Supplemental data

Figure S1. Multiple sequence alignment of the REPULS domains. Positions where hydrophobic and aromatic characters are particularly conserved are shaded green and orange, respectively. Other striking similarities are in blue and pink (basic and acidic residues, respectively), as well as in yellow (small residues or loop-forming residues). Predicted secondary structures are reported up to the alignment. REPULS domains which were particularly difficult to detect are reported at bottom. UniProt identifiers : *Saccharomyces cerevisiae* (Q08562), *Candida glabrata* (Q6FSM2), *Kazachstania africana* (H2AY18), *Naumovozyma dairensis* (G0W9C7), *Naumovozyma castelli* (G0VIR1), *Tetrapisispora phaffii* (G8BZA4), *Vanderwaltozyma polyspora* (A7TPE3)), *Zygosaccharomyces rouxii* (C5DWI1), *Torulospora delbrueckii* (G8ZXX1), *Kluyveromyces waltii* (Kwal14.1868 (from YOGB, Byrne and Wolfe 2005)), *Lachancea thermotolerans* (C5E2Q8), *Kluyveromyces lactis* (Q6CUF0), *Ashbya gossypii* (Q75EC7), *Eremothecium cymbalariae* (G8JR28).

The central region of Uls1 (aa 550 to 900), located after the SIMs and before the Swi2/Snf2-like translocase domain, harbors typical features of globular domains, as deduced from Hydrophobic Cluster Analysis (HCA) (Callebaut et al. 1997). Indeed this region contains ~1/3 of strong hydrophobic amino acids (V I L F M Y W), which are distributed on the HCA two-dimensional plot into hydrophobic clusters, which mainly correspond to the regular secondary structures forming the core of globular domains. Using this Uls1 fragment as query, we searched the sequence databases (non redundant (nr) database at NCBI, 17,919,084 sequences) using PSI-BLAST (Altschul et al. 1997). We deduced from the PSI-BLAST results, obtained at convergence by iteration 3, that Uls1 contains a repeated domain, included between amino acids 677 and 761 (first repeat) and 773 and 855 (second repeat). We named these repeats REPULS after REPeat in ULS1.

REPULS domains are present in Uls1 sequences from yeasts of the *Saccharomyces* complex, also named *Saccharomycetaceae*. *Saccharomycetaceae* are hemiascomycetes that have HML/HMR-like cassettes, which appear after the split from the *Yarrowia lipolytica* and *Debaromyces hansenii/ Candida albicans* branch (Génolevures Consortium 2009, Dujon 2010). No REPULS domains could indeed be detected in these last yeast species, belonging to other subdivisions of hemiascomycetes, or in other more distant species. Strict tandem repeats are

observed in Uls1 from *Saccharomyces cerevisae* to *Zygosaccharomyces rouxii*, whereas *Kluyveromyces waltii* and *Lachancea thermotolerans* Uls1 have only one REPULS domain, and *Kluyveromyces lactis*, *Ashbya gossypii* and *Eremothecium cymbalariae* have multiple copies.

Secondary structure predictions indicate that the REPULS domains are likely made of four alpha-helices. No obvious similarity has been detected with known 3D structures, using sequence similarity searches (see before) or fold recognition programs (*e.g.* Phyre : Bennett-Lovsey et al. 2008). Positions in which the hydrophobic character is conserved (in green) likely participate in the hydrophobic core of the domain. Positions mainly occupied by aromatic amino acids (orange) might play a role either in the packing of the hydrophobic core or in a specific function, at the surface of the domain. REPULS domains contain several conserved basic amino acids and are characterized by a striking sequence signature (G-[LV]-K-M-[PDEN]), located in the loop linking helices C and D. Worth noting is that these sequence features of REPULS domains are well conserved in Uls1 proteins of species containing only two (tandem) or one (single) REPULS domain(s). In species having more than two domains, some of the additional REPULS domains lose some of these sequence characteristics, so that they are difficult to detect. This is particularly the case for the degenerated REPULS repeats D of *Ashbya gossypii* and *Eremothecium cymbalariae* and repeat F of *Kluyveromyces lactis*. The sequence of the repeat D of *Eremothecium cymbalariae* was the only one that could be detected from the PSI-BLAST results. The relationships between the REPULS repeat D of *Ashbya gossypii* and *Eremothecium cymbalariae* and the REPULS repeat F of *Kluyveromyces lactis* were then detected using HCA.

Figure S2. Telomere length in cells lacking Uls1, Rif2 and Sir4. Genomic DNA from strains Lev346 (WT), 184-48c (*uls1-Δ*), 183-30d (*rif2-Δ*), Lev575 (*sir4-Δ*), 184-14c (*rif2-Δ sir4-Δ*), 183-10c (*uls1-Δ sir4-Δ*), 183-2d (*uls1-Δ rif2-Δ*) and 183-25a (*uls1-Δ rif2-Δ sir4-Δ*) was digested with XhoI, separated in a 0.9% agarose gel and probed with a Y' distal fragment.

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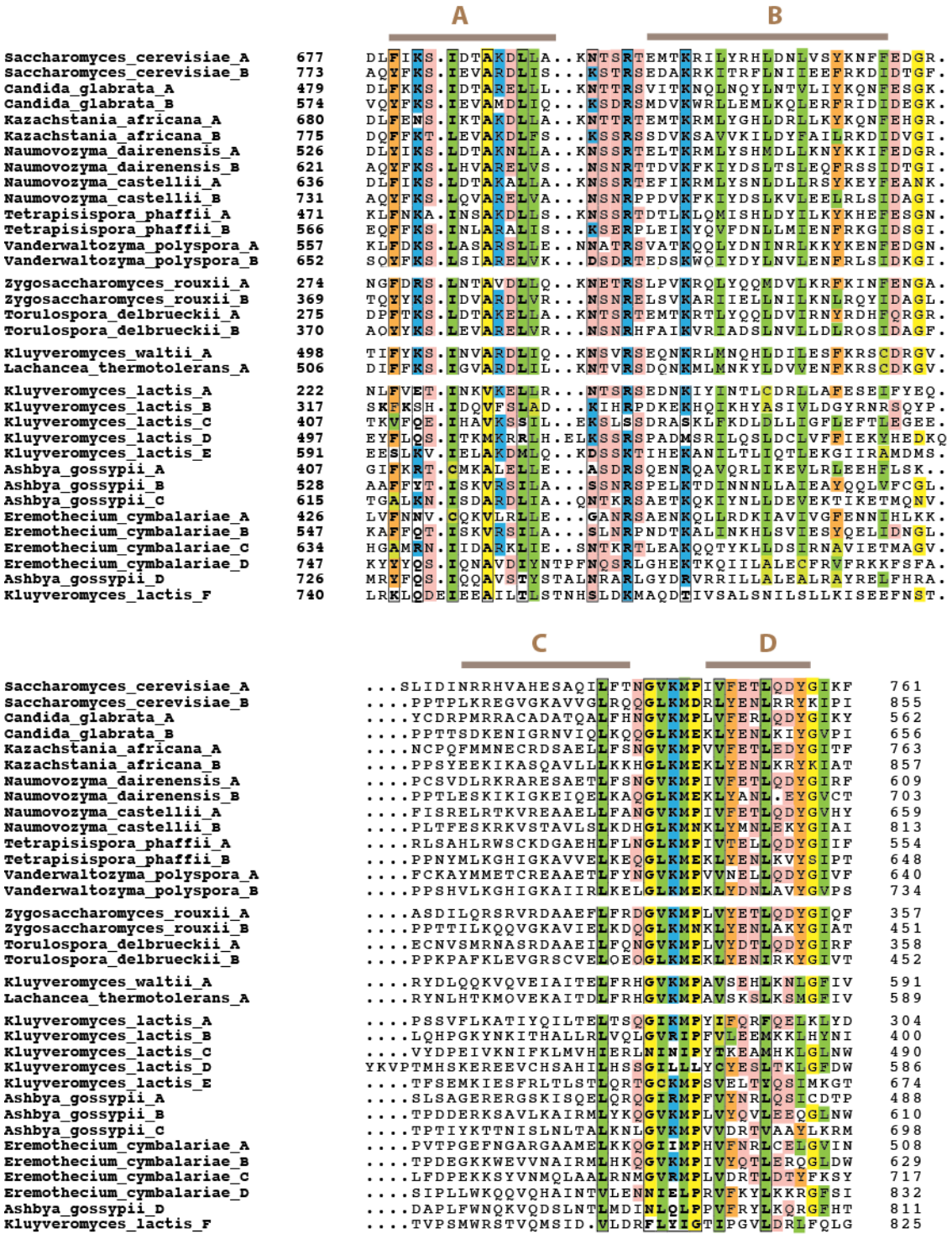
A
B
C
D
Saccharomyces_cerevisiae_A 677 DLFIKS.IDTAKDLLA..KNTSRTEMTKRILYRHLDNLVSYKNFFEDGR.
Saccharomyces_cerevisiae_B 773 AQYFKS.IEVARDLIS...KSTRSEDAKRKITRFLNIIEEFRKDIDTGF.
Candida_glabrata_A 479 DLFKKS.IDTARELLL..KNTTRSVITKNQLNQYLNTVLIYKQNFESGK.
Candida_glabrata_B 574 VQYFKS.IEVAMDLIQ...KSDRSMDVKWRLLEMLKQLERFRIDIDEGK.
Kazachstania_africana_A 680 DLFENS.IKTAKDLLA..KNTTRTEMTKRMLYGHLDRLLKYKQNFEHGR.
Kazachstania_africana_B 775 DQFFKT.LEVAKDLFS...KSSRSSDVKSAVVKILDYFAILRKDIDVGI.
Naumovozyma_dairenensis_A 526 DLYIKS.LDTAKNLLA..KNSSRTELTKRMLYSHMDLLKNYKKIFEDGR.
Naumovozyma_dairenensis_B 621 AQYFKS.LHVARELVS...NSNRTTDVKFKIYDSLTSLEQFRSSIDTGI.
Naumovozyma_castellii_A 636 DLFIKS.LDTAKALLA..KNSSRTEFIKRMLYSNLDLLRSYKEYFEANK.
Naumovozyma_castellii_B 731 AQYFKS.LQVARELVA...NSNRPPDVKFKIYDSLKVLEELRLSIDAGI.
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Vanderwaltozyma_polyspora_B 652 SQYFKS.LSIARELVK...DSDRTEDSKWQIYDYLNVLENFRLSIDKGI.
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Lachancea_thermotolerans_A 506 DIFFKS.IGVARDLIL..KNTVRSDQNKMLMNKYLDVVENFKRSCDKGV.
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Kluyveromyces_lactis_B 317 SKFKSH.IDQVFSLAD...KIHRPDKEKHQIKHYASIVLDGYRNRSQYP.
Kluyveromyces_lactis_C 407 TKVFQE.IHAVKSSIL..EKSLSSDRASKLFKDLDLLIGFLEFTLEGEE.
Kluyveromyces_lactis_D 497 EYFLQS.ITKMKRRLH.ELKSSRSPADMSRILQSLDCLVFFIEKYHEDKQ
Kluyveromyces_lactis_E 591 EESLKV.IELAKDMLQ..KDSSKTIHEKANILTLIQTLEKGIIRAMDMS.
Ashbya_gossypii_A 407 GIFKRT.CMKALELLE...ASDRSQENRQAVQRLIKEVLRLEEHFLSK..
Ashbya_gossypii_B 528 AAFFYT.ISKVRSILA...SSNRSPELKTDINNNLLAIEAYQQLVFCGL.
Ashbya_gossypii_C 615 TGALKN.ISDARDLIA..QNTKRSAETKQKIYNLLDEVEKTIKETMQNV.
Eremothecium_cymbalariae_A 426 LVFNNV.CQKVLRLLE...GANRSAENKQLLRDKIAVIVGFENNIHLKK.
Eremothecium_cymbalariae_B 547 KAFFQT.ISKVRSILA...SLNRPNDTKALINKHLSVIESYQELIDNGL.
Eremothecium_cymbalariae_C 634 HGAMRN.IIDARKLIE..SNTKRTLEAKQQTYKLLDSIRNAVIETMAGV.
Eremothecium_cymbalariae_D 747 KYYYQS.IQNAVDIYNTPFNQSRLGHEKTKQIILALECFRVFRKKFSFA.
Ashbya_gossypii_D 726 MRYFQS.IQQAVSTYSTALNRARLGYDRVRRILLALEALRAYRELFHRA.
Kluyveromyces_lactis_F 740 LRKLQDEIEEAILTLSTNHSLDKMAQDTIVSALSNILSLLKISEEFNST.
Saccharomyces_cerevisiae_A ...SLIDINRRHVAHESAQILFTNGVKMPIVFETLQDYGIKF 761
Saccharomyces_cerevisiae_B ....PPTPLKREGVGKAVVGLRQQGLKMDRLYENLRRYKIPI 855
Candida_glabrata_A ....YCDRPMRRACADATQALFHNGVKMPLVFERLQDYGIKY 562
Candida_glabrata_B ....PPTTSDKENIGRNVIQLKQQGLKMEKLYENLKIYGVPI 656
Kazachstania_africana_A ....NCPQFMMNECRDSAELLFSNGVKMPVVFETLEDYGITF 763
Kazachstania_africana_B ....PPSYEEKIKASQAVLLLKKHGLKMEKLYENLKRYKIAT 857
Naumovozyma_dairenensis_A ....PCSVDLRKRARESAETLFSNGVKMPIVFETLQDYGIRF 609
Naumovozyma_dairenensis_B ....PPTLESKIKIGKEIQELKAQGLKMEKLYANL.EYGVCT 703
Naumovozyma_castellii_A ....FISRELRTKVREAAELLFANGVKMPIVFETLQDYGVHY 659
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Zygosaccharomyces_rouxii_B ....PPTTILKQQVGKAVIELKDQGLKMNKLYENLAKYGIAT 451
Torulospora_delbrueckii_A ....ECNVSMRNASRDAAEILFQNGVKMPLVYDTLQDYGIRF 358
Torulospora_delbrueckii_B ....PPKPAFKLEVGRSCVELQEQGLKMEKLYENIRKYGIVT 452
Kluyveromyces_waltii_A ....RYDLQQKVQVEIAITELFRHGVKMPAVSEHLKNLGFIV 591
Lachancea_thermotolerans_A ....RYNLHTKMQVEKAITDLFRHGVKMPAVSKSLKSMGFIV 589
Kluyveromyces_lactis_A ....PSSVFLKATIYQILTELTSQGIKMPYIFQRFQELKLYD 304
Kluyveromyces_lactis_B ....LQHPGKYNKITHALLRLVQLGVRIPFVLEEMKKLHYNI 400
Kluyveromyces_lactis_C ....VYDPEIVKNIFKLMVHIERLNINIPYTKEAMHKLGLNW 490
Kluyveromyces_lactis_D YKVPTMHSKEREEVCHSAHILHSSGILLLYCYESLTKLGFDW 586
Kluyveromyces_lactis_E ....TFSEMKIESFRLTLSTLQRTGCKMPSVELTYQSIMKGT 674
Ashbya_gossypii_A ....SLSAGERERGSKISQELQRQGIRMPFVYNRLQSICDTP 488
Ashbya_gossypii_B ....TPDDERKSAVLKAIRMLYKQGVKMPLVYQVLEEQGLNW 610
Ashbya_gossypii_C ....TPTIYKTTNISLNLTALKNLGVKMPVVDRTVAAYLKRM 698
Eremothecium_cymbalariae_A ....PVTPGEFNGARGAAMELKKQGIIMPHVFNRLCELGVIN 508
Eremothecium_cymbalariae_B ....TPDEGKKWEVVNAIRMLHKQGVKMPIVYQTLERQGLDW 629
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Ashbya_gossypii_D ....DAPLFWNQKVQDSLNTLMDINLQLPPVFRYLKQRGFHT 811
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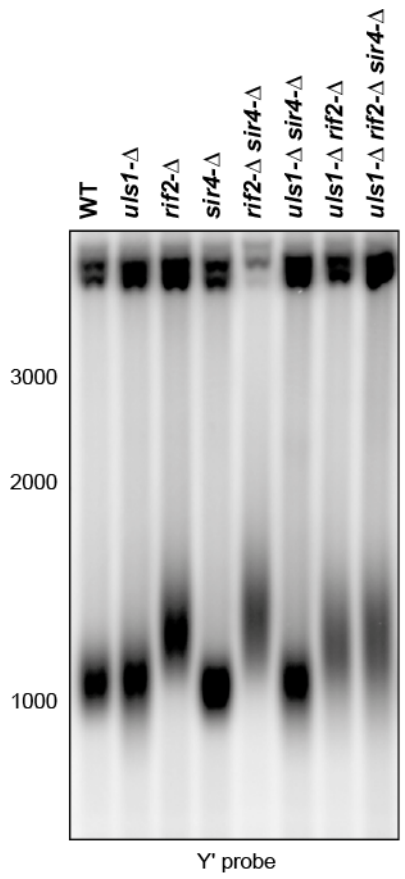
Figure S1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uls1-Δ
rif2-Δ
sir4-Δ
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3000
2000
1000
Y' probe

Figure S2

Supplementary table 1. Yeast strains used in this study. All strains are from the W303-1a background (*ade2-1 trp1-1 ura3-1 leu2-3,112 his3-11,15 can1-100 RAD5*).

Strains	Genotype
Lev346	*MAT**a***
RL71	*MAT**a** uls1-Δ::klLEU2*
212-12a	*MAT**a** hml-Δ::NAT*
213-4a	*MAT**a** hml-Δ::NAT*
213-7b	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
213-9c	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
195-28a	*MAT**a** uls1-Δ::klLEU2 lif1-Δ::skHIS3*
210-3d	*MAT**a** hml-Δ::NAT*
211-1a	*MAT**a** hml-Δ::NAT*
211-8d	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
211-10b	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
209-1c	*MAT**a** hml-Δ::NAT pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr*
209-2b	*MAT**a** pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr*
206-2b	*MAT**a** hml-Δ::NAT*
205-9a	*MAT**a** hml-Δ::NAT*
206-1d	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
205-14c	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
199-3a	*MATalpha lys2::pGAL1-ISCEI uls1-C1333S*
200-2c	*MATalpha uls1-K975R*
200-5d	*MAT**a** uls1-K975R*

196-11c *MATalpha bar1-Δ*

196-13b *MATalpha bar1-Δ*

196-5a *MATalpha bar1-Δ uls1-Δ::klLEU2*

196-6a *MATalpha bar1-Δ uls1-Δ::klLEU2*

Lev791 *MATalpha bar1-Δ uls1-Δ677-855*

Lev792 *MATalpha bar1-Δ uls1-Δ677-855*

210-5b *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*

RL179 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI*

RL183 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI uls1-Δ::klLEU2*

RL185 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI uls1-Δ::klLEU2 smt3-3R::TRP1*

RL181 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI smt3-3R::TRP1*

RL266 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI uls1-Δ::klLEU2 siz1-Δ::HPH*

RL267 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI uls1-Δ::klLEU2 siz2-Δ::NAT*

RL268 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI siz1-Δ::HPH*

RL269 *MAT**a** bar1-Δ lys2::GAL-ISCEI ISceI-URA3-ISceI siz2-Δ::NAT*

169-1c *MAT**a** pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr*

169-15c *MAT**a** bar1-Δ pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr*

169-6b *MAT**a** pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr smt3-3R::TRP1*

169-11a *MAT**a** bar1-Δ pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr smt3-3R::TRP1*

195-19d *MATalpha*

195-3a *MATalpha rap1-DAmp::KANr*

195-7b *MATalpha uls1-Δ::klLEU2*

195-8a *MATalpha rap1-DAmp::KANr uls1-Δ::klLEU2*

195-6b *MATalpha rap1-DAmp::KANr uls1-Δ::klLEU2 lif1-Δ::skHIS3*

195-1d *MATalpha rap1-DAmp::KANr uls1-Δ::klLEU2 smt3-3R::TRP1*

198-16a *MAT**a** uls1-Δ::klLEU2 rap1-Δ2-228*

198-19a *MAT**a** uls1-Δ::klLEU2 rap1-Δ2-228*

196-1a *MATalpha bar1-Δ uls1-Δ::klLEU2 rap1-Δ2-309*

196-7c *MATalpha bar1-Δ uls1-Δ::klLEU2 rap1-Δ2-309*

210-2d *MAT**a** hml-Δ::NAT*

210-3d *MAT**a** hml-Δ::NAT*

210-5b *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*

210-4c *MATalpha hml-Δ::NAT uls1-Δ::klLEU2*

210-10b *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 rap1-K246R*

210-5d *MATalpha hml-Δ::NAT uls1-Δ::klLEU2 rap1-K246R*

210-1b *MAT**a** hml-Δ::NAT rap1-K246R*

210-7b *MAT**a** hml-Δ::NAT rap1-K246R*

212-2d *MAT**a** hml-Δ::NAT*

212-10b *MAT**a** hml-Δ::NAT*

212-1a *MATalpha hml-Δ::NAT uls1-Δ::klLEU2*

212-8a *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*

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212-17d *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 rap1-K240R, K246R*

212-13c *MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 rap1-K240R, K246R*

212-3b *MAT**a** hml-Δ::NAT rap1-K240R, K246R*

212-4c *MAT**a** hml-Δ::NAT rap1-K240R, K246R*

184-48c *MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2*

184-9d *MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2*

183-30d *MATalpha bar1-Δ hmr-Δ::NAT rif2-Δ::HPH*

Lev575 *MAT**a** hml-Δ::NAT sir4-Δ::HPH*

Lev576	*MATalpha hmr-Δ::NAT sir4-Δ::HPH*
184-14c	*MATalpha bar1-Δ hmr-Δ::NAT rif2-Δ::HPH sir4-Δ::HPH*
Lev601	*MAT**a** hml-Δ::NAT rif2-Δ::skHIS3 sir4-Δ::HPH*
183-10c	*MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH*
183-2d	*MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2 rif2-Δ::HPH*
183-25a	*MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2 rif2-Δ::HPH sir4-Δ::HPH*
184-49a	*MATalpha bar1-Δ hmr-Δ::NAT uls1-Δ::klLEU2 rif2-Δ::HPH sir4-Δ::HPH*
205-9a	*MAT**a** hml-Δ::NAT*
205-14c	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2*
212-3c	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH*
212-4b	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH*
205-16a	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH smt3-3R::TRP1*
212-1b	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH rap1-K240R, K246R*
212-2a	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH rap1-K240R, K246R*
207-14d	*MAT**a** hml-Δ::NAT uls1-Δ::klLEU2 sir4-Δ::HPH rap1-Δ2-309*