

Evaluation of long-term fermentation performance with engineered *Saccharomyces cerevisiae* strains

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SUPPLEMENTARY FIGURES

CONSTRUCT linear DNA ADH6

DEFINITION ADH6

LENGTH 4200

FEATURES Location/Qualifiers

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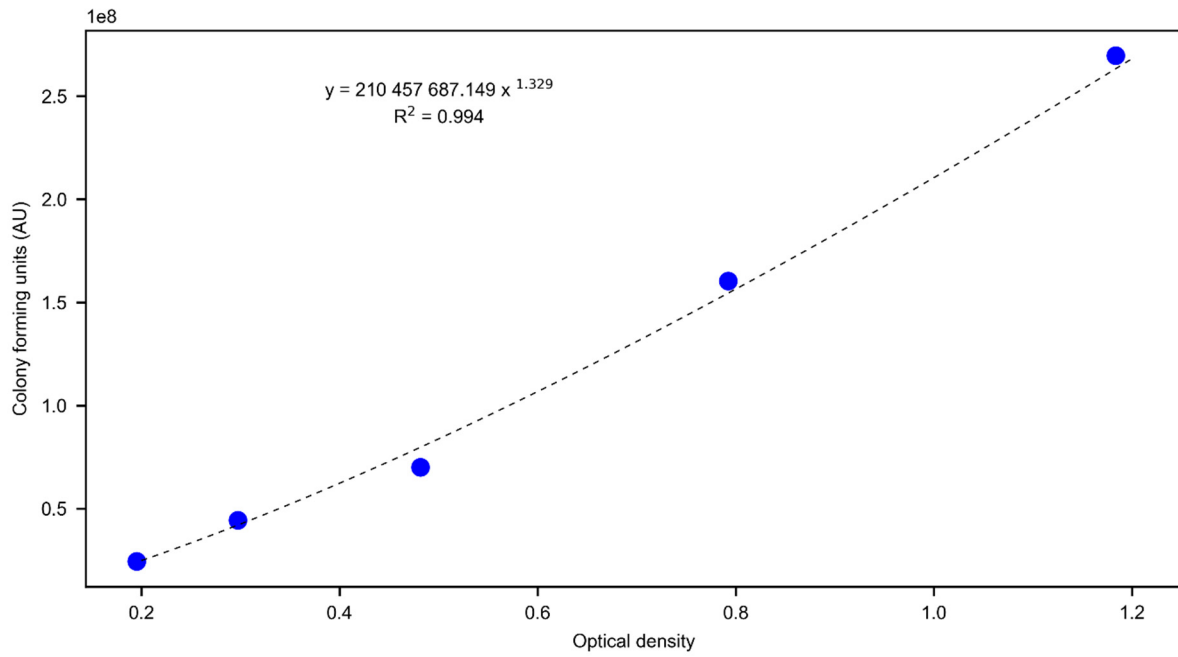
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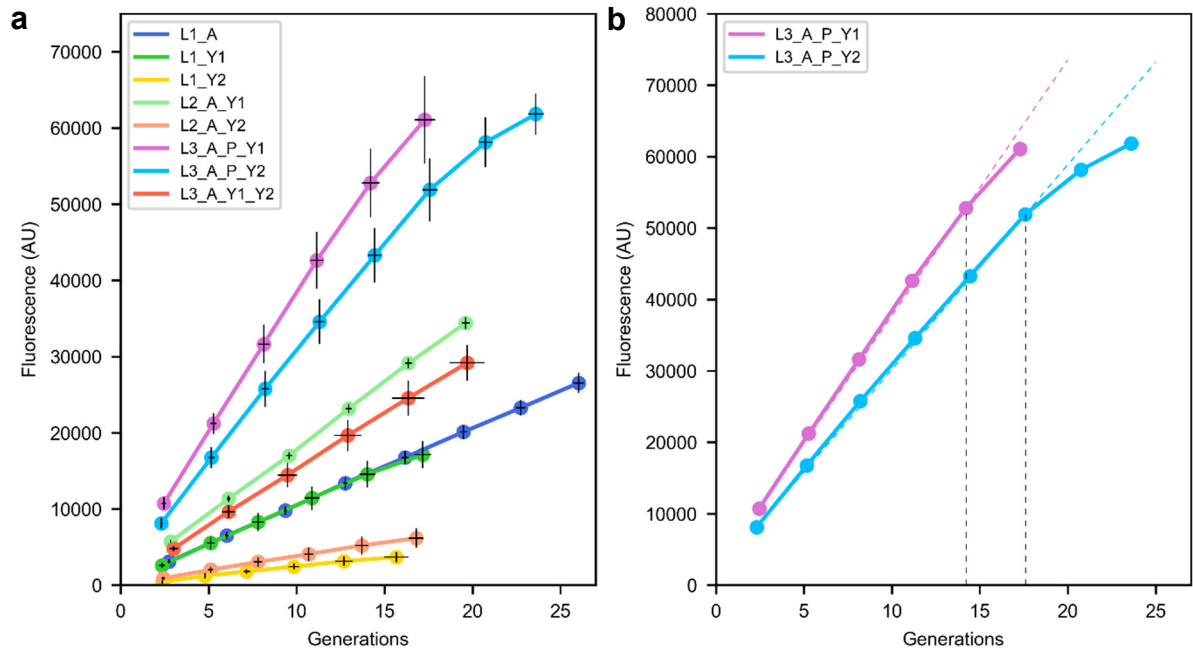
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Supplementary Figure S1 Annotated Genbank file of the integration cassette for the genomic modification of baker's yeast at the *ADH6* locus. Similar integration cassettes for other genomic locations were made, for which the homologous regions of *ADH6* were replaced by the once of the corresponding integration locus (sequence given in Supplementary Table S2). The selection marker present between the LoxP sites for the integration at other genomic locations, was *LEU2* instead of *HIS5*.



Supplementary Figure S2. Correlation between the optical density (OD) at wavelength 600 nm and the colony forming units (CFU) of the wild-type yeast used in this study. As for some samples the OD was above 1.0, an exponential standard curve was established according to the spectrophotometer's specifics.



Supplementary Figure S3. Cumulative yECFP output profiles of the first sequential batch experiment. (a) Profile for each strain examined. The error bars represent the standard deviation. (b) Cumulative yECFP output for strains L3_A_P_Y1 and L3_A_P_Y2 with their estimated maximal production capacity (dotted colour lines). The difference of output between these maximal production capacities and their respective actual output indicates potential product loss (PPL). The black dotted lines represent the G_{ST} points on which the maximal production capacity is calculated.

SUPPLEMENTARY TABLES

Supplementary Table S1. Homologous regions for genomic integration used in this study.

Genomic locus	Sequence
<i>ADH6</i>	<p>Upstream</p> <p>GTAGCGATCGACCTTAGAACTTTTATTTAGTTTGTACATATACCTCACCTGAGTTTT GCTTTTTTCTCTGGGAGCCTAAACCATTAAAAATGATATATAATAGATAATAAATCCAGG ATAAAATGTGGCTAATTGATCTTTTTTCATTTCAACTTGGTAATGACGTACTGGATACT TTCGACGCTCTTTTTTAGTCCCCGATCCCCGCTCTCCAGGACCTTGACGTGGAATTCCGA TCACAGCCACTCTCGTCACGGCTCCGTTAAAATGAATGGTTTTCCGTTACATTTACTGGT CTTTTTATCTTTTTACAGTAAATGGGTGATATACTGTGACACAATTTGTGTCTCTACTGT GTGAACTTCCATTGCTGACTAAAGATTCCCCGCTCCGCTTATATGTCCGGTCCGCTCTTG ACCGAAGATCACATTGCCAATTTTTACATCTGGAAGCGATACGACAATATAGGAGAAAA AGAAAAGTGAAAGGCAAAAAAGCACCAACAGTTCTCGAGGTGAAGTGCCGTCAATCTTCT GTATAAATTCGGCCAATTCAATCTAATTTAATAGATTTGCGACAGACTTTCACATCCACA TTCGAGGAAGAAATTCAACACAACAACAAGAAAAGCCAAAATC</p> <p>Downstream</p> <p>GTTGTCAAGCTCTTGATAAATGTAGCTCCTTTCTTTTAACTGCTCCATGTTTTGGGTCT GTATATAGGAGTGCTGTTTTAATCGATATAGGTTACATTTGAAACTTTTTTTATGATTA TAAGGTACTATTTAAATATTTACAACCTCGTACAGTTCTCTTTGTTTTTTCTCTTTTTCT CCTTGCTAAGCACCTTTAAGGTGAAAAGAAACACATATCAATACACTAACAAAAAAGGTG CACGTTTATAGGGTATCGGACAATACTATATTACTAAGCTACCAAAGCAATAGCGCCAA TAACAAAAGCTTTCATGTTAATGTTTCAGGGATGCACCGGTATTTCTTTTGCCGTGCTGA CAGTATATGTTGTGCTTTGTTTAGCGGATGAGGATGTTCCATGAATCGCTGCCGTAGGTT TGGTGGTAGATCTGTTTACCTTTTCAGTGGATGAACTTAGTTGTGATGTTAATAGCGACT TTTGCTCGAGACAATTATCGTTTCAAGGGTAGTTTACCCCTCCCGATCGGACTTTTCAG TTGAAGATTTGCAACAGTTGATGTGTTTTTCGCTTAGTGTTGATACTCCTTCTGAAT TTTTAGTTGACTTGCTTTTTGCTCCCTC</p>
<i>YGLC3</i>	<p>Upstream</p> <p>GTATGCGGTGTTTCCTCTGTCCATTAAAAGCAAGTGTCTTTTCTTTAGAGTCTCGGATTA TACTATCTTGGAAGTGAGTTTTTAAAGAACCAGAATGGCAGGACACTGGAATACAGATTG GCTTTATATCGTTACTGCCGAGGGAAGACTCTCATAATATCTAAATTTTCTGTTCCGATT CTCTTACTTGCAAAATATTTTACACTCTGTGTCAGGAATAATCAGTAATTGCCTCATTGAATT ATCTTAGGTAGTTCTTTCAACTGCTATTGTATTTCAATCTTGGGGACTACAAAACGTT GTAGCAATTGTTGACGATGGTTCCTACCTGAAATATGGCCTTAAGAATTTACCGCGAGCA ACGGGAGGGATAATGTCCAACACGGCTTTTTATTATTATTTTCCGAACCTATAAAATC CATCAGTATTCCTTCAAGAGTTTTTCCGCTCTCTGGCTGAAGGCTCATTTCCATGATGGG GTCACAATTATTATCGCACACAATTTTGGTGGCGTTGAAATTGATGCCGGAATTTGCCCC AGTGCTATAGGTGTTTTACTTAACACATAATTTCTGAACCCATAATAAATGACACGTAAA</p>

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TGTTGCTTGATTTGCCCTGTTTGTCTTATGTGTTGAAGAGAAATTAGAATATTATGTGGC
GTATCAATGCCAAAGTATT

Downstream

AAAGAGCTTAGCTGTCTTAGTTAGCTTCGCTTTTTATCATAACTACACCTCCTTTAGGTA
AAAGAGAAGTACCTCTAACTTACACTATTTCTTTCGTGGAAAGAAGCTGCACTAAAACAG
GTTTTAAAACAGACCGATTTATTTACCATAGGATACCTACATATTTTTGTCCCCTCTACA
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YIRCΔ6

Upstream

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AAAGTTTAATATCTTGTTTAGCACATAATTTTCGATGTACTTTTCTTTTAAATCGAAT
TATCAGCGATTATTCAG

Downstream

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CGATAAGCTTCGCTTTTCTCAACCAATATATTTACTAAAGATTAATGCTCTTACCGCAGA
TTACACTGCATCGCTTCAATTATTCCTGAGAAGTATTAATAAGTACGCAGAATGGTTTTA
GCCGTGTGTTCTTCTCATATTGATTAAAGTAGAGCTAATCAGTAATACTTTTCATACAGA
CAAGTGTCAGATAGAACCATCCAAGAATGAGTGGCTTAGTTAATTTTACAGCCGTGGTCA
ATACAATTAGAATATGGAGAATTTGCTTTGCTTACTGGTAGAAGATACGTAAGTAATCGC
TAATATTATTACCATATATAACGAGAGGTTTACGGAAGCGACATTGTTGATTTGAATCAT
TGCAGTACGGTCTAAGGCGGGAAGCTTAACCTCGGATTTGGTAGGTATAATATCGCAGA
TATTGTTTCATGTCCAACGCCGCGCACTTATCGT

PGM2

Upstream

TAGAGGACGTTCCCTGTATGTACTAAAATCATACTATCATCCGTTGAGGTCGAGCGGTTT
TCTAATCTCAACACACGCTGCTGCCCCTGAGCTTGCAGCTAAGGACAACAGGGCCAACT
TGTTCTTTCTAGGGGGGAGAGGCCCAACTTAGGCTCGGTGAAGAGAAGCGCGGAGCTCAC
GAAAAGGGGTGGTGTGCGCACTACTTTTTCTGTTGGGGAACCTACGTGAAAGGGGACGTT
ATACTTTTGTTATGTGCAGTTCCAGGAACAAACAGGGGTTGAAAAGTGTAAGAAATAG

TTGATTCTTTCAGAATCTGGATGACTGCCGCCCCGAACAATGTGGGAAATATAAAAAGGTA
CCGTTAATATTGTTTCATTTTCGAAGATAGTGTTCCATGTCTTCTTCAGCGTTGCTTGA
ACAATAGGATAATAAGAAGAAGATCAACCAATCTTCTCAGTAAAAAAGTAACAAAAGT
TAACATAAC

Downstream

AACGAATGATTTACTAATGGCTTAATGATTTTCACCTTTTCAATGAATATTAACGGTAA
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TATACAAATATACATATAGTAACATGCATTCTGGCGAATATCCA

Supplementary Table S2. Calculated relative expression strengths between strains (at generation 5). This data is visualised as a heatmap in Figure 1.

	L1_A	L1_Y1	L1_Y2	L2_A_Y1	L2_A_Y2	L3_A_P_Y1	L3_A_P_Y2	L3_A_Y1_Y2
L1_A	1,00	1,21	5,72	0,59	3,26	0,32	0,37	0,69
L1_Y1	0,83	1,00	4,74	0,48	2,70	0,26	0,30	0,57
L1_Y2	0,17	0,21	1,00	0,10	0,57	0,06	0,06	0,12
L2_A_Y1	1,71	2,06	9,78	1,00	5,57	0,54	0,63	1,18
L2_A_Y2	0,31	0,37	1,75	0,18	1,00	0,10	0,11	0,21
L3_A_P_Y1	3,15	3,81	18,05	1,85	10,28	1,00	1,16	2,17
L3_A_P_Y2	2,73	3,30	15,62	1,60	8,90	0,87	1,00	1,88
L3_A_Y1_Y2	1,45	1,75	8,31	0,85	4,74	0,46	0,53	1,00