Supplementary materials

Integration sites designed for two model lager yeast

We designed five integration sites for *Saccharomyces pastorianus* lager yeast. All the integrations sites are identical in both model lager yeast LG160 and LG230. These integration sites are distributed in two chromosomes represent as Sp_X and Sp_XI. The integration sites denominated as Sp_X-1, Sp_X-2, and Sp_X-3 are more than 97% similar to the homologous regions of the *Saccharomyces eubayanus* chromosome X; whereas, Sp_XI-1 and Sp_XI-2 are completely identical to homologous regions of *Saccharomyces cerevisiae* chromosome XI.

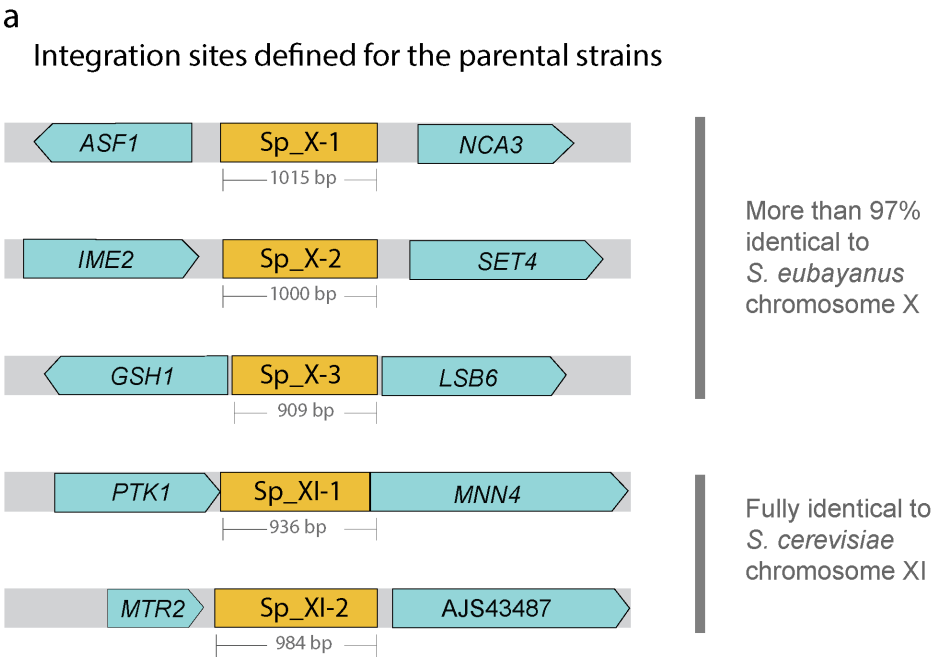

Figure S1. Representation of the location of the integration sites designed for the model lager yeast LG160 and LG230. The integrations sites are distributed in two chromosomes, which are homologous to chromosomes X and XI of *S. eubayanus* and *S. cerevisiae*, respectively.

Nucleotide sequences of the integration sites

Table S1. Nucleotide sequences of lager yeast integration sites

Integration site	Nucleotide sequence
Sp_X-1	GGAACAGATTTACGTGTTTCGGGCGACAAAAAAAAAAACGCCAGAAAAAGTAAAACGCGTAAAAGTCGATAATGGTCCATTTTA AAACCCAAACCAACCTTGTGAAACCAGAATCTTACCGAGGCGCTGCCGCCCGATTATATGCAAGAAAACCAACCACAGAGCAGG GGGGTGTTCAAGGTTAGGCACGGCACAGAGCCGAAAAAAAAGAAAGGACAAGGACATGGGCAAGATGAGACCGGACAAGGAA AAGAACTCGATGGAACTAATCTCTGCTAAAGGCAACACAAGGGCCAGGCAACACTACGGCATCATGTATGCCAGCCAAAACCCT ATTTGCCTCCGTCTTGTGGTTTTCAAACACCGACCATAGCAGCCTGGGGAAAGACACACTTGAAGACTCGGATGAGGAAAGAAAC AACGGGCCACTCAATGTCTTCGTCACTCATGCTCACCGCCAAGTATTCCAAAGCTTAATTGGTGGGCTCTTACCAAACACGCACAC ACCCCGCCAGAAAAAGAAACGGCCGGCGCAAGTGGGCTGATGCGCAGTCGATGCGTGGGGTAACCGGCCTGCAAGTCAGGGCC GGTTCGGTAGGCCTAGACCCGCACGTGACCGGTTCAAGGTACGGTTGCCTGCGGTGTTCTTTGCCAGAGGAAAAAAGAAACGGAA GCAATTTACTCTATGCGGAAAAAGAGGATCCTGTGGCAGCAAATAGGTGCGCCATCTCCGAAAAAAAGTCTAGACAATACCATG GCAATTAACCCAAAGAGGATATTAGATCGAACATGAAATTTTGACATAAGTGGAATCGGTGGCGATGTTACGGTATAGTGTTTTC AAGAGTAGGTCGAGTGAGAAAAGGTGGAGTTTGTTGAAACTCCGGAACTAAGTACTTGTCACGGGTGGCGAAATGGAGATGCGT TTTTTTGATATATAATCTGTAGACGCCATGACCAATGTACCCTTTCTTTCTGTCTTGTTTCCTGTATATGTTTTGGTAGTGTTTGCCGC
Sp_X-2	GACAGAATACTAGTGTATTTATCGTTAACTGGGAAACACAGCAGCGATGGACCCGCTAATTTACCAAAACCCTATTACTGCATAC GAAGAAAAGGCAGAAGAAAATTTTCGGTCGAAGCAAACCACATACAAAGAGGTTCCATGGACCAATAAAATACGACACGTATTT TATTGGGAGTCTAGGCTATATGCAAAGAAAGATGAGTCTACATGAAAACATTCCTTTCCCTAAAAGGTGGTGTACGTATTTGAAGT CAGTAGTAAACACCACCCACAGTTGTTTTTTTTCTAAACGACGAGCTTGGCTCTCCTTTGTTTTGATTTTGATGTCTTTCGTTTGTGAC TTCACCCAGGAAAGCGAGATCGCCGAATGCCACGCGAGAGAAGGGATATAAGGTTTCAGGCACGAAAACAATAGGCAAGAAGC AAAGGAGAACCGACATACGAGACTGGCGTGCCAGCGTTTACGGAAGTTATACCTAACTAGCCGGACATTAACAAAAGGCCAAGA CCGACGGCGCATATACACGCAATCACATTCGTTTTTCATATACCAAGAACTCACCTGAGTTTTTTACTTCTCTCTACCATTTTTTGCA CGTTACATTATGCAGCTTGAAGAGTTTTAACGAGATGAAATAGTCCGCCTACATCAGGCACGAAGGCACCGTCGTATATGCAGTG GAACTCTATCGCACTGGACAATCAAATCACACAGGTATAGGTAACAAAAAAAAAGCTGCGTCTCAATCGAGAGTCGATCTCTTCG TTTGGCTTTGCTTGGCTGGCGCCGTCGCTTTTCTTCGCTTATACCCGCCCTTTGACGCTCGACCTCGTTCATCGAGGTACCTTTAATCT TGCACATTTTCTGGCTTTTTTCTCGTCCGCACCTCGAGTACATGTAATCATGCACACAGAAGGTGCTGCAGCCAGGCGTTCCTTTCT ACTAGTCGTTTGCTTTTGATGCCAAACTTCCTGGTGATCAAGAACCACCTCCTTT

Sp_X-3	ACTCTAAAGCAGTTCTTTTCTGGCAAAACGATGAAAGAGAAAAGCCTAACTCTGAAATTTAGCCTTTTTTTATATCAACTTTTTCAG TTTCTTGCAAGTAACTCAAGAACAGATGGAAAAATCCAGCAACTCAGGCGCAACAATATCACTCTTCTCGATCGTTCAAGATTCA AGAAGTGAACGAAAGACATCCAGGCTCCCCCCCCCTCCCATTCTGGCCTTTATAAATAAGAAAGACACCCATTGTCAAGGGAGGC ACATGGGTCTTTGGCTCAGTCTGCTTTTAGGAGTGAAAAATAAAAAAAAAAGGACTCTATTCCAGTCGCCTCGCTGAAAAACCGGA GGCCTTCAGCCACAGTTGCGATCACGTGCACCCGATGCTGACTAATGACAGATGCCGCTGGGAAGAGAAGGTTTAGATGCATGCC ACAAGAACAACAGCTGCGTTCTCTAGAGACTGTTGGAGCGTGGAGGGTACACGTTGTCAACAAGCTGCTACATGCAGGACGACG GCTCTTGTATCGTCTTTGGGTTGGCCAGATGGCAGACACTGTACACACTTGCAAGGATGCGAATAGAAACGTATATGTACAGACTC GCAGCTGGATGGTTTGAGGTTACATGAACACATGGATATATTCATGAATATATTTATTTATAACTACGTGCATATGTAGATTTCTCC CTCTCGAGCAGCATTCTATTAAGCTGGAGCACCTTACTTGTATGTAACAAGACGCTTTTTTTCTGTTCCTAGTTAGTTATCTTTCGCA TCAATTTTCCTTTTGACCGATAAAATCTCTTGGGTTTCTTAGATTAGACAATTGATGCGATCAAAGAAACCCAGTGAAATAATATGT GTATGATTCGGTGCAAAACGTATTGGAAACATTAATGAAGTTGGTGGAGC
Sp_XI-1	CCTTCCGCTCGCGTTCGTGGCCACTCCCCGCACAGGGTAGTGCATCACCATCTAAACATTGTCAACAGCTTGGTCCATAGTTCTAG CGCGGCGTCTTCTCAGGTTCCGGCTTCGACCTAGCCAGAATGTGCAGCAGAGTAAATAATATTCACTTCCCTGTTCCTAAAACGTA CAATATACAACACCAACCTCATATCAACTACCAGAAACAGTATCATACGTAATAGTTATTTTTTATTTTTTTAATTTTTTTTTTTTTTG CCACTGACCAGTCTTCGCGGCGGTGTGAGTTTCCGTCTGTACGCAGCATTTAGCAGAGATTTGCCAATGCCAAGAAACTCCACTAA ACTAACATCGCATTGCAATGGGCTTGGTATTCCGTGGAAGAAAATGAAAACCGGCGCAAAGAATCCTCCGAGAAAGTTTGATGC GGTGCACGGATTTCAGCACGGATTTTATTTTTTTTGCACCCCCCCCAATTTCTCGGCGATGATAAACATACTGCATCTAAAACCGTAT ACAGGAGGCTACGGTACAGAAGCCAAGATTAACAAAAAAAAAAAAAAAGAAAAAAAAAAGAAAAAAGAAAAATGTCAAGTGTT ATTCCTTGTCCCCTCTGACCTACGTAGTTACTTGCGTGGCTTCAAATTTAGATATCAGCGCTCGAGACGTCCTGATCTCTATACCTTA TTGCTTGCTTTAGGTCGAGGCGATAAAAAAATAATTACATCTTCGGTTCTTTCCCCCTTTTTATATATCTGCAACAATATTAAACAA TTAAATTAAAGAAGCACGTTTCCGTTTTTTATCATATACCATTCCCCTAACCGCCGAACTCACCGCATCACAACGTCACTATTCCTT CACACAAATAAACTAATTAGTTATGCTTCAGCGAATATCAACTAAACTGCACAGGCGGTTCTTATCTGGCCTGC

Sp_XI-2	CCTGTGCCCTTTCTATTTTTATGGTTGAAAAGGTTACAGAGGATCCTCTGGAGCGGTGACAACAGTTGACGCAATCATGGTCCCTG GCGGTCCCTAAGGATATCTCGGAGGAACCGAGGCACGGAAACGGCTGAACGGGTCCGCCAGATAAGGATAATTCAGCCGTCACG CATGAACAGAAGGATGAGCATTTGCTCACATTCCTAGACTTAACTTACATGAATGAAAAGAAGAACACCAATGTTGGAGTAGGG CTTCGCACCAAACTTTAATCAAGAAATCCGAGGAGAAATGATTCTAAGTCTAGCCAAGAAAACTGCAAAGCGCGCCAAAAGTAA GAAAACGACAAAGTTTTTCTAGCAATTTTTTTTTTTCATCAACAGTAGAAACTAGATTTATATAACTGTTACCGCTAAAACCTACAT CTAAACTTTTTAATACCTGAAAGCACTAGTCGTGTGTACCCCCCTCAACTGATCAACTGATCAGCGAGCTTTGCTTGTGGAAGTTC ATGGCAGACGCTCCTTGGCTTTTGCCTAGTCTTGTTCTATGGCACATTTTTCTGTTGAGTTATACCCCGCGTGCTAAAAAAGGGGTG AACCATCAGAAAGCTTATTACTGAGTTTTGCGGAGCATCGCTCGGAGCGGCGGAATTGAATCGAACCGCCGTGCTATTACCGAAC AAAAAAATTCGAAAGCATAAACTCAGTAGTGAAAAACTTGAGAATTTTCAGATGAGTGGCGACTTTCCAGTCCTTGCGGTTTTGTC ACCTTAGTCAGCTAGTAAGGAGGCCGTGTGGGTTAGAGTGGCTACAATGCTCAAAGGGCACTTCTAGAACCCACGGTAAATTTTTT TTGGCATGATAAATCGGTAGAATCGGTGAAGTAATTACCCAAAAAAGGATCGGGATTGTGTTTCTCGTAATTCCGTATTATTGCCG ATGGCATCGACTACTTCTTTTTTCAGAAACCCCAACAAGGGTC

List of primers used in this study

Table S2. List of primers used in this study. USER™ overhangs are underlined and KOZAK sequences are shown in italics/bold letters

Primer_ID	Sequence	Description
CP0001	ATTAAGTCCUCAGCGAGCTCG	Used to amplify biobrick bCP001
CP0002	ATTAATGCCUCAGCACTAGTCCTG	Used to amplify biobrick bCP001
CP0003	AGACCTCAGUGCGGCCGCAAATTTA AATAAAATGAAG	Used to amplify biobrick bCP002
CP0004	ATTAAACCCUCAGCGCGG	Used to amplify biobrick bCP002
CP0017	AGGGTTTAAUCCTTCCGCTCGCGTTC	Used to amplify biobrick bCP009
CP0018	AGGACTTAAUCATCAAACTTTCTCG GAGGATTCTTTG	Used to amplify biobrick bCP009
CP0019	AGGCATTAAUCGGTGCACGGATTTC AG	Used to amplify biobrick bCP010
CP0020	ACTGAGGTCUGCAGGCCAGATAAG AACCG	Used to amplify biobrick bCP010
CP0021	AGGGTTTAAUGGTTACAGAGGATCC TCTGGAG	Used to amplify biobrick bCP011
CP0022	AGGACTTAAUCTCGCTGATCAGTTG ATCAGTTG	Used to amplify biobrick bCP011
CP0023	AGGCATTAAUCTTTGCTTGTGGAAGT TCATGGC	Used to amplify biobrick bCP012
CP0024	ACTGAGGTCUGACCCTTGTTGGGGTT TCTG	Used to amplify biobrick bCP012
CP0071	CAGCGTTTCTGGGTGAGC	Used for sequencing of the assembled plasmids
CP0072	GGGGATTTAAATGCGGCCG	Used for sequencing of the assembled plasmids
CP0073	GATCGCACGCATTCCGT	Used for sequencing of the assembled plasmids
CP0074	ACGGAATGCGTGCGATC	Used for sequencing of the assembled plasmids
CP0075	CAGTTATGAGGGTACTGTCGTTCC	Used for sequencing of the assembled plasmids
CP0076	CATCATCGAATTCCAGCTGACC	Used for sequencing of the assembled plasmids
CP0077	ACCTCCAGACCTCAGUGC	Used for sequencing of the assembled plasmids
CP0078	CAGAATCAGGGGATAACGCAG	Used for sequencing of the assembled plasmids
CP0079	TCCCGTCAAGTCAGCGT	Used for sequencing of the assembled plasmids

CP0094	ACCTGCACUTTTTAGGCTGGTATCTTGATTCTAAATCG	Used to amplify biobrick bCP043
CP0095	ATGACAGAUTTGTTTTATATTTGTTGTAAAAAGTAGATAATTACTTCCTTG	Used to amplify biobrick bCP043
CP0096	ATCTGTCAUAAAACAATGAAAAATATCATTTCATTGGTAAGCAAGAAG	Used to amplify biobrick bCP044
CP0097	CACGCGAUTTAACATTTATCAGCTGCATTTAATTCTCGC	Used to amplify biobrick bCP044
CP0096_b	CGTGCGAUTTAACATTTATCAGCTGCATTTAATTCTCGC	Used to amplify biobrick bCP049
CP0097_b	AGTGCAGGU***AAAACA***ATGAAAAATATCATTTCATTGGTAAGCAAGAAG	Used to amplify biobrick bCP049
CP0098	CGTGCGAUCACCCATGAACCACACGG	Used to amplify biobrick bCP045
CP0099	ATGACAGAUTTATTGATATAGTGTTTAAGCGAATGACAGAAGA	Used to amplify biobrick bCP045
CP0100	ATCTGTCAU***AAAACA***ATGACTATTTCTTCTGCACATCCAGAG	Used to amplify biobrick bCP046
CP0101	CACGCGAUTCATTCGCTGATATATATTCTTCCTTCCC	Used to amplify biobrick bCP046
CP0108	CCCTATTTACACTTAGGGAAACCC	Used to verify the insertion in the Int. Site XI-1
CP0109	CAAGTCCGTTACGCTGCGG	Used to verify the insertion in the Int. Site XI-1
CP0110	GGAAGATTCCGCTCTACCAG	Used to verify the insertion in the Int. Site XI-2
CP0111	CGTATGAGGATTTTCGATGGAGC	Used to verify the insertion in the Int. Site XI-2
CP0113	CGTGCGAUATTCAGTTTTCTGACTGAGTTAAAAACTAATG	Used to amplify biobrick bCP051
CP0114	CGTGCGAUATTCAGTTTTCTGACTGAGTTAAAAACTAATG	Used to amplify biobrick bCP051

Biobricks used in this study.

Table S3. List of biobricks used in this study.

Biobrick_ID	Expected length	Description
bCP001	2160	Part of [1] plasmids amplified using primers CP0001 and CP0002
bCP002	2800	Part of [1] plasmids amplified using primers CP0003 and CP0004
bCP009	450	Amplification of *S. pastorianus* XI_I-UP integration site from gDNA_LG232 using primers CP0017 and CP0018 for USER™ cloning
bCP010	526	Amplification of *S. pastorianus* XI_I-DW integration site from gDNA_LG232 using primers CP0019 and CP0020 for USER™ cloning
bCP011	482	Amplification of *S. pastorianus* XI_II-UP integration site from gDNA_LG232 using primers CP0021 and CP0022 for USER™ cloning
bCP012	511	Amplification of *S. pastorianus* XI_II-DW integration site from gDNA_LG232 using primers CP0023 and CP0024 for USER™ cloning
bCP043	2000	Amplification of the double promoter P_{PGI1}-P_{PGK1} from plasmid pCfB3479 using primers CP0094 and CP0095 for USER™ cloning
bCP044	1800	Amplification of Seq19 using primers CP0096 and CP0097 for USER™ cloning
bCP045	430	Amplification of promoter P_{CCW12} from plasmid Ant_E113 using primers CP0098 and CP0099 for USER™ cloning
bCP046	1800	Amplification of Seq15 using primers CP0100 and CP0101 for USER™ cloning
bCP049	1800	Amplification of Seq19 using primers CP0096_b and CP0097_b for USER™ cloning
bCP051	1016	Amplification of promoter PPGI1 using primers CP0113 and CP0114 for USER™ cloning

List of plasmids used in this work.

Table S4. List of plasmids used in this study.

Plasmid_ID	Description	Reference
Ant_E113	Used for the amplification of various biobricks	Plasmid kindly provided by Helen Olsson from DTU-Biosustain
pCfB3479	Used for the amplification of various biobricks	Plasmid kindly provided by Irina Borodina from DTU-Biosustain
pX-2	Used for the amplification of various biobricks	[1]
pCP0005	Integrative plasmid, X3-DR-KlURA3	This study
pCP0011	Integrative plasmid, XI_1-DR-KlURA3	This study
pCP0015	Integrative plasmid, XI_2-DR-KlURA3	This study
pCP0032	Integrative plasmid, XI_1, DR-KlURA3, bCP045 (P_{CCW12} ->), bCP046 (*IMA1* ->)	This study
pCP0044	Integrative plasmid, XI_1, DR-KlURA3, bCP049 (*AGT1* <-), bCP043 (<- P_{PGI1}-P_{PGK1}->),bCP046 (*IMA1* ->)	This study
pCP0047	Integrative plasmid, XI_2, DR-KlURA3, b_CP049 (*AGT1*<-), bCP043 (<- P_{PGI1}-P_{PGK1}->),bCP046 (*IMA1* ->)	This study
pCP0066	Integrative plasmid, X3, DR-KlURA3, bCP051 (P_{PGI1}->), bCP044 (*AGT1* ->)	This study

Synthetic gene sequences

Table S5. Nucleotide sequences of the synthetic isomaltase and maltose transporter genes

Gene	Sequence
IMA1	ATGACTATTTCTTCTGCACATCCAGAGGCAGAACCAAAGTGGTGGAAAGAGGCCACGTTCTATCAAATTTACCCAGCAAGTTTCA AAGACTCTAATGACGATGGCTGGGGTGATATGAAGGGTATTTCCTCCAAGTTGGAGTATATCAAGGAGCTTGGTGCCGATGCCAT TTGGATCTCACCATTCTACGACTCGCCACAAGATGATATGGGTTACGATATTGCCAACTACGAAAAGGTCTGGCCAACATATGGT ACGAATGAAGACTGCTTTGCCTTGATCGAAAAGACACATAAGCTTGGTATGAAATTCATCACCGACTTGGTCATCAATCACTGTTC CAGCGAACATGAATGGCTCAAAGAGAGCAGATCCTCGAAGACCAATCCGAAGCGTGACTGGTTCTTCTGGAGACCTCCTAAGGG TTATGACGCCGAAGGCAAGCCAATTCCTCCAAATAATTGGAAGTCCTATTTTGGTGGTTCCGCATGGATCTTCGATGAAAAGACAC AAGAATTCTACTTGCGTTTGTTTTGCTCCACTCAACCTGATTTGAATTGGGAGAATGAAGACTGTAGAAAGGCAATCTACGAAAGT GCCGTTGGATACTGGTTAGACCATGGTGTAGACGGCTTTAGAATTGATGTCGGAAGTTTGTACTCCAAAGTTGTAGGTTTACCAGA TGCCCCTGTTGTTGACAAAAACTCGACTTGGCAATCCAGTGATCCATACACATTGAATGGACCACGTATTCACGAGTTCCATCAAG AAATGAATCAATTCATCAGAAACAGAGTGAAGGATGGCAGGGAGATTATGACAGTCGGTGAAATGCAACATGCTTCCGACGAAA CTAAGAAACTTTATACGAGTGCTTCAAGACACGAACTTAGTGAGTTATTTAACTTTTCCCACACTGATGTGGGGACTTCACCTTTGT TCCGTTACAACTTGGTCCCATTTGAACTGAAGGATTGGAAGATTGCCCTTGCTGAGCTGTTCAGGTTCATTAATGGTACAGATTGTT GGTCAACAATCTATCTGGAAAATCACGACCAACCTCGTTCAATTACGAGATTTGGTGACGATTCTCCTAAGAACCGTGTTATTTCT GGTAAGTTACTCTCTGTGTTGCTAAGTGCCTTGACCGGTACTCTATATGTGTATCAGGGACAAGAGCTTGGCCAAATCAATTTCAA GAACTGGCCTGTTGAAAAGTACGAGGATGTCGAAATCAGAAACAACTACAATGCAATTAAAGAAGAGCATGGGGAAAACTCAG AGGAGATGAAAAAGTTTTTAGAAGCCATTGCCCTTATCTCCAGGGACCATGCTAGAACACCTATGCAATGGTCTCGTGAGGAGCC AAATGCTGGTTTTTCTGGTCCTAGTGCTAAACCATGGTTTTACTTGAACGACTCTTTCAGAGAAGGCATTAACGTCGAAGATGAAA TCAAGGATCCCAACTCGGTTTTGAACTTCTGGAAGGAGGCCTTGAAGTTTAGAAAGGCGCATAAAGACATTACTGTGTACGGATA CGATTTCGAGTTTATTGATTTAGACAATAAGAAGTTGTTTAGCTTCACAAAGAAGTACAACAATAAAACATTGTTTGCGGCTTTGA ACTTTAGCTCTGATGCGACAGATTTCAAGATTCCAAATGATGATTCATCGTTCAAGTTAGAGTTTGGAAACTATCCAAAGAAGGAG GTAGATGCCTCTTCCAGAACATTGAAGCCATGGGAAGGAAGAATATATATCAGCGAATGA

AGT1

ATGAAAAATATCATTTCATTGGTAAGCAAGAAGAAGGCTGCCTCAAAAAATGAGGATAAAAACATTTCTGAGTCTTCAAGAGAT
ATTGTAAACCAACAGGAGGTTTTCAATACTGAAAATTTTGAAGAAGGGAGAAAGGATAGTGCCTTTGAGCTAGACCACTTAGAGT
TCACCATCAATTCAGCCCAGTTAGGAGATTCTGACGAAGATAACGAGAATGTGATTAATGAGACGAACACTACTGATGATGCAA
ATGAAGCTAACAGCGAGGAAAAAAGCATGACTTTAAAGCAGGCGTTGCTAATATATCCAAAAGCAGCCCTGTGGTCCATATTAG
TGTCTACTACCCTGGTTATGGAAGGTTATGATACCGCACTACTGAACGCACTGTATGCCCTGCCAGTTTTTCAGAGAAAATTCGGT
ACTTTGAACGGGGAGGGTTCTTACGAAATTACTTCCCAATGGCAGATTGGTTTAAACATGTGTGTCCAATGTGGTGAGATAATTGG
TTTGCAAATCACGCCTTATATGGTTGAATTTATGGGGAATCGTTATACGATGATTACAGCACTTGGTTTGTTAACTGCTTATGTCTTT
ATCCTCTACTACTGTAAAAGTTTAGCTATGATTGCTGTGGGACAAGTTCTCTCAGCTATGCCATGGGGTTGTTTCCAGGGTTTGACT
GTTACTTATGCTTCGGAAGTTTGCCCTTTAGCATTAAGATATTATATGACCAGTTACTCCAACATTTGTTGGTTATTTGGTCAAATCT
TCGCCTCTGGTATTATGAAAAACTCACAAGAGAATTTAGGGAACTCTGACTTGGGCTATAAATTGCCATTTGCTTTACAATGGATT
TGGCCTGCTCCTTTAATGATCGGTATCTTTTTCGCTCCTGAGTCGCCCTGGTGGTTGGTGAGAAAGGATAGGGTCGCTGAGGCAAG
AAAATCTTTAAGCAGAATTTTGAGTGGTAAAGGCGCCGAGAAGGACATTCAAATTGATCTTACTTTAAAGCAGATTGAATTGACT
ATTGAAAAAGAAAGACTTTTAGCATCTAAATCAGGATCATTCCTTGATTGTTTCAAGGGAGTTAATGGAAGAAGAACGAGACTTG
CATGTTTAACTTGGGTAGCTCAAAATACTAGCGGTGCCTGTTTACTTGGTTACTCGACATATTTTTTTGAAAGAGCAGGTATGGCCA
CCGACAAGGCGTTTACTTTTTCTGTAATTCAGTACTGTCTTGGGTTAGCGGGTACACTTTGCTCCTGGGTAATATCTGGCCGTGTTG
GTAGATGGACAATACTGACCTATGGTCTTGCATTTCAAATGGTCTGCTTATTTATTATTGGTGGAATGGGTTTTGGTTCTGGAAGCG
GCGCTAGTAATGGTGCCGGTGGTTTATTGCTGGCTTTATCATTCTTTTACAATGCTGGTATCGGTGCAGTTGTTTACTGTATCGTAAC
TGAAATTCCATCAGCGGAGTTGAGAACTAAGACTATAGTGCTGGCCCGTATTTGCTACAATATCATGGCCGTTATCAACGCTATAT
TAACGCCCTATATGCTAAACGTGAGCGATTGGAACTGGGGTGCCAAAACTGGTCTATACTGGGGTGGTTTCACAGCAGTCACTTTA
GCTTGGGTCATCATCGATCTGCCTGAGACAAGTGGTAGAACCTTCAGTGAAATTAATGAACTTTTCAACCAAGGGGTTCCTGCCAG
AAAATTTGCATCTACTGTGGTTGATCCATTCGGAAAGGGAAAAACTCAACATGATTCGCTAGATGATGAGAGTATCAGTCAGTCC
TCAAGCATAAAACAGCGAGAATTAAATGCAGCTGATAAATGTTAA

Estimation of the growth parameters

The growth parameters were estimated using the growth parametric model Baranyi and Roberts[2]. The integrated format of the two differential equations in which this model is based on can be written as:

$$lnN = \ln N0 + \mu_{max} \cdot A - \ln(1 + \frac{\exp(\mu_{max}A)-1}{\exp\ (lnN_{max}-\ln N_0)})$$

Where N is the cell number at time t, N_0 is the initial cell number, and μ_{max} is the maximum growth rate.

$$A = t + \left(\frac{1}{\mu_{max}}\right) \cdot \ln(\frac{\exp(\mu_{max} \cdot t) + q_0}{1 + q_0})$$

Where q represents the physiological state of the cell population, and q_0 represents the initial state of the population. The lag phase was estimated as follows:

$$lag\ time = \frac{q_0}{\mu_{max}}$$

All the calculations were performed in R Studio version 1.4.1106 (RStudio Inc., Boston, MA, USA)[3] software using the package growthrates v0.8.2[4]. We utilized the first 120 h of the data points to feed the model. Only the data with a fitness to the model with r^2>0.9 were considered to estimate the growth parameters.

Table S6. Growth parameters

Carbon source	strain	μ (h-1)	Carrying capacity	Lag Time (h)	r2
	LG160	0,057	0,318	0,02	0,967
	LG160	0,049	0,335	0,02	0,901
	LG160	0,061	0,354	0,02	0,931
	LG230	0,040	0,322	0,03	0,904
	sCP034	0,067	0,287	0,01	0,935
	sCP034	0,034	0,331	0,03	0,955
	sCP047	0,033	0,314	0,03	0,953
glucose	sCP101	0,087	0,432	8,23	0,995
	sCP101	0,108	0,456	14,41	0,992
	sCP101	0,128	0,469	15,02	0,989
	sCP101	0,110	0,490	11,29	0,984
	sCP107	0,082	0,499	6,39	0,994
	sCP107	0,114	0,474	11,70	0,991
	sCP107	0,109	0,530	12,08	0,990
	sCP107	0,140	0,503	13,75	0,993
	LG160	0,194	0,322	25,20	0,968
isomaltose	LG160	0,109	0,325	20,56	0,978
	LG160	0,067	0,335	21,74	0,973

	LG160	0,050	0,359	16,34	0,968
	LG230	0,083	0,270	15,54	0,939
	LG230	0,030	0,304	3,73	0,951
	sCP034	0,035	0,299	25,79	0,956
	sCP041	0,077	0,558	13,63	0,996
	sCP041	0,066	0,534	16,73	0,996
	sCP041	0,092	0,580	17,38	0,997
	sCP041	0,062	0,571	16,25	0,997
	sCP047	0,026	0,567	0,04	0,980
	sCP047	0,017	0,636	0,06	0,969
	sCP047	0,016	0,802	0,06	0,993
	sCP101	0,049	0,419	14,35	0,991
	sCP101	0,062	0,415	29,29	0,978
	sCP101	0,047	0,460	6,74	0,994
	sCP107	0,214	0,312	16,78	0,946
	sCP107	0,162	0,325	20,15	0,973
	sCP107	0,084	0,318	6,00	0,954
panose	sCP034	0,075	0,325	18,40	0,901
	sCP034	0,064	0,298	9,29	0,904
	sCP041	0,028	0,475	0,04	0,980
	sCP041	0,048	0,613	19,56	0,985
	sCP041	0,030	0,434	0,03	0,930
	sCP041	0,020	0,517	0,05	0,962
	sCP047	0,198	0,445	14,03	0,973
	sCP047	0,164	0,494	12,92	0,985
	sCP101	0,040	0,325	0,02	0,988
	sCP101	0,095	0,308	12,47	0,916
	sCP101	0,050	0,341	0,02	0,978
	sCP101	0,048	0,360	0,02	0,972

Summary of the genome assemblies

Table S7. Summary of the statistics of the genome assemblies.

Strain	**LG160**	**LG230**
Source of the reads	Paired-end reads from Illumina HiSeq	Paired-end reads from Illumina HiSeq and long reads from PacBio
# contigs	466	413
# contigs (>= 0 bp)	3030	413
# contigs (>= 1000 bp)	374	413
# contigs (>= 5000 bp)	264	413
# contigs (>= 10000 bp)	238	394
# contigs (>= 25000 bp)	194	212
# contigs (>= 50000 bp)	136	141
Largest contig	473310	546161
Total length	22263347	26883767
Total length (>= 0 bp)	22668571	26883767
Total length (>= 1000 bp)	22196358	26883767
Total length (>= 5000 bp)	21963635	26883767
Total length (>= 10000 bp)	21775258	26720695
Total length (>= 25000 bp)	21071091	23499906
Total length (>= 50000 bp)	18882533	21003994
N50	138865	140211
N75	71167	62600
L50	45	55
L75	99	126
GC (%)	39.1	38.5
Mismatches		
# N's	8207	0
# N's per 100 kbp	36.86	0

Phylogenetic tree of the several isomaltases

The nucleotide sequences of *IMA1* and *AGT1* used as a query in this study are part of the list of the upregulated genes reported by [5] First, to verify endogenous gene copies of the genes *IMA1* and *AGT1*, we performed a BLAST analysis against the genomes of the model lager yeast LG160 and LG230, and two *S. eubayanus* strains CBS12357 (assembly: ASM332760v1[6]), and FM1318 (assembly: SEUB3.0[7]). From BLAST results, matching nucleotide sequences carrying truncated domains, incomplete TIM barrel´s amino acid (aa) sequence, or holding premature stop codons, were excluded for further analysis. Then, using MAFFT[8] software, we employed a multiple sequence alignment MSA, in which, besides the isomaltases found in the model lager yeast, we included the isomaltases identified in the two available genomes of *S. eubayanus*, five isomaltases (*IMA1-5*), *MAL32* and *MAL12* from *S. cerevisiae* S288c[9], and *IMA1* from nine different *S. cerevisiae* strains. Then, an alignment curation using trimAI[10] software was performed, followed by the construction of phylogenetic trees using PhyML[11] and SMS[12] software, see ***Figure S2***.

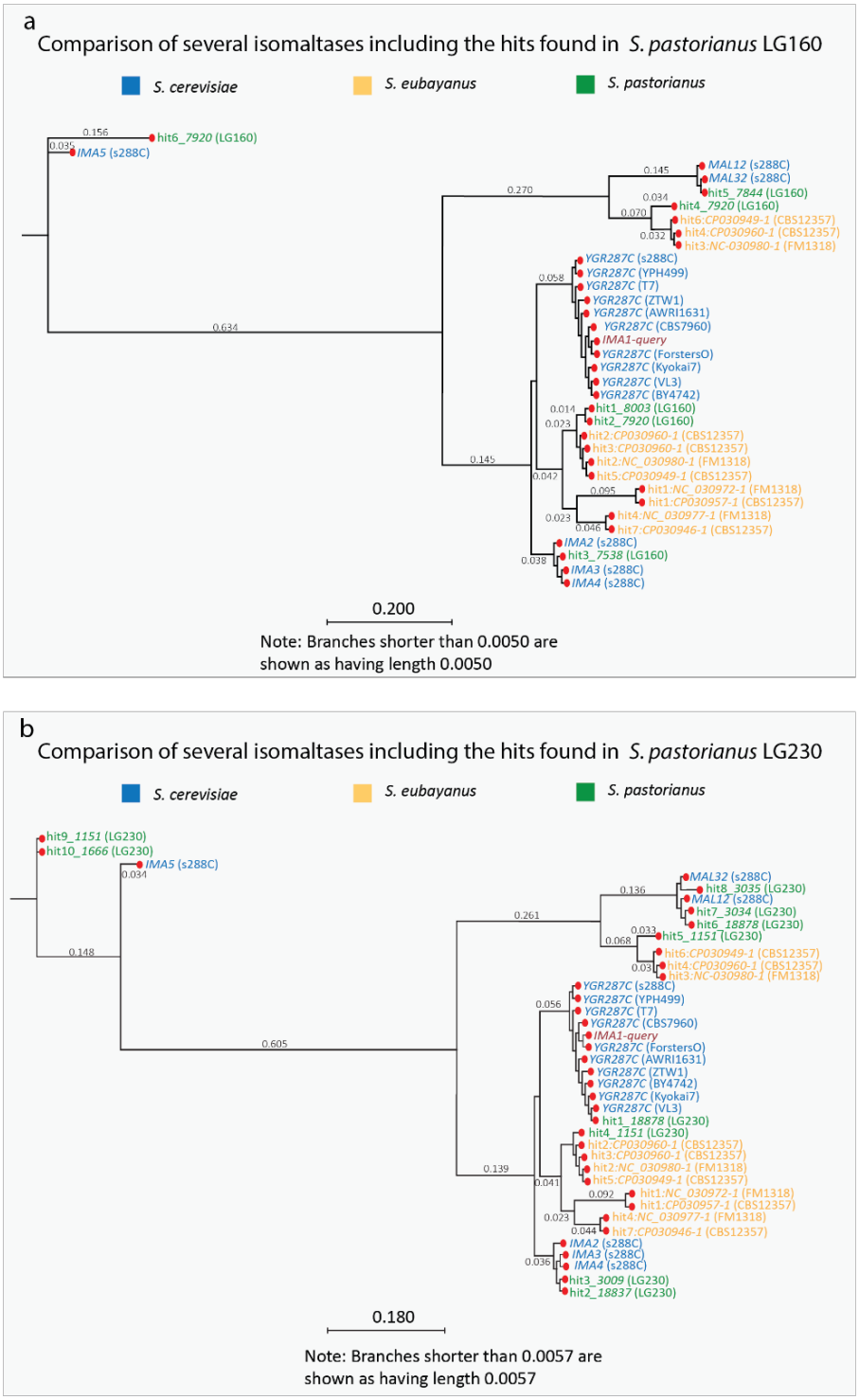

Figure S2. Neighbor joining phylogenetic trees of several isomaltases. The phylogenetic three was build using PhyML[11] and SMS[12]. The bootstrap values are indicated on the branches. IMA-query indicates the query sequence used in this study. In green are highlight the hits obtained for (**a**) *S. pastorianus* LG160 and (**b**) *S. pastorianus* LG230.

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