HERP cassettes can be used to replace a single loci on both chromosomes simultaneously. A) Eight S. cerevisiae double replacement transformants were sporulated by first patching culture onto ultra-rich medium (2% yeast extract, 5% peptone, 6% glucose, 1.8% agar) at 30° for 24 hours, then spreading onto sporulation media (0.1% potassium acetate, 0.05% zinc acetate, 1.8% agar) at room temperature for six days. Twenty tetrads for each transformant were dissected onto YPD, and three fully-viable tetrads were picked and struck to YPD plates. The ADE2 locus of each of spore-derived streaks was amplified by colony PCR (cPCR); all 96 spores exhibited a band whose size was consistent with replacement of the HERP cassette, and all 96 bands, when sequenced, possessed the S. uvarum ADE2 sequence. Lanes labeled with the same number and a letter A-D are derived from four different spores from the same dissected tetrad. The lane labeled "M" is 1 kbp DNA ladder from New England Biolabs. B) The three S. uvarum double replacement candidates were sporulated by direct plating to sporulation media. 20 tetrads were dissected for each transformant, and three fully-viable tetrads were struck to YPD plates for analysis via cPCR and sequencing as above. All 36 strains possessed the PTDH3-yEGFP-TCYC2 construct (HITTINGER and CARROLL 2007) at the targeted locus on chromosome V.