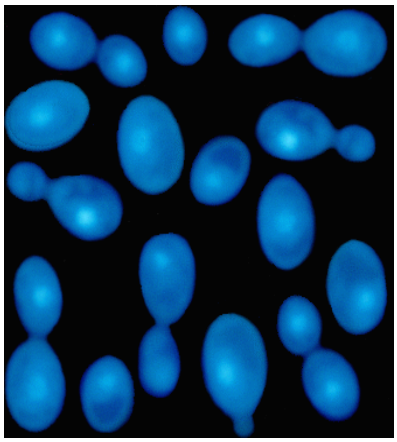
Transformación en levaduras

- El primer reporte de transformación en hongos fue en el año 1973 en el laboratorio de E.L. Tatum en la Universidad de Rockefeller y la especie fue *Neuropora crassa* inl^{-}.



Procedimientos para la Transformación

Preparación de las células

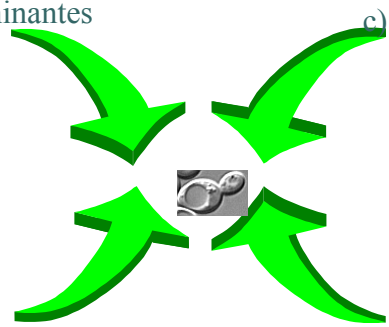
Protoplastos	**Sales de Litio**	**Electroporación**
Glucuronidasa Zimoliasa	Acetato de Litio	Dependiente del Aparato

Selección de los transformantes

Existen varios métodos de selección de transformantes

a) Marcadores Dominantes

c) Selección visual



b) Selección en dos etapas

d) Purificación de los transformantes

Tipos de transformación

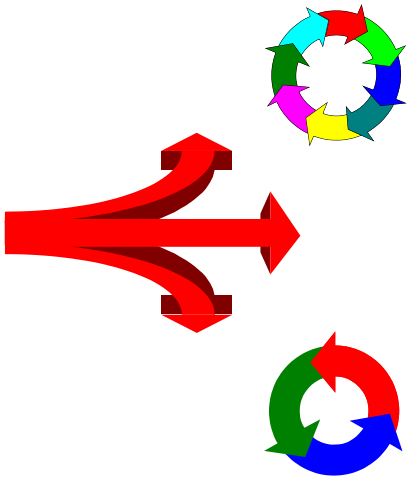
Por Integración

Por reemplazo

Por replicación autónoma

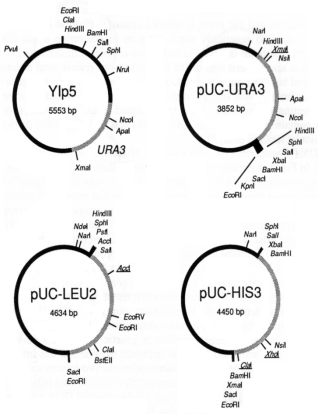
El tipo de transformación depende del vector

- Principales vectores:
  - Integrativos: Ex. YIp5
  - Replicativos
  - a) Episómicos : YEp13
  - b) Autónomos : YRp7
  - Centroméricos : YCp50
  - Lineales : YLp
  - Cromosomas Artificiales YAC



Vectores de Integración

- Los vectores de integración se caracterizan porque sólo llevan un gen estructural de *Saccharomyces cerevisiae* en un vector bacteriano.
- Ej. YIp5



Vectores replicativos

- Los vectores replicativos se caracterizan por tener un origen de replicación que puede ser un ARS o un ori del plásmido 2μ de *Sacharomyces cerevisiae*
- Ex: *YRp7 ; YEp13*

Reemplazo de genes en *Saccharomyces*

Vectores centroméricos

- Se caracterizan porque llevan un replicador, un gen marcador y un centrómero.
- Transforman en alta frecuencia y se mantienen en bajo número de copias por célula

Propiedades de la Transformación de levaduras

	Chromosomal integration	Gene conversion	Episomal replicator	Chromosomal replicator	Mini-chromosome	Linear DNAs
Vector	I	I	E	R	C	L
Transformation frequency	10	1	10,000	10,000	10,000	10,000
Autonomous replication	None	None	Circular	Circular	Circular	Linear
Copies per cell	1	1	5–40	3–30	1	5–30
Vector sequences	Yes	No	Yes	Yes	Yes	Yes
Integration frequency	1	1	Variable	10^{-5}	10^{-7}	NT
Required elements	Yeast DNA	Yeast DNA	2μ *ARS*	*ARS*	*ARS* *CEN*	*ARS* *TEL*
Mitotic loss	0.1%	0	30%	30%	1%	30%
Meiotic loss	1–10%	0	90%	90%	30%	90%

Each column represents a particular mode of yeast transformation. To use their distinct properties, yeast shuttle vectors were developed; for simplicity they are categorized as I, E, R, C and L. It should be noted that these distinctions, being somewhat arbitrary, break down in complex situations. The transformation frequency is measured in colonies per μg of transforming DNA. In cases involving high rates of mitotic loss, the number of copies per cell represents an average. Except for I vectors, the integration frequency is calculated as events per generation; for I vectors, the frequency of 1 indicates that all transformation events require integration. (Chromosomal integration of E vectors is discussed in refs 14, 94 and 95.) Mitotic loss is measured on a per generation basis. Meiotic loss is determined by tetrad analysis and thus the values also reflect mitotic instability. *CEN*, centromeric DNA; *TEL*, telomeric DNA; *NT*, not tested.

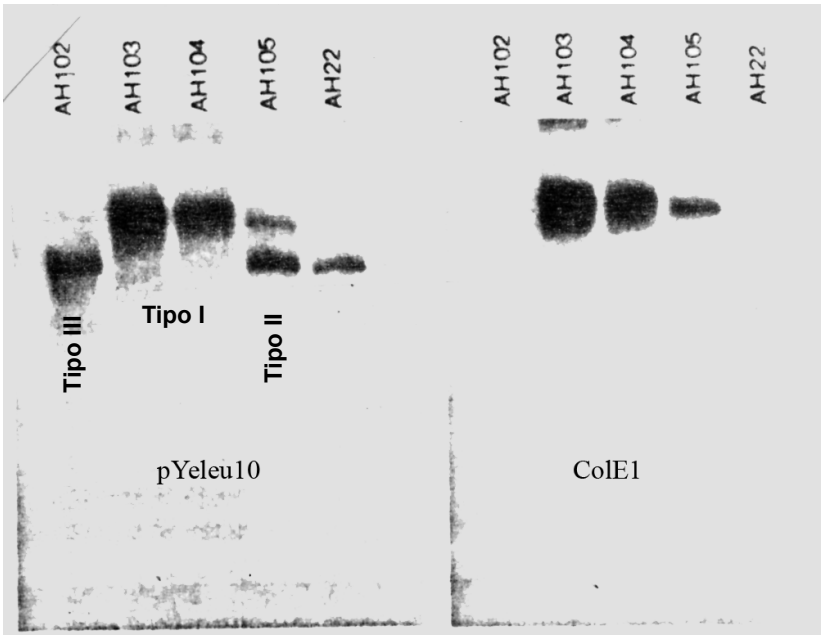


Table 1. Linkage of *LEU2*$^+$ to *his4* and *trp1**

	leu2–his4			*leu2–trp1*		
	PD	NPD	TT	PD	NPD	TT
Expected	50	<1	50	45	45	10
Observed:						
AH-102 × MC-333	18	0	22	17	22	10
AH-103 × MC-333	17	1	15	17	16	6
AH-104 × MC-333	31	0	22	27	25	6
AH-105 × MC-333	2	6	31	10	7	27

Ligados

25.7%

20.7%

No ligados

10% Ligados al centrómero

7.7% 1:1<4

5.2%

These data represent the results of crosses between the transformants (*LEU2*$^+$ *his4*$^-$ *TRP1*$^+$ *MET8*$^+$) by strain MC333 (*leu2*$^-$ *HIS4*$^+$ *trp1*$^-$ *met8*$^-$). In nontransformed strains, *his4* and *leu2* give a ratio parental ditype (PD)/nonparental ditype (NPD)/tetratype (TT) of 1:0.01:0.7, indicating 20% linkage. Thus, in the first three crosses above, *LEU2*$^+$ is linked to *his4*. If PD:NPD:TT is approximately 1:1:4, then two markers are considered unlinked. In the last cross, *his4* and the *LEU2*$^+$ region are unlinked. If the ratio PD:NPD:TT is 1:1:<4, two markers are considered linked to their centromeres. *leu2* and *trp1* in untransformed strains show centromere linkage. In the first three crosses the new *LEU2*$^+$ region shows centromere linkage, whereas in the AH-105 × MC333 cross it does not.

* Numbers represent both complete asci and three-spored asci that could be scored unambiguously.

Table 2. Segregation of *LEU2*$^+$ in transformants × wild type (S288C) crosses

	Leu$^+$:Leu$^-$ 4:0 DP	3:1 TT	2:2 DNP	
Expected:				
New Leu$^+$ at *leu2*	100	0	0	
New Leu$^+$ unlinked to *leu2*	17	66	17	
Observed:				
AH-102 × S288C	41	0	0	
Ligados: AH-103 × S288C	38	2	0	2.5%
Ligados: AH-104 × S288C	42	2	0	2.3%
AH-105 × S288C	4	19	6	53.0%

All strains used in these crosses are phenotypically Leu$^+$. The appearance of Leu$^-$ segregants therefore means that the original *leu2* region of the recipient is still present in the transformed strain. The frequency of Leu$^-$ segregants (3:1 and 2:2 asci) is a measure of the linkage between the old *leu2*$^-$ region and the new *LEU2*$^+$ region introduced by transformation. In the last three crosses, the *leu2*$^-$ region was clearly present. In the second and third crosses, *LEU2*$^+$ and *leu2*$^-$ were closely linked, whereas in the last cross they segregated independently.

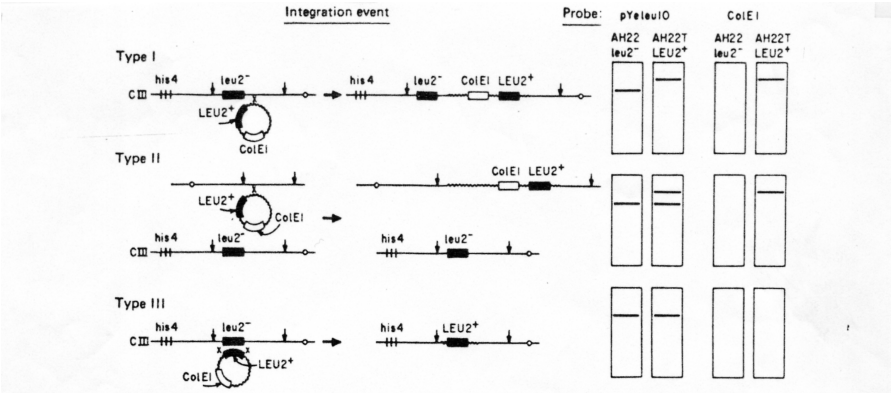


FIG. 2. Schematic interpretation of the integration events proposed for transformant types I, II, and III. Each type of integration event (*Left*) gives rise to a unique chromosome structure (*Center*) that can be visualized by hybridization of pYe*leu*10 and ColE1 DNA to *Hin*dIII restriction digests (*Right*). The arrows (↓) represent *Hin*dIII restriction sites. Type I: integration of plasmid pYe*leu*10 into chromosome III at a sequence complementary to a yeast sequence carried by the plasmid. Type II: integration of plasmid pYe*leu*10 into a chromosomal location genetically unlinked to the *leu2* region of chromosome III. Type III: integration of yeast DNA sequences of plasmid pYe*leu*10 into the *leu2* region by a double crossover event. These different integration events lead to predictable patterns when *Hin*dIII restriction digests of these strains are hybridized with pYe*leu*10 or ColE1 DNA. These hypothetical hybridization patterns are in agreement with the actual patterns shown in Fig. 1.

Table 1. Segregation of Markers *leu2*, *his3*, *ade2*, and the Mating Type Locus (MAT) in Genetic Crosses

(a) Normal arrangement

his3-leu2				*leu2-MAT*				*his3-ade2*			
P	N	T		P	N	T		P	N	T	
12	16	38	(unlinked)	31	1	37	(linked)	31	1	72	(linked)

(b) LEU2$^+$ *Transposed to chromosome XV*

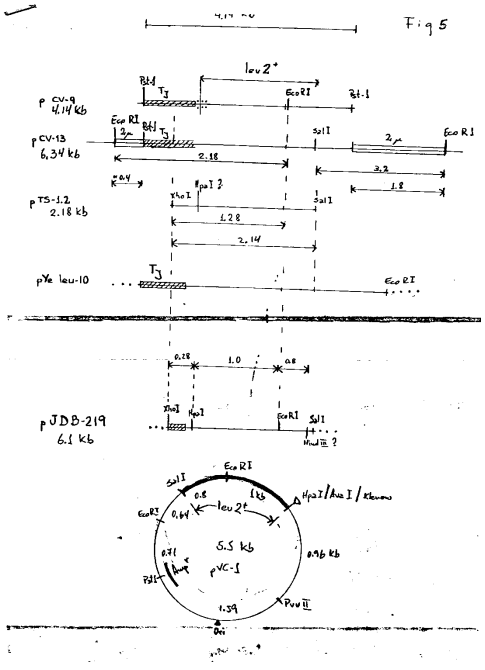
his3-leu2				*leu2-MAT*				*leu2-ade2*			
P	N	T		P	N	T		P	N	T	
74	0	0	(linked)	11	13	50	(unlinked)	20	3	54	(linked)

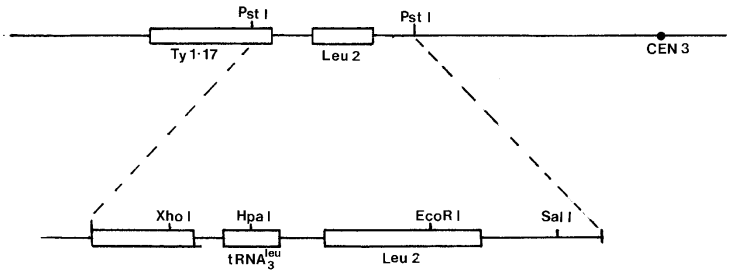
(c) HIS3$^+$ *Transposed to chromosome III*

his3-leu2				*his3-MAT*				*his3-ade2*			
P	N	T		P	N	T		P	N	T	
70	0	2	(linked)	26	0	30	(linked)	7	10	47	(unlinked)

Numbers shown here represent the types of meiotic tetrads obtained for each pair of markers. Genetic linkage is determined by the ratios of parental (P), nonparental (N), and tetratype (T) arrangements of the markers in these tetrads. Linked genes yield an excess of parental over nonparental segregants (e.g., *leu2-MAT* in *a*); P : N : T ratios approaching 1 : 1 : 4 indicate no linkage between markers.

(*a*) Crosses involving normal (untransformed) strains of genotypes *MATa leu2 his3* × *MATα ade2*; *leu2* is linked to *MAT* on chromosome III, and *his3* and *ade2* are linked on chromosome XV. In *b*, a strain transformed to *LEU2*$^+$ was crossed with a normal strain: *MATa leu2 his3*(*LEU2*$^+$) × *MATα leu2 ade2*. Tetrad data from this cross clearly show that the newly introduced *LEU2*$^+$ allele resides at the *his3* locus linked to *ade2* on chromosome XV. Conversely, *c* demonstrates the integration of a *HIS3*$^+$ allele at the *leu2* locus on chromosome III. This cross is of the type *MATa leu2*(*HIS3*$^+$)his3$^-$ × *MATα his3 ade2*.





Organización de la región del cromosoma III donde se encuentra el gen leu 2 de levadura.

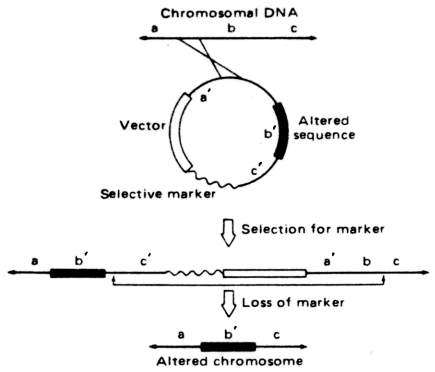


FIG. 1. Replacement of a chromosome segment with an altered DNA sequence. The transforming DNA (propagated by using the attached vector sequences) recombines with the homologous DNA sequence in the chromosomes and results in a direct, nontandem duplication separated by the vector and marker DNAs. A second recombinational event, on the other side of the sequence alteration relative to the first, generates a chromosome with the altered sequence replacing those in the untransformed cell. The vector and marker DNAs are lost. Homologous sequences on both sides of the alteration must be present in the transforming DNA but the molecule need not be capable of autonomous replication in the organism where the replacement will occur. A classical prokaryotic example of this type of manipulation would be transduction of mutant galactose genes with phage λ as the vector and immunity as the selection (1).

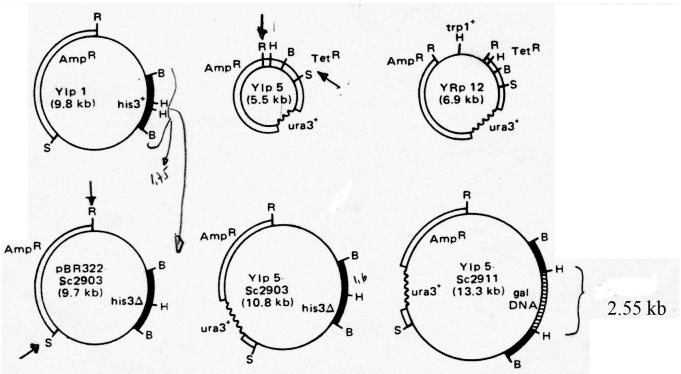


FIG. 2. Structures of plasmids used in these experiments. YIpl contains 6.1 kilobases (kb) of DNA from the *his*3 region of yeast replacing a portion of the tetracycline-resistance genes of pBR322 (3). The *his*3 gene is contained within the 1.75-kb *Bam*HI fragment (8). YIp5 has a 1.1-kb fragment containing the *ura*3 gene inserted by dG/dC homopolymer extensions into the *Ava* I site of pBR322 (3). Genotypes and DNA structures of the plasmids constructed for this work were verified by transformation of appropriate *E. coli* strains and preparation of small quantities of DNA by a sodium dodecyl sulfate lysis procedure similar to that used for phage λ (14). Cleavage sites: R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal* I. Double lines, pBR322 DNA sequences; wavy lines, yeast *ura*3$^+$ gene; thick lines, yeast *his*3 DNA sequences; striped bar, *gal* DNA; thin lines, other yeast DNA sequences.

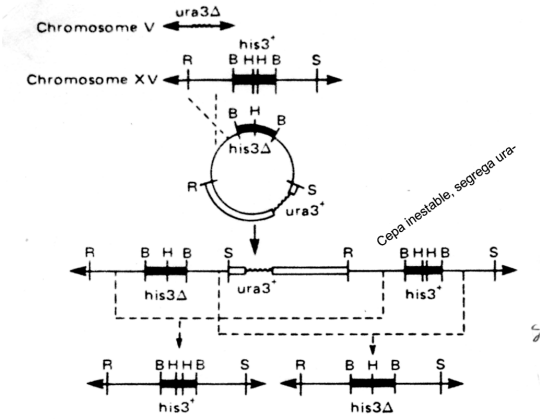


FIG. 3. Introduction of a deletion into the *his*3 gene. SX1-2 was transformed with YIp5-Sc2903 DNA and a transformant integrated on chromosome XV was identified. Two classes of segregants arise from the duplicated structure. One is indistinguishable from the untransformed strain; the other class has a deletion of *his*3 identical in size to the transforming DNA and has lost its vector sequences. The DNA structures of the strains are shown in Fig. 4. Cleavage sites: R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal* I. The fragments shown as thick lines or double lines are homologous to the hybridization probe used in Fig. 4. Double lines, *E. coli* DNA vector sequences; wavy line, yeast *ura*3$^+$ gene used for selection; thin lines, other yeast DNA sequences not homologous to the hybridization probe used in Fig. 4; thick lines, *Bam*HI fragments containing *his*3 sequences.

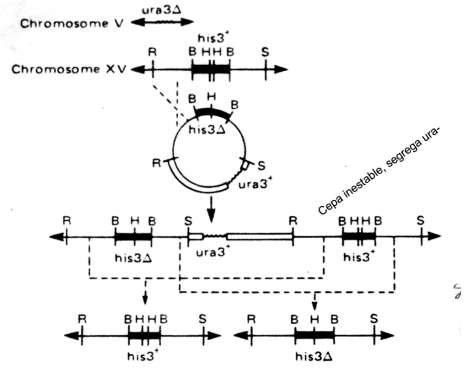


FIG. 3. Introduction of a deletion into the *his3* gene. SX1-2 was transformed with YIp5-Sc2903 DNA and a transformant integrated on chromosome XV was identified. Two classes of segregants arise from the duplicated structure. One is indistinguishable from the untransformed strain; the other class has a deletion of *his3* identical in size to the transforming DNA and has lost its vector sequences. The DNA structures of the strains are shown in Fig. 4. Cleavage sites: R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal* I. The fragments shown as thick lines or double lines are homologous to the hybridization probe used in Fig. 4. Double lines, *E. coli* DNA vector sequences; wavy line, yeast *ura3*+ gene used for selection; thin lines, other yeast DNA sequences not homologous to the hybridization probe used in Fig. 4; thick lines, *Bam*HI fragments containing *his3* sequences.

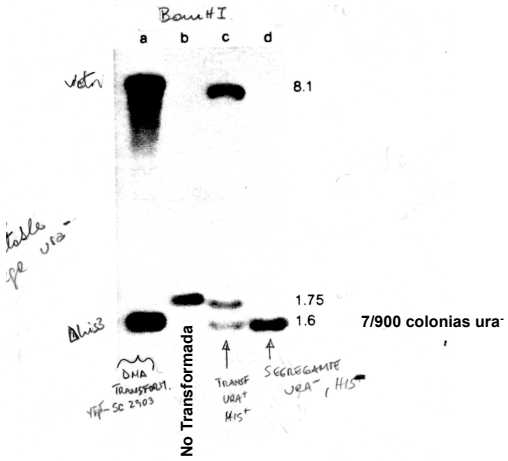


FIG. 4. DNA structures of strains in the insertion of *his3*Δ into chromosome XV. All DNA samples were cleaved with *Bam*HI, co-electrophoresed in a 1% agarose/Tris acetate/EDTA gel at 0.6 V/cm for 36 hr, transferred to nitrocellulose, probed with nick-translated pBR322-Sc2676 (the *his3 Bam*HI fragment), and exposed at −70°C with Cronex 4 film and a Du Pont Lightning plus intensifying screen for a few hours. Lanes: a, YIp5-Sc2903; b, untransformed wild type; c, transformant integrated at *his3*; d, His⁻ segregant. Rapid lysate DNA (lanes c and d) migrated anomalously fast due to overloading with large amounts of contaminating RNA oligonucleotides. Size shown at right in kb.

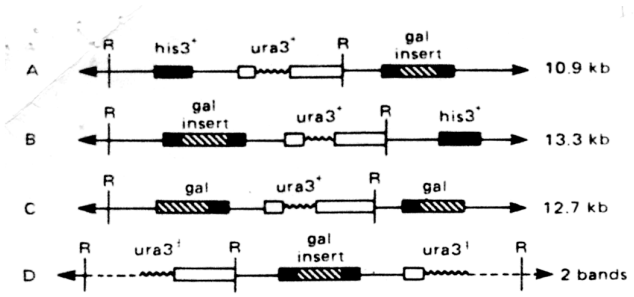


FIG. 5. Possible structures of transformants generated with YIp5-Sc2911. (*A*) Integration to the right of *his3*. (*B*) Integration to the left of *his3*. (*C*) Integration at *gal*. (*D*) Integration at *ura3*. The sizes given are for an *Eco*RI hybridization spectrum probed with pBR322 DNA. The location and orientation of the 2.55-kb *Hin*dIII fragment is known with respect to the Sc2903 sequences and the neighboring *Eco*RI sites at *gal*. Clones in both orientation have been constructed. The orientation used here gives more easily resolved DNA fragments. Thick lines, *his3 Bam*HI DNA fragment sequences (Sc2676); striped bar, *gal* DNA; wavy line, *ura3* DNA; double line, pBR322 DNA; thin unbroken lines, other yeast DNA segments of known size; thin broken lines, yeast DNA sequences adjacent to the *ura3* gene of undetermined size.

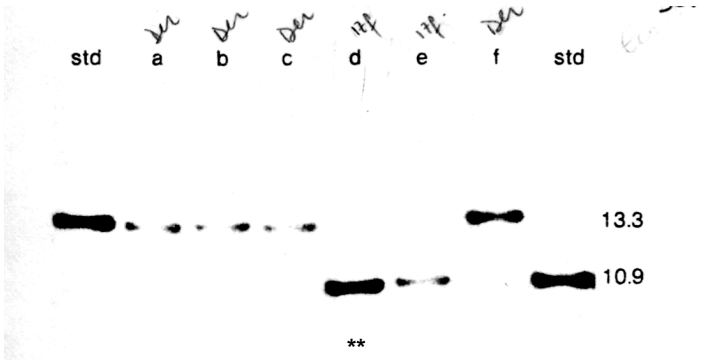


FIG. 6. Location of transformants of SX2-2 with YIp5-Sc2911. std, Lanes, at the left and right, containing standard DNA fragments, 13.3 and 10.9 kb, respectively. The other lanes carried six independent transformants analyzed as described in Fig. 5. Transformants a, b, c, and f have the DNA sequences integrated to the right of *his*3. Transformants d and e have the DNA sequences integrated to the left of *his*3. Transformant d was used to introduce the *gal* sequences stably into *his*3. Electrophoresis was for 36 hr at 0.6 V/cm in 0.4% agarose.

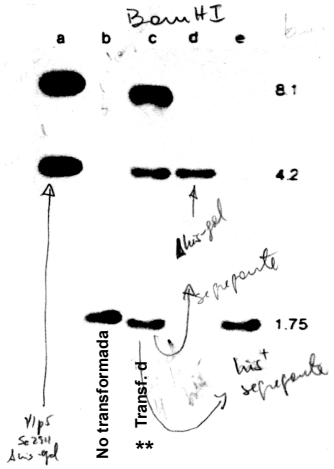


FIG. 7. DNA structures of strains used to insert *gal* sequences into *his*3. Samples were treated as in Fig. 3. Lanes: a, YIp5-Sc2911 DNA, showing a vector band at 8.1 kb and the *his*3 deletion and *gal* insertion band at 4.2 kb; b, untransformed wild type showing the wild-type *his*3 DNA band at 1.75 kb; c, transformant d (see Fig. 6), a composite of the DNA shown in a and b; d, His$^-$ segregant, showing that it does not contain vector sequences (8.1-kb band) or wild-type *his*3 sequences (1.75-kb band) but does contain the *his*3 deletion and *gal* insertion sequences (4.2-kb band); e, His$^-$ segregant identical to the untransformed strain (lane b). Lanes c, d, and e contained DNA from a rapid lysate procedure and showed slightly higher mobilities than the purified DNA contained in tracks a and b.

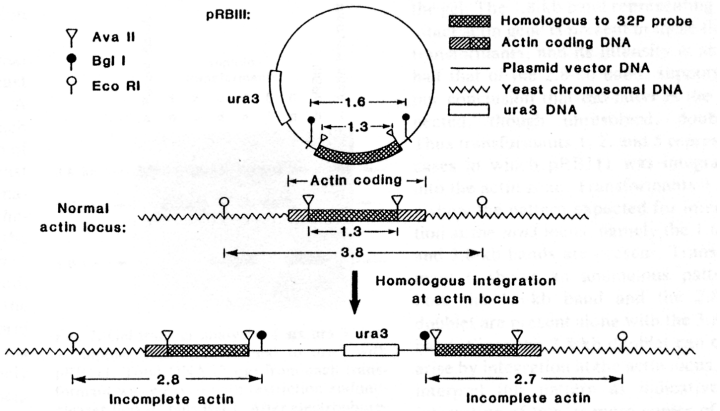


Fig. 1. Structure of the yeast actin locus before and after recombination with the hybrid plasmid pRB111. Positions of the Eco RI and Ava II restriction sites are taken from (*2*). Distances between relevant restriction sites are indicated in kilobase pairs. The structure predicted for the actin locus after recombination with pRB111 is confirmed by the data shown in Fig. 2. Plasmid construction involved ligation of the 1.3-kb Ava II fragment of the cloned actin gene (which contains the entire protein-encoding sequence except for deletion of the first 3⅓ codons plus 50 base pairs of intervening sequence from the amino terminal end and the last nine codons from the carboxyl terminal end) into the yeast integrative cloning vector YIp-5 which had been linearized by Ava II cleavage in the presence of ethidium bromide (100 μg/ml). From among the amp^r tel^s bacterial transformants with this ligation mixture, pRB111 was identified as a hybrid plasmid with a single 1.3-kb fragment inserted into the Ava II site at position 1135 in the tetracycline resistance gene of pBR322.

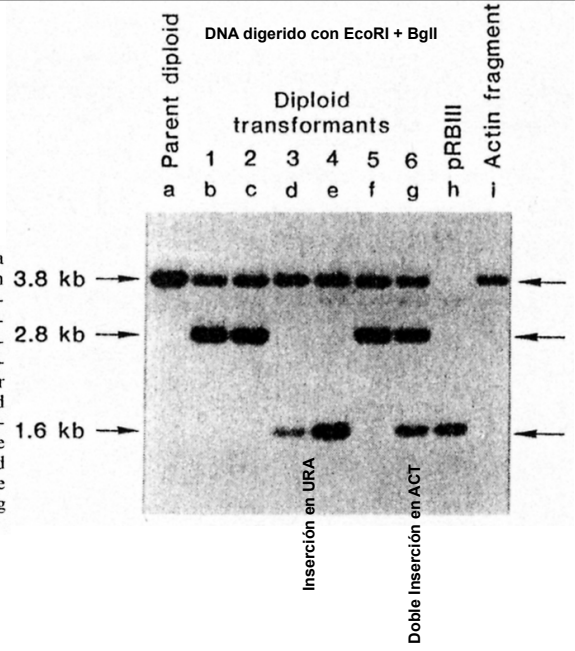
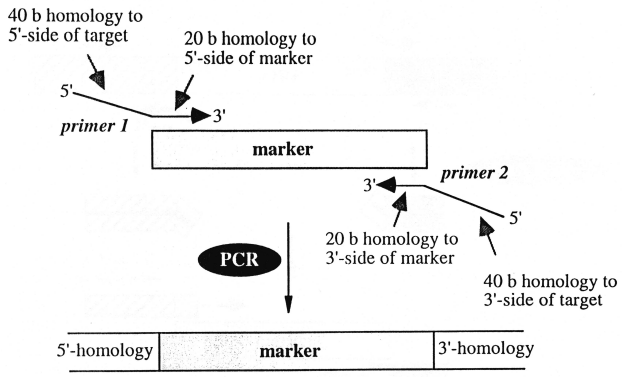
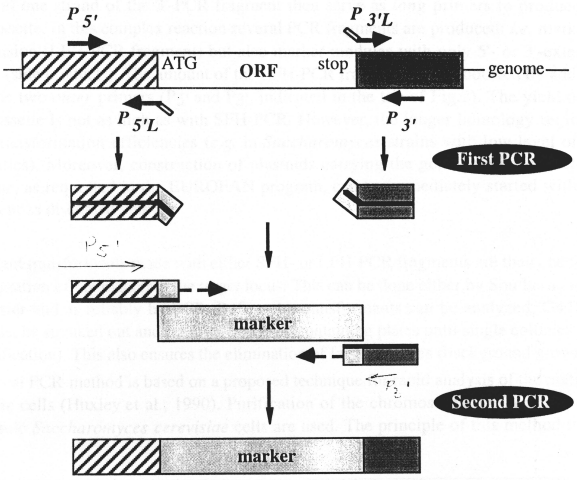


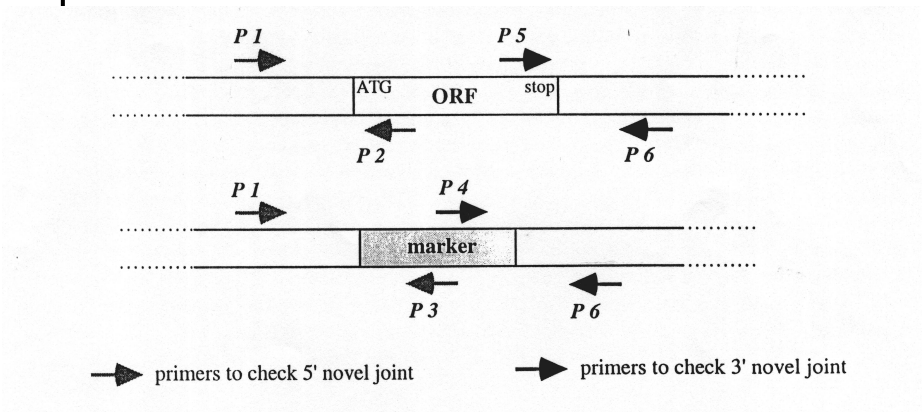
Fig. 2. Gel transfer analysis of six ura 3^-/ura 3^- diploid yeast transformed to ura^+ with pRB111. Total DNA (2 μg) from each transformant was cleaved with restriction endonucleases Eco RI plus Bgl I. After electrophoresis on a 1.2 percent agarose gel, DNA fragments were transferred to nitrocellulose filter paper (*10*) and hybridized with a ^{32}P-labeled (10^6 count/min) 1.3-kb Ava II fragment derived from the cloned yeast actin gene. (Lane h) 100 pg of plasmid pRB111 DNA cleaved with Eco RI and Bgl I; (lane i) 40 pg of the 3.8-kb Eco RI actin fragment cloned by Ng and Abelson (*2*).

Table 1. Results of tetrad analysis on the six yeast diploids transformed with pRB111. Procedures for growth of cells, sporulation, micromanipulation, and scoring of genetic markers were carried out by standard methods (*15*).

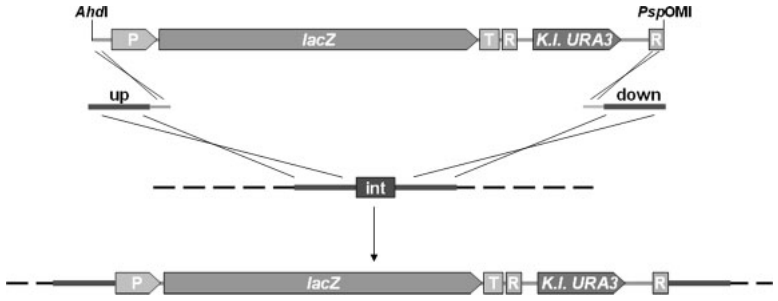
Trans-formant	Locus of inte-gration	Viable spores per tetrad				Ratio of spores
		4	3	2	1	$ura^+:ura^-$
1	Actin	0	1	11	3	0:28
2	Actin	0	0	16	1	0:33
5	Actin	0	0	9	0	0:18
6	Actin	0	0	10	1	0:21
3	Ura 3	13	3	0	0	30:31
4	Ura 3	5	3	0	0	15:14



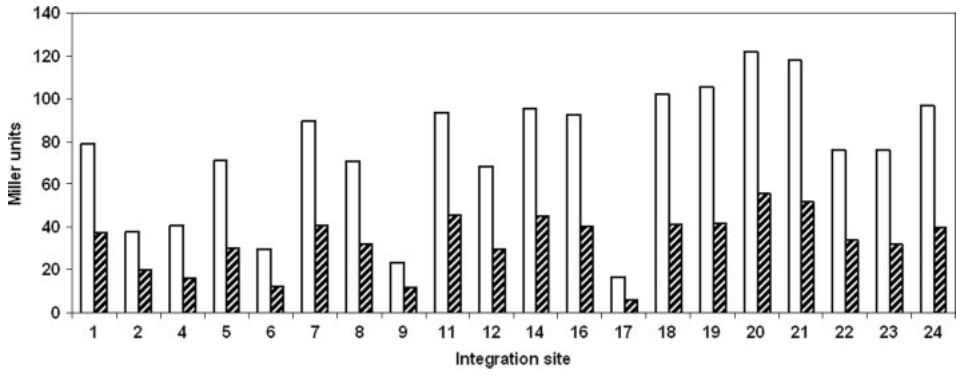




PCR-análisis de células de *S. cerevisiae* diploides para identificar clones con DNA-marcador correctamente ubicado.

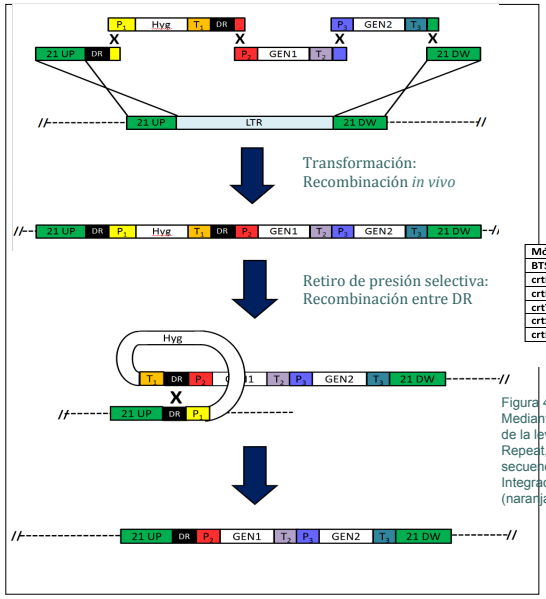


Bai et al., 2009 Yeast 26:545.



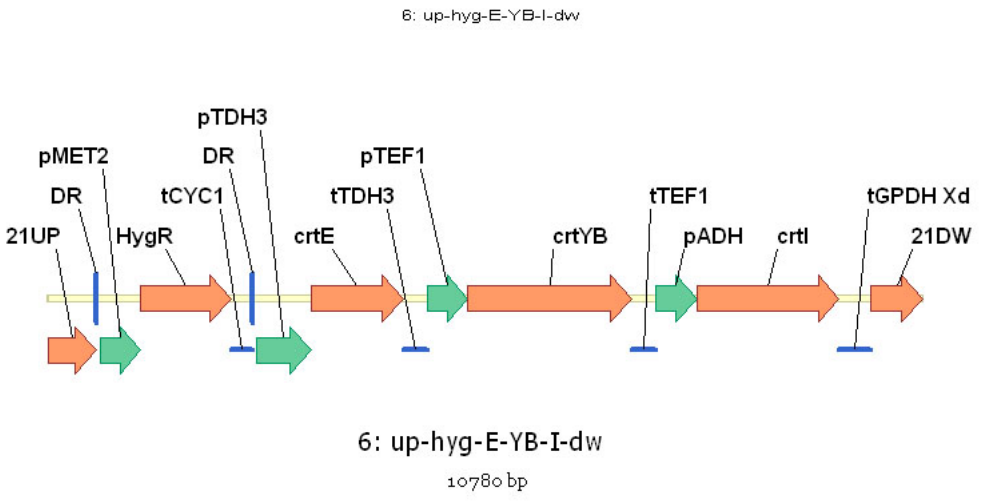
Expresión beta-galactosidasa en *S. cerevisiae*.

Bai et al., 2009 Yeast 26:545.



Módulo	Promotor	Gen	Terminador
BTS1	THD3 (853106)	BTS1 (856036)	THD3 (853106)
crtE	THD3 (853106)	*crtE* (DQ016502.1)	THD3 (853106)
crtI	ADH (854068)	*crtI* (AY177424.1)	ADH (854068)
crtYB	TEF (8044980)	*crtYB* (AY177204.1)	TEF (8044980)
crtS	HXT7 (851943)	*crtS* (DQ002007.1)	PGK1 (850370)
crtR	PGK1 (850370)	*crtR* (EU884134.1)	HXT7 (851943)

Figura 4. **Adaptación de Método "DNA assembler"**
Mediante recombinación homóloga es posible introducir más de un gen en el genoma de la levadura huésped y luego retirar el marcador de resistencia. LTR: Long Terminal Repeat, sitio de integración flanqueado por 21 UP (verde) y 21 DW (verde). 21 UP: secuencia río arriba del sitio de Integración. 21. DW: secuencia río abajo del sitio de Integración. P_n: Promotor (amarillo, rojo y azul respectivamente). T_n: Terminador (naranja, gris y verde mar respectivamente). DR: Direct Repeat (negro).



Panel cepa industrial

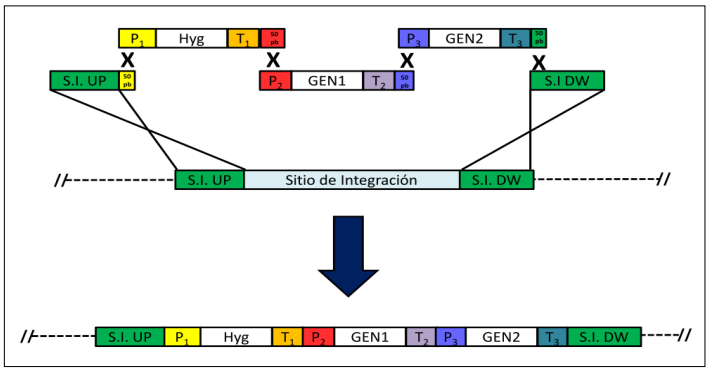
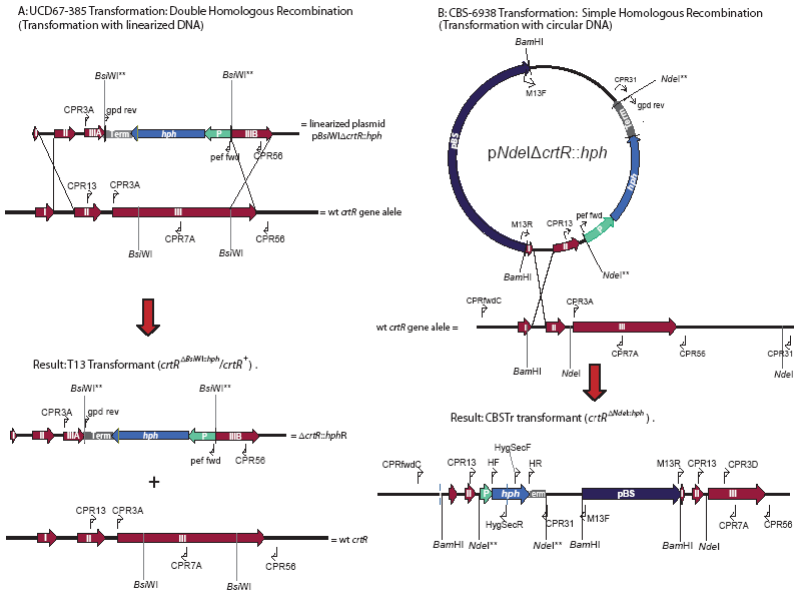


Figura 3. Esquema de estrategia de "*DNA assembler*" adaptada para la levadura *X. dendrorhous*.
Mediante recombinación homóloga es posible introducir más de un gen en el genoma de la levadura huésped.
S.I.: Sitio de Integración. S.I. UP: secuencia río arriba del sitio de Integración. S.I. DW: secuencia río abajo del sitio de Integración.
P_n: Promotor. T_n: Terminador.



Panel MIG1

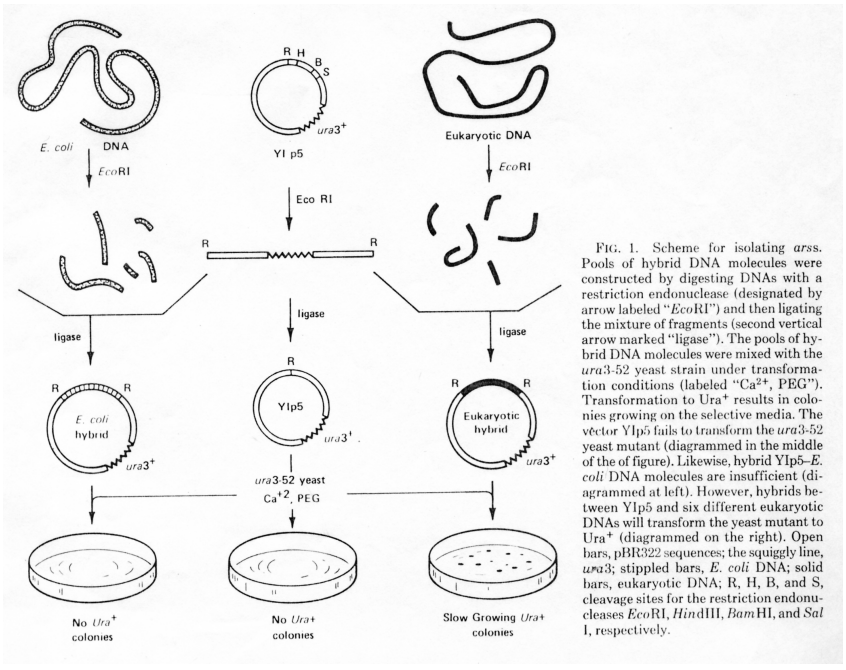
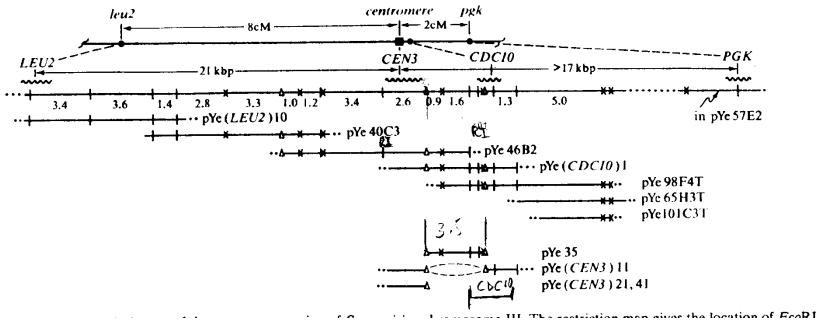
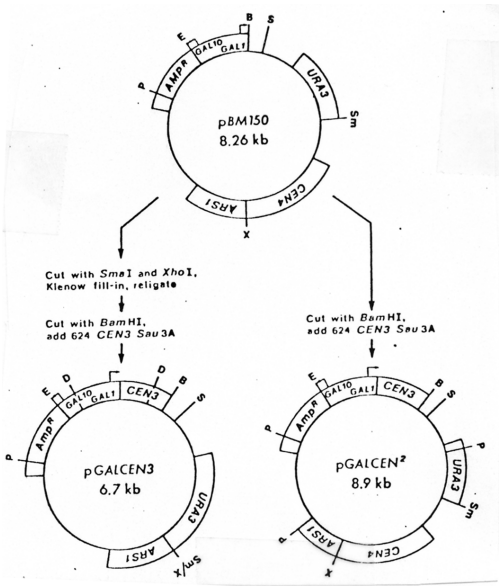


FIG. 1. Scheme for isolating *ars*s. Pools of hybrid DNA molecules were constructed by digesting DNAs with a restriction endonuclease (designated by arrow labeled "*Eco*RI") and then ligating the mixture of fragments (second vertical arrow marked "ligase"). The pools of hybrid DNA molecules were mixed with the *ura*3-52 yeast strain under transformation conditions (labeled "Ca^{2+}, PEG"). Transformation to Ura^+ results in colonies growing on the selective media. The vector YIp5 fails to transform the *ura*3-52 yeast mutant (diagrammed in the middle of the of figure). Likewise, hybrid YIp5–*E. coli* DNA molecules are insufficient (diagrammed at left). However, hybrids between YIp5 and six different eukaryotic DNAs will transform the yeast mutant to Ura^+ (diagrammed on the right). Open bars, pBR322 sequences; the squiggly line, *ura*3; stippled bars, *E. coli* DNA; solid bars, eukaryotic DNA; R, H, B, and S, cleavage sites for the restriction endonucleases *Eco*RI, *Hin*dIII, *Bam*HI, and *Sal* I, respectively.


leu2
8cM
centromere
2cM
pgk
LEU2
CEN3
CDC10
PGK
21 kbp
>17 kbp
3.4 3.6 1.4 2.8 3.3 1.0 1.2 3.4 2.6 0.9 1.6 1.3 5.0
in pYe 57E2
pYe (LEU2) 10
pYe 40C3
pYe 46B2
pYe (CDC10) 1
pYe 98F4T
pYe 65H3T
pYe101C31
pYe 35
pYe (CEN3) 11
pYe (CEN3) 21, 41


pBM150
8.26 kb
AMPR
GAL10
GAL1
URA3
CEN4
ARS1
Cut with SmaI and XhoI,
Klenow fill-in, religate
Cut with BamHI,
add 624 CEN3 Sau3A
Cut with BamHI,
add 624 CEN3 Sau3A
pGALCEN3
6.7 kb
pGALCEN2
8.9 kb
CEN3

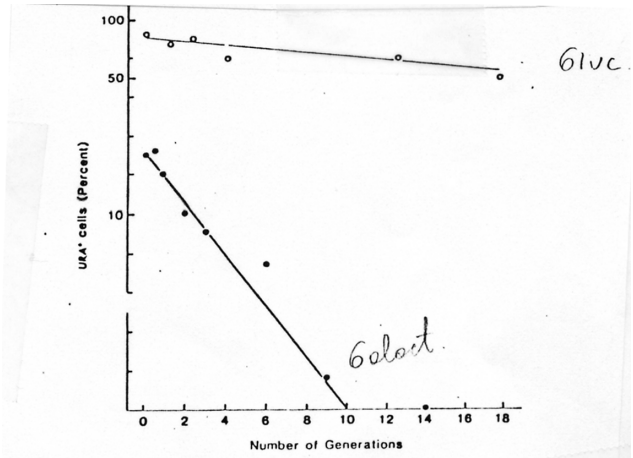


FIG. 2. Mitotic stability of pGALCEN3 in media containing either glucose or galactose as the carbon source. The mitotic stability of pGALCEN3 in glucose growth conditions is indicated by the open circles and in galactose growth conditions by the solid circles.

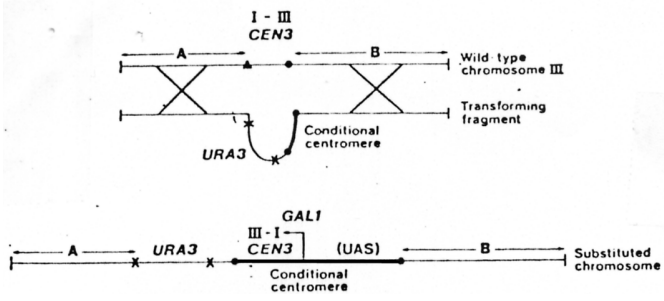


FIG. 4. Substitution of the centromere region of chromosome III with the conditional centromere. The conditional centromere was constructed by isolating the 1.3-kb *Eco*RI-*Bam*HI restriction fragment from pGALCEN3 (Fig. 1) and digesting it with *Dde*I. *Dde*I digestion generates an 865-bp DNA fragment that does not include the *GAL10* transcription initiation site. The four elements of the upstream activating sequences (UAS_G) are present as well as the complete centromere elements I to III. The ends of the *Dde*I fragment were filled in with Klenow polymerase, and *Bam*HI linkers were attached by blunt-end ligation. The resulting GALCEN *Bam*HI fragment was inserted into the *Bam*HI site of the centromere III substitution vector pJC3-13 (8). When pJC3-13 is restricted with *Eco*RI a transforming fragment is generated that contains at its ends DNA sequence from the chromosomal flanking regions of CEN3, A (1.9 kb) and B (2.5 kb). In addition the *URA3*$^+$ gene is present as a selectable marker. The diagram indicates the recombination event that must occur between the transforming fragment and the centromere region of chromosome III to generate a chromosome substituted with the conditional centromere. The location of the conditional centromere is indicated by the heavy line, which in the diagram of the substituted chromosome has been expanded for clarity. The direction of *GAL1* transcription is indicated by the arrow, and the position of the UAS_G is indicated. The orientation of the *CEN3* elements in the conditional centromere is reversed from that of the wild type. It has been demonstrated that the stabilizing properties of *CEN3* are orientation independent (8). Restriction sites: *Eco*RI, |; *S. u*3A, ▲; *Bam*HI; ●; *Hin*dIII, ×.

TABLE 1. Quantitative mating experiments[a]

Strain	Carbon source	Fraction of mated cells in population
YGALCEN3	Glucose	1.8×10^{-4}
	Glycerol plus lactate	2.0×10^{-4}
	Galactose (five generations)	1.8×10^{-1}
J178-1D × J17	Glucose	3.5×10^{-4}
	Glycerol plus lactate	4.5×10^{-4}
	Galactose	4.7×10^{-4}

[a] Mating experiments were done to determine the frequency with which mating-competent cells arise from a diploid YGALCEN3 population cultured in various carbon sources.

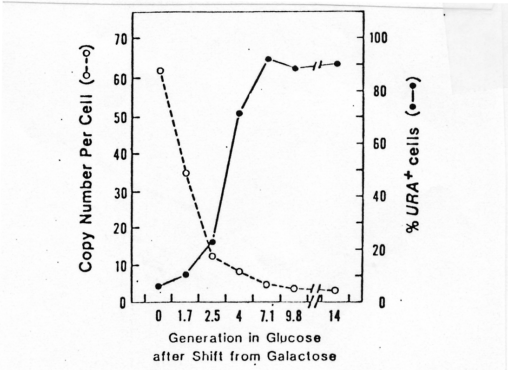
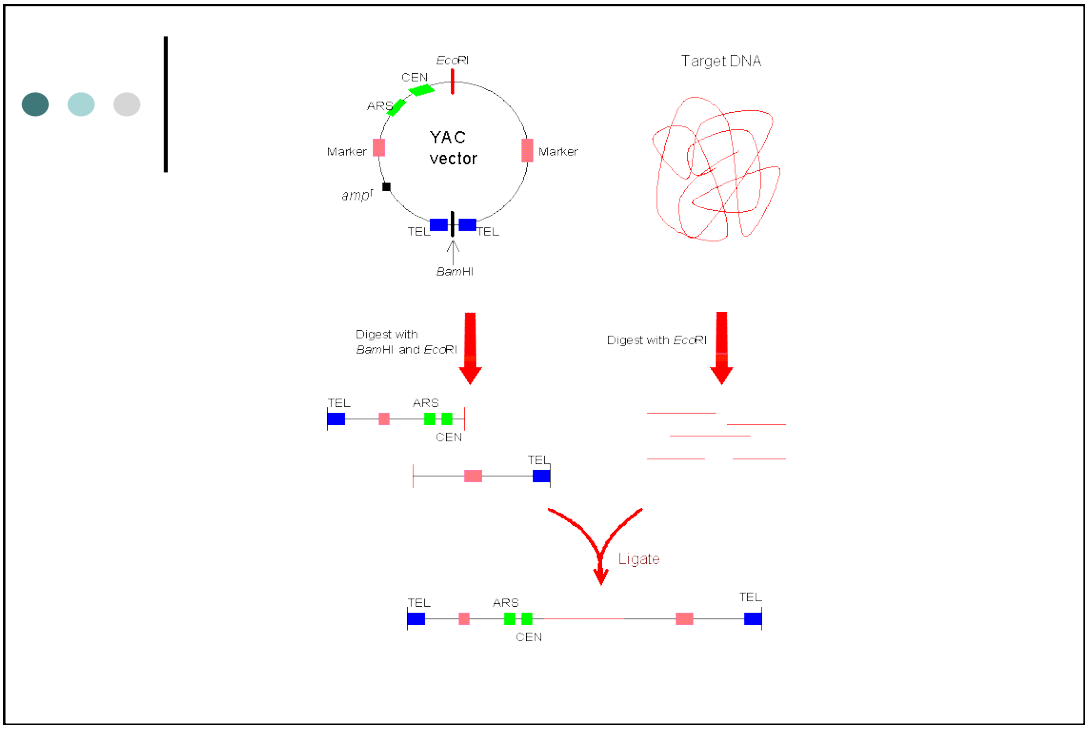


FIG. 8. Mitotic stability and copy number of pGALCEN3 in subsequent generations in glucose-containing medium after switching from galactose-containing medium. J17 cells transformed with pGALCEN3 were grown selectively for the presence of the plasmid in medium containing 2% galactose as the sole carbon source. Cells were spun down, washed twice in sterile water, and suspended in the same medium except that the 2% galactose was replaced with 2% glucose. Samples were removed at subsequent generations and used to determine the percentage of *URA*$^+$ cells and plasmid copy


EcoRI
CEN
ARS
YAC
vector
Marker
Marker
ampr
TEL
TEL
BamHI
Target DNA
Digest with
BamHI and EcoRI
Digest with EcoRI
TEL
ARS
CEN
TEL
Ligate
TEL
ARS
CEN
TEL