Supporting information

The Functional Promiscuity of the COG0720 Family

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This Supporting Information Contains

Supplemental data 1.

PTPS-VI sequences identified

gi|229578027|ref|YP_002836425.1| hypothetical protein YG5714_0210 [Sulfolobus islandicus Y.G.57.14]
MKVRVGIEGITMDSAHYTLSSYADSQIHGHTYIINVEVEGEVNEKSGFVVDFNLLKKMV
REVIQEWDHKL
IIPKVDLDKSRFEGPFRVDYKVIDAPFPTAEYIGIEIAKDIYLKLNKKYRILLKIYEGKDSY
AIIEYP

>gi|227826615|ref|YP_002828394.1| hypothetical protein M1425_0206 [Sulfolobus islandicus M.14.25]
MKVRVGIEGITMDSAHYTLSSYADSQIHGHTYIINVEVEGEVNEKSGFVVDFNLLKKMV
REVIQEWDHKL
IIPKVDLDKSRFEGPFRVDYKVIDAPFPTAEYIGIEVAKDIYLKLNKKYRILLKIYEGKDSY
AIIEYP

>gi|227829256|ref|YP_002831035.1| hypothetical protein LS215_0237 [Sulfolobus islandicus L.S.2.15]
MKVKVGIEGITMDSAHYTLSSYADSQIHGHTYIINVEVEGEVNEKSGFVVDFNLLKKMV
REVIQEWDHKL
IIPKVDLDKSRFEGPFRVDYKVIDAPFPTAEYIGIEIAKDIYLKLNKKYRILLKIYEGKDSY
AIIEYP

>gi|15899165|ref|NP_343770.1| hypothetical protein SSO2412 [Sulfolobus solfataricus P2]
MKVRVGIEGITMDSAHYTLSSYADSQIHGHTYIVNVEVEGEVNEKSGFVVDFNLLKKIIK
ETIQEWDHKL
IIPKVDLDKSRFEGPFRVDYKVIDAPFPTAEYIGIEIAKDIYLKLNKKYRILLKIYEGKDSY
AIIEYP

>gi|330835333|ref|YP_004410061.1| putative 6-pyruvoyl tetrahydropterin synthase [Metallosphaera cuprina Ar-4]
MKIKVGIEGFTIDSAHYTLSSPKDDQLHGHTYTVSVEVEGDINEDNGFVVDFNKLRNLV
NQTISLWDHKF
IVPKKDFEKINIASPFRVDVKLIEAPYPTVEYIGIEIAKHIYNSLGNNFKISVKIYEGRDSYA
VVEYP

>gi|332797876|ref|YP_004459376.1| putative 6-pyruvoyl tetrahydropterin synthase [Acidianus hospitalis W1]
MKVKIGIEGFTIDSAHYTLSSPGDSQLHGHTYIINVEVEGNINPANGFVIDFNKLKDEIGKI
IKEWDHKL
IVPKKDLEKISISGPFRNEIKIIEEDYPTAEYIGFELAKEIYNRINMPIKIKIYEGKDSYAIIEY
P

>gi|15920762|ref|NP_376431.1| hypothetical protein ST0543 [Sulfolobus tokodaii str. 7]
MKVKVGLEGLSFDSAHYTLSSEGNQQIHGHTYKLSIEIEGNSIDENTGFVIDFEILKKIIND
IVKDWDHK
LIIPSEDLNQIYFKGPFKLDIKVIPYKYPTAEYIGLEIAKSIYEKLQKKYKITVKIYEGENNY
AIIEYP

>gi|70606719|ref|YP_255589.1| hypothetical protein Saci_0934 [Sulfolobus acidocaldarius DSM 639]
MKVVIGVEGFSFDSAHYTLSSKGNDQIHGHTYKLSVEIEGQDVDKNSGFVIDFMILTKIIH
EVIREWDHK
LIIPSRDARKIELKGPFKVEYKIIEEDFPTVEYIGSEIAKDIYEKLEKKFKVRVKIYEGENSY
ALIEYP

>gi|146303412|ref|YP_001190728.1| putative 6-pyruvoyl tetrahydropterin synthase [Metallosphaera sedula DSM 5348]
MKVRVGIEGFSIDSAHYTLSSPKDNQLHGHTYRVTVEVEGEVHEENGFVVDFNKLRELI
KEAISSWDHKF
IVPRRDMDKISITSPFKLEVKIIEAPYPTVEYIGMEIAKYVFTRLGSNFKVFVKIYEGSDSY
ALIEYP

PTPS-V sequences identified

>gi|126459676|ref|YP_001055954.1| hypothetical protein Pcal_1063 [Pyrobaculum calidifontis JCM 11548]
MRTCVELRGSISVAHKPSFSPGWARVHGHDYFITVGICVEGYRDLVVDADEASKKFREA
LARMDGKYLAS
PQEKVALDAGEIYVVPCNLPGVSGECLAKHIADLVGAAWVRVCESSLGGPCFYFSKS

>gi|18313125|ref|NP_559792.1| hypothetical protein PAE2137 [Pyrobaculum aerophilum str. IM2]
MRTCVELRGSISVAHKPAFSPTWSRVHGHDYYITVGICKEGYHEVVVDASAAGDRFKE
LLAKLDGKYLAS
SSEQTPLSADEVYIVPCEAKGVSGECLAKHIADLMGATWVRVCESAYASPCFYFER

>gi|171185792|ref|YP_001794711.1| hypothetical protein Tneu_1339 [Thermoproteus neutrophilus V24Sta]
MRTCVELKGSVSVAHNPSFSPKWARIHGHDYVITVGICREGYTDVVVDAAEAGDKFRA
LLASIDGKYLAS
PLENPPLEPSGVYTVPCGQPGVSGECLAKYIAELMGASWVRVCESGLGSPCFYFER

>gi|119872458|ref|YP_930465.1| hypothetical protein Pisl_0946 [Pyrobaculum islandicum DSM 4184]
MRTCVELRGSISVAHNPSFSPKWARIHGHDYVITVGICRDGYTDVVVDAAEVSEKFRKV
LTSMDGRYLAS
PFEKLPPDIKDVYIVPCSLSGVSGECLARHIAELMRASWVRICESGFGSPCFYYES

>gi|145591060|ref|YP_001153062.1| hypothetical protein Pars_0824 [Pyrobaculum arsenaticum DSM 13514]
MRTCVELRGSISVAHKPSFSTAWGRVHGHDYIITASICRDGFHDVVVDAGEAGERLREL
LSKMDGKYLAS

SAEQVDLPQEEVYVVPCNAAGLSGECLAKHIADLLGASWVRVCESGYGAPCFLYER

>gi|325969372|ref|YP_004245564.1| hypothetical protein VMUT_1861 [Vulcanisaeta moutnovskia 768-28]
MCIIASTVVETTCLSMRFMFSGAHRVRFSGVYGNIHGHNYDITIRLCVNGRRTLIMDIDK
VRHEIQGIIS
AFDGKYIKSMNEETRELTPNEYYVEIPCIPEGATGECLAWYLMDKVLDVLRNMGISEITET
QVVVCDSPIN
CFESSSLLST

>gi|307595147|ref|YP_003901464.1| hypothetical protein Vdis_1024 [Vulcanisaeta distributa DSM 14429]
MKFMFSGAHRVRFSDIYGNVHGHNYDVTIRLCVRGRRMLVTDIDKIRREVQGIVNTFD
GKYIKSSMESAQ
GLMPGELVEIPCIPEGATGECVAWYFIDSVMGILSRLGINDVVEVQVIVCDSPMNCFEAR
SS

Supplemental Table 1. Organisms predicted to contain COG0720 enzymes with dual PTPSI/III activities.

Organism	Accession numbers	Motif
Desulfuromonas acetoxidans	ZP_01312295.1	GDCENLHGHNWK
Geobacter_sulfurreducens	NP_952770.1	GDCENLHGHNWR
Pirellula_sp.	NP_868552.1	DICERIHGHNYGV
Thermotoga_maritima	NP_227854.1	GKCERLHGHTYR
Geobacter_metallireducens	YP_384613.1	GDCENLHGHNWK
Thermoanaerobacter_tengcongensis	NP_623903.1	GKCEELHGHTYRL
Desulfovibrio_desulfuricans	YP_388689.1	GKCEALHGHNFG
Dehalococcoides_ethenogenes	YP_182300.1	GKCENLHGHRYE
Blastopirellula_marina	ZP_01091150.1	GTCERVHGHNYR
Solibacter_usitatus	YP_822324.1	GKCENVHGHNYR
Syntrophobacter_fumaroxidans	YP_844142.1	GKCENLHGHNWK
Syntrophus_aciditrophicus	YP_462286.1	GNCEHLHGHNWA
Clostridium_botulinum	YP_001383205.1	GKCERLHGHTYG
Desulfovibrio_vulgaris	YP_010571.1	GKCENLHGHNFA
Bacteroides_vulgatus	YP_001299367.1	SKCENLHGHNWI
Anaeromyxobacter_sp	YP_001378545.1	GKCERLHGHNW
Anaeromyxobacter_dehalogenans	YP_465721.1	GKCERLHGHNWRV
Thermotoga_petrophila	YP_001244479.1	GKCEKLHGHTYR
Herpetosiphon_aurantiacus	YP_001545606.1	GKCERLHGHNYR
Fervidobacterium_nodosum	YP_001411270.1	GKCEKLHGHTYK
Pelobacter_propionicus	YP_901456.1	GDCENLHGHNWK
Caldicellulosiruptor_saccharolyticus	YP_001179049.1	GKCERLHGHTYK
Thermoanaerobacter_pseudethanolicus	YP_001664327.1	GKCEELHGHTYK
Desulfococcus_oleovorans	YP_001529374.1	HKCENLHGHNWK
Dethiosulfovibrio_peptidovorans	ZP_06392544.1	GKCEALHGHTYR
Planctomyces_limnophilus	YP_003630433.1	NICERLHGHNWR
Denitrovibrio_acetiphilus	YP_003504095.1	GKCENLHGHNWK

Supplemental Table 2. Distribution of QueD2 in sequenced organisms.
* gene predicted to be regulated by Zur

Organism	QueD (Accession)	Motif	QueD2 (Accession)	Motif
Acinetobacter baylii sp. ADP1			YP_046954.1	CKRSIHGH
Acinetobacter baumannii ATCC 17978			YP_001707113.1	CKRSIHGH
Psychrobacter sp. PRwf-1			YP_001279719.1	CKRSIHGH
Psychrobacter cryohalolentis K5			YP_580760.1	CKHSIHGH
Sulfurovum sp. NBC37-1			YP_001357532.1	CSENIHGH
Cupriavidus metallidurans CH34	YP_583724.1	CRNIHGH	YP_583256.1*	CSHSIHGH
Burkholderia cenocepacia AU 1054	YP_622375.1	CRNLHGH	YP_622905.1*	CSHSIHGH
Azotobacter vinelandii	ZP_00091843.1	CGRLHGH	YP_002800062.1*	CSRSLHGH
Thiomicrospira denitrificans ATCC 33889			fig|326298.3.peg.328	CRSSIHGH
Campylobacter concisus 13826			YP_001466043.1	CRTSIHGH
Campylobacter jejuni subsp. jejuni CF93-6			ZP_01067834.1	CKSSIHGH
Campylobacter coli RM2228			ZP_00367800.1	CKSSIHGH
Campylobacter upsaliensis RM3195			ZP_00371720.1	CKTSIHGH
Campylobacter lari RM2100			YP_002575991.1	CKTSIHGH
Campylobacter hominis ATCC BAA-381			YP_001405930.1	CRESIHGH
Helicobacter mustelae 43772			YP_003516807.1	CSRSLHGH
Helicobacter acinonychis str. Sheeba			YP_664840.1,	CAQNIHGH
			YP_664846.1	CAQNIHGH
Helicobacter pylori J99			NP_223585.1	CAQNIHGH

Supplemental Table 3. Strains and Plasmids

Strains	Genotype/Comments	Reference/Source
E.coli		
JW2735	*rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1ΔygcM*	(*1 single-gene knockout mutants: the Keio collection*)
GM2163	F$^-$ *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL 136 dam13::Tn9 xylA5 mtl-1 thi-1 mcrB1 hsdR2*	New England Biolab
INV110	F′{*tra*Δ*36 proAB lacIq lacZ*ΔM15} *rpsL* (StrR) *thr leu endA thi-*1 *lacY galK galT ara ton*A *tsx dam dcm supE*44 Δ*(lac-proAB)* Δ*(mcr*C-*mrr)*102::Tn*10* (TetR)	Invitrogen
Topo 10	(F- *mcrA* Δ(*mrr-hsdRMSmcrBC*) Φ80*lacZ*ΔM15 Δ*lacX*74 *recA1 araD*139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G)	Invitrogen
DH5α	F$^-$ *recA1 endA1 hsdR17*(r_k^- m_k^+) *supE44 thi-1 gyrA relA1*	Life Technologies
MG1655	*F$^-$ λ$^-$ ilvG- rfb-50 rph-1*	(*2*)
VDC2043	MG1655 Δ*PTPSI$_{Ec}$*	This study
VDC3267	MG1655 Δ*folB*::*Kan^r*	This study
VDC3321	VDC2043 pBAD24	This study
VDC3325	VDC2043 pBAD24::*PTPS-I*	This study
VDC3331	VDC2043 pUC19::*PTPS-II$_{rat}$*	This study
VDC3335	VDC2043 pBAD24::*PTPS-I/III$_{Sa}$*	This study
VDC3337	VDC2043 pBAD24::*PTPS-I/III$_{Sa}$* E27A	This study
VDC3339	MG1655 pBAD24	This study
VDC3365	VDC2043 pBAD24::*PTPS-I/III$_{Sa}$* Cys26Ala	This study
VDC3441	VDC2043 pBAD24::*PTPS-I/III$_{Sa}$* Glu27Cys	This study
VDC3447	VDC2043 pUC19::*PTPS-II$_{Rn}$* Cys42Ala and Asn44Cys	This study
VDC3452	VDC2043 pBAD24::*PTPS-I$_{Ec}$* Lys23Cys and Cys24Ser	This study
VDC4660	VDC2043 pBAD24::*PTPS-I$_{Ab\ ADPI}$*	This study
VDC3524	VDC2043 pBAD24 :: *SSO2412*	This study
VDC3516	VDC2043 pBAD24 :: *Pcal_1063*	This study
VDC3473	VDC3276 pBAD24	This study
VDC3475	VDC3276 pBAD24::*PTPS-I/III$_{Sa}$* Cys26Ala	This study
VDC3477	VDC3276 pBAD24::*PTPS-I/III$_{Sa}$* Glu27Cys	This study
VDC3465	VDC2043 pBAD24::*PTPS-I$_{Li}$*	This study
VDC3467	VDC2043 pBAD24::*PTPS-III$_{Li}$*	This study
VDC3469	VDC2043 pBAD24::*PTPS-I$_{Cb}$*	This study
VDC3471	VDC2043 pBAD24::*PTPS-I/III$_{Cb}$*	This study
VDC3479	VDC3267 pBAD24: *PTPS-I/III$_{Sa}$*	This study
VDC3481	VDC3267 pBAD24:: *PTPS-I/III$_{Sa}$* Glu27Ala	This study
VDC3484	VDC3267 pUC19::*PTPS-II$_{Rn}$*	This study
VDC3485	VDC3267 pUC19:: *PTPS-II*$_{Rn}$ Cys42Ala and Asn44Cys	This study
VDC3487	VDC3267 pBAD24::*folB$_{Ec}$*	This study
VDC3495	VDC3267 pBAD24::*PTPS-I$_{Cb}$*	This study
VDC3493	VDC3267 pBAD24::*PTPS-I/III$_{Cb}$*	This study
H. volcanii		
H26	DS70 *ΔpyrE2*	(*3*)
VDC3290	H26 Δ*HVO_1718*	This study
VDC3241	H26 Δ*folE2*	(*4*)
VDC3453	H26 Δ*HVO_1718* pJAM202::*VNG6306*	This study
VDC3455	H26 Δ*HVO_1718* pJAM202c	This study
VDC3342	H26 Δ*HVO_1282*	This study
VDC3405	H26 Δ*HVO_1282* pJAM202c	This study

VDC3401	H26 Δ*HVO_1282* pJAM202::*HVO_1282*	This study
VDC3442	H26 Δ*HVO_1284*	This study
VDC3226	H26 pJAM202c	(*5*)

Plasmids	**Description**	**Reference or source**
pBAD24	Amp^r; colE1	(*6*)
pTA131	Amp^r; pMB1; pyrE2 under ferredoxin promoter	(*7*)
pBY158	pTA131 derivative containing the $attCm^RCcd^R$ cassette	(*4*)
pGP058	pBY158 derivative containing 1000bp Upstream and downstream of *HVO_1718*	This study
pGP044	pCR8/GW containing 1000bp Upstream and downstream of *HVO_1718*	This study
pGP325	pBAD24::$PTPSI_{Ec}$	This study
pSTV28MPS	pSTV28 containing *mtrA*, $PTPS\text{-}II_{Rn}$ and *spr*	(*8, 9*)
pBY148	pUC19::$PTPS\text{-}II_{Rn}$ cloned between *Eco*RI and *Bam*HI from pSTV28MPS	This study
pGP447	pUC19::$PTPS\text{-}II_{Rn}$ Cys42Ala and Asn44Cys	This study
pGP452	pBAD24::$PTPS\text{-}I_{Ec}$ Lys23Cys and Cys24Ser	This study
pGP365	pBAD24::$PTPS\text{-}I/III_{Sa}$ Cys26Ala	This study
pGP441	pBAD24::$PTPS\text{-}I/III_{Sa}$ Cys26Ala and Glu27Cys	This study
pJAM202	Amp^r Nov^r; pBAP5010 containing $P2_{rrn}$-*psmB*-his_6; β-His_6 expressed in *H. volcanii*	[46]
pJAM202c	Amp^r; Nov^r; pJAM202-derived control plasmid	(*10*)
pGP401	pJAM202:: *HVO_1282*	This study
pGP425	pJAM202::*VNG6306*	This study
pCH129	pBAD24::$PTPS\text{-}I2A_b$	This study
pIKB272	pTA131::*HVO_1282*	This study
pIKB306	pTA131::*HVO_1284*	This study
Sa PTPS-III E27A – pBAD24	pBAD24::$PTPS\text{-}I/III_{Sa}$ Glu27Ala	(*11*)
Sa PTPS-III– pBAD24	pBAD24::$PTPS\text{-}I/III_{Sa}$	(*11*)
Li PTPS-I– pBAD24	pBAD24::$PTPS\text{-}I_{Li}$	(*11*)
Li PTPS-III– pBAD24	pBAD24::$PTPS\text{-}III_{Li}$	(*11*)
Cb PTPS-I – pBAD24	pBAD24::$PTPS\text{-}I_{Cb}$	(*11*)
Cb PTPS-III– pBAD24	pBAD24::$PTPS\text{-}I/III_{Cb}$	(*11*)
pGPP522	pBAD24::$PTPS\text{-}VI_{Ss}$	This study
pGP505	pBAD24::$PTPS\text{-}V_{Pc}$	This study

Genome abbreviation. Ab: *Acinetobacter baylyi* ADP1, Cb: *Clostridium botulinum*, Ec: *Escherichia coli*, Li: *Leptospira interrogans L1-130*, Pf: *Plasmodium falciparum*, Sa: *Synthrophus aciditrophicus*, *Rn: Rattus norvegicus, Ss: Sulfolobus solfataricus P2, Pc: Pyrobaculum calidifontis.*

Supplemental Table 4. List of primers used in this study

Primer name	Sequence	Use
Site directed mutagenesis		
mutSyacCtoA_Fw	AACTATCCGGGCAAC**GCC**GAACAT	Construction of plasmid pGP365
mutSyacCtoA_Rev	GCCATGCAGATGTTC**GGC**GTTGCC	
SyacmutAEtoAC_Fw	GGCAACGCC**TGC**CATCTGCAT	Construction of plasmid pGP441
SyacmutAEtoAC_Rev	CATGCAGATG**GCA**GGCGTTGC	
YgcMmutCX3toCX4_Fw	CCGGAAGGGCAT**TGTA**GTGGTCGCCTG	Construction of plasmid pGP452
YgcMmutCX3toCX4_Rev	GTGCAGGCGACCAC**TACA**ATGCCCTTCC	
PTPSIImutCX5toCX3_Fw PTPSIImutCX5toCX3_Rev	GGGAAA**GCC**AAC**TGT**CCGAATGGC ATGGCCATTCGG**ACA**GTT**GGC**TTTCCC	Construction of plasmid pGP447
Cloning		
HsQueD_NdeI_Fw	GAGCGCATATGCGTTCAGCACAGTCCAA	Construction of plasmid pGP425
HsQueD_BlpI_Rev	GAGCGGCTCAGCTCAGTTCGGGCCAGCAGT	
QueDADP1_EcoRI_Fw	TTGATCGGAATTCACCATGTTAATTCGTAAGTTATTTA AGTTTG	Construction of plasmid pCH129
QueDADP1_XbaI_Rev	TTGATCTCTAGATTAAATATGAACTTGCAATTCCAC	
ygcM_EcoRI_Fw	ATATGAATTCTGTAGAGAAATTATGATGTCCAC	Construction of plasmid pGP325
ygcM_KpnI_Rev	ATATGGTACCTCATTCGCCGCGATAGATACA	
HvPTPSIV_NdeI_Fw	CATATGTACCGCGTCTCGGTTCG	Construction of plasmid pGP401
HvPTPSIV_BlpI_Rev	GCTCAGCTCAGAGCGGAGTCTCGTAGGT	
PcQueD2WHGH_Fw	AGGAGCCATGGGGACGTGTGTAGAATT	Construction of plasmid pGP505
PcQueD2WHGH_Rev	AGGAGGGATCCTTAACTCTTGCTGAAGTAGAAGCA	
SsQueD2QHGH_Fw	AGGAGGAATTCACCATGAAAGTTAGAGTTGGTATCG AAG	Construction of plasmid pGP522
SsQueD2QHGH_Rev	TTATGGATACTCTATAATTGCATATGA	
ECygcmS	GGTATTGAGGGTCGCATGATGTCCACCACG	Construction of plasmid pBL-I41
ECygcmAS	AGAGGAGAGTTAGAGCCTCATTCGCCGCG	
MJ1272-Fwd	GGTCATATGATGTTGGAGTTAAAT	Construction of plasmid pMJ1272
MJ1272-Rev	GCTGGATCCTTATTTCACCTCTAAG	
***H. volcanii* deletions**		
EntUPDNHvqueD_Fw	CGGATTCGTAGATGAGCGTGAT	Construction of plasmid pGP044
EntUPDNHvqueD_Rev	CGGTCGTCGCGCTGTCCATG	
UDqueDD_Fw	GAGCTCTGCGCCGGCTTCT	Construction of plasmid pGP058
UDqueDD_Rev	CGTTCGCCTGCTCGGGACA	
GTPCHIII_N_Fw	CGGGCCCCCCCTCGAGCTTTCGTCGGGTTGTTCG	Construction of plasmid pIKB306
GTPCHIII_N_Rev	GACGCGTTCATATGCTGCGTATTCGTCACGGTAGA	
GTPCHIII_C_Fw	GCATATGAACGCGTCAGACGACTGAGCGATGGAG	
GTPCHIII_C_Rev	CGGGCTGCAGGAATTCAAGCGACTCGGGATGGAC	

PTPS4_N_Fw	CGGGCCCCCCCTCGAGCGCTCAACTTCGTGATGGAC	Construction of plasmid pIKB272
PTPS4_N_Rev	GACGCGTTCATATGCGTTCGGAACCGTCAGGTG	
PTPS4_C_Fw	GCATATGAACGCGTCACCTACGAGACTCCGCTCTG	
PTPS4_C_Rev	CGGGCTGCAGGAATTCCGGTTAGCTTCCAGTCGTC	
***H. volcanii* verifications of deletions**		
intckqueD_Fw	CTCCACCACGACGGCAAGTGT	Internal check for deletion of *HVO_1718*
intckqueD_Rev	CGCTGACCGAGACCGAGACC	
Chk_HvqueD_Fw	ACCCGAAGATATCAGTATTATAAC	External check for deletion of *HVO_1718*
Chk_HvqueD_Rev	GTTCGTAGCCGCGTCGCGTT	
HvGTPCHIIIintck_Fw	AACATGGTCGCGGTCACCAA	Internal check for deletion of *HVO_1284*
HvGTPCHIIIintck_Rev	ACAGTCTTCGAGCGCGTGTT	
HvGTPCHIIIck_Fw	GGAGGTCGTCGTAGTGGAAC	External check for deletion of *HVO_1284*
HvGTPCHIIIck_Fw	CGTCTTCGAGGAGTCGGTAG	
HvPTPS4intck_Fw	TCTCGGTTCGCAGGGACCTC	Internal check for deletion of *HVO_1282*
HvPTPS4intck_Rev	CGCTCGCCACGTCGTCCTC	
HvPTPS4ck_Fw	AGCCGCGTCTTCGCGTTCAA	External check for deletion of *HVO_1282*
HvPTPS4ck_Rev	GATAGCGAGCGACTGCAACC	

c Restriction sites are underlined; point mutations are in bold.

Supplemental Material and Methods

Preparation of *M. jannaschii* Cell Extracts.

M. jannaschii cells were grown as previously described (*12*). Cell extracts of *M. jannaschii* were prepared by sonicating 5 g of a frozen cell pellet suspended in 10 ml of TES extraction buffer (50 mM TES/K^+, 10 mM $MgCl_2$, 10 mM DTT, pH 7.5) under Ar for 5 min at 3°C. A W-385 Sonicator with a microtip from Heat Systems-Ultrasonics, Inc was used. The resulting mixture was centrifuged under Ar (27000 × *g*, 10 min) and was stored frozen under Ar at –20°C until used. The protein concentration of the *M. jannaschii* extract was ~30 mg/ml. Protein concentrations were measured using the BCA total protein assay (Pierce) with bovine serum albumin as a standard.

Assay of pterin, biopterin and hydroxymethylpterin in *M. jannaschii*

To 0.5 g of frozen cell pellet is added 0.5 mL of water and the sample shaken and heated at 100°C for 5 min. To the suspension is added 200 μL of 50% TCA and the mixture heated an addition 100°C for 10 min and then centrifuged (14000*g*, 10 min). The pellet was washed with 100 μL of 5% TCA centrifuged (14000*g*, 10 min) and the combined supernates mixed with iodine in MeOH (50 mg/mL) to give an intensity purple solution. After 1 hr at room temperature the color was dispelled by the addition of a saturated solution of sodium bisulfite in water. An equal volume of methanol was then added, and the samples were centrifuged (14,000*g*, 10 min) to remove the precipitate. After removal of the evaporation methanol the sample was applied to a Dowex 50W-8X-H^+ column (3 x 10 mm) that, after washing with water, the bound pterins were eluted with 6 M NH_4OH. The pterins purified by preparative LC using the acetonitrile-water-formic acid (88%), 40:10:5 v/v/v as the developing solvent. The pterin, biopterin and hydroxymethylpterin containing spot was then removed (they all have the same TLC R_f) and eluted with 50% methanol. After removal of the methanol by evaporation the samples were assayed by HPLC using the Varian PursuitXRs C18 and Varian Pursuit-PFP HPLC columns as described in the methods section of the text. These two columns were required for the separation of the pterin from the 6-hydroxymethypterin. Of the three-targeted pterins only the 6-hydroxymethylpterin, an intermediate in methanopterin biosynthesis, was detected. The absence of biopterin indicated that this pterin was not produced by *M. jannaschii* and that these cells would not contain PTPS-II.

Supplemental Results

Measuring the incorporation Deuterium into CPH_4 produced by the MJ1272 derived enzyme.

When a incubation with sepiapterin and H_2neoterin-P was done in the presence of 50% D_2O the amount of deuterium incorporated into the recovered pterin product was 48% of the molecules with one deuterium. This would have been incorporated in the last (retroaldol) reaction leading to 6-carboxytetrahydropterin product (see Figure 6 of main manuscript). Subsequent oxidative decarboxylation would lead the C-6 labeled pterin that was isolated and measured.

Testing proton exchange of pterin and dihydropterin

To establish the extent of chemical proton exchange of pterin and dihydropterin each were dissolved in D_2O and heated for different periods under different conditions and then analyzed for deuterium incorporation by mass spectrometry. After this treatment the dihydropterin were oxidized to pterin prior to analysis as described above and the pterins purified by retention on a Dowex 50W-8X-H^+ column followed by elution with aqueous ammonia. The resulting pterins were analyzed for deuterium exchange either by direct insertion (DI) mass spectral of the pterin, M^+ = 163 *m/z* or by DI or GC-MS analysis of the pterin $(TMS)_2$ derivative having intense ions at M^+ = 307 *m/z* and M^+ -15 = 292 *m/z*. 7-8-Dihydropterin was prepared either by reduction of pterin with Adams catalysis (*13*) or by reduction with zinc metal in 1 N NaOH (*14*). The dihydropterin in dilute phosphate buffer was evaporated to dryness with a stream of nitrogen gas and place in D_2O under argon and heated at 100°C. After 17 hr the sample was cooled and oxidized with I_2 in methanol followed by bisulfite reduction. The pterin was then isolate on a Dowex 50W-8X-H^+ column and converted into the $(TMS)_2$ derivative as described above. Mass spectral analysis showed that the pterin had incorporated in 15% of the molecules a single deuterium that we consider to be incorporated at C-7. Pterin heated under the same conditions

did not incorporate any deuterium. These results show that the label from the incubations with substrates and D_2O would not result from the chemical incorporation into pterin or dihydropterin.

Involvement of cysteine in the catalytic mechanism of the MJ1272 derived enzyme.

Incubation of the enzyme (10 μg) with 2 mM iodoacetamide for 1 hr resulted in the alkylation of the one conserved cysteine producing an enzyme that exhibited 38% of the activity compared to the untreated control. These results indicated that the cysteine in the PTPS-I signature motif {C-X(3)-H-G-H} was either involved in the enzymatic mechanism and/or substrate binding of to this enzyme.

Metal binding analysis of MJ1272

Metal analysis

Metal analysis of the MonoQ purified MJ1272 gene product was performed at the Virginia Tech Soil Testing Laboratory using inductively coupled plasma emission spectrophotometry. Instrumentation included a Spectro CirOS VISION made by Spectro Analytical Instruments equipped with a Crossflow nebulizer with a Modified Scott spray chamber, nebulizer rate was 0.75 L/min. A 50 mg/L yttrium internal standard was introduced by peristaltic pump. Samples were analyzed for iron, manganese and zinc.

Purified MJ1272 was also analyzed for iron, manganese, nickel, and zinc by ICP spectroscopy. Manganese, and nickel were not detected, however a 0.6 molar ratio of zinc and a 0.1 molar ratio of iron per MJ1272 monomer was measured. To test the effect of different metals on activity MJ1272 was incubated with different metals and assayed by HPLC for the different products obtained from sepiapterin. The MJ1272 gene product had the following order of activity with the divalent metal indicated: Fe>>>Co>>Zn, Cu, Ni, Mn, Mg. The ratio of 6-carboxypterin and

pterin varied between metals. Additional minor peaks were observed in some of the reactions but were not identified. Incubations were conducted in the typical assay conditions with 2 mM metal added.

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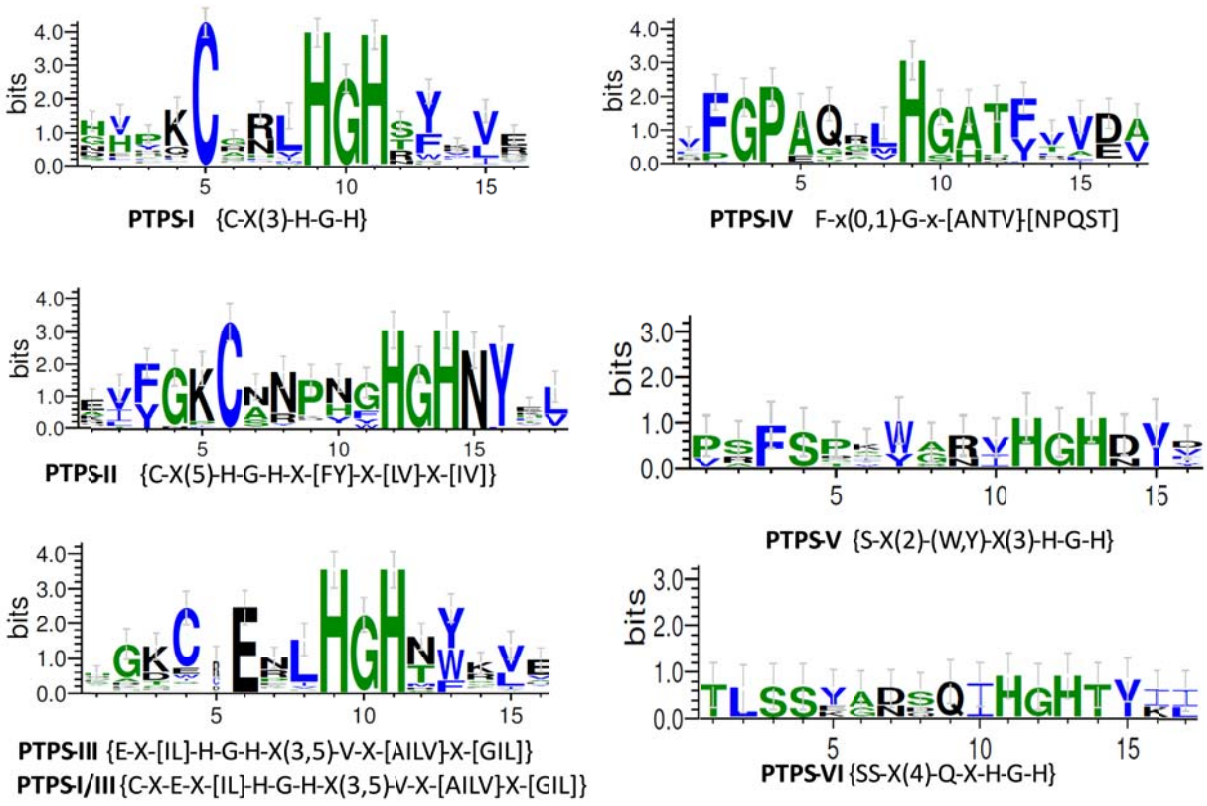
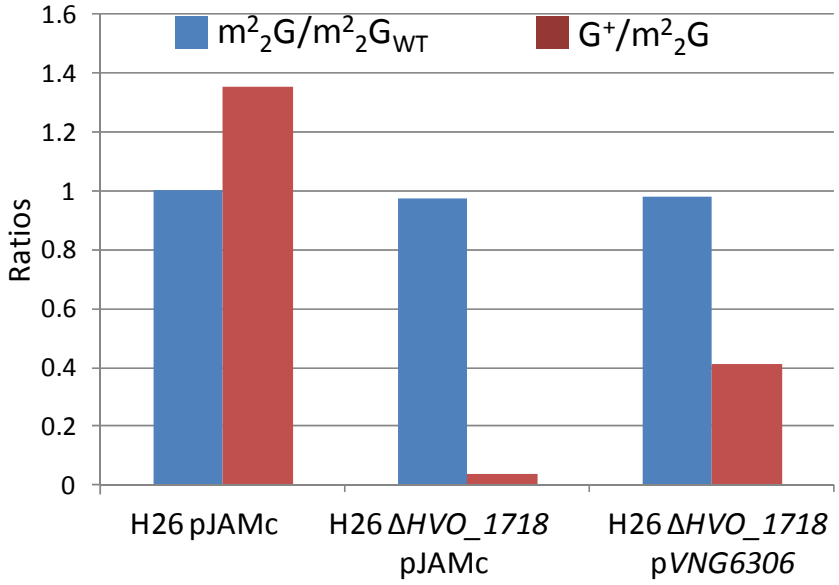
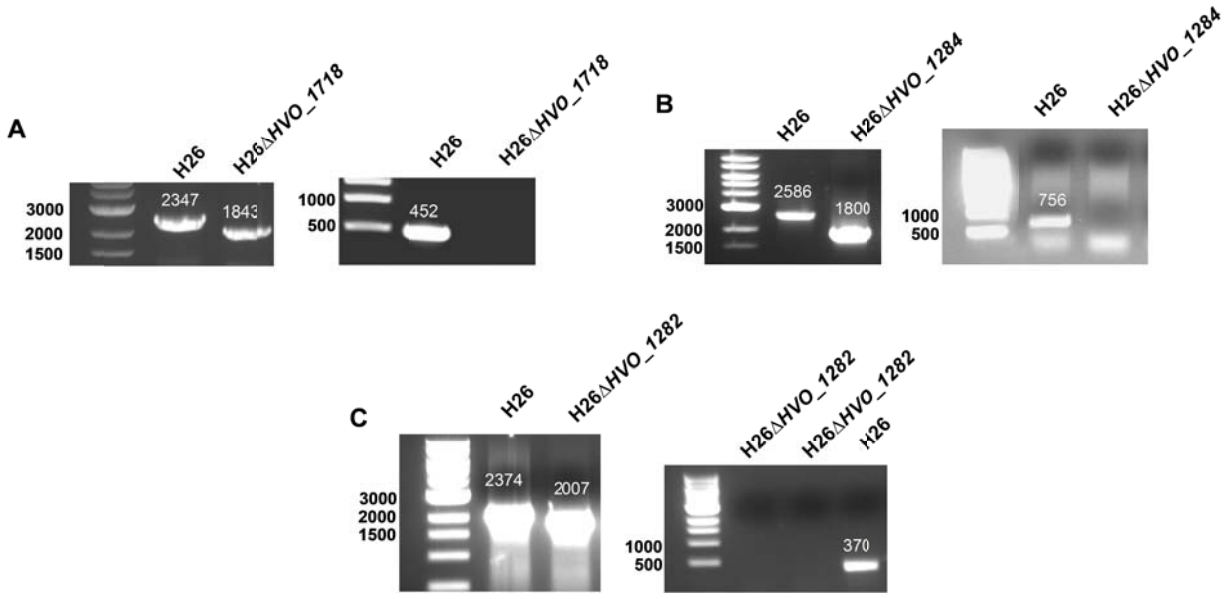


Supplemental Figure 1. Sequence Logos and signatures. Signature motifs obtained using Prosite and logos for the PTPS-I to IV family members. The {C-X(3)-H-G-H} motif is found in PTPS-I member involved in Q biosynthesis encoded by the *queD* gene. The {C-X(5)-H-G-H-X-[FY]-X-[LV]-X-[IV]} motif is found in PTPS-II involved in BH_4 biosynthesis, The {E-X-[IL]-H-G-H-X(3,5)-V-X-[AILV]-X-[GIL]} motif is found in PTPS-III involved in folate biosynthesis, the {C-E-X-[ILPV]-H-G-H-X-[FWY]-X(3)-[AILV]} motif is found in PTPS-I/III involved in both queuosine and folate biosynthesis. The {F-X(0,1)-G-X-[ANTV]-[NPQST]} motif was found in PTPS-IV sequences.



Supplemental Figure 2. Complementation of G^+ deficient phenotype by *H. salinarum* QueD homolog. The G^+ deficient phenotype was complemented by the in trans expression of *H. salinarum* Vng6306, *HVO_1718* homolog, in the mutant strain. To control for the amount of tRNA, the m^2_2G content in the complemented strains was compared with the m^2_2G content in the WT control (blue bars). The ratios of G^+/m^2_2G of tRNA extracted from the *H. volcanii* Δ*HVO_1718* derivatives strains are shown by the red bars.



Supplemental Figure 2. PCR verifications of *H. volcanii* gene deletions of (A) *HVO_1718*, (B) *HVO_1284* and (C) *HVO_1282.* The gene deletions were verified by locus-specific PCR using primer pairs designed to anneal upstream and downstream the deletion cassette and primers pairs designed to anneal within the deleted gene (as indicated in Supplementary Table 2). Predicted amplicon sizes are indicated above each PCR band.