

SUPPLEMENTAL MATERIAL

Table S1 presents a list of Bioneer deletion strains selected for the second and third mutant screens and Table S2 a list of potential false positives. Figure S1 to S8 show the phenotypes of mutants examined in the second screen; Figure S9 to S11 show the phenotypes of mutants examined in the third screen. Table S3 contains a list of strains and their genotypes; Table S4 contains oligonucleotide sequences.

Table S1. Bioneer deletion strains selected for second and third screens. -Plate positionørefers to the plate images in Figure S1-8 showing the results of the second screen.

| Deletion strains used in 2 nd screen | | | | used in 3 rd screen | Final list |
|---|-----------|--------------|--|--------------------------------|------------|
| Plate position | Bioneer # | ID | Gene description | | |
| A1 | V3-P01-05 | SPAC11E3.15 | 60S ribosomal protein L22 (predicted) | | |
| A2 | V3-P01-17 | SPAC1687.23c | sequence orphan | | |
| A3 | V3-P02-16 | SPAPJ691.03 | mitochondrial conserved eukaryotic protein | | |
| A4 | V3-P02-40 | SPBC1711.01c | mating-type m-specific polypeptide mi 1 | | |
| A5 | V3-P02-41 | SPBC1711.02 | mating-type m-specific polypeptide mc 1 | | |
| A6 | V3-P02-75 | SPBC4B4.11 | conserved fungal protein | | |
| A7 | V3-P03-81 | SPAC1687.05 | SUMO E3 ligase Pli1 | • | • |
| A8 | V3-P03-83 | SPAC1687.15 | serine/threonine protein kinase Gsk3 | | |
| A9 | V3-P04-23 | SPAC1851.03 | CK2 family regulatory subunit Ckb1 | • | • |
| A10 | V3-P04-49 | SPAC227.10 | prefoldin subunit 2 (predicted) | | |
| A11 | V3-P05-23 | SPAC2F7.09c | mitochondrial GTPase related protein (predicted) | • | |
| A12 | V3-P05-24 | SPAC2F7.10 | palmitoyltransferase (predicted) | | |
| A13 | V3-P05-33 | SPAC31G5.19 | ATPase with bromodomain protein Abo1 | • | • |
| A14 | V3-P05-52 | SPAC3C7.10 | peroxin 13 (predicted) | | |
| A15 | V3-P05-57 | SPAC3G6.04 | RNA-binding protein Rnp24 | | |
| A16 | V3-P05-67 | SPAC3H5.12c | 60S ribosomal protein L5 (predicted) | | |
| B1 | V3-P06-02 | SPAC57A7.12 | heat shock protein Ssz1(predicted) | • | • |
| B2 | V3-P06-05 | SPAC5D6.06c | UDP-GlcNAc transferase associated protein Alg14 (predicted) | | |
| B3 | V3-P06-14 | SPAC664.01c | chromodomain protein Swi6 | • | • |
| B4 | V3-P06-19 | SPAC694.06c | mediator of replication checkpoint 1 | • | • |
| B5 | V3-P06-41 | SPAC824.08 | guanosine-diphosphatase Gda1 | | |
| B6 | V3-P06-95 | SPBC12C2.02c | Rictor homolog, Ste20 | | |
| B7 | V3-P07-42 | SPBC1709.14 | peptide N-glycanase (predicted) | | |
| B8 | V3-P07-44 | SPBC1718.06 | mitochondrial dynamin family GTPase Msp1 | | |
| B9 | V3-P07-46 | SPBC1734.06 | Rad18 homolog Rhp18 | • | |
| B10 | V3-P07-80 | SPBC19G7.10c | topoisomerase II-associated deadenylation-dependent mRNA-decapping factor Pdc2 (predicted) | • | |
| B11 | V3-P08-10 | SPBC23E6.08 | Golgi membrane exchange factor subunit Sat1 (predicted) | • | |
| B12 | V3-P08-53 | SPBC336.14c | serine/threonine protein kinase, PAN complex subunit, Ppk26 | | |
| B13 | V3-P08-63 | SPBC365.04c | RNA-binding protein, involved in ribosome biogenesis (predicted) | • | |

| | | | | | |
|-----|-----------|---------------|---|----------------|----------------|
| B14 | V3-P09-27 | SPBP16F5.05c | ribosome biogenesis protein Nop8 (predicted) | | |
| B15 | V3-P09-30 | SPBP22H7.05c | ATPase with bromodomain protein Abo2 (predicted) | • ^a | • ^a |
| B16 | V3-P09-32 | SPBP35G2.10 | SHREC complex subunit Mit1 | • | • |
| C1 | V3-P09-52 | SPCC1183.02 | glutathione S-transferase (predicted) | | |
| C2 | V3-P09-54 | SPCC11E10.08 | silencing protein Rik1 | • | • |
| C3 | V3-P09-69 | SPCC1450.11c | serine/threonine protein kinase Cek1 | | |
| C4 | V3-P10-07 | SPCC553.08c | GTPase Ria1 (predicted) | | |
| C5 | V3-P10-23 | SPCC74.06 | histidine kinase Mak3 | | |
| C6 | V3-P10-35 | SPAC10F6.11c | autophagy associated protein kinase activator Atg17 | | |
| C7 | V3-P10-52 | SPAC17H9.10c | damaged DNA binding protein Ddb1 | • | • |
| C8 | V3-P10-53 | SPAC1805.10 | sequence orphan | | |
| C9 | V3-P11-19 | SPAPYUG7.03c | medial ring protein Mid2 | | |
| C10 | V3-P11-68 | SPBC800.03 | histone deacetylase (class II) Clr3 | • | • |
| C11 | V3-P11-94 | SPCC338.16 | F-box protein Pof3 | • | • |
| C12 | V3-P12-13 | SPCPB1C11.03 | cysteine transporter (predicted) | | |
| C13 | V3-P12-29 | SPAC13A11.06 | pyruvate decarboxylase (predicted) | | |
| C14 | V3-P12-43 | SPAC1B2.03c | GNS1/SUR4 family protein (predicted) | | |
| C15 | V3-P12-46 | SPAC1B3.17 | chromatin silencing protein Clr2 | • | • |
| C16 | V3-P12-69 | SPAC25A8.02 | sequence orphan | | |
| D1 | V3-P12-78 | SPAC30D11.05 | AP-3 adaptor complex subunit Aps3 (predicted) | | |
| D2 | V3-P13-41 | SPBC18E5.01 | cycloisomerase 2 family | | |
| D3 | V3-P14-03 | SPCC11E10.05c | nucleoside diphosphatase Ynd1 | • | |
| D4 | V3-P14-10 | SPCC1322.02 | sequence orphan | | |
| D5 | V3-P14-25 | SPCC188.07 | telomere maintenance protein Ccq1 | • | |
| D6 | V3-P14-50 | SPAC11G7.01 | sequence orphan | | |
| D7 | V3-P14-52 | SPAC12G12.09 | conserved fungal protein | | |
| D8 | V3-P14-53 | SPAC13F5.04c | endosomal sorting protein (predicted) | | |
| D9 | V3-P14-69 | SPAC17H9.19c | WD repeat protein Cdt2 | • | • |
| D10 | V3-P14-80 | SPAC22F8.07c | replication termination factor Rtf1 | • | |
| D11 | V3-P15-44 | SPBC14F5.03c | karyopherin Kap123 | | |
| D12 | V3-P15-63 | SPBC2D10.17 | cryptic loci regulator Clr1 | • | • |
| D13 | V3-P15-77 | SPBC428.08c | histone H3 methyltransferase Clr4 | • | • |
| D14 | V3-P15-80 | SPBC4F6.11c | asparagine synthase (predicted) | | |
| D15 | V3-P16-15 | SPCC306.11 | sequence orphan | | |
| D16 | V3-P16-20 | SPCC4G3.15c | CCR4-Not complex subunit Not2 (predicted) | • | • |
| E1 | V3-P16-29 | SPCC965.05c | uracil DNA N-glycosylase Thp1 | • | |
| E2 | V3-P16-66 | SPAC31G5.09c | MAP kinase Spk1 | | |
| E3 | V3-P17-85 | SPCC757.09c | RNA-binding protein that suppresses calcineurin deletion Rnc1 | • | |

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|-----|-----------|---------------|--|---|---|
| E4 | V3-P18-15 | SPAC19D5.02c | peroxisomal membrane protein Pex22 (predicted) | | |
| E5 | V3-P18-50 | SPAPB24D3.08c | NADP-dependent oxidoreductase (predicted) | | |
| E6 | V3-P18-68 | SPBC28F2.02 | mRNA export protein Mep33 | | |
| E7 | V3-P18-94 | SPCC1442.07c | ubiquitin/metalloprotease fusion protein | | |
| E8 | V3-P19-39 | SPAC18G6.04c | serine hydroxymethyltransferase Shm2 (predicted) | | |
| E9 | V3-P19-50 | SPAC24C9.12c | glycine hydroxymethyltransferase (predicted) | • | |
| E10 | V3-P19-56 | SPAC30C2.07 | sequence orphan | | |
| E11 | V3-P19-82 | SPBC14F5.11c | sorting nexin Snx41 (predicted) | | |
| E12 | V3-P20-37 | SPCC70.10 | sequence orphan | | |
| E13 | V3-P20-41 | SPAC1002.12c | succinate-semialdehyde dehydrogenase (predicted) | | |
| E14 | V3-P20-59 | SPAC21E11.03c | transcription factor Pcr1 | | |
| E15 | V3-P20-82 | SPAPYUG7.04c | DNA-directed RNA polymerase II complex subunit Rpb9 | | |
| E16 | V3-P21-16 | SPBC83.09c | GYF domain protein | | |
| F1 | V3-P21-34 | SPCC569.02c | S. pombe specific UPF0321 family protein 2 | | |
| F2 | V3-P21-50 | SPAC1F7.10 | hydantoin racemase family (predicted) | | |
| F3 | V3-P21-57 | SPAC29B12.06c | RNA-binding protein, CCR4-NOT complex subunit Rcd1 | • | • |
| F4 | V3-P21-63 | SPAC589.10c | ribosomal-ubiquitin fusion protein Ubi5 (predicted) | | |
| F5 | V3-P21-64 | SPAC5H10.02c | ThiJ domain protein | | |
| F6 | V3-P21-90 | SPBC2G5.01 | DUF1682 family protein | | |
| F7 | V3-P22-54 | SPBC215.03c | COP9/signalosome complex subunit Csn1 | • | • |
| F8 | V3-P23-38 | SPBC106.01 | dual specificity protein kinase Mph1 | | |
| F9 | V3-P23-49 | SPBC36B7.06c | sequence orphan | | |
| F10 | V3-P23-58 | SPCC613.11c | cell surface glycoprotein (predicted), DUF1773 family protein 2 | | |
| F11 | V3-P23-75 | SPAPB8E5.10 | conserved fungal protein, with meiosis specific splicing | | |
| F12 | V3-P23-80 | SPBC3B8.10c | Nem1-Spo7 phosphatase complex catalytic subunit Nem1 (predicted) | | |
| F13 | V3-P24-07 | SPAC869.07c | alpha-galactosidase, melibiase | | |
| F14 | V3-P24-57 | SPAC1687.07 | conserved fungal protein | | |
| F15 | V3-P24-62 | SPAP27G11.06c | AP-1 adaptor complex sigma subunit Aps1 | | |
| F16 | V3-P24-80 | SPAC7D4.08 | sequence orphan | | |
| G1 | V3-P25-45 | SPAC15E1.07c | meiotic cohesin complex associated protein Moa1 | | |
| G2 | V3-P25-50 | SPAC2E1P5.02c | Rab GTPase binding protein upregulated in meiosis II (predicted) | | |
| G3 | V3-P25-57 | SPBC365.11 | GRIP domain protein | | |
| G4 | V3-P25-58 | SPBC4B4.03 | RSC complex subunit Rsc1 | | |

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|-----|-----------|---------------|---|---|---|
| G5 | V3-P25-74 | SPBC21C3.19 | SBDS family protein Rtc3 (predicted) | | |
| G6 | V3-P25-91 | SPBPJ4664.05 | conserved fungal protein | | |
| G7 | V3-P25-93 | SPCPJ732.01 | retromer complex subunit Vps5 | | |
| G8 | V3-P25-94 | SPAC1142.01 | DUF654 family protein | | |
| G9 | V3-P25-95 | SPAC15A10.15 | shugoshin Sgo2 | | |
| G10 | V3-P26-29 | SPAC29A4.05 | myosin I light chain Cam2 | | |
| G11 | V3-P26-47 | SPBC428.07 | meiotic chromosome segregation protein Meu6 | • | • |
| G12 | V3-P26-71 | SPCC594.07c | bouquet formation protein Bqt3 | • | |
| G13 | V3-P26-77 | SPAC2E1P3.01 | dehydrogenase (predicted) | | |
| G14 | V3-P26-91 | SPBC16A3.12c | triglyceride lipase-cholesterol esterase (predicted) | | |
| G15 | V3-P26-94 | SPBC609.05 | FACT complex component Pob3 | • | • |
| G16 | V3-P27-16 | SPBP8B7.21 | ubiquitin C-terminal hydrolase Ubp3 | | |
| H1 | V3-P27-23 | SPAC26A3.02 | adenine DNA glycosylase Myh1 | | |
| H2 | V3-P27-24 | SPAC2G11.06 | AAA family ATPase Vps4 (predicted) | | |
| H3 | V3-P27-26 | SPAC6C3.03c | sequence orphan | | |
| H4 | V3-P27-29 | SPAC926.02 | conserved fungal protein | | |
| H5 | V3-P27-31 | SPBC21B10.06c | myosin binding vezatin family protein involved in peroxisome inheritance Inp2 (predicted) | | |
| H6 | V3-P27-38 | SPAC11H11.01 | ESCRT I complex subunit Vps23 | | |
| H7 | V3-P27-53 | SPBC6B1.09c | Mre11 complex subunit Nbs1 | | |
| H8 | V3-P27-56 | SPAC31F12.01 | zds family protein phosphatase type A regulator Zds1 (predicted) | | |
| H9 | V3-P27-64 | SPAC140.03 | argonaute binding protein 1 | | |
| H10 | V3-P27-69 | SPBC83.11 | ER triose phosphate transmembrane transporter (predicted) | | |
| H11 | V3-P27-95 | SPBC1289.11 | splicing factor Spf38 | | |
| H12 | V3-P28-23 | SPCC794.02 | wtf element Wtf5 | | |
| H13 | V3-P28-34 | SPAC29E6.07 | sequence orphan | | |
| H14 | V3-P28-69 | SPAC1687.21 | phosphoglycerate mutase family (predicted) | | |
| H15 | V3-P28-70 | SPAC6F6.17 | telomere length regulator protein Rif1 | • | • |
| H16 | V3-P28-71 | SPBC800.02 | cell cycle transcriptional repressor Whi5 (predicted) | | |
| I1 | V3-P28-91 | SPBC365.20c | nicotinamidase (predicted) | | |
| I2 | V3-P29-18 | SPBC13G1.12 | vacuolar sorting protein Did2 (predicted) | • | |
| I3 | V3-P29-20 | SPBC17D11.02c | ubiquitin-protein ligase Hrd1, synviolin family | | |
| I4 | V3-P29-23 | SPBC1A4.03c | DNA topoisomerase II | | |
| I5 | V3-P29-39 | SPAC4G9.19 | DNAJ domain protein DNAJB family (predicted) | | |
| I6 | V3-P29-55 | SPCC1223.15c | DASH complex subunit Spc19 | • | |
| I7 | V3-P29-74 | SPAC16A10.01 | DUF1212 family protein | | |

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|-----|-----------|---------------|---|---|---|
| I8 | V3-P29-75 | SPAC16C9.05 | Clr6 histone deacetylase associated PHD protein-1 Cph1 | • | • |
| I9 | V3-P29-80 | SPAC1952.16 | RhoGAP, GTPase activator towards Rho/Rac/Cdc42-like small GTPases (predicted) | | |
| I10 | V3-P29-89 | SPAC19D5.03 | poly(A) polymerase Cid1 | | |
| I11 | V3-P30-11 | SPAC25A8.01c | fun thirty related protein Fft3 | • | • |
| I12 | V3-P30-31 | SPAC607.06c | metallopeptidase | | |
| I13 | V3-P30-41 | SPAPB24D3.09c | ABC transporter Pdr1 | | |
| I14 | V3-P30-46 | SPBC1347.02 | FKBP-type peptidyl-prolyl cis-trans isomerase (predicted) | • | • |
| I15 | V3-P30-81 | SPBC3B8.06 | conserved fungal protein | | |
| I16 | V3-P30-86 | SPBC530.01 | GTPase activating protein Gyp1 (predicted) | | |
| J1 | V3-P30-89 | SPBC557.04 | Ark1/Prk1 family protein kinase Ppk29 | | |
| J2 | V3-P31-01 | SPBC725.06c | serine/threonine protein kinase Ppk31 (predicted) | | |
| J3 | V3-P31-18 | SPCC1322.14c | vacuolar transporter chaperone (VTC) complex subunit (predicted) | | |
| J4 | V3-P31-22 | SPCC162.10 | serine/threonine protein kinase Ppk33 (predicted) | | |
| J5 | V3-P31-44 | SPCC965.06 | potassium channel subunit (predicted) | | |
| J6 | V3-P31-47 | SPCC970.07c | Rik1-associated factor Raf2 | • | • |
| J7 | V3-P31-64 | SPAC24H6.03 | cullin 3 | | |
| J8 | V3-P31-66 | SPAC323.07c | MatE family transporter (predicted) | | |
| J9 | V3-P31-75 | SPBC25D12.05 | N2,N2-dimethylguanosine tRNA methyltransferase | | |
| J10 | V3-P31-78 | SPBC359.03c | amino acid transporter Aat1 (predicted) | | |
| J11 | V3-P31-83 | SPCC1322.12c | serine/threonine protein kinase Bub1 | | |
| J12 | V3-P31-89 | SPCC285.16c | MutS protein homolog | • | |
| J13 | V3-P31-92 | SPCC584.11c | Svf1 family protein Svf1 | | |
| J14 | V3-P32-21 | SPAC977.16c | dihydroxyacetone kinase Dak2 | | |
| J15 | V3-P32-46 | SPCC4B3.03c | mitochondrial morphology protein (predicted) | | |
| J16 | V3-P32-63 | SPAC3G6.06c | FEN-1 endonuclease Rad2 | | |
| K1 | V3-P32-77 | SPBC428.17c | Wings apart-like homolog Wpl1 | • | • |
| K2 | V3-P32-78 | SPBC4F6.16c | ER oxidoreductin Ero1a | | |
| K3 | V3-P33-11 | SPAC2E1P3.02c | ammonium transporter Amt3 | | |
| K4 | V3-P33-12 | SPAC30D11.07 | DNA endonuclease III | | |
| K5 | V3-P33-28 | SPBC646.02 | complexed with Cdc5 protein Cwf11 | | |
| K6 | V3-P33-45 | SPAC1F8.03c | siderophore-iron transporter Str3 | | |
| K7 | V3-P33-49 | SPAC23C11.06c | hydrolase (inferred from context) | | |
| K8 | V3-P33-53 | SPAC869.06c | HHE domain cation binding protein | | |
| K9 | V3-P33-61 | SPBC16G5.15c | fork head transcription factor Fkh2 | • | • |
| K10 | V3-P33-72 | SPCC16A11.03c | DUF2009 protein | | |

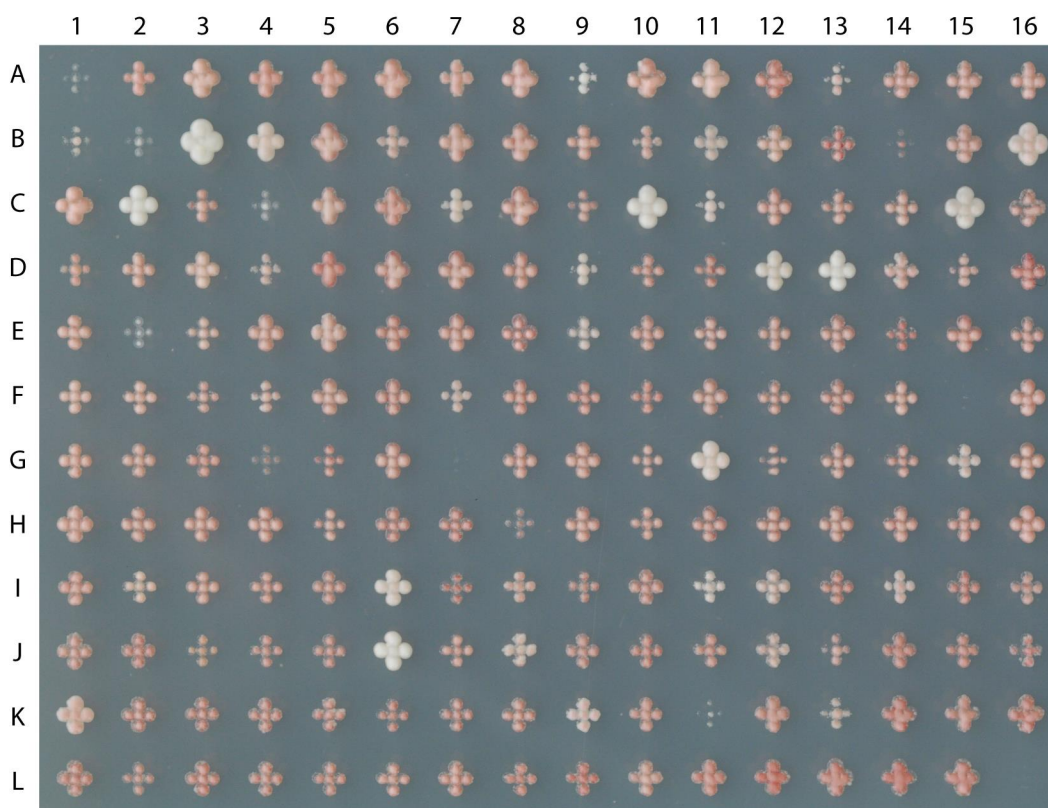
| | | | | | |
|--|--------------------|---------------|--|---|---|
| K11 | V3-P33-82 | SPCC794.09c | translation elongation factor EF-1 alpha Ef1a-a | | |
| K12 | V3-P33-84 | SPCPB16A4.02c | conserved fungal protein | | |
| K13 | V3-P34-37 | SPAC4F10.14c | Basic transcription factor Btf3 | • | • |
| K14 | V3-P34-51 | SPAC4G9.12 | gluconokinase | | |
| K15 | V3-P34-60 | SPBC3H7.05c | sequence orphan | | |
| K16 | V3-P34-61 | SPBC3H7.14 | BRCT domain protein | | |
| L1 | V3-P34-86 | SPBC3E7.02c | heat shock protein Hsp16 | | |
| L2 | V3-P34-90 | SPCC417.02 | DASH complex subunit Dad5 | | |
| L3 | V3-P35-03 | SPAC3H1.08c | DUF1640 family protein | | |
| L4 | V3-P35-05 | SPBC1539.07c | glutathione-dependent formaldehyde dehydrogenase (predicted) | | |
| L5 | V3-P35-25 | SPAC27D7.04 | 4-alpha-hydroxytetrahydrobiopterin dehydratase (predicted) | | |
| L6 | V3-P35-44 | SPCC1020.13c | DDHD family phospholipase (predicted) | | |
| L7 | V3-P35-48 | SPAC13G6.13 | sequence orphan | | |
| L8 | V3-P35-62 | SPBC16G5.09 | serine carboxypeptidase (predicted) | | |
| L9 | V3-P35-81 | SPAC12G12.11c | DUF544 family protein | | |
| L10 | V3-P35-84 | SPCC4B3.05c | uroporphyrinogen decarboxylase Hem12 (predicted) | | |
| L11 | V3-P36-01 | SPCC613.08 | CDK regulator, involved in ribosome export (predicted) | | |
| L12 | V3-P36-27 | SPBC1198.09 | ubiquitin conjugating enzyme Ubc16 (predicted) | | |
| L13 | V3-P36-35 | SPAC3H1.07 | arginase Aru1 | | |
| L14 | V3-P36-44 | SPBC3E7.06c | vacuolar membrane amino acid uptake transporter Fnx2 | | |
| L15 | V3-P36-54 | SPCC364.01 | calnexin independence factor Cif1 | | |
| Deletion strains independently re-tested and added to final list | | | | | |
| - | V3-P11-59 | SPBC354.10 | RNAPII degradation factor Def1 (predicted) | • | • |
| - | V3-P19-76 | SPAPB1E7.02c | DNA polymerase alpha accessory factor Mcl1 | • | • |
| - | V3-P20-56 | SPAC1952.05 | SAGA complex histone acetyltransferase catalytic subunit Gcn5 | • | • |
| - | V3-P30-44 | SPBC1198.11c | RNA polymerase I transcription termination factor Reb1 | • | • |
| - | V3-P03-26 | SPAC1071.02 | Dos2 silencing complex subunit Mms19 | • | • |
| - | added ^b | SPBC216.06c | replication fork protection complex subunit Swi1 | • | • |
| - | added ^b | SPBC30D10.04 | replication fork protection complex subunit Swi3 | • | • |
| - | added ^b | SPCC613.12c | CLRC ubiquitin E3 ligase complex specificity factor Clr8/Raf1/Dos1 | • | • |

^a Abo2 was examined for comparison with Abo1.

^b These strains were not in the V3 Bioneer library.

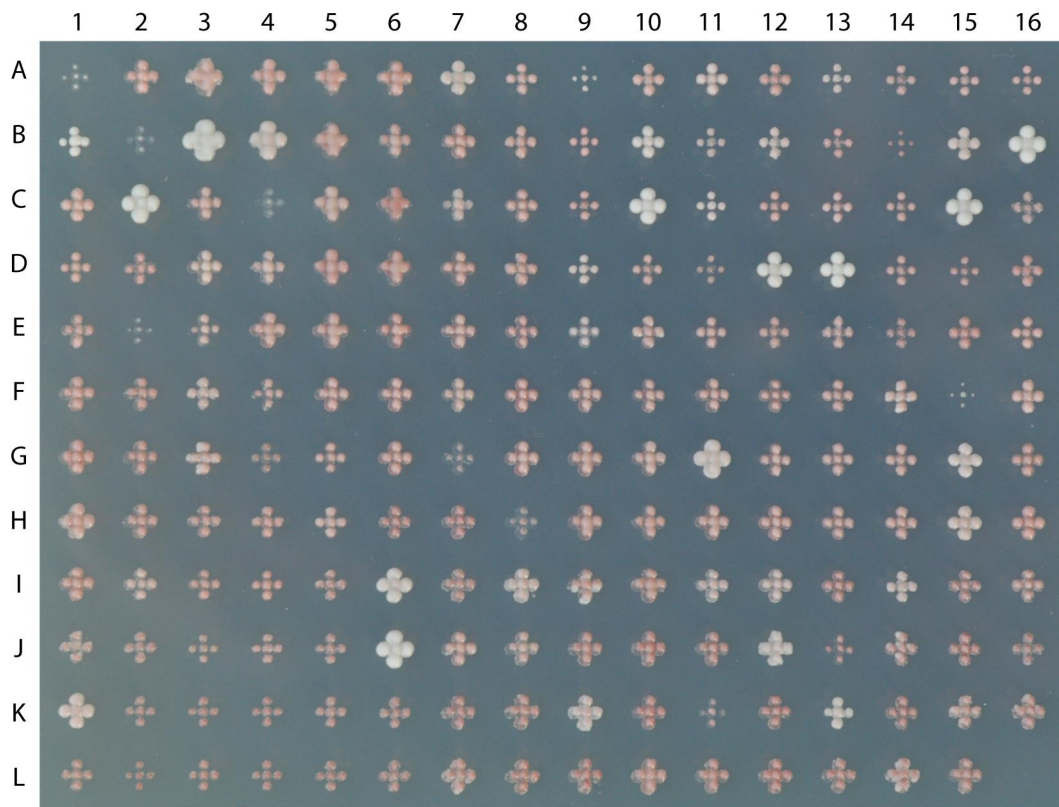
Table S2. Potential false positives. The strains listed in this Table produced Ade⁺ colonies in the absence of crossing indicating the Ade⁺ phenotype following crosses is not likely to reflect (*EcoRV*)::*ade6*⁺ expression.

| | | |
|-----------|---------------|---|
| V3-P02-69 | SPBC3D6.09 | DNA polymerase epsilon subunit Dpb4 |
| V3-P11-45 | SPBC23E6.09 | transcriptional co-repressor Ssn6 |
| V3-P15-60 | SPBC25D12.02c | nucleolar protein Dnt1 |
| V3-P17-26 | SPBC1A4.09 | pseudouridine synthase (predicted) |
| V3-P17-46 | SPBC577.05c | meiotic recombination protein Rec27 |
| V3-P17-49 | SPBC776.06c | Arf3/6 docking factor (predicted) |
| V3-P19-93 | SPBC216.03 | conserved fungal protein |
| V3-P19-94 | SPBC21C3.12c | DUF953 family protein |
| V3-P20-13 | SPBC56F2.01 | F-box protein Pof12 |
| V3-P21-03 | SPBC21.03c | DUF55 family protein |
| V3-P21-93 | SPBC582.06c | horsetail movement protein Hrs1/Mcp6 |
| V3-P23-51 | SPBC887.06c | sorting nexin Snx3 (predicted) |
| V3-P28-66 | SPBC16E9.18 | phosphatidylserine decarboxylase Psd1 |
| V3-P28-92 | SPBC13G1.14c | RNA-binding protein (predicted) |
| V3-P29-55 | SPCC1223.15c | DASH complex subunit Spc19 |
| V3-P34-57 | SPBC21C3.07c | actin binding methyltransferase (predicted) |
| V3-P35-71 | SPBC21.02 | TLDc domain protein 2 |



crosses with strain B19 (*IR-R*⁺)

Figure S1. Second screen with *IR-R*⁺ boundary. Strain B19 was crossed with strains from the Bioneer collection as described. *(EcoRV)::ade6*⁺ expression was assayed in progeny combining *(EcoRV)::ade6*⁺*IR-R*⁺ with each ORF deletion listed in Table S1. In the image shown, the recombinant progeny was propagated on NBA medium supplemented with leucine to assess *(EcoRV)::ade6*⁺ expression.



crosses with strain B15 (*STAR1*)

Figure S2. Second screen with *STAR1* boundary. Same as Figure S1, with strain B15 in which *IR-R⁺* is replaced with the *STAR1* element.

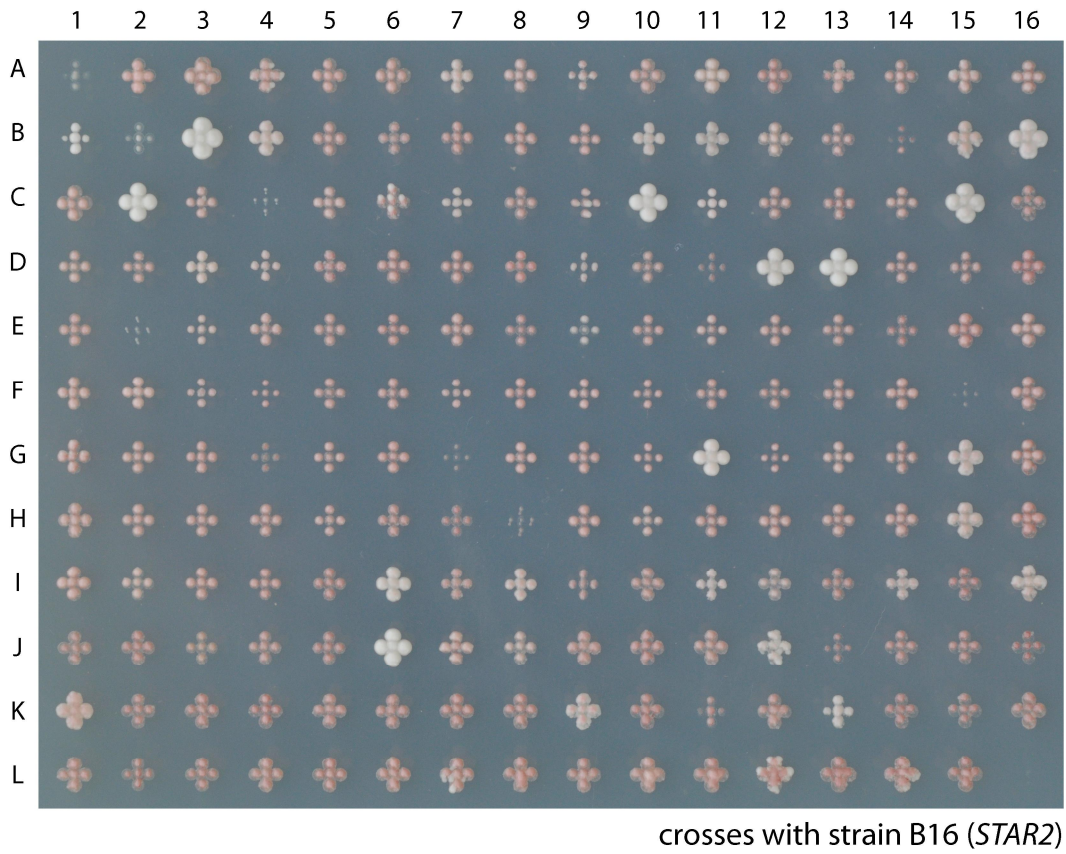


Figure S3. Second screen with *STAR2* boundary. Same as Figure S1, with strain B16 in which *IR-R⁺* is replaced with the *STAR2* element.

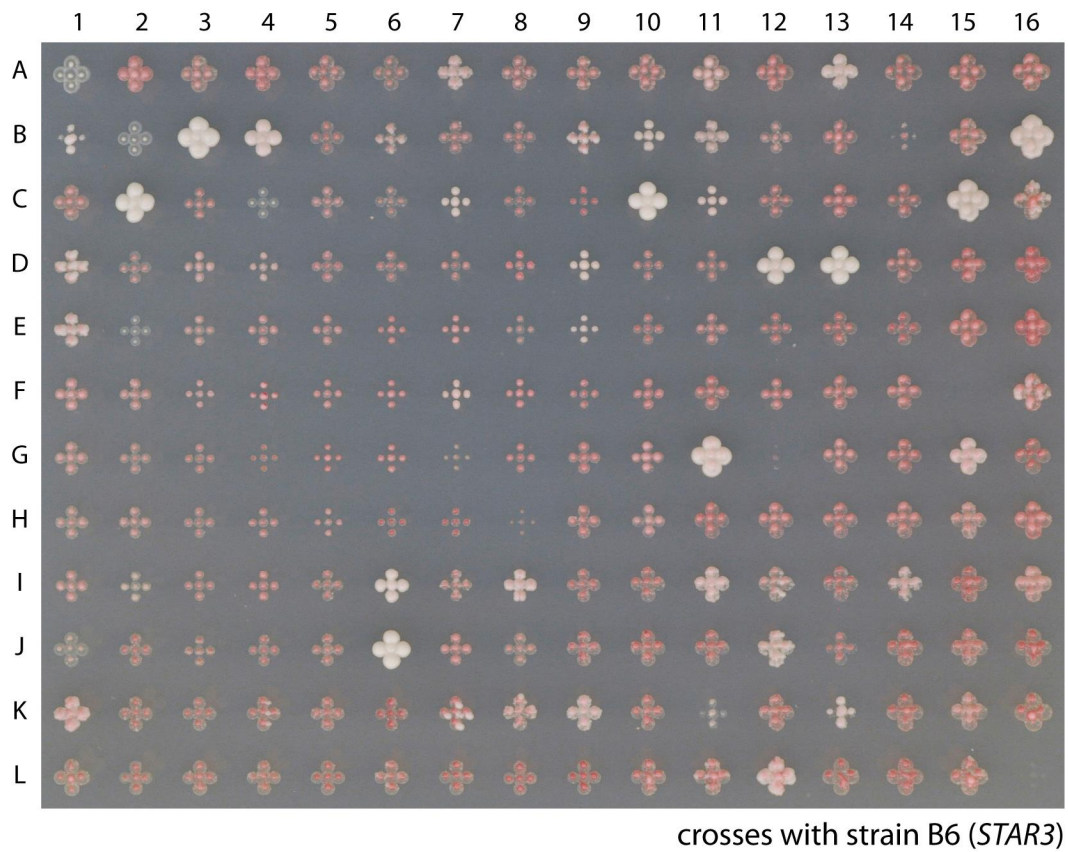


Figure S4. Second screen with *STAR3* boundary. Same as Figure S1, with strain B6 in which *IR-R⁺* is replaced with the *STAR3* element.

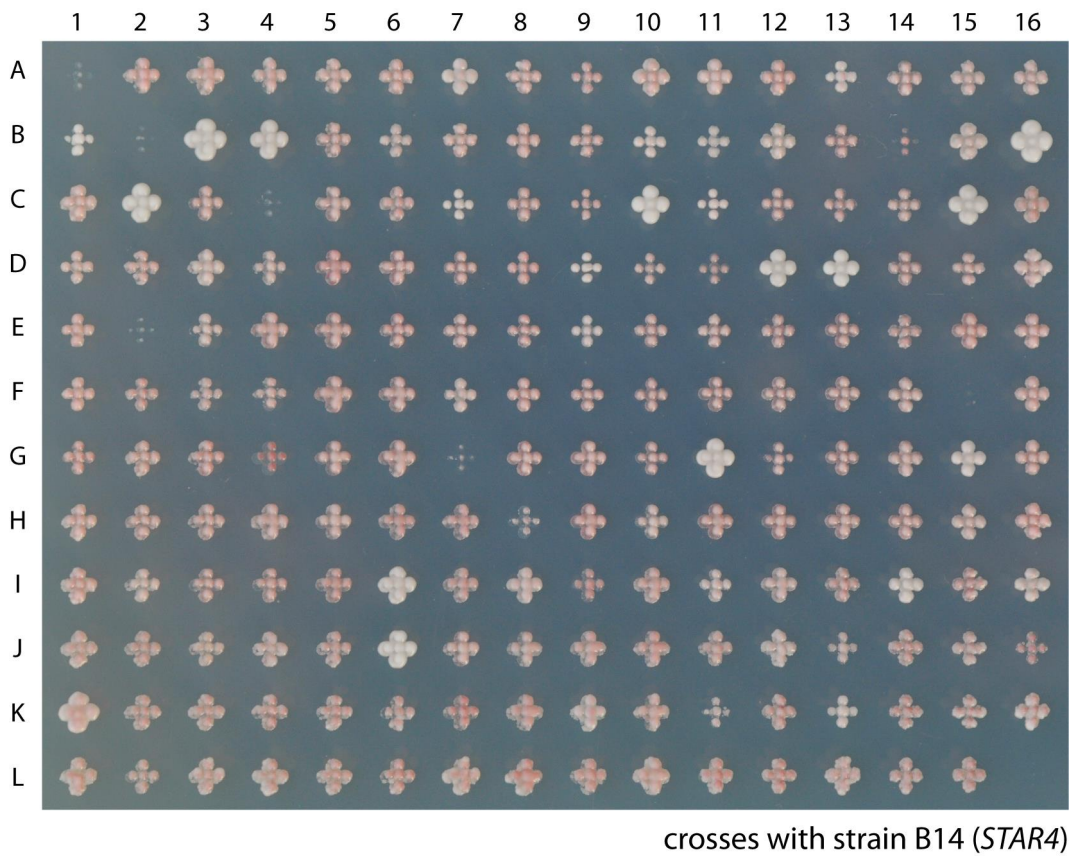
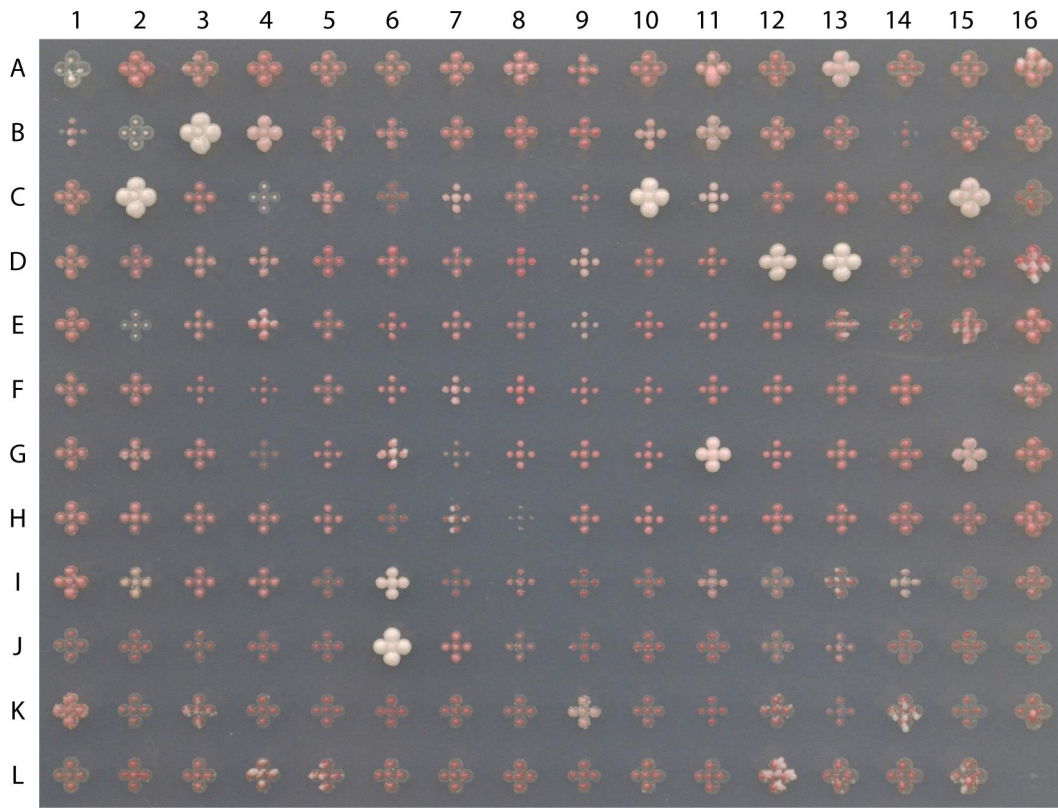
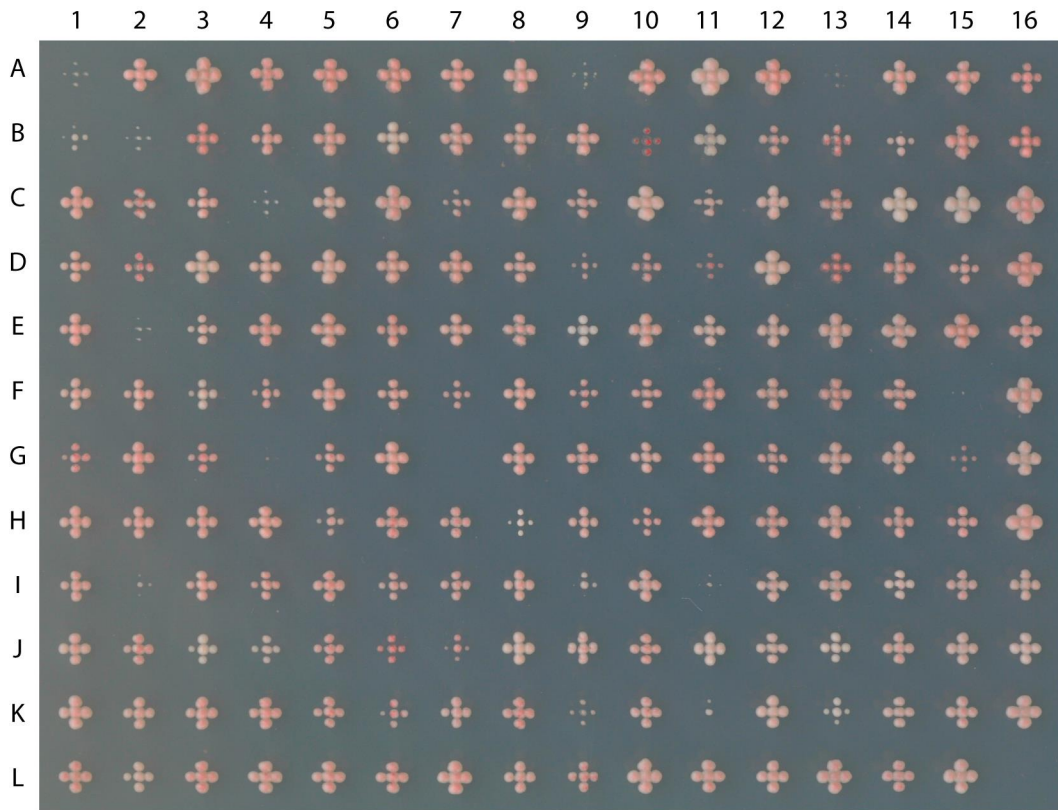


Figure S5. Second screen with *STAR4* boundary. Same as Figure S1, with strain B14 in which *IR-R⁺* is replaced with the *STAR4* element.



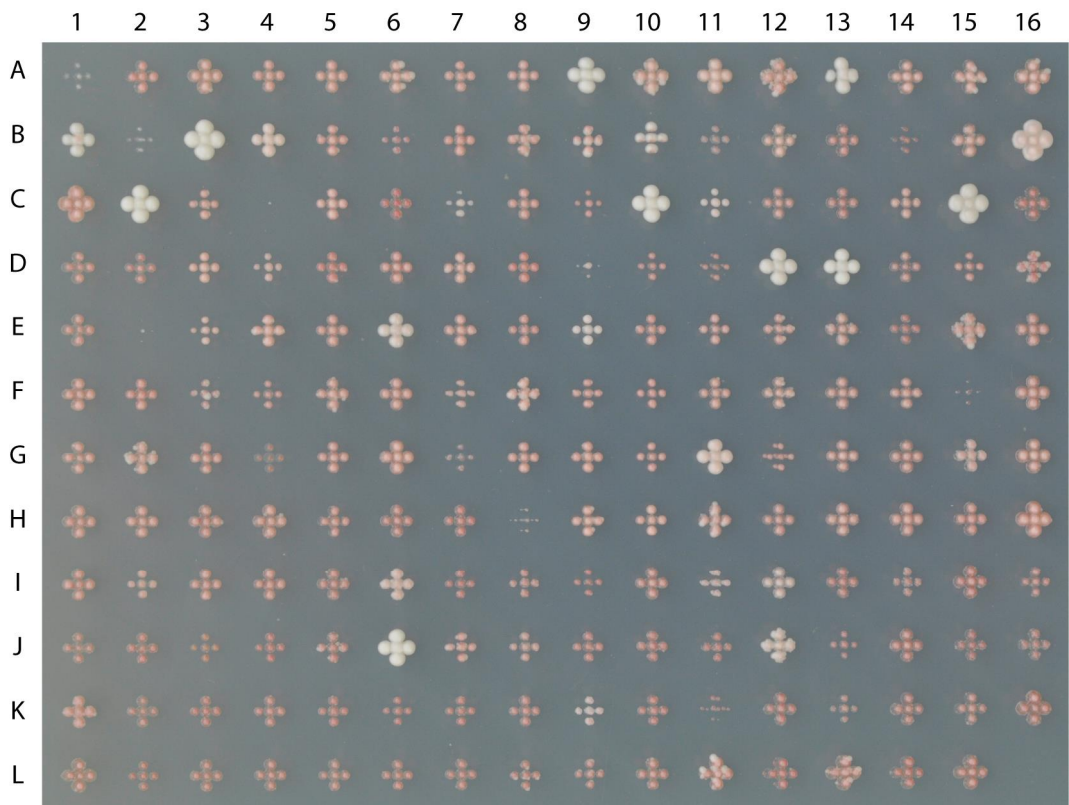
crosses with strain B7 (*BTH1*)

Figure S6. Second screen with *BTH1* boundary. Same as Figure S1, with strain B7 in which *IR-R⁺* is replaced with the *BTH1* element.



crosses with strain B17 (*rDNA*)

Figure S7. Second screen with *rDNA* boundary. Same as Figure S1, with strain B17 in which *IR-R⁺* is replaced with an *rDNA* repeat.



crosses with strain B18 (*cen1*)

Figure S8. Second screen with *cen1* boundary. Same as Figure S1, with strain B18 in which *IR-R*⁺ is replaced with the *cen1* element.

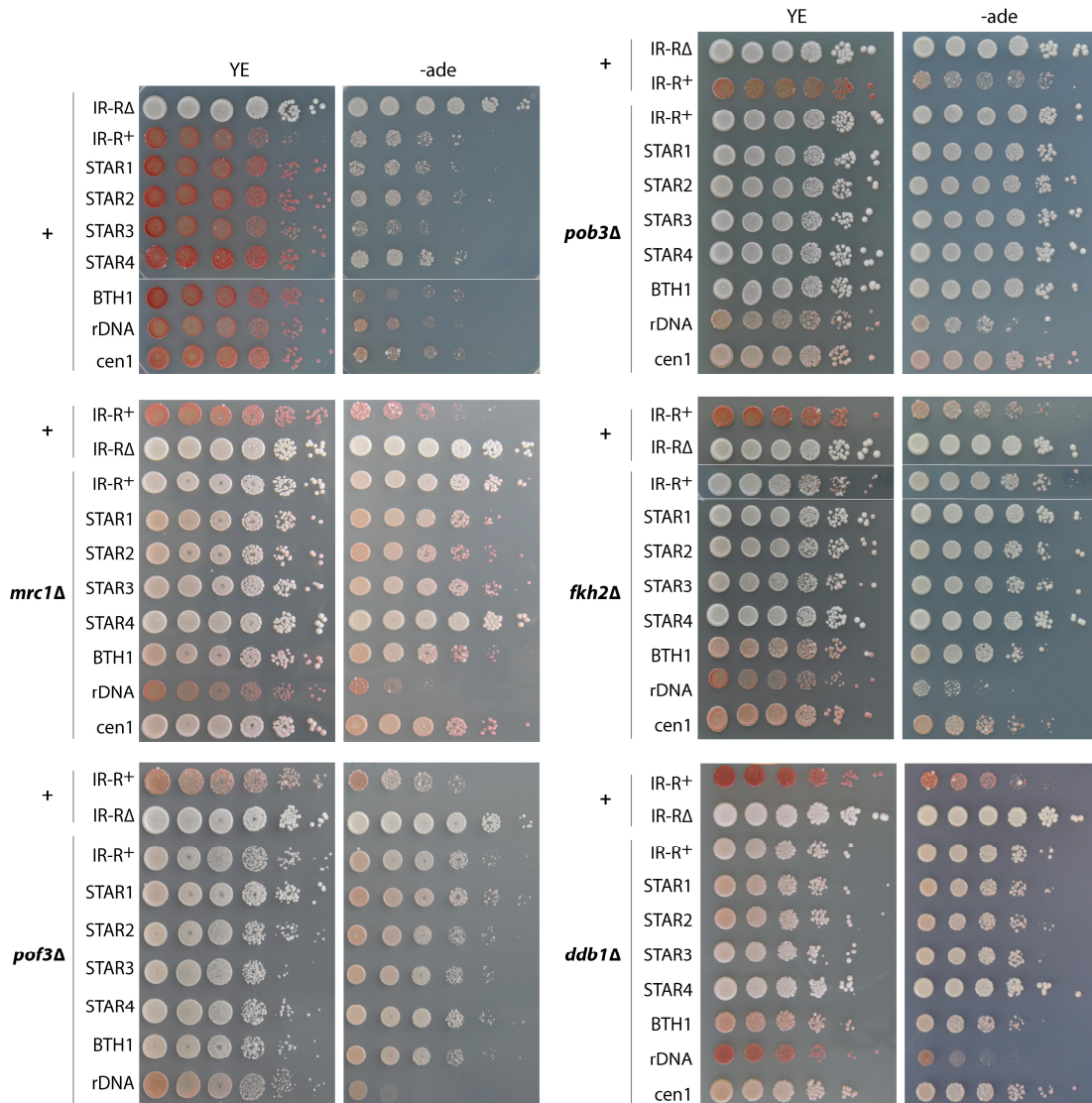


Figure S9. Third screen: effect of Pob3, Mrc1, Fkh2, Pof3, and Ddb1 on heterochromatic silencing at *(EcoRV)::ade6⁺*. Ten-fold serial dilutions of cell suspensions were spotted on medium with a low adenine concentration (YE) or no adenine. The wild-type series (+) shows PG3947 (IR-R⁻) together with the query strains used in the screen: B19, B15, B16, B6, B14, B7, B17, and B18. In the following panels, PG3950 (IR-R⁺) and PG3947 (IR-R⁻) were spotted on all plates as standards for *(EcoRV)::ade6⁺* repression and derepression respectively. Strains used for *pob3Δ* series: DN173, DN169, DN170, DN166, DN168, DN167, DN171, DN172; strains used for *mrc1Δ* series: LJ102, LJ117, LJ118, LJ114, LJ116, LJ115, LJ119, LJ120; strains used for *fkh2Δ* series: DN129, DN125, DN126, DN122, DN124, DN123, DN127; strains used for *pob3Δ* series: LJ220, LJ224, LJ225, LJ221, LJ223, LJ222, LJ226; strains used for *ddb1Δ* series: DN105, DN101, DN102, DN98, DN100, DN101, DN103.

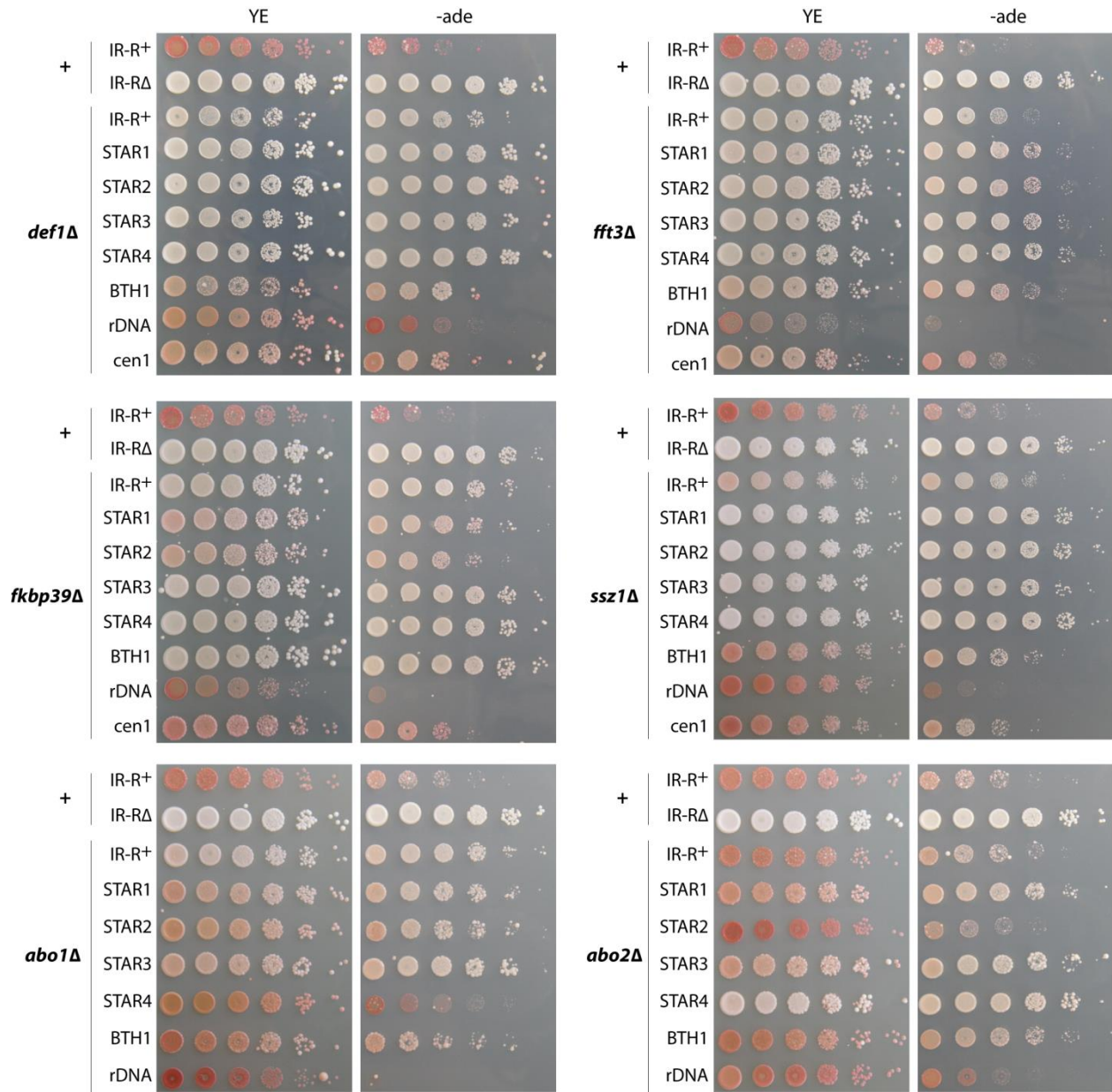


Figure S10. Third screen: effect of Def1, Fft3, Fkbp39, Ssz1, Abo1 and Abo2 on heterochromatic silencing at *(EcoRV)::ade6⁺*. As in Figure S9, PG3950 (IR-R⁺) and PG3947 (IR-R⁻) were spotted on all plates as standards for *(EcoRV)::ade6⁺* repression and derepression respectively. Strains used for *def1Δ* series: LJ100, LJ152, LJ153, LJ149, LJ151, LJ150, LJ154, LJ155; strains used for *fft3Δ* series: LJ103, LJ124, LJ125, LJ121, LJ123, LJ122, LJ126, LJ127; strains used for *fkbp39Δ* series: LJ104, LJ131, LJ132, LJ128, LJ130, LJ129, LJ133, LJ134; strains used for *ssz1Δ* series: LJ203, LJ202, LJ111, LJ107, LJ109, LJ108, LJ112, LJ113; strains used for *abo1* series: LJ199, DB44, DB45, DB41, DB43, DB42, DB46; strains used for *abo2Δ* series: LJ105, DB52, DB53, DB49, DB51, DB50, DB54. See Figure S9 for control strains with ectopic boundary elements in wild-type background.

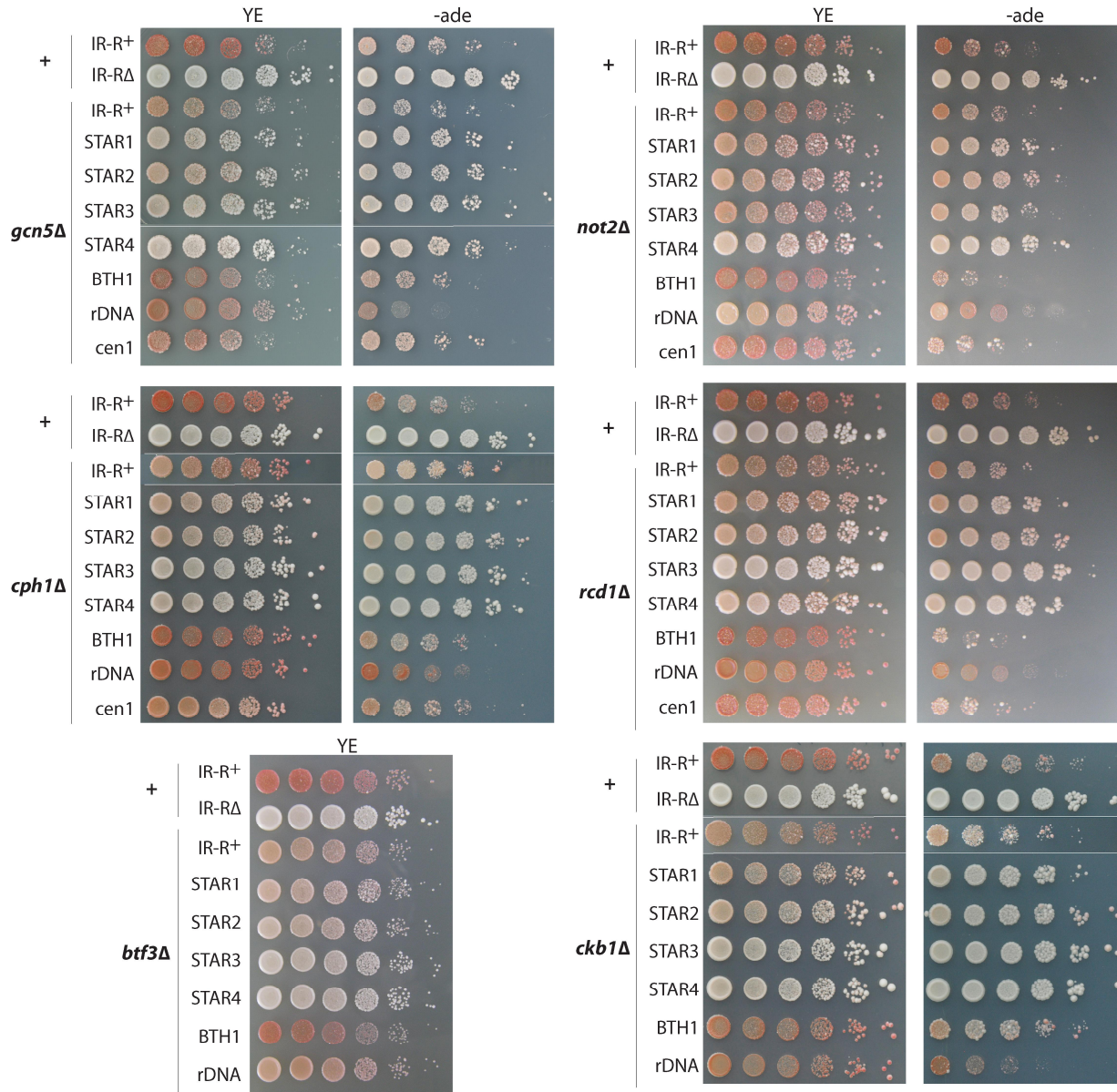


Figure S11. Third screen: effect of Gcn5, Not2, Cph1, Rcd1, Btf3, and Ckb1 on heterochromatic silencing at *(EcoRV)::ade6⁺*. As in Figure S9, PG3950 (*IR-R⁺*) and PG3947 (*IR-R⁻*) were spotted on all plates as standards for *(EcoRV)::ade6⁺* repression and derepression respectively. Strains used for *gcn5Δ* series: PA91-PA98; strains used for *not2Δ* series: DB105, DB4, DB5, DB1, DB3, DB2, DB6, DB7; strains used for *cph1Δ* series: DN121, DN117, DN118, DN114, DN116, DN115, DN119, DN120; strains used for *rcd1Δ* series: DB112, DB60, DB61, DB57, DB59, DB58, DB62, DB63; strains used for *btf3Δ* series: DB16, DB20, DB21, DB17, DB19, DB18, DB22, DB23; strains used for *ckb1Δ* series: DN97, DN93, DN94, DN90, DN92, DN91, DN95. See Figure S9 for control strains with ectopic boundary elements in wild-type background.

Table S3. Strains and their genotypes.

| Strain | Genotype |
|---|--|
| Strains used for strain constructions and plating controls | |
| PG3947 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N</i> |
| PG3950 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N</i> |
| PC152 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N</i> |
| PT600 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2-S) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N</i> |
| Query strains used in library screens | |
| B6 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(BTH1) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B7 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR3) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B14 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR4) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B15 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR1) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B16 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B17 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ R-Δ (SpeI)::(rDNA) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B18 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(cen1) (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| B19 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| Strains in Figure 4 | |
| LJ99 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 clr4Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PG3950 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ102 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ100 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ220 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ203 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ104 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ103 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ199 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 abo1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ105 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 abo2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PM20 | <i>mat3-M(EcoRV)::ade6⁺ swi1::kanR leu1⁺ CO3-CFP ura4-D18 ade6-DN/N his7⁺::lacI-GFP his2[::hph1-ura4⁺-lacOp]</i> |
| PM24 | <i>mat3-M(EcoRV)::ade6⁺ swi3::kanR leu1⁺ CO3-CFP ura4-D18 ade6-DN/N his7⁺::lacI-GFP his2[::hph1-ura4⁺-lacOp]</i> |
| Strains in Figure 6 | |
| LJ177 | <i>mat3-M (EcoRV)::ura4⁺ clr4Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| PG1899 | <i>mat3-M (EcoRV)::ura4⁺ leu1-32 ura4-DS/E ade6-216</i> |
| LJ176 | <i>mat3-M (EcoRV)::ura4⁺ mrc1Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| LJ178 | <i>mat3-M (EcoRV)::ura4⁺ pof3Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| LJ179 | <i>mat3-M (EcoRV)::ura4⁺ def1Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |

| | |
|----------------------------|---|
| LJ175 | <i>mat3-M (EcoRV)::ura4⁺ ssz1Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| LJ196 | <i>mat3-M (EcoRV)::ura4⁺ fkbp39Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| LJ197 | <i>mat3-M (EcoRV)::ura4⁺ fft3Δ::kanR leu1-32 ura4-DS/E ade6-210</i> |
| Strains in Figure 7 | |
| PA71 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 mcl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA72 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (SpeI)::(STAR2) (BplI)::LEU2 mcl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA73 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (SpeI)::(STAR2-S) (BplI)::LEU2 mcl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA74 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (BplI)::LEU2 mcl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA82 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 wpl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA83 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (SpeI)::(STAR2) (BplI)::LEU2 wpl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA84 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (SpeI)::(STAR2-S) (BplI)::LEU2 wpl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA85 | <i>mat3-M (EcoRV)::ade6⁺ IR-RA (BplI)::LEU2 l wpl1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| Strains in Figure 8 | |
| LJ171 | <i>h^A clr4Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| FY520 | <i>h⁺ leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4-TEL(72)</i> |
| LJ163 | <i>h^A mrc1Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ172 | <i>h^A pof3Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ173 | <i>h^A def1Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ162 | <i>h^A ssz1Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ174 | <i>h^A fkbp39Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ165 | <i>h^A fft3Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ170 | <i>h^A bt3Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ168 | <i>h^A rif1Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ161 | <i>h^A abo1Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4⁺-TEL(72)</i> |
| LJ166 | <i>h^A abo2Δ::kanR leu1-32 ura4-DS/E ade6-210/216 Chr16 m23:ura4-TEL(72)</i> |
| LJ185 | <i>h^A clr4Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| FY648 | <i>h⁺ leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ180 | <i>h^A mrc1Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ186 | <i>h^A pof3Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ190 | <i>h^A def1Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ192 | <i>h^A ssz1Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ187 | <i>h^A fkbp39Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ183 | <i>h^A bt3Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ181 | <i>h^A rif1Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ191 | <i>h^A abo1Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |
| LJ189 | <i>h^A abo2Δ::kanR leu1-32 ura4-DS/E ade6-210 otr1(SphI)::ura4⁺</i> |

| Strains in Figure S9 | |
|-----------------------------|---|
| <i>mrc1Δ</i> series | |
| LJ102 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ117 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR1) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ118 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ114 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR3) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ116 | <i>mat2-M (Xmnl)::ura4⁺ mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR4) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ115 | <i>mat2-M (Xmnl)::ura4⁺ mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(BTH1) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ119 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(rdNA) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ120 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(edge cen1) (BlnI)::LEU2 mrc1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| <i>pof3Δ</i> series | |
| LJ220 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ224 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR1) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ225 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ221 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR3) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ223 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR4) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ222 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(BTH1) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ226 | <i>mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(rdNA) (BlnI)::LEU2 pof3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| <i>pob3Δ</i> series | |
| DN173 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN169 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR1) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN170 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR2) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN166 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR3) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN168 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(STAR4) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN167 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(BTH1) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN171 | <i>mat2-M (Xmnl)::ura4⁺ mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(rdNA) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN172 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-Δ (SpeI)::(cen1 NheI) (BlnI)::LEU2 pob3Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |

| <i>fkh2Δ</i> series | |
|------------------------------|--|
| DN129 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN125 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN126 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN122 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN124 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN123 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN127 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 fkh2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| <i>ddb1Δ</i> series | |
| DN105 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN101 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN102 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN98 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN100 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN101 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN103 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 ddb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| Strains in Figure S10 | |
| <i>def1Δ</i> series | |
| LJ100 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| LJ152 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ153 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ149 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ151 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ150 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ154 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| LJ155 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BlnI)::LEU2 def1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |

| fft3Δ series | |
|-----------------------|--|
| U103 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| U124 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U125 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U121 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U123 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U122 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BplI)::LEU2 fft3Δ::kanR ura4-leu1-32 D18 ade6-DN/N arg12Δ::natR</i> |
| U126 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U127 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge of cen1 RHS NheI) (BplI)::LEU2 fft3Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| fkbp39Δ series | |
| U104 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| U131 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U132 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U128 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U130 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BplI)::LEU2 fkbp39Δ::kanR ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U129 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U133 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U134 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BplI)::LEU2 fkbp39Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| ssz1Δ series | |
| U203 | <i>mat3-M (EcoRV)::ade6⁺ (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| U202 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR?</i> |
| U111 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U107 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U109 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U108 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U112 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |
| U113 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BplI)::LEU2 ssz1Δ::kanR leu1-32 ura4-D18 ade6-DN/N arg12Δ::natR</i> |

| abo1Δ series | |
|------------------------------|---|
| LJ199 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 abo1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB44 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| DB45 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| DB41 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| DB43 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| DB42 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI):(BTH1) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| DB46 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI):(rDNA) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo1Δ::kanR</i> |
| abo2Δ series | |
| LJ105 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 abo2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB52 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| DB53 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| DB49 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| DB51 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| DB50 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI):(BTH1) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| DB54 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6 IR-RΔ (SpeI):(rDNA) (BlnI)::LEU2 leu1-32 ura4-D18 ade6-DN/N abo2Δ::kanR</i> |
| Strains in Figure S11 | |
| gcn5Δ series | |
| PA91 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA92 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA93 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA94 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA95 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA96 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA97 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| PA98 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BlnI)::LEU2 gcn5Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |

| not2Δ series | |
|---------------------|---|
| DB105 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DB4 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB5 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB1 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB3 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB2 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB6 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB7 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BlnI)::LEU2 not2Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| cph1Δ series | |
| DN121 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN117 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN118 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN114 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN116 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN115 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN119 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN120 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(edge cen1) (BlnI)::LEU2 cph1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| rcd1Δ series | |
| DB112 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DB60 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB61 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB57 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB59 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB58 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB62 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB63 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(cen1 NheI) (BlnI)::LEU2 rcd1Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |

| btf3Δ series | |
|---------------------|---|
| DB16 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB20 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB21 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB17 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB19 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB18 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB22 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| DB23 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(cen1 NheI) (BlnI)::LEU2 btf3Δ::kanR leu1-32 ura4-D18 ade6-DN/N</i> |
| ckb1Δ series | |
| DN97 | <i>mat3-M (EcoRV)::ade6⁺ (BlnI)::LEU2 ckb1Δ::kanR leu1-32 ura4-D18 or DS/E ade6-DN/N</i> |
| DN93 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR1) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |
| DN94 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR2) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |
| DN90 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR3) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |
| DN92 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(STAR4) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |
| DN91 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(BTH1) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |
| DN95 | <i>(Xmnl)::ura4⁺ mat2-P mat3-M (EcoRV)::ade6⁺ IR-RΔ (SpeI)::(rDNA) (BlnI)::LEU2 ckb1Δ::kanR ura4-D18 or DS/E ade6-DN/N</i> |

Table S4. Oligonucleotide sequences.

| Oligonucleotide | Sequence |
|---|-------------------------------|
| Confirmation of Bioneer ORF deletions | |
| Cp-N10 (GTO-596) | GATGTGAGAAGTGTATCCTAGCAAG |
| Cp-C3 (GTO-597) | GGCTGGCCTGTTGAACAAGTCTGGA |
| Cp5-mcl1D | CGAGAATCTCACACAGTTCTACAGC |
| Cp3-mcl1D | GCCAGAACTTTGGGCGTTAGCTC |
| Cp5-wpl1D | GGTGGCTCAATAATGGCCGCATTCCG |
| Cp3-wpl1D | GGCGATAACACAACCCTTTCCATC |
| Cp5-dpb4D | GTTCGAGGAGATGGTTAGAAGCAAGTG |
| Cp3-dpb4D | CTGGACAGTAAACACCGATTAGGATG |
| GTO-598 pli1 (03-81) c | GCTGCCCGTAGTCTAATCAATCATT |
| GTO-599 ckb1 (04-23) c | CTACAAAGTGTCACTGTCACACCTG |
| GTO-600 abo1 (05-33) | TACACGCGCATATCCTGTGTGCGTG |
| GTO-601 pdr13 (06-02) | GCATGTGGTGATAACACAGATAGTAG |
| GTO-602 mrc1 (06-19) c | GGCAACGTTTATGGTGATATGACTG |
| GTO-603 rhp18 (07-46) | GGTACATCTAGAAATCTTCACATGC |
| GTO-604 ppo1 (07-80) c | GCATTGCCAGATTGATGTAGCTTGC |
| GTO-605 mit1 (09-32) c | GTATCCGCAAATACCTATATGGCTC |
| GTO-606 ddb1 (10-52) c | GAATATCCTGTTAATGCAATCAC |
| GTO-607 pof3 (11-94) c | GCTTCAGTGCAAAATAGTAAACACC |
| GTO-608 ccq1 (14-25) c | CCTTATAAGAGCCAGAATGAATTGC |
| GTO-609 cdt2 (14-69) c | GGGTCCTGCAAGGTGAACAGCTAAC |
| GTO-610 rtf1 (14-80) c | GATATGAGGCAGATACCTGTAAACG |
| GTO-611 rnc1 (17-85) c | CTTCCAAAGATATAACGACTGAGGC |
| GTO-612 csn1 (22-54) c | GAATACATGGGGTTCTAATCTTGG |
| GTO-613 meu6 (26-47) c | CGCAGACAGCTCGATTCCAGAAG |
| GTO-614 pob3 (26-94) c | CGTAGGAGACAATATGGGAATCCCG |
| GTO-615 did2 (29-18) c | GGTTGAAGAGCGGTGTCCTTTGC |
| GTO-616 spc19 (29-55) | CCGAAGGATTGTGTAGTTTACACAGG |
| GTO-617 cph1 (29-75) c | GCGTTTTAAACCATTGATATGGCTG |
| GTO-618 fft3 (30-11) c | CTAGGTGAAGTGCTTTCCTCTGTC |
| GTO-619 fkbp39 (30-46) c | CTGCAATCCCTACTGGGCGTCGACGC |
| GTO-620 msh6 (31-89) c | CAGAGGATTGTTGGCAGGATCTTGG |
| GTO-621 wpl1 (32-77) c | GCTGTAAACGAGTACATAGCCATGG |
| PCR amplification of <i>mcl1Δ::kanR</i>, <i>wpl1Δ::kanR</i> and <i>dpb4Δ::kanR</i> | |
| GTO-1332 | CCTGTGAGGCTCCACTAGGTTTCTC |
| GTO-1333 | CTGTTGCTCGTTCAATTCATCGCCTAC |
| GTO-1363 | CTAGTTATAAAGGGAAGTCGGTCTTCAC |
| GTO-1364 | CTTCTGCTTTCACTGGAATAGCAACATC |
| GTO-1365 | GAGCGTGTGCGTTTGTGACGCGTCGC |
| GTO-1366 | CGTTATGGCGAGCTGAAGCTTCTGTCC |
| RT-QPCR for <i>ade6</i> and <i>act1</i> | |
| GTO-218 | GGCTCGAGCCCGGGCATGGAAATTGCAG |
| GTO-219 | GGGGATCCATGGCAAAGGGTTTGAGCAGC |
| TJO-55 | CTGTTTTGTCTTTGTATGCC |
| TJO-58 | TAAGGTAGTCAGTCAAGTCA |

| Identification of <i>ade6-DN/N</i> allele | |
|--|-------------------------|
| GTO-232 | CATTGGCTTACGACGGTCGTGG |
| GTO-233 | CCACATATGGCCCGTAAGTGAGC |