



1 **Supplementary Table S1.** Hybrid sporulation assay. Strains sporulating on ACM medium after 3 and 7 days
 2 were scored as + and w (weak), respectively, while no sporulating strains were scored as -.

Crosses ¹	Hybrids	Sporulation (days)		
		3	7	15
Sc x Su	Y23.7A x RC2-10.4A	-	-	-
	Y23.10B x RC2-10.7B	-	-	-
	Y23.10D x RC2-10.7D	+	+	+
Sc x Sbay	Y15.2B.3A x NBRC 1948.3A	-	+	+
	Y19.11B x NBRC1948.4B	+	+	+
	Y19.12B x NBRC1948.5B	+	+	+
	Y19.12C x NBRC1948.5C	+	+	+
	Y19.13C x NBRC1948.6C	+	+	+
	Y21.7B x NBRC1948.8B	+	+	+
	Y21.9C x NBRC1948.10C	+	+	+
	Y21.10A x NBRC1948.11A	-	w	w
Sc x Scar	Y19.8A x CBS 8841.2A	-	w	w
	Y19.8C x CBS 8841.2C	+	+	+
	Y19.5A.1B x CBS 8841.4B	+	+	+

3 ¹ Abbreviation: Sc, *S. cerevisiae*; Su, *S. uvarum*; Sbay, *S. eubayanus* x *S. uvarum*; Scar, *S. cariocanus*.

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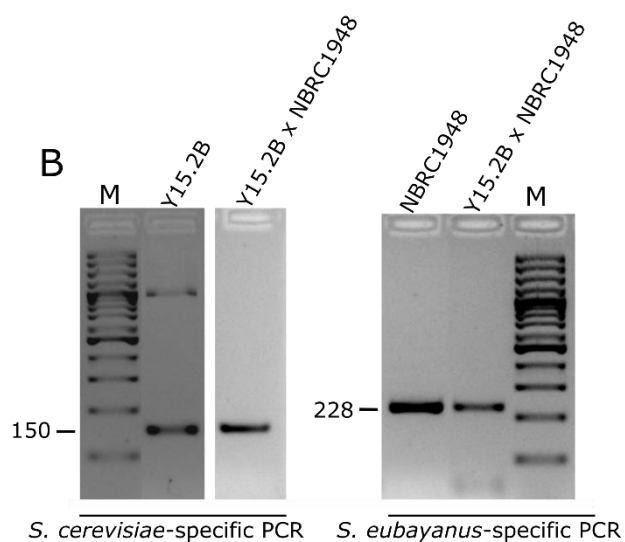
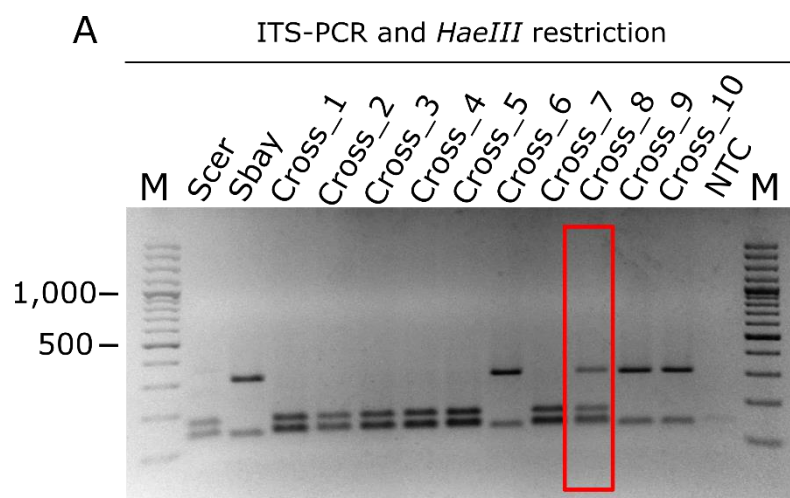
5 **Supplementary Table S2.** Wort fermentation parameters in laboratory scale trials (15 °P, 20°C).

Species ¹	Strains	Ethanol (% v/v)	pH	Viability (%)	Dry mass (g/L)
Su	RC2-10	5.8 ± 0.03	4.4 ± 0.00	43.3 ± 5.01	0.164 ± 0.011
H	Y23 x RC2-10	6.6 ± 0.01	4.5 ± 0.02	85.0 ± 1.97	0.169 ± 0.001
Sc	Y23	7.2 ± 0.01	4.5 ± 0.01	58.1 ± 14.65	0.156 ± 0.006
Sc	Y15.2B	7.2 ± 0.09	4.4 ± 0.01	72.9 ± 0.78	0.07 ± 0.01
Sc	Y15.2B x NBRC 1948	7.2 ± 0.02	4.4 ± 0.01	72.6 ± 5.62	0.07 ± 0.01
Sbay	NBRC 1948	7.1 ± 0.01	4.5 ± 0.01	70.8 ± 11.59	0.09 ± 0.01
Sc	Y19	7.3 ± 0.03	4.5 ± 0.01	73.8 ± 11.35	0.155 ± 0.005
H	Y19 x NBRC 1948	7.0 ± 0.01	4.4 ± 0.05	77.1 ± 3.36	0.179 ± 0.008
Sc	Y21	7.2 ± 0.04	4.3 ± 0.02	80.7 ± 1.86	0.165 ± 0.010
H	Y21 x NBRC 1948	7.0 ± 0.03	4.3 ± 0.03	79.8 ± 1.07	0.208 ± 0.001
Sc	3002	6.00 ± 0.01	4.3 ± 0.03	76.0 ± 19.0	0.11 ± 0.05
H	LS3	6.02 ± 0.04	4.5 ± 0.01	44.6 ± 8.22	0.13 ± 0.03
Su	7877	6.03 ± 0.01	4.4 ± 0.01	34.1 ± 4.19	0.06 ± 0.04

6 ¹ Abbreviations: Sc, *S. cerevisiae*; Sbay, *S. eubayanus* x *S. uvarum*; Su, *S. uvarum*; H, hybrid.

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8 **Supplementary Figure S1.** Confirmation of hybridization by (A) ITS1 PCR-RFLP with endonuclease
 9 *HaeIII* and (B) amplification of *FSY1* and *MEX67* genes using species-specific primers.



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