ADDITIONAL FILE 1

List of tables

List of figures

Table S1. Primers used for cloning of *A. pleuropneumoniae gshF* gene optimized according to codon usage of *S. cerevisiae.*

Primers	Oligonucleotide sequences (5'-3')
APF1_1	GAATTCCATATGAAATTACAACAGTTGATTAAAACTCA TCACTTAGGTTTGCTATT
APF1_2	CATCACTTAGGTTTGCTATTTCAACAGGGTAAATTTGG CATCGAAAAGGAATCTCAAAG
APF1_3	CGAAAAGGAATCTCAAAGAATTGATAATAAAGGTAAT ATTGTTACTACAGCCCATCCTT
APF1_4	TTACTACAGCCCATCCTTCTGTTTTTGGTAACAGATCAT ATCATCCATATATTCAGACC
APF1_5	CATCCATATATTCAGACCGATTTTGCAGAAAGTCAGTT AGAATTAATTACACCACCTAA
APF1_6	ATTAATTACACCACCTAACGATACATTGGAAGACACAT ATAGATGGCTATCTGCTATTC
APF1_7	GATGGCTATCTGCTATTCATGAGGTAACATTAAGATCA TTGCCAGATGATGAATATATT
APF1_8	CCAGATGATGAATATATTTTCCCATTTTCTATGCCTGCC GGTTTACCGCCAGAATTTGA
APF1_9	TTTACCGCCAGAATTTGAAATTAAAGAGGCACAATTAG ATAACGAATGGGACGTGAAAT
APF1_10	TTGTATTTGCCATAAATGGCAGACAAATGTTCTCTATA TTTCACGTCCCATTCGTTATCGATAACGAATGGGACGT GAAATATAGAGAACATTTGTCTGCCATTTATGGCAAAT ACAA
APF1_11	CAGAAATTTGGAAATTATAGTGAATACCTGACACCATT TGCTTGTATTTGCCATAAATGCATTTATGGCAAATACA AGCAAATGGTGTCAGGTATTCACTATAATTTCCAAATT TCTG
APF1_12	GTATTCCGTTTGTAATGCAAATGTAGATTCGACAAACT CTTCAGAAATTTGGAAATTATATAATTTCCAAATTTCT GAAGAGTTTGTCGAATCTACATTTGCATTACAAACGGA ATAC
APF1_13	AATTCCATATATAACGCATTTCTGAACGCAATTTTATCT CTGTATTCCGTTTGTAATGCGCATTACAAACGGAATAC AGAGATAAAATTGCGTTCAGAAATGCGTTATATATGGA ATT
APF1_14	AATAAACTAAAATCCATTGGTATCTTAAAAAGTTATTG GCTAATTCCATATATAACGCATGCGTTATATATGGAAT TAGCCAATAACTTTTTAAGATACCAATGGATTTTAGTT TATT
APF1_15	ACCGAAATATTGCGCTTCTACAGTTGGGGTTGCGGCTA ACAAATAAACTAAAATCCATTAATGGATTTTAGTTTAT TTGTTAGCCGCAACCCCAACTGTAGAAGCGCAATATTT CGGT
APF1_16	AAACTTCTTACTAATTGACCTTCTGCCAATGGTGAGTTT TTACCGAAATATTGCGCTTCGAAGCGCAATATTTCGGT AAAAACTCACCATTGGCAGAAGGTCAATTAGTAAGAA

	GTTT
APF1_17	CAATATGTGGCGCATTTACATAACCATAAGGGCCAGAC CTTAAACTTCTTACTAATTGATCAATTAGTAAGAAGTT TAAGGTCTGGCCCTTATGGTTATGTAAATGCGCCACAT ATTG
APF1_18	AAAGAGGTCGACATATTGTTGCAAGCTGTCGTGGTTGA TCACAATATGTGGCGCATTTATAAATGCGCCACATATT GTGATCAACCACGACAGCTTGCAACAATATGTCGACCT CTTT
APF2_1	GAATTCCTCGAGTCTTTAGAACATTTTGTAGCAACCGG CGAT
APF2_2	AACATTTTGTAGCAACCGGCGATTTGTTGGCAGAAAAA GAATTTTATTCAAACGTTAGA
APF2_3	TTTTATTCAAACGTTAGATTAAGGGGTGCGAAAAAGGC AAGAAAATTGTTAGAGAAGGG
APF2_4	AAAATTGTTAGAGAAGGGTGTTAAATATGCGGAATTTA GATTATTTGATTTGAATCCTT
APF2_5	TATTTGATTTGAATCCTTTTTCTCCTTACGGTATCGAAT TAGCAGACGCGAAATTTATT
APF2_6	GCAGACGCGAAATTTATTCATTTGTTCTTATTGGCGAT GTTGTGGATGGATGAAACATC
APF2_7	GTGGATGGATGAAACATCTGGTCAAAGAGAAGTCGAA ATTGGTACACAAAAATTATACC
APF2_8	GTACACAAAAATTATACCAAGTTGCCTTGGAAGATCCT AGATCACACACTGCGTTCCAA
APF2_9	TCACACACTGCGTTCCAAGCAGAGGGTGAGGCGATCTT GAACTTAATGTTGGCAATGTT
APF2_10	CTTAATGTTGGCAATGTTAGACGATTTGTCTGTACCAC AAAACGAGAAAGATTTATTGC
APF2_11	ACGAGAAAGATTTATTGCAACAAAAATTGGCACAATTT GCCGATCCTTCACAAACTGTA
APF2_12	TAAGAGCCGGCTTGTTCGACTGCGGCCAATAATCTACC GTTTACAGTTTGTGAAGGATCGATCCTTCACAAACTGT AAACGGTAGATTATTGGCCGCAGTCGAACAAGCCGGC TCTTA
APF2_13	GCGCTTTATATTGTTGAGCTAATTGTGCACCCAATGCTT TATAAGAGCCGGCTTGTTCGCGAACAAGCCGGCTCTTA TAAAGCATTGGGTGCACAATTAGCTCAACAATATAAA GCGC
APF2_14	ATTATCGAAAGCAGAAATCGCATAAAATCTCTCGAATG CTTGCGCTTTATATTGTTGAGCTCAACAATATAAAGCG CAAGCATTCGAGAGATTTTATGCGATTTCTGCTTTCGA TAAT
APF2_15	TGGATCGCATCAAATAACAAAGCCTGTGTAGACAACTC CATATTATCGAAAGCAGAAATATTTCTGCTTTCGATAA TATGGAGTTGTCTACACAGGCTTTGTTATTTGATGCGA TCCA
APF2_16	ACTGATCGTTTTCATCTAACAATTCGATCTGTAAGCCTT GTTGGATCGCATCAAATAACGTTATTTGATGCGATCCA

	ACAAGGCTTACAGATCGAATTGTTAGATGAAAACGAT CAGT
APF2_17	TTTCACATATTCCAAATGATCGCCAAATTTTAATGCCA AGAACTGATCGTTTTCATCTATAGATGAAAACGATCAG TTCTTGGCATTAAAATTTGGCGATCATTTGGAATATGT GAAA
APF2_18	AATGGCGAAATATACTGATCGTGAGAGGTCATATTGCC GTTTTTCACATATTCCAAATGCATTTGGAATATGTGAA AAACGGCAATATGACCTCTCACGATCAGTATATTTCGC CATT
APF2_19	TCGCCAACACCTTTTTGGTTACGACTTTGTTTTCCATAA TTAATGGCGAAATATACTGATCAGTATATTTCGCCATT AATTATGGAAAACAAAGTCGTAACCAAAAAGGTGTTG GCGA
APF2_20	AGAGGTAAATTCAACAGATTTTGGCACATTAAAACCG GCTTTCGCCAACACCTTTTTGGCCAAAAAGGTGTTGGC GAAAGCCGGTTTTAATGTGCCAAAATCTGTTGAATTTA CCTCT
APF2_21	TTACCTTCAAATAATGGATAGTGTGCCACCGCTTGTTC TACAGAGGTAAATTCAACAGATCTGTTGAATTTACCTC TGTAGAACAAGCGGTGGCACACTATCCATTATTTGAAG GTAA
APF2_22	ATCATGGTTAACCACCGCTTTACCTTCAAATAATGGAT AGT
APF3_1	GATATAAATATTAAGCCTAAATCAACTAATTACGGCTT AGGT
APF3_2	AAATCAACTAATTACGGCTTAGGTATTACAATTTTCCA GCAAGGCGTGACGGATAAAGC
APF3_3	AGGCGTGACGGATAAAGCCGACTTTGCCAAAGCGATT GAAATTGCGTTCAGAGAAGATA
APF3_4	TTGCGTTCAGAGAAGATAAAGAAGTGATGGTGGAAGA CTATTTAGTCGGCACCGAATAC
APF3_5	TTAGTCGGCACCGAATACAGATTCTTTGTGTTAGGCGA TGAAACATTGGCGGTATTGTT
APF3_6	AACATTGGCGGTATTGTTAAGAGTGCCAGCAAATGTGA AAGGTGATTGTATACATACAG
APF3_7	GTGATTGTATACATACAGTGAGAGAATTGGTGGAAGC GAAAAACTCAGATCCATTGAGA
APF3_8	AACTCAGATCCATTGAGAGGTGACGGCTCAAGATCAC CATTGAAGAAAATCGCCTTAGG
APF3_9	GAAGAAAATCGCCTTAGGTGATATTGAATTGTTACAAT TGAAAGAGCAAGGTTTAACGC
APF3_10	TCTTAATTGTACGATTTGACCATCAGCAGGAATAGAAT CAGGCGTTAAACCTTGCTCTTAAGAGCAAGGTTTAACG CCTGATTCTATTCCTGCTGATGGTCAAATCGTACAATT AAGA
APF3_11	ATATCAATTGAATCACCGCCGGTAGAAATATTAGAGTT GGCTCTTAATTGTACGATTTGCAAATCGTACAATTAAG AGCCAACTCTAACATTTCTACCGGCGGTGATTCAATTG ATAT

APF3_12	CGACCGCTAATTGTTTATAACTGTCATGCATTTGATCA GTCATATCAATTGAATCACCGCGGTGATTCAATTGATA TGACTGATCAAATGCATGACAGTTATAAACAATTAGCG GTCG
APF3_13	ATCCACACCGCAGACTTTTGCACCCATTTCTTTGGCAA TACCGACCGCTAATTGTTTATATAAACAATTAGCGGTC GGTATTGCCAAAGAAATGGGTGCAAAAGTCTGCGGTG TGGAT
APF3_14	AAAGAAGGTTCAGCGGCTTTGGTTAAATCTGGAATGAT TAAATCCACACCGCAGACTTTAAAGTCTGCGGTGTGGA TTTAATCATTCCAGATTTAACCAAAGCCGCTGAACCTT CTTT
APF3_15	TCATAGGATTAAAGTTTGCTTCAATCACACCCCATGAT CTCAAAGAAGGTTCAGCGGCTAGCCGCTGAACCTTCTT TGAGATCATGGGGTGTGATTGAAGCAAACTTTAATCCT ATGA
APF3_16	CCTTCTTGATTTTCCTTGGTAAGGGAAAATATGCATCA TCATCATAGGATTAAAGTTTGCAAACTTTAATCCTATG ATGATGATGCATATTTTCCCTTACCAAGGAAAATCAAG AAGG
APF3_17	GGCAATTCTGGAAACAACATTTTTAACACGGCTTTGGT CAACCTTCTTGATTTTCCTTGCAAGGAAAATCAAGAAG GTTGACCAAAGCCGTGTTAAAAATGTTGTTTCCAGAAT TGCC
APF3_18	GGATCCGGCTAGCTTAAGGCAATTCTGGAAACAACATT TT

Table S2. Primers used for cloning of *S. cerevisiae GSH2* gene.

Primers	Oligonucleotide sequences (5'-3')
GSH2_1	GATCCTCCAAAGGTAGCAAAGTG
GSH2_2	AAGTTCTAGCATCATCTTCCTAGC
GSH2_3	AAGGATATCATATGGCACACTATCCACCTTCC
GSH2_4	AGGATCACTAGTTGTAGAAGAACTT
GSH2_5	TCTACGGCTAGCGATCCTATTGTCGCATTCATT
GSH2_6	CCTATTGTCGCATTCATTGTGCAAAGAAACGAGAGAAA TGTGTTTGATCAAAAGGTCTT
GSH2_7	GTTTGATCAAAAGGTCTTGGAATTGAATTTATTGGAAAA ATTCGGTACTAAATCTGTTA
GSH2_8	TCGGTACTAAATCTGTTAGGTTGACTTTTGATGATGTTAA TGATAAATTGTTTATTGAT
GSH2_9	TCCTGCTCTGTGTCCCTAATGAATAATTTTCCAGTTTTAT CATCAATAAACAATTTATC
GSH2_10	CAGTGGTTGTGTAACCCGTTCTGTAATAAACCACCGCTA TTTCCTGCTCTGTGTCCCTA
GSH2_11	CAAGAATAGTCTTGCCTCCCAGTCCTTTTCAGAAGTGTA ATCAGTGGTTGTGTAACCCG
GSH2_12	TGCGAAACTTTTTTCCAAGAATAGTCTTGCCTCC
GSH2_13	TCTTGGAAAAAAGTTTCGCAATAAAGGCCCCAGATTTAT TGACTCAATTATCTGGCTCC
GSH2_14	CTCTTGCCTTCCCTGCCAAGTTTCGTATCATCCAAGGGA TA
GSH2_15	ACTTGGCAGGGAAGGCAAGAG
GSH2_16[a]	TACACGGGA<u>TCCGCCACCTCCACCTCC</u>GTAAAGAATAAT ACTGTCCAAA

[a] Nucleotide sequence encoding the linker is underlined.

Table S3. Primers used for cloning of *S. cerevisiae GSH1* gene.

Primers	Oligonucleotide sequences (5'-3')
GSH1_1	GAGCAGATTTAGTATAGGGCTAC
GSH1_2	ATTCCAGGATCCATGGGATTGTTAGCTTTGGGCACGCCTTTGC
GSH1_3	CTTGTCATGGCAAACGTCCAACATAGAATTTCTCTCCTT
GSH1_4	GACGTTTGCCATGACAAGATATTAACTGAGCTTAATAT
GSH1_5	AGCGGAAGATCTTTAATGTTAATAAAGTCGGGGC
GSH1_6	CATTAAGGATCCGTGGAATCAT
GSH1_7	GCCCATGCCAAAACCCATAGAATCCATATAAATGAAAC
GSH1_8	CTATGGGTTTTGGCATGGGC
GSH1_9	GATATCCCCGGGGCCTTATCATTCTCTAATAGTCTTCCTAATACTTTTTCATT
GSH1_10	GATATCAATATTGGACTATGATCTTGCTAAACATTTTGCGCATTTGTACATAAGAGATC
GSH1_11	CACTTCAAATGGTCTGAACTCCACTCTCCAACCAGGAG
GSH1_12	AGTTCAGACCATTTGAAGTG
GSH1_13	TTCCCATACTTTGGACATGTGAATATATGCGTTGATATTATCGGAAAAGGTC
GSH1_14	CACATGTCCAAAGTATGGGAA
GSH1_15	TCTAGAACTAGCTTAACATTTGCTTTCTATTGAAG
GSH1_16	CAATCACCGTGTCACCCAAATCG

Table S4. Primers used for cloning of *S. cerevisiae PRO1* gene.

Primers	Oligonucleotide sequences (5'-3')
Pro1_1	GGTCATTTTATCAGCTACTGTAATATAG
Pro1_2	AAAGGATCCATATGAAGGATGCTAATGAGAGTAAATC
Pro1_3[a]	GAATTCATGGATCCGCCGCCACCGCCACCACGAGGTGG GAATGCCAAATTTTC
Pro1_4	AAGAAACAGCTGCTCTGCACAATTCTTC
Pro1_5	TTCGTTGGCTAGCTCAACGAGGTGGGAATGCCAAATTT

[a] Nucleotide sequence encoding the linker is underlined.

Table S5. Primers used for cloning of *E. coli proB* mutant optimized according to codon usage of *S. cerevisiae.*

Primers	Oligonucleotide sequences (5'-3')
ProB1_1	GGTATCCATATGTCAGACTCTCAGACTTTGGTGGTAAAA TTAGGTACTTCAGTTTTAAC
ProB1_2	AGGTACTTCAGTTTTAACAGGCGGATCAAGACGTTTGA ACAGAGCTCATATCGTTGAAT
ProB1_3	GAGCTCATATCGTTGAATTAGTTAGACAATGCGCTCAAT TACATGCTGCCGGTCATAGG
ProB1_4	CATGCTGCCGGTCATAGGATTGTTATTGTGACTTCTGGC GCAATTGCAGCTGGAAGAGA
ProB1_5	AATTGCAGCTGGAAGAGAACACTTGGGTTACCCAGAAT TGCCAGCTACTATCGCCTCGA
ProB1_6	TGAATCAATCTTGATTGACCTACAGCTGCCAACAATTGT TTCGAGGCGATAGTAGCTGG
ProB1_7	CGACATGAATGCCATAAATAGAAAACAACTGTTCCCAC AATTGAATCAATCTTGATTGA
ProB1_8	TTCTCTGTCTTCCATATCAGCTCTGGTCAACAACATTTG ACCGACATGAATGCCATAAA
ProB1_9	TCTAACAAAGCTCTCAAAATATCTCTAGCGTTCAAGAAC CTTTCTCTGTCTTCCATATC
ProB1_10	TCCATGGTTAACGATATTGTTATCTAACAAAGCTCTCAA
ProB2_1	TCTAGAAGGCCTGTAATTAATGAAAACGATGCTGTTGCT ACAGCAGAAATTAAGGTCGG
ProB2_2	AGCAGAAATTAAGGTCGGTGATAACGATAATTTGTCTGC TTTGGCTGCAATTTTGGCTG
ProB2_3	TGGCTGCAATTTTGGCTGGTGCTGATAAATTATTGTTATT GACCGATCAAAAAGGTTTG
ProB2_4	ACCGATCAAAAAGGTTTGTATACCGCTGATCCAAGATCA AATCCACAGGCAGAATTGAT
ProB2_5	TCCACAGGCAGAATTGATTAAAGATGTTTACGGCATTGA TGACGCATTGAGAGCTATTG
ProB2_6	ACGCATTGAGAGCTATTGCTGGTGATTCTGTTTCAGGCT TAGGAACTGGTGGCATGTCA
ProB2_7	GGAACTGGTGGCATGTCAACTAAATTGCAGGCCGCTGA CGTGGCTTGCCGTGCTGGTA
ProB2_8	TGGCTTGCCGTGCTGGTATCGACACTATTATTGCCGCTG GTTCTAAGCCAGGTGTTATT
ProB2_9	TCTAAGCCAGGTGTTATTGGTGATGTGATGGAAGGCATT TCTGTTGGTACGTTGTTCCA
ProB2_10	AAATCCATCTTTTTCTGTTTTCTAATGGAGTAGCCTGAG CATGGAACAACGTACCAACA
ProB2_11	CTTCATCTACAGTGATTTCACCAGCAGGTGGAGCACCG AAAATCCATCTTTTTCTGTTT
ProB2_12	CAACAAAGATGAACCTCTTTCCAAAATAGCGGCAGTTG CACCTTCATCTACAGTGATTT
ProB2_13	CCTCTTGAAAAATTACCAGTCACAGATTTAATACCTTTT GGCAACAAAGATGAACCTCT

ProB2_14	CGATGTCTCTACCTTCCAAGTTACAAATTCTAATAACTTC ACCTCTTGAAAAATTACCA
ProB2_15	TCTCCTTAATGCATCAGAATTATATCTTGAGACGCCATGA GCGATGTCTCTACCTTCCA
ProB2_16	CCCAAAATTGCATCAATTTCTTGAGAGTGATGTCCGGCA ATTCTCCTTAATGCATCAGA
ProB2_17	TCATGTCATCTCTATGAACAGCAACTGGGCCGTATTCATA TCCCAAAATTGCATCAATT
ProB2_18[a]	GAATTCATGGATCCGCCGCCACCGCCACCTCTGGTAATC ATGTCATCTCTATGAAC

[a] Nucleotide sequence encoding the linker is underlined.

Table S6. Primers used for cloning of *E. coli gshB* mutant optimized according to codon usage of *S. cerevisiae.*

Primers	Oligonucleotide sequences (5'-3')
GSHB1_1	GATATCAAGATCTATGATTAAATTGGG
GSHB1_2	AAGATCTATGATTAAATTGGGTATTGTTATGGACCCAATC GCTAATATTAATATTAAGA
GSHB1_3	CTAATATTAATATTAAGAAAGATTCTTCTTTTGCTATGTT GTTAGAAGCACAAAGAAGA
GSHB1_4	TTAGAAGCACAAAGAAGAGGTTATGAATTGCATTATATG GAAATGGGTGATTTGTATTT
GSHB1_5	AATGGGTGATTTGTATTTGATTAATGGTGAAGCTAGAGC ACATACTAGAACTTTGAATG
GSHB1_6	ATACTAGAACTTTGAATGTTAAACAAAATTATGAAGAGT GGTTTTCTTTCGTTGGTGAA
GSHB1_7	TTTTCTTTCGTTGGTGAACAAGATTTGCCATTGGCTGAT TTAGATGTTATTTTGATGAG
GSHB1_8	AGTAGCATAAATAAATTCAGTATCAAAAGGTGGATCTTT TCTCATCAAAATAACATCTA
GSHB1_9	ACAATCAAAGTACCTTTCTCTTCAGCTCTTTCCAAAATA TAAGTAGCATAAATAAATTC
GSHB1_10	ACAATTTTTCATTACAATCTCTCAAAGATTGTGGTTTATT AACAATCAAAGTACCTTTC
GSHB1_11	ACCAATGTTTCTGGAGTTAAATCAGAAAACCAAGCAGT AAACAATTTTTCATTACAATC
GSHB1_12	GTTTTTCCCAAAAAGCCTTTAATTGAGCTTTATTTCTAGT AACCAATGTTTCTGGAGTT
GSHB1_13	TCCACCCATACCATCCAATGGTTTCAAAATTATATCAGAA TGTTTTTCCCAAAAAGCCT
GSHB2_1	CCATTGGATGGTATGGGTGGAGCTTCAATTTTTAGAGTT AAAGAAGGTGATCCAAATTT
GSHB2_2	AGAAGGTGATCCAAATTTGGGTGTTATTGCTGAAACTTT GACTGAACATGGTACTAGAT
GSHB2_3	CTGAACATGGTACTAGATATTGTATGGCTCAAAATTATTT GCCAGCTATTAAAGATGGT
GSHB2_4	CCAGCTATTAAAGATGGTGATAAAAGAGTGTTGGTTGTA GATGGTGAACCAGTTCCATA
GSHB2_5	TGGTGAACCAGTTCCATATTGTTTGGCTAGAATACCACA AGGTGGAGAAACTAGAGGTA
GSHB2_6	GTGGAGAAACTAGAGGTAATTTGGCTGCCGGTGGTAGA GGTGAACCAAGACCATTGACT
GSHB2_7	AAAGTTGGACCAATTTGTCTAGCAATTTTCCAATCAGAT TCAGTCAATGGTCTTGGTTC
GSHB2_8	TATAATATCCAAACCAACAAAAATCAAACCTTTTTCTTT CAAAGTTGGACCAATTTGTC
GSHB2_9	CATGTTGGAGAAGTAACATTAATTTCAGTCAATCTATCA CCTATAATATCCAAACCAAC
GSHB2_10	CAGTAATAGAAACTGGAAATTCTGCTTCAATTTCTCTAA TACATGTTGGAGAAGTAACA

GSHB2_11	TTGCTGTTGTAATCTTGCTTCAATAGCATCCATTAACATT CCAGTAATAGAAACTGGAA
GSHB2_12	GAATTCGGCTAGCTTATTGCTGTTGTAATCTTGCTTC

Table S7. Primers used for real-time PCR to verify copy number of *gshF* gene.

Primers	Oligonucleotide sequences (5'-3')
GshF_RT1	GCCAGCAAATGTGAAAGG
GshF_RT2	TGACCATCAGCAGGAATAGA
ACT_RT1[a]	TGTGATGTCGATGTCCGTAA
ACT_RT2	AAGAAGCCAAGATAGAACCA

[a] Primers used for amplification of endogenous reference *β-actin* gene.

Table S8. Relative quantitation of copy number of *gshF* gene.

Strains	C_T of *gshF* (target)	C_T of *β-actin* (reference)	relative copy number of *gshF*
W303-1b/F (positive control)	14.97	15.09	1
W303-1b/FF 19#	15.83	17.06	2.16±0.05
W303-1b/FF 24#	14.24	15.38	2.01±0.07
W303-1b/FF 33#	13.86	15.04	2.09±0.04

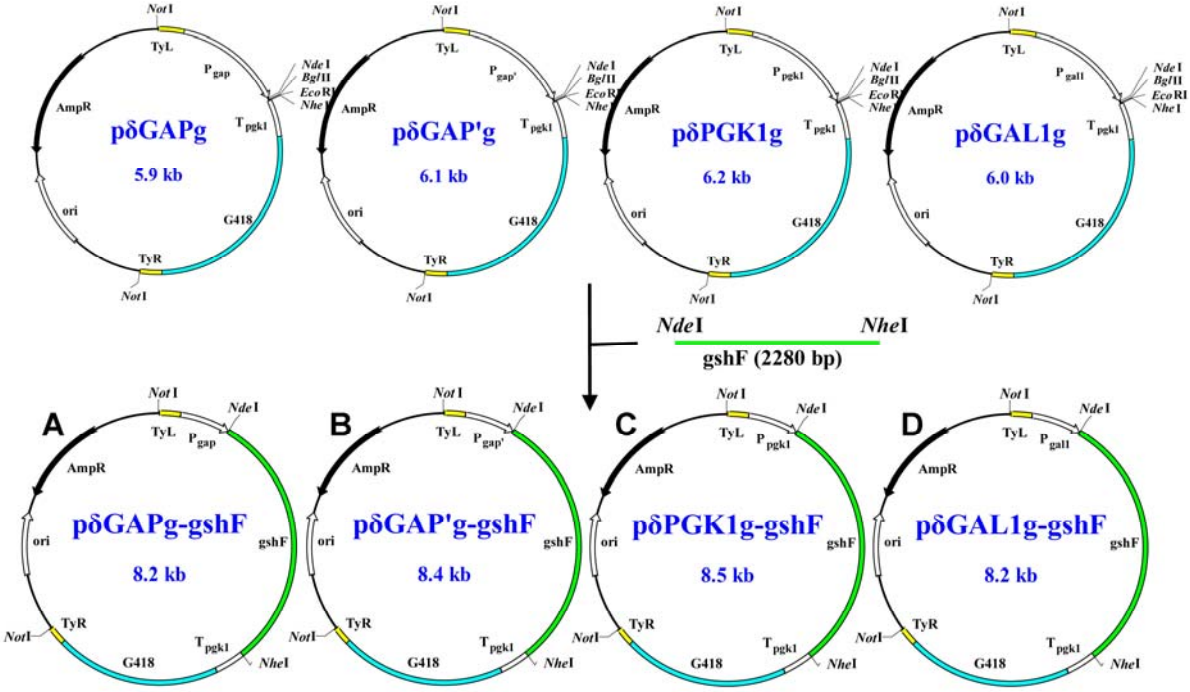

Figure S1. Construction of integrative expression vectors pδGAPg-gshF, pδGAP'g-gshF, pδGAL1g-gshF and pδPGK1g-gshF used to evaluate effects of different promoter strengths on the activity of GshF. The DNA fragment (2280 bp) of *gshF* gene obtained by double restriction digestions of pUC19-GshF with *Nde*I and *Nhe*I was inserted into plasmid pδGAPg constructed previously in our lab, generating the expression vector pδGAPg-gshF (A). Following the construction of plasmid pδGAPg-gshF, the expression vectors pδGAP'g-gshF (B), pδPGK1g-gshF (C) and pδGAL1g-gshF (D) with different promoters were constructed.

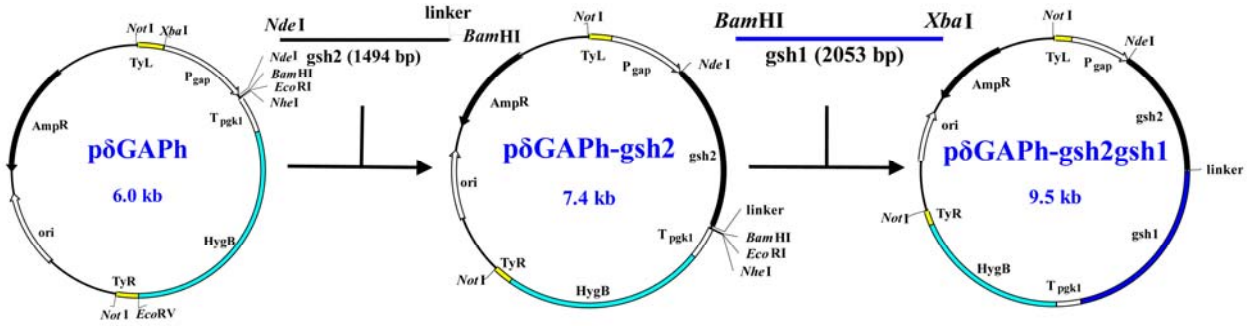

Figure S2. Construction of integrative expression vector pδGAPh-gsh2gsh1 for overexpression of Gsh2-Gsh1 fusion protein. The plasmid pδGAPh-gsh2 was generated by subcloning of *GSH2* DNA fragment (1494 bp) in plasmid EZ-GSH2 into plasmid pδGAPh constructed previously in our lab. The *GSH1* DNA fragment (2053 bp) obtained by double digestions of EZ-GSH1 with *Bam*HI and *Xba*I was ligated into plasmid pδGAPh-gsh2 digested by *Bam*HI and *Nhe*I to create the expression vector pδGAPh-gsh2gsh1.

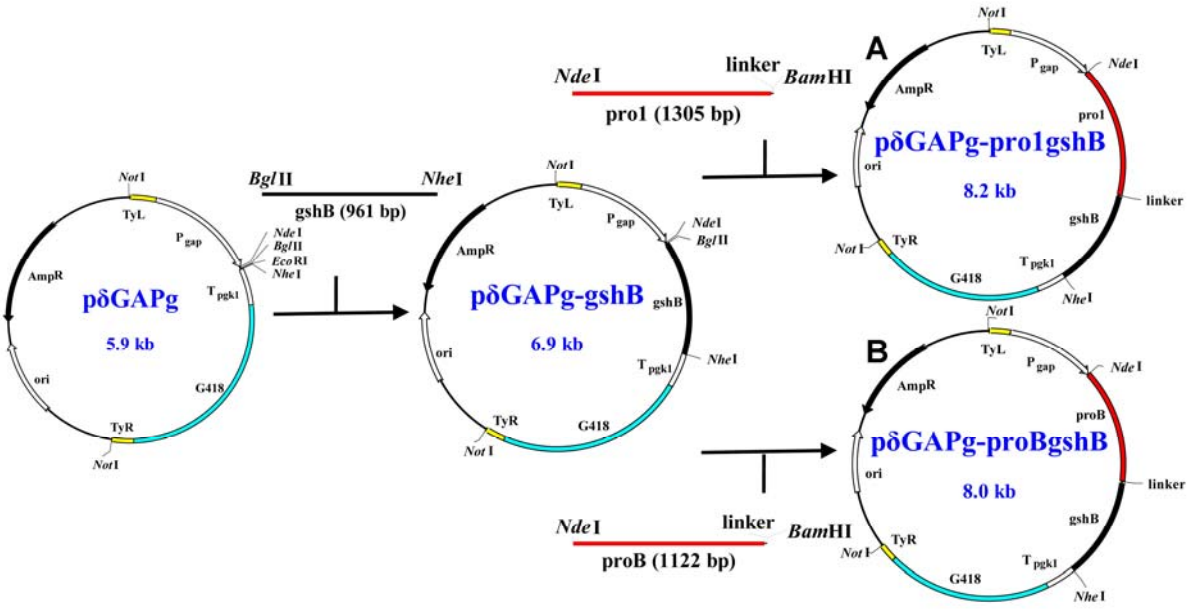

Figure S3. Construction of integrative expression vectors pδGAPg-pro1gshB and pδGAPg-proBgshB for overexpression of Pro1-GshB and ProB-GshB fusion proteins. The intermediate plasmid pδGAPg-gshB was firstly generated by subcloning of *gshB* DNA fragment (961 bp) in plasmid EZ-GSHB into plasmid pδGAPg constructed previously in our lab. Two DNA fragments, the *PRO1* DNA fragment (1305 bp) amplified by PCR and the *proB* DNA fragment (1122 bp) in plasmid EZ-PROB, were cleaved by restriction enzymes *Nde*I and *Bam*HI and inserted into plasmid pδGAPg-gshB digested by *Nde*I and *Bgl*II to generate the expression vector pδGAPg-pro1gshB (A) and pδGAPg-proBgshB (B), respectively.

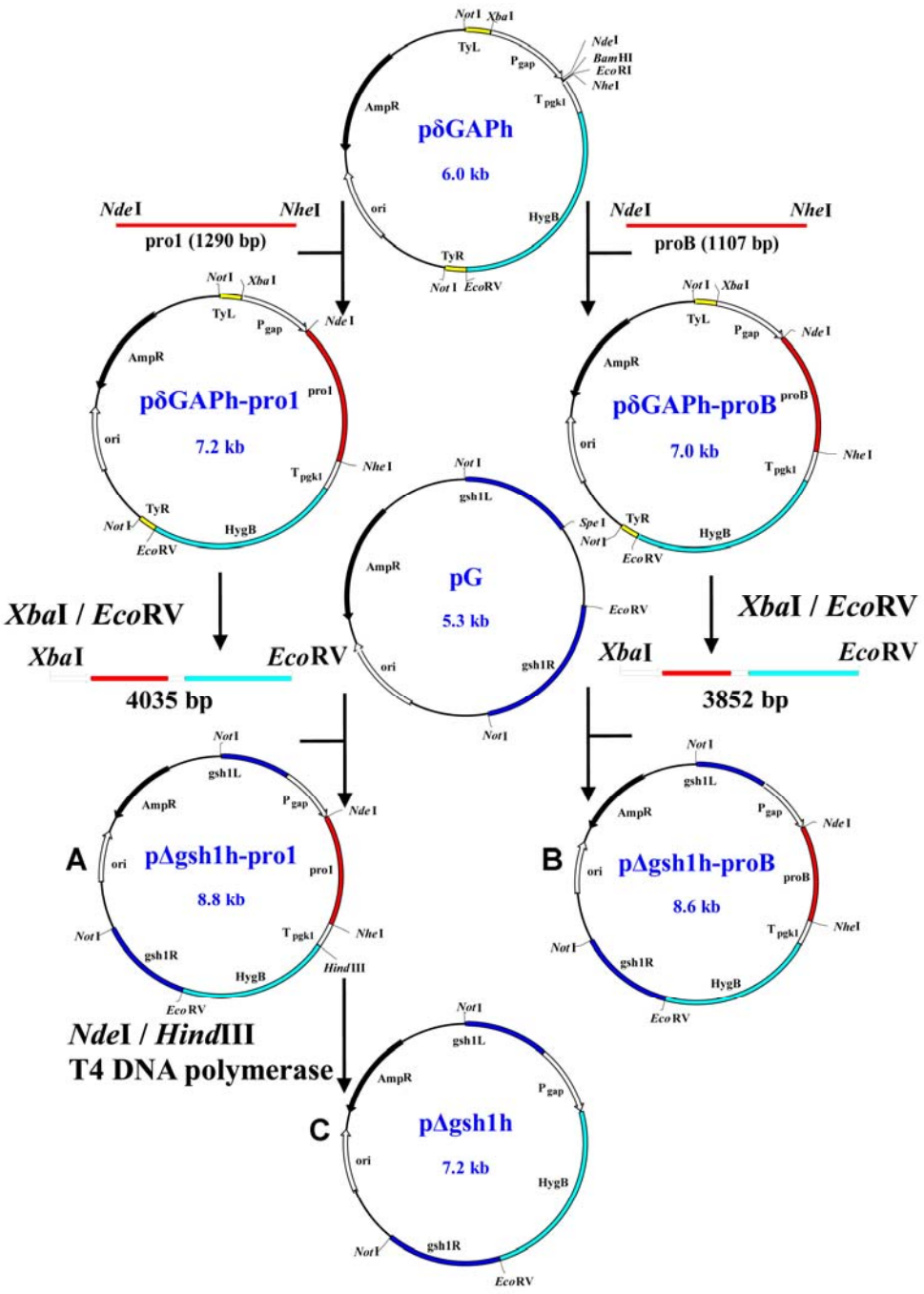

Figure S4. Construction of plasmids pΔgsh1h-pro1, pΔgsh1h-proB and pΔgsh1h used for deletion of *GSH1* gene. (A) The *PRO1* DNA fragment (1290 bp) was amplified by PCR with a pair of primers Pro1_2 and Pro1_5 (TableS4) for introducing *Nde*I and *Nhe*I sites and plasmid template of pδGAPg-pro1gshB, and then inserted into plasmid pδGAPh to generate plasmid pδGAPh-pro1. The recombinant construct of PRO1 (4035 bp) containing GAP promoter, PGK1 terminator and selective marker HygB was obtained by double digestions of plasmid pδGAPh-pro1 with *Xba*I and *Eco*RV, and directly cloned into plasmid pG

constructed previously at the *Spe*I-*Eco*RV sites, yielding plasmid pΔgsh1h-pro1 for replacement of *GSH1* by *PRO1*. (B) The plasmid pΔgsh1h-proB for replacement of *GSH1* by *proB* was created as constructed pΔgsh1h-pro1. (C) The plasmid pΔgsh1h was derived from the self-ligation of plasmid pΔgsh1h-pro1 digested with *Nde*I and *Hind*III and then filled in with T4 DNA polymerase.

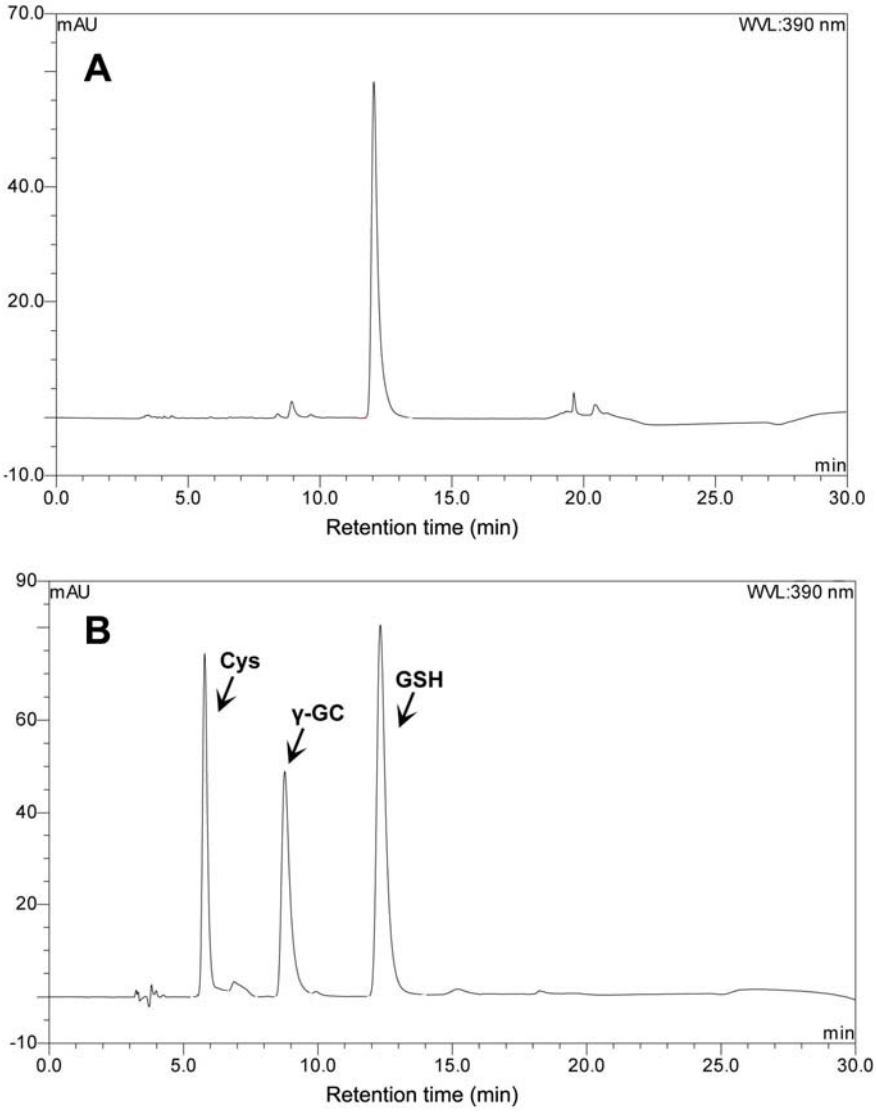

Figure S5. Chromatograms of standard thiols and samples labelled with ABD-F. (A) Intracellular thiols of engineered strain W303-1b/FGP. (B) Thiol standards of Cys, γ-GC and GSH.

Methods

1. Cloning of related genes used in this work

Cloning of *A. pleuropneumoniae gshF* gene optimized according to codon usage of *S. cerevisiae*

To functionally express heterologous *A. pleuropneumoniae gshF* (GenBank No.: **GU138097**) in *S. cerevisiae*, the codon-conformed *gshF* DNA fragment was resynthesized through splice overlap extension PCR (SOE-PCR) with the synthesized primers (Table S1). The whole DNA fragment was divided into three parts used to assemble the open reading frame (ORF) of *gshF*.

5'-terminal DNA fragment APF1 of *gshF* was obtained by SOE-PCR with primers from APF1_1 to APF1_18. Primers APF1_9 and APF1_10 were used to run a complementary reaction by PCR. Then, the fragment APF1 of the expected length (740 bp) was further obtained by eight-round SOE-PCRs with the subsequent pairs of primers APF1_8/APF1_11, APF1_7/APF1_12, APF1_6/APF1_13, APF1_5/APF1_14, APF1_4/APF1_15, APF1_3/APF1_16, APF1_2/APF1_17 and APF1_1/APF1_18. Fragment APF1 was cloned into a T-vector EZ-T (derivative of pBluescript II KS(+), GenStar, China) via TA cloning to give EZ-APF1 and verified by DNA sequencing. Middle *gshF* DNA fragment APF2 (870 bp) in plasmid EZ-APF2 and 3'-terminal *gshF* DNA fragment APF3 (709 bp) in EZ-APF3 were synthesized as amplified fragment APF1. After that, fragment APF1 obtained by digestion of EZ-APF1 with *Nde*I and *Eco*RI was inserted into plasmid pUC19, generating pUC19-APF1. Fragment APF2 isolated by digestion of EZ-APF2 with *Xho*I and *Hind*III was inserted into the *Sal*I-*Hind*III sites of plasmid pUC19-APF1 to yield pUC19-APF1-APF2. Then APF3 obtained by digestion of plasmid EZ-APF3 with *Ssp*I and *Hind*III was ligated into plasmid pUC19-APF1-APF2 digested with *Hpa*I and *Hind*III to create plasmid pUC19-GshF with a complete ORF of *A. pleuropneumoniae gshF*.

Cloning of *S. cerevisiae GSH2* encoding glutathione synthetase

To remove restriction sites *Hind*III, *Kpn*I and *Spe*I in DNA sequence of *GSH2* and sub-clone conveniently for the construction of recombinant *GSH2-GSH1* gene, primers (Table S2) were designed and synthesized according to the nucleotide sequence of *S. cerevisiae* S288C *GSH2* (GenBank No.: **NC_001147**). The whole DNA fragment was broken into three parts used to form the ORF of *GSH2*.

5'-terminal DNA fragment F1 of *GSH2* was amplified by Nested-PCR. Primers GSH2_1 and GSH2_2 were used for the first-step amplification with the genomic DNA of *S. cerevisiae* W303-1b, then fragment F1 of the expected size (665 bp) was obtained by Nested-PCR from the first-round PCR product with primers GSH2_3 and GSH2_4. Fragment F1 was cloned into a T-vector via TA cloning to give EZ-Gsh2-F1 and verified by DNA sequencing.

Middle DNA fragment F2 of *GSH2* was obtained by SOE-PCR with primers from GSH2_5 to GSH2_12. The primers GSH2_8 and GSH2_9 were used to run a complementary reaction by PCR. Then fragment F2 of *GSH2* of the expected length (228 bp) was further synthesized by three-round SOE-PCRs with the subsequent pairs of primers GSH2_7/GSH2_10, GSH2_6/GSH2_11 and GSH2_5/GSH2_12.

3'-terminal DNA fragment F3 of *GSH2* was obtained through Nested-PCR and SOE-PCR. Primers GSH2_1 and GSH2_2 were used for the first-step amplification with the genomic DNA of *S. cerevisiae* W303-1b, then two fragments of the expected size (199 bp and 391 bp) were amplified by Nested-PCR from the first-round PCR product using two pairs of primers GSH2_13/GSH2_14 and GSH2_15/GSH2_16. A further complementary reaction between 199 bp and 391 bp fragments was carried out. Then, fragment F3 of the expected length (590 bp) was amplified by SOE-PCR with primers GSH2_13 and GSH2_16 from the PCR product of complementary reaction. At last, the recombinant fragment F23 (864 bp) was generated by SOE-PCR from the PCR product of the complementary reaction between two fragments F2 and F3 with primers GSH2_5 and GSH2_16, in which an in-frame sequence encoding six-glycine linker was incorporated into the 3' terminal of *GSH2* during the PCR reaction.

The resulting fragment F23 was cloned into a T-vector via TA cloning to generate EZ-GSH2-F23 and confirmed by DNA sequencing. The plasmid EZ-GSH2 with the complete *GSH2* ORF was obtained by ligation of fragment F1 in EZ-GSH2-F1 cleaved by *Eco*RV and *Spe*I into plasmid EZ-GSH2-F23.

Cloning of *S. cerevisiae GSH1* encoding γ-glutamylcysteine synthetase

Sixteen primers (Table S3) derived from the nucleotide sequence of *S. cerevisiae* S288C *GSH1* (GenBank No.: **X85021**) were designed and synthesized. The whole DNA fragment was divided into three parts for re-assembling the complete *GSH1* ORF in order to eliminate restriction sites of *Bam*HI, *Eco*RI, *Nco*I, *Nde*I and *Ssp*I and

conveniently construct recombinant *GSH2-GSH1* gene.

5'-terminal DNA fragment F1 of *GSH1* was obtained by Nested-PCR. Primers GSH1_1 and GSH1_16 were used to run the first-step amplification with the genomic DNA of *S. cerevisiae* W303-1b, then two fragments of the expected length (231 bp and 323 bp) were amplified by Nested-PCR with the two pairs of primers GSH1_2/GSH1_3 and GSH1_4/GSH1_5. A complementary reaction between 231 bp and 323 bp fragments was carried out. Then, fragment F1 of the expected length (536 bp) was obtained by SOE-PCR with the primers GSH1_2 and GSH1_5 from the PCR product of the complementary reaction, and cloned into a T-vector via TA cloning to generate EZ-GSH1-F1 for verifying by DNA sequencing.

Middle DNA fragment F2 (705bp) of *GSH1* in the plasmid EZ-GSH1-F2 was created through Nested-PCR and SOE-PCR with the two pairs of primers GSH1_6/GSH1_7 and GSH1_8/GSH1_9 as amplified 5'-terminal DNA fragment F1 of *GSH1*.

The procedure for cloning 3'-terminal DNA fragment F3 of *GSH1* was different from that of middle DNA fragment F2. Three intermediate fragments (235 bp, 137 bp and 442 bp) were amplified by PCR using the three pairs of primers GSH1_10/GSH1_11, GSH1_12/GSH1_13 and GSH1_14/GSH1_15 and then used to carry out the complementary reaction. The resulting fragment F3 of the expected length (853 bp) was amplified by SOE-PCR with the primers GSH1_10/GSH1_15 and the PCR product of the complementary reaction. Plasmid EZ-GSH1-F3 was generated by TA cloning and verified by DNA sequencing.

The complete ORF of *GSH1* in plasmid EZ-GSH1 was obtained by two consecutive DNA ligation that the fragment F3 (847 bp) isolated by digestion of EZ-GSH1-F3 with *Ssp*I and *Xba*I was inserted into plasmid EZ-GSH1-F2 cleaved by *Sma*I and *Xba*I, and the fragment F1 (523 bp) in plasmid EZ-GSH1-F1 with the *Eco*RI-*Bgl*II digestion was ligated into the intermediate EZ-GSH1-F23 digested by *Bam*HI and *Eco*RI.

Cloning of *S. cerevisiae PRO1* encoding γ-glutamyl kinase

According to the nucleotide sequence of *S. cerevisiae* S288C *PRO1* (GenBank No.: **M85293**), four primers from Pro1_1 to Pro1_4 (Table S4) were synthesized and used to clone the *PRO1* gene. The complete *PRO1* ORF (1284 bp) was amplified by Nested-PCR with the genomic DNA of *S. cerevisiae* W303-1b, and an in-frame

sequence encoding six-glycine linker was added into the 3' terminal of *PRO1* during the PCR reaction.

Cloning of *E. coli proB* mutant optimized according to codon usage of *S. cerevisiae*

On the basis of the nucleotide sequence of *E. coli* BL21 *proB* (GenBank No.: **AM946981**), the synthesized primers (Table S5) were used to clone the whole DNA fragment of *proB* mutant which was divided into two parts for DNA fragment assembly.

5'-terminal DNA fragment proB1 of *proB* mutant was amplified by SOE-PCR with primers from ProB1_1 to ProB1_10. Primers ProB1_5 and ProB1_6 were used to run a complementary reaction by PCR. Then, fragment proB1 of the expected length (408 bp) was obtained by four-round SOE-PCRs with the subsequent pairs of primers ProB1_4/ProB1_7, ProB1_3/ProB1_8, ProB1_2/ProB1_9 and ProB1_1/ProB1_10, and cloned into a T-vector via TA cloning to generate plasmid EZ-ProB1 for verifying by DNA sequencing.

3'-terminal DNA fragment proB2 (735 bp) of *proB* mutant was synthesized as amplified fragment proB1. Using the primer ProB2_18, an in-frame sequence encoding six-glycine linker was added into the 3' terminal of *proB* during the PCR reaction. The resulting plasmid EZ-ProB2 was verified by DNA sequencing after TA cloning. At last, fragment proB1 obtained by digestion of plasmid EZ-ProB1 with *Hind*III and *Hpa*I was inserted into plasmid EZ-ProB2 digested by *Hind*III and *Stu*I, generating plasmid EZ-ProB used for the construction of recombinant *proB-gshB* gene.

Cloning of *E. coli gshB* mutant optimized according to codon usage of *S. cerevisiae*

Based on the encoding sequence of *E. coli* BL21 *gshB* (GenBank No.: **NP_417422**), the synthesized primers (Table S6) were used to clone the whole DNA fragment of *gshB* mutant which was divided into two parts for DNA fragment assembly.

5'-terminal DNA fragment gshB1 of *gshB* mutant was obtained by SOE-PCR with primers from GSHB1_2 to GSHB1_13. Primers GSHB1_7 and GSHB1_8 were used for a complementary reaction by PCR. Then, the fragment gshB1 of the expected length (508 bp) was synthesized by five-round SOE-PCRs with the subsequent pairs

of primers GSHB1_6/GSHB1_9, GSHB 1_5/GSHB 1_10, GSHB1_4/GSHB1_11, GSHB1_3/GSHB1_12 and GSHB1_2/GSHB1_13.

3'-terminal DNA fragment gshB2 (484 bp) of *gshB* mutant was constructed as amplified fragment gshB1. And then, two fragments gshB1 and gshB2 were carried out a complementary reaction by PCR. At last, the whole *gshB* ORF (977 bp) was amplified by SOE-PCR with the primers GSHB1_1/GSHB2_12 and the PCR product of complementary reaction. The resulting plasmid EZ-GshB was used for DNA sequencing and further construction of expression vector.

2. GSH production of the engineered strains harbouring *gshF* gene under the control of four different promoters

Three positive colonies of each engineered strain of W303-1b/F/GAP, W303-1b/F/GAP', W303-1b/F/GAL1 and W303-1b/F/PGK1 was cultured at 30°C in shake flasks containing 20 mL of liquid YPD medium with agitation at 250 rpm for 18-24 h. An adequate volume of fresh cultures were then inoculated into a set of 250 mL flasks containing 50 mL of liquid YPD medium (W303-1b/F/GAP, W303-1b/F/GAP' and W303-1b/F/PGK1) or YPGal medium (10 g/L yeast extract, 20 g/L tryptone and 20 g/L galactose, W303-1b/F/GAL1) to keep an initial optical density at 600 nm (OD600) value of 0.2. The cultures were incubated at 30°C with agitation at 250 rpm for additional 96 h. Intracellular GSH amount was monitored after 24 hours fermentation and measured every 12 hours.