SUPPLEMENTARY METHODS

Construction of the Plasmids

The integrative plasmids are based on pRS303, pRS305, or pRS306 (1). First, the multicloning sites (MCS) of these plasmids were destroyed, followed by insertion of the drug resistance marker in the HIS3, LEU2, or URA3 gene.

pRS303X series. The plasmid pRS303 was digested with Ecl136II and XhoI, treated with mung bean nuclease, and self-ligated to obtain plasmid pRS303nomcp. Plasmid pRS305K was created by ligation of the 1.5-kb EcoRI/PvuII fragment from pFA6a-kanMX4 into pRS305nomcp opened with EcoRI/EcoRV. The plasmid pRS305H was created by ligation of the 1.7-kb BamHI/Ecl136II fragment from pFA6a-hphNT1 into BamHI/Ecl136II-digested plasmid pRS305K. The plasmid pRS305N was created by ligation of the 1.3-kb BamHI/Ecl136II fragment from pFA6a-natNT2 into BamHI/Ecl136II-digested plasmid pRS305K.

pRS306X series. The plasmid pRS306 was digested with Ecl136II and XhoI, treated with mung bean nuclease, and self-ligated to obtain plasmid pRS306nomcp. Plasmid pRS306K was created by ligation of the 1.5-kb EcoRI/PvuII fragment from pFA6a-kanMX4 into pRS306nomcp opened with EcoRI/EcoRV. The plasmid pRS306H was created by ligation of the 1.7-kb BamHI/Ecl136II fragment from pFA6a-hphNT1 into BamHI/Ecl136II-digested plasmid pRS306K. The plasmid pRS306N was created by ligation of the 1.3-kb BamHI/Ecl136II fragment from pFA6a-natNT2 into BamHI/Ecl136II-digested plasmid pRS306K.

The centromeric and episomal plasmids were derived from plasmids pRS416 and pRS426, respectively (1,2). The URA3 gene and the flanking promoter and terminator regions in these plasmids were replaced by the three different dominant selection markers. The selection markers were amplified by PCR using the oligonucleotide pairs pRS-nat/hph/kan1 and pRS-nat2 with plasmid pFA6a-kanMX4 (7), pRS-nat/hph/kan1 and pRS-hph2 with plasmid pFA6a-hphNT1 (5), as well as pRS-nat/hph/kan1 and pRS-nat2 with plasmid pFA6a-natNT2 (5). The oligonucleotides are given in Supplementary Table S1. The PCR products contained the drug resistance markers flanked by regions homologous to DNA sequences flanking the URA3 regions in pRS416 and pRS426. The plasmids pRS416 and pRS426 were digested with StuI and transformed into yeast strain ESM356 (17), together with the PCR products. After selection of drug resistance, yeast clones, and plasmid rescue into Escherichia coli, the plasmids pRS41H, pRS41K, pRS41N, pRS42H, pRS42K, and pRS42N were obtained. They were verified by sequencing and restriction digest.

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Figure S1. Detailed maps of the new centromeric and episomal plasmids. Restrictions sites shown in the maps are unique in the vectors. The sites marked in red are unique in the multicloning site of all six vectors. Full plasmid sequences can be obtained upon request from the authors.
Figure S2. Detailed maps of the new integrative plasmids. Restrictions sites shown in the maps are unique in the vectors. The unique restriction enzyme sites for the introduction of foreign DNA in the multicloning site are shown in blue. The sites, which can be used in addition downstream of the drug resistance marker, are shown in green. It is also possible to use restriction sites that lay within the auxotrophic marker gene. However, the remaining part of the auxotrophic marker has to be long enough to allow integration into the yeast genome by homologous recombination. Full plasmid sequences can be obtained upon request from the authors.