

Table S1. Yeast strains used in this study.

Strain	Relevant genotype	Genetic background	Description	Source
Diploids:				
JAY270	<i>MATa/MATα, ACE2/ace2-A7</i>	JAY270	single colony isolate from PE-2 strain stock	Argueso <i>et al.</i> , 2009
JAY663	<i>MATa/MATα, ace2-A7/ace2-A7</i>	JAY270	Spontaneous rough colony isolate	This study
JAY664	<i>MATa/MATα, ace2-A7/ace2-A7</i>	JAY270	Spontaneous rough colony isolate	This study
JAY665	<i>MATa/MATα, ace2-A7/ace2-A7</i>	JAY270	Spontaneous rough colony isolate	This study
JAY912	<i>MATa/MATα, ace2-A7/ace2-A7</i>	JAY270	Spontaneous rough colony isolate	This study
JAY913	<i>MATa/MATα, ace2-A7/ace2-A7</i>	JAY270	Spontaneous rough colony isolate	This study
JAY585 (FGY50)	<i>MATa/MATα, ACE2/ace2-A7, ura3Δ0/ura3Δ0</i>	JAY270	Ura ⁻ derivative of JAY270	Argueso strain collection
JAY1101, 1102, 1103	<i>MATa/MATα, ACE2::CORE2/ace2-A7, ura3Δ0/ura3Δ0</i>	JAY270	Hemizygous CORE2 insertion at Chr12-P	This study
JAY1099, 1100, 1104	<i>MATa/MATα, ACE2/ace2-A7::CORE2, ura3Δ0/ura3Δ0</i>	JAY270	Hemizygous CORE2 insertion at Chr12-M	This study
JAY865, 866	<i>MATa/MATα, ACE2/ace2-A7, ura3Δ0/ura3Δ0, SSF2::CORE2/SSF2</i>	JAY270	Hemizygous CORE2 insertion at Chr4	This study
JAY867	<i>MATa/MATα, ACE2/ace2-A7, ura3Δ0/ura3Δ0, MAL13::CORE2/MAL13</i>	JAY270	Hemizygous CORE2 insertion at Chr7	This study
JAY868	<i>MATa/MATα, ACE2/ace2-A7, ura3Δ0/ura3Δ0, ADH6::CORE2/ADH6</i>	JAY270	Hemizygous CORE2 insertion at Chr13	This study
JAY859, 860	<i>MATa/MATα, ACE2/ACE2, ura3Δ0/ura3Δ0, ADH6::CORE2/ADH6</i>	CG379*	Hemizygous CORE2 insertion at Chr13	This study
JAY861, 862	<i>MATa/MATα, ACE2/ACE2, ura3Δ0/ura3Δ0, SSF2::CORE2/SSF2</i>	CG379*	Hemizygous CORE2 insertion at Chr4	This study
JAY863, 864	<i>MATa/MATα, ACE2/ACE2, ura3Δ0/ura3Δ0, MAL13::CORE2/MAL13</i>	CG379*	Hemizygous CORE2 insertion at Chr7	This study
JAY1105,1106	<i>MATa/MATα, ACE2::CORE2/ACE2, ura3Δ0/ura3Δ0</i>	CG379*	Hemizygous CORE2 insertion at Chr12	This study

Table S1 (continued). Yeast strains used in this study.

Strain	Relevant genotype	Genetic background	Description	Source
Haploids:				
JAY289	<i>MATa, ACE2</i>	JAY270	Sibling spores from a JAY270 tetrad	Argueso <i>et al.</i> , 2009
JAY290	<i>MATα, ACE2</i>	JAY270		
JAY291	<i>MATa, ace2-A7</i>	JAY270		
JAY292	<i>MATα, ace2-A7</i>	JAY270		
JAY293	<i>MATα, ACE2</i>	JAY270	Sibling spores from a JAY270 tetrad	Argueso strain collection
JAY294	<i>MATα, ACE2</i>	JAY270		
JAY295	<i>MATa, ace2-A7</i>	JAY270		
JAY296	<i>MATa, ace2-A7</i>	JAY270		
JAY325	<i>MATα, ace2-A7</i>	JAY270	Sibling spores from a JAY270 tetrad	Argueso strain collection
JAY326	<i>MATa, ace2-A7</i>	JAY270		
JAY327	<i>MATa, ACE2</i>	JAY270		
JAY328	<i>MATα, ACE2</i>	JAY270		
JAY2176	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2177	<i>MATa, ace2-A7</i>	JAY270		
JAY2178	<i>MATα, ace2-A7</i>	JAY270		
JAY2179	<i>MATα, ace2-A7</i>	JAY270		
JAY2180	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2181	<i>MATa, ace2-A7</i>	JAY270		
JAY2182	<i>MATα, ace2-A7</i>	JAY270		
JAY2183	<i>MATα, ace2-A7</i>	JAY270		
JAY2184	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2185	<i>MATa, ace2-A7</i>	JAY270		
JAY2186	<i>MATα, ace2-A7</i>	JAY270		
JAY2187	<i>MATα, ace2-A7</i>	JAY270		
JAY2176	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2177	<i>MATa, ace2-A7</i>	JAY270		
JAY2178	<i>MATα, ace2-A7</i>	JAY270		
JAY2179	<i>MATα, ace2-A7</i>	JAY270		
JAY2180	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2181	<i>MATa, ace2-A7</i>	JAY270		
JAY2182	<i>MATα, ace2-A7</i>	JAY270		
JAY2183	<i>MATα, ace2-A7</i>	JAY270		
JAY2184	<i>MATa, ace2-A7</i>	JAY270	Sibling spores from a JAY663 tetrad	This study
JAY2185	<i>MATa, ace2-A7</i>	JAY270		
JAY2186	<i>MATα, ace2-A7</i>	JAY270		
JAY2187	<i>MATα, ace2-A7</i>	JAY270		
JAY1039	<i>MATα, ACE2, ura3-154, CEN5::HphMX4</i>	JAY270	ACE2 allele replacement in JAY292	This study
JAY1051	<i>MATa, ACE2, ura3-W256G, CEN5::HphMX4</i>	JAY270	ACE2 allele replacement in JAY291	This study

* CG379 diploid strains are also homozygous for: *ade5-1, his7-2, leu2-3,112, Leu⁺, trp1-289, cup1 Δ , RSC30, sfa1 Δ ::hisG*.

Table S2. Oligonucleotides used in this study.

Name	5'-3' sequence *	Description
JAO904	ACACTCAAGATGAGGAGTAT	ACE2 primer Fwd, pAR1 construction
JAO905	TCGCATGAATACGGTCTATC	ACE2 primer Rev, pAR1 construction
JAO906	TACTTCAATAATGGTTCAC	ACE2 primer Fwd, sequencing of A8/A7 tract
JAO907	TGATATTGTCGAGACCGTGG	ACE2 primer Rev, sequencing of A8/A7 tract
JAO912	TAGCACAAGATATGAACT	Chr12 409,200 distal SNP sequencing Fwd
JAO913	TATATCATCTACAATGAC	Chr12 409,200 distal SNP sequencing Rev
JAO944	ATACTGGAGAGTGTGGG	Chr12 400,451 proximal SNP sequencing Fwd
JAO945	TCTAGCGACCAATGGCC	Chr12 400,451 proximal SNP sequencing Rev
JAO1077	CCATTCTTTATCCGCATTC	Chr12 14,795 SNP HhaI RFLP Fwd
JAO1078	CACCTTGCTACGCTCCTTA	Chr12 14,795 SNP HhaI RFLP Rev
JAO1079	GCCTCGTCACAGTTTTTT	Chr12 85,625 SNP HhaI RFLP Fwd
JAO1080	CGTTTGGATTGCCCCTT	Chr12 85,625 SNP HhaI RFLP Rev
JAO1081	TCCTCGCTGTTTCGTTTT	Chr12 148,252 SNP Apol RFLP Fwd
JAO1082	AGGGGTACTGGTATTGTC	Chr12 148,252 SNP Apol RFLP Rev
JAO1083	CGGAATGAGACACTGTTA	Chr12 227,328 SNP HinfI RFLP Fwd
JAO1084	GTTGAAGTGAAGAAGGA	Chr12 227,328 SNP HinfI RFLP Rev
JAO1085	CGACAAACCGTGGTACAA	Chr12 292,284 SNP XbaI RFLP Fwd
JAO1086	CGAACATTCTACTCCAT	Chr12 292,284 SNP XbaI RFLP Rev
JAO1087	CACAGTCAAGAGAAAGAACA	Chr12 364,388 SNP HhaI RFLP Fwd
JAO1088	GCACACACAAAAGGAACTAA	Chr12 364,388 SNP HhaI RFLP Rev
JAO1089	CACTGAACACCAACATCT	Chr12 392,257 SNP HhaI RFLP Fwd
JAO1090	TCCTCAACAAGCAAGCAA	Chr12 392,257 SNP HhaI RFLP Rev
JAO1091	CCTACGTAAAAGATGACC	Chr12 419,261 SNP HhaI RFLP Fwd
JAO1092	CATTACCGCAACAGATCC	Chr12 419,261 SNP HhaI RFLP Rev
JAO1093	CGTGTGGATGATCTGATT	Chr12 450,044 SNP Apol RFLP Fwd
JAO1094	GAAAAGAAATGACTACGGTG	Chr12 450,044 SNP Apol RFLP Rev
JAO14	AGGAGGGTATTCTGGGCTCCATG	Inside Mx4 region Fwd
JAO15	ATGCGAAGTTAAGTGCGCAGAAAG	Inside Mx4 region Rev
JAO1073	GGGCAATGTACCCATAAGGTTGTG	Proximal of ACE2::CORE2 insertion Fwd
JAO1074	CTTTAGGGTTATGTCCCTATAAAGCATGACTATTGCCTTTTTTGGCCCTTAAGACTACAGTGACGTAAATC	Insertion of CORE2 proximal of ACE2 Fwd
JAO1075	GAACATTATGCTATGCATGATATTAACATAATAAATAATAGTAACAATAATATAATACATTATTCTTTAC	Insertion of CORE2 proximal of ACE2 Rev
JAO957	AGCGTACCAAAAGAGAAT	Inside KIURA3 Fwd
JAO1076	GATCAACAAGAACTTACATCTCCC	Distal of ACE2::CORE2 insertion Rev
JAO501	GTCCGATACCCATGAACGTG	Proximal of ADH6::CORE2 insertion Fwd
JAO502	TTGTTAGTGTATTGATATGTGTTTCTTTTACCTTAAAGGTGCTTAGCAAGGAG	Insertion of CORE2 proximal of Chr13 ADH6 Fwd
JAO503	TTTTTATGATTATAAGGTACTATTTAAATATTTACAACCTCGTACAGTTCTC	Insertion of CORE2 proximal of Chr13 ADH6 Rev
JAO504	GGTCTGTATATAGGAGTGCTG	Distal of ADH6::CORE2 insertion Rev
JAO505	GTGACTTATTCAGTGAAGTAG	Proximal of SSF2::CORE2 insertion Fwd
JAO506	CCTCCGTACGTAACATCACTATCCATATAGTAGCCATGACTCCGATGGAC	Insertion of CORE2 distal of Chr4 SSF2 Fwd
JAO507	TTGAGGTGTTCCCTCACCTATGAATAAACAGACACTTCCTGGTTCTTTAA	Insertion of CORE2 distal of Chr4 SSF2 Rev
JAO508	TTTGTCTTTTCCATGATGCCG	Distal of SSF2::CORE2 insertion Rev
JAO1442	AAGAGGTCCGCTAATTCTGGAG	MAT locus Fwd
JAO1371	AGAACAAGAAGGATGCTAAG	MAT locus Rev
JAO1761	ATCCTACCATACCCCCACCC	Inside ACE2, Fwd, digital droplet PCR
JAO1762	CCCGTCTTAGGAGAAGGCAC	Inside ACE2, Rev, digital droplet PCR
JAO1765	AAGGAACTCTGTGGTGGTGC	Inside DNM1, Fwd, digital droplet PCR
JAO1766	TGTAGGACGGGGACCACTAG	Inside, DNM1, Rev, digital droplet PCR
JAO1769	GGGACCAAAACCAGACCAA	Inside YLR001C, Fwd, digital droplet PCR
JAO1770	TTGACAAAGCAATGGCGCTC	Inside YLR001C, Rev, digital droplet PCR

* In the case of long oligonucleotides used for PCR-based integrations, the nucleotides in the 5' end (**bold**) are targeting tails with homology to the respective chromosomal insertion sites; the 3' end nucleotides (*italicized*) correspond to primer sequences used to amplify the selectable marker sequence from the specific template plasmids.

Table S3. List of phased JAY270 HetSNPs and hemizygous sequences and respective detection methods.

Marker SGD coordinates		PCR primers	Polymorphism detection method	SGD Watson bases ¹	JAY270 Watson phased bases	
Chromosome	Nucleotide				Chr12-P	Chr12-M
Chr12	14,795	JAO1077 + JAO1078	<i>HhaI</i>	<u>C</u>	A	<u>C</u>
Chr12	85,625	JAO1079 + JAO1080	<i>HhaI</i>	<u>G</u>	A	<u>G</u>
Chr12	148,252	JAO1081 + JAO1082	<i>ApoI</i>	<u>A</u>	<u>A</u>	G
Chr12	227,328	JAO1083 + JAO1084	<i>HinfI</i>	<u>C</u>	T	<u>C</u>
Chr12	292,284	JAO1085 + JAO1086	<i>XbaI</i>	I	C	I
Chr12	364,388	JAO1087 + JAO1088	<i>HhaI</i>	<u>G</u>	A	<u>G</u>
Chr12	392,257	JAO1089 + JAO1090	<i>HhaI</i>	<u>G</u>	A	<u>G</u>
Chr12	400,451	JAO944 + JAO945	Sanger seq	G	A	G
Chr12	405,711 to 405,718	JAO906 + JAO907	Sanger seq	8x T	8x T	7x T
Chr12	409,200	JAO912 + JAO913	Sanger seq	C	T	C
Chr12	419,261	JAO1091 + JAO1092	<i>HhaI</i>	T	T	<u>C</u>
Chr12	450,044	JAO1093 + JAO1094	<i>ApoI</i>	C	C	I

1. Nucleotides that are underlined indicate the sequences that are cut by the respective restriction endonucleases, whereas lack of underlining corresponds to no cut site.