

**Table S1.** Organisms, cloned homologues of xylose reductase (XR), xylitol dehydrogenase (XDH), and xylulose kinase (XKS) and primer sequences used for cloning into gene expression cassettes. Primers contained the sequences homologous to each gene (underlined), promoters, and terminators.

Organism	Abbreviation	NCBI accession number	Primer sequence	
1 <i>Aspergillus flavus</i>	afXR	XM_002374429	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGCTTCTCCACCGTCAAGC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGAAGATAGGAACGTACATA -3'
2 <i>Aspergillus nidulans</i>	anidXR	XM_652935	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGTCCCCTCCACCGTCAAAC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGAAGATAGTGATTGGCAGA -3'
3 <i>Aspergillus niger</i>	anXR	XM_001388767	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGCCTCTCCACAGTAAAGC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTAGAAAATAGGAGCGTAGAGT -3'
4 <i>Aspergillus oryzae</i>	aoXR	FJ957890	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGCTTCTCCACCGTCAAGC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGAAGATAGGAACGTACATA -3'
5 <i>Pichia guilliermondii</i>	pgXR	AF020040	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGTCTATTACTTTGAACTCAG</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTACACAAAAGTTGGAATCTTG -3'
6 <i>Candida parapsilosis</i>	cpXR	XM_002492928	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGTCTACTGCTACTGCTTCC</u> -3'
			Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAACAAAAACTGGAATGTTGGAG-3'
7 <i>Candida shehatae</i>	csXR	EF042296	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGAGCCCAAGCCCAATTCCAGC</u> -3'
			Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAACGAAGATTGGAATGTTGTCC-3'
8 <i>Candida tropicalis</i>	ctXR	EU273285	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGTCTACTACTGTTAATACTCC</u> -3'
			Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAACAAAAGATTGGAATGTTGTCC-3'
9 <i>Kluyveromyces lactis</i> NRRL Y-1140	klXR	XM_454929	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGACGTACTTAGCAGAAACAG</u> -3'
			Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGATGAAAGTTGGGAATTCGTTG-3'
10 <i>Magnaporthe grisea</i> 70-15	mgXR	XM_361105	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGCGTCGCCAACGATGAAGC</u> -3'
			Rev	5'-TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATCAACCAAAGATCCAGAGCTTG-3'
11 <i>Pichia guilliermondii</i>	pgXR	DQ297454	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGTCTATTACTTTGAACTCAG</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTACACAAAAGTTGGAATCTTG -3'
12 <i>Neurospora crassa</i>	ncXR	XM_958838	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGTTCTGCTATCAAGCTCAAC</u> -3'
			Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTAACCGAAAATCCAGAGGTTCTC-3'
13 <i>Penicillium chrysogenum</i>	pcXR	XM_002561226	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGTTGCCCTACTGTCAAGC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATCAATACTCCACGCAAACCAA -3'
14 <i>Phaeosphaeria nodorum</i> SN15	pnXR	XM_001802990	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGTTGCCGTCGCTTTTGCC</u> -3'
			Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTAAGCGAAGACGTAGCAAGGG -3'
15 <i>Pichia pastoris</i>	ppXR	XM_002492928	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCA <u>ATGGCTACTCTATTAATAATTGA</u> -3'

				Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTAGGCAAATATAGGGATCTTG -3'
16	<i>Podospora anserine</i>	paXR	XM_001912551	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCAATGGCCCCCGTCATCAAGCTCA -3'
				Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGCCAAAGATCCAGAGGAGC -3'
17	<i>Scheffersomyces stipitis</i> ( <i>Pichia stipitis</i> )	psXR	XM_001385144	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCAATGCCTTCTATTAAGTTGAACTC-3'
				Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGACGAAGATAGGAATCTTGTC-3'
18	<i>Scheffersomyces stipitis-mutant</i>	psXR-mut <sup>a</sup>	XM_001385144	Fwd	5'-TATTTCAAGCTATACCAAGCATACAATCAACTCCAATGCCTTCTATTAAGTTGAACTC-3'
				Rev	5'-GGAGACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGACGAAGATAGGAATCTTGTC-3'
19	<i>Talaromyces stipitatus</i>	tsXR	XM_002484006	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCAATGTCTTCTCCCACCGTCAAGC -3'
				Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTATGCGAAAATAGGAATGTAG -3'
20	<i>Zygosaccharomyces rouxii</i>	zrXR	XM_002494601	Fwd	5'- TATTTCAAGCTATACCAAGCATACAATCAACTCCAATGGCAAGTGTGGTCGCTTTGA -3'
				Rev	5'- TGGAGACTTGACCAAACCTCTGGCGAAGAAGTCCACTAATCAAAAATAGGAATGGA -3'

Continued (Table S1)

Organism	Abbreviation	NCBI accession number	Primer sequences	
1 <i>Arxula adenivorans</i>	aaXDH	AJ748124	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCTGCACAAGTCGAGGAAC -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACTCAGGTCCATCAATAATC -3'
2 <i>Aspergillus nidulans</i>	anidXDH	BN001306	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGAGCTCACAGACCCCAACAG -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTATGCGGCGCCAGGGCCAGCA -3'
3 <i>Aspergillus niger</i>	anXDH	AJ854041	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGAGCACCCAGAACACCAA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATCTATGAATCGACACCAGCAA -3'
4 <i>Aspergillus oryzae</i>	aoXDH	AB109101	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGGTGCGCCACCCAAAACCG -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTCATACATCAACACCAGCAAT -3'
5 <i>Candida albicans</i>	caXDH	XM_714341	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACAAACCCTTCTTTAGTG -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTATTCTGGGCCATCGATTAAC -3'
6 <i>Candida dubliniensis</i> CD36	cdXDH	XM_002420506	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGTCAATTCCAGCTTCAAT -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTCAACTGGTCAAATCAAAAA -3'
7 <i>Candida shehatae</i>	csXDH	FJ040172	Fwd	5'-AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACTGCTAACCCCTTCGCTC -3'
			Rev	5'-CGTGAATGTAAGCGTGACATAACTAATTACATGATCTATTCCAGGGCCATCAATGA -3'
8 <i>Candida tropicalis</i>	ctXDH	DQ220745	Fwd	5'-AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACTGCAAACCCATCATT -3'
			Rev	5'-CGTGAATGTAAGCGTGACATAACTAATTACATGATCTATTCTGGACCATCAATTA -3'
9 <i>Trichoderma longibrachiatum</i>	tlXDH	AF428150	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCGACTCAAACGATCAACA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACACCTTCTCGTTGGGCCCG -3'
10 <i>Kluyveromyces lactis</i> NRRL Y-1140	kIXDH	XM_453306	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGTCAGGCACACAAAAGGCGG -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATCTATTCCGGGCCATCGATCATT -3'
11 <i>Pichia guilliermondii</i>	pgXDH	XM_001481913.1	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGCTTTGTAATTTTACTTCAA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACTCGGGACCATCTATAATA -3'
12 <i>Nectria haematococca</i>	nhXDH	XM_003053919.1	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCCAGCAACCTGTCTTTC -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACTCCTCATTGGGACCCCTTG -3'
13 <i>Neurospora crassa</i>	ncXDH	XM_959714.1	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCTACCGACGGCAAGTCTA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTAGCAGCAGCCAGAACCACCG -3'
14 <i>Neurospora crassa-mutant</i>	ncXDH-mut <sup>b</sup>	XM_959714.1	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCTACCGACGGCAAGTCTA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTAGCAGCAGCCAGAACCACCG -3'
15 <i>Pachysolen tannophilus</i>	ptXDH	ACD81475.1	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGCATGGAAAGGTGATTGGCC -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACCTTGAAGTATCAACAACG -3'
16 <i>Penicillium chrysogenum</i>	pcXDH	XM_002568139	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGGCCACAGCTCAGGTAGA -3'
			Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACACATCAATACCACCAA -3'

17	<i>Phaeosphaeria nodorum</i> SN15	pnXDH	XM_001801582	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACGACAAAGACAGCTAC-3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTCACTCCTCAGGTCCTCAATAAG-3'
18	<i>Pichia angusta</i>	paXDH	AB185335	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGAAAGGTTTACTTTATTACG-3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTAGGAAACCTCGTTCGGCGTC-3'
19	<i>Pichia pastoris</i> GS115	ppXDH	XM_002489888	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGTCCGATAACCCAAGTGT-3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACTCTGGGCCGTCATGATAG-3'
20	<i>Scheffersomyces stipitis</i> ( <i>Pichia stipitis</i> )	psXDH	X55392.1	Fwd	5'-AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACTGCTAACCCCTTCCTTG-3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACTCAGGGCCGTCATGAG-3'
21	<i>Talaromyces stipitatus</i>	tsXDH	XM_002488189	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGTCTCTCACGGAACCAA -3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTACAAACCCACAATGATAG -3'
22	<i>Zygosaccharomyces rouxii</i>	zrXDH	XM_002497263	Fwd	5'- AGTAATTATCTACTTTTTACAACAAATATAAAACAATGACAAAGCAAGACGCAATTGTTTC-3'
				Rev	5'- CGTGAATGTAAGCGTGACATAACTAATTACATGATTTATTCTGGGCCGGTGATAATG-3'

Continued (Table S1)

Organism	Abbreviation	NCBI accession number	Primer sequences	
1 <i>Aspergillus flavus</i>	afXKS	XM_002383656	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGCAAGGCCCATTTGTACATC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCACC GCGTCTCACCTCCTG -3'
2 <i>Aspergillus fumigatus Af293</i>	afugXKS	XM_748563	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGACTTCTCAGGGCCCTCTATAC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTATTTCTGGCTGCCTCCTG -3'
3 <i>Aspergillus nidulans</i>	anidXKS	XM_676967	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGTCCTCGCGCTCCTCTTCTC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTATTTCTTCCCTCCTGCTC -3'
4 <i>Aspergillus niger</i>	anXKS	XM_001391360	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGCAAGGTCCTCTATACATC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCAGTGCTTCCCTTCTG -3'
5 <i>Aspergillus oryzae</i>	aoXKS	XM_001824842	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGCAAGGCCCATTTGTACATC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCACC GGTCTCACCTCCTG -3'
6 <i>Candida albicans</i>	caXKS	XM_706361.1	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGTACAGTTTCACTTTCACTATC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCAAATAATTTTCCAATATATTC-3'
7 <i>Candida dubliniensis CD36</i>	cdXKS	XM_002420098.1	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGACCGATTATTCTAATTC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTATTGTTTTAACAAATGTTTCTTCC-3'
8 <i>Candida tropicalis</i>	ctXKS	XP_002549576	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGACTACTGATTATTCTGAAAAAC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTATTGTTTTAATAAAGTCTCTTCC-3'
9 <i>Kluyveromyces lactis NRRL Y-1140</i>	kIXKS	XM_454390	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGAGTGAATCAGGTTATTATTAG -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTAGTGTGATGACGCTTCTC -3'
10 <i>Pichia guilliermondii</i>	pgXKS	XM_001482293	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGACTTCTAAATCCTCAGCTA -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTAATCATGCTTCAAATTCTC-3'
11 <i>Nectria haematococca</i>	nhXKS	XM_003048919	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGCCTTTCTTGGCTCGATC -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTAAACCTTTTCTCTGAAC -3'
12 <i>Neurospora crassa</i>	ncXKS	XM_001728085	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGGACGTTCAAGCAATCGTAATC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTACTTATTCTCGCCACCTCC-3'
13 <i>Penicillium chrysogenum</i>	pcXKS	XM_002557373	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGGCATCAGACAGTCCCCTCTAC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCAGTAATCATCTCCATCGCCCTTC-3'
14 <i>Pichia pastoris GS115</i>	ppXKS	XM_002489890	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGGTTACCAAAGAAATCC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTACTTCTCTAGAGTTTGTCTATTTC-3'
15 <i>Scheffersomyces stipitis</i> ( <i>Pichia stipitis</i> )	psXKS	AF127802	Fwd	5'-CAAGACACCAATCAAAACAAATAAAACATCATCACAATGACCACTACCCCATTTGATGC-3'
			Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTAGTGTTCATTCACCTTCCATC-3'
16 <i>Podospora anserine</i>	paXKS	XM_001907740	Fwd	5'- AAGACACCAATCAAAACAAATAAAACATCATCACAATGACCGACAACGGACCCCTTT -3'
			Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTACGTATTCTTCGCAACTTTC -3'

17	<i>Saccharomyces cerevisiae</i>	scXKS	AAFW02000100	Fwd	5'-CAAGACACCAATCAAACAATAAAACATCATCACAATGTTGTGTTTCAGTAATTCAAAG-3'
				Rev	5'-AAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTTAGATGAGAGTCTTTTCCAG-3'
18	<i>Talaromyces stipitatus</i>	tsXKS	XM_002484215	Fwd	5'- AAGACACCAATCAAACAATAAAACATCATCACAATGGCACCCGGGCCGTTGTATATC -3'
				Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTGATTCTTGAATCTGCTGACATC -3'
19	<i>Zygosaccharomyces rouxii</i>	zrXKS	XM_002498463	Fwd	5'- AAGACACCAATCAAACAATAAAACATCATCACAATGACTGAAACCAACGATTCATTC -3'
				Rev	5'- TAAAGTCAATCATTTGGCAAGCTTCTCAGCAAACCTCAATGCTTTGGTAATGATGCTTC -3'

<sup>a</sup> Engineered xylose reductase (K270R mutant) for altered cofactor specificity from NADPH to NADH (2)

<sup>b</sup> Engineered xylitol dehydrogenase (D211S/I212R/S348T triple mutant) for altered cofactor specificity from NAD<sup>+</sup> to NADP<sup>+</sup> (1)

**Table S2.** Primer sequences used to amplify the assembly fragments from the expression cassettes.

Gene	Annealing region		Sequence
Xylose reductase	ADH1p	Fwd	5'- CGAGGTGCCGTAAAGCACTAAATC -3'
	ADH1t	Rev	5'- GAGAGAGTAACAGTACGATCG -3'
Xylitol dehydrogenase	PGK1p	Fwd	5'- TGGACTTCTTCGCCAGAGGTTTG -3'
	CYC1t	Rev	5'- CGTTGCGAGAGATGGAAAAC -3'
Xylulose kinase	PYK1p	Fwd	5'- ATCATGTAATTAGTTATGTCACG -3'
	HXT7t	Rev	5'- GGAAGCGGAAGAGCGCCCAATACG -3'

**Table S3.** Combinations of the three xylose pathway genes in randomly picked 16 and 12 clones from two independent test libraries.

Clone #	XR	XDH	XKS
1	<i>K. lactis</i>	<i>P. tannophilus</i>	<i>S. stipitis</i>
2	<i>N. crassa</i>	<i>A. oryzae</i>	<i>S. stipitis</i>
3	<i>A. oryzae</i>	<i>C. tropicalis</i>	<i>S. stipitis</i>
4	<i>A. oryzae</i>	<i>C. tropicalis</i>	<i>P. chrysogenum</i>
5	<i>C. tropicalis</i>	<i>P. tannophilus</i>	<i>P. chrysogenum</i>
6	<i>K. lactis</i>	<i>T. stipitatus</i>	<i>S. stipitis</i>
7	<i>P. guilliermondii</i>	<i>T. stipitatus</i>	<i>A. niger</i>
8	<i>N. crassa</i>	<i>N. crassa</i>	<i>S. Stipitis</i>
9	<i>P. guilliermondii</i>	<i>T. stipitatus</i>	<i>P. chrysogenum</i>
10	<i>N. crassa</i>	<i>C. shehatae</i>	<i>C. tropicalis</i>
11	<i>K. lactis</i>	<i>A. oryzae</i>	<i>S. stipitis</i>
12	<i>A. oryzae</i>	<i>K. lactis</i>	<i>P. chrysogenum</i>
13	<i>S. stipitis</i>	<i>S. stipitis</i>	<i>P. chrysogenum</i>
14	<i>S. stipitis</i>	<i>K. lactis</i>	<i>P. chrysogenum</i>
15	<i>C. shehatae</i>	<i>C. tropicalis</i>	<i>A. niger</i>
16	<i>K. lactis</i>	<i>S. stipitis</i>	<i>A. niger</i>
17	<i>A. oryzae</i>	<i>P. tannophilus</i>	<i>C. albicans</i>
18	<i>S. stipitis</i>	<i>S. stipitis</i>	<i>P. chrysogenum</i>
19	<i>A. oryzae</i>	<i>N. crassa</i>	<i>P. chrysogenum</i>
20	<i>P. guilliermondii</i>	<i>C. shehatae</i>	<i>P. chrysogenum</i>
21	<i>K. lactis</i>	<i>C. tropicalis</i>	<i>P. chrysogenum</i>
22	<i>A. oryzae</i>	<i>S. stipitis</i>	<i>P. chrysogenum</i>
23	<i>A. oryzae</i>	<i>K. lactis</i>	<i>A. niger</i>
24	<i>S. stipitis</i>	<i>N. crassa</i>	<i>A. niger</i>
25	<i>A. oryzae</i>	<i>S. stipitis</i>	<i>P. chrysogenum</i>
26	<i>K. lactis</i>	<i>C. shehatae</i>	<i>P. chrysogenum</i>
27	<i>S. stipitis</i>	<i>A. oryzae</i>	<i>P. chrysogenum</i>
28	<i>K. lactis</i>	<i>K. lactis</i>	<i>S. cerevisiae</i>

**Table S4.** Combinations of the three xylose pathway genes in the 10 fast and selected slow growers of each strain screened by the growth rate on xylose. “F” – fast grower, “S” – slow grower.

	XR	XDH	XKS
<b>INVSc1</b>			
F1	<i>Aspergillus nidulans</i>	<i>Scheffersomyces stipitis</i>	<i>Podospora anserina</i>
F2	<i>Aspergillus nidulans</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
F3	<i>Scheffersomyces stipitis</i>	<i>Pichia pastoris</i>	<i>Podospora anserina</i>
F4	<i>Neurospora crassa</i>	<i>Zygosaccharomyces rouxii</i>	<i>Aspergillus niger</i>
F5	<i>Zygosaccharomyces rouxii</i>	<i>Arxula adenivorans</i>	<i>Kluyveromyces lactis</i>
F6	<i>Aspergillus nidulans</i>	<i>Scheffersomyces stipitis</i>	<i>Podospora anserina</i>
F7	<i>Neurospora crassa</i>	<i>Arxula adenivorans</i>	<i>Saccharomyces cerevisiae</i>
F8	<i>Neurospora crassa</i>	<i>Neurospora crassa</i>	<i>Aspergillus niger</i>
F9	<i>Zygosaccharomyces rouxii</i>	<i>Zygosaccharomyces rouxii</i>	<i>Aspergillus nidulans</i>
F10	<i>Scheffersomyces stipitis</i>	<i>Aspergillus oryzae</i>	<i>Nectria haematococca</i>
<b>ATCC 4124</b>			
F1	<i>Aspergillus flavus</i>	<i>Podospora anserine</i>	<i>Aspergillus flavus</i>
F2	<i>Pichia guilliermondii</i>	<i>Pencillium chrysogenum</i>	<i>Aspergillus oryzae</i>
F3	<i>Neurospora crassa</i>	<i>Aspergillus oryzae</i>	<i>Pichia pastoris</i>
F4	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Zygosaccharomyces rouxii</i>
F5	<i>Aspergillus flavus</i>	<i>Scheffersomyces stipitis</i>	<i>Pichia guilliermondii</i>
F6	<i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i>	<i>Kluyveromyces lactis</i>
F7	<i>Aspergillus flavus</i>	<i>Zygosaccharomyces rouxii</i>	<i>Kluyveromyces lactis</i>
F8	<i>Aspergillus nidulans</i>	<i>Candida dubliniensis</i>	<i>Zygosaccharomyces rouxii</i>
F9	<i>Talaromyces stipitatus</i>	<i>Kluyveromyces lactis</i>	<i>Zygosaccharomyces rouxii</i>
F10	<i>Aspergillus flavus</i>	<i>Scheffersomyces stipitis</i>	<i>Zygosaccharomyces rouxii</i>
<b>CTY</b>			
F1	<i>Aspergillus flavus</i>	<i>Candida dubliniensis</i>	<i>Nectria haematococca</i>
F2	<i>Aspergillus flavus</i>	<i>Candida dubliniensis</i>	<i>Saccharomyces cerevisiae</i>
F3	<i>Aspergillus nidulans</i>	<i>Aspergillus niger</i>	<i>Pencillium chrysogenum</i>
F4	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>
F5	<i>Neurospora crassa</i>	<i>Aspergillus niger</i>	<i>Zygosaccharomyces rouxii</i>
F6	<i>Aspergillus flavus</i>	<i>Candida shehatae</i>	<i>Nectria haematococca</i>
F7	<i>Aspergillus flavus</i>	<i>Aspergillus oryzae</i>	<i>Aspergillus niger</i>
F8	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Candida tropicalis</i>
F9	<i>Aspergillus flavus</i>	<i>Pichia guilliermondii</i>	<i>Candida dubliniensis</i>
F10	<i>Talaromyces stipitatus</i>	<i>Candida shehatae</i>	<i>Candida dubliniensis</i>



Continued (Table S4)

	XR	XDH	XKS
INVSc1			
S1 <sup>b</sup>	<i>Neurospora crassa</i>	<i>Aspergillus nidulans</i>	n.a. <sup>a</sup>
S5	<i>Aspergillus flavus</i>	<i>Neurospora crassa</i>	<i>Aspergillus fumigatus</i>
S10	<i>Pichia guilliermondii</i>	<i>Candida shehatae</i>	<i>Podospora anserina</i>
ATCC 4124			
S1	<i>Zygosaccharomyces rouxii</i>	<i>Candida shehatae</i>	<i>Pichia guilliermondii</i>
S5	<i>Pichia guilliermondii</i>	<i>Zygosaccharomyces rouxii</i>	<i>Kluyveromyces lactis</i>
S8	<i>Candida shehatae</i>	<i>Candida shehatae</i>	<i>Aspergillus oryzae</i>
S10	<i>Scheffersomyces stipitis</i>	<i>Scheffersomyces stipitis</i>	<i>Nectria haematococca</i>
CTY			
S1	<i>Candida dubliensis</i>	<i>Nectria haematococca</i>	<i>Aspergillus niger</i>
S5	<i>Zygosaccharomyces rouxii</i>	<i>Aspergillus niger</i>	<i>Nectria haematococca</i>
S10	<i>Candida dubliensis</i>	<i>Scheffersomyces stipitis</i>	<i>Podospora anserina</i>

<sup>a</sup> n.a.: could not be sequenced correctly.

**Table S5.** Enzyme activities of XR, XDH, and XKS homologues in the INVSc1 strain

	Specific activity (U/mg protein)			Specific activity (U/mg protein)			Specific activity (U/mg protein)	
	Average <sup>a</sup>	Stdev <sup>b</sup>		Average	Stdev		Average	Stdev
neg <sup>c</sup>	0.0078	0.0021	neg	0.0009	0.0001	Neg	0.0279	0.0135
afXR	0.0211	0.0029	aaXDH	0.0199	0.0012	afXKS	0.0218	0.0055
anidXR	0.0344	0.0012	anidXDH	0.0915	0.0032	afugXKS	0.0075	0.0195
anXR	0.0269	0.0013	anXDH	0.1546	0.0073	anXKS	-0.0080	0.0072
aoXR	0.0151	0.0030	aoXHD	0.0669	0.0091	anidXKS	-0.0200	0.0069
pgXR	-0.0022	0.0050	caXDH	0.2230	0.0280	aoXKS	0.0967	0.0122
cpXR	0.0829	0.0026	cdXDH	0.1658	0.0131	bfXKS	-0.0004	0.0011
csXR	0.0930 (0.0858) <sup>d</sup>	0.0145 (0.0043) <sup>d</sup>	csXDH	0.1982	0.0042	caXKS	-0.0047	0.0142
ctXR	0.1120	0.0111	ctXDH	0.2392	0.0020	cdXKS	0.1520	0.0301
klXR	0.0665	0.0080	klXDH	0.0289	0.0016	ctXKS	0.2266	0.0031
mgXR	-0.0005	0.0020	ncXDH	0.0900	0.0047	klXKS	0.0965	0.0065
paXR	-0.0015	0.0022	ncXDH-mut	0.0010	0.0001	ncXKS	0.0132	0.0030
pcXR	-0.0001	0.0017	nhXDH	0.1968	0.0113	nhXKS	0.0329	0.0005
pgXR	0.1106	0.0242	paXDH	0.0755	0.0083	paXKS	0.0549	0.0015
pnXR	0.0752	0.0116	pcXDH	-0.0002	0.0001	pcXKS	0.0595	0.0020
pnXR	0.0922	0.0050	pgXDH	0.0127	0.0004	pgXKS	0.2477	0.1468
ppXR	0.0200	0.0000	pnXDH	0.0759	0.0020	pnXKS	0.2910	0.1624
psXR	0.1080	nd <sup>e</sup>	ppXDH	0.0518	0.0012	psXKS	0.1930	0.0483
psXR-mut	0.0469 (0.0755) <sup>d</sup>	0.0108 (0.0058) <sup>d</sup>	psXDH	0.2286	0.0132	scXKS	0.2236	0.0795
tsXR	0.0129	0.0014	ptXDH	0.0001	0.0001	tsXKS	0.0567	0.0045
zrXR	0.0117	0.0022	tlXDH	0.1264	0.0052	zrXKS	0.2071	0.0044
			tsXDH	0.1019	0.0033			
			zrXDH	0.1569	0.0034			

<sup>a, b</sup> Average and standard deviation (Stdev) (n = 2)

<sup>c</sup> Negative control indicates background enzyme activities measured in the cells transformed with an empty vector. All enzyme activities were reported after subtraction with negative control.

<sup>d</sup> Values in the parenthesis indicate the activities with NADH

<sup>e</sup> Not determined (n = 1)

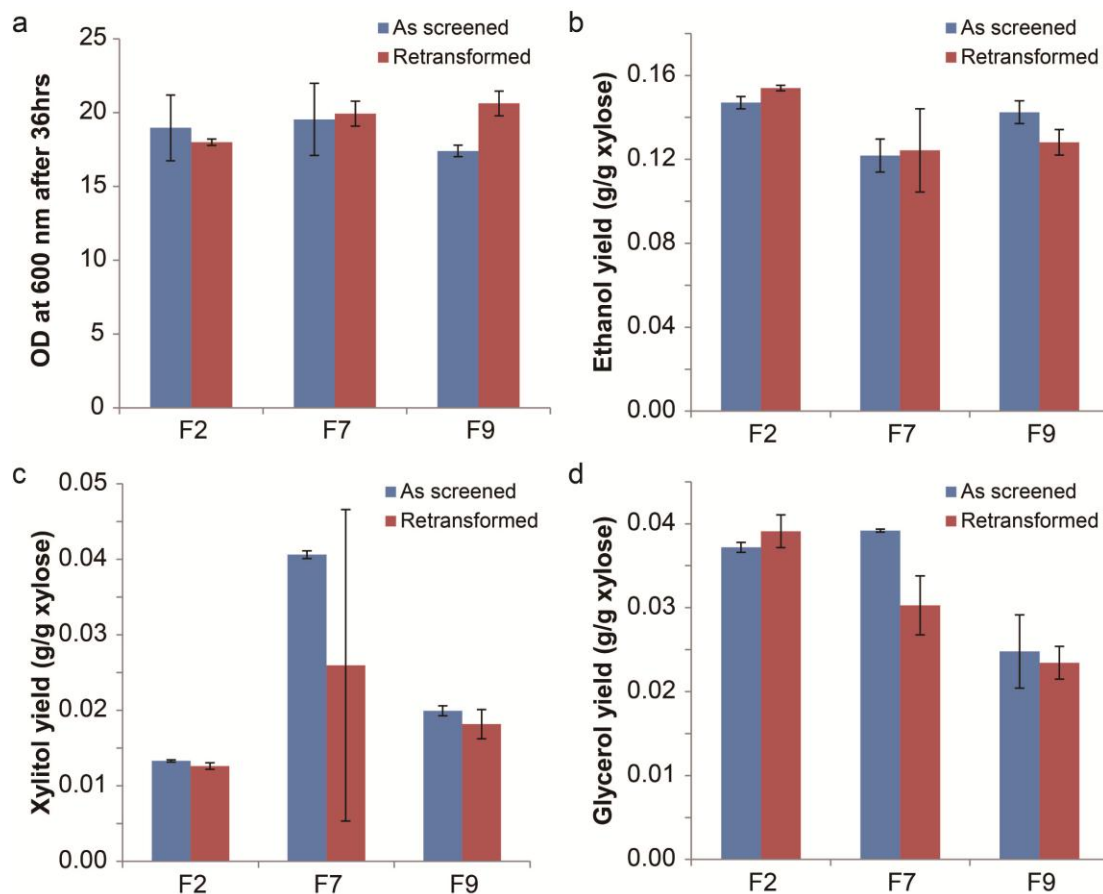
**Table S6.** Xylose and mixed sugar fermentation properties of the screened recombinants and comparison with the strains reported in literature.

Xylose (40 or 45 g/L)										
Host strain	Strain	Genotype	OD <sub>0</sub> <sup>a</sup>	Sugar consumption rate (g/L/hr)	Final biomass (cdw, g/L)	Y <sub>etoh</sub> <sup>b</sup> (g/g)	Y <sub>xyI</sub> <sup>c</sup> (g/g)	Y <sub>gly</sub> <sup>d</sup> (g/g)	Y <sub>acet</sub> <sup>e</sup> (g/g)	Reference
D452-2	MA-D4		~15	0.56	4.5	0.35	0.08	na <sup>f</sup> (<3 g/L)	na (<0.8 g/L)	
INVSc1	MA-N4	psXR, psXDH, scXKS1 single-copy integration	~15	0.56	4.5	0.36	0.10	na (<3 g/L)	na (<0.8 g/L)	(5)
Sc Type II	MA-T4		~15	0.50	7.8	0.34	0.09	na (<3 g/L)	na (<0.8 g/L)	
IR-2	MA-R4	psXR, psXDH, scXKS1 single-copy integration	~15	1.07	7.8	0.35	0.05	0.1	0.01	(6)
IR-2	MA-R5	psXR, engineered psXDH, scXKS1 single-copy integration	~15	1.49	7.8	0.37	0.04	0.07	0.01	(6)
INVSc1	INVSc1-F2	anidXR, caXDH, scXKS1 single-copy plasmid	10	0.48	11.0	0.24	0.01	0.04	0.02	This study
CTY	CTY-F3	anidXR, anXDH, pcXKS single-copy plasmid	10	0.51	11.6	0.19	0.07	0.01	0.00	This study
ATCC 4124	ATCC4124-F2	pgXR, pcXDH, aoXKS single-copy plasmid	10	0.93	13.4	0.27	0.07	0.01	0.00	This study
INVSc1	INVSc1-F2	anidXR, caXDH, scXKS single-copy plasmid	1	0.39	9.8	0.23	0.02	0.04	0.02	This study
CTY	CTY-F3	anidXR, anXDH, pcXKS single-copy plasmid	1	0.54	12.6	0.22	0.08	0.03	0.00	This study
ATCC 4124	ATCC4124-F2	pgXR, pcXDH, aoXKS single-copy plasmid	1	0.54	11.5	0.23	0.06	0.01	0.00	This study
ATCC 4124	ATCC4124-S10	psXR, psXDH, nhXKS single-copy plasmid	1	0.62	11.2	0.27	0.03	0.01	0.00	This study

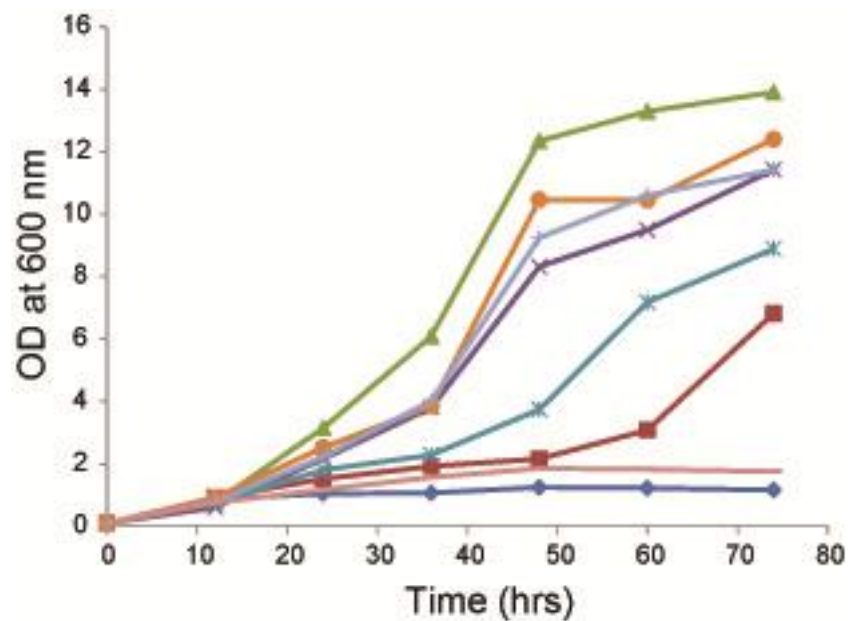
Continued (Table S6)

Xylose (80 g/L)											
Host strain	Strain	Phenotype/Genotype	OD <sub>0</sub>	Sugar consumption rate (g/L/hr)	Final biomass (cdw, g/L)	Y <sub>etoh</sub> (g/g)	Y <sub>xyl</sub> (g/g)	Y <sub>gly</sub> (g/g)	Y <sub>acet</sub> (g/g)	Reference	
D424-2	DA24	pxXR, engineered psXR, psXDH, scXKS1, single-copy integration	1	1.16	~6	0.34	na	na	na	(4)	
DA24	DA24-16	Evolved isolate from DA24	1	1.32	~6	0.35	na	na	na	(4)	
ATCC 4124	ATCC4124-S10	psXR, psXDH, nhXKS single-copy plasmid	1	0.88	~13.8	0.31	0.02	0.03	0.01	This study	
Glucose (70 g/L) and xylose (40 g/L)											
Host strain	Strain	Phenotype/Genotype	OD <sub>0</sub>	Sugar consumption rate (g/L/hr)	Final biomass (cdw, g/L)	Y <sub>etoh</sub> (g/g)	Y <sub>xyl</sub> <sup>g</sup> (g/g)	Y <sub>gly</sub> (g/g)	Y <sub>acet</sub> (g/g)	Reference	
Saccharmyces sp.1400	424A (LNH-ST)	psXR, psXDH, scXKS multi-copy integration	~4	2.29	9.5	0.43	na	na	na	(3)	
D424-2	DA24	psXR, engineered psXR, psXDH, scXKS1, single-copy integration	1	1.45	Na	0.39	na	na	na	(4)	
D424-2	DA24-16	Evolved isolate from DA24	1	1.78	Na	0.42	na	Na	na	(4)	
CTY	CTY-F3	anidXR, anXDH, pcXKS single-copy plasmid	5	1.63	13.8	0.39	0.05	0.04	0.02	This study	

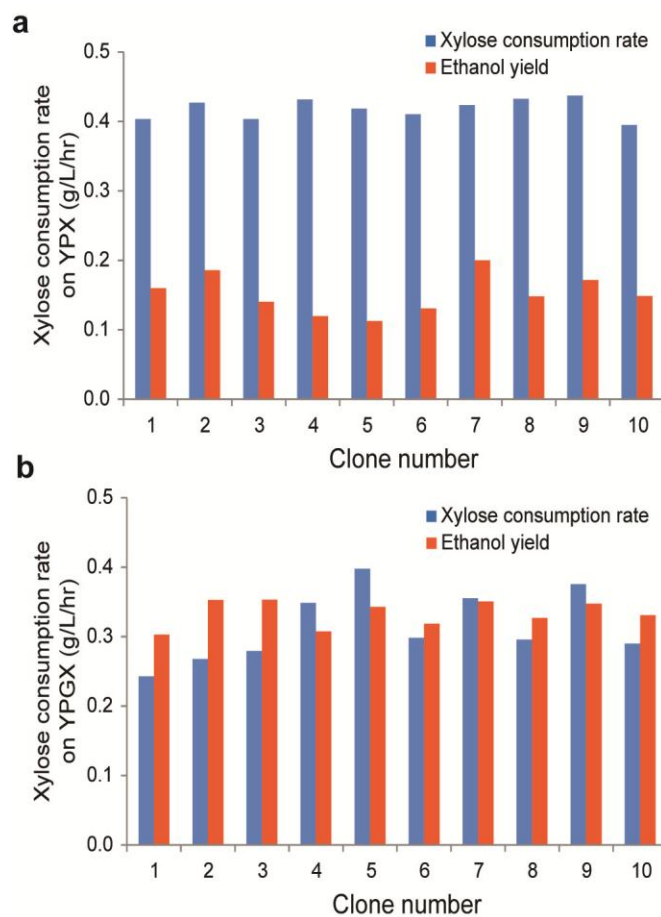
<sup>a</sup> Initial OD (600nm)<sup>b, c, d, e</sup> Ethanol, xylitol, glycerol, and acetate yield per consumed sugar, respectively.<sup>f</sup> Value was not reported.<sup>g</sup> Xylitol yield was calculated per consumed xylose in co-fermentation.



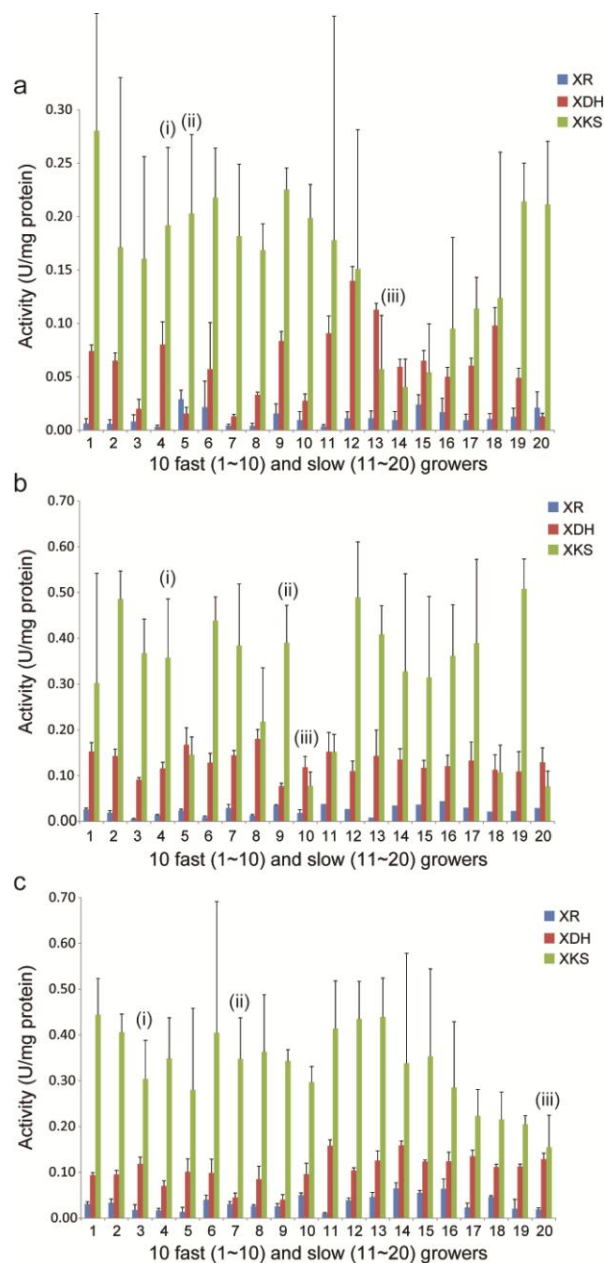
**Figure S1. Growth and xylose utilization of the selected recombinants as screened and after retransformed.** Error bar indicates standard deviation (n=2). Among 10 fast grower screened from INVSc1 library, three recombinants (F2, F7, and F9) were tested for xylose utilization (2% YPX) as screened and after retransformed with fresh host strain. Error bar indicates standard deviation (n=2).



**Figure S2. Diversity in the growth on xylose as a sole carbon source.** Eight clones were randomly picked from the library plate and their growth was determined on complex media containing 2% xylose as a sole carbon source.

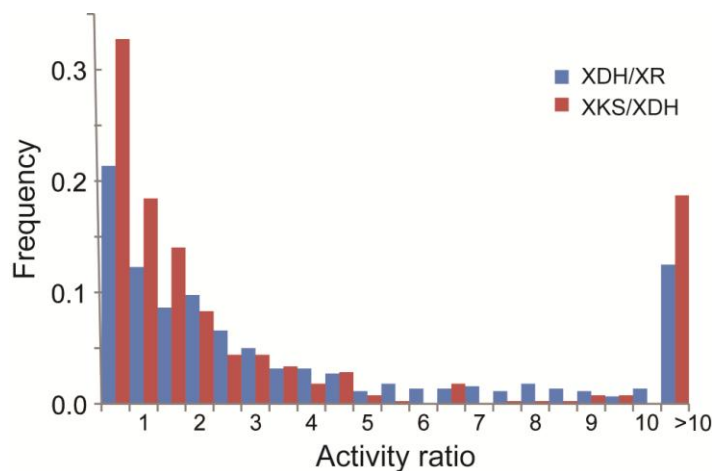


**Figure S3. Screening for single and mixed sugar fermentation.** Volumetric xylose consumption rates and ethanol yields of the fast 10 growers of INVSc1 strain on xylose (2% YPX) and mixed sugar of glucose and xylose (4/4% YPX).

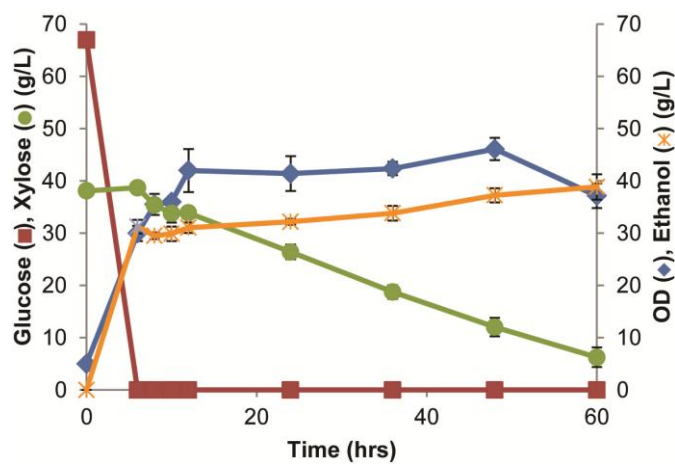


**Figure S4. XR, XDH, and XKS activities in 10 fast and 10 slow growers of INVSc1 (a), ATCC 4124 (b), and CTY strain (c).** Three different activity ratios found more than once were large XDH to XR (>5) and XKS to XDH (>2) ratios, small XDH to XR (<2) and large XKS to XDH (>2) ratios, and large XDH to XR (>5) and small XKS to XDH (<2) ratios. An example of each case is indicated with (i), (ii), and (iii), respectively. Error bar indicates standard deviation (n=3).





**Figure S5.** The distribution of the enzyme activity ratios in a theoretical library consisting of all possible combinations.



**Figure S6. Glucose and xylose co-fermentation of CTY-F3.** Cofermentation of 70 g/L glucose and 40 g/L xylose by CTY-F3. Cofermentation results were summarized in Table S5. Error bar indicates standard deviation (n=3).

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