High-frequency transformation of yeast by plasmids containing the cloned yeast ARG4 gene

(RECOMBINANT DNA/YEAST REPLICON/YEAST PLASMID/ARGININOSUCCINATE L YASE)

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ABSTRACT Hybrid CoE1 plasmids, containing cloned DNA from the yeast ARG4 region [e.g., pYe(arg4)1], transform yeast arg4 mutants to ARG4 + with a frequency of 10^-4 (about 10^5 transformants per mg of plasmid DNA) and can replicate autonomously without integrating into the yeast genome. The yeast transformants are genetically unstable when grown on nonselective medium, but can be readily grown and maintained on minimal medium lacking arginine. The existence of unintegrated replicating plasmid DNA in the yeast transformants was demonstrated by Southern gel hybridization and by transformation of Escherichia coli argH mutants with DNA preparations from yeast transformants and subsequent recovery of intact plasmid DNA from the bacterial transformants. Plasmid DNAs recovered from the E. coli–yeast–E. coli "shuttle" remain essentially unchanged, as judged by DNA restriction fragment patterns. Some plasmid mutations leading to increased efficiency of expression of the ARG4 gene in E. coli do not appear to affect expression of the cloned ARG4 gene in yeast. Appropriate derivatives of these ARG4 plasmids are of potential usefulness as vectors for cloning genes in yeast and for studying the mechanism of yeast DNA replication.

The transformation of yeast with exogenous DNA was first demonstrated by Hinnen et al. (1). They succeeded in transforming a yeast leu2– strain to LEU2 + by using a derivative of bacterial plasmid CoE1 carrying the yeast LEU2 gene, designated pYe(leu2)10 (2). With plasmid pYe(leu2)10 DNA, the transformation frequency was quite low (10^-6 to 10^-7) and the transforming plasmid DNA was found to be integrated into the yeast genome at the leu2 locus and at several other genetic sites (1). High-frequency transformation of yeast has been accomplished by use of a hybrid plasmid that contains as a selective marker the same LEU2 gene fused to a yeast plasmid (2μ DNA) and an Escherichia coli vector (pMB9), thus enabling the hybrid plasmid to be replicated autonomously in both yeast and E. coli (3). Struhl et al. (4) have also described high-frequency transformation of yeast by using bacterial plasmid vectors containing a yeast DNA restriction fragment that includes the TRP1 gene or by using hybrid plasmids containing segments of 2μ plasmid DNA.

We have been able to transform yeast arg4– strains to ARG4 + with a frequency of approximately 10^-4 by using hybrid CoE1 plasmids containing yeast DNA that includes the ARG4 region. In the experiments described here, we show that the transforming plasmid, pYe(arg4)1 (ref. 2), can replicate autonomously in yeast and can be recovered in E. coli without apparent modification by the yeast cells. We also show that certain plasmid mutations in pYe(arg4)1 that lead to increased efficiency of expression of the ARG4 gene in E. coli do not appear to affect the expression of the same cloned ARG4 gene in yeast. These mutant plasmids maintain their altered structures and phenotypic characteristics when "shuttled" back into E. coli by transformation of the appropriate argH strains.

MATERIALS AND METHODS

Strains. All yeast strains (Saccharomyces cerevisiae) used in this study were obtained from the Yeast Genetic Stock Center (Donner Laboratory, University of California, Berkeley, CA). The arg4 strains used are the following: S2072A (a arg4 gal2 leu1 trp1), X3656-7D (a leu1 ade6 ura1 arg4-1 thr1), and Z136-1-13c (a arg4-2 leu1 ade1 trp5).

Plasmids. Plasmids pYe(arg4)1, pYe(arg4)2, pYe(arg4)3, and pYe(arg4)4 have been described (2).

Media. YPD (rich medium) and SD (minimal medium) for yeast (5) and M9 (minimal medium) and LB (rich medium) for E. coli (6) were prepared and used as described.

DNA Preparations. Plasmid DNA was isolated as described by Ratzkin and Carbon (7). Yeast DNA was prepared by the following procedure. Spheroplasts were prepared from a fresh logarithmic phase yeast culture in minimal medium (250 ml) according to the method of van Soolingen and van der Plaat (8). The spheroplasts were resuspended in 2 ml of 0.9 M sorbitol and then lysed by osmotic shock with the addition of 20 ml of 50 mM sodium citrate (pH 5.8) containing 20 mM EDTA and 0.1 mg of proteinase K per ml. After 1 hr of incubation at 37°C, 2 ml of 1 M Tris-HCl (pH 8.0) was added and the lysate was extracted twice with 10 ml of phenol (that had been saturated with 10 mM Tris-HCl, pH 8.0/10 mM NaCl/1 mM EDTA). The total yeast nucleic acids were then precipitated with ethanol and used directly for E. coli transformations without further purification.

Transformation of Yeast. A modification of the procedure of Hinnen et al. (1) was used for yeast transformation (Louise Clarke, personal communication). A 40-ml aliquot of fresh logarithmic phase yeast culture (1-2 X 10^7 cells/ml) was harvested by centrifugation. The cells were resuspended in 4 ml of 0.8 M sorbitol and treated with 40 μl of Zymolyase 60,000 (Kirin Brewery Co., Tokyo, Japan) (0.5 mg/ml) at 30°C for 1 hr. The spheroplasts were washed twice with 0.8 M sorbitol and resuspended in 10 ml of 1.0 M sorbitol in 67% YPD medium. After 1 hr of incubation at 30°C, the spheroplasts were centrifuged and resuspended in 0.4 ml of 0.9 M sorbitol/67% YPD medium/10 mM Tris-HCl, pH 7.5/10 mM CaCl_2. Plasmid DNA (5-10 μg) was added to 0.1 ml of the spheroplast suspension and the mixture was incubated at room temperature for 15 min. Finally, 1 ml of 40% polyethylene glycol 4000 (BDH Chemicals Ltd., Poole, England)/10 mM Tris-HCl/10 mM CaCl_2, pH 7.5, was added and the mixture was shaken briefly by hand. After 30 min, 0.2-ml aliquots were added to 10 ml of regeneration minimal agar and poured on minimal agar plates.

Abbreviations: bp, base pair; kb, kilobase pair.
as described (8). Regeneration agar contains 0.67% Difco yeast nitrogen base, 0.9 M sorbitol, 2% glucose, 3% Difco Bacto-agar, plus other nutrients as required.

DNA-DNA Hybridization. Yeast DNA preparations and their restriction digests were fractionated on 0.8% agarose horizontal slab gels in a Tris borate buffer system (9) and transferred to nitrocellulose strips (Millipore HAWP) according to the method of Southern (10). Hybridization was performed with plasmid DNA labeled in vitro by nick translation (11) as described by Chinault and Carbon (12).

Biohazard Considerations. This work was carried out under the containment conditions specified by the National Institutes of Health Guidelines for Recombinant DNA Research (June 1976) and the National Institutes of Health memorandum, dated March 28, 1978, entitled "Recombinant DNA Experiments Involving Yeast Hosts."

RESULTS

Transformation of Yeast arg4 Mutants. We have previously described the molecular cloning and characterization of a 12-kilobase-pair (kb) segment of yeast DNA that was assumed to contain the yeast ARG4 gene (specifies argininosuccinate lyase) (2). Hybrid CoEl1 plasmids containing this yeast DNA segment (e.g., pYe(arg4)1) are capable of transforming E. coli argH deletion mutants to Arg+ with high frequency (12 transformants per μg of DNA). The resulting E. coli transformants contain detectable levels of an argininosuccinate lyase activity, whereas the original argH strains were devoid of this enzyme (2).

Recently, Hinnen et al. (1) described the successful transformation of a yeast leu2 double mutant with hybrid CoEl1 plasmid DNA containing the yeast LEU2 gene [pYe(leu2)10]. The transformation procedure involved exposing yeast spheroplasts to the plasmid DNA under conditions favoring spheroplast fusion (8) and plating the spheroplasts in regeneration agar selecting for the desired phenotype. Because transformation of yeast with pYe(leu2)10 DNA occurred at a very low frequency (10-6 to 10-7), it was advantageous to use a leu2- double mutant as recipient to minimize the occurrence of Leu+ revertants (1).

In contrast to transformation using pYe(leu2)10 DNA, the transformation of several yeast arg4- strains to Arg+ with pYe(arg4)1 DNA occurred reproducibly at a relatively high frequency (about 10-4), about 2–3 orders of magnitude higher than the frequency of the leu2 transformation (see Table 1). Because a thousand or more ARG4+ transformants could be obtained per μg of plasmid DNA and the frequency was appreciably above the reversion background (<10-7), it was unnecessary to use arg4- double mutants in this transformation system. The Arg+ transformants formed visible colonies after 3–4 days of incubation at 30°C, while on control plates supplemented with arginine the spheroplasts regenerated to form colonies in 2–3 days. The slower growth of the Arg+ transformants is apparently a result of segregation of the transforming DNA (see below).

Table 1. Transformation of yeast by pYe(arg4)1 DNA

<table>
<thead>
<tr>
<th>Yeast arg4- strain</th>
<th>ARG4+ transformants/μg DNA</th>
<th>Transformation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>X3656-7D</td>
<td>940</td>
<td>1.8 × 10^-4</td>
</tr>
<tr>
<td>S2072A</td>
<td>1278</td>
<td>3.0 × 10^-4</td>
</tr>
<tr>
<td>Z136-1-13C</td>
<td>608</td>
<td>2.1 × 10^-4</td>
</tr>
</tbody>
</table>

Arg+ revertants appeared on control plates with a frequency of less than 10^-7 after mock transformations carried out in the absence of DNA. Strain genotypes are given in Materials and Methods. Plasmid pYe(arg4)1 DNA was prepared as described (2).

Properties of ARG4+ Transformants. Yeast LEU2+ strains obtained by transformation of leu2- mutants with pYe(leu2)10 were relatively stably transformed, and the transforming DNA could be shown to be integrated into the yeast chromosomes (1). However, the ARG4+ transformants obtained with pYe(arg4)1 DNA were genetically unstable, especially when grown on rich nonselective medium. As shown in Table 2, more than 90% of the cells lost the Arg+ phenotype after growth in YPD medium for 12 generations. On selective medium in the absence of arginine, the transformants could be readily grown to reasonably high cell concentrations (10^7–10^8 cells per ml), however, more than 50% of the resulting cells failed to form colonies in minimal medium in the absence of arginine. The latter observation is probably a result of two factors: cells are constantly budding off that contain no transforming DNA (as plasmid, see below), and the plating efficiency of the transformant cells could be relatively low on minimal medium in the absence of arginine.

The generation times of various ARG4+ transformants in minimal medium without arginine are shown in Table 3. As would be predicted from the rather high segregation rate of the ARG4 gene, the transformants grew somewhat more slowly in the absence of arginine than did the parent in supplemented medium. Growth rates of the transformants on selective me-

Table 2. Genetic instability of ARG4+ transformants

<table>
<thead>
<tr>
<th>Transformant</th>
<th>Arg+ cells (%) remaining after 12 generations</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Grown in YPD</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)1</td>
<td>0.6</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)1</td>
<td>1.2</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)2</td>
<td>4.0</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)3</td>
<td>6.6</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)3</td>
<td>21</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)4</td>
<td>2.7</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Transformants were colony purified on minimal medium without arginine, inoculated into either YPD or minimal medium (SD) without arginine, and grown for 12 generations. The fraction of cells remaining Arg+ was determined by plating aliquots on YPD agar plates and on minimal agar in the absence of arginine. The plating efficiency of an ARG4+ control strain (X2180-IAna) on the minimal agar plates lacking arginine averaged 90–95% of that obtained on YPD agar.

Table 3. Relative growth rates of various yeast transformants on selective media

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>Arginine</th>
<th>Doubling time, hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z136-1-13C</td>
<td>+</td>
<td>2.0</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)1</td>
<td>+</td>
<td>2.0</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)2</td>
<td>+</td>
<td>3.2</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)3</td>
<td>+</td>
<td>3.8</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(arg4)4</td>
<td>+</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Fresh subcultures were inoculated into yeast minimal medium (SD) plus glucose (2%), leucine (30 μg/ml), tryptophan (20 μg/ml), and adenine (20 μg/ml). The cultures were incubated at 30°C with shaking and the growth rates were followed by turbidity measurements at 660 nm. Strain Z136-1-13C (arg4-), was grown in the presence of arginine (20 μg/ml). The transformant cultures reached stationary phase at a concentration of about 2 × 10^7 cells per ml, whereas wild-type ARG4+ strains grew to about 6 × 10^7 cells per ml under the same conditions.
diurnum without arginine averaged about 50–60% slower than the same strains, or the *arg4*− parent, grown in the presence of arginine.

Although the *ARG4* gene on the original plasmid isolate, pYe(*arg4*), is expressed relatively poorly in *E. coli* host cells, mutationally altered plasmids can be easily selected that are capable of expressing the heterologous gene at greatly increased levels (2). In extracts prepared from these variants, pYe(*arg4*2), pYe(*arg4*3), and pYe(*arg4*4), the yeast argininosuccinate lyase activity is comparable to the normal endogenous activity seen in wild-type (*argH*+) *E. coli* extracts. These mutations occur within the cloned yeast DNA and, in one case [pYe(*arg4*3), a 450-base-pair (bp) deletion occurred within a 2.8-kbp *HindIII* restriction fragment that contained the *ARG4* gene (2).

Transformations of yeast *arg4*− strains with the genetically altered pYe(*arg4*) plasmid DNAs yielded Arg*+* transformants at approximately the same frequency as with pYe(*arg4*)1 DNA, and the resulting transformants grew in selective medium with generation times only slightly greater than that of the pYe(*arg4*)1 transformants (Table 3). This result is somewhat surprising since it might be anticipated that DNA structural alterations affecting yeast gene expression in *E. coli* could exert detrimental effects on the expression of the altered DNA in yeast.

Transforming DNA Replicates Autonomously in Yeast Cells as Circular DNA. Because the *ARG4*+ yeast transformants are genetically unstable and readily lose their transforming DNA, it was suspected that the transforming plasmid DNA might not be integrated into the host chromosomes. Therefore, the intracellular molecular state of the transforming DNA was investigated by isolating total cellular DNA from the transformants, fractionating the DNA by electrophoresis on agarose gels, and hybridizing to a labeled CoE1 DNA probe by the Southern blot-through technique (10). The results, shown in Fig. 1, indicated that the transforming DNA was present in the cells as a circular supercoiled DNA of the same size as the original CoE1 hybrid plasmid used for the transformation. For example, cells transformed with pYe(*arg4*)3 DNA [about 450 bp smaller than pYe(*arg4*)1] contained a DNA species that hybridized with the CoE1 probe and migrated in an agarose gel with the same mobility as authentic pYe(*arg4*)3 supercoiled DNA (lanes a and h). Cells transformed with the larger pYe(*arg4*)1 DNA contained a CoE1 hybridizing plasmid with a mobility somewhat slower than pYe(*arg4*)3 DNA, but identical to the pYe(*arg4*)1 plasmid DNA control (lanes b and g). All DNA preparations from the transformed strains also contained DNA species with the same mobility as nicked or relaxed plasmid DNA.

The pYe(*arg4*) plasmids contain a single EcoRI site and are therefore converted to unit-length linear molecules by treatment with this enzyme (1, lanes e and f). After EcoRI digestion of DNA isolated from the *ARG4*+ yeast transformants and fractionation on agarose gels, the Southern hybridizations with 32P-labeled CoE1 DNA as probe revealed single strong radioactive bands with mobilities identical to the appropriate unit-length linear pYe(*arg4*) DNA (lanes c and d). The above results suggest that the transforming DNA replicates autonomously and remains unaltered in the recipient yeast host cells.

Recovery of Plasmids from Yeast Transformants. If the transforming plasmid DNA exists as a supercoiled circular DNA in the yeast host cells, it should be possible to reisolate the plasmid by transforming an *E. coli* *argH* strain with DNA isolated from the transformed yeast cells, selecting for Arg*+* and colicin E1 resistance. Relatively large amounts of hybrid plasmid DNA could then be isolated from the bacterial transformant culture by amplification with chloramphenicol (13).

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** The transforming pYe(*arg4*) DNA is maintained as a supercoiled circular DNA in the yeast transformants. Autoradiogram of 32P-labeled CoE1 DNA hybridized to total yeast DNA fractionated by agarose gel electrophoresis by the method of Southern (10). Shown are gel fractionations of total DNA from: lane a, strain Z136-1-13C/pYe(*arg4*)3; lane b, Z136-1-13C/pYe(*arg4*)1, partial EcoRI digest; lane c, as in lane a, EcoRI digest; lane d, as in lane b, EcoRI digest; lane e, pYe(*arg4*)1, EcoRI digest; lane f, pYe(*arg4*)3, EcoRI digest; lane g, pYe(*arg4*)1; and lane h, pYe(*arg4*)3. Arrows indicate nicked (1), supercoiled (2), and linear (3) forms of the plasmid DNAs.

Crude DNA preparations from several different pYe(*arg4*) yeast transformants were used to transform *E. coli* strain JA228 (*argH hsm*+ *hsr*−), selecting for growth on minimal medium without arginine. As shown in Table 4, Arg*+* *E. coli* transformants were readily detected when the source of DNA was yeast cells previously transformed with pYe(*arg4*)1, pYe(*arg4*)2, or pYe(*arg4*)3 DNAs. Yeast DNAs from the untransformed *arg4*− strain or from an *ARG4*+ wild-type (X2180-1A) were incapable of transforming *E. coli* strain JA228 to Arg*+*.

Plasmid DNAs were isolated from several of the *E. coli* transformants obtained as described above and in Table 4. These plasmid DNAs were digested with the *HindIII* restriction endonuclease and fractionated by gel electrophoresis, and the fragment band patterns were compared with those obtained from *HindIII* digests of authentic pYe(*arg4*)1 and 3 DNAs (Fig. 2). The band patterns of the "shuttled" plasmid DNAs were identical to those of the parent plasmids used for the original yeast transformations, even to the extent of maintaining the 450-bp deletion in *HindIII* fragment B of pYe(*arg4*)3 (see Fig. 2).

![Table 4](https://example.com/table4.png)

**Table 4.** Transformation of *E. coli* with DNA from pYe(*arg4*) yeast transformants.

<table>
<thead>
<tr>
<th>DNA source</th>
<th>Arg*+* transformants/μg DNA</th>
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<tbody>
<tr>
<td>Z136-1-13C/pYe(<em>arg4</em>)1</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(<em>arg4</em>)2</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Z136-1-13C/pYe(<em>arg4</em>)3</td>
<td>38 ± 18</td>
</tr>
<tr>
<td>Z136-1-13C</td>
<td>0</td>
</tr>
<tr>
<td>X2180-1A</td>
<td>0</td>
</tr>
</tbody>
</table>

Yeast DNA was used to transform *E. coli* strain JA228 (W3110 *argH hsm*+ *hsr*−) by standard methods (14). The Arg*+* transformants were all colicin E1 resistant.
of the presence of the ARG4 gene on plasmids pYe(arg4)1, 2, 3, and 4.

The transformation frequencies obtained with the pYe(arg4) hybrid plasmids are quite high (about 10^6 transformants per µg of DNA), 2–3 orders of magnitude higher than can be obtained with transforming DNAs, such as pYe(leu2)10, that integrate into the yeast genome (1). The pYe(arg4) plasmids replicate autonomously in the host yeast cell, arguing for the presence of an active origin of replication or replicon somewhere on the cloned yeast DNA segment. Because DNA replication origins are thought to be distributed randomly along the chromosome arms (17), it is possible that many randomly cloned segments of yeast DNA would be able to replicate autonomously when introduced back into yeast cells. For example, various plasmids containing a 1.4-kbp yeast DNA EcoRI restriction fragment that includes the yeast TRP1 gene (YEp7 and pLC544) are also found to replicate autonomously in trp1– yeast host cells (4, 18).

Plasmids containing the TRP1 gene have not been found to integrate into the yeast genome (4, 18); however, stable integrated forms of pYe(arg4)1 transformants occur at low frequency and have been isolated and characterized (unpublished results). Tetrad analysis of crosses involving these stable transformants indicate that the integration occurs at the arg4 locus on chromosome VIII.

Because trp1 and arg4 are both centromere-linked genes in yeast (19), one possibility that must be considered would be the presence of centromeric DNA sequences within the cloned DNA segments in plasmids YEp7 (containing TRP1) and pYe(arg4)1, thereby permitting the transforming DNAs to replicate as minichromosomes in the transformed host cells. The trp1 locus has been mapped at a distance of 0.5 centimorgan from the centromere on chromosome IV, whereas arg4 is approximately 8.4 centimorgans from the centromere on chromosome VIII (19). The relationship between genetic map distances, measured by recombinational frequencies, and actual physical distances in terms of kbp of DNA could average about 2–5 kbp per centimorgan (12), although this value could vary markedly in regions near the centromere where recombination frequencies may be atypical. The DNA sequence necessary for autonomous replication of the TRP1 plasmids is quite close to the TRP1 gene on the same 1.4-kbp EcoRI restriction fragment (4), whereas the yeast replicon on pYe(arg4)1 is about 3 kbp from the ARG4 gene (unpublished results). Thus, it seems unlikely that the pYe(arg4) plasmids could contain centromeric DNA.

Finally, the ability of pYe(arg4) plasmids to replicate autonomously in yeast suggests that appropriate plasmid derivatives might eventually be useful as cloning vehicles in yeast and E.coli. Conceivably, the replicon on pYe(arg4)1 could be isolated on a small restriction fragment and incorporated into an E.coli plasmid vector along with a selective marker (such as LEU2 or ARG4) for yeast cloning. These plasmids also offer a unique opportunity for studying yeast origins of replication, their arrangement on the yeast chromosomes, and the molecular mechanism of replication of yeast DNA.

We thank Dr. Robert Mortimer for many helpful discussions. This work was supported by Grant CA-11034 from the National Cancer Institute.


**DISCUSSION**

It is significant that the relative efficiency of expression in E.coli of the ARG4 gene on the various plasmids remains unchanged after transfer into yeast and recovery in E.coli; the mutationally altered plasmids, pYe(arg4)2 and pYe(arg4)3, still produce relatively high levels of argininosuccinate lyase in E.coli, whereas pYe(arg4)1 is still a low level producer of active enzyme (2). It is clear that there are no apparent modifications of the transforming plasmid by the yeast cells.

**FIG. 2.** The pYe(arg4) plasmids are recovered unchanged after passage through the E.coli–yeast–E.coli “shuttle.” Lanes a and e, agarose gel (0.8%) electrophoresis of HindIII digests of pYe(arg4)1 DNA; lane b, pYe(arg4)3 DNA; lane c, plasmid DNA obtained from yeast transformant Z136-1-13C/pYe(arg4)1 via E.coli strain JA228; lane d, plasmid DNA from Z136-1-13C/pYe(arg4)3 via E.coli strain JA228. Arrow indicates the HindIII B fragment that contains the ARG4 gene (2).

Various arg4– yeast strains can be readily transformed to ARG4+ by hybrid CoEI–yeast DNA plasmids that were originally isolated by their ability to complement mutations at the argH locus of E.coli. Both ARG4 and argH specify argininosuccinate lyase activities in their respective organisms (15, 16). Thus, these transformation results offer definitive proof that the presence of the ARG4 gene on plasmids pYe(arg4)1, 2, 3, and 4.

The transformation frequencies obtained with the pYe(arg4) hybrid plasmids are quite high (about 10^6 transformants per µg of DNA), 2–3 orders of magnitude higher than can be obtained with transforming DNAs, such as pYe(leu2)10, that integrate into the yeast genome (1). The pYe(arg4) plasmids replicate autonomously in the host yeast cell, arguing for the presence of an active origin of replication or replicon somewhere on the cloned yeast DNA segment. Because DNA replication origins are thought to be distributed randomly along the chromosome arms (17), it is possible that many randomly cloned segments of yeast DNA would be able to replicate autonomously when introduced back into yeast cells. For example, various plasmids containing a 1.4-kbp yeast DNA EcoRI restriction fragment that includes the yeast TRP1 gene (YEp7 and pLC544) are also found to replicate autonomously in trp1– yeast host cells (4, 18).

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