Supplementary material to:

CRISPR-based multi-gene integration strategies to create *Saccharomyces cerevisiae* strains for consolidated bioprocessing

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Table S1: Primers used in the study.

Primer Name	Primer sequence (in 5'-3' direction)	T_A used	Application
CHX_ENO-L	GCAGTTATCTCTGTGTCCAGATCCCTTTGA AGTAAAGTTTATTCAATTTTCTTCTAGGCG GGTTATCTACTG	57°C	Amplification of the genes flanked by *ENO1* promoter and terminator to provide ChX homology 3'and 5' ends
CHX_ENO-R	CTACAGTAATTGTGCGGTGCAGGGAGGC AATGTTTAGTGCATCTCCTCCGTCGAACA ACGTTCTATTAGG		
ChrXI_ENO-L	TGTAAAACAGGTATTGGCTGCTTCATAGTA CACCCAATTGCTTCTAGGCGGGTTATCTACTG	55°C	Amplification of the genes flanked by *ENO1*promoter and terminator to provide CHXI homology 3'and 5' ends
ChrXI_ENO-R	GCAACTCTGAAATGTCAAAACGGTCGTGTATA AATAAATGCCGTCGAACAACGTTCTATTAGG		
ChrXII_ENO-L	GCGTCCTACAGCGTGATGAAAATTTCGCCTGC TGCAAGATCTTCTAGGCGGGTTATCTACTG	53°C	Amplification of the genes flanked by *ENO1* promoter and terminator to provide CHXII homology 3'and 5' ends
ChrXII_ENO-R	CTGTCAAACTTCTGAGTTGCCGCTGATGTGACA CTGTGACCCGTCGAACAACGTTCTATTAGG		
DELTA-ENO1-L	CTTAAGATGCTCTTCTTATTCTATTAAAAATAGA AAATGACTTCTAGGCGGGTTATCTACTG	53°C	Amplification of the genes flanked by *ENO1* promoter and terminator with DELTA homology 3'and 5' ends
DELTA-ENO1-R	GTTTGTTTGCGAAACCCTATGCTCTGTTGTTCGG ATTTGACGTCGAACAACGTTCTATTAGG		
Ch11_SEDp-L	TGTAAAACAGGTATTGGCTGCTTCATAGTACACC CAATTGATTGGATATAGAAAATTAACGTAAGGCAGTATC	53°C	Amplification of *A.a.bgl1* flanked by *SED1* promoter and *DIT1* terminator with CHXI homology 3'and 5' ends
CH11_DITt-R	GCAACTCTGAAATGTCAAAACGGTCGTGTATAAATAAAT GTTACTCCGCAACGCTTTTCTG		
ENO1-L	GTAACATCTCTCTTGTAATCCCTTATTCCTTCTAGC	57°C	Confirmation of the *eg2* cassette in transformants
ENO1-R	GCAACCCTATATAGAATCATAAAACATTCGTGA		
EGR-Rev	ATCTGGATTAGTAACTTGAGACAAAGCAG	59°C	To confirm presence of *eg2*, use with ENO1-L
CBH1R-Rev	TGTTGAGAGAAGTCGTCGGTGTCAC	59°C	To confirm presence of *cbh1*, use with ENO1-L
SED1p_check-L	GACAAGCAAAATAAAATACGTTCGCTC	60°C	To check transformants containing genes under SED1p and DIT1t
DIT1t_check-R			
Ch.10Check-L	GCAGTTATCTCTGTGTCCAGATCC	57°C	To confirm if genes were integrated into the correct CRISPR targeted site, use with ENO1-R
Ch.11Check-L	GCCTTCGATTTGACACATCTCTAAGC	55°C	
Ch.12Check-L	GCCATTGAGTCAAGTTAGGTCATCC	53°C	
DeltaCheck-L	CTGTTGGAATAAAAATCCACTATCGTC	53°C	
ALG9L	TGCATTTGCTGTGATTGTCA	60°C	qPCR primers for amplification of α-1,2-mannosyltransferase gene (ALG9) in the yeast genome as internal reference gene.
ALG9R	GCCAGATTCCTCACTTGCAT		
Eg2_L	TCTGCTGCTGCTTTGTCTCAAG	60°C	qPCR primers for amplification of *T.r.eg2*
Eg2_R	CTCAACCAAGTAGCCAATGGAG		
CBH_L	TCTAACAACGCTAACACTGGCA	60°C	qPCR primers for amplification of *T.e.cbh1*
CBH_R	TAAGTACCACCACAGTCATCGC		

Table S2: Gene integration target sites on different chromosomes

Chromosome sites	gRNA targeting sequence (5`-3`)
Chr. X	GTAGCTACAAGAACATATGG
Chr. XI	GCACCTCTAAAACTGCTCCG
Chr. XII	GTCACTGACAGCCACCGCAG
Delta	GGAATATTGGGTCAGATGAA

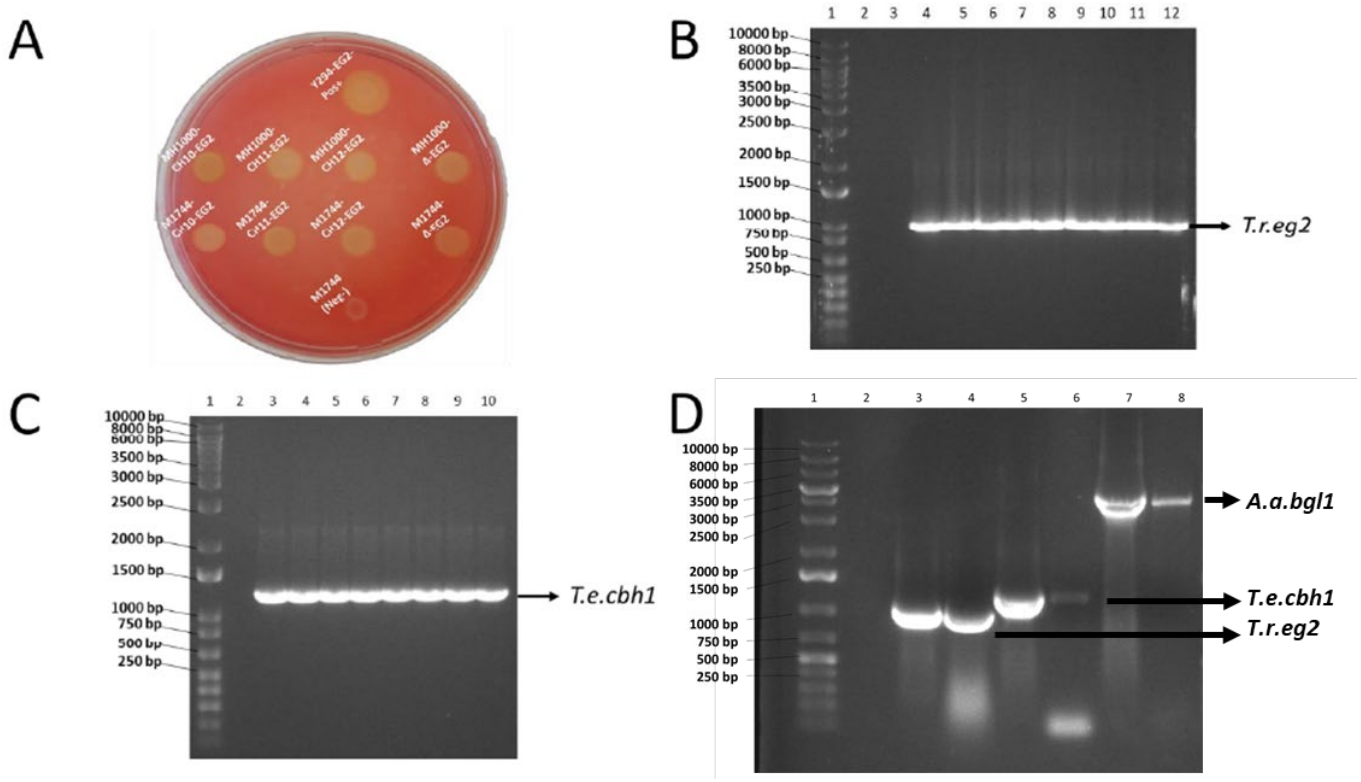

Figure S1: Confirmation of the cellulase genes integrated into haploid and diploid *S. cerevisiae* isolates. (a) Screening of *T.r.eg2* activity in EG2 yeast transformants on a 1% CMC agar plate. The plate was stained with 0.1% Congo Red, and the generated halos represented the EG2 active transformants. Y294 +pRDH147::fur1 and M1744 were used as positive and negative controls, respectively. This plate represents an example as several transformants for each integration locus was screened (b) Electrophoresis of *T.r.eg2* PCR products from CMC selected yeast transformants on a 1% agarose gel. Lane 1: 1kb Plus DNA Ladder (Invitrogen); Lane 2 and 3: M1744 and MH1000 (negative controls), respectively; Lane 4: Positive control (pRDH180); Lane 5 to 12: *T.r.eg2* yeast transformants. (c) Electrophoresis of *T.e.cbh1* PCR products from selected yeast transformants on a 1% agarose gel. Lane 1: 1kb Plus DNA Ladder (Invitrogen); Lane 2: M1744 (negative control); Lane 3: Positive control (pMI529); Lane 4 to 10: *T.e.cbh1* yeast transformants. (d) Electrophoresis of three distinct PCR products from the CBP MH1000 strains on a 1% agarose gel. Lane 1: 1kb Plus DNA Ladder (Invitrogen); Lane 2: MH1000 (negative control); Lane 3: pRDH180 (*T.r.eg2* positive control), Lane 4: *T.r.eg2* in CBP MH1000; Lane5: pMI529 (*T.e.cbh1* positive control); Lane 6: *T.e.cbh1* in CBP MH1000; Lane 7: pMUSD1 (*A.a. bgl1* positive control); Lane 8; *A.a. bgl1* in CBP MH1000