Table S1. Strain genotypes and constructions.

Strain name	Genotype	Strain construction (reference)
S1	*MATa gal2*	S288c background, Engel *et al.*, 2014
W303-1A	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 RAD5*	Thomas and Rothstein 1989
YJM789	*MATα lys2 gal2 ho::hisG*	Wei *et al.*, 2007
YJM790	*MATa lys2 gal2*	John McCusker, Duke University
JSC10-1	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::natMX4 RAD5*	W303-1A background, St. Charles and Petes 2013
JSC19-1	*MATα ade2-1 ura3 gal2 ho::hisG can1Δ::natMX4*	YJM789 background, St. Charles and Petes 2013
YZ08	*MATa gal2 V31539::kanMX4*	S1 transformed with PCR fragment amplified from plasmid pFA6a-kanMX4 using primers V31539::DR F and V31539::DR R; insert *kanMX4* next to *CAN1*
YZ10	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::natMX4 RAD5 VIII212140::hphMX4*	JSC10-1 transformed with PCR fragment amplified from plasmid pAG32 using primers VIII212140::HYG F and VIII212140::HYG R; insert *hphMX4* at *VIII212140*
YZ15	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::natMX4 RAD5 VIII212140::hphMX4 VIII218090::CAN1-kanMX4*	YZ10 transformed with PCR fragment amplified from YZ08 using primers VIII218090::CAN1-KANMX F and VIII218090::CAN1-KANMX R; insert *CAN1-kanMX4* at *VIII218090*
YZ18-10	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::natMX4 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4*	YZ15 transformed with PCR fragment amplified from S1 using primers VIII214177::URA3 F and VIII214177::URA3 R; insert *URA3* into one of the *CUP1* repeats at VIII214177; the *CUP1* repeat with *URA3* inserted was physically confirmed to be at the center of the cluster
YZ17	*MATα ade2-1 ura3 gal2 ho::hisG can1Δ::natMX4*	A derivative of JSC19-1 with enhanced copper resistance; constructed by selecting 0.4 mM copper sulfate-resistant derivatives of JSC19-1 on SDC + 0.4 mM copper sulfate; the *CUP1* cluster in this strain is physically measured to be 30 kb
YZ26	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::natMX4 RAD5*	A *MATα* derivative of JSC10-1; constructed by mating type switching of JSC10-1 using the plasmid pGAL-HO
YZ41	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4*	YZ18-10 transformed with PCR fragment amplified from S1 using primers CAN1::HIS3 F and CAN1::HIS3 R; replace *can1-100Δ::natMX4* with *HIS3*
YZ42	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5*	YZ26 transformed with PCR fragment amplified from S1 using primers CAN1::HIS3 F and CAN1::HIS3 R; replace *can1-100Δ::natMX4* with *HIS3*
YZ43	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD52::NAT F and RAD52::NAT R; replace *RAD52* with *natMX4*

	kanMX4 rad52Δ::natMX4	
YZ44	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 rad52Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD52::NAT F and RAD52::NAT R; replace *RAD52* with *natMX4*
YZ45	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 rad51Δ::natMX4*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD51::NAT F and RAD51::NAT R; replace *RAD51* with *natMX4*
YZ46	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 rad51Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD51::NAT F and RAD51::NAT R; replace *RAD51* with *natMX4*
YZ47	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 mre11Δ::natMX4*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers MRE11::NAT F and MRE11::NAT R; replace *MRE11* with *natMX4*
YZ48	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 mre11Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers MRE11::NAT F and MRE11::NAT R; replace *MRE11* with *natMX4*
YZ49	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 rad1Δ::natMX4*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD1::NAT F and RAD1::NAT R; replace *RAD1* with *natMX4*
YZ50	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 rad1Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD1::NAT F and RAD1::NAT R; replace *RAD1* with *natMX4*
YZ51	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 cup2Δ::natMX4*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers CUP2::NAT F and CUP2::NAT R; replace *CUP2* with *natMX4*
YZ52	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 cup2Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers CUP2::NAT F and CUP2::NAT R; replace *CUP2* with *natMX4*
YZ60	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 can1-100Δ::HIS3 RAD5*	YZ42 transformed with PCR fragment amplified from S1 using primers IV461633 F and IV462765 R; replace *trp1-1* with *TRP1*
HB1	*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 rad5Δ::natMX4*	YZ41 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD5::NAT F and RAD5::NAT R; replace *RAD5* with *natMX4*
HB3	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 rad5Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers RAD5::NAT F and RAD5::NAT R; replace *RAD5* with *natMX4*
HB5	*MATa leu2-3,112 his3-11,15 ura3-1*	YZ41 transformed with PCR fragment amplified

	ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 VIII212140::hphMX4, VIII214177::URA3, VIII218090::CAN1-kanMX4 mms2Δ::natMX4	from plasmid pAG25 using primers MMS2::NAT F and MMS2::NAT R; replace *MMS2* with *natMX4*
HB7	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100Δ::HIS3 RAD5 mms2Δ::natMX4*	YZ42 transformed with PCR fragment amplified from plasmid pAG25 using primers MMS2::NAT F and MMS2::NAT R; replace *MMS2* with *natMX4*
YZ103	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::natMX4/can1-100Δ::natMX4 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090*	YZ18-10 x YZ26
YZ104	*MATa/MATα HO/ ho::hisG leu2-3,112/LEU2 his3-11,15/HIS3 ura3-1/ura3 ade2-1/ade2-1 trp1-1/TRP1 can1-100Δ::natMX4/can1Δ::natMX4 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090*	YZ18-10 x YZ17
YZ113	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::can1-kanMX4/VIII218090 rad52Δ::natMX4/ rad52Δ::natMX4*	YZ43 x YZ44
YZ114	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 rad51Δ::natMX4/ rad51Δ::natMX4*	YZ45 x YZ46
YZ115	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 mre11Δ::natMX4/ mre11Δ::natMX4*	YZ47 x YZ48
YZ116	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3*	YZ49 x YZ50

	RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 rad1Δ::natMX4/rad1Δ::natMX4	
HB9	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 rad5Δ::natMX4/rad5Δ::natMX4*	HB1 x HB3
HB13	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 mms2Δ::natMX4/ mms2Δ::natMX4*	HB5 x HB7
YZ117	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 cup2Δ::natMX4/cup2Δ::natMX4*	YZ51 x YZ52
YZ118	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 can1-100Δ::HIS3/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090 cup2Δ::natMX4/cup2Δ::natMX4* with plasmid p425-GPD-CUP1.	YZ117 transformed with plasmid p425-GPD-CUP1
MD692	*MATa/MATα leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/TRP1 can1-100Δ::natMX4/can1-100Δ::HIS3 RAD5/RAD5 VIII212140::hphMX4/VIII212140 VIII214177::URA3/VIII214177 VIII218090::CAN1-kanMX4/VIII218090*	YZ18-10 x YZ60

Table S2. PCR primer names and sequences used in strain constructions and analysis.

Primer name	Sequence (5' to 3')	Strain constructed /analyzed
V31539::DR F	AGGTCTGAAGGAGTTTCAAATGCTTCTACTCCGTCTGCTTTCTTTTCGGGACGTACGCTGCAGGTCGAC	YZ08 (insert *kanMX4* into S1 at *V31539*)
V31539::DR R	CTATAATCTCAGTATATTTATTTTTATCGATGATTCACCAACAATCTTGCTATCGATGAATTCGAGCTCG	YZ08 (insert *kanMX4* into S1 at *V31539*)
EXT V31539 F	TGGTTCTAGGTTCGGGTGACG	YZ08 (confirm *V31539::kanMX4* insertion)
EXT V31539 R	CACATTTCCACGCCATTTCG	YZ08 (confirm *V31539::kanMX4* insertion)
VIII212140::HYG F	ATATATGTTTGAAGTGTATATTAAAAATAAAGTCATTATTTGAATATTGGTTTCTCGGTCTAAGAGCTTACGTACGCTGCAGGTCGAC	YZ10 (insert *hphMX4* into JSC10-1 at *VIII212140*)
VIII212140::HYG R	TGTTATCCCCGTCACTTTCAATGATCTATTTATTTGAAGCGGATAGTACAACAGATCAGTCTAAAACGTAATCGATGAATTCGAGCTCG	YZ10 (insert *hphMX4* into JSC10-1 at *VIII212140*)
EXT VIII211913 F	AGATCTTCTAGCGAGCTTG	YZ10 (confirm *VIII212140::hphMX4* insertion)
HYG INT R	CCTCCTACATCGAAGCTG	YZ10 (confirm *VIII212140::hphMX4* insertion)
VIII218090::CAN1-KANMX F	TTATATTCAAAATGATATGATTTCCCCAATTAATACTTGCTGTTGCATTTTGAATCGGATGTAACTGGGACGTACGCTGCAGGTCGAC	YZ15 (insert *CAN1-kanMX4* into YZ10 at *VIII218090*)
VIII218090::CAN1-KANMX R	TAGAGAACGTGGGAACAGTTCCGCACTGTGACCACCGGAACGTACGTCTGGTTAGCGCAGCATTA GCGAACTTGAAGAATAACCAAGGTC	YZ15 (insert *CAN1-kanMX4* into YZ10 at *VIII218090*)
EXT VIII217877 F	CAACGACAATGCTCCTCTCATAA	YZ15 (confirm *VIII218090::CAN1-kanMX4* insertion)
CAN1 INT R	GGTGTTAGCTTTGCTGCCG	YZ15 (confirm *VIII218090::CAN1-kanMX4* insertion)
VIII214177::URA3 F	GCAAGTAGAAAGGAACACCTTGCAGAATATTTATCAATTTTGCTTGCGTTTCCAGTAATTTTAAATCGTTAATGTGGCTGTGGTTTCAGG	YZ18 (insert *URA3* into YZ15 at *VIII214177*)
VIII214177::URA3 R	GGTACCCGCTGCTGAAAACCTATCTCCGATACCTGCCTCTATTGATACGAACGACATTCCTTTAATTGCTAGATTCCCGGGTAATAACTG	YZ18 (insert *URA3* into YZ15 at *VIII214177*)
VIII214038 F	AAGTCTACTGTATTACTCACGCC	YZ18 (confirm *VIII214177::URA3* insertion)
URA3 INT R (V116511 R)	CCATTCTGCTATTCTGTATACACC	YZ18 (confirm *VIII214177::URA3* insertion)
CAN1::HIS3 F	ATTAGTATTAGCGTGAAAATGTACACATATACATATATATATATATATATATATACTTGAAGGTCTGAAGGACTAGCATGTACGTGAGCG	YZ41/YZ42 (use *HIS3* to replace *CAN1* at its endogenous locus in YZ18-10/YZ26)
CAN1::HIS3 R	CAGCAAAGGCCACAGAACCGTATTCATGTTACTTCTGCAATATCAATCACTTACTGGCAAGTGCGTATATACCACTTGCCACCTATCACC	YZ41/YZ42 (use *HIS3* to replace *CAN1* at its endogenous locus in YZ18-10/YZ26)
HIS3 INT R	GACTTCCCTGACTAATGCCG	YZ41/YZ42 (confirm *can1Δ::HIS3* replacement, use with primer EXT

		V31539 F)
RAD52::NAT F	ATATAGGAAGAAATACAAATAAAATCAGAATTGAAAGAGTAACTAGAGGATTTTGGAGTAATAAATAATGATCGTACGCTGCAGGTCGAC	YZ43/YZ44 (use *natMX4* to replace *RAD52* at its endogenous locus in YZ41/YZ42)
RAD52::NAT R	ATGGAAATGAAGATAAAAATGTACGGAACGCAACCTAAGAAAAAGAGGAGAATTGAAAGTAAACCTGTGTAATCGATGAATTCGAGCTCG	YZ43/YZ44 (use *natMX4* to replace *RAD52* at its endogenous locus in YZ41/YZ42)
XIII212091 F (RAD52)	GTTGGGAATGGATAGGTCCGA	YZ43/YZ44 (use with INT NAT R to confirm *rad52Δ::natMX4* replacement)
INT NAT R (AMC067)	CCGGTAAGCCGTGTCGTC	YZ43/YZ44/YZ45/YZ46/YZ47/YZ48/YZ49/YZ50/YZ51/YZ52 (to confirm the existence of *natMX4*)
INT RAD52 R	TGGGAATCAAGTACCGCGT	YZ43/YZ44 (use with XIII212091 F to confirm the deletion of *RAD52*)
RAD51::NAT F	CTTCTTCTATCTTCCGTAGTTTCCATATACTAGTAGTTGAGTGTAGCGACAAAGAGCAGACGTAGTTATTTG CGTACGCTGCAGGTCGAC	YZ45/YZ46 (use *natMX4* to replace *RAD51* at its endogenous locus in YZ41/YZ42)
RAD51::NAT R	ATGGAAATGAAGATAAAAATGTACGGAACGCAACCTAAGAAAAAGAGGAGAATTGAAAGTAAACCTGTGTAATCGATGAATTCGAGCTCG	YZ45/YZ46 (use *natMX4* to replace *RAD51* at its endogenous locus in YZ41/YZ42)
V349750 F (RAD51)	GACTAGGCCACACTTCGTTAC	YZ45/YZ46 (use with INT NAT R to confirm *rad51Δ::natMX4* replacement)
INT RAD51 R	CTATGGATACCAATCTTACCGG	YZ45/YZ46 (use with V349750 F to confirm the deletion of *RAD51*)
MRE11::NAT F	TCATGAATTTAGAATGCAAATTTGCTCCTCTCAAAATGGCATACCTTGTTGTTCGCGAAGGCAAGCCCTTGGCGTACGCTGCAGGTCGAC	YZ47/YZ48 (use *natMX4* to replace *MRE11* at its endogenous locus in YZ41/YZ42)
MRE11::NAT R	TGGAAACAACATTAAGAGAATGCAGACAATTGACGCAAGTTGTACCTGCTCAGATCCGATAAAACTCGACTATCGATGAATTCGAGCTCG	YZ47/YZ48 (use *natMX4* to replace *MRE11* at its endogenous locus in YZ41/YZ42)
MMS2::NAT F	GAAATTCTTATTCTGTATATGCAACGTAGAAGAAAGCAGCGTTTACACAAAACGTACGCTGCAGGTCGAC	HB5/HB7 (use *natMX4* to replace *MMS2* at its endogenous locus in YZ41/YZ42)
MMS2::NAT R	GTATATATGAGTGGCTTGGAATGCTGCAAATACTGTTTAGGAAAAAGTAGATAAATGGATGAATTCGAGCTCG	HB5/HB7 (use *natMX4* to replace *MMS2* at its endogenous locus in YZ41/YZ42)
MMS2-forward	AAGATCAACTTCCTACACAATCT	Used to confirm deletion of *MMS2*
MMS2-reverse	AAGGTGTGGAAGTCAGTTTGG	Used to confirm deletion of *MMS2*
RAD5::NAT F	CATTATCAAAAGGCCTTAGAAACACACCTAAAGTCTTACAGTATCACAATCGTACGCTGCAGGTCGAC	HB1/HB3 (use *natMX4* to replace *RAD5* at its endogenous locus in YZ41/YZ42)
RAD5::NAT R	GGTTGAAAATAATAATAAATAAAGTCTTTATATATGAGTATGTGGTATGAATCGATGAATTCGAGCTCG	HB1/HB3 (use *natMX4* to replace *RAD5* at its endogenous locus in YZ41/YZ42)
RAD5-forward	CTGCTAAGCGCATTGCTCACTTGAAAG	Used to confirm deletion of *RAD5*
RAD5-reverse	GGACAAGGTTGAATTTGAGGTATC	Used to confirm deletion of *RAD5*
XIII718186 F (MRE11)	CATATTCCTATCACAGTTAACGC	YZ47/YZ48 (use with INT NAT R to confirm *mre11Δ::natMX4* replacement)
INT MRE11 R	AGACATGGTTGTACAGTCCGG	YZ47/YZ48 (use with XIII718186 F to confirm the deletion of *MRE11*)

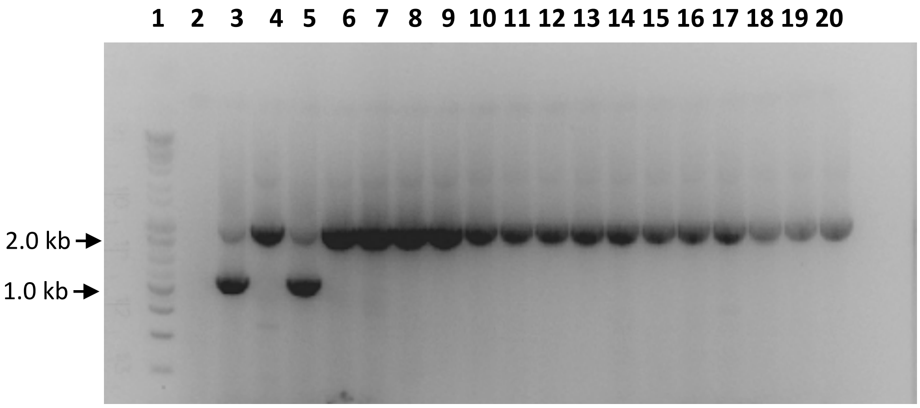
RAD1::NAT F (AMC050)	GAGAGAGCACAGGTGTACTGGAGGGTTCAGGACGTTGGTAGAGCATTTGCCGTACGCTGCAGGTCGAC	YZ49/YZ50 (use *natMX4* to replace *RAD1* at its endogenous locus in YZ41/YZ42)
RAD1::NAT R (AMC051)	AAGATTCAAAGAGCATGTCTAACTTATAACATATACGGTCGAAGTCACCAATCGATGAATTCGAGCTCG	YZ49/YZ50 (use *natMX4* to replace *RAD1* at its endogenous locus in YZ41/YZ42)
AMC052	AGTAAGCTATAGCCACAG	YZ49/YZ50 (use with INT NAT R to confirm *rad1Δ::natMX4* replacement)
INT RAD1 R (AMC053)	TAACACTGCATCGTCATC	YZ49/YZ50 (use with AMC052 to confirm the deletion of *RAD1*)
CUP2::NAT F	GTAAGTATTAAGCTGTGGACGAAATAGCAGTAGCCATGGCGATGTAATTTATTAGACGGCGGCTTGATAAAACGTACGCTGCAGGTCGAC	YZ51/YZ52 (use *natMX4* to replace *CUP2* at its endogenous locus in YZ41/YZ42)
CUP2::NAT R	ATCTCGGCCCGACTGCCAGCTTGCCGGGAGAACAAACAACCGCCAATATATGTATATGTATATTTATATAGATCGATGAATTCGAGCTCG	YZ51/YZ52 (use *natMX4* to replace *CUP2* at its endogenous locus in YZ41/YZ42)
VII190919 F (CUP2)	CCTTCCACCACTACGATCG	YZ51/YZ52 (use with INT NAT R to confirm *cup2Δ::natMX4* replacement)
INT CUP2 R	GAGCGACCATTTAGGCCC	YZ51/YZ52 (use with VII190919 F to confirm the deletion of *CUP2*)
CUP1 amp5	CGAGATGAAATGAATAGCAACGG	Synthesize DIG probes that hybridize to both 2.0 kb and 1.2 kb *CUP1* repeats
CUP1 amp3	TTCATTTCCCAGAGCAGCATGAC	Synthesize DIG probes that hybridize to both 2.0 kb and 1.2 kb *CUP1* repeats
CUP1 amp5*	GTTCAGCGAATTAATTAACTTCC	Primer to prepare probe to detect YIp5-*CUP1* plasmid
CUP1 amp3*	CATTTCCCAGAGCAGCATGAC	Primer to prepare probe to detect YIp5-*CUP1* plasmid
bla-F	ATGCTTAATCAGTGAGGCACC	Primer to prepare probe to detect vector sequences
bla-R	ATTCAACATTTCCGTGTCGCC	Primer to prepare probe to detect vector sequences
CUP1 W303 spec amp5	AGATTCTGTTCAGTTCACTAACGG	Synthesize DIG probes that hybridize specifically to the 2.0 kb *CUP1* repeats
CUP1 W303 spec amp3	GTTCAAAACGAATCCTCTTTGTCC	Synthesize DIG probes that hybridize specifically to the 2.0 kb *CUP1* repeats
IV461633 F	CAGTTATGACGCCAGATGGC	YZ60 (replace *trp1-1* with*TRP1*)
IV462765 R	TCATTGTAGCGTATGCGCC	YZ60 (replace *trp1-1* with*TRP1*)
IV461811 F	CGTGATTAAGCACACAAAGGC	YZ60 (use with IV462578 R to confirm that *trp1-1* was replaced by *TRP1*)
IV462578 R	TTTCAAAAGGCCTGCAGGC	YZ60 (use with IV461811 F to confirm that *trp1-1* was replaced by *TRP1*)
HindIII-CUP1 stop ligate	TCGAGGTCGACGGTATCGATAAGCTTTCATTTCCCAGAGCAGCA	p425-GPD-CUP1 construction
CUP1 start-BamHI ligate	GTGGATCCCCCGGGCTGCAGGAATTCATGTTCAGCGAATTAATTAACTTC	p425-GPD-CUP1 construction

VIII 108799-F	ACAGTTCTTAAGTATGACGCG	Primer used to detect LOH at *CEN8*
VIII 109229-R	AACCACTCACCAACCTTCTCC	Primer used to detect LOH at *CEN8*
F1	CATTGGCACTCATGACCTTCA	Primer used to detect inter-homolog gene conversion
R1’	ATTCATGTAGCTGCCCAACGG	Primer used to detect inter-homolog gene conversion
2172R	GCGGATAGTACAACAGATCAGTC	Primer used to detect inter-homolog gene conversion
4038F	AGTCTACTGTATTACTCACGCC	Primer used to detect inter-homolog gene conversion

Table S3. Plasmids used in this study.

Plasmid name	Relevant features	Source
pFA6a – kanMX4	*kanMX4*	Wach *et al.*, 1994
pAG25	*natMX4*	Goldstein and McCusker, 1999
pAG32	*hphMX4*	Goldstein and McCusker, 1999
pGAL-HO	Galactose-regulated HO gene	Herskowitz and Jensen, 1991
p425-GPD-CUP1	*CUP1* gene regulated by *GPD* promoter; *LEU2*	Constructed in this study
YIp5-*CUP1*	*CUP1* repeat cloned in YIp5 vector; *URA3*	Constructed in this study

A. PCR with primers F1 and R1’



B. PCR with primers 4038F and 2172R

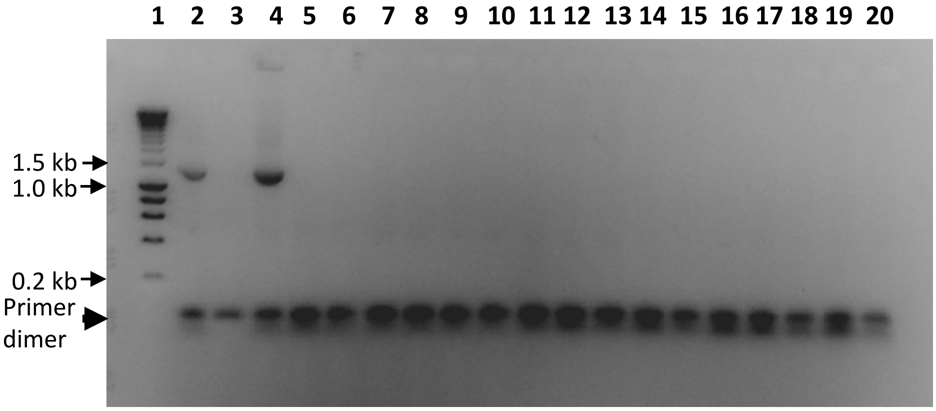


Figure S1. PCR analysis of inter-homolog gene conversion. As discussed in the main text, inter-homolog gene conversion would be expected to produce a *CUP1* array with a mixture of 2 kb *CUP1* repeats (derived from W303-1A) and 1.2 kb repeats (derived from YJM789). **A.** Using primers F1 and R1’ (shown in Fig. 4), we amplified DNA from spores derived from the strain with the putative gene conversion event. These primers result in bands that are the same size as the *CUP1* repeats. Lane 1 contains the HyperLadder I (Bioline), Lanes 3 and 4 contain DNA from a control spore in which we selected for recombination between *CUP1* clusters on the two homologs, Lane 4 has DNA from S288c, a strain with the same *CUP1* repeats as W303-1A, and Lanes 6-20 have DNA derived from independent putative gene conversion events; none have a mixture of the two types of repeats. **B.** PCR analysis using primers 4038F and 2172R (Fig. 4). These primers produce a 1.2 kb fragment only if there is a mixture of two types of repeats in the spore DNA samples. Such a fragment was produced only in the control samples (Lanes 3 and 5). Lane 1 has the size ladder, and Lane 3 has S288c DNA. Lanes 5-20 have samples from independent putative conversion events. The samples in Lanes 5-19 are the same as in Lanes 6-20 of Fig. S1A. The band below 200 bp in all samples is likely to represent primer dimers.

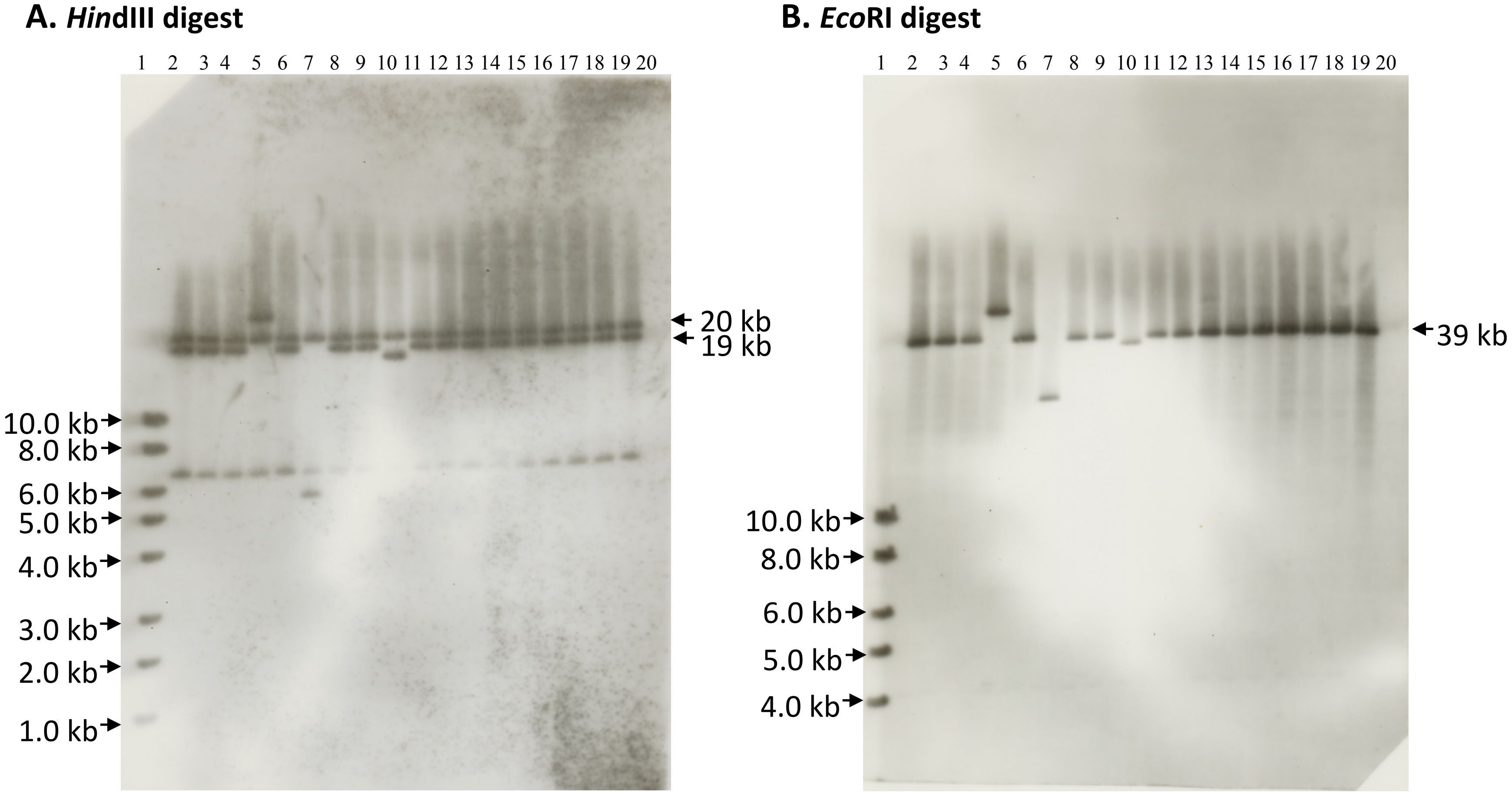


Figure S2. Southern analysis of duplications of the *URA3* gene in Ura+ sectors. As described in the text, if loss of *URA3* in the Ura- sector is a consequence of unequal crossing-over, the Ura+ sector should have two *URA3* insertions. We tested this prediction by treating DNA samples with *Hin*dIII (which cuts within the *URA3* gene, but not within the *CUP1* repeats) and *Eco*RI (which cuts neither the *URA3* insertion nor the *CUP1* repeats). **A. Southern analysis of *Hin*dIII-treated samples.** Lane 1 has HyperLadder I (Bioline), and Lane 20 has the HyperLadder VI. The ladder in Lane 20 was visualized by straining with ethidium bromide and has DNA fragments in the size range of 10-48.5 kb. Lanes 2-18 have independently-isolated samples from Ura+ sectors. Lane 19 has DNA from the starting control strain YZ18-10. The gel was hybridized to a *CUP1* probe. With the exception of a cross-hybridizing ribosomal DNA fragment at 6.3 kb, all of the samples show only two strongly-hybridizing fragments as expected for a single *URA3* insertion at the *CUP1* array. In Lanes 5, 7, and 10, alterations in the number of *CUP1* repeats were observed, two deletions and one addition. **B. Southern analysis of *Eco*RI-treated samples.** The lanes were loaded in the same order and with comparable samples as in Fig. S2A. The observed fragment sizes were as expected based on the previous gel.