

Identification of mutations responsible for improved xylose utilization in an adapted xylose isomerase expressing *Saccharomyces cerevisiae*

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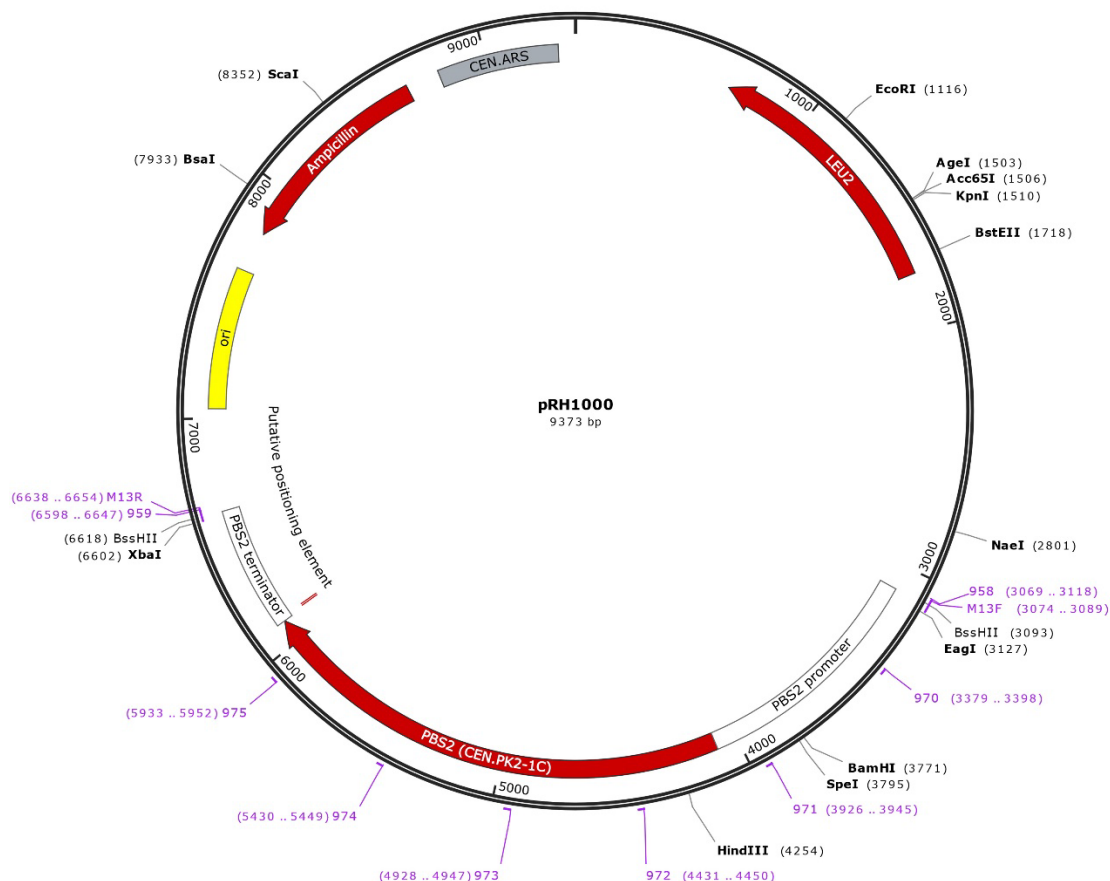
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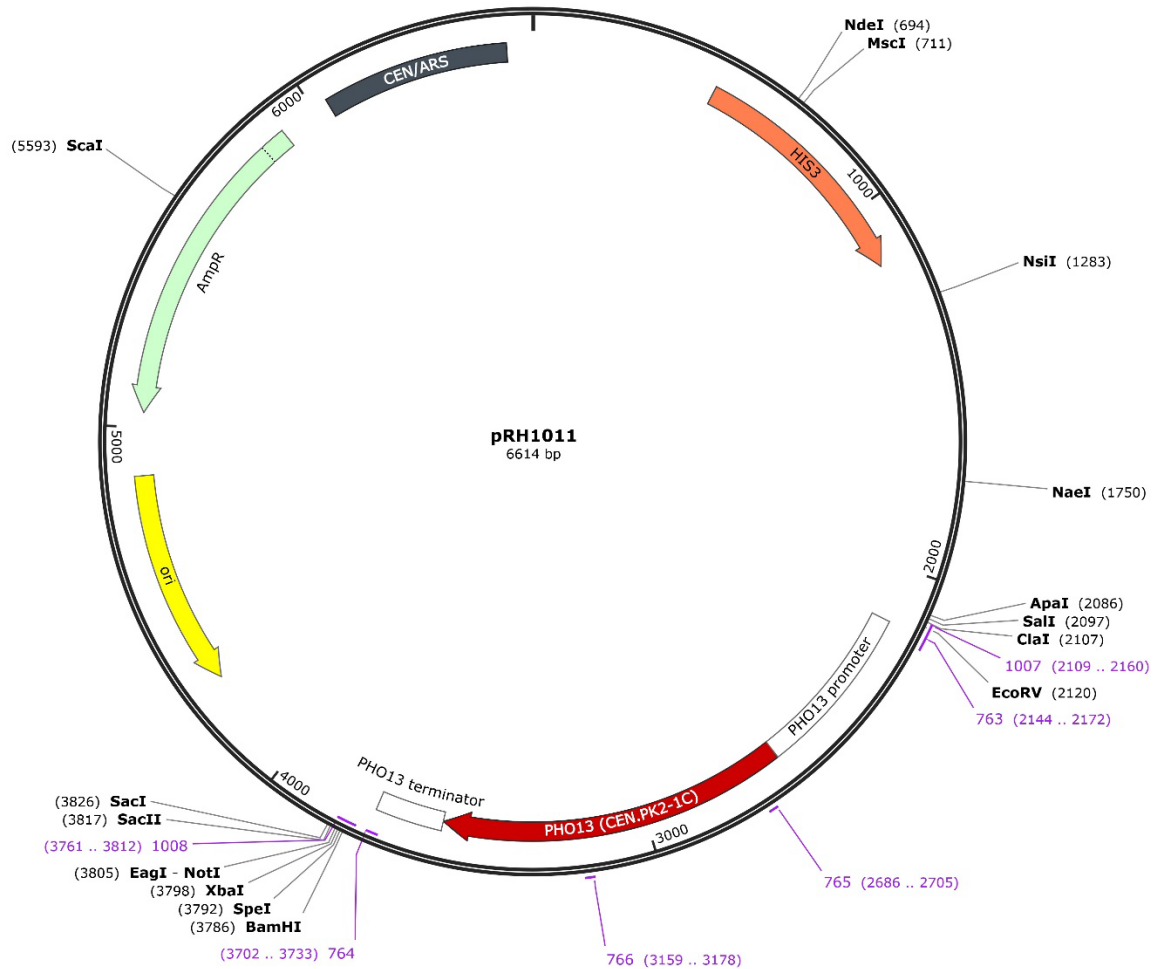
Supplemental materials:

Construction of plasmids for expressing *PBS2* and *PHO13*

PBS2 DNA sequence, including *PBS2* promoter and termination regions, was amplified from CEN.PK2-1C genomic DNA using Phusion polymerase and oligos 958 and 959. To generate pRH1000, the DNA fragment (bp -992/ORF/+520) was cloned into BssHII cut pRS415 using NEBuilder HiFi DNA assembly. DNA sequence homologous to the flanking BssHII sites of pRS415 was included in primers 958/959 to direct integration of the P_{PBS2} -*PBS2*- T_{PBS2} fragment. Prior to transformation into *S. cerevisiae*, plasmid pRH1000 was sequenced using the primers shown below to confirm that no mutations were present.



PHO13 DNA sequence, including *PHO13* promoter and termination regions, was amplified from CEN.PK2-1C genomic DNA using Phusion polymerase and oligos 1007 and 1008. To generate pRH1011, the DNA fragment (bp -466/ORF/+180) was cloned into *Sma*I cut pRS413 using NEBuilder HiFi DNA assembly. DNA sequence homologous to the flanking *Sma*I site of pRS413 was included in primers 1007/1008 to direct integration of the P_{PHO13} -*PHO13*- T_{PHO13} fragment. Prior to transformation into *S. cerevisiae*, plasmid pRH1011 was sequenced using the primers shown below to confirm that no mutations were present.



Construction of PBS2 and PHO13 deletion strains

To delete *PBS2*, primers 989 and 990 were used to amplify the *LEU2* gene from plasmid pRS405. To direct integration of the *LEU2* gene to create *pbs2Δ::LEU2* strains, primers 989 and 990 also contained DNA sequence homologous to the sequence flanking the *PBS2* gene. Each *pbs2Δ::LEU2* strain was confirmed by PCR amplification of DNA fragments using primers that hybridize to sequences adjacent to the *PBS2* gene and within the *LEU2* gene. Hot-start Taq polymerase was used with primer pairs 991/36 and 992/35 to confirm gene deletion.

To delete *PHO13*, primers 761 and 762 were used to amplify the *HIS3* gene from plasmid pRS403. To direct integration of the *HIS3* gene to create *pho13Δ::HIS3* strains, primers 761 and 762 also contained DNA sequence homologous to the sequence flanking the *PHO13* gene. Each *pho13Δ::HIS3* strain was confirmed by PCR amplification of DNA fragments using primers that hybridize to sequences adjacent to the *PHO13* gene and within the *HIS3* gene. Hot-start Taq polymerase was used with primer pairs 763/59 and 764/60 to confirm gene deletion.

Supplementary Table S1. DNA oligonucleotides used in this study

Oligo#	Description/Use	Sequence (5' to 3')
35	LEU2 primer for gene deletion confirmation	CCAACGTGGTCACCTGGCAA
36	LEU2 primer for gene deletion confirmation	GTACCACCGAAGTCGGTGAT
761	PHO13 deletion oligo	AACTTTCCGGTTTTCTTTTTTCGGTGAATGTTCTTCCGTTTTAGTGAAGATTGTACTGAGAGTGCAC
762	PHO13 deletion oligo	TATTTTCTCTTTCAAAAAGTAATTCTACCCCTAGATTTGCATTGCTCCCTGTGCGGTATTCACACCG
763	PHO13 deletion confirmation oligo	AAGTGGCTTGAGCTGTGGATAAGAAAAGC
764	PHO13 deletion confirmation oligo	TAATCGTCATCATTTTATTCACACCTCCGGAT
765	PHO13 sequencing oligo	CACGTTTCTGTTCGATTGTG
766	PHO13 sequencing oligo	TGCAGAAGGATTCTGTTAC
958	PBS2-F2, for cloning P_{PBS2} -PBS2- T_{PBS2}	ACGTTGTAAACGACGCGCCAGTGAGCGCGCTTTACTGCGGGATTAAC TTC
959	PBS2-R2, for cloning P_{PBS2} -PBS2- T_{PBS2}	CAGCTATGACCATGATTACGCCAAGCGCGCTAGTTCCTGGTCTAGACTCC
970	PBS2 sequencing oligo	AATCTTATTGCGTACCACTC
971	PBS2 sequencing oligo	AGGGTAGCTGCTATTGTGAG
972	PBS2 sequencing oligo	TCGTGCAAGCGTCCTCCAAG
973	PBS2 sequencing oligo	AGCACATCCACCTCATCAAG
974	PBS2 sequencing oligo	ACGACGAATCATCTGAAATC
975	PBS2 sequencing oligo	GCAGCTTTAACAGAGCATCC
981	SAS3-WT primer	GGTAACGTATCACTTTGCAATCATAGTG
982	SAS3-SNP-primer	GGTAACGTATCACTTTGCAATCATAGTT
983	PBS2-WT primer	CCATGACCCAGTTCATACA
984	PBS2-SNP-primer	CCATGACCCAGTTCATACT
989	PBS2 deletion oligo	TATAGATACATTATTATAATTAAGCAGATCGAGACGTTAATTTCTCAAAGAGATTGTACTGAGAGTGCAC
990	PBS2 deletion oligo	TTGTTGTTATATTACGTCCTGTTTGCTTTTATTGGATATTAACGCTACTGTGCGGTATTCACACCG
991	PBS2 deletion confirmation oligo	GATTCGTGAGCCATACACGTTT
992	PBS2 deletion confirmation oligo	GTCCACATCGCTTCACTTGC
993	PHO13-WT primer	CCGCAAAAGGGTTATACATTTACG
994	PHO13-SNP primer	CCGCAAAAGGGTTATACATTTCACT
995	PHO13-R primer	CTCATTATTGGTTAAGGTGTAGATGTCAC
996	HSP104-WT primer	AATTGGCTAAAAAGTTGCTGGATTCTT
997	HSP104-SNP primer	AATTGGCTAAAAAGTTGCTGGATTCTC
998	HSP104-R primer	GATAAATTGAGCACCTAGATTGGAAGTC
999	PHO81-SNP primer	CCTTAAGTGGAAACAACCCAATTTCTT
1000	PHO81-WT primer	CCTTAAGTGGAAACAACCCAATTTCCC
1001	PHO81-R primer	GTAGATCCCGCTGTAACCTCCA
1002	STE24-WT primer	CAGAGGATCTACATTATGGTGGACAG
1003	STE24-SNP primer	CAGAGGATCTACATTATGGTGGACAA
1004	STE24-R primer	CCATGAAATCGGTCACTGGCAA
1007	PHO13-F, for cloning P_{PHO13} -Pho13- T_{Pho13}	GATAAGCTTGATATCGAATTCCTGCAGCCCGATATAAGTGGCTTGAGCTGTG
1008	PHO13-R, for cloning P_{PHO13} -Pho13- T_{Pho13}	GGCGGCCGCTCTAGAACTAGTGGATCCCCGTTCAAAGTGGCAGAGAATTGAG