

Supplementary

Tolerance of *Yarrowia lipolytica* to inhibitors commonly found in lignocellulosic hydrolysates

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Supplementary figures

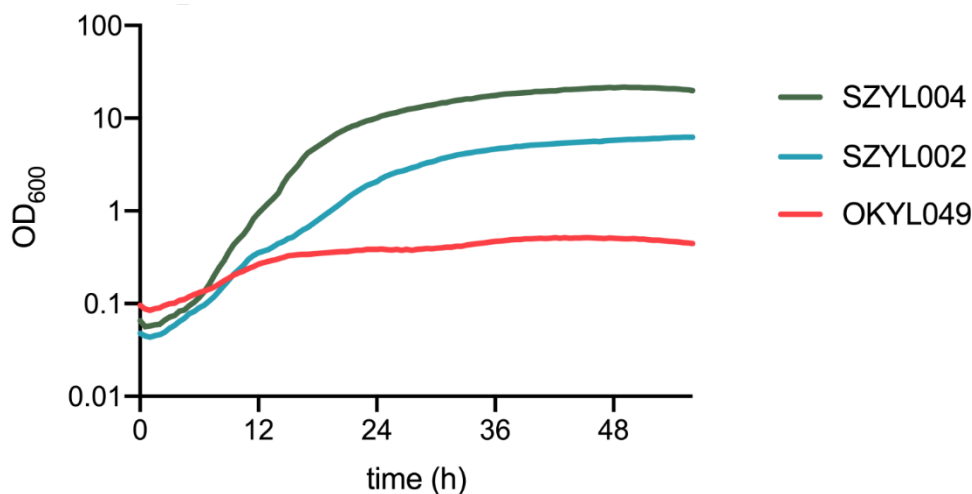


Figure S1: Growth curves of *Y. lipolytica* strains expressing xylose genes. Strains were precultured for 18 hours in LPU media with 10% glucose and then grown in LPU media with 10% xylose as sole-carbon source. Cells were cultured in 96-well plates and the OD₆₀₀ was measured with the growth profiler every 30 minutes. The curves represent the average of triplicates.

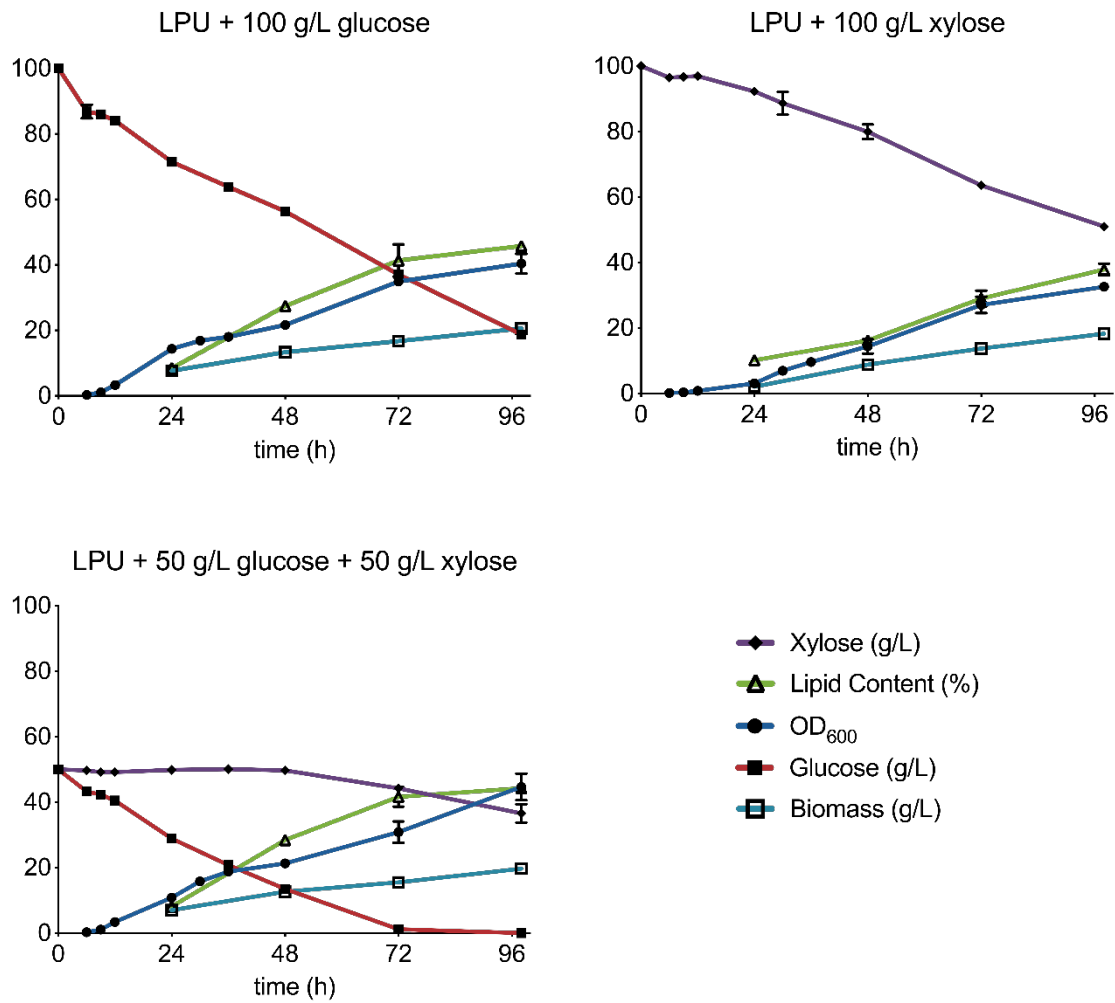


Figure S2: Shake flask cultivation of SZYL004 on different carbon sources. Glucose, xylose, OD₆₀₀, biomass and lipid content curves of SZYL004 on LPU containing different percentages of glucose and xylose as carbon sources. Strains were cultured in shake flasks for 96 hours. The curves represent the average, and error bars the standard deviation of triplicates, respectively.

Supplementary list 1: List of plasmids used in the study - Konzock, Zaghen, Norbeck 2021

Name	Plasmid contains	Yarrowia marker	Purpose of plasmid	Reference
pCfB6630	gRNA C3 locus	nourseothricin	expresses gRNA for cut in C3 locus of YaliEASYCLONE kit	EasyCloneYALI: CRISPR/Cas9-Based Synthetic Toolbox for Engineering of the Yeast <i>Yarrowia lipolytica</i>. Holkenbrink C, Dam MI, Kildegaard KR, Beder J, Dahlin J.
pCfB6633	gRNA E1 locus	nourseothricin	expresses gRNA for cut in E1 locus of YaliEASYCLONE kit	
SZ_EC_01	(pTef1+XK+tPex20)- (pGAPDH+XDH+tLip2) in Int_C3	marker-free	cut with NotI to linearize repair fragment for C3 locus, overexpressing XK, and XDH	this paper
SZ_EC_02	(pTef1+XK+tPex20)- (pGAPDH+XDH+tLip2)- (pPYK1+XR+tPex16) in Int_C3	marker-free	cut with NotI to linearize repair fragment for C3 locus, overexpressing XK, XDH, and XR	this paper, available on Addgene
OKEC012	pTef1in+DGA1+tPEX20 in Int_E1	marker-free	cut with NotI to linearize repair fragment for E1 locus, overexpressing DGA1	this paper

Supplementary list 2: List of primer sequences used in the study - Konzock, Zaghen, Norbeck 2021

	Name	Sequence	Target
repair fragments for deletion	are1_deletion-box-1	ggggcaaaaaggatacaagtcgttcttacaagactctccgcacacacagcagctcgcttcaccac ttgtcctatagaatgtcaaccactgtcaac	fused together to form repair fragment for ARE1 deletion
	are1_deletion-box-2	gttgacagtgttgacattctataggcacaagtggtgaagcgactgtgctgtgtgctgagagtgctt gtaagaacgactgtatcctttttgcccc	
	MHY1_deletion_box1	cgccaaaaccatccaatcgaaaaaataaaagtcatagtacattatcgccaaaaggtagagcgccttc tagtctccgctccattttttatgttaaccag	fused together to form repair fragment for MHY1 deletion
	MHY1_deletion_box2	ctggttacaataaaaaaatggagcggagactagaaggcctctaccttttggcgataatgtactatgac ttttgttttcgattgggatggtttggcg	
cut sites to build gRNA plasmids *	ARE1_Cut-site_FW	gaagagctcaatgtgcccgagttttagagct	cut sites for ARE1
	ARE1_Cut-Site_RV	tcgggcacattgagctcttcaaccaacct	
	ARE1_Cut-site_2_FW	gtttctgcagcatggccaggttttagagct	
	ARE1_Cut-Site_2_RV	ctggccatgctgcagaaaaataaccaacct	
	cut sites for MHY1	MHY1_cut-site_1_FW	aggcgacagcatgtaaagggttttagagct
		MHY1_cut-site_1_RV	ccatttacatgctgctccttaaccaacct
		MHY1_cut-site_2_FW	ctcgagaggcgagtaagtggttttagagct
		MHY1_cut-site_2_RV	ccacttactgcctctcgagtaaccaacct
screening primer for deletions	are1-del_FW_Screen	tgttgctgggtaagtcaaacc	ARE1 deletion screening: 616 bp for deletion / 2248 bp for WT
	are1-del_RV_Screen	aacttcagtgaagcattgtagc	
	MHY1-del_FW_Screen	tcttcaaccgtcccacacc	MHY1 deletion screening: 675 bp deletion / 1533 bp MHY1 WT
	MHY1-del_RV_Screen	caaagtggacaagcgactgc	
primer to build plasmid with xylose pathway (SZ_EC_02)	XK_Gibson_FW	ACACTTCAACGGAATGCGTGCATtatttctccaggcaggcg	Primers with overhangs used to create the fragments for the gibbon assembly of SZ_EC_02
	XK_Gibson_RV	agaatcattcaaatgtatctcggactggatctttcg	
	Tef1_Gibson_FW	gtccgagatacatttgaatgattcttatactcagaaggaaatgc	
	Tef1_Gibson_RV	attcaaccgcatcgagagaccgggtggcggcg	
	GAPDH_Gibson_FW	ccggtctctgcatcgggttgaatgaatcggccg	
	GAPDH_Gibson_RV	ggttagaagacattgtgatgtgtttaaattc	
	XDH_Gibson_FW	acacacatcaacaatgtcttcaaccgtcattg	
	XDH_Gibson_RV	CCGAACAGAAGGAATGCACGCGTActactctctcggaccgtc	
	pPYK1_Gibson_FW	gcttgaaggacattgtaactgtggtgtaattctcc	
	pPYK1_Gibson_RV	gccgattcattcaaccGCGattgctgcttctgtttg	
	XR_Gibson_FW	aatcatcaatccttaggcgaaatgggaaggttag	
	XR_Gibson_RV	caccacagttacaatgtcctcaagctgcctc	

	pex16_Gibson_FW	cgccgccaacccggctctgataagcttcgagaagcccgaac	
	pex16_Gibson_RV	catttcgcctaagcgattgatgattggaacac	
screening primers for integration	PR-14838	aaacctgtcgtatgagctac	screening of C3 locus: positive integration: RP-14838 + RP-8859 = 915 bp; negative: RP-14838 + RP-14588 = 1106 bp
	PR-8859	aagtgtgatggggaagtgag	
	PR-14588	acgcgaaattattgcacaggacacac	screening of E1 locus: positive integration RP-14442 + RP-8859 = 891 bp; negative RP-14442 + RP-14398
	PR-14442	agtttgaccaagacaaatg	
	PR-14398	cacgcgaugttagaagcaattggagaag	

* Marked in orange are USER cloning overhangs, black are the 20 nt CRISPR recognition sites.

Terminator	tPEX20			AAGTGTGGATGGGGAAGTGAGTGCCCGTTCTGTGTGCACAATTGGCAATCCAAGATGGATGGATTCAACACAGGGATATAGCGAG CTACGTGGTGGTGCAGGATATAGCAACGGATATTTATGTTTGACACTTGAGAATGTACGATACAAGCACTGTCCAAGTACAATACTA AACATACTGTACATACTCATACTCGTACCCGGCAACGGTTTCACTTGAGTGCACTGGCTAGTGCTCTTACTCGTACAGGTGCAATAC TGGTATCATAGTCTTTGATGTATATCGTATTCAATCATGTTAGTTGCGT
	tLIP2			CTTCTGTTCCGGAATCAACCTCAAGGTTAACGGCCACGATCCCTCGTTGTTACTCTTGGTCAGCCCATTGTGCGTAACGCTGGCTTTGCT AACTGGGTCGATAAACTCTTCTTTGGCCAGGAGAACCCCGATGTCTCAAGGTGTCAAAGACCGAAAGCTTACCGAATCACCCACC GAGGAGATATCGTCCCTCAAGTGCCCTTCTGGGACGGTTACCAGCACTGCTCTGGTGAGGTCTTTATTGACTGGCCCTGATCCACCCT CCTCTCTCAACGTTGTCATGTGCCAGGGCCAGAGCAATAAACAGTGCTCTGCCGGTAACACTCTGCTCCAGCAGGTCAATGTGATTG GAAACCATCTGCAGTACTTCGTACCCGAGGGTGTCTGTGGTATCTAAGCTATTTACTCTTTACAACCTTCTACCTCAACTATCTACTTT AATAAATGAATATCGTTTATTCTCTATGATTACTGTATATGCGTTCCTCTAAGACAAATCGAAACCAGCATGCGATCGAATGGCATA AAAGTTTCTCCGAAGTTGATCAATGTCTGATAGTCAGGCAGCTTGAGAAGATTGACACAGGTGGAGGCCGTAGGGAAACCGATCAA CCTGTCTACCAGCGTTACGAATGGCAAATGACGGGTTCAAAGCCTTGAATCCTTGCAATGGTGCCTTGGATACTGATGTCACAACTTA AGAAGCAGCCGTTGCTCTTCTCGAAACTCTCAAACACAGTCCAGAAGTCTTTATAGTTTGATCTGTATCCAGATAGCCTCCGTA ATTGGTGTGTCTTCAAATCCAGACGTCCACATTGGCATGTCTCCACTGATAAGCATTGAAAGTTCATCTGCGTTGAACATTGAGA CCCACGAAGGGTCAATGAGCTGGTATAGACCCCAAGAATGCATCTG
	tPEX16			GCGATTGATGATTGGAAACACACACATGGGTTATATCTAGGTGAGAGTTAGTTGGACAGTTATATATTAATCAGCTATGCCAACGGT AACTTCATTCATGTCAACGAGGAACCAAGTACTGCAAGTAATATAGAATTTGACCACCTTGCCATTCTCTTGCACTCCTTTACTATATCT CATTTATTTCTTATATACAAATCACTTCTTCTCCAGCATCGAGCTCGGAAACCTCATGAGCAATAACATCGTGGATCTCGTCAATAGA GGGCTTTTGGACTCCTTGCTGTTGGCCACCTTGCTCTTGTCTGGCTCATTCTGTTTCAACGCCCTTTCGCGCCAGACCATCAACCTT GTTGAGCTCTCCGTGAGCAGCTCGACCAGATCATCAAACCAAGACCTTGGCTCGAGTTCGGGCTTCTCGAAGCTT

Gene	XDH	YALIOE12463g	YALI1_E15452g	<p>ATGTCCTTAACCCGTCATTTGTTCTTCGAAAGCCATTGGATCTCGTCTTTGAGGATCGGCCCGACCCCAAGATCCAGGACCCCACTCC GTC AAGGTGGCAGTCAAAAAGACCGGAGTTTGGCGCTCGGATGTCCACTACTATCTGCATGGAGGAATCGGCGACTTCATTGTCAAG GCTCCCATGGTTCTAGGCCATGAAAGTGCCGGAGAGGTGGTTGAGTTGGTCTGAAAGTCAAGGACCTCAAGGTGGGAGATCGAGT GGCTCTCGAGCCGGAGTGCCGTCTCGATTGTACAGGAGTACAAGGAGGGACGATACAACCTGTGTCTTGCATGGTGTGGTCTCGAGGA ACCCCTCCCTACGACGGTACTCTGTGTCGTCACATACATCATTCCCGAGGACTTTTGTGTCAAGCTGCCTGATCATGTGTCTCTCGAGGA GGGAGCTCTTGTGGAGCCTGTCCGTGGCTGTCCACTGCAACAAGCTGGCCAAGACCACTGCCAGGACGTGGTTATTGATTTTGGGA GCTGGCCAGTCGGACTGTAGCCGTGGGAGTGGCCAATGCCTTTGGATCATCTACCATTGTGTGTGTTGATCTTGTCCCGAGAAGC TGGAGCTCGCCAAGAAGTTCGGTGCCACTCATACGTTTGTACCACCTAAGGGAGACAGTCCCAACGAGTCTGCTGACAAGATCCGAGC TCTGATCAAGGGCGCTGGTCTCTGACTCGCCAATGTGGCTTTGGAGTGCACCGGAGCTGAGCCTTCTATTCAAGACTGCTGTTTCTG TGCTGGCCACTCCGGTCGACTTGTGACAGTGGCATGGGCAAGGATGACGTCAACTTCCCTATCACCAAATGCATTGTAAAGGAGAT TACCGTCTCGGATCGTTCCGATACTGCCATGGTACTATCCCTGGCTGTTGAGCTGGTTGCTTCTGGCAAGATTGACGTCGAAGAAGC TGGTGACCAACCGGTTACCTTCAAGGAGGCTGAGCAGGCGTACAAGACGGCGGCCGAGGGCAAGGCCATCAAGATCATCATTGAC GGTCCCGAGGAGGAGTAG</p>
	XK	YALIOF10923g	YALI1_F14583g	<p>ATGTATCTCGGACTGGATCTTTGACTCAACAGCTCAAGGGCATCATTCTGGACACAAAACGCTGGACACGGTCACACAAGTCCATG TGGACTTTGAGGACGACTTGCCGAGTTCAACACCGAAAAGGGCGTCTTTCACAGCTCTACAGTGGCCGGAGAAATCAATGCTCTGT GGCAATGTGGGGGCGAGCTGTGGACTTGTGATAGAGCGTCTGTCAAAGGAAATAGACCTTCCACGATCAAGTTTGTGCGGGCTC GTGCCAGCAACACGGCTCTGTTTATCTCAACAGCAGCTACAAGGAGGGCCTGGGTTCTCTGGACAAACACAAAGACTTGTCTACAGGA GTGTCATCCTTACTGGCGCTCGAAGTCAGCCCAATTGGCAGGATGCAAGCACGGAGAAGGAGTGTGCGCAGTTTGGAGCTGCAGTC GGCGTCCCGAGCAGTGGCTGAGATCACTGGCTCTCGAGCACATACTCGTTTACCAGGGCCCAAGATTCTCAAGTCAAGGAACGC AACCCCAAGGATTCAAGGCCACGTACGGGTCCAGCTCATATCCAATTTCTAGCATCTGTTTGGCCGCAAGGGCTGCCCTTTGA TCTTGCTGACGCCTGTGGAATGAATCTGTGGGACATCCAGAATGGCCAGTGGTGAAGAACTCACAGATCTCATACCGATGACACC CACTCGGTGAGTCCCTCCTTGGAGACGTGGAACAGACCCCAAGGCTCTACTGGGCAAAATCTCGCCCTATTTGCTCTCAAGGGCT TCTCTCCCTCTTGTGAGGTGGCACAGTTTACAGGCGACAACCCAGGCACTATGCTGGCTCTCCCTTACAGGCCAATGACGTGATTGTG TCTTTGGGAACATCTACGACCGCCCTGTCGTAACAAACAAGTACATGCCGACCCCGATACCATGTGTTCAACACCCCATGGAGG GATACATGGGCATGCTGTGCTACTGCAACGGAGGTCTAGCACGAGAGAAGATCCGAGACGAGCTTGGAGGCTGGGACGAGTTAAT GAGGCGGCCGAGACCACCAACACAGTGTCTGCTGACGATGTCATGTTGGCATCTACTTTCACTACGAGAAATCCTTCTCGAGCAG GTCCCTTTGAACGACGTTTCTATCTACAACAGACAAAGTGAACAGCTTACAGAGATGGCTTCTCCAGAGGACTCACTGGCAACCGAACA CAAACCGCAGGCTCAAATCTCAAGGACAGTGGCCGCCACAAATGGACGCCACTGCCATCATTCAAAGCCAGGCCCTCAGTATCAAA ATGAGACTCCAACGCATGATGATGGCGATATTGAAAGGTGATTTTGTGGGAGGCGCTCGGTCAACACTGCTATCTGCAGCGTAA TGTCTGCCATCTTAAACCAACAAGGGCGCTGGAGATGTGGTCTGGAAATGGCAAACGCTTGTGCCATTGGAAGTGGCCATCACGC CTGGCTTTCGACCCCAACAAGACAGGCCAGGTACAGGTTACGAAGAAGAGGTCAAATACAAGAATGTGGACACAGACGTGCTACT CAAGGCGTTCAAGCTGGCCGAAAACGCCTGCCTGGAGAAATAA</p>

Gene	XR	YALI0D07634g	YALI1_D09870g	<p>ATGTCCTTCAAGCTCGCCTCCGGAAAGTCCATGCCAAGGTCGGATTGGCCTGTGGAAGGTCCCCGTGACAAGACCGCCGACCCG TCTACGGAGCCATCAAGAACGGTTACAGACTGTTTGACGGCGCCTTCGACTACCAGAACGAGCGAGAGGCCGGGAAGGTATCCGAC GAGCCATCAAGGATGGCTGGTCAAGCGAGAGGACATCTTCATACCACCAAGTTGTGGAACACCTTCCACTCAAAGGAGCACGCTCT GCAGATCGCCAAGGAGCAGAACGAGTGGTGGGGACTCGACTACATCGATCTCTACCTCATCCACTTCCCATCCCATGCAGTACATT CCCATCTCCGAGAAGGAGTGGGCTGGATGGACCAACGCCACTGACTCGGGTCTAACCCTCTGGCCAAGATCCCTACCCGAGAGACCT GGGAGGCTCTCGAGGAGCTAGTTGATACCAGGAATCGCCAAGTCCATTGGTGTCTCCAATTACCCGCCAGAACATTTACGACGTCCA GACCTACAACAAGCACCCATTCTGCTCTGCAGATTGAGCACCACCCCTACCTGGTGCAGCCCCAGCTGACCCAGCTCGAAAGGAC AACACATCCAGGTCACTGCCTACTTTTTCGGCCCCGCTCCTTTGTGGAGATTGGCATGGACCAGAAGTCCCTCTCTTTTCGA GAACGAGACCATCACAAGATCGCCAAGGCTCACAACAAGACCCCTCCCAGGTTCTGCTGCGATGGGCTACCCAGCGAGGCATTGC CGTCATCCCCAAGTCCAACAACGTCGAGCGACAACTCAGAACCTCGAATCTCTGGACTTTGACCTGACCCGAGGCCGAGATTAAGGAG ATCTCCAACCTCAACAAGAACCTGCGATTCAACGATCCCGGTGTCTACGCTAACCTTCCCATTTTCGCTAA</p>
	DGA1	YALI0E32769g	YALI1_E38810g	<p>(ATG)ACTATCGACTCACAATACTACAAGTCGCGAGACAAAAACGACACGGCACCCAAAATCGGGGAATCCGATATGCCCGCTATCG ACACCATTACTCAACCGATGTGAGACCTTCTCTGGTCTGGCACAATTTTACGATTCCCACTTCTCACAATTTTCATGCTATGCTGCG CAATTCACCTGCTCTGGCCATTTGTGATTGCGTATGTAGTGTACGCTGTTAAAGACGACTCCCGTCCAACGGAGGAGTGGTCAAGCG ATACTCGCCTATTTCAAGAACTTCTTCATCTGGAAGCTCTTTGGCCGCTACTTCCCATAACTCTGCACAAGACGGTGGATCTGGAGC CCACGCACATACTACCTCTGGACGTCCAGGAGTATCACCTGATTGCTGAGAGATACTGGCCGAGAACAAGTACCTCCGAGCAAT CATCTCCACCATCGAGTACTTTCTGCCGCTTCATGAAACGGTCTTTCTATCAACGAGCAGGAGCAGCCTGCCGAGCGAGATCCTC TCCTGTCTCCGTTTCTCCAGCTCTCCGGTCTCAACTGACAAGTGGATTAACCACGACAGCAGATATAGCCGTGGAGAATCATCT GGTCCAACGGCCACGCTCGGGTCCGAACCTAACGGCAACGGCAACAATGGCACCCTAACCGACGACCTTTGTGCTCCGCTCTG CTGGCTCCACTGCATCTGATTCCACGCTTCTAACGGGTCCTCAACTCCTACGCCAACCCAGATCATTGGCGAAAACGACCCACAGCTG TCGCCCACAAAACCAAGCCCACTGGCAGAAAATACATCTTCGGTACCACCCACGGCATTATCGGCATGGGAGCCTTTGGTGGAA TTGCCACCGAGGGAGCTGGATGGTCCAAGCTTTTCCGGGCATCCCTGTTTCTTATGACTCTCACAACAACCTCCGAGTGCCTCTCT ACAGAGAGTACCTCATGAGTCTGGGAGTCGCTTCTGTCTCAAGAAGTCTGCAAGGCCCTCCTAAGCGAAAACCACTCATCTGCAT TGTCGTTGGTGGAGCACAGGAAAGTCTTCTGGCCAGACCCGGTGTGATGGACCTGGTACTCAAGCGAAAAGGTTTTGTTGACTT GGTATGGAGGTCGGAAATGTCGCCCTTGTCCCATCATGGCCTTTGGTGGAGAACGACCTCTATGACCAGGTTAGCAACGACAAGTCGT CCAAGCTGTACCGATTCCAGCAGTTTGTCAAGAATTCCTTGGATTACCCTTCTTTGATGCATGCCGAGGGCTTCAACTACGAT GTCGGTCTTGTCCCTACAGGCGACCCGTCAACATTGTGGTGGTTCCTTACTTACTTATCTCCACACCCACCCGACGAAGA AGTGTCCGAATACCACGACCGATACATCGCCGAGCTGCAGCGAATCTACAACGAGCACAAGGATGAATATTTTCATCGATTGGACCGA GGAGGGCAAAGGAGCCCCAGAGTTCGAATGATTGAGTAA</p>