco). Required amino acids were added to 50 μg/ml.

Hybridization conditions. Restriction endonuclease fragments of *S. cerevisiae* DNA were transferred from agarose gels to nitrocellulose membranes and hybridized to radioactive probe as described by Southern (18). ^{32}P-labeled plasmid DNA for probes was prepared by the nick-translation method with *E. coli* DNA polymerase I (13).

Restriction endonucleases and T4 ligase. Restriction endonucleases and T4 ligase were purchased from New England Biolabs, Boston, Mass. Digestion of DNA with restriction endonucleases was as recommended by the supplier. The DNA ligase reaction was carried out under the following conditions: 50 mM Tris-hydrochloride (pH 7.5)–10 mM $MgCl_2$–20 mM dithiothreitol–1.0 mM ATP (ligation buffer). Because of the presence of NaCl in the DNA solution after digestion with restriction endonucleases, the final volumes of the T4 ligase reactions were adjusted so as to reduce the NaCl concentration below 5 mM. In some instances DNA was precipitated with two volumes of 95% ethanol after endonuclease digestion and was dissolved at a concentration of 250 μg/ml in ligation buffer. The ligation reaction mixtures were incubated for 12 h at 15°C with 0.05 U of T4 ligase per μg of DNA; then the DNA was used to transform *E. coli*. DNA fragments were analyzed by 0.7% agarose gel electrophoresis as described elsewhere (17). Restriction fragment sizes were determined with reference to lambda DNA fragments of known size. These analyses form the basis for the restriction maps shown in Fig. 1 through 4.

Transformation. The procedure for transformation of *E. coli* has been described elsewhere (12). Transformation of *S. cerevisiae* was carried out essentially as described by Hinnen et al. (9), except that 20% polyethylene glycol (4000) was used. In our hands, this significantly increased the transformation frequency per microgram of DNA.

DNA preparation. Covalently closed circular plasmid DNA was isolated from *E. coli* essentially as described by Clewell and Helinski (6). *S. cerevisiae* DNA was isolated from small cultures (100 ml or less) by the method of Cryer et al. (7). Large-scale preparation of *S. cerevisiae* DNA from cultures of 1 to 10 liters was purified by a modification of the "MUP" method (15). Cells grown to stationary phase in YEPD were pelleted, washed by centrifugation with 10 mM Tris-hydrochloride–1 mM EDTA, pH 8.0 (TE), and resuspended in 50 ml of TE. Cells were then converted to spheroplasts by the addition of 4 mg of Zymolyase 5000 (Kirin, Japan) per ml and incubation for 2 h at 30°C. Spheroplasts were lysed by the addition of 1% (final concentration) sodium dodecyl sulfate. DNA was isolated from the lysates by two cycles of chloroform-octanol (9:1, vol/vol) extraction (14) and then precipitated by raising the NaCl concentration to 50 mM and adding 2 volumes of ethanol. The DNA precipitate was dissolved in TE. The chloroform-octanol extraction and ethanol precipitation was repeated. The final DNA precipitate was dissolved in 25 ml of 0.2 M Na_2HPO_4–8 M urea and applied under slight pressure to a hydroxyapatite (Sigma Chemical Co., St. Louis, Mo.) column (8-mm diameter, 2-cm bed height). RNA and other impurities were eluted from the column with 100 ml of 0.012 M Na_2HPO_4. The Na_2HPO_4 concentration was then raised to 0.48 M; the DNA usually eluted immediately after the void volume (approximately 2 ml). Eluted DNA was precipitated with 2 volumes of ethanol and dissolved in TE.

RESULTS

Cloning the *S. cerevisiae his3* and *leu2* genes on pBR313. The first step in the construction of a yeast cloning vehicle was to clone the *his3* and *leu2* genes from *S. cerevisiae* on the *E. coli* plasmid pBR313. This was achieved by preparing two "shotgun" banks of *S. cerevisiae* DNA, one each derived from complete digests with either *Hin*dIII or *Bam*HI. The fragments were ligated in the appropriate site of the tetracycline (Tc) gene of pBR313 (3). These ligated DNA preparations were used to transform *E. coli* strain JF1161. Approximately 6,000 independent ampicillin-resistant, tetracycline-sensitive clones derived from each of the *Hin*dIII or *Bam*HI shotgun clonings were picked from agar and grown separately in microtiter wells. The *Hin*dIII or *Bam*HI shotgun clones were also pooled in separate cultures. Plasmid DNA prepared from each of the two pools was used to transform *E. coli* strain JF1184 *hisB* or JF1125 *leuB*, selecting for ampicillin resistance and either the $HisB^+$ or $LeuB^+$ phenotype. For each of these two auxotrophic markers, only the DNA derived from the *Bam*HI pool of *S. cerevisiae* DNA yielded plasmids capable of complementing the *E. coli* mutants. Plasmid DNA was prepared from clones of each of the complemented *E. coli* strains and was verified by a second transformation in the appropriate host strain of *E. coli*.

The plasmid carrying the gene which complements *hisB* mutants of *E. coli* (*his3* of *S. cerevisiae* [19]) is called pYF40 and is carried in *E. coli* strain YF40. That which complements *leuB* mutants of *E. coli* (*leu2* of *S. cerevisiae* [16]) is called pYF36 and is carried in *E. coli* strain YF36.

To demonstrate that the *hisB*- and *leuB*-complementing genes carried on the plasmids were derived from *S. cerevisiae*, covalently closed circular DNA prepared from *E. coli* strains YF40 and YF36 was radioactively labeled by the nick-translation method (13) and hybridized by the Southern technique (18) to fragments of total *S. cerevisiae* DNA digested with *Bam*HI. These results are not shown here, but they indicated that pYF36 DNA hybridized to a *Bam*HI restriction fragment of total yeast DNA that is the

same size as the smaller *Bam*HI fragment from pYF36 itself. This presumably is the fragment that carries the *S. cerevisiae leu2* gene. A similar finding was made for pYF40 (data not shown). In neither case was there any detectable annealing to *E. coli* DNA.

Construction of three chimeric plasmids carrying portions of the 2-μm circle inserted in pBR322. The endogenous *S. cerevisiae* plasmid, the 2-μm circle, was used as a yeast replicon. The source of 2-μm DNA was a plasmid, 82-6B, given us by John Donelson. This plasmid contains a partially reiterated R-form (11) of the 2-μm circle inserted with adenine and thymine tails in the *Eco*RI site of pMB9 (J. Donelson, personal communication; Fig. 1). Fragments resulting from a partial *Eco*RI digest of DNA from plasmid 82-6B were ligated in the *Eco*RI site of pBR322. The products of this ligation were then used to transform *E. coli* strain JF1161, selecting for both ampicillin and tetracycline resistance. The DNA from 20 of the resulting drug-resistant clones was analyzed and one of these, strain YF86, yielded a plasmid, pYF86, carrying one complete copy of the 2-μm circle.

To construct a smaller plasmid capable of efficient transformation of *S. cerevisiae*, plasmid pYF86 was subjected to partial *Eco*RI digestion followed by ligation and transformation in *E. coli* strain JF1161. This step yielded two additional plasmids. One, pYF84, contained the 1.5-megadalton (MD) *Eco*RI fragment of the 2-μm (3.9-MD) circle and a second, pYF85, contained the 2.4-MD *Eco*RI fragment of the 2-μm circle.

Construction of composite plasmids carrying the *his3* gene of *S. cerevisiae*. Three related plasmids containing the *his3* gene were constructed as follows. DNA from each of the three plasmid types described in the preceding section (pYF86, pYF84, and pYF85) was digested with *Bam*HI and then ligated to *Bam*HI fragments derived from pYF40 (the composite pBR313-*his3* plasmid described earlier). pYF40 DNA had also been digested with *Sal*I to minimize the probability of reconstituting that plasmid in the ligation step. After ligation of the *Bam*HI fragments, DNA was used to transform strain JF1184 (*hisB*), selecting for ampicillin resistance and *hisB* complementation. Finally, covalently closed circular plasmid DNA was isolated from these transformants. The structures of the three types of plasmids derived from this construction (pYF87, pYF88, and pYF92) are shown in Fig. 1. Each of the three types of *his3* cloning vehicles (pYF87, pYF88, and pYF92) contains unique *Sal*I, *Xho*I and *Kpn*I restriction sites. Of these, the *Sal*I restriction site lies outside the *his3* gene and is thus ideal as a cloning site. A detailed restriction map of pYF92 is presented in Fig. 2.

Construction of composite plasmids carrying the *leu2* gene of *S. cerevisiae*. Composite plasmids containing *leu2* were constructed as follows (Fig. 3). The first step was to insert the *leu2* gene derived from pYF36 (described above) in the *Bam*HI site of plasmid 82-6B. This plasmid is called pYF81. pYF81 has suffered spontaneous loss of an as yet undetermined portion of the 2-μm circle sequences. DNA from pYF81 was digested with *Bam*HI and ligated to DNA from a complete *Bam*HI digest of pYF88. The ligated mixture was used to transform *E. coli* strain JF1125 (*leuB*); ampicillin resistance and leucine prototrophy were selected. This operation excised the *his3*-carrying *Bam*HI fragment from pYF88 and exchanged it for the *leu2*-carrying *Bam*HI fragment of pYF81. The fact that pYF88 carries Ap^r and pYF81 does not allows us to detect the *leu2* insert in the newly constructed plasmid. This plasmid (called pYF90) has a unique *Xho*I site. Plasmid pYF90 was further modified by digestion with *Hin*dIII, ligation, and transformation in strain JF1125 (*leuB*), once again selecting ampicillin resistance and leucine prototrophy. This operation yielded plasmid pYF91 carrying the *leu2* DNA fragment of *S. cerevisiae* lacking a small *Hin*dIII fragment that was present in pYF90. The final plasmid, pYF91, has unique sites for *Bam*HI, *Hin*dIII, and *Xho*I (Fig. 4).

Table 1 summarizes the characteristics of the plasmids constructed in this study.

Transformation of *S. cerevisiae* with composite plasmids. Plasmids pYF36, pYF81, pYF90, and pYF91 carrying the *leu2* gene were used to transform *S. cerevisiae* strain LL20. The results of a typical transformation are shown in Table 2. These four plasmids fall into three categories with respect to transformation frequency. pYF36 has no 2-μm circle sequences and transforms with a frequency of only 6.1×10^{-8}. pYF81 has an almost complete copy of the 2-μm circle, but lacks sequences in the repetitive portion of the 2.4-MD *Eco*RI fragment (unpublished data) and transforms with an intermediate frequency of 2.6×10^{-5}. pYF90 and pYF91 contain the complete 2.4-MD *Eco*RI fragment of the 2-μm circle and transform the most efficiently of all (approximately 10^{-4}).

A similar experiment was done with a set of *his3* plasmids, pYF40, pYF87, pYF88, and pYF92 (Table 2). Again it was observed that the inclusion of the 1.5-MD *Eco*RI fragment of the 2-μm circle raised the transformation frequency by at least two orders of magnitude (pYF87

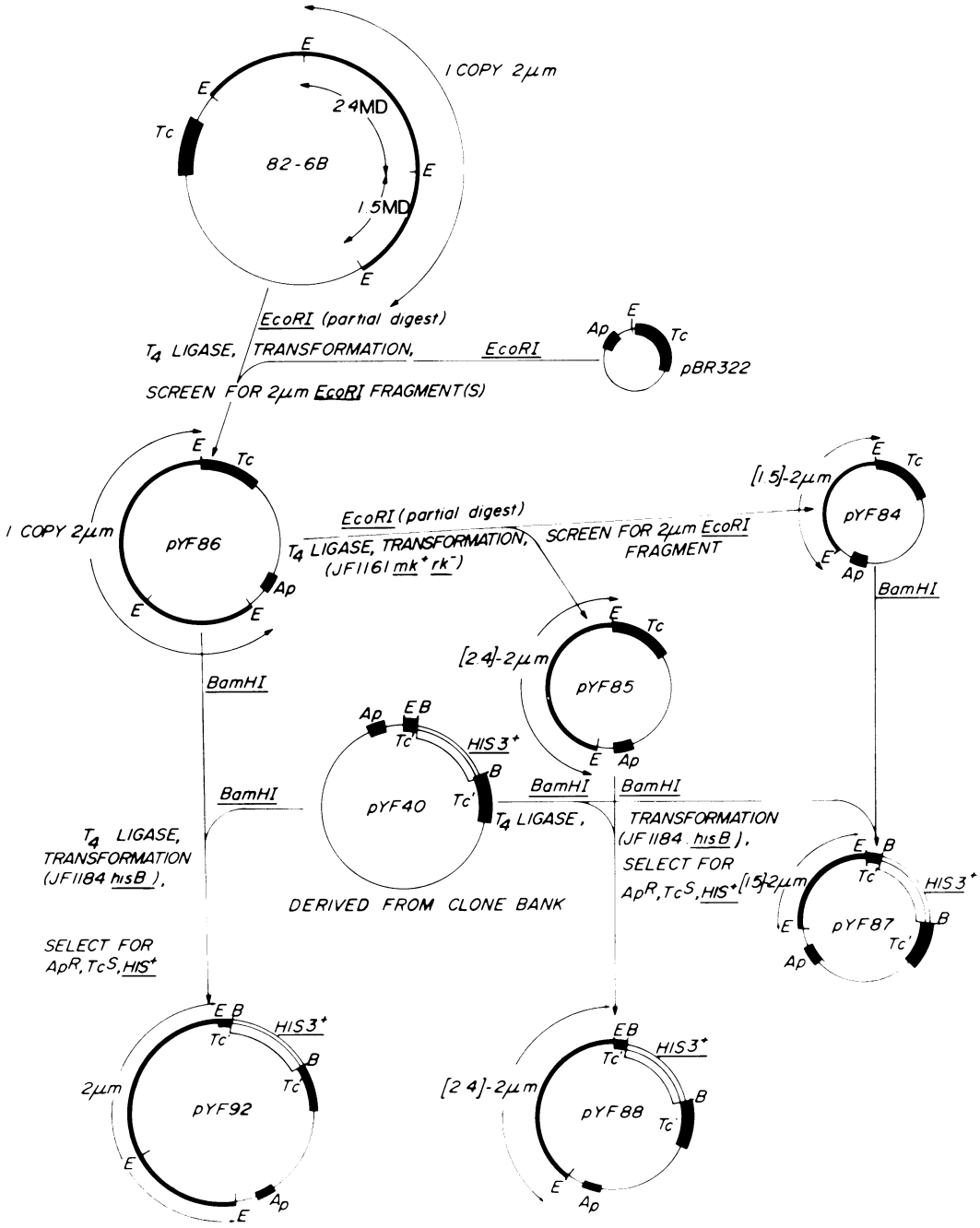

FIG. 1. *Schematic diagram of construction of the chimeric plasmids pYF84, pYF85, pYF86, pYF87, pYF88, and pYF92. Thin-lined segments represent DNA from an E. coli plasmid. Intermediate thickness of segments represent DNA from the S. cerevisiae 2-μm circle. Triple segments represent DNA from the S. cerevisiae genome. Thick segments represent plasmid genes. Abbreviations used: E, EcoRI; B, BamHI; H, HindIII; Ap, ampicillin resistance gene; Tc, tetracycline resistance gene(s).*

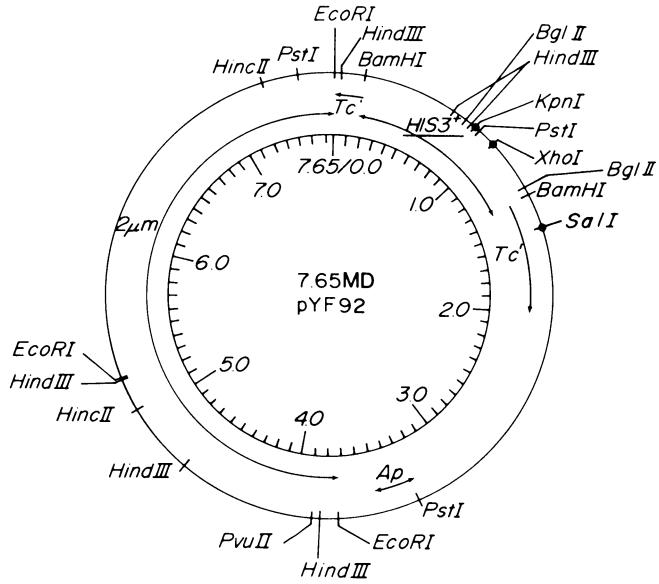

FIG. 2. *Detailed restriction map of pYF92. The dots indicate positions of unique restriction endonuclease sites. See legend to Fig. 1 for abbreviations and text for details.*

compared with pYF40); inclusion of the 2.4-MD *Eco*RI fragment raised the frequency by an additional 2- to 10-fold.

We have observed day-to-day variation in the transformation frequency with the same DNA stock but independently prepared competent cells of a particular host strain. However, experiments with the same preparation of competent host cells consistently yielded lower transformation frequencies by those plasmids with the 1.5-MD fragment of the 2-μm circle than by those with the 2.4-MD fragment. To verify these observations, six independently prepared batches of competent LL20 cells were transformed with pYF87 and pYF88 DNA, selecting for histidine prototrophy. In each of the six transformations the plasmid carrying the 2.4-MD fragment (pYF88) transformed yeast at a higher frequency than the plasmid carrying the 1.5-MD fragment (pYF87). A *t* test analysis of the data indicated this result to be significant, with $P = 0.02$. These observations suggest that the large *Eco*RI fragment contains nucleotide sequences that are especially important for high-frequency transformation.

Hybridization analysis of yeast transformants. *S. cerevisiae* strain LL20 was transformed with pYF88, which carries *his3* plus the 2.4-MD fragment of the 2-μm circle. Ten independently transformed colonies were selected for further study. DNA was isolated from them by the method of Cryer et al. (7) and digested with *Bam*HI. Southern blots (18) were prepared from agarose gels of these digests, and the DNA was hybridized with ^{32}P-labeled pBR322. Hybridization of pBR322 thus provides a marker for those *S. cerevisiae* DNA fragments which carry at least a portion of the original cloning vehicle. Two typical examples of results obtained for strain LL20 transformed with pYF88 are shown in Fig. 5, lanes 2 and 3. Two major bands of hybridization to pBR322 are seen. The lower band in lanes 2 and 3 corresponds to the *Bam*HI fragment of pYF88 which includes pBR322 (Fig. 5, lane 1). This represents sequences of pBR322 which either are resident on pYF88 or are integrated in the yeast chromosome (9). The upper band in lanes 2 and 3 of Fig. 5 might represent *Bam*HI fragments from larger plasmids that result from recombinational integration of resident 2-μm DNA with the transforming DNA. Further evidence in support of this notion is presented below.

The mobility of the *Bam*HI fragment derived from the plasmids is not exactly coincident with the corresponding fragment from transformed *S. cerevisiae*. (For example, compare lane 1 with the lower band of lanes 2 and 3 in Fig. 5.) The reason for this is not known, but is probably a gel artifact, since data to be presented below indicate that the *Bam*HI fragments represented by the lower band of Fig. 5, lanes 2 and 3, are unaltered in comparison with the original transforming plasmid.

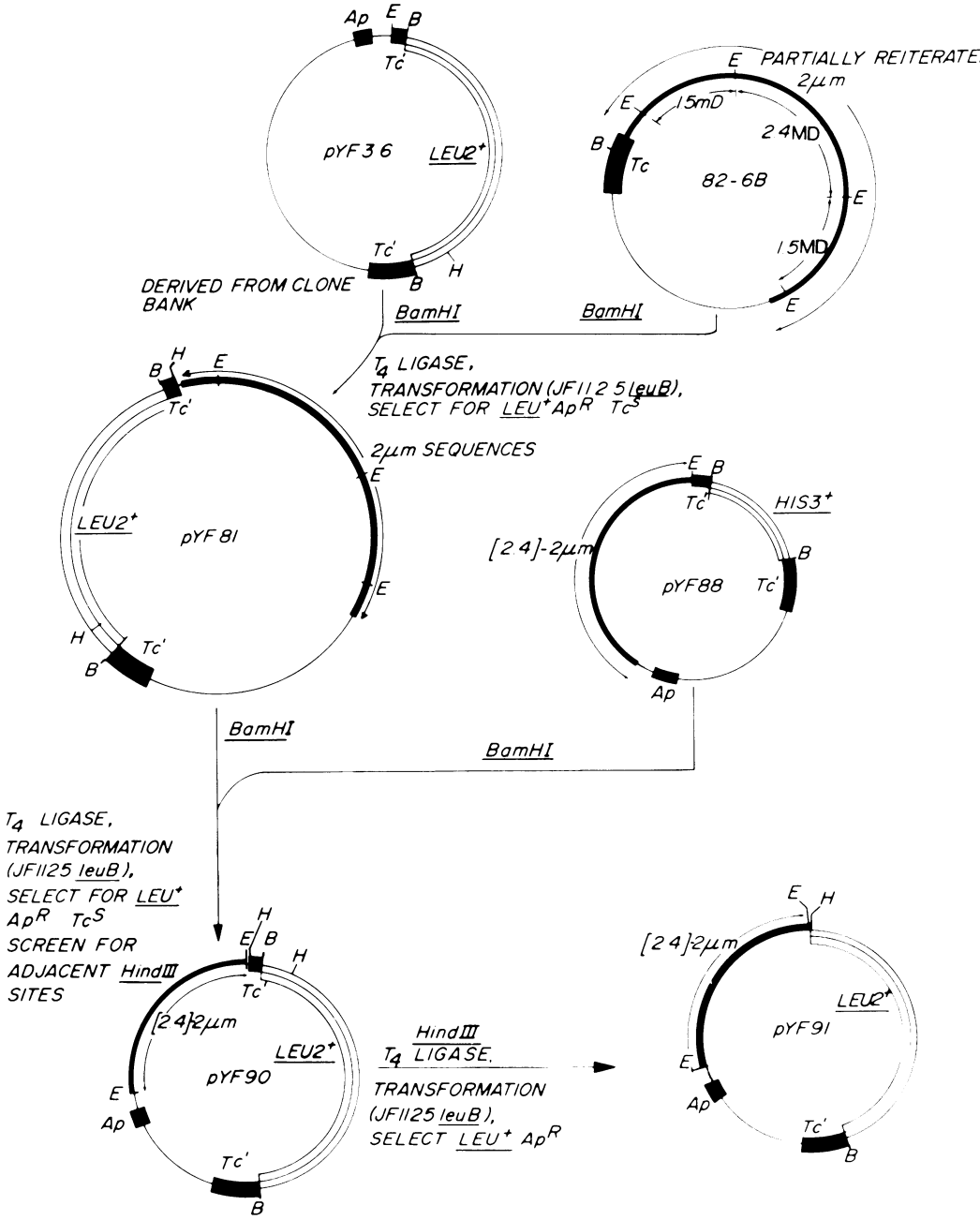

FIG. 3. *Schematic diagram of the construction of the chimeric plasmids pYF81, pYF90, and pYF91. See legend to Fig. 1 for abbreviations and text for details.*

A similar experiment was carried out with pYF90 (*leu2*). Figure 5, lanes 5 and 6, shows results indicating that pBR322 sequences from this plasmid also are resident in at least two locations in *S. cerevisiae*.

Recovery of composite plasmids from *S. cerevisiae*. DNA was prepared from 10 independent isolates of *S. cerevisiae* strain LL20 that had been transformed with pYF90 (*leu2*). All 10 of these preparations were used independently to transform *E. coli* strain JF1125, selecting ampicillin resistance. Several transformed bac-

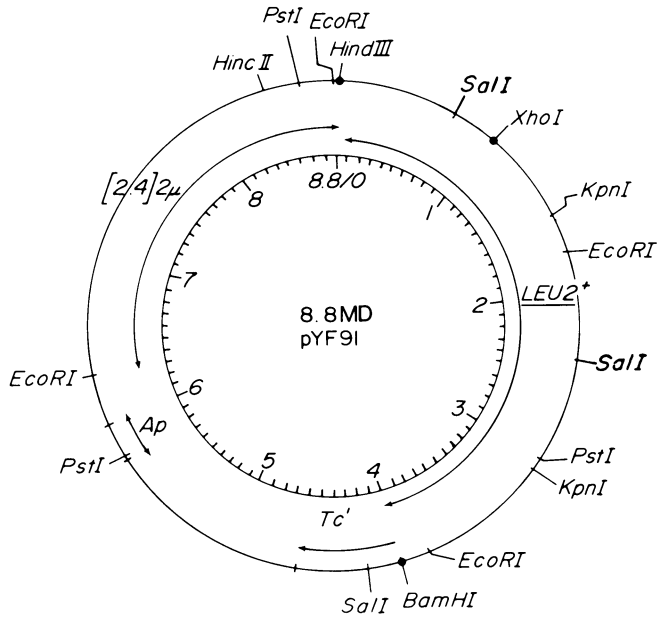

FIG. 4. *Detailed restriction map of pYF91. The dots indicate positions of unique restriction endonuclease sites. See legend to Fig. 1 for abbreviations and text for details.*

TABLE 1. *Constructed chimeric plasmids*

Chimeric plasmid	*E. coli* plasmid	Drug resistance	Yeast gene	2-μm circle *Eco*RI fragments
pYF36	pBR313	Ap^r Tc^s	*leu2*	
pYF40	pBR313	Ap^r Tc^s	*his3*	
pYF84	pBR322	Ap^r Tc^r		1.5 MD
pYF85	pBR322	Ap^r Tc^r		2.4 MD
pYF86	pBR322	Ap^r Tc^r		1.5 MD + 2.4 MD (intact 2-μm circle)
pYF87	pBR322	Ap^r Tc^s	*his3*	1.5 MD
pYF88	pBR322	Ap^r Tc^s	*his3*	2.4 MD
pYF92	pBR322	Ap^r Tc^s	*his3*	1.5 MD + 2.4 MD (intact 2-μm circle)
pYF81	pMB9	Ap^s Tc^s	*leu2*	(Approx 1 copy of 2-μm circle)
pYF90	pBR322	Ap^r Tc^s	*leu2*	2.4 MD
pYF91	pBR322	Ap^r Tc^s	*leu2*	2.4 MD

TABLE 2. *Transformation frequencies using chimeric plasmids*[a]

Plasmid	Transformants per μg of DNA added	Transformants per surviving spheroplast
leu2		
pYF36	30	6.1×10^{-8}
pYF81	12,600	2.6×10^{-5}
pYF90	55,000	1.1×10^{-4}
pYF91	45,500	9.3×10^{-5}
his3		
pYF40	5	1×10^{-8}
pYF87	15,880	3.2×10^{-5}
pYF88	49,500	1.0×10^{-4}
pYF92	19,300	3.9×10^{-5}

[a] Transformation of strain LL20 was carried out as described in the text. The data presented in this table are from a single experiment in which a single batch of spheroplasts was divided into aliquots and transformed with the different plasmids.

terial clones from each of the 10 sets (36 in all) were checked for leucine requirement; all were found to be prototrophic. Covalently closed circular DNA isolated from all 36 of these *E. coli* tranformants was digested with *Eco*RI or *Bam*HI and analyzed by agarose gel electrophoresis (Fig. 6a). Thirty-three of these covalently closed circular DNA preparations proved to have the same digestion pattern as the parent plasmid pYF90. (For one example of this class, compare lanes 1 and 2 with lanes 3 and 4 in Fig. 6a.) Three plasmids, each retrieved from a different initial yeast transformant, showed two extra *Eco*RI fragments (lane 5) and an altered *Bam*HI fragment (lane 6). These new bands are indicated by white arrowheads placed to their left in lanes 5 and 6. In lane 5 the uppermost of the two marked bands coincides with two 2.4-MD bands already present in the original plasmid. We believe that in lane 5 this band is now actually a triplet, one of whose components originates from an acquired single copy of the 2-μm circle (see below). This notion is based on the size difference between the upper band of lane 6 (8.8 MD) and the upper band of lane 4 (5.0 MD), which equals 3.8 MD and corresponds to the size of a single 2-μm circle. This additional

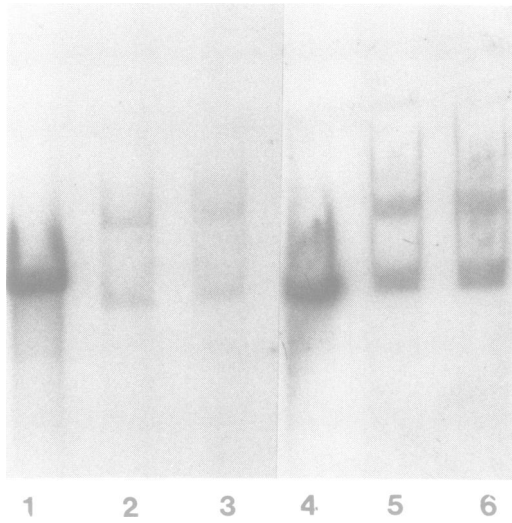

FIG. 5. *Autoradiography of ^{32}P-labeled pBR322 DNA hybridized to Southern blots of BamHI restriction digests of DNA from: lane 1, pYF88 (his3); lanes 2 and 3, strain LL20 transformed with pYF88; lane 4, pYF90 (leu2); lane 5 and 6, strain LL20 transformed with pYF90; DNA from strain LL20 did not hybridize with the probe (data not shown).*

DNA appears in lane 5 as additional fragments of 1.5 MD (lower arrow) and 2.4 MD (upper arrow).

The *Eco*RI and *Bam*HI restriction patterns of these altered plasmids are those to be expected if the original plasmid, pYF90, had acquired an additional copy of the 2-μm circle through a single recombination event along the lines suggested by Campbell (5). Recombination between any portion of the 2-μm sequences of pYF90 and a homologous sequence of a resident 2-μm circle could result in the observed structures. This finding is under further investigation.

A somewhat different experiment was carried out with *S. cerevisiae* strain LL20 that had been transformed with pYF88 (*his3*). DNA from a single yeast transformant was used to transform *E. coli* strain JF1161, selecting ampicillin resistance. Crude lysates were prepared from 60 of these bacterial transformants, the DNA digested with *Eco*RI or *Sal*I and displayed on an agarose gel (Fig. 6b). Fifty of the sixty were found to have the same structure as the parent plasmid, an example of which is shown in Fig. 6b, lanes 3 and 4. Ten had alterations in structure that were consistent with the recombinational insertion of a 2-μm circle (lanes 1 and 2, where the extra or altered bands are indicated with white arrows).

Isolation of cloned genes by direct selection in *S. cerevisiae*. A clone bank of *S. cerevisiae* genes has been prepared by subjecting *S. cerevisiae* DNA to partial *Hin*dIII digestion and ligating this in the *Hin*dIII site of pYF91. The yeast DNA inserts in this library range from 1 to 10 MD in size. Transformation of *S. cerevisiae* with this *Hin*dIII library has enabled the isolation of plasmids with the following DNA fragments: a 1.0-MD *Hin*dIII fragment carrying the *ura3* gene, a 3.3-MD *Hin*dIII fragment carrying the *tyr-1* gene, and a 4.4-MD *Hin*dIII fragment carrying the *met-2* genes (data not shown). These plasmids are currently being characterized further.

DISCUSSION

We have constructed four composite plasmids suitable for use as cloning vehicles in either *S. cerevisiae* or *E. coli*. They are based on the bacterial plasmid pBR322 linked to a portion or all of the yeast plasmid, the 2-μm circle, and also carrying a genetic marker selectable in either host organism. These chimeric plasmids are capable of transforming both *E. coli* and *S. cerevisiae* at a high frequency. Two of these plasmids, pYF88 and pYF92, carry the *his3* gene of *S. cerevisiae* and have unique *Sal*I, *Kpn*I and *Xho*I restriction sites. The other two, pYF90 and pYF91, carry the *leu2* gene of *S. cerevisiae* and have a unique *Xho*I restriction site; pYF91 also has unique *Bam*HI and *Hin*dIII restriction sites. Thus, unique cut sites enable the cloning of additional DNA in all of these plasmids.

The most useful restriction sites for cloning purposes are those that do not destroy the function of the *his3* or *leu2* structural genes. For pYF91 these are the *Hin*dIII and *Bam*HI sites; for pYF92 it is the *Sal*I site.

We have found, as has Beggs (2), that our composite plasmids can be recovered from transformed *S. cerevisiae*. The majority of the 96 plasmids recovered from pYF88- and pYF90-transformed *S. cerevisiae* were found to be structurally identical to the parental plasmid; interestingly, 13 of them were altered. These altered plasmids have acquired an additional complete copy of 2-μm circle DNA and may have arisen by interplasmid recombination events, involving the 2-μm circle portion of pYF90 or pYF88 and a homologous sequence of a resident 2-μm plasmid. This finding is consistent with the observation that pBR322 hybridizes to two *Bam*HI fragments of DNA extracted from pYF88- and pYF90-transformed *S. cerevisiae*. Recombination between 2-μm-circle-based plasmids has also recently been reported by Gerbaud et al. (8).

Plasmids that carry the 2.4-MD *Eco*RI fragment of the 2-μm circle recombine with the resident 2-μm circle relatively infrequently (13/

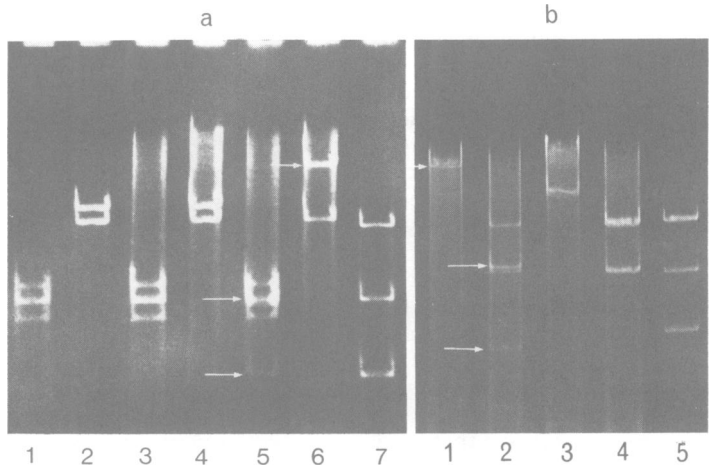

FIG. 6. *Restriction endonuclease digestion patterns of plasmids recovered from transformed S. cerevisiae. DNA was isolated from transformed S. cerevisiae and used to transform E. coli. Covalently closed circular DNA was recovered from the transformed bacteria, digested with restriction endonuclease, and analyzed by agarose gel electrophoresis. (a) Analysis of plasmids retrieved from a pYF90-transformed LL20. Lane 1, EcoRI digest of pYF90. From top to bottom, the bands correspond to the following masses: 2.6 MD, a doublet of 2.4 MD, and 2.1 MD. Lane 2, BamHI digest of pYF90. From top to bottom, the bands correspond to the following masses: 5.0 and 4.4 MD. Lane 3, EcoRI digest of an unaltered plasmid retrieved from pYF90-transformed S. cerevisiae. From top to bottom, the bands correspond to the following masses: 2.6 MD, a doublet of 2.4 MD, and 2.1 MD. Lane 4, BamHI digest of the same plasmid as shown in lane 3. From top to bottom the bands correspond to the following masses: 5.0 and 4.4 MD. Lane 5, EcoRI digest of a plasmid retrieved from pYF90-transformed S. cerevisiae that shows the acquisition of an extra copy of the R-form of the 2-μm circle (arrows). From top to bottom, the bands correspond to the following masses: 2.6 MD, a triplet of 2.4 MD, and 2.1 MD. Lane 6, BamHI digest of the plasmid shown in lane 5. From top to bottom, the bands correspond to the following masses: 8.8 and 4.4 MD. Lane 7, EcoRI digest of 82-6B. From top to bottom, the bands correspond to the following masses: 4.2, 2.4, and 1.5 MD. (b) Analysis of plasmids retrieved from pYF88-transformed LL20. Lane 1, SalI digest of a plasmid retrieved from pYF88-transformed S. cerevisiae that has acquired an extra copy of the 2-μm circle. The single band corresponds to a mass of 10.1 MD. Lane 2, EcoRI digest of the same plasmid as shown in lane 1. This plasmid has acquired an additional copy of the L-form (11) of the 2-μm circle as indicated by the presence of 1.4- and 2.5-MD EcoRI fragments (white arrows). From top to bottom, the bands correspond to the following masses: 3.8, 2.5, 2.4, and 1.4 MD. Lane 3, SalI digest of an unaltered pYF88 plasmid. The single band corresponds to a mass of 6.2 MD. Lane 4, EcoRI digest of the same plasmid as shown in lane 3. From top to bottom the bands correspond to the following masses: 3.8 and 2.4 MD. Lane 5, EcoRI digest of 82-6B. From top to bottom the bands correspond to the following masses: 4.2, 2.4, and 1.5 MD.*

96). We have also analyzed the plasmids recovered from *S. cerevisiae* that had been transformed with one of the composite plasmids carrying the smaller (1.5-MD) *Eco*RI fragment of the 2-μm circle such as pYF87 and found, to our surprise, that almost 50% of these carried additional 2-μm plasmid sequences (unpublished results). This observation, and the fact that pYF87 transforms *S. cerevisiae* 2- to 10-fold less efficiently than pYF88, suggest that for a plasmid like pYF87 to transform *S. cerevisiae* it must first recombine with a resident 2-μm circle or homologous sequences in the nuclear genome. The unaltered plasmids recovered from cells transformed with pYF87 could arise as a reversal of the original recombination event. Alternatively, the 1.5-MD fragment may contain a low-efficiency replication origin.

Our results are in general agreement with those of Struhl et al. (20) with two exceptions. First, we find that the 2.4-MD *Eco*RI fragment of the 2-μm circle is necessary for maximum transformation frequency, whereas Struhl et al. (20) suggest that the inverted repeat sequences are essential. Second, Struhl et al. (20) suggest that, in hybridization to the Southern blots of DNA from transformed *S. cerevisiae*, bands which do not correspond to the original transforming plasmid are due to recombination of the plasmid with a homologous region of the host chromosome. In contrast, we postulate that these extra bands arise from recombination between the transforming plasmid and the resident 2-μm plasmid; as evidence, we point to our observation that such recombination can take place. Experiments to resolve these differences

are in progress.

In principle, any high-transforming cloning vehicle, such as pYF88, pYF92, pYF90, or pYF91, can be useful for the direct isolation in *S. cerevisiae* of DNA sequences from a variety of sources. For example, a pool of such vectors carrying "shotgunned" fragments of the *S. cerevisiae* genome could be used as a source of genetic probes for specific yeast genes. However, in practice this approach is near the limit of practicability. A calculation of the product of the number of *S. cerevisiae* transformants per surviving spheroplasts (at best, 3×10^{-3}) and the average number of fragments (several thousand) derived from a complete digest of the *S. cerevisiae* genome with the commonly used restriction endonucleases convinces one that for a particular gene the transformation frequency is likely to be very near the spontaneous reversion frequency. This limitation can be overcome by the judicious use of such vectors as pYF91 or pYF92. For example, by cloning *S. cerevisiae* DNA in the appropriate restriction site of either of these plasmids and selecting *his3*$^+$ or *leu2*$^+$ transformants and a second gene of interest, one can carry out genetic screenings at a level far above the typcial spontaneous reversion frequency. In fact, it is now possible to screen for nonselectable genes. The isolation of *S. cerevisiae* sequences coding for the *tyr1*, *ura3*, and *met2* genes by using a *S. cerevisiae* DNA library cloned in pYF91 demonstrates the usefulness of this system.

ACKNOWLEDGMENTS

We thank Isobel Matthews for technical assistance, Joan Sheehy for editorial assistance, and Gordon Temple for photographic expertness.

This work was supported by the National Research Council (grants A5734 and A0627) and the National Cancer Institute of Canada. R.K.S. is the holder of a National Research Council postdoctoral fellowship.

LITERATURE CITED

1. **Anderson, E. H.** 1946. Growth requirements of virus resistant *Escherichia coli* strain B. Proc. Natl. Acad. Sci. U.S.A. **32**:120–122.
2. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
3. **Bolivar, F., R. L. Rodriguez, M. C. Betlach, and H. W. Boyer.** 1977. The circular restriction map of pBR313, p. 684–685. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. The circular restriction map of pBR322, p. 686–687. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Campbell, A.** 1962. Episomes. Adv. Genet. **11**:101–145.
6. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428–4440.
7. **Cryer, D. R., R. Eccleshall, and J. Marmur.** 1975. Isolation of yeast DNA. Methods Cell Biol. **12**:39–44.
8. **Gerbaud, C., P. Fournier, H. Blanc, M. Aigle, H. Heslot, and M. Guerineau.** 1979. High frequency yeast transformation by plasmids carrying part or entire 2-μm yeast plasmid. Gene **5**:233–253.
9. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**: 1929–1933.
10. **Lennox, E. S.** 1975. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
11. **Livingston, D. M., and H. L. Klein.** 1977. Deoxyribonucleic acid sequence organization of a yeast plasmid. J. Bacteriol. **129**:472–481.
12. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **73**:453–471.
13. **Maniatis, T., A. Jeffery, and D. Kleid.** 1975. Nucleotide sequences of the rightward operator of phage lambda. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
14. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**: 208–218.
15. **Meinke, W., D. A. Goldstein, and M. R. Hall.** 1974. Rapid isolation of mouse DNA from cells in tissue culture. Anal. Biochem. **58**:82–88.
16. **Ratzkin, B., and J. Carbon.** 1977. Functional expression of cloned yeast DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:487–491.
17. **Sharp, P. A., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenza* using analytical agarose-ethidium bromide electrophoresis. Biochemistry **12**: 3055–3063.
18. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol. **98**:503–517.
19. **Struhl, K., J. R. Cameron, and R. W. Davis.** 1976. Functional genetic expression of eukaryotic DNA in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **73**:1471–1475.
20. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. U.S.A. **76**:1035–1039.