

SUPPLEMENTARY MATERIAL

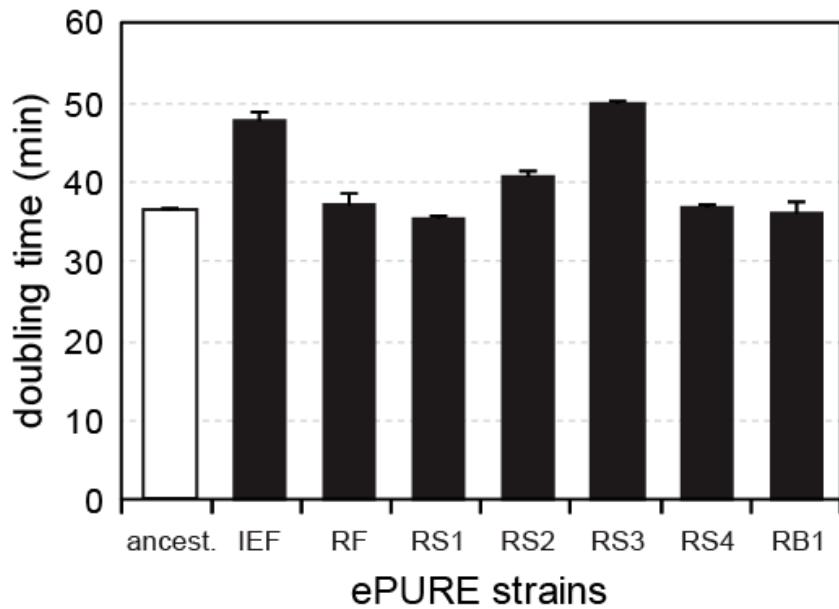
Multiplexed *in vivo* His-tagging of enzyme pathways for *in vitro* single-pot multi-enzyme catalysis

Harris H. Wang^{1,2*}, Po-Yi Huang^{1,3*}, George Xu^{1,4,5}, Wilhelm Haas⁶, Adam Marblestone^{2,4}, Jun Li¹, Steven Gygi⁶, Anthony Forster⁷, Michael C. Jewett⁸, George Church^{1,2}

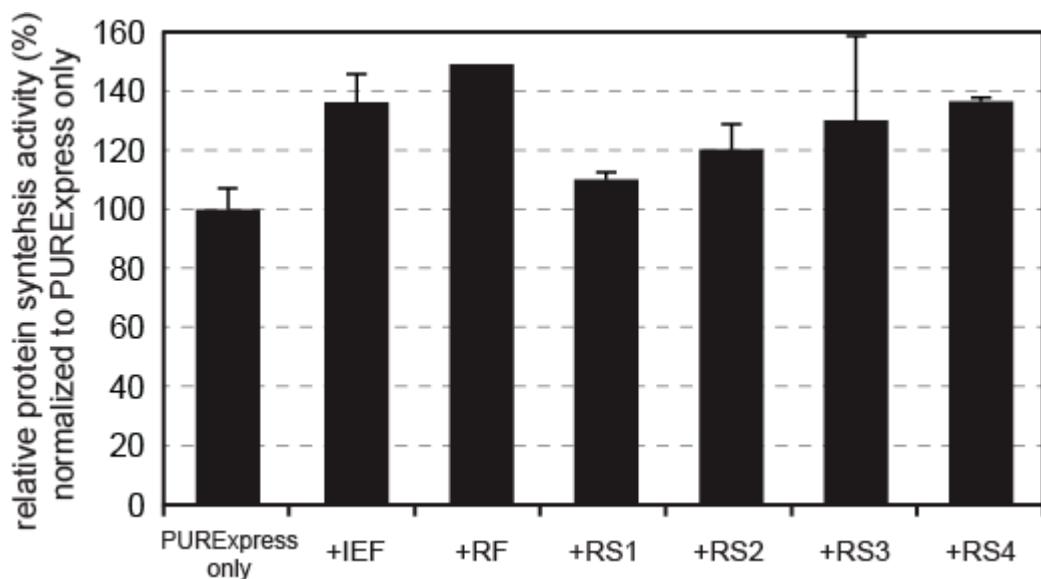
1. Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.
2. Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA.
3. Program in Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA.
4. Program in Biophysics, Harvard University, Cambridge, MA 02138, USA.
5. Program in Medical Engineering Medical Physics, Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA.
6. Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA.
7. Department of Cell and Molecular Biology, Uppsala University, Uppsala 75124, Sweden
8. Department of Chemical and Biological Engineering and Chemistry of Life Processes Institute, Northwestern University, Evanston, IL 60208, USA

* These authors contributed equally to this work.

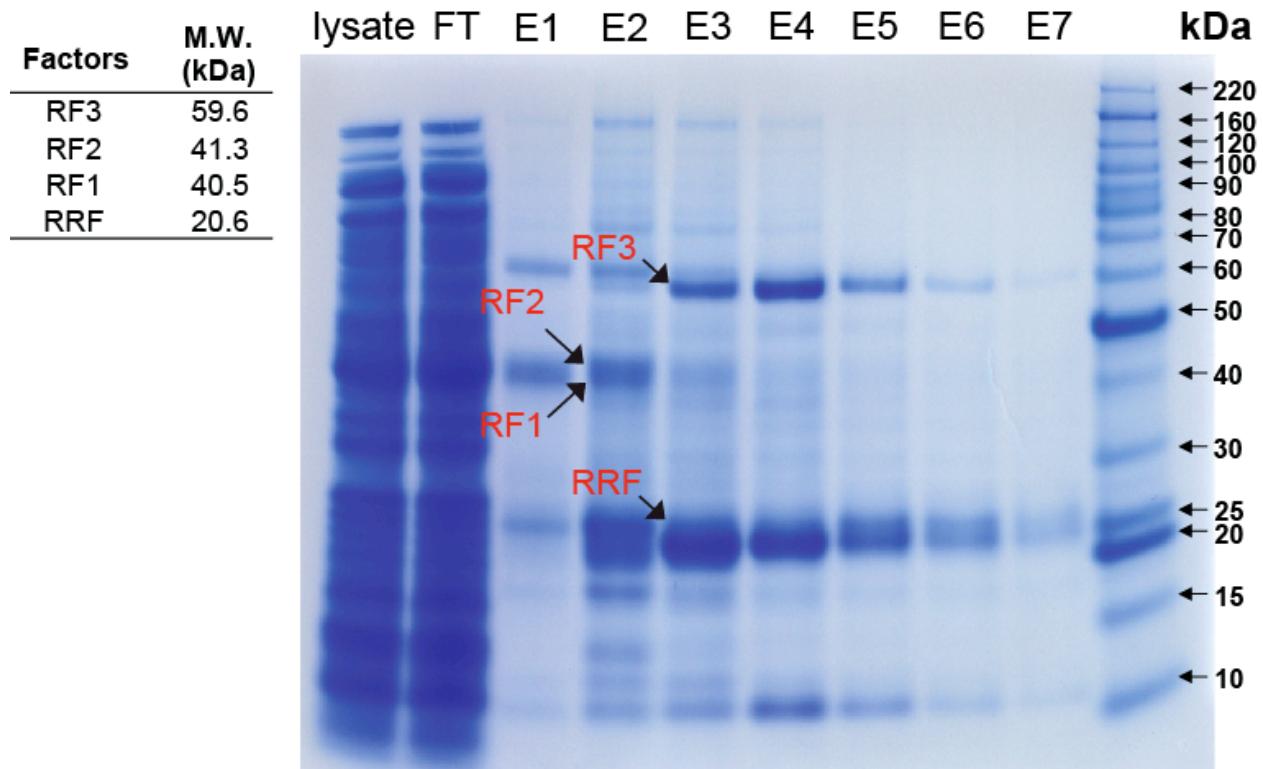
Correspondences should be addressed to H.H. Wang at harris.wang@wyss.harvard.edu or G. M. Church at gmc@harvard.edu.



Supplementary Figure 1. Growth rates of strains (black bar) in comparison to the ancestral strain (white bar) as determined by time-course spectrophotometry in a 96-well plate. Error bars are \pm standard deviations, with n=3. RB2 and RB3 growth rates are not shown since they were not used for subsequent ePURE systems.



Supplementary Figure 2. Supplement PURE in vitro translation reaction with each ePURE pool of factors to examine the presence of any hypothetical inhibitory contaminants. Translation reactions producing luciferase are performed in 10 μ L PURExpress reaction with the following amount of ePURE factors added: 0.372 mg/mL of IEF, 0.311 mg/mL of RF, 0.176 mg/mL of RS1, 0.663 mg/mL of RS2, 0.172 mg/mL of RS3, and 0.116 mg/mL of RS4. Activities are measured by luminescence and PURExpress reaction without supplement is set as standard for comparison and normalized to 100%. Error bars are \pm standard deviations, with n=3



Supplementary Figure 3.

Protein gel of His-tagged RF factors purified from Ni-NTA resin. The resin is washed with lower concentration of 35mM Imidazole in order to prevent RF factors detaching during wash step. Factors eluted consecutively with a linear gradient from 35mM to 400mM Imizadole.

Supplementary Table 1. Oligos and primers for ePURE experiments

MAGE Oligos		
gene	terminus	oligo sequence (* = phosphorothioate bond)
tsf	C	a*g*a*gccggccctcaggcggtcttttgcataattaaATGGTGTatGTTGatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
infA	N	t*c*a*attacggtagtgatgaccccagaggatgtCACcatCACCATGCCATgc当地aaagaacaatattgaaatgc当地acgttaccgtt
infC	N	g*t*a*ggccggccgttgtatcgcttgc当地atcgatgtttccgc当地ttATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
lepA	C	g*a*t*caggccaaacatccggccatccaaactctaaagggttaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
infB	N	t*c*g*c*ttcaatcaaactaattaaacgtgtatattaaATGGTGTatGTTGatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
tufA	C	g*c*c*ttcaatcaaactaattaaacgtgtatattaaATGGTGTatGTTGatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
fusA	C	g*a*g*a*gccggggactgttgtatccatccggccatccaaalgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
tufB	C	g*a*t*g*c*ttccatccggccatccaaalgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
frr	C	a*t*c*actgacggccgttgttgc当地aaagaataatATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
prfA	N	a*t*g*c*agggtccatgttgc当地aaacatgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
prfB	N	g*t*c*t*ggatcgatgtttatccggccatccaaatATTGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
prfC_C	C	g*c*g*c*ggccggccctcgaggccaaataatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
prfC_N	N	g*c*g*c*ttccatccggccatccaaalgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
ileS	N	t*g*t*g*c*ttccatccggccatccaaalgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
proS	C	g*a*t*g*ggatcatgtccatatacttgc当地aaacatgtttatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
cysS	C	g*g*a*t*gccaaaatccgc当地aaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
leuS	C	t*a*c*g*accatgttgc当地aaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
glnS	N	a*c*t*g*accatgttgc当地aaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
serS	C	g*c*a*g*ggcccttttagatcgccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
asnS	N	t*t*a*t*tccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
tyrS	C	g*t*a*t*gccatccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
pheT	C	t*c*t*g*accatgttgc当地aaatcgccatcaATGGTGTatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
pheS	N	a*t*g*g*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
thrS	N	a*t*g*c*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
aspS	C	g*c*a*a*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
argS	N	t*t*t*t*gccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
metG	C	a*t*g*a*t*tccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
gltX	C	t*t*a*t*ccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
hisS	C	t*c*t*g*accatgttgc当地aaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
alaS	N	g*a*g*a*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
lysS	C	a*c*a*t*tccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
fmt	C	t*c*t*g*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
trpS	C	a*t*g*a*ccaaacggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
glyS	C	t*g*c*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
glyQ	C	c*c*a*c*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
valS	C	a*a*a*c*t*gatgtccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
rpsB	C	c*t*c*ccggccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
rplC	C	g*c*g*t*ccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa
rplL	C	c*t*g*a*t*tccatccggccatccaaatcgccatcaATGGTGTatGTTGatGTTGatGactgttgtggacatcgccagaaccttcgtctgcaaa

MasPCR Forward Primers			MasPCR Reverse Primers		
gene	amplicon	primer sequence	gene	amplicon	primer sequence
tsf	700	TGTCCAAGCAGTCCTCACCAT	tsf	700	CGCCGGATCAGGGG
infA	600	CCAGAGGATTAGATGCACCAT	infA	600	ACGAGCTCTTAAATAGCGC
infC	500	AATTGGGAGATAAGTTTCCACCAT	infC	500	GACCAACTGCGATTTCTTC
lepA	400	GGCAAAGACAACACACCAT	lepA	400	GCGTTTCTGATAGATAGGATCTTA
infB	300	AAAGGAACAGCATGCACCAT	infB	300	CGTTTCAAAGGTGCGTT
tufA	250	CTAAAGTCTCTGGGACCAT	tufA	250	GAACCCCTAACATGAATTAAATTACGCC
fusA	200	GGCCGTGGTAAACACCAT	fusA	200	GTACGGTGGTATTCCAGC
tufB	150	GCAAAAGTCTCTGGGACCAT	tufB	150	CCTGTCAGCAATGATTATGACA
frr	500	GAACTGTGCACTTCACCAT	frr	500	GTATTCTTATTGGCCAGCAAA
prfA	400	GCATTTACGCCCTATGCACCAT	prfA	400	AAAGCGCCGCTTCG
prfB	300	AAGAATCAGACATGCACCAT	prfB	300	CGTGTAGCTTACAGC
prfC_C	250	CCGCAGCATCACCAT	prfC_C	250	GTGAAATGACAATCGACGGC
prfC_N	200	CTCAAGAAGAAAATTATGCACCAT	prfC_N	200	GTCCGACTTAGCTGCTG
ileS	600	CCGAGAATCTGATGCACCAT	ileS	600	CAGAACCGCAGTCACGC
proS	500	TGAAACAGATTAAGGCCACCAT	proS	500	TGGCTTGTGCGGG
cysS	400	GGCGTGTAAAGCACCAT	cysS	400	ACCCATTGCGTGGTITTG
leuS	300	CTGGTCGTGGCACCAT	leuS	300	ATCGGTATCTTCGCGAT
glnS	250	GAATCCACGATGCACCATCAC	glnS	250	ACTAACATACTCGATATCTTCTTACC
serS	200	GACTGGAATATATTGGCCACCAT	serS	200	GCAGTCCGCTATAGGCTTGT
asnS	600	ACTTAAACAGAGAGAATTATATGCACCAT	asnS	600	GTTACGGGGCAGGTTTC
tyrS	500	TCTGATTTGCTGAAACACCAT	tyrS	500	AGGCAAACCGTGGCC
pheT	400	GCATCATTTGAGGGATCACCAT	pheT	400	GATTTACCGTGAETCTTGAC
pheS	300	ATGAGGAAAACCATGCACCAT	pheS	300	ATCAATCGTTCGCGCC
thrS	250	TGCAATAAAGGATATAAACACCAT	thrS	250	AGGAGTACGAATGATCTCCA
aspS	200	GAAGGCTGAGAAACACCAT	aspS	200	GTGCGTAGATGACCAACTAACGTC
argS	600	AGTATTCCGGTGCACCAT	argS	600	CGGTAGAAAACCTTCAGGTCAG
metG	500	GGTCATCGGTAAACACCAT	metG	500	AGCATCTGTATTTTGTCAAGC
gltX	400	CGAAAATCAGCAGCACCAT	gltX	400	GTTAACCGTGGATCAGAGAAAAG
hisS	300	CACGTTACTGGGTACCAT	hisS	300	ATAACTTATTTTATTTTCAAGCAGCAAATTTTC
alaS	250	CTTATTTCAGGATAATTATGCACCAT	alaS	250	GTTTACCCACCGCAGC
lysS	200	GCCTCGGTAACACCAT	lysS	200	CAATGGTGTATTAGCAGATGTATTAC
fmt	500	AACCGTCTGGTACCAT	fmt	500	AGATAACGTCATACTGGC
trpS	400	GGAAAGCCGCACCAT	trpS	400	TTTCAATCGATTACCGCAGTTTC
glyS	300	TCGCTTGTGCAACACCAT	glyS	300	GTCACACTTTGGGGTCACTGA
glyQ	250	GTGCAACAAAGATAAGCACCAT	glyQ	250	TTCGATTTCACGATCCGGT
valS	200	CCGGCTGTGACCAT	valS	200	CAAAAATACGTATTGCTGGG
rplC	300	GCTGAAGTGAAGTAAACACCAT	rpsB	300	GCGGTAATTTCAGGCCATTCTAAAT
rpsB	300	TCGAGAAGCTGAGCACCAT	rplC	300	CCGGAACCAGTAACTCAGC
rplL	200	CTGTAAGGCGCACCAT	rplL	200	CAGTTGTGACACTTTGTTG

Sequencing Verification Forward Primers		Sequencing Verification Reverse Primers	
gene	primer sequence	gene	primer sequence
tsf.f	gtaaaaacgacggcaGGCCGCATGAAGAAATTCA	tsf.r	GTTCAACAGCTTTGATTCCTG
infA.f	gtaaaaacgacggcaTTTTCGCGTCAGGTAA	infA.r	AATAAAAAGGCCGTTAACCG
infC.f	gtaaaaacgacggcaTTGGCTTAAATCCGCGAG	infC.r	TTTCCCTAACCTGGATAACTTTTG
lepA.f	gtaaaaacgacggcaACGCACATCATGGCG	lepA.r	ACCTGACGGGATCTGGAA
infB.f	gtaaaaacgacggcaAAGGGGAGATCGTGTATT	infB.r	GATCGCGTTCACAAAGGT
tufA.f	gtaaaaacgacggcaCCGAGTTACTTCGTAC	tufA.r	CCCTTGTGACGTTAAACTGC
fusA.f	gtaaaaacgacggcaCGCAGTTACTTCGTAC	fusA.r	GTGTCGTATTCAACGTGAGAAGT
tufB.f	gtaaaaacgacggcaCCGAGTTACTTCGTAC	tufB.r	CACTTCATCGCTTCCAGGC
frr.f	gtaaaaacgacggcaGAAGCAGAACAGCGC	frr.r	AGAAGTTTCGCACTCGCTT
prfA.f	gtaaaaacgacggcaCTTGAGCAGGCCG	prfA.r	ATTGCTGTCCAGTTGCTCG
prfB.f	gtaaaaacgacggcaGGCCGATAACCGCG	prfB.r	GGTTTCTTCGTCGTGAGCT
prfC_C.f	gtaaaaacgacggcaGAGTCAGTCAACGTTCCA	prfC_C.r	AAAAGCGATGAGTGTGAATAACG
prfC_N.f	gtaaaaacgacggcaTTGGACCTGCTG	prfC_N.r	AGAAGTCTTGTGCCCCG
ileS.f	gtaaaaacgacggcaATGTTCAATGACCTTAC	ileS.r	CCAGCCAGGCACATAC
proS.f	gtaaaaacgacggcaGTGCTGCTGGATGACCG	proS.r	CCCCAAGGCTTGAGGAGA
cysS.f	gtaaaaacgacggcaCAAGAACCGGAAGCGTCC	cysS.r	GTTTTAACCGTCTCGTTGCTATC
leuS.f	gtaaaaacgacggcaATGGTGGAAAGACTCCACG	leuS.r	GGAATACCGATGCGGTATCTT
glnS.f	gtaaaaacgacggcaTGCTTATGCTGATGCCA	glnS.r	TACCAAGCAGTGAACACCTAAC
serS.f	gtaaaaacgacggcaTCCAGGCACGTCATG	serS.r	CGACTCACCTCAGCAGC
asnS.f	gtaaaaacgacggcaTCGACCTTCCGATATTAAAAAG	asnS.r	TGCTGGCCTGAATTCAATTG
tyrS.f	gtaaaaacgacggcaCTGATGAGGCACTGTC	tyrS.r	CAGGTGTGAAATTATCAATGGCG
pheT.f	gtaaaaacgacggcaGTTGGCGTAAATCAGGTAGTT	pheT.r	GCGTTTCGACCAGG
pheS.f	gtaaaaacgacggcaTTGAAAATAAAAGCCTGTCATCA	pheS.r	GAGAGACATCAATGTTCCCG
thrS.f	gtaaaaacgacggcaAGGTACAACTCCCTTTTTAGTGT	thrS.r	GGCCGATTGCCATTGTTGG
aspS.f	gtaaaaacgacggcaACTCGCCGCACG	aspS.r	TTCGCGCATGGCAGC
argS.f	gtaaaaacgacggcaTTCAGTAAAGCGTTAATTACCTG	argS.r	CATGTTCAGCCAGGAATGC
metG.f	gtaaaaacgacggcaGTCCTCGCTATTGTTCTG	metG.r	CCGCTGCCGAGC
gltX.f	gtaaaaacgacggcaGTTCATCACCTATTAGGC	gltX.r	CAAAGTTGCAGTTATTCAAGACA
hisS.f	gtaaaaacgacggcaAAATTGATGACCAACCGC	hisS.r	CCAGCGCACCATAAAGTATTTTATT
alaS.f	gtaaaaacgacggcaCCAGCGTTCTGCTCTATCG	alaS.r	TGCCCAGCATTGCAAGAA
lysS.f	gtaaaaacgacggcaTTCTGGATCAGGTTGC	lysS.r	GCTACCCGCCAAC
fmt.f	gtaaaaacgacggcaAAAGCATCGGTATGATACGG	fmt.r	CAGGTAATGCACGGTCCG
trpS.f	gtaaaaacgacggcaATGTATGGTCATCTGAAAGGTGAAG	trpS.r	AATTTCAGTAAATTGAATATTATTCAGGAATT
glyS.f	gtaaaaacgacggcaAACGTTGGAGCCGTACTTAC	glyS.r	TGTTAACCTGTCACATTGTTGG
glyQ.f	gtaaaaacgacggcaAATCCGCTGCCGCTG	glyQ.r	TTCTGCCGCTTGCTCG
valS.f	gtaaaaacgacggcaGAAGATGAGCTGGCGCG	valS.r	CGTACCAACGGTATGCTTTATCG
rpsB.f	gtaaaaacgacggcaGCAAACAACCTGGTATTCC	rpsB.r	TTACCAAGGGATGCCGTAAATTTC
rplC.f	gtaaaaacgacggcaGGTTCTATCGGTCAAACCG	rplC.r	TGGTCTCGCGACGAG
rplL.f	gtaaaaacgacggcaCTCAGATCGACCGCCTG	rplL.r	GAGCAGCCGGTCAG