

Overexpression of LAS21 in cellulase-displaying *Saccharomyces cerevisiae* for high-yield ethanol production from pretreated sugarcane bagasse

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Table S1 List of primers used in this study.

Primers	Sequences (5'-3')
Cloning of genes encoding GPI-biosynthesis and remodeling related proteins	
BST1_SacI_F	AATTGAGCTCATGGGTATCAGGAGATTAGTTAGTGTTA
BST1_NotI_R	AATTGCGGCCGCGCATGTATTGTTTCGAAAAATAGCAACCAGG
CWH43_BamHI_F	AATTGGATCCATGCTGATCATCAATGGGAAGATCATC
CWH43_NotI_R	AATTGCGGCCGCGCTAAGTAATAACGTGGCTCATCAAAAACATG
ERI1_BamHI_F	AATTGGATCCATGAGACCACGTGACCAAGGCTT
ERI1_NotI_R	AATTGCGGCCGCGCTTCATCTTTACTACTTGCGAACATTTTAC
GPI1_BamHI_F	AATTGGATCCATGCCAAATTACATTTTCTGGCCATAC
GPI1_NotI_R	AATTGCGGCCGCGCTGCCTGAATAGTCAGCCTTTTGTATAAATC
GUP1_BamHI_F	AATTGGATCCATGTCGCTGATCAGCATCCTGTC
GUP1_NotI_R	AATTGCGGCCGCGCGCATTTTAGGTAAATTCCGTGCCTCTT
LAS21_BamHI_F	AATTGGATCCATGAACTTGAAGCAGTTCACGTGCC
LAS21_NotI_R	AATTGCGGCCGCGCATCAAGAGCGCAAAGGAGGGCC
PER1_BamHI_F	AATTGGATCCATGAGGTTAGCTGTGGTTGTGACC
PER1_NotI_R	AATTGCGGCCGCGCGTACAATTGTCTATTACCCCAATAGGC
Single guide RNA (For cloning to pML104-kan)	
sgRNA-URA3-F	GATCCTGGAGTTAGTTGAAGCATTGTTTTAGAGCTAG
sgRNA-URA3-R	CTAGCTCTAAAACAATGCTTCAACTAACTCCAG
sgRNA-LEU2-F	GATCGATTTTCGAAAATCATTTAATGTTTTAGAGCTAG
sgRNA-LEU2-R	CTAGCTCTAAAACATTAAATGATTTTCGAAATC
sgRNA-ADE2-F	GATCGGAGCCATTAACGTGGTCATGTTTTAGAGCTAG
sgRNA-ADE2-R	CTAGCTCTAAAACATGACCACGTTAATGGCTCC
sgRNA-TRP1-F	GATCCACAGGTAGTTCTGGTCCATGTTTTAGAGCTAG
sgRNA-TRP1-R	CTAGCTCTAAAACATGGACCAGAACTACCTGTG
sgRNA-HIS3-F	GATCCAGATTGCGATCTCTTTAAAGTTTTAGAGCTAG
sgRNA-HIS3-R	CTAGCTCTAAAACCTTAAAGAGATCGCAATCTG
Nonsense mutation oligonucleotides (For gene disruption by CRISPR-Cas9)	
Oligo-URA3-F	CATTGGATGTTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGG TCCCTAAATTTGTTTACTAAAACACATGTGGATATCTTG
Oligo-URA3-R	CAAGATATCCACATGTGTTTTAGTAAACAAATTTAGGGACCTAATGCTTCAA CTAACTCCAGTAATTCCTTGGTGGTACGAACATCCAATG
Oligo-LEU2-F	GCTATTTCTGATGTTTCGTTCCAATGTCAAGTTCCATTTTCGAAAATCATTTAAT TGGTGGTGTCTATCTAGGCTACAGGTGTTCACTTC
Oligo-LEU2-R	GAAGTGAACACCTGTAGCCTAGATAGCAGCACCACCAATTAAATGATTTTCG AAATGGAACCTTGACATTGGAACGAACATCAGAAATAGC

Oligo-ADE2-F	GAAAATTCTCCTGCCAAACAAATAAGCAACTTCAATGACCACGTTAATGGCT CCTTTTCAATCCTCTTGATATCGAAAACTAGCTGAA
Oligo-ADE2-R	TTCAGCTAGTTTTTCGATATCAAGAGGATTGAAAAGGAGCCATTAACGTGGT CATTGAAGTTGCTTATTTGTTTGGCAGGAGAATTTTC
Oligo-TRP1-F	ACACAAAGGCAGCTTGGAGTATGTCTGTTATTAATTTACAGGTAGTTCTGG TCCATATTGAAAGTTTGCGGCTTGCAGAGCACAGAGGC
Oligo-TRP1-R	GCCTCTGTGCTCTGCAAGCCGAAACTTTCAATATGGACCAGAACTACCTGT GAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGT
Oligo-HIS3-F	GTAAAGCGTATTACAAATGAAACCAAGATTCAGATTGCGATCTCTTTAAACT TGGTCCCCTAGCGATAGAGCACTCGATCTTCCCAG
Oligo-HIS3-R	CTGGGAAGATCGAGTGCTCTATCGCTAGGGGACCAAGTTTAAAGAGATCGC AATCTGAATCTTGGTTTCATTTGTAATACGCTTTAC

Table S2 Recombinant vectors used in this study.

Recombinant vector	Cellulase/Gene	Source	Selective marker	Description	References
For screening of cellulase sources and GPI-biosynthesis and remodeling related proteins in <i>S. cerevisiae</i> INVSc1					
pYES3/CT (pY)	-	-	<i>TRP1</i>	Expression vector with <i>GAL1</i> promoter	Invitrogen
pYES3-Ura (pY-U)	-	-	<i>URA3</i>	pY with <i>URA3</i> selective marker	This study
pSS _{SUC2} -PBGL1-21_Sc	β -glucosidase 1	<i>Periconia</i> sp.	<i>TRP1</i>	pY containing PBGL1-21_Sc	Phienluphon et al., 2019
pSS _{SUC2} -PBGL1-6_K1	β -glucosidase 1	<i>Periconia</i> sp.	<i>TRP1</i>	pY containing PBGL1-6_K1	Phienluphon et al., 2019
pSS _{SUC2} -AaCBHB-6_K1	Cellobiohydrolase B	<i>Aspergillus aculeatus</i>	<i>TRP1</i>	pY containing AaCBHB-6_K1	This study
pSS _{SUC2} -AaEG1-6_K1	Endoglucanase 1	<i>Aspergillus aculeatus</i>	<i>TRP1</i>	pY containing AaEG1-6_K1	This study
pSS _{SUC2} -AaEG2-6_K1	Endoglucanase 2	<i>Aspergillus aculeatus</i>	<i>TRP1</i>	pY containing AaEG2-6_K1	This study
pSS _{SUC2} -AaBGL1-6_K1	β -glucosidase 1	<i>Aspergillus aculeatus</i>	<i>TRP1</i>	pY containing AaBGL1-6_K1	This study
pSS _{SUC2} -ChCBH2-6_K1	Cellobiohydrolase 2	<i>Cochliobolus heterostrophus</i>	<i>TRP1</i>	pY containing ChCBH2-6_K1	This study
pSS _{SUC2} -ChEG2-6_K1	Endoglucanase 2	<i>Cochliobolus heterostrophus</i>	<i>TRP1</i>	pY containing ChEG1-6_K1	This study
pSS _{SUC2} -ChBGL1-6_K1	β -glucosidase 1	<i>Cochliobolus heterostrophus</i>	<i>TRP1</i>	pY containing ChBGL1-6_K1	This study
pSS _{SUC2} -CiCBH2-6_K1	Cellobiohydrolase 2	<i>Chrysosporium lucknowense</i>	<i>TRP1</i>	pY containing CiCBH2-6_K1	This study
pSS _{SUC2} -HiEG1-6_K1	Endoglucanase 1	<i>Humicola insolens</i>	<i>TRP1</i>	pY containing HiEG1-6_K1	This study
pSS _{SUC2} -PdBGL1-6_K1	β -glucosidase 1	<i>Penicillium decumbens</i>	<i>TRP1</i>	pY containing PdBGL1-6_K1	This study
pSS _{SUC2} -TrCBH1-6_K1	Cellobiohydrolase 1	<i>Trichoderma reesei</i>	<i>TRP1</i>	pY containing TrCBH1-6_K1	This study
pSS _{SUC2} -TrCBH2-6_K1	Cellobiohydrolase 2	<i>Trichoderma reesei</i>	<i>TRP1</i>	pY containing TrCBH2-6_K1	This study
pSS _{SUC2} -TrEG2-6_K1	Endoglucanase 2	<i>Trichoderma reesei</i>	<i>TRP1</i>	pY containing TrEG2-6_K1	This study
pSS _{SUC2} -AtCBH1-6_K1	Cellobiohydrolase 1	<i>Acremonium thermophilum</i>	<i>TRP1</i>	pY containing AtCBH1-6_K1	This study

pSS _{SUC2} - <i>TtEG1</i> -6_K1	Endoglucanase 1	<i>Thielavia terrestris</i>	<i>TRP1</i>	pY containing <i>TtEG1</i> -6_K1	This study
pY-U-BST1	BST1	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene BST1	This study
pY-U-CWH43	CWH43	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene CWH43	This study
pY-U-ERI1	ERI1	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene ERI1	This study
pY-U-GPI1	GPI1	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene GPI1	This study
pY-U-GUP1	GUP1	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene GUP1	This study
pY-U-LAS21	LAS21	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene LAS21	This study
pY-U-PER1	PER1	<i>Saccharomyces cerevisiae</i>	<i>URA3</i>	pY-U containing enhancing gene PER1	This study
For auxotrophic gene disruption by CRISPR/Cas9 in <i>S. cerevisiae</i> TISTR5088					
pML104	-	-	<i>URA3</i>	CRISPR/Cas9 expression vector	Laughery et al., 2015
pML104-kan	-	-	<i>kan</i>	pML104 with <i>kan</i> selective marker	This study
pML104-kan-ADE2	-	-	<i>kan</i>	ADE2 gene disruption by CRISPR/Cas9 system	This study
pML104-kan-LEU2	-	-	<i>kan</i>	LEU2 gene disruption by CRISPR/Cas9 system	This study
pML104-kan-HIS3	-	-	<i>kan</i>	HIS3 gene disruption by	This study

				CRISPR/Cas9 system	
pML104-kan-TRP1	-	-	<i>kan</i>	TRP1 gene disruption by CRISPR/Cas9 system	This study
pML104-kan-URA3	-	-	<i>kan</i>	URA3 gene disruption by CRISPR/Cas9 system	This study
For multi-copy integration in <i>S. cerevisiae</i> TISTR5088					
pASB	-	-	<i>URA3</i>	Integrative vector to rDNA	Laboratory stock
pASB- <i>TrEG2</i> -Ura3	Endoglucanase 2	<i>Trichoderma reesei</i>	<i>URA3</i>	Vector containing <i>TrEG2</i> -6_K1 gene cassette	This study
pASB- <i>C/CBH2</i> -Leu2	Cellobiohydrolase 2	<i>Chrysosporium lucknowense</i>	<i>LEU2</i>	Vector containing <i>C/CBH2</i> -6_K1 gene cassette	This study
pASB- <i>PBGL1</i> -Ade2	β -glucosidase 1	<i>Periconia</i> sp.	<i>ADE2</i>	Vector containing <i>PBGL1</i> -21_Sc gene cassette	This study
pASB-LAS-Ade2	LAS21	<i>Saccharomyces cerevisiae</i>	<i>ADE2</i>	Vector containing LAS21 gene cassette	This study
pASB- <i>C/CBH2</i> -Trp1	Cellobiohydrolase 2	<i>Chrysosporium lucknowense</i>	<i>TRP1</i>	Vector containing <i>C/CBH2</i> -6_K1 gene cassette	This study
pASB- <i>TrEG2</i> -His4	Endoglucanase 2	<i>Trichoderma reesei</i>	<i>HIS4</i>	Vector containing <i>TrEG2</i> -6_K1 gene cassette	This study

Table S3 Primers for real-time PCR based determination of gene copy number.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Actin</i>	CCCAGGTATTGCCGAAAGAATGC	TGGAAGGTAGTCAAAGAAGCCAAGA
<i>CICBH2</i>	ACAACAATGGGGTGACTGGT	GGTTGTAAAGCATCAGACAAACCAC
<i>TrEG2</i>	ACATCTTCATCTACACCACCAACTTC	TGTTGCATTTGACCGATACCATCTG
<i>PBGL1</i>	GATATCATCTACGAACCGACAGC	CGAGTAGGAGAAGTTGGTGTACG
<i>LAS21</i>	CTACGCCTCTATTCCAGCACAAG	CAGCAATCCCATCACAAAATTCCAC

Table S4 Determination of gene copy number in *S. cerevisiae* ECBLCCE5.

Gene	Gene copy number
<i>CICBH2</i>	16
<i>TrEG2</i>	9
<i>PBGL1</i>	15
<i>LAS21</i>	2

Table S5 Enzyme activity profile of commercial cellulase.

Enzyme	Cellulase C	Ctec2
FPase (U/FPU)	1	1
CMCase (U/FPU)	34.8	38.5
β-glucosidase (U/FPU)	7.2	61.8
Xylanase (U/FPU)	791	45.2

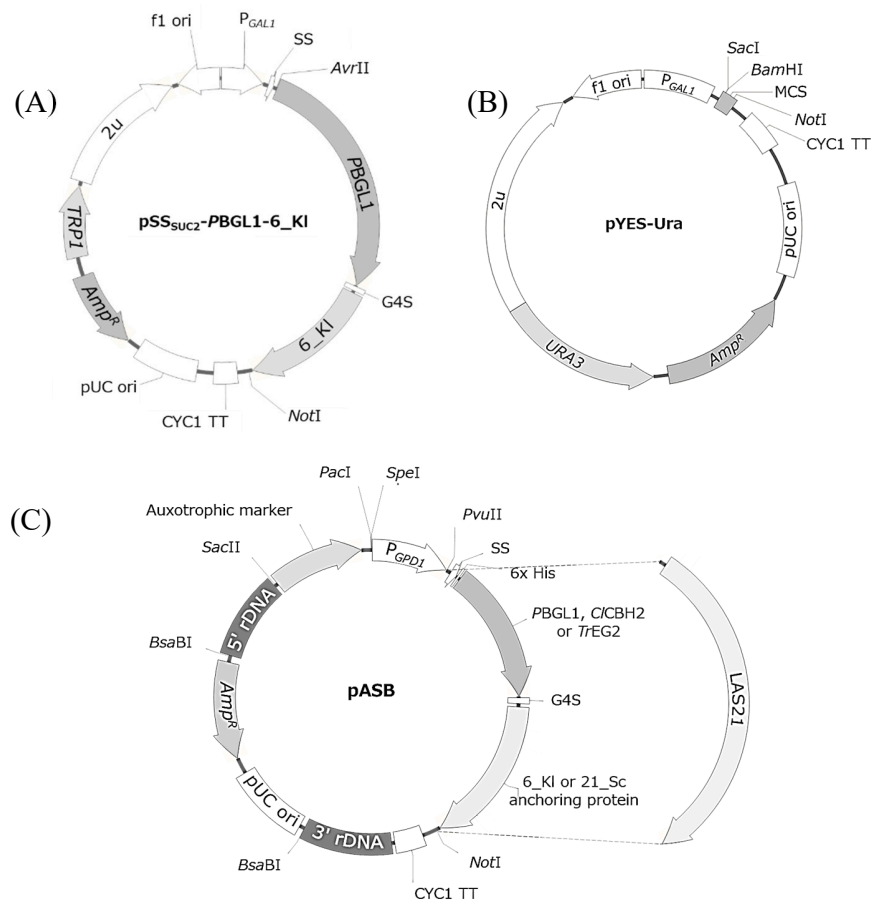


Figure S1 Feature maps of plasmids pSS_{SUC2}-PBGL1-6_KI (A), pYES3-Ura (B), and pASB (C) series.