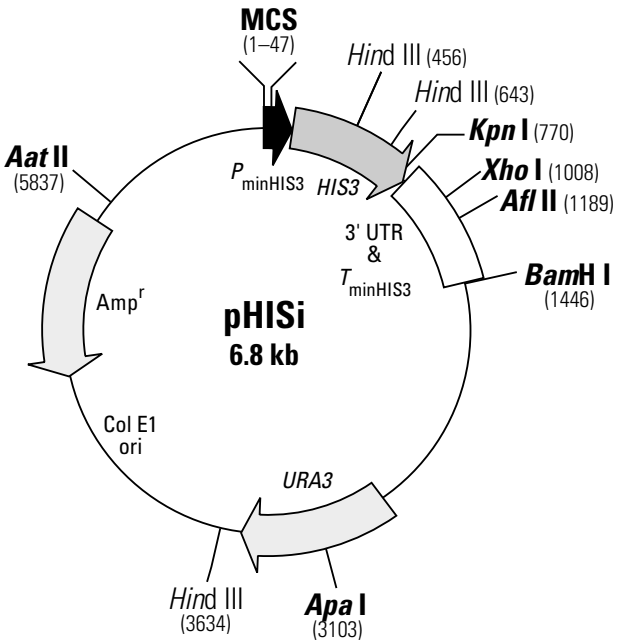
Appendix A. MATCHMAKER One-Hybrid Vectors



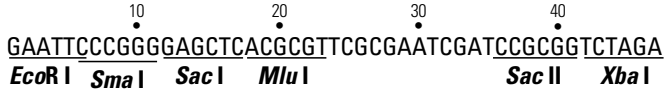
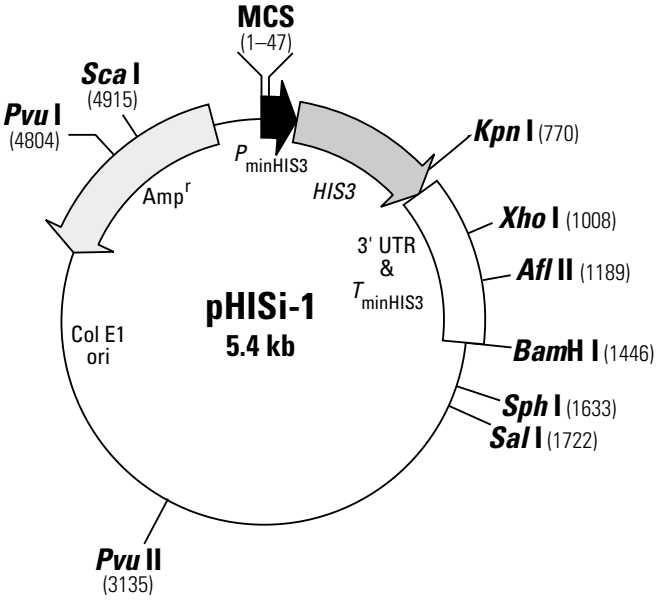


Figure 5. Map and multiple cloning site (MCS) of pHISi. pHISi is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. pHISi contains the yeast *HIS3* gene downstream of the MCS and the minimal promoter of the *HIS3* locus (P_{minHIS}). *Cis*-acting sequences of interest (i.e., target elements) can be inserted into the MCS. Without activation by a target element, constitutive *HIS3* expression from P_{minHIS} is very low in yeast, but allows enough growth to select for integration when constructing *HIS3* reporter strains. During library screening, the leaky expression of *HIS3* is controlled by adding 3-AT to the medium.

The yeast *URA3* and *HIS3* genes of pHISi can be used as selectable markers for integration into the nonfunctional *ura3* and *his3* loci, respectively, of the YM4271 host strain. Before integrating, the vector is linearized at the *Xho* I or *Afl* II sites (*his3* locus) or at the *Apa* I site (*ura3* locus). The *Kpn* I site cannot be used for integration because it cuts within the coding region of the *HIS3* gene, and that region is deleted in YM4271. pHISi cannot replicate autonomously in yeast. The plasmid contains a bacterial Col E1 origin (ori) and the ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli.* Unique restriction sites are in bold.

Appendix A. MATCHMAKER One-Hybrid Vectors *continued*



GAATTCCCGGGGAGCTCACGCGTTCGCGAATCGATCCGCGGTCTAGA

*Eco*R I *Sma* I/ *Xma* I *Sac* I *Mlu* I *Sac* II *Xba* I

Figure 6. Map and multiple cloning site (MCS) of pHISi-1. pHISi-1 is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. pHISi-1 contains the yeast *HIS3* gene downstream of the MCS and the minimal promoter of the *HIS3* locus (P_{minHIS}). *Cis*-acting sequences of interest (i.e., target elements) can be inserted into the MCS. Without activation by a target element, constitutive *HIS3* expression from P_{minHIS} is very low in yeast, but allows enough growth to select for integration when constructing *HIS3* reporter strains. During library screening, the leaky expression of *HIS3* is controlled by adding 3-AT to the medium. pHISi-1 was constructed by transferring the *HIS* reporter gene from pHISi to the *Eco*R I/*Bam*H I sites of pBR322. Leaky *HIS3* expression in pHISi-1 is generally lower than that in pHISi, presumably due to differences in the flanking vector sequence.

The yeast *HIS3* gene is used as a selectable marker for integration into the nonfunctional *his3* locus of the YM4271 host strain after linearizing the vector at the *Xho* I or *Afl* II sites. The *Kpn* I site cannot be used for integration because it cuts within the coding region of the *HIS3* gene, and that region is deleted in YM4271. Because it does not carry the *URA3* marker, pHISi-1 can be used (together with pLacZi) to construct a dual *HIS3/lacZ* reporter strain. pHISi-1 cannot replicate autonomously in yeast. pHISi-1 contains a bacterial Col E1 origin (ori) and the ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*. Unique restriction sites are in bold.

Appendix A. MATCHMAKER One-Hybrid Vectors *continued*

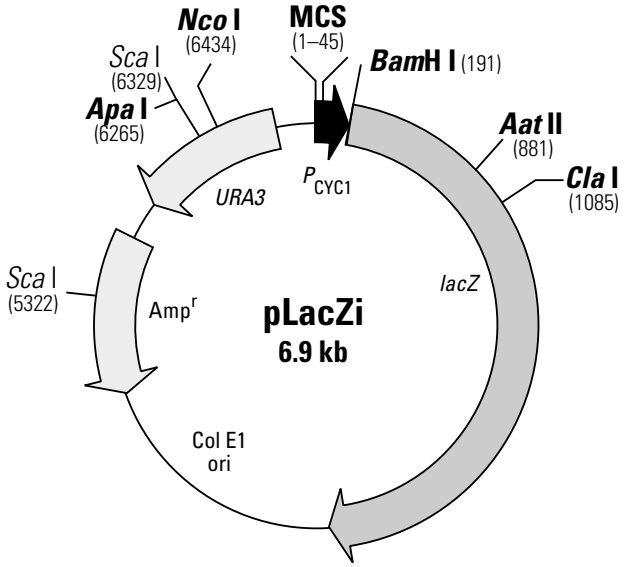


Figure 7. Map and multiple cloning site (MCS) of pLacZi. pLacZi is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. This plasmid contains the bacterial *lacZ* gene downstream of the minimal promoter of the yeast iso-1-cytochrome C gene (P_{CYC1}). Target elements can be inserted into the MCS upstream of the P_{CYC1}-*lacZ* reporter. Without activation from a *cis*-regulatory element, *lacZ* expression is very low when the vector is integrated into the yeast genome. The yeast *URA3* gene is used as a selectable marker for integration into the nonfunctional *ura* locus of the YM4271 host strain after linearizing the vector at the *Nco* I or *Apa* I site. pLacZi cannot replicate autonomously in yeast. This plasmid contains the ampicillin resistance gene (Amp^r) and the Col E1 origin for selection and propagation in *E. coli*. Unique restriction sites are in bold.