A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in *Saccharomyces cerevisiae*

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ABSTRACT

A series of yeast shuttle vectors and host strains has been created to allow more efficient manipulation of DNA in *Saccharomyces cerevisiae*. Transplacement vectors were constructed and used to derive yeast strains containing nonreverting *his3*, *trp1*, *leu2* and *ura3* mutations. A set of YCp and YIp vectors (pRS series) was then made based on the backbone of the multipurpose plasmid pBLUESCRIPT. These pRS vectors are all uniform in structure and differ only in the yeast selectable marker gene used (*HIS3*, *TRP1*, *LEU2* and *URA3*). They possess all of the attributes of pBLUESCRIPT and several yeast-specific features as well. Using a pRS vector, one can perform most standard DNA manipulations in the same plasmid that is introduced into yeast.

PLASMID cloning vectors that can "shuttle" DNA between yeast and bacteria are fundamental tools in the molecular genetic analysis of *Saccharomyces cerevisiae*. They are used extensively to manipulate cloned yeast genes and to define *cis*-acting DNA elements such as promoters and autonomously replicating sequences. Of the several types of shuttle vectors that have been created (PARENT, FENIMORE and BOSTIAN 1985), the Yeast Integrating Plasmid (YIp) and Yeast Centromere Plasmid (YCp) types seem to have the most general applicability. However, despite their broad utility and routine usage, most YIp and YCp vectors in common use today remain the original prototypes, plasmids that were not designed for cloning efficiency or versatility. Cloning in these prototypes (YCp50, YIp5, etc.) is hampered by the availability of few useful cloning sites, the lack of a visual screen for recombinants, the limited variety of yeast selectable markers, and the relatively low copy number of pBR322 replicons. Improved yeast vectors have been constructed (BALDARI and CESARENI 1985; HILL *et al.* 1986; ELLEDGE and DAVIS 1988), but to date no uniform set of multipurpose YIp and YCp vectors has been made that overcomes all of these drawbacks.

In contrast to yeast vectors, *Escherichia coli* cloning vectors have been systematically modified and have evolved into efficient, multipurpose cloning vehicles (BALBAS *et al.* 1986). Perhaps the most sophisticated of these is the phagemid pBLUESCRIPT (Stratagene). It contains an f1 phage origin of DNA replication for the *in vivo* production of single stranded (ss) DNA and T3/T7 phage promoters for the *in vitro* production of RNA. It also contains a large polylinker region and a bacterial gene that allows a color screen for recombinants so that standard cloning is greatly simplified.

In an effort to incorporate these advanced features into yeast cloning vectors, we have constructed a set of YIp and YCp vectors based on the backbone of pBLUESCRIPT. These new vectors are small (≤6 kb), contain many unique cloning sites, replicate to high copy number in bacteria, and offer a choice of four yeast selectable markers. The vectors make cloning efficient in that most DNA manipulations (sequencing, unidirectional deletions, mutagenesis, etc.) can be performed in the same plasmid that is introduced into yeast. In addition, we have made yeast strains that contain nonrevertible (deletion) auxotrophic mutations that can serve as host to any or all of these plasmids. Together, the vectors and yeast strains provide the basic materials for a wide variety of molecular genetic manipulations in *S. cerevisiae*.

MATERIALS AND METHODS

Bacterial strains and media: *Escherichia coli* strains DH5α [F-, *endA1*, *hsdR17*(*rk-mk+*), *supE44*, *thi-1*, λ, *recA*, *gyrA96*, *re1A1*, Δ(*argF-laczya*), *U169*, *Ø80 lac ZΔM15*], from Bethesda Research Laboratories; and MH1066 [*ΔlacX74*, *hsr-*, *rpsl*, *pyrF:Tn5*, *leuB600*, *trpC9830*, *galE*, *galK*], from MIKE HALL via J. Boeke (Johns Hopkins University); were used as plasmid hosts. Recombinant plasmids containing the yeast *URA3*, *TRP1*, or *LEU2* genes were selected in MH1066 by complementation on M9 medium with uracil (20 μg/ml), tryptophan (30 μg/ml), or leucine (40 μg/ml) added as required.

Yeast transformations and media: Yeast transformations were performed by the LiAc procedure of ITO *et al.* (1983). Media components were from Difco or Sigma. Yeast media were as described by SHERMAN, FINK and LAWRENCE (1979) and HIETER *et al.* (1985a).

DNA manipulations: Restriction enzymes and DNA polymerases were purchased from Boehringer Mannheim and used as instructed. T4 DNA ligase was purchased from New England Biolabs. Standard recombinant DNA techniques

TABLE 1

Yeast strains

Name	Genotype						
YNN214	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*			
YNN215	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*			
YNN216	**a**/α	*ura3-52* / *ura3-52*	*lys2-801^{amber}* / *lys2-801^{amber}*	*ade2-101^{ochre}* / *ade2-101^{ochre}*			
YPH4	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*		
YPH5	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*		
YPH45	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trpl-Δ1*		
YPH47	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trpl-Δ1*		
YPH266	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*		
YPH267	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*		
YPH52	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*	*trp1-Δ1*	
YPH54	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*	*trp1-Δ1*	
YPH98	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*	*trp1-Δ1*	
YPH102	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*	*his3-Δ200*	
YPH258	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*	*leu2-Δ1*	
YPH259	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*his3-Δ200*	*leu2-Δ1*	
YPH262	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*	*trp1-Δ1*	
YPH263	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*leu2-Δ1*	*trp1-Δ1*	
YPH250	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trp1-Δ1*	*his3-Δ200*	*leu2-Δ1*
YPH252	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trp1-Δ1*	*his3-Δ200*	*leu2-Δ1*
YPH274	**a**/α	*ura3-52* / *ura3-52*	*lys2-801^{amber}* / *lys2-801^{amber}*	*ade2-101^{ochre}* / *ade2-101^{ochre}*	*trp1-Δ1* / *trp1-Δ1*	*his3-Δ200* / *his3-Δ200*	*leu2-Δ1* / *leu2-Δ1*
YPH499	**a**	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trp1-Δ63*	*his3-Δ200*	*leu2-Δ1*
YPH500	α	*ura3-52*	*lys2-801^{amber}*	*ade2-101^{ochre}*	*trp1-Δ63*	*his3-Δ200*	*leu2-Δ1*
YPH501	**a**/α	*ura3-52* / *ura3-52*	*lys2-801^{amber}* / *lys2-801^{amber}*	*ade2-101^{ochre}* / *ade2-101^{ochre}*	*trp1-Δ63* / *trp1-Δ63*	*his3-Δ200* / *his3-Δ200*	*leu2-Δ1* / *leu2-Δ1*

were used (MANIATIS, FRITSCH and SAMBROOK 1982). For ligations of DNA with incompatible end structures, the ends were first made flush with Klenow DNA polymerase (5′ extensions) or T4 DNA polymerase (3′ extensions). Recombinant plasmids were identified by colony hybridization (BENTON and DAVIS 1977) using oligonucleotide-labeled probes (FEINBERG and VOGELSTEIN, 1983) or by direct complementation of *E. coli* mutations in JBe181. A rapid DNA isolation procedure (HOLMES and QUIGLEY 1981) was used to characterize putative clones. CsCl purified DNA was used for all cloning steps.

Construction of yeast strains: All strains listed in Table 1 were derived from the diploid strain YNN216 (JOHNSTON and DAVIS 1984; original source: M. CARLSON, Columbia University), which is congenic with S288C. Nonreverting deletion alleles of the *TRP1, HIS3*, and *LEU2* genes were constructed *in vitro* in the vector YRp14 (ST. JOHN *et al.* 1981) and introduced into the YNN216 background via gene replacement techniques (SCHERER and DAVIS 1979). The *trp1-Δ1* allele was constructed by inserting a 1.5-kb *Hin*dIII/*Eco*RI fragment (immediately centromere proximal to the 1.45-kb *Eco*RI/*Eco*RI *TRP1* gene containing fragment) and a 2.7-kb *Eco*RI/*Eco*RI fragment (immediately centromere distal to the *TRP1* gene containing fragment) into the *Hin*dIII/*Eco*RI sites of YRp14. The 2.7-kb fragment was inserted in the orientation that preserves the genomic orientation of the two fragments (STINCHCOMB, MANN and DAVIS, 1982). The resultant YRp14/*trp1-Δ1* plasmid was digested to completion with *Xho*I (which cuts uniquely in the 2.7-kb fragment) and 3 μg used to transform strain YNN216 to Ura$^+$ using the LiAc procedure (ITO *et al.* 1983). A single pink diploid transformant (*i.e.*, carrying a single integrated copy of the *URA3/SUP11* plasmid) (HIETER *et al.* 1985b) was sporulated and dissected. One white *MAT***a** (*ura3 SUP11*) haploid strain and one white *MATα* (*ura3 SUP11*) haploid strain were picked, each was grown nonselectively in liquid YPD medium for 5–10 generations, and each was plated onto YPD plates for single colonies (10,000 total colonies for each). Several red (*SUP11* gene lost) segregants of each were picked and tested for simultaneous loss of the *URA3* and *TRP1* markers. YPH45 (*MAT***a** *ura3 trp1*) and YPH47 (*MATα ura3 trp1*) strains were isolated and genomic DNA was prepared from each. Genomic Southern blots confirmed the presence of the *trp1-Δ1* deletion on chromosome *IV* (data not shown). The *his3-Δ200* allele was constructed and kindly provided by M. FASULLO (FASULLO and DAVIS 1988; STRUHL and HILL 1987) and consists of a 1.0-kb deletion that deletes the entire coding region of the *HIS3* gene within the genomic *HIS3* containing *Eco*RI/*Sal*I fragment cloned into the *Eco*RI and *Sal*I sites of YRp14. An aliquot of 3 μg of the YRp14/*his3-Δ200* plasmid was used to transform YNN216 to Ura$^+$ to yield a stable "pink" diploid integrant. YPH4 (*MAT***a** *ura3 his3*) and YPH5 (*MATα ura3 his3*) were isolated as described above and the presence of the *his3-Δ200* allele on chromosome *XV* confirmed by genomic Southern evaluation (data not shown). The *leu2-Δ1* allele was constructed by introducing a 0.6-kb *Eco*RI/*Cla*I deletion (Klenow fill-in followed by ligation of blunt ends) into a 2.2-kb *Sal*I/*Sal*I *LEU2* gene containing fragment cloned into pUC8 (this fragment corresponds to the genomic 2.2-kb *Sal*I/*Xho*I fragment in which the *Xho*I site was converted to a *Sal*I site by a synthetic linker; M. SMITH, personal communication). The resultant *leu2-Δ1 Sal*I/*Sal*I fragment was inserted into the *Sal*I site of YRp15

(ST. JOHN *et al.* 1981) [Note: YRp15 is identical to YRp14 except for the orientation of the *SUP11* gene with respect to the *URA3* gene.] The resultant YRp15/*leu2-Δ1* plasmid was transformed into YPH45 and YPH5 and stable *URA3 SUP11* (white) integrants colony purified. Ura$^-$ segregants were selected using the 5-fluoro-orotic acid selection procedure (BOEKE, LACROUTE and FINK 1984) and several *ura3* segregants tested for simultaneous acquisition of red and Leu$^-$ phenotypes. YPH98 (*MAT***a** *ura3 trp1 leu2*) and YPH102 (*MATα ura3 his3 leu2*) were isolated and shown to contain the *leu2-Δ1* allele on chromosome *III* by genomic Southern evaluation (data not shown). YPH98 and YPH102 were mated, the diploid was sporulated and tetrads were dissected and scored for their Ura, Lys, Ade, His, Trp, Leu and mating phenotypes. Strains YPH266, YPH267, YPH258, YPH259, YPH262, YPH263, YPH250 and YPH252 are all haploid strains isolated from this cross. YPH274 was isolated by microdissection of a zygote from a mating between YPH250 and YPH252. The alternative *trp1* deletion allele (*trp1-Δ63*; G. YELLEN and P. HIETER, unpublished data) was constructed by inserting the 1.5-kb *Eco*RI/*Hin*dIII fragment (immediately centromere proximal to the *TRP1* gene) into the *Eco*RI/*Hin*dIII sites of YRp14. This recombinant was linearized with *Eco*RI, made blunt-ended by fill in with Klenow fragment polymerase, and ligated to the 0.8-kb *Hin*dIII/*Eco*RI fragment (made blunt-ended with Klenow fragment) that includes the 3′ end of the *TRP1* gene. Recombinant plasmids were selected in which the genomic orientation of the fragments with respect to one another was preserved. The final construct represents a 0.6-kb *Eco*RI/*Hin*dIII deletion that deletes the promoter and 5′ portion of the *TRP1* gene. The *trp1-Δ63* deletion was introduced into YPH102 as described above for the *leu2-Δ1* deletion. The resultant yeast strain (GY36) was mated to YPH1, the diploid was sporulated, and tetrads were dissected. YPH499 and YPH500 are spore clones derived from this cross. YP501 was isolated by microdissection of a zygote from a mating between YP499 and YP500.

Construction of new yeast integrating plasmids (pRS300 series): The pBLUESCRIPT (Stratagene) plasmid was modified by ligating a *Pvu*I fragment (bp 498-2412) of pBLUESCRIPT (KS,M13+) to a *Pvu*I fragment (bp 2580-730) of pBLUESCRIBE (M13+; Stratagene). This hybrid, pRSS56, contains the KS polylinker from pBLUESCRIPT (see note added in proof) and the f1(+) phage DNA replication origin and unique *Aat*II and *Nde*I restriction sites (located between the f1 origin and β-lactamase gene) from pBLUESCRIBE. To make a set of YIp vectors, pRSS56 was digested with *Nde*I, made blunt ended with Klenow polymerase (referred to as "blunt, Klenow"), and blunt-end ligated to minimal DNA segments that encode the yeast *HIS3*, *TRP1*, *LEU2* and *URA3* genes to make pRS303, pRS304, pRS305 and pRS306, respectively.

pRS303 carries a 1184-bp *Eco*RI (blunt, Klenow) to *Bam*HI (blunt, Klenow) fragment containing the *HIS3* gene. The *Eco*RI site was the result of a synthetic linker placed at −296 with respect to the first HIS3 mRNA start site (K. STRUHL, personal communication). The *Bam*HI site was a synthetic linker placed at the genomic *Xho*I site.

pRS304 carries a 1002-bp *Hin*cII to *Pst*I (blunt, T4 DNA polymerase) fragment containing the *TRP1* gene. Both sites were genomic. The *Eco*RI site in this fragment (external to coding sequence) was destroyed by *Eco*RI digestion, Klenow fill-in and religation.

pRS305 carries a 2235-bp *Sal*I (blunt, Klenow) to *Sal*I (blunt, Klenow) fragment containing the *LEU2* gene. The 3′ *Sal*I site was genomic. The 5′ *Sal*I site corresponds to the genomic *Xho*I site after modification by the addition of a synthetic linker (M. SMITH, personal communication).

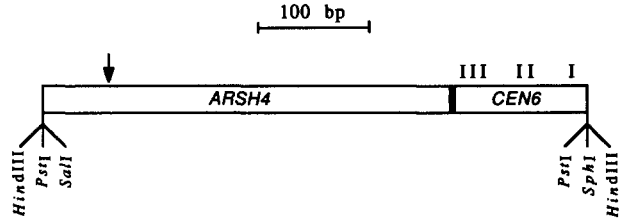

FIGURE 1.—Organization of the *CEN6/ARSH4* DNA cassette. The map is from pRSS84, but pUC sequences are omitted. The arrow points to the location of the *ARS* consensus sequence. The Roman numerals refer to the conserved centromere elements.

pRS306 carries a 1112-bp *Sac*I (blunt, T4 DNA polymerase) to *Sma*I fragment containing the *URA3* gene. The *Sac*I site was a modified genomic *Hin*dIII site. This modification was necessary to destroy the *Hin*dIII site in the final construct, since ligation of the Klenow blunted *Hin*dIII and *Nde*I sites would have regenerated the *Hin*dIII site. The *Sma*I site was genomic.

Construction of new yeast centromere plasmids (pRS310 series): The above integrating plasmids were converted into mitotically stable yeast replicating plasmids by the addition of a yeast centromere sequence (*CEN*) and autonomously replicating sequence (*ARS*). These two elements were first combined to make a 518 bp *CEN/ARS* DNA cassette (Figure 1). A minimal centromere DNA segment was obtained from pUC19-CEN6:32 which has the 125 bp *Mbo*II/*Mbo*II fragment of *CEN6* cloned into the *Hin*cII site of pUC19. This small DNA fragment is sufficient to confer complete mitotic and meiotic stability when tested on a yeast chromosome fragment (G. COTTAREL, J. SHERO, P. HIETER and H. HEGEMANN, unpublished data). The *ARS* DNA was obtained from pAB9, which has a 374 bp *Sau*3AI fragment containing the histone H4 associated *ARS* cloned into the *Bam*HI site of pUC8 (BOUTON and SMITH 1986). A *Sca*I/*Eco*RI fragment from pUC19-CEN6:32 and a *Sca*I/*Eco*RI fragment from pAB9 were isolated and ligated together such that the *CEN6* and *ARSH4* sequences would be juxtaposed in the polylinker region of the resulting plasmid, pRSS83. The restriction sites between *CEN6* and *ARSH4* were removed by digesting pRSS83 with *Sma*I and *Xba*I, treating with Klenow to make blunt ends, and ligating to recircularize. This was done so that the cassette would not contain any restriction sites that are present in the pBLUESCRIPT polylinker. The *CEN6/ARSH4* cassette was then isolated from this plasmid, pRSS84, as a *Hin*cII/*Pst*I fragment, made blunt with T4 DNA polymerase, and ligated into the *Aat*II (blunt, T4 DNA polymerase) sites of plasmids pRS303, pRS304, pRS305 and pRS306 to create plasmids pRS313, pRS314, pRS315 and pRS316, respectively. For uniformity, clones were selected that had the cassette oriented in the same direction, that is, with *CEN6* closest to the β-lactamase gene. Restriction maps of the new YIp and YCp vectors were created (Figure 2) based on the known sequences of all component parts [*HIS3*: STRUHL (1985); *TRP1*: TSCHUMPER and CARBON (1980) and DOBSON *et al.* (1983); *LEU2*: ANDREADIS *et al.* (1982, 1984); *URA3*: ROSE, GRISAFI and BOTSTEIN (1984); *CEN6*: PANZERI and PHILIPPSEN (1982); *ARSH4*: SMITH and ANDRESSON (1983); pBLUESCRIPT/pBLUESCRIBE: Stratagene]. All restriction sites labeled in these maps were checked and shown to be correct by restriction enzyme digestion and agarose gel electrophoresis for all members of the YCp series. As would be predicted from the sequences, all sites at the junctions of blunt end ligations were destroyed.

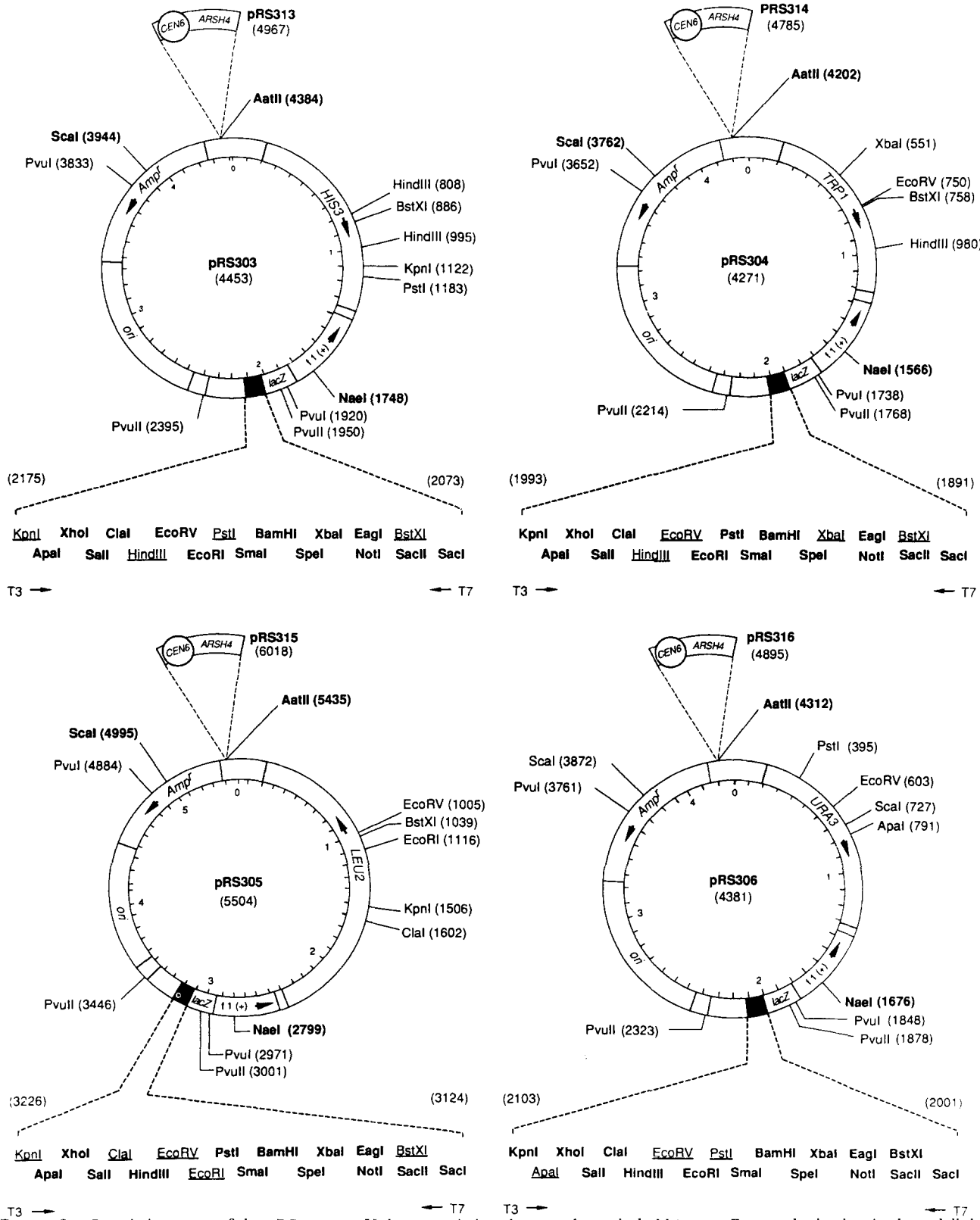

FIGURE 2.—Restriction maps of the pRS vectors. Unique restriction sites are shown in bold letters. For emphasis, sites in the polylinker that are no longer unique have been underlined. Maps of the YIp set may be converted to YCp maps by the insertion of the *CEN/ARS* cassette (the *Aat*II site is destroyed in the YCp derivatives). The numbering system is the same for both sets. The direction of T3 or T7 polymerase transcription is as labeled. Numbers in parentheses refer to base pairs.

Qualitative assessment of CEN and ARS function in the CEN/ARS cassette: The *Cen6/ARSH4* cassette was subcloned as a *Sal*I/*Hin*dIII fragment from pRSS84 into the *Sal*I/*Hin*dIII sites of pYCF5 (HEGEMANN *et al.* 1988), a YIp vector that contains the yeast *SUP11* and *URA3* genes, to make pRSS93. The parent vector pYCF5 is highly unstable mitotically, since it carries no *CEN* DNA and a very weak *ARS* (*ARS3*). About 5 μg of pRSS93 or the control plasmid YRp14/*ARS1*/*CEN4* (HIETER *et al.* 1985a) were transformed into YPH274. Transformants were selected on the appropriate minimal medium and replated onto nonselective plates containing limiting amounts of adenine (6 μg/ml) as described (HIETER *et al.* 1985a). The color sectoring frequencies of the colonies were then compared.

Quantitative assessment of CEN and ARS function in the CEN/ARS cassette: The mitotic stability of the four pRS YCp vectors was quantitated as follows. Strain YPH274 was transformed with the test plasmid and plated to selective medium. Five colonies from each transformation were picked to liquid selective medium and grown to stationary phase. The percentage of cells in this culture that contained the plasmid was determined by plating a dilution to YPD plates and replica-plating the resulting colonies to selective plates. The stationary phase culture was then diluted 1:1024 in minimal complete medium and growth without selection until stationary phase (approximately 10 generations). Again, the percentage of cells carrying the plasmid was determined. YCp19, a 10.6 kb pBR322 based vector containing *CEN4*, *ARS1*, *TRP1* and *URA3* (C. MANN and R. DAVIS, unpublished), served as a control.

RESULTS

Construction of an isogenic set of yeast host strains: A series of yeast strains carrying various combinations of nonreverting *ura3*, *his3*, *leu2* and *trp1* alleles were constructed for efficient use with the pRS vector series. All strains are derived from YNN216, which is a strain congenic to S288C (original source: Dr. M. CARLSON, Columbia University). This strain carries the nonreverting *ura3-52* mutation (ROSE and WINSTON 1984), sporulates well, has good DNA-mediated transformation properties, is Gal$^+$, and carries an amber (*lys2-801*) and ochre (*ade2-101*) mutation. The *ade2-101* mutation is useful, since it allows use of the cloned *SUP11* gene as a colony color marker (HIETER *et al.* 1985a). In addition, the copy number of the *SUP11* gene (0, 1 or 2 copies) present in the colonies can be visually distinguished (for discussion see KOSHLAND and HIETER 1987). As described in detail in MATERIALS AND METHODS, we have introduced nonreverting deletion alleles of the *HIS3*, *TRP1* and *LEU2* genes via gene replacement techniques, using the transplacement vectors listed in Table 2. These strains represent an isogenic set with all combinations of four nonreverting auxotrophic markers in both mating types. The *his3-Δ200* and *trp1-Δ1* alleles eliminate essentially all homology to the *HIS3* and *TRP1* markers present in the pRS vector series. Therefore, targeted integration and/or gene conversion at these sites is totally eliminated. The *ura3-52*, *leu2-Δ1*, and *trp1-Δ63* alleles are also nonreverting but retain homology to the *URA3*, *LEU2* and *TRP1* markers present on the pRS vector series. It is therefore possible to target homologous integrative recombination events to the *URA3*, *LEU2* or *TRP1* loci using pRS306, pRS305 or pRS304, respectively.

There are two important differences between the *trp1* deletion alleles that should be considered before choosing between them for use in particular experiment. The first, mentioned above, is that *trp1-Δ63* retains homology to the *TRP1* selectable marker gene present in the pRS vectors, whereas *trp1-Δ1* does not. As a consequence, the possibility of integration and/or gene conversion at the *TRP1* locus exists for *trp1-Δ63* but not for *trp1-Δ1*. Therefore, the *trp1-Δ1* allele is preferable in experiments in which recombination between the vector and the genomic *TRP1* locus presents a problem. The second difference is that the *trp1-Δ1* deletion also deletes the UAS elements required for expression of the adjacent *GAL3* gene, whereas the *trp1-Δ63* deletion does not (BAJWA, TORCHIA and HOPPER 1988). *trp1-Δ1* strains are therefore simultaneously *gal3*$^-$, whereas *trp1-Δ63* strains remain *GAL3*$^+$. Although *gal3* yeast are able to utilize galactose as a carbon source and induce galactose regulatable promoters fully, the kinetics of induction is slow relative to *GAL3* strains. Therefore, the *trp1-Δ63* allele is preferable in experiments that require rapid transcriptional induction by galactose.

Construction of a uniform set of new yeast integrating plasmids: A set of four YIp vectors (pRS303, pRS304, pRS305 and pRS306) that differ in sequence from each other only in the yeast selectable marker gene was constructed (Figure 2). A modified version of the versatile plasmid pBLUESCRIPT (see MATERIALS AND METHODS) served as the backbone for all pRS plasmids. Modification of pBLUESCRIPT was necessary to create unique cloning sites that were external to the f1 origin, polylinker, and *LacZ* gene. One of these sites was then used to insert minimal DNA sequences that contained the yeast *HIS3*, *TRP1*, *LEU2* or *URA3* genes. Following linearization within a cloned insert DNA, and integrative transformation into the appropriate yeast host strain, pRS303, pRS304, pRS305 and pRS306 confer prototrophy to yeast strains carrying *his3*, *trp1*, *leu2* or *ura3* mutations, respectively. Greatly reduced (approximately 1000-fold) numbers of transformants are produced if linearization is omitted. This is comparable to results using conventional YIp vectors (for example, YIp5) and demonstrates the lack of detectable *ARS* activity in the modified pBLUESCRIPT sequence.

Construction of a uniform set of new yeast centromere plasmids: A set of YCp vectors was created by the addition of yeast *ARS* and *CEN* sequences to plasmids of the pRS YIp series (Figure 2). *ARSH4* and *CEN6* were chosen because of their small size (374 bp

TABLE 2

List of plasmids

Plasmid	Composition	Reference
Transplacement vectors		
YRp14/*trp1-Δ1*	pBR322, *URA3*, *SUP11*, *trp1* deletion (1.45 kb)	This paper
YRp15/*leu2-Δ1*	pBR322, *URA3*, *SUP11*, *leu2* deletion (0.6 kb)	This paper
YRp14/*his3-Δ200*	pBR322, *URA3*, *SUP11*, *his3* deletion (1.0 kb)	FASULLO and DAVIS (1988)
YRp14/*trp1-Δ63*	pBR322, *URA3*, *SUP11*, *trp1* deletion (0.6 kb)	G. YELLEN (unpublished data)
Cloning vectors		
pRS303	pBluescript, *HIS3*	This paper
pRS304	pBluescript, *TRP1*	This paper
pRS305	pBluescript, *LEU2*	This paper
pRS306	pBluescript, *URA3*	This paper
pRS313	pBluescript, *HIS3*, *CEN6*, *ARSH4*	This paper
pRS314	pBluescript, *TRP1*, *CEN6*, *ARSH4*	This paper
pRS315	pBluescript, *LEU2*, *CEN6*, *ARSH4*	This paper
pRS316	pBluescript, *URA3*, *CEN6*, *ARSH4*	This paper
Other		
pRSS56	pBluescript/Bluescribe	This paper
pRSS84	pUC, *CEN6/ARSH4* Cassette	This paper

and 125 bp, respectively) and paucity of restriction enzyme recognition sites. For ease in manipulation, *CEN6* and *ARSH4* were first combined into a 518 bp DNA cassette (Figure 1). Because the proximity or organization of the two DNA elements might have adversely affected their function, we first assayed the combined function of *CEN* and *ARS* in a transformation, colony-color assay (HIETER *et al.* 1985a). The *CEN6/ARSH4* cassette was cloned into a YRp14 derivative (pYCF5) and transformed into a yeast strain containing an ochre supressible allele of *ade2*. (This vector contains a very weak *ARS* (*ARS3*) and no *CEN* sequence and is consequently highly unstable.) Transformants were produced that grew at normal rates and had a colony sectoring frequency similar to the control plasmid YRp14/*ARS1/CEN4*, which has larger *CEN* and *ARS* sequences (data not shown). The *CEN6/ARSH4* cassette was then inserted into pRS303, pRS304, pRS305, and pRS306 to make the YCp derivatives pRS313, pRS314, pRS315 and pRS316, respectively. Circular forms of these new YCp vectors efficiently transform yeast at frequencies comparable to those obtained using conventional YCp vectors (for example, YCp50; data not shown). As a more definitive test of the *CEN* and *ARS* function, we quantitated the stability of the new YCp vectors in yeast transformants. It is clear from the data in Table 3 that all pRS YCp vectors are as mitotically stable as the standard YCp vector YCp19. Since mitotic stability requires *ARS* and *CEN*, we assume that both are functional in the new vectors.

TABLE 3

Mitotic stability of the pRS YCp vector series

Vector	Marker scored	Percentage of cells containing vector after nonselective growth: 0 Generations	10 Generations
pRS313	*HIS3*	73 ± 9	48 ± 4
pRS314	*TRP1*	74 ± 17	64 ± 14
pRS315	*LEU2*	77 ± 8	58 ± 5
pRS316	*URA3*	71 ± 8	57 ± 9
YCp19	*URA3*	81 ± 6	57 ± 13

The calculated value is the average and standard deviation obtained from five individual cultures started from five independent transformants. Zero generations refers to a stationary phase culture grown in selective medium. See MATERIALS AND METHODS for details.

DISCUSSION

We have used minimal, defined segments of yeast DNA (*CEN6*, *ARSH4*, *HIS3*, *TRP1*, *LEU2* and *URA3*) to convert the multipurpose *E. coli* plasmid pBLUESCRIPT into a set of YIp and YCp vectors. All members of the set are uniform in structure, and one need only be familiar with the cloning sites and features of pBLUESCRIPT to fully manipulate any of the pRS vectors. We have also made a set of isogenic yeast host strains containing mutations (*his3*, *trp1*, *leu2* and *ura3*) that will allow for selection of any or all of these vectors. In addition to the general features afforded the pRS vectors by the pBLUESCRIPT backbone, such as ssDNA production, high plasmid DNA yields, an extensive polylinker, unidirectional deletion formation, and simplified cloning (blue/white

screening for recombinants), these new vectors offer unique yeast-specific features.

The YCp vectors allow one to perform almost all routine yeast DNA manipulations in the same plasmid. A typical scheme for the characterization of a newly isolated gene can be seen in the recent analysis of *CHL1* (S. GERRING and P. HIETER, unpublished results). First, the gene was cloned by complementation of a yeast mutation and a pRS YCp vector recombinant was obtained. This was done by subcloning the gene from an existing library clone, but this step can obviously be bypassed if the library is made directly in the pRS vector. Next, a series of unidirectional, exonuclease III generated deletions were made by taking advantage of the large polylinker and flanking 3′ overhang restriction sites (*Exo*III does not digest 3′ overhang structures). Since these deletion constructs can be transformed directly into yeast, the borders of the gene were easily identified by assaying several representative deletions for loss of complementing activity. The deletions beyond the gene border were directly converted into ssDNA templates for sequencing. Deletion series generated from two clones (direct and inverted gene orientations) yielded sequencing data from both DNA strands as well as the functional 5′ (promoter) and 3′ borders of the gene.

The unidirectional deletions generated in a pRS vector have several uses in addition to sequencing. The 3′ deletions can be used to define activity domains (such as DNA binding regions in DNA binding proteins) or they can serve as potential dominant negative (HERSKOWITZ 1987) mutant alleles. The 5′ deletions can be cloned into bacterial expression vectors (*e.g.*, pATH) for the production of fusion proteins. Appropriate 5′ deletions (within the 5′ untranslated leader) can also be used directly in an *in vitro* coupled transcription/translation reaction (HOPE and STRUHL 1985) using the phage promoter on the vector. In addition, by combining 5′ and 3′ deletions one can construct null alleles (deletions), which can then be recombined into the yeast chromosome.

The extensive polylinker region makes the YIp vectors ideal for a gene disruption procedure that we have termed "γ transformation" (a variation of "Ω transformation"; ROTHSTEIN, 1983). Sequences flanking the DNA to be deleted are cloned into the polylinker of a pRS YIp vector in tandem but reverse order leaving a unique restriction site between them (Figure 3). Transformation of the linearized construct results in a deletion mutation at the chosen locus and the insertion of plasmid DNA. This method has been used with a pRS vector to disrupt the yeast *CKA1* gene (C. GLOVER, personal communication) and to introduce a centromere deletion/substitution allele onto chromosome *VI* (H. HEGEMANN, personal communication). γ transformation is especially useful

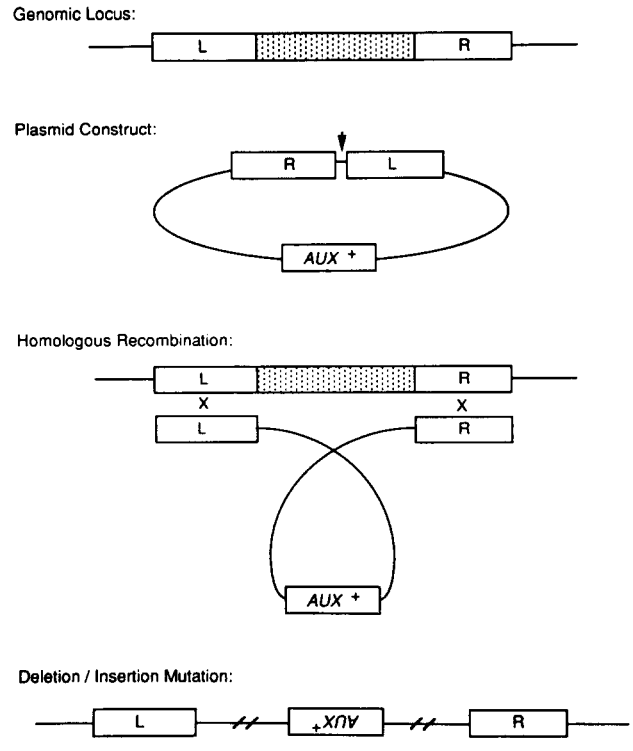

FIGURE 3.—Modification of a genomic locus by "γ-transformation." DNA segments flanking the segment that is to be deleted are cloned into a pRS YIp in tandem but reverse order. After linearization at the site designated with an arrow and transformation, the construct recombines as shown (when diagrammed, this structure resembles the Greek letter γ). The overall result is a deletion of the chosen DNA segment and an insertion of the entire plasmid. *AUX*$^+$ refers to the selectable marker gene.

when the restriction sites required to create a particular deletion are not unique in the cloned locus. In such cases (which in our experience are quite common), making the deletion by an Ω transformation construct would require complex, partial restriction enzyme digestion followed by insertion of a yeast selectable marker DNA fragment. In contrast, making such a deletion by a γ transformation construct (a two-step cloning procedure) will often be simple due to the extensive polylinker in the pRS vectors.

The streamlined design of the pRS vectors makes them well suited to serve as the starting point for construction of other yeast vectors. High copy 2μ type plasmids (YEp) are useful for overexpressing gene products, but the standard 2μ vectors are also not optimized for DNA manipulations. Insertion of a small DNA fragment containing the 2μ origin and *flp* site into the AatII site of a pRS YIp vector makes it high copy in yeast, and several pRS 2μ derivatives have already been made (J. SHERO and P. HIETER, unpublished results). Genes manipulated in pRS YIp or YCp vector recombinants can readily be converted to pRS YEp recombinants by exchanging *Pvu*I-*Pvu*I segments between these plasmids. For example, with pRS303 this would mean replacing *Pvu*I (3833)-*Pvu*I (1920) with the corresponding segment from a pRS

2μ plasmid. Since recognition sites for *Pvu*I (double CG, six base pair) are very rare in yeast DNA, this will usually be possible. (Alternatively, flanking sites in the polylinker can be used with some inserts.) In fact, exchange of *Pvu*I segments can be used to conveniently shuffle DNA segments between all pRS vectors when a different marker gene or vector type is needed. Overexpression vectors can also be designed using heterologous promoters. Since the polylinker region contains a continuous open reading frame with no ATG start codons in any frame, it can serve as untranslated leader sequence or coding sequence, depending on the vector. For example, we have inserted a cassette containing the inducible *GAL1/GAL10* promoters into the *Kpn*I site in two orientations. One orientation has an mRNA start but no ATG; the other orientation has an mRNA start and an ATG that is in frame with the *LacZ* gene. Interestingly, *E. coli* colonies carrying this latter plasmid remain blue on X-gal plates (R. S. SIKORSKI, unpublished observation).

Because the yeast strains that we have made will allow the use of four different selectable markers, they have the potential to accommodate up to four different plasmids. This can be useful in those experiments requiring the modification of several genetic loci in the same cell, for example, when a gene family is being studied. The gene replacement vectors listed in Table 2 can be used to introduce *trp1*, *leu2* and *his3* deletion mutations into any existing *ura3* strain. The resultant strains are perfectly isogenic to the original parent and become competent for use of the set of pRS vectors and for the DNA manipulations made possible by them.

Addendum regarding production of LacZ′ fusion proteins: We have been notified by Stratagene that all pBLUESCRIPT KS plasmids contain a cryptic single base pair deletion of one of the three Gs (GCTGGGTACC) immediately upstream of the *Kpn*I site of the polylinker and downstream of the *LACZ′* initiating ATG codon. The only consequence of this missing nucleotide is a change in the polylinker-*LacZ′* reading frame. *E. coli* colonies carrying this plasmid, however, still remain (by an unknown mechanism) blue on X-gal medium, and inactivation of the blue color phenotype by insertion of cloned DNA *can still be used* to identify recombinants. All applications of the pRS vectors described in this paper are unaffected by this base deletion. However, if one plans to use the *LacZ* promoter and the polylinker open reading frame to make fusion proteins one must be aware of this frame shift and adjust the predicted reading frame accordingly. Recently, a new set of pRS vectors has been created that utilizes the polylinker of pBLUESCRIPT II (which does contain the G residue mentioned above and also contains flanking *Bss*HII sites). These new plasmids, pRS403, 404, 405, 406, 413, 414, 415, and 416, which are structurally similar to pRS 303, 304, 305, 306, 313, 314, 315, and 316, respectively, are recommended if expression of a *LacZ′* fusion protein is intended.

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