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A Stable Plasmid Carrying the Yeast *LEU2* Gene and Containing Only Yeast Deoxyribonucleic Acid

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The plasmid pSLe1 is a deletion derivative of the yeast-*Escherichia coli* hybrid plasmid pJDB219, obtained by *Hin*dIII digestion, ligation, and transformation directly into *Saccharomyces cerevisiae*. pSLe1 has only yeast sequences; it contains one of the inverted repeated sequences of plasmid 2μDNA and the *LEU2* gene. pSLe1 is stably maintained in yeast cells without selective pressure. pSLe1 is about half as large as 2μDNA, but pSLe1 does not displace the normal 2μDNA.

Saccharomyces cerevisiae has, per cell, 50 to 100 copies of a circular DNA plasmid called 2μDNA (3, 4, 12, 14, 16, 20, 21, 22), whose replication control (17) resembles that of nuclear DNA (9, 10). 2μDNA has an inverted repeat sequence (8, 13, 16).

With the discovery of transformation in yeast (11), hybrid plasmids containing the 2μDNA replicon, selectable yeast genes, and an *Escherichia coli* plasmid vector have been constructed and introduced into yeast (1, 23). These plasmids are unstable under nonselective conditions, making them unsuitable for certain studies. We have isolated a stable, selectable fragment of one of these plasmids which contains only yeast DNA and can be used for genetic studies of 2μDNA replication without the need for containment procedures.

Beggs (1) constructed a hybrid plasmid (pJDB219) consisting of the entire 2μDNA yeast plasmid, the *E. coli* ColE1-related plasmid pMB9, and a 1.2-kilobase (kb) insert containing the yeast *LEU2* gene. Beggs showed that this plasmid transforms yeast with high frequency, replicates in yeast or *E. coli*, and may be amplified in *E. coli* (1). One *Hin*dIII fragment of pJDB219 (about 3.2 kb) should contain the *LEU2* gene and one of the inverted repeat sequences of 2μDNA, but no *E. coli* DNA, based on the published restriction data (1). Since one of the 2μDNA inverted repeat sequences is sufficient to allow replication in yeast (23; Hinnen, Hicks, and Fink, cited in 23), this *Hin*dIII fragment should replicate autonomously in yeast cells.

To construct such a plasmid, either (i) *Hin*dIII endonuclease digest of pJDB219 was self-ligated (24) and used to transform AH22 to *leu*$^+$, or (ii) the 3.2-kb *Hin*dIII fragment described above was purified from 100 μg of digested pJDB219 by preparative electrophoresis on a 1% agarose gel, self-ligated, and used for transformation into yeast. The procedure for transformation of yeast was Begg's modification (1) of the procedure of Hinnen et al. (11), except that 0.1 mg of Zymolyase 60,000 (Kirin Brewery Co.) per ml was used instead of helicase. The experiments were carried out in a P2 laboratory, as specified by National Institutes of Health Guidelines for Recombinant DNA Research. The strains used are listed in Table 1. Two transformants isolated by method (i) (AT416 and AT417) and six isolated by method (ii) (YAT277 through YAT282) were analyzed further.

Genetic properties of *leu*$^+$ transformants. The *leu*$^+$ transformants were all stable, since *leu* segregants were rarely seen after growth on nonselective medium. The stability of the *leu*$^+$ trait of AT416 was compared with that of AH22 carrying pJDB219. Both strains were grown from small inocula on −Leu medium and plated for single colonies on YPAD, and these colonies were tested for leucine requirement. For strain AT416, 5 of 1,134 colonies (0.5%) had become *leu*, whereas for AH22 (pJDB219) 74 of 599 (12.5%) had become *leu*. In another experiment, these two strains were grown in YPAD broth. After 24 generations, 52% of the cells of strain AH22 (pJDB219) had become *leu*, whereas after 32 generations, less than 20% of the cells of strain AH22 (pSLe1) had become *leu*. Each *leu*$^+$ transformant was crossed with a *leu2* strain (YAT242), and 12 tetrads were analyzed in each cross. The *leu* phenotype always segregated 4+:0−, whereas *his4* and *cyh2* showed regular 2+:2− segregation. The *leu*$^+$ character could also be transferred by cytoplasmic mixing without nuclear fusion by using the *kar1* mutant described by Conde and Fink (7). A respiratory-

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TABLE 1. *Strains*

Organism	Strain	Genotype	Source
S. cerevisiae	AH22	a *leu2-3, 112 his4-519 can1*	Hinnen et al. (11)
	YAT242	α *leu2 cyh2 phoC phoS*	This work
	YAT227	α *leu2 cyh2 kar1* ρ^0	This work
	YAT233	α *leu2 lys10 his4 cyh2 kar1* ρ^0	This work
	YAT234	a *leu2 his4*(pSLe1)	This work
	AT416	AH22(pSLe1)	This work
E. coli	JA221	*recA1 leuB6 ΔtrpE5 hsdR hsdM⁺ lacY*(pJDB219)	J. D. Beggs (1)

competent *leu*$^+$ cycloheximide-sensitive segregant, YAT234, from the cross AT416 × YAT242, was used as the donor of cytoplasm. The α *kar1 cyh2 leu2 rho*^{0} strains, YAT227 and YAT233, were used as recipients. After overnight mating, cycloheximide-resistant, respiratory-competent cells were selected and tested for *leu*. Since *cyh2* is a recessive marker, respiratory-competent, cycloheximide-resistant colonies (cytoductants) received a nucleus from the recipient and mitochondria from the donor of cytoplasm. Forty-eight cytoductants were selected from each cross. Each isolate had α mating type, and all isolates from the YAT234 × YAT233 cross required lysine, confirming that these isolates are true cytoductants. A total of 16 clones out of 48 cytoductants from cross YAT234 × YAT227 were *leu*$^+$, and 22 out of 48 cytoductants from the YAT234 × YAT233 cross were *leu*$^+$. These results show that the *leu2* gene is located on a plasmid. A similar partial transfer in cytoduction was first described by Livingston and Klein for the intact 2μ plasmid (16).

Plasmids in *leu*$^+$ transformants. Plasmid DNA from AT416, AT417, and YAT277–YAT282 was prepared and analyzed on agarose gels. AT416 and YAT277 through YAT282 had a small DNA band not present in AH22, as well as a band migrating like 2μDNA (Fig. 1). The small band is called pSLe1. AT417 had a DNA band the same size as pJDB219.

Seven of seven *leu* mitotic segregants of AT416 had lost the pSLe1 band, but retained the band migrating with 2μDNA, indicating that the smaller plasmid, pSLe1, is responsible for the *leu*$^+$ phenotype of AT416 and that the 2μDNA-size band is not a dimer of pSLe1, but is the original 2μDNA. Thus, the smaller pSLe1 does not displace the normal 2μDNA. Although the two plasmids share the 2μDNA replicon, they are compatible.

Characterization of pSLe1 DNA. Plasmid DNA was extracted from a 2-liter culture of AT416 in YPAD as described previously (2, 23),

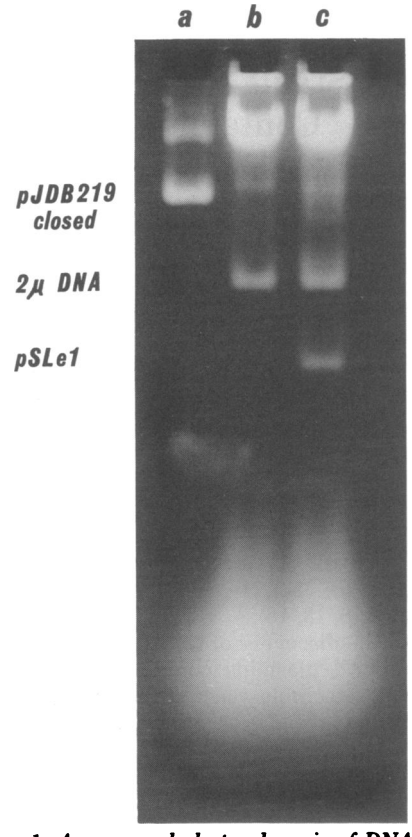

FIG. 1. *Agarose gel electrophoresis of DNA from AH22 and the leu*$^+$ *transformant, AT416. (a) pJDB219; (b) DNA preparation from AH22; (c) DNA preparation from AT416. Bacterial plasmids were isolated as described (5, 6). The yeast plasmids, 2μDNA and its derivatives, were isolated by published methods (2, 23, 27). DNA was analyzed on 1% agarose gels, stained, and photographed as described (25). The minor bands seen in lanes b and c at about the position of pJDB219 are the dimers of the 2μDNA plasmid (22).*

with a minor modification: insoluble material was removed after the addition of 5 M potassium acetate by centrifugation at 20,000 rpm in a 35 Beckman rotor for 1 h at 4°C, and two cycles of standard phenol extraction and one cycle of acid phenol extraction (27) were added. The pSLe1 DNA was purified by preparative agarose gel electrophoresis and analyzed by *Hin*dIII and *Eco*RI digestion (Fig. 2). The gel pattern of the *Hin*dIII digest showed one band with the same electrophoretic mobility as the 3.2-kb fragment of pJDB219. Three DNA bands can be seen after incomplete digestion by *Eco*RI; one is the same size as that of the linear DNA of pSLe1, the second barely separates from the 2.5-kb fragment in the *Eco*RI digest of pJDB219, and the third is the 0.8-kb fragment of the *Eco*RI digest

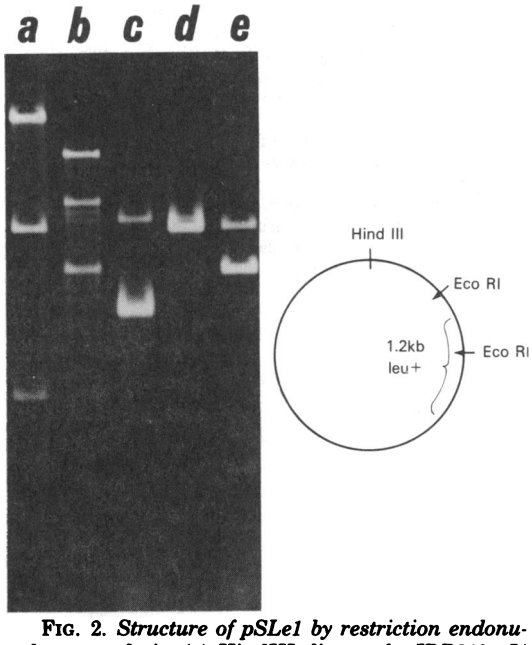

FIG. 2. *Structure of pSLe1 by restriction endonuclease analysis. (a) HindIII digest of pJDB219; (b) EcoRI digest of pJDB219; (c) undigested control of pSLe1, (d) HindIII digest of pSLe1; (e) partial EcoRI digest of pSEe1. Samples were analyzed on a 1% agarose slab gel and stained with ethidium bromide. The structure of pSLe1 is shown diagramatically. The location of the LEU2 gene is inferred from the results of Beggs (1).*

of pJDB219. All these results are consistent with the structure of pSLe1 shown in Fig. 2. DNA was isolated from another *leu*$^+$ transformant, YAT282, and DNA showing the same size as pSLe1 was purified. *Hin*dIII digestion of this DNA gave the same digestion pattern shown in Fig. 2. Thus, the plasmid in YAT282 also has the same structure as the pSLe1 plasmid.

The 3.2-kb plasmid, pSLe1, is stably maintained in yeast cells even under nonselective conditions and is inherited like the 2μDNA from which its replicon was derived. Whether pSLe1 can be integrated into a chromosome remains to be tested. pSLe1 and 2μDNA are compatible although pSLe1 is about half the size of 2μDNA, unlike most deletion mutants of other yeast plasmids and animal viruses which generally are preferentially replicated when in competition with the wild-type genome.

The pSLe1 plasmid can be used as a vector to clone yeast DNA into yeast cells because it can replicate stably in yeast, has an easily selectable marker, and has at least one cloning site, *Hin*dIII. Because pSLe1 contains only yeast DNA, it falls outside the National Institutes of Health Guidelines for Recombinant DNA. Because of its stability, pSLe1 is also useful for genetic studies on the maintenance of 2μDNA.

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LITERATURE CITED

1. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
2. **Cameron, J. R., P. Philippsen, and R. W. Davis.** 1977. Analysis of chromosomal integration and deletions of yeast plasmids. Nucleic Acids Res. **4**:1429–1448.
3. **Clark-Walker, G. D.** 1972. Isolation of circular DNA from a mitochondrial fraction from yeast. Proc. Natl. Acad. Sci. U.S.A. **69**:388–392,
4. **Clark-Walker, G. D., and G. L. G. Miklos.** 1974. Localization and quantification of circular DNA in yeast. Eur. J. Biochem. **41**:359–365.
5. **Clewell, D. B.** 1972. Nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
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. **Conde, J., and G. R. Fink.** 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. U.S.A. **73**:3651–3655.
8. **Guerineau, M., C. Grandchamp, and P. P. Slonimski.** 1976. Circular DNA of a yeast episome with two inverted repeats: structural analysis by a restriction enzyme and electron microscopy. Proc. Natl. Acad. Sci. U.S.A. **73**:3030–3034.
9. **Hartwell, L. H.** 1976. Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. J. Mol. Biol. **104**:803–817.
10. **Hereford, L. M., and L. H. Hartwell.** 1974. Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. J. Mol. Biol. **84**:445–461.
11. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**: 1929–1933.
12. **Hollenberg, C. P., P. Borst, and E. F. J. van Bruggen.** 1970. Mitochondrial DNA. V. A 25-μ closed circular duplex DNA molecule in wild-type yeast mitochondria. Structure and genetic complexity. Biochim. Biophys. Acta **209**:1–15.
13. **Hollenberg, P., A. Degelmann, B. Kustermann-Kuh, and H. D. Royer.** 1976. Characterization of 2-μm DNA of *Saccharomyces cerevisiae* by restriction fragment analysis and integration in an *Escherichia coli* plasmid. Proc. Natl. Acad. Sci. U.S.A. **73**:2072–2076.
14. **Leth, B., C. Christiansen, and G. Christiansen.** 1972. Circular, repetitive DNA in yeast. Biochim. Biophys. Acta **269**:527–530.
15. **Livingston, D. M.** 1977. Inheritance of the 2μmDNA plasmic from *Saccharomyces*. Genetics **86**:73–84.
16. **Livingston, D. M., and H. L. Klein.** 1977. Deoxyribonucleic acid sequence organization of a yeast plasmid. J. Bacteriol. **129**:472–481.
17. **Livingston, D. M., and D. M. Kupfer.** 1977. Control of *Saccharomyces cerevisiae* 2μmDNA replication by cell division cycle genes that control nuclear DNA replication. J. Mol. Biol. **116**:249–260.
18. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Mortimer, R. K., and D. C. Hawthorne.** 1975. Genetic mapping in yeast. Methods Cell Biol. **11**:221–223.
20. **Petes, T. D., and D. H. Williamson.** 1975. Replicating

circular DNA molecules in yeast. Cell 4:249–253.
21. **Sinclair, J. H., R. J. Stevens, P. Sanghavi, and M. Rabinowitz.** 1967. Mitochondrial-satellite and circular DNA filaments in yeast. Science **156**:1234–1237.
22. **Stevens, B. J., and E. Moustacchi.** 1971. Adn satellite γ et molecules circulaires torsadées de petite taille chex la levure *Saccharomyces cerevisiae*. Exp. Cell Res. **64**: 259–266.
23. **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.
24. **Tanaka, T., and B. Weisblum.** 1975. Construction of a colicin E1-R factor composite plasmid in vitro: means for amplification of deoxyribonucleic acid. J. Bacteriol. **121**:354–362.
25. **Toh-e, A., P. Guerry, and R. B. Wickner.** 1978. Chromosomal superkiller mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **136**:1002–1007.
26. **Wickner, R. B., and M. J. Leibowitz.** 1976. Two chromosomal genes required for killing expression in killer strains of *Saccharomyces cerevisiae*. Genetics **82**:429–447.
27. **Zasloff, M., G. D. Ginder, and G. Felsenfeld.** 1978. A new method for the purification and identification of covalently closed circular DNA molecules. Nucleic Acids Res. **5**:1139–1152.