Supporting Information

Diversity of Archaeosine Synthesis in Crenarchaeaota

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Content of Supporting Information

1. Methods

Media.

Escherichia coli derivatives were routinely grown at 37°C in LB (BD Diagnostic System) supplemented with 0.2% glycerol as a carbon source. Growth media were solidified with 15 g/L agar (BD Diagnostic System) for the preparation of plates. Ampicillin (Amp^r, 100 µg/mL), Thymidine (dT, 300 µM), Kanamycin (Kan^r, 50 µg/mL) and L-arabinose (0.2%) were added when needed. Transformations of *E. coli* were performed following standard procedures (*1*).

Growth of *Haloquadratum walsbyi.*

H. waslbyi C23 (*2*) was grown at 37°C static in defined media (DBCM2) (*3*) containing 200 g NaCl, 29.1 g $MgSO_4$•$7H_2O$, 25g $MgCl_2$•$6H_2O$, 5.8 g KCl, 5 mM, 5.0 mM NH_4Cl, 1mM K_2HPO_4 pH 7.5, 0.25% HCl, 0.015 g $FeCl_2$•$4H_2O$, 0.19 mg $CoCl_2$•$6H_2O$, 0.1 mg $MnCl_2$•$4H_2O$, 0.07 mg $ZnCl_2$, 0.006 mg H_3BO_3, 0.036 Na_2MoO_4•$2H_2O$, 0.024 mg $NiCl_2$•$6H_2O$, 0.002 mg $CuCl_2$•$2H_2O$, 0.04 mg 4-aminobenzoate, 0.003 mg biotin, 0.09 mg nicotinic acid, 0.05 mg calcium panthotenate, 0.15 mg pyridoxamine hydrochloride, 0.09 mg thiamine chloride hydrochloride, 0.05 cyanocobalamine, 0.03 mg lipoic acid, 0.03 mg riboflavin, 0.012 mg folic acid, and 10 mM pyruvate. The cells were collected by centrifugation (5000 rpm for 5 min at RT) and stored at -20°C for further use.

Growth of *Haloferax volcanii.*

H. volcanii was grown at 45°C and 200 rpm in Hv-YPC rich medium (*4*) containing: 144 g NaCl, 21 g $MgSO_4$•$7H_2O$, 18 g $MgCl_2$•$6H_2O$, 4.2 g KCl, 10mM Tris HCl (pH 7.5), 0.5% yeast extract, 0.1% peptone, and 0.1% casamino acids (w/v). The cells were collected by centrifugation (5000 rpm for 5 min at RT) and stored at -20°C for further use.

Archaeal tRNA extraction.

To extract tRNA, the frozen cells were thawed and resuspended in 50 mM Na acetate buffer pH 5.8 (3 mL buffer per 1g of cells). Equal volume of phenol saturated with mildly acid buffer (50 mM NaOAc pH 5.8) was immediately added to the cell suspension and shaken overnight at room temperature. The aqueous phase was recovered by centrifugation (20 min at 5,000 rpm), and another one volume of buffered saturated phenol was added. The phenol:buffer was vigorously shaken again for 2 minutes at room temperature. After centrifugation, as above, one volume of chloroform was added and mixed vigorously again for 2 minutes at room temperature. The supernatant was recovered by centrifugation and adjusted to 20% isopropanol followed by one hour incubation at -20°C. The pellet containing genomic DNA and long RNA (mRNA and rRNA) was spun down, and the amount of isopropanol was adjusted to 60% final concentration. After one overnight standing at -20°C, the precipitated small RNAs (mostly 'soluble' RNA = tRNA) were recovered by centrifugation at 4°C, washed twice with cold 70% ethanol (to remove the salts from the cellular extract) and then once with cold 80% ethanol, dried and finally resuspended in 5000 µL water. Further purification steps were achieved on DEAE®-cellulose (Fisher Scinetific cartridge/5 mL) column or on Nucleobond® AXR-400 (Clontech Laboratories); both last chromatography steps were performed according to the manufacturer's protocols. All tRNA extractions and analysis were performed at least twice, independently.

Bulk tRNA digestion for LC-MS/MS analysis.

Four hundred µg of bulk tRNA was resuspended in 100 µL water. To this solution were added 0.1 volume of 0.01 M ammonium acetate (pH 5.3) and 0.2 units of Nuclease P1. The solution was incubated at 45°C for 2 hours and then briefly cooled on ice. Then, 0.1 volume of ammonium bicarbonate (1.0 M at pH 7.0) was added along with 0.02 units of Phosphodiesterase I and 5.0 units of *E. coli* alkaline phosphatase. The resulting solution was incubated for 2 hours at 37°C. LC-MS/MS analysis was done on a high performance liquid chromatography (HPLC) system coupled to a hybrid triple quadrupole ion trap MS (4000 Q-TRAP; Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray (TIS) interface operated in the positive ion mode at the Donald Danforth Plant Science Center - Mass Spectrometry and Proteomics Facility (St. Louis, MO)

tRNA^{Asp} purification.

tRNA^{Asp} (GUC) was purified from bulk tRNA using biotinylated primers on Streptavidin sepharose resin (GE Healthcare, Pittsburgh, PA) according to Rinehart et al. (*5*). Four hundred µg of 5'-biotinylated specific primers (5'biotin-CCCTGCGTGACAGGCAGG-3') in 6X NTE solution (20X NTE solution is 4.0 M NaCl, 0.1 M Tris-HCl pH 7.5, 50 mM EDTA, 5.0 mM 2-BME) were added to the Hitrap Strepaptavidin® sepharose HP R-10 1 mL column (GE Healthcare). Then, 4.0 mg of total tRNA (10 mg/mL in 6X NTE) were added and incubated at 65°C for 30 min. After incubation, the temperature of the mixture was decreased slowly to 30°C. The tRNA was washed three times with 3X NTE, 1X NTE, and 0.1X NTE until the absorbance (λ=260 nm) of the wash was zero. The tRNA^{Asp} retained on the beads was eluted with 1 mL of 0.1X NTE at 65°C. 1.0 M NaCl and 80% isopropanol was added to precipitate the tRNA. The pellet was washed with 85% ethanol and dried. The tRNA was resuspended in 50 µL sterile water.

Strains and plasmids constructions.

The Δ*queC*::Kan^R allele was transferred by P1 transduction as described by (*6*) from the Keio collection strain JW0434-2 (*7*) into *E. coli* K12 MG1655 to create strain VDC7012 (MG1655 Δ*queC* ::Kan^R). The Kan^R marker was then excised as described by (*8*) to create VDC2047 (MG1655 Δ*queC*). The deletion of the *queC* gene was verified by PCR using primers chkybaX.ol1 (5'-AGCATACGCACATCTACAAT-3') and chkybaX.ol2 (5'-CATAACATCGCCTTCGTCGT-3'). The Δ*queF*::Kan^R allele was transferred by P1 transduction from the from the Keio collection strain JW2765-2 (*7*) into VDC2047 and MG1655 to create VDC3274 and VDC3275, MG1655 Δ*queC* Δ*queF*::Kan^R and MG1655 Δ*queF*::Kan^R

respectively. The Kan^R markers were excised from both strains to create VDC3280 (MG1655 Δ*queC* Δ*queF*) and VDC2043 and (MG1655 Δ*queF*). The deletion of *queF* gene was verified by PCR using primers ckyqcD.ol1 (5'-CAAGGCGTTCCACGCAGCC-3') and ckyqcD.ol2 (TCAGCAGGTGGCTGACAAG).

The plasmid expressing SSO0016 (NP_341593.1) under the P_{BAD} promoter was a pMH4 derivative (Kan^R, ColE1) gift from the Joint Center for Structural Genomics (La Jolla, CA). *P. calidifontis* JCM 11548 genomic DNA was extracted using Nucleobond® AXR-400 (Clontech Laboratories) according to manufacturer instructions. The *Pcal_0221* (YP_001055124.1) gene was amplified from genomic DNA by PCR using primers QueFLikepbad24.ol1 (5'-GGCG<u>CCATGG</u>TCAAGGTCTCCAAGTC-3') and QueFLikepbad24.ol2 (5'-GGCG<u>GCATGC</u>TTAGATGTAGACCGGCGGGA-3'). The obtained PCR fragment was digested with *Nco*I and *Sph*I and subsequently ligated into pBAD24 (Amp^R, ColE1) (*9*) after digestion with appropriate endonucleases to give plasmid pGPP358. VDC3280 (Δ*queC* Δ*queF*) was transformed by pMH4::*SSO0016* and pBAD24 to give VDC3282 and VDC3281 respectively. VDC2043 (Δ*queF*) was transformed with pGP358 and pBAD24 to give VDC3368 and VDC3367 respectively

PCR conditions.

Polymerase chain reactions (PCRs) were performed using Phusion™ Hot Start, (New England Biolabs, Beverly, MA). For each PCR reaction, 100 ng of template DNA, 0.2 µM forward primer, 0.2 µM reverse primer, GC reaction buffer to 1X concentration, 200 µM dNTP, nuclease free water and 1 – 2 units DNA polymerase per 100 µl reaction were used. The thermocycling conditions for PCR were: 1 cycle of initial denaturation at 95°C for 1 minute, 30 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C, extension at 72°C for 30 seconds per kb, and a last cycle of final extension at 72°C for 10 minutes.

RNase T1 Digestion of tRNA and LC-MS/MS.

Purified tRNA^{Asp} was digested with RNase T1 by mixing 1 μg of the sample with 50 U of RNase T1 in 20 mM ammonium acetate and incubating for 2 h at 37 °C. Digestion products were separated using a Thermo Surveyor HPLC system with a Xterra C18 1.0 x 150 mm column (Waters) at room temperature at a flow rate of 40 μL/min. Before each run the column was equilibrated for 15 min at 95% Buffer A (200 mM HFIP, 8.15 mM TEA pH 7.0) and Buffer B (200 mM HFIP, 8.15 mM TEA:methanol, 50:50 v:v, pH 7.0). The gradient used was 5% B for 5 min, 30% B at 7 min and 95% B at 50 min and held at 95% B for 5 min. The eluent was directed into an LTQ-XL (Thermo Scientific) linear ion trap mass spectrometer. Analysis conditions were a capillary temperature of 275 °C, spray voltage of 4.5 kV, sheath gas, auxiliary gas, and sweep gas at 25, 14, and 10 arbitrary units, respectively. Collision induced dissociation (CID) tandem mass spectrometry (normalized collision energy 42%) was used in data-dependent mode to obtain sequence information from the tRNA^{Asp} RNase T1 digestion products. Data-dependent MS/MS, with a Q value of 0.35 for 30 msec, was recorded based on the most abundant ion, and each ion was analyzed for 15 scans or 30 s before being placed on a dynamic exclusion list for 30 s.

2. Supplemental data 1

Gat-QueC Sequences

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>gi|327310688|ref|YP_004337585.1| exsB protein [Thermoproteus uzoniensis 768-
20]
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List of QueF-like and QueF sequences used for alignment

QueF-like sequences

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>gi|118431468|ref|NP_147965.2| putative GTP cyclohydrolase I [Aeropyrum
pernix K1]
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VCVKLTGSHGRVGMVVERCEDTSPTEDMGPI

>gi|124027916|ref|YP_001013236.1| GTP cyclohydrolase I [Hyperthermus
butylicus DSM 5456]
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ERVKAAVNPPYVKVVVKSIYMGVEVEVIAESGGVPPVYI

>gi|307596129|ref|YP_003902446.1| GTP cyclohydrolase I/Nitrile oxidoreductase
[Vulcanisaeta distributa DSM 14429]
MIRTIKAGPVDYVHLETSFSAICPVDHNIDNYVIEVDYKPTCSDGGCIYMELNSLREYLDGFKDRVIYHE
DLINELINEFVRTLNPIEITVTLTSNYKGIKYVIRRSINKRDSQHAP
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7-cyano-7-deazaguanine reductase -NADPH dependent– oxidoreductase (QueF)

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>gi|16130701|ref|NP_417274.1| 7-cyano-7-deazaguanine reductase (NADPH-
dependent) [Escherichia coli str. K-12 substr. MG1655]
MSSYANHQALAGLTLGKSTDYRDTYDASLLQGVPRSLNRDPLGLKADNLPFHGTDIWTLYELSWLNAKGL
PQVAVGHVELDYTSVNLIESKSFKLYLNSFNQTRFNNWDEVRQTLERDLSTCAQGKISVALYRLDELEGQ
PIGHFNGTCIDDQDITIDNYEFTTDYLENATCGEKVVEETLVSHLLKSNCLITHQPDWGSLQIQYRGRQI
DREKLLRYLVSFRHHNEFHEQCVERIFNDLLRFCQPEKLSVYARYTRRGGLDINPWRSNSDFVPSTTRLV
RQ

>gi|15598002|ref|NP_251496.1| 7-cyano-7-deazaguanine reductase [Pseudomonas
aeruginosa PAO1]
MQHPAEHSPLGKTSEYVSSYTPSLLFPISRTAKWAELGLSAETLPYRGVDIWNCYELSWLTPAGKPVVAI
GEFSIPADSPNIIESKSFKLYLNSLNQSAFDSREALRAVLQKDLSAAVGAPVGVRLRSLDEVAEEGIGRL
PGRCIDELDIAVDGYEQPRPELLRCDAGRIVEEQLYSHLLKSNCPVTGQPDWGTLVVDYRGPALDPASLL
AYLVSFRQHQDFHEQCVERIFLDLQRLLQPQALSVYARYVRRGGLDINPYRSLAEVAPDNRRLVRQ

>gi|37524663|ref|NP_928007.1| 7-cyano-7-deazaguanine reductase [Photorhabdus
luminescens subsp. laumondii TTO1]
MSLYRDHQALEQLTLGKTTLYRDQYDASLLQAVPRNMNREPLEIFPDNLPFHGADIWTLYELSWLNNRGL
PQVAVGHVSLNAASTNLIESKSFKLYLNSFNQTRFENWQAVEETLQRDLAACAEGEVEVALHHLDHFNNQ
PISTFTGECIDDQDIEVTEYDFNRHYLQNAAQGPQVEEVLVSHLLKSNCLITHQPDWGSVQIHYKGSKIN
REALLRYLISFRHHNEFHEQCVERIFSDLQQLCAPEKLSVYARYTRRGGLDINPWRTNSEGFVPATGRLA
RQ

>gi|22127023|ref|NP_670446.1| 7-cyano-7-deazaguanine reductase [Yersinia
pestis KIM 10]
MSSYQNHKALAELTLGKPTAYCDYYDATLLQAVPRSMNREPLGLYPDNLPFHGADIWTLYELSWLNSNGL
PQVAVGEISLNADSINLIESKSFKLYLNSFNQTIFADKESVRMTLQRDLAACAQGNVSVALYDLDEITGQ
PISNFNGECLDKQDIRIDSYEFNADYLQGAAGKDHVEESLVSHLLKSNCLITHQPDWGSVQIHYRGPQID
HEALLRYLVSFRHHNEFHEQCVERIFNDIMRFCQPETLTVYARYTRRGGLDINPWRSNTDFVPLTGRLAR
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Q

>gi|229846813|ref|ZP_04466920.1| 7-cyano-7-deazaguanine reductase
[Haemophilus influenzae 7P49H1]
MNYQDNSLKSLKLGQKTEYASQYDRTLLQPVPRALNRDGLGITQNQPFTIGADIWTAYEISWLNEKGLPQ
VAIADIYLDYQSQNLIESKSFKLYLNSFNQSKFTDFNAVQQTMQRDLIECAQGDVKVRLNPVAVYDAQKI
EHLQGDCIDEQDIEITSYEFNANWLKDCVSDEIVEEKLVSHLLKSNCLITNQPDWGTLHIHYVGKKINQE
KLLRYVVSFRQHNEFHEQCVERIFCDLMHYAKPEKLTVYARYTRRGGLDINPFRSNFENLPENLRLARQ

>gi|121588138|ref|ZP_01677885.1| conserved hypothetical protein [Vibrio
cholerae 2740-80]
MNRLKNMSKYSDAKELASLTLGKKTEYANQYDPSLLQPVPRSLNRNDLHLSATLPFQGCDIWTLYELSWL
NQKGLPQVAIGEVSIPATSANLIESKSFKLYLNSYNQTRFASWDEVQTRLVHDLSACAGETVTVNVKSLN
EYTAEPIVTMQGECIDDQDIEIANYEFDDALLQGAAQGEEVSEVLHSHLLKSNCLITNQPDWGSVEIAYH
GAKMNREALLRYLVSFREHNEFHEQCVERIFTDIMRYCQPQSLTVYARYTRRGGLDINPFRSSHQSAPNH
NQRMARQ

>gi|24114078|ref|NP_708588.1| 7-cyano-7-deazaguanine reductase [Shigella
flexneri 2a str. 301]
MSSYANHQALAGLTLGKSTDYRDTYDASLLQGVPRSLNRDPLGLKADNLPFHGTDIWTLYELSWLNAKGL
PQVAVGHVELDYTSVNLIESKSFKLYLNSFNQTRFNNWDEVRQTLERDLSTCAQGKISVALYRLDELEGQ
PIGHFNGTCIDDQDITIDNYEFTTDYLENATSGEKVVEETLVSHLLKSNCLITHQPDWGSIQIQYRGRQI
DREKLLRYLVSFRHHNEFHEQCVERIFNDLLRFCQPEKLSVYARYTRRGGLDINPWRSNSDFVPSTTRLV
RQ

>gi|16078439|ref|NP_389258.1| 7-cyano-7-deazaguanine reductase [Bacillus
subtilis subsp. subtilis str. 168]
MTTRKESELEGVTLLGNQGTNYLFEYAPDVLESFPNKHVNRDYFVKFNCPEFTSLCPKTGQPDFATIYIS
YIPDEKMVESKSLKLYLFSFRNHGDFHEDCMNIIMNDLIELMDPRYIEVWGKFTPRGGISIDPYTNYGKP
GTKYEKMAEYRMMNHDLYPETIDNR

>gi|15923718|ref|NP_371252.1| 7-cyano-7-deazaguanine reductase
[Staphylococcus aureus subsp. aureus Mu50]
MAHGRQQDELQDITLLGNQDNTYNFDYRPDVLESFDNKHQGRDYFVKFNCPEFTSLCPITGQPDFATIYI
SYIPNVKMVESKSLKLYLFSFRNHGDFHEDCMNIIMNDLIELMDPHYIEVWGKFTPRGGISIDPYTNYGR
PNSKYEKMAEHRLMNHDLYPEKIDNR

>gi|15606257|ref|NP_213635.1| hypothetical protein aq_931 [Aquifex aeolicus
VF5]
MEAKEKKYGEIEIEKAQLEAWPNPNPERDYMIEITFPEFTCLCPRSGYPDFATIKIRYIPDKYIVELKSL
KLWLNKFRNRYISHEAATNEIYQALYDLLKPRFLEVVGDFHPRGNVHTVVRVRSDENYG
>gi|30250215|ref|NP_842285.1| 7-cyano-7-deazaguanine reductase [Nitrosomonas
europaea ATCC 19718]
MTTQPSKQLETFENPVQTRDYRIHMEIPEFTCLCPKTGQPDFARLTLDYIPDKKCIELKSLKLYIWSYRD
EGAFHEAVTNRILDDLVAAMKPRFIRLTSKFYVRGGIFTNVVAEHRKKGWQPQPPVLLEVFEQQFNTHG

>gi|57236909|ref|YP_179862.1| 7-cyano-7-deazaguanine reductase [Campylobacter
jejuni RM1221]
MRYGEKEIKEFDVENMEIWPNDAKNDYIIKITLPEFMCCCPRSGYPDFATIYLEYMPNKFVVELKAIKLY
INTFMYRNVSHEASINEIYNTLKDKLKPKWIKVVGDFNPRGNVHTVIECRSDMVVPK

>gi|291276630|ref|YP_003516402.1| NADPH-dependent 7-cyano-7-deazaguanine
reductase QueF [Helicobacter mustelae 12198]
MKDLSLLGKQDVEYAFHYNPKILETFENRHKENDYFVKFNCPEFTSLCPMTGQPDFATIYINYIPQHKMV
ESKSLKLYLFSFRNHGDFHENCVNVIMKDLIGVMEPKFIEVWGKFLPRGGISIDPYANYGLPNTKYEEMA
HYRLLHHDLSFEKIDNR
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3. Supplemental data 2

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gi|14521321|ref|NP_126796.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Pyrococcus abyssi GE5]
MSRGDKMLKFEVKARDGAGRIGKLEVNGKKIETPAIMPVVNPKQLIVEPKELEKMGFDIIITNSYIIYKD
RELREKALEVGIHKLLGYDGIIEVDSGSFQLMRYGNVDVSNREIVEFQHRIGVDIGTFLDIPTPPDAPKE
KAMEDLKITLERAREAEEIKEIAMNAAIQGSTYTDLRRYAARRLSSMNFEIHPIGGVVPLLEAYRFREVV
DIVISSKMALRPDRPVHLFGAGHPMVFALAVAMGVDLFDSASYALYAKDDRYLTPEGTKRLDELEYFPCS
CPVCSRYTPQELREMPKEERARLLAIHNLWVIKEEIERIKQAIREGELWRLVDERARSHPKLYSAYKRLL
DHYTFLEEFEPVTKKSAVFKISHESLRWPLVRRARERAERVNSKFGDLVEHPIFGKVTKYLTLTYPFAQS
EAEDEFSIEKPTRENAIRYVMAIAEYQFGENASKAFEGAEVELARTGMPRQVKLNGKRLATVRAEDGFLT
LGIEGAKRLHKVLEYPRMRVVVSEEAEPFARKGKDVFAKFVLFADPGIRPYDEVLVVNEKDELLATGQAL
MSGREMIVFQYGRAVKVRRGISGG

>gi|14590946|ref|NP_143020.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Pyrococcus horikoshii OT3]
MSRGDKMLKFEIKARDGAGRIGKLEVNGKKIETPAIMPVVNPKQMVVEPKELEKMGFEIIITNSYIIYKD
EELRRKALELGIHRMLDYNGIIEVDSGSFQLMKYGSIEVSNREIIEFQHRIGVDIGTFLDIPTPPDAPRE
QAVKELEITLSRAREAEEIKEIPMNATIQGSTYTDLRRYAARRLSSMNFEIHPIGGVVPLLESYRFRDVV
DIVISSKMALRPDRPVHLFGAGHPIVFALAVAMGVDLFDSASYALYAKDDRYMTPEGTKRLDELDYFPCS
CPVCSKYTPQELREMPKEERTRLLALHNLWVIKEEIKRVKQAIKEGELWRLVDERARSHPKLYSAYKRLL
EHYTFLEEFEPITKKSALFKISNESLRWPVVRRAKERAKSINERFGELVEHPIFGRVSRYLSLTYPFAQS
EAEDDFKIEKPTKEDAIKYVMAIAEYQFGEGASRAFDDAKVELSKTGMPRQVKVNGKRLATVRADDGLLT
LGIEGAKRLHRVLPYPRMRVVVNKEAEPFARKGKDVFAKFVIFADPGIRPYDEVLVVNENDELLATGQAL
LSGREMIVFQYGRAVKVRKGVE

>gi|18977418|ref|NP_578775.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Pyrococcus furiosus DSM 3638]
MSRGDSMLRFEIKDRDAAGRIGKLEVNGKKIETPAIMPVVNPKQLIVEPKELKRMGFDIIITNSYIIYKD
KKLREKALEKGIHRLLDYDGIIEVDSGSFQLMRYGKVEVTNREIVEFQHKIGVDIGTFLDIPTPPDAPRE
KAEQDLKITLERAKEAESIKQIPMNATVQGSTYLDLRKLAARKLSEMNFEIHPIGAVVPLLESYRFKDVV
DIVIASKMGLRPDRPVHLFGAGHPMVFALAVAMGVDLFDSASYALYAKDDRYLTPQGTKRLEELEYFSCS
CPVCSKYTPQELREMPKEEREKLLALHNLWVIREEINRVKQAIKEGELWRLVDERARAHPKLYAAYKRLL
EYYHYLEEYEPITKKSAFFKISEESLKWPIARRAKERAEKVKAKFPESIPHPIFGEIPKYLSLTYPFAQS
ESEEDFQIEKPTRENAILYIMAIAEYQFGEGAGEAFRDAEVEIAKTGMPRQVKKNGKRLATVRAEDGLLT
LGIEGAKRLHELLPYPVMRVVVNKEAEPFARKGKDVFAKFVEFADPKIRPYDEVLIVNENDELLATGQAL
LSGREMVLFSSGRAVKTRRGVEEKK

>gi|57640695|ref|YP_183173.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Thermococcus kodakarensis KOD1]
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MVDFRFEVKARDASGRIGKLTVNGKTVETPAIMPVINPKQLIVTPKELKEMGFGMIITNSYIIYKTPELR
EKALEVGIHKLLDYDGIIEVDSGSFQLMRYGGVEVTNREIVEFQHEIGVDIGTFLDIPTPPDAPREKAEE
DLRITLERAKEAEEIKEIAMNAAVQGSTYPDLRTYAARELSRMNFEIHPIGAVVPLMESYRYRDLVDVVI
ASKVGLRPDRPVHLFGAGHPMIFALAVAMGIDLFDSASYALYAKDDRYMTPEGTKRLEELEYFPCSCPVC
SRYTPQELREMPKEERTRLLAIHNLWVIREELNRVKQAIKEGELWRLVDERARSHPKLYAAYKRLLEYRE
YLEKNEPVTKASAFFKVSEEALRWPIVERARERAERVRSKFPETISHPIFGEIPKYLSLSYPFAQSEGEE
DFTVEKPEKGEARKYVMAVAEYQFGEGAGEAFKDAFVELSRKTGMPRQIKAKGKHLATFRAEDGLLTLGI
EGAKRLHEVLPFPRMRVVVDEDAEPFARRGKNVFAKFVVDADLNIRPYDEVLVVNRNDELLATGQTLLNG
EELKIFQQGLAVKVRRGVEK

>gi|15678204|ref|NP_275319.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanothermobacter thermautotrophicus str. Delta H]
MISLISRGNTLLMTLWEDFMFEIKSKDGLGRTGILKTEHGTVRTPALMPVIHPGKQTIDVKGPGAEIVIT
NAYIIYRNPELRERALSDGVHRLIDFDGPIMTDSGSFQLSEYGDIEVENPEIIRFQDEIGTDIGTSLDIP
TPPGVSHRRAIEEVEVTLERARESIEYRERMMLNAVVQGSTHPDLRRYCASRLAELPVELHPIGAVVPLM
ESYRYRELVDAVLSSVSELPPSRPRHLMGAGHPMLFALAVSMGCDLFDSAAYILYAEDDRLLSTEGTYKL
ENLQEMPCSCSVCTDYTPSELMGMDREERRNLIAEHNLHVSFAEIRKVRQAIHDGNLMELVEERCRAHPR
LLEGYRRMSEYLDLIEKFEPRSKRSAFFYTGPESLGRVEVHRHLKRVKEHLGERLALVAPSRRPYSSSLP
ARIGGFSSLRPQSGGPWRVVVVDLPFGIIPLELDQVYPLAQSDAPGIMDLDGEEFLRGLVRDLMDGDAIV
DDALCSELGIELPYKYMGEVETTVDDLDRVRMVADYQFGMGAGELLFTDDVRIERSRNTGKIRHIYAGDE
LICTMRASDGLLVLGAEGAVRLHKGTDYPAWRVAVNEESEPFARKGKSVFAKFIIDCDNNIRANDEVLIV
NADDELLATGKALLCAEEMMDLNHGQAVKTRKGGF

>gi|45358173|ref|NP_987730.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanococcus maripaludis S2]
MFEIKARDAMGRLGSITINGKKIETPTIMPVIHPNPKKQTVSMDLINKMADVVITNSYITYTTPELREIA
ETKGIHELIDFKNVVVTDSGSFQLSVYGDVNVGPMEIIDFQEKIGVDVGTILDIPTGPDVSREKAESDLI
ETFKRAEDSIKRRKEMGYKLALNGTIQGSKYLDLRQKSAEVMGKMDFDIYPIGAVVPLMEDYRYREVAEV
ILNSKMHLPTNKPVHLFGCGHPMLFALSVALGCDLFDSAAYALYAKNGRYLTADGTLHLKDMKDLKSFPC
TCKVCSEYTPKQLYNLEEKEKTRLLAEHNLYVTFEEIDRIKNAIKEGNLWELVEERCRSHPKLLNGLRVI
SKYMDFIEKHDPVSKKSGFFYTGYESMNRPEIYRHKQRLDRIQYDKIYVTSVSENTSKPYSENLSNVPCD
VDVLVKDSVFGLVPLNIDTMYPLAQNEVPDLYDFEKKYNNEFVSEFNEKHAEKILDISTYNYYINHYGKK
KECDKINPDIFRIGKMLEYQYGAKILDEELMEKVKSRRSKNTGRIRNLLLEKEVLFTLRANDNFLIPAKS
GAELLHEKLEFPKYRIVIDSSVEEFARAGKSVYSKFVKDCDPELRPFEEVLIVNSDDELLAYGTTILNGR
ELMEFDYGVAATLRGGLKK

>gi|159904673|ref|YP_001548335.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Methanococcus maripaludis C6]
MFEIKARDAMGRLGVITINGKKIETPTIMPVIHPNPKKQTVSMDLINKMADVVITNSYITYTTPELREIA
ETKGIHELIDFKNVVVTDSGSFQLSVYGDVNVGPMEIIDFQEKIGVDVGTILDIPTGPDVSREKAESDLL
ETFKRAEDSIKRRKEMGYKLALNGTIQGSKYLDLRQKSADVMGKMDFDIYPIGAVVPLMEDYRYREVAEV
ILNSKMHLPTNKPVHLFGCGHPMLFALSVALGCDLFDSAAYALYAKNGRYLTADGTLHLEDMKDLKNFPC
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TCKVCSEYTPKQLFNLEEKEKTRLLAEHNLYVTFEEIDRIKNAIKEGNLWELVEERCRSHPKLLNGLRVI
SKYMDFIEKHDPVSKKSGFFYTGYESMNRPEIYRHKQRLERIQYDKIYVTTVSENTSKPYHENLDNVPCD
VDVLIKDSVFGLVPLNIDTMYPLAQNEVPDLYDFEKKYNNEFVSNFMEKNSEKILDISTYNYYINHYGTK
KECDKINPDVFRVGKMLEYQYGAKILDDELMEKVKTRRSKNTGRIRNLLLEKEVLFTLRANDNFLIPAKS
GAELLHEKLEFPKYRIVIDSSVEEYARAGKSVYSKFVKDCDPELRPFEEVLIVNSDDELLAYGTTILNGL
ELMEFDYGVAATLRGGLKK

>gi|134046031|ref|YP_001097517.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Methanococcus maripaludis C5]
MFEIKARDAMGRLGVITINGKKIETPTIMPVIHPNPKKQTVSMDLINKLADVVITNSYITYTTPELREIA
ETKGIHELIDFKNVVVTDSGSFQLSVYGDVNVGPMEIIDFQEKIGVDVGTILDIPTGPDVSREKAESDLL
ETFKRAKDSIKRRKEMGYKLALNGTIQGSKYLDLRQKSAEVMGKMDFDIYPIGAVVPLMEDYRYREVAEV
ILNSKMHLPTNKPVHLFGCGHPMLFALSVALGCDLFDSAAYALYAKNGRYLTAEGTLHLEDMKDLKSFPC
TCKVCSEYTPKQLFNLEEKEKTRLLAEHNLYVTFEEIDRIKNAIKEGNLWELVEERCRSHPKLLNGLRVI
SKYMDFIEKHDPVSKKSGFFYTGYESMDRPEIYRHKQRLERIQYDKIYVTSVSENTSKPYHENLSNVPCD
VDVLIKDSVFGLVPLNIDTMYPLAQNEVPDLYDFEKKYNNEFVSEFREKHTEKILDISTYNYYINHYGKK
KDCDKINPDIFRIGKMLEYQYGAKILDDELMGKVKSRRSKNTGRIRNLLLEKEVLFTLRANDNFLIPAKY
GAELLHEKLEFPNYRIVIDSSVEEFARAGKSVYSKFVKDCDHELRPFEEVLIVNSDDDLLAYGTTILNGQ
ELMEFDYGVAATLRGGIKK

>gi|15668612|ref|NP_247410.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanocaldococcus jannaschii DSM 2661]
MEIIEFQERIGVDVGTILDIPTPPDVDRERAEKELEETLKRAKASIELKEERGFKLLLNGTVQGSTYLDL
RQKSAKEMAKLGFDIYPIGAVVPLMEQYRYRDVAEIIINSKMYLPTNKPVHLFGCGHPMFFALAVALGCD
LFDSAAYALYAKDDRYLTERGTLHLEEIKDLKAFPCSCPVCSSYTPKELASLNKKERERLLAEHNLYVTF
EEINRIKQAIRDGSLWELVEERVRCHPKLLEAYRVVRKYIDYIEKFDPVTKKSAFFYTGIESMFRPEVLR
HKKRLKRLRYEKVYITTVSSSIEKPYHEHLNVVETDVDILIKDPVFGFIPYYIDTVYPLSQHEIPELFDY
EKEINKRFVDEFIDWLKKKIGEDNILDIMTYNYYINYFSANKKINADALRIRKMLQYQYGFDIIDDELMN
KIKVVRSKTTGRLRQVLDENGEILFSVRSNDNLLIPSEKGAKLLWKKIPFPKYRVVVNKEAEEFAREGRN
VFAKFVIDCDEELRPYEEVLVVNEDDELLAYGTTILNGIELREFNYGLAVKVRGGLKINK


>gi|170290974|ref|YP_001737790.1| tRNA-guanine transglycosylase [Candidatus
Korarchaeum cryptofilum OPF8]
MGLYFRVRKSDASARLSELKTKSGTLILPEFFPVYNPNKPVITPREMSEMGIKAIITNSYLIYRSPELRE
AAIERGIHSLLGFDGVVMTDSGAYQIYRYGRVDVTNSEILRFQHSIGSDIGSILDVPMSSEIGREEAESG
VERTIRNAEEWASMREELSNTLWVGTPQGSIYRDLVIKCSERIRELDFDYNGVGSIKVALEKYDFVTQVD
HFMSIRSILRAGKPFHFWGIGHPSTFAFFAAIGADSFDSASYSLYAEQGRYMTPHGTLLLDEIEEFPCSC
PVCSSHDPKEVKAMSKEERTKLLAKHNLYISISEIKKVREAIRGEWLWELVQERSRFHPNLYFALMHLLR
RYSSLLEAREPLFKSSGLQCSGPESFLRPEVVRARNRLKYIHYNGKFRRVLYGDVPLGLKYLYPFGQTIC
PYDEEVQDEPEDDEIITCVLSYQYEFPFPKLPAIMRRSKSTGTLREVSLEGKVIGHFRPNDGAFIPTLDG
ASLILSHLPYPKGRVVVKGLFSDTVARGTTVFVKFVKEADPSIRPKSEVIVVNESDELLATGKAVLSGVE
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YHQYHPDHPFIIIRRHVKPRSEEKPPEVDS

>gi|20093628|ref|NP_613475.1| queuine/archaeosine tRNA-ribosyltransferase
[Methanopyrus kandleri AV19]
MNVAFEVKDRDVAGRLGRLEVNGRRLKTPALLPVVNPNKPTLDPREISKLGFDGVITNAYIIRKHEHLRE
QALEEGVHGLLGFDGFVMTDSGSFQLAEYGDVEVSNEEIVRFQAKIGSDVGTILDVPTPPDAPRSRVERD
LETTLKRAREAVELDEHPPLALTVQGSTYEDLRRLCAEKLAELPAAVYPVGGVVPLLEEYRFVDVVRVVL
AAKSSLPPHRPVHLFGCGHPLAIPLAVAMGCDLFDSASYAIYARSDRYMSILGTLKLEELETFPCSCPAC
TRHDPDDVREMEPRERTRVLATHNLYELRRVIETTRQAIVSGELWELAESVCRAHPRAWAGMVELARRGG
ELERWCPAVKRSVFVCDEVSKGRPELRLYRRRLRDRFGELSGRKVVKGISRPYAEIVEWLEPWELAFADE
WLGVVPGELSWSYPCHCLVEPSGDDEGEDRRRGEEGRRR

>gi|16082455|ref|NP_394944.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Thermoplasma acidophilum DSM 1728]
MKIEERDGLARIAKFETPHGPIETPTVLPVINPNIMNITPQEMKPLGLQGIITNSYIILRTPELRERALR
EGLHSLIGYDGPIMTDSGTFQSYVYGSIEFNNREVVDFQRRIGSDISTILDVFTTPGTPKPEAEKAVIET
YNRMLEVNDEEGIIAGPVQGGVYPDLRQKSAELMNSTNAGYHPIGGVVPLLETYDYSTLVDIIINSKINL
SFNKPVHLFGGGHPMFFAFSVYLGVDLFDSASYIKYAKDDRLIYPDGTRDLARITELPQWSPLYDRYTVK
EIRDLDKERRSLEIARHNLKAIFMEISEIKERIYEEGLAQYVAQKARSHPSLMKAYSRIMSYSNILEKYE
DLSKKTAYFFYDSFSTRNPYVSRINRFTESYLSSNKKDTYAFTYRAWNPGYTNSEFVRDVYQKIDCNALI
SWSGTFVPAELENTYPIEQTVSSGFEPDPDFSRAKDLIAPFRVDMYKGEKFEGEQVRSFNLNKIRMVADY
QFGSGVGRMIFRDDVRINVSKTGRIRGILSKEGRQIATMRNDGFFTLTYYGASLIHSQLKPPAMRVTVSK
ESAEYNAKGYSVFFKFILGSDENIIAKNDVLVVDEDDVLAAVGKAMVSGRELREYTEGIAVKVHEGRDQS
EK

>gi|13542328|ref|NP_112016.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Thermoplasma volcanium GSS1]
MEIRERDGLARIARFDTPHGTIETPTVLPVINPNIMDITPEEMKKYGVHGVITNSYIILRNDRLREEAEK
YGVHSLIGYDGPVMTDSGTFQSYVYGSVEFNNRQVVEFQKTIGSDILTILDIFTTPSSSRQEVENAITET
YRRMLEVNDAGGMIAGPIQGGIYPDLRKRSAELMNSTNASYLPIGGVVPLLESYEYDKLVDIILNSKLNV
SFGKPIHLFGGGHPMFFAFAVYLGVDLFDSASYVKYAKDDRLIYPDGTRDLARIIEIPEWSPLFDKYTVK
ELKELPKEQRSVELSRHNLKAIFMEISEIRERIYEESMDQYLAQKAKSHPSLLKAYVKVMQYSKMLEKYQ
DLFKKAAYFFYDSFSTKNTYVARLEKFTSKYLTSKKKETYVFSRKDWLPGYTNLNFVRDVYERTECNALI
PWSGIMVPAELENTYPIEQTVSSGLEPDPDVSAISESISPFDIRVYKGESVDSDKIRSFDLEKIRTIADY
QFGYGIGKDFFKDDVRIFKSKTGRIRGVFDKGNKLIATLRNDGFFTLTFHGATLLYNVSKSPNLRVFVKN
ESAEYNAKGYSVFFKFILDADPDIIAKNETLVVNENGELVAVGKATVSGKELREYSDGIAVKIHEGRDQS
AK

>gi|48477526|ref|YP_023232.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Picrophilus torridus DSM 9790]
MEILFRENLARIARFKTPHGEIETPTVMPVINPNLNFLDESTLRSYGVQAVITNSYIIKRNQRLNEDALR
HGLHSLIKFSGPIMTDSGTFQSHVYGDIEYSNKEIVDFQKAIGSDIITILDVFTEPDESYNSARSKVIET
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YKRLKEIDFEDKIIAGPVQGSIYPDLRRLSAYLMSDALYLPIGGVVPLLESYRYSDLVKIIFNSKVSSDF
SRPVHLFGGGHPMFFAFAVMLGVDLFDSASYIKYAKDNRLLYSEGTRALNDIREFPEWSPIHGKYTPQEL
LHEESEKRTRMLALHNLKSIFIEINEIKERIYENTLYNYVEEKARSHPALFKAFMSMINYDTSDYSPLSY
KSPFFYYDKTSLNHPIIKRIMKFTENYISNSRHTLIISSKYWRPGVKNENVIKNIVECTDFNLLVSWNGI
YIPLFLEDSYPVQQLVSSGLNDKKLEEDYLKRLKSINNDIEFYEGEHYDKRLRDYDTEKINTIAMFQFNI
NERFFDKSNIIKSKSTGHIRNIIEDNNIIATMRNDGYLTLSIKGAYRLLSMKPWPGLRVVVDDESGRFNA
NGYNVFFKFIKSFDTGIIPGNETLVVSEDDDLYAVGKAAVSGIEMYYYKSGVAVKVHEGVNKKAA

>gi|257076281|ref|ZP_05570642.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Ferroplasma acidarmanus fer1]
MFFREGLARIGKFSTPHGDIETPTVMPVINPNLNFLTKEELKSIGVQAVITNSYIIKRTASLEQDALKHG
VHRLINFDGPIMTDSGTFQSYVYGDIEYGNKEIVQFQKDIGSDIITILDIFTKPQDSYEQAKAAVYETSR
RLQEVNTPDSIIAGPIQGSIYPDLRRESARLMSEASYLPIGGVVPLLESYRYSDLVNIIINSKLNSDFSK
PVHLFGGGHPMFFAFSVLLGVDIFDSASYIKYAKDNRVLYTEGTRNLKEIRDFPEWSPLFNKYSPVELIK
ADEKTRLKLLSLHNLKAIFNEITEIKERIYENTLYQYVEEKSMAHPALYRAYLEMLDHNLEPFWNISLKS
PLYFFNSSTYKNTFIKRLVKFTEDYISNGKKTVLVSYRQWRHGFPVSDDILRSYETTDYNFLIEWNDIFI
PMELENTYPVSQMVPSGVLDKIYRDDYIAWLKSINNDISFYDPSITGSEKIRNYSHSKINEVFHLQFKAR
KNLFVNSDKIIKSKATGHIRNIKRGEKIIATMRNDGFLTLSIYGGKLLNDMLDFPASRVIVTDDSGEFNS
QGYNVFFKFVEDCDREIIAGNDVMVTSASGDLYAVGHATVSGKEMGFYRNGIAVKVHEGINKL

>gi|84489330|ref|YP_447562.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanosphaera stadtmanae DSM 3091]
MVDIIVDFEIKYKDAMARVGKFKTPHGTVTTPALMPVVHPGKQTLDVKKLGAQIVITNSYIIYKNEELKK
KALEEGVHSLIDFPNTIETDSGSFQLSVYGDIDITNEEVIKFQEAIKTDIGTSLDIPTAPYVKREEAEND
LEITIERAKEAANVKSDLLLNSVVQGSTFPDLREKCAKEISKYDADIYPIGAVVPLMEMYRYADLVDAVM
YSMRGLPENKPRHLMGAGHPMVFALATAMGCDLFDSAAYILYANKDRFMMPDGTLRLEDLIEMPCSCRVC
CEYTVDELKQMDQKKRAKLIAEHNLHISFAEIRRIRQAIVDGELMKLVELRCRSHPFLLDGLRRLMEYKE
DMERLNPSSKKSAFFYTGYESLARSEVPKHLKQLENIKPKNKNLVILPHTRKPYTKYVNREYIKKYTPKI
PTYYSNTTNTDYSNSDVVVADIPFGIIPLGLDEFYPLAQNESPSIHDLDSKRFIRDIINNYSKKYDNVLI
HRKVIEKFDITNFNLIEDELQLPEAKISDFNRLNDIADYQFGCGAGNALFGGDEDKITIEKSRKTKKIRH
VFEDNENIVNMRANDGFLILSDLGAKRLHKFLEYPHNRVVVSEDSEPFALKGKSVFNKFVLDCDENIRRN
DEVLIVNKDDKLLAFGKALLSSYEIKDFNTGQAIKTRKWKKEIE

>gi|240103198|ref|YP_002959507.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Thermococcus gammatolerans EJ3]
MRGESMEFRFEVKARDAAGRIGKLTVNGKSIETPAIMPVINPKQLIVTPKELEEMGFGMIITNSYIIYKT
PELKEKALELGIHRLLDYDGIIEVDSGSFQLMRYGEVEVTNREIIEFQEKIGVDIGTFLDIPTPPDAPRE
KAEEDLRITLERAKEAESIKNIAMNAAVQGSTYPDLRTHAAQELSKMNFEIHPIGAVVPLMESYRYRDLV
DVVVASKLGLRPDRPVHLFGAGHPMIFALAVAMGVDLFDSASYALYAKDDRYLTPEGTKRLEELEYFPCS
CPVCSRYTPQELREMPKEERTRLLAIHNLWVIREELNRVKQAIKEGELWRLVDERARSHPKLYSAYKRLL
EYRDYLEKNEPITKASAFFKVSEEAMRWPIVYRAKERAERVAKKFPERIRHPIFGEIPKYLSLSYPFAQS
EGEEDFTIEKPRKGEARKYVMAVAEYQFGEGAGEAFKDAFVELSRKTGMPRQVKAKGKHLATFRAEDGLL
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TLGIEGAKRLHALLPFPKMRVVVNEDAEPFAKRGKNVFAKFVVDADPSIRPYDEVLVVNEKDELLATGQT
LLNGEELKVFQSGLAVKVRRGIEKG

>gi|91772941|ref|YP_565633.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanococcoides burtonii DSM 6242]
MSSIFEITHKDAAGRIGKLRTPHGTVETPTVMPVINPNLQVIKPSEMRDFGAQMLITNSYIISRRDKLRE
KALKDGLHSLLDFDGPIMTDSGSFQLSVYGDIEVTNEQIIEFQKTIGSDVGVPLDIPTPPDVPRSRAESE
METTIERLIEARSMVNDEMLLAGPVQGSTYTDLREKCASTISEHKFDVYPLGAVVPLMESYRYAELVDVI
VSSKKGLDPTVPVHLFGAGHPMMFALAVALGCDLFDSAAYALYAKDRRYITSKGTYHIDNLSYLPCSCPI
CVSHTAEEVKKADNCSDLLARHNLYVTFEEIRLIKQSIKEGNLLELVEMRCRSHPRMLEALKRMYSYSDW
IEKYDPASKSTFFYCGPESSQRPEVLRFSKRLERFTIKGTAVIRPFSMRTYPESDNDLMFKPPFGTFPAE
LSEVYPFNAEVIEDTDIESLGKALENTIRLIELNPDAEFTFIKGKELEHPLFEKLEKIADVKE

>gi|21227203|ref|NP_633125.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanosarcina mazei Go1]
MSAIFEILDKDAGGRIGKLRTPHGVVETPTVMPVINPNIQLISPKEMRSFGAEILITNSYIIYRKEELRT
VALEKGLHELLGFDGPIMTDSGSFQLSVYGSVEVTNEEILGFQEKIGSDIIVPLDIPTPPDVHFRRAEEE
LATTAERLEAARKFIQSKQLLAGPVQGSTYPELREKAASHLKDLNFEVYPLGAVVPLMESYRYAELVDVI
AASKKGLSPTSPVHLFGAGHPMMFALAVALGCDLFDSAAYALYAKDGRYITSNGTYHLEKLNYLPCSCPV
CSRYTAEELRKAKNKEELLGRHNLYATFAEIRLIKQSIKDGKLLELVEQRCRAHPKLLDGLKRLYTHSAW
LEQFDPATKGTYFYCGPESASRPEVLRFGKRLERFSIEGSAIIRTSPVKGEKDYDRILTFKAPFGTFPAE
MEEVYPFNAEVPKFPDYEALSTSLSNTIKLMDLNPGAEFTFICEKEFQHPLIEEIGKKAKLVYREAWKKE

>gi|20093206|ref|NP_619281.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanosarcina acetivorans C2A]
MSAIFEILDKDAGGRIGKLRTPHGIVETPTVMPVINPNIQLISPKEMKSFGAEILITNSYIIYRKEELRT
VALEKGLHGLLGFDGPIMTDSGSFQLSVYGSVEVTNEEILGFQQKIGSDIIVPLDIPTPPDVHFRRAEEE
LAVTAERLEAARKFIQGEQLLAGPVQGSTYPELREKAASRLRDLNFEVYPLGAVVPLMEAYRYAELVDVI
AASKKGLSPTSPVHLFGAGHPMMFALAVSLGCDLFDSAAYALYAKDGRYITANGTYHLEKLNYLPCSCPV
CSKYTADELRKAKNKEELLGKHNLYATFAEIRLIKQSIKDGKLLELVEQRCRAHPKLLDGLKRLYTHSAW
LEQFDPATKGTYFYCGPESSFRPEVLRFEKRLDRFSLEGSAIIRTAPVKGEKDYDRVLTFKAPFGSFPAE
MEEVYPFNAEVPKFPDYETLSTALSNTLKLMELNPGAEFTFICEKEFEHPLIEEIGKKAKLVYRAAWKKE

>gi|73668621|ref|YP_304636.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanosarcina barkeri str. Fusaro]
MSAIFEILDKDAGGRIGRLRTPHGTVETPTVMPVINPNIQLIPPKEMRNFGAEILITNSYIIYRKEELKS
VALEKGLHGLLGFDGPIMTDSGSFQLSVYGSVEVTNEEILGFQQKIGSDIIVPLDIPTPPDVHYRRAEEE
LAITAERLEAARKFIQSEQLLAGPVQGSTYPELREKAASHLKDLNFEVYPLGAVVPLMEAYRYAELVDVI
AASKKGLSPASPVHLFGAGHPMMFALAVAMGCDLFDSAAYALYAKDGRYITVNGTYHVEKLNYLPCSCPV
CSKYTAEELKKADNREELLGKHNLYATFAEIRLIKQCIKDGKLLELVEQRCRAHPKLLDGLKKLYTHSSW
LEQLDPATKGTFFYCGPESSSRPEILRFGKRLDRFSLQGSVIIRTGSVKGEKDYDQILTFKAPFGAFPVE
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MEEVYPFNAEVPKFPDYESLNTALSNTLKLIDLNPEAEFTFICEEEFKHPLIEEIRKRAKLVYRKDWKKE

>gi|73671775|gb|AAZ80293.1| archaeosine tRNA-guanine transglycosylase
catalytic subunit [Methanosarcina barkeri]
MSAIFEILDKDAGGRIGRLRTPHGTVETPTVMPVINPNIQLIPPKEMRNFGAEILITNSYIIYRKEELKS
VALEKGLHGLLGFDGPIMTDSGSFQLSVYGSVEVTNEEILGFQQKIGSDIIVPLDIPTPPDVHYRRAEEE
LAITAERLEAARKFIQGEQLLAGPVQGSTYPELREKAASHLKDLNFEVYPLGAVVPLMEAYRYAELVDVI
AASKKGLSPASPVHLFGAGHPMMFALAVAMGCDLFDSAAYALYAKDGRYITVNGTYHIEKLNYLPCSCPV
CSRYTAEELKKADNREELLGKHNLYATFAEIRLVKQCIKDGKLLELVEQRCRAHPKLLDGLKKLYTHSSW
LEQLDPATKGTFFYCGPESSSRPEVLRFGKRLDRFSLQGSVIIRTGPVKGEKDYDQILTFKAPFGAFPVE
MEEVYPFNAEVPKFPDYESLNSALSNTLKLIDLNPEAEFTFICEEEFKHPLIEEIRKRAKLVYRKDWKKE

>gi|116754948|ref|YP_844066.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanosaeta thermophila PT]
MQGCFEILHKDLAGRIGRLHTPHGIVETPALMPVVNPHVLLLSPQELADLGAEMIITNSYIIHQDPVLRD
TALERGVHGLLNFDGPVMTDSGAFQLSVYGSVDIEPLEILKFQQKIRSDVSVPLDIPTAPDAPREQAERE
LAETERRLREAVDHRSESLLLAGPVQGGIYPDLRESSARRLRDLGFDLYPIGAVVPLMESYRFRELVDVV
VASKTGLGPGVPVHLFGAGHPMVFALAAALGCDLFDSAAYALYARDGRYLTPRGTYRLSEMKYLPCSCDV
CRKHTPESLNEDPKRVELLARHNLAVSFQEIRAVRQSIHEGSLWEHLERRCRSHPRMYQAFKHLSRYVEY
FERLDRVSKSTLFYLSTESARRPEVLRYRKRICRLELRGRVLVTDRRPAEEDRSKYDHILGFLPPFGPYP
LGLEEMYPLNAELPDEMDENAMKEALDTLRLLIRENPDAEFHFEIRCLEGHRDAVL

>gi|55379810|ref|YP_137660.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Haloarcula marismortui ATCC 43049]
MTNFEVRQYDAAGRLGELTVPRAGVTVETPTILPVVNPHVQTVAPATLASEFGAEILITNSYILHGSDDL
REPVLEQGLHDLLGFDGAIMTDSGSFQLAEYGDIDVTTEEILEFQHEIGSDIGTPVDIPTPPDVDRERAT
EELKTTQERLEHAATVDTGEMLVSAPVQGATYPDLRERAAADAVSTGLDVFPLGAVVPLMNEYRYADLAD
VVAACKRGLGEVGPVHLFGAGHPMMFAMAAALGCDLFDSAAYALYARDDRYLTVQGTELLDELSYFPCHC
PVCTDHTPAELDAMDADAREELLARHNLHVTYGEIRTVKQAIRSGNLMELVDSRARGHPEMLDGYRALLD
HSEQLERTDPVSKDAFFYTSTESARRPEVRRHQDRLERLPVEGEEVLLTEGSSSAQYDESWGVLPPFGPY
PRELADTYPLTAETPDRTDRAAYEAAATGVRRLVELHPDVSFTLVHDDWPATALDRVPEGVRLRDLHARD


>gi|88602410|ref|YP_502588.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Methanospirillum hungatei JF-1]
MTLSFEILEKDIAGRIGKLSGQGKIVKTPTLLPVINPHLNLIPPKEMQEMGVEAVITNAYIFSRSEEYRE
RALTDGLAKTLDFDGVIMTDSGSFQLSVYGEVEITNAQTIAFQQAIKSDIIVPLDIPTHPDSPREQVEQE
LSVTMDRIMEAKDIADHEHHTLAGPVQGGLFPDLREETGRRLSEAGFRFCPIGAVVPLMESYRYAELVKV
VMAAKRGLSPAVCVHLFGAGHPSMFALAVAMGCDLFDSAAYALYAKDGRYMTTHGSYHLNELSYLPCPCP
VCVGHTAKELNESPDRERLLAMHNLRVSLAEINRVRQAIRDGVLWELVDERCRSHPALLRGYRTLLGYNE
ELTALDRETKRRFFYRGDESCKRTEVVRYHQMVGRLTAGERTLISFSRHIKKKQTEEYDSVFYFKPPFGP
FPAELTETFPIGQSEIPDFDEEMIKTGCIGIARLMETNPDSHFTIRCRPVWKDLITQILPTVEVQDEGS
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>gi|76801897|ref|YP_326905.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Natronomonas pharaonis DSM 2160]
MRDIFERRDGDAAGRIGELSVPRAGTTVETPALLPVVNPHIRTIAPAELESTFGAQILITNGYILYGSDE
YRERALADGLHDLFDFSGAIMTDSGSFQLSEYGEISVTNEEILRFQRDIGSDIGTPVDIPTPPEASREQT
AADLETTKARLEAAETVDTGEMLVNAPVQGGTYPDLREEAAEHAYGTTLDVFPVGAVVPLMNSYRYGDMI
EAILGAKRGLGADAPVHLFGAGHPMMFALAVAAGCDLFDSAAYALYARDDRYLTVAGTDHLDDLEYLPCS
CPVCADHTPAELQAEDDTERERLLARHNLHVSFQELRTIKQAIKKGNLLELVERRARGHPAMVDGYRALL
EAADQLERDDPVSKGSFFYLSGESARRPEVKRHHDRLSRLSVDGDRVLLSEGGDNSRFDETWRLQPPFGP
FPPALSDSYPLTAELPERLDDRAYEAAAKGVRRLVDDHPETEFAVAHWGWPDAALDSLPDGVELLRLGAD
SDHPERDSAQSDMDGDEPKEDYPPGEDENA

>gi|126179234|ref|YP_001047199.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Methanoculleus marisnigri JR1]
MAITFEVIHKDIAGRVGKLRVNDKTVRTPALLPVVNPHLPLVTPREMREMGVEALITNAYIFRRSTEFHD
RALAEGLHGVLDFDGVIMTDSGSFQLSVYGEVEVSNRDTLEFQQAIKSDIVVPLDLPTPPDAGPGRAARE
LAVTMERIREAQALFPDANLAAPVQGGIFTDLREEAGRAVRDLDFTFAPIGAVVPLMESYRYKELVQVVL
AAKRGLSPGTAVHLFGAGHPSMFALAVAMGCDLFDSAAYALFAREGRYITPHGSLKIDELAELPCACRVC
RSMTADELRKSEDRERLLALHNLHVTLAEIARIRQAIQDGTLWELVDERCRSHPRLLDGYRELLAHVAEL
ERDDPVSKRRFFYRGSETCRRTEVLRFHEVIPRIPLGERVLVSFDGQGAPGFDTVLNFKPPFGPYPVELA
ETFPVGQSEVPEWDDDMVRSGCAGIRSLMEAHPESRFTVQCGEVWTRLVLEEVPDAEVLHEQV

>gi|124486027|ref|YP_001030643.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Methanocorpusculum labreanum Z]
MAITFEVIHKDIAGRVGKLKAGDKAIRTPALLPVVNPHLQIIPPSEMKKMGVEGIITNAYIFSKSEEFRG
PALEKGLHEVLDFDGLIMTDSGSFQMSVYGSVDITNEQTLSFQRDIGSDIWVPLDIPTHPDTERDEVIAQ
MEITMARMKEAKELFGDDAPISGPVQGAVFEDLREYAGKTVSDMGFAYCPVGAVVPLMESYRYRELVDVI
LAAKKGLNPGACVHLFGAGHPSMFALAAALGCDVFDSAAYALYAKEGRYITTYGTLKLDEMSELPCACPV
CRSHTVEELKKSPDKQKLLAYHNLAVTMAEISRIRAAIQDGTLWELVDERCRAHPKLLDGYRRLLERVEE
IEHLDRASKRRFFYRGSESCRRTEVTNYHAMIPRVKLSDVSLIAAGGPVPSRFEEVIEFKPPFGPLPYEL
AETFPAGPAEVPTWDEEMMKYGIKGLKGLLATNPDTKVTISTTAKWADLFRAEFPAAEVIT

>gi|15790835|ref|NP_280659.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Halobacterium sp. NRC-1]
MRDVFEVGAQDGLARIGELDVPRAGVTVETPTLMPVVNPNLITVEPSRFVDEFGAELLITNSYIINSDDD
LRERALDEGLHEMLGFDGAIMTDSGSFQLAEYGEIDTDTEAILRFQHDIGSDIGTPVDIPTPPDADREQA
AAELETTQQRLELAETVDVGDMLVNAPVQGATQPDLREQAGAHAYGTALDLFPVGAVVPLMNQYRYDDMT
EAVLAAKRGLGRDAPVHLFGAGHPMMFALAAALGCDLFDSAAYAIYARDDRYLTVSGTEHLDDLHYFPCD
CPVCAEHSPQAVRGMAAGDRERLLAEHNLHVSFGEIRRVKQAIKSGTLMELVAARAHAHPSTLDGYRALL
DHSDQLEASDPASKDAFFYTGAGSARRPEVHRHHQRLDRLDVDGDDVLLTEGDSNHRYDESWNVLPPFGP
YPSALATTYPLTAETPARMDRAGYEAAAEGVCRLAEANPDTAFTLAHDDWPESALEAVPQRVSLYNAVRG
E
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>gi|11498196|ref|NP_069422.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Archaeoglobus fulgidus DSM 4304]
MQRFEILDKDAMGRICRIETPHGRIETPTILPVINPNIPFIRAEEMKKFGAQAVITNSYIIYRSMREEAL
EKGVHGILETDMPVMTDSGSYQLMVYGDVEIKNAEIVEFQRHIGSDIIVPLDIPTPPDADYATAESDLRI
TLEREREAKELLKGAENLLAVPVQGSTHPDLRRFAAGEARKIGGDIYPIGAVVPLMDAYRFRDLARVILE
VRSALPVEPIHLFGCGHPMLFAMAVALGCDLFDSAAYALYAKDDRYLTVYGTKKLSELNYFPCKCPVCSN
HDPEELRRMEKNERERLIAEHNLYVSFQEIETIKQAIKENSLFELVEKRVRAHPNMLAGWRQVKHYWELL
EKADPKMKRKFLYTGIDSLYRPAVRRHVKAIKNVELPEEVLVSTDFGIYANIYLRPVFGPVPAEMLETYP
AGHAEIPEEDVVEEEALKAASEALMELMNSHPEKRFKVYVSKVWMKHLQNLPPNGELNVLS

>gi|154150856|ref|YP_001404474.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Candidatus Methanoregula boonei 6A8]
MAITFESLDSDIAGRTGKLGAGKRIIKTPALLPVINPHLMLVTPKELRAMGVEALITNAYIFSQSRQYSE
RVKEEGLHKLLDFDGLIMTDSGSFQLSVYGQVSFTNKQTLSYQRDIGSDIWVPLDIPTSPDADRPTTERE
LAITMERLREAKELFGNDAPLAGPVQGGIFPDLRERAGKEVGDLGFSFCPIGAVVPLMESYRYRELVDVV
MAAKSTIPRSACVHLFGAGHPAMFALAAAMGCDLFDSAAYALYAKDGRYLTTHGSFRIDELTDLPCACAV
CRSHTAEELCTAKDRTRLLALHNLAVTLAEISRIRQAIADGTLWELVDERCRTHPQLLSGYRRLLSHAAD
LELYDRASKRRFFYRGDESCARTEVLRYQRQLDRLRLGKTVLVACDGKEREGYDDVLFFKPPFGPYPPAL
KETFPIGPAEIPPWDEAMVRQGCRGIRMLAESHKESRIMVSGLLNWEEIFTQEAGDAAELVR

>gi|119719273|ref|YP_919768.1| tRNA-guanine transglycosylases, various
specificities [Thermofilum pendens Hrk 5]
MVWSEVFEIKEVDLLGRIGSISTRRGNVETPTLTPVINPSKPVLEPREIQQMGFNLIMTNSYIIKRQYGD
LAKEVKVHQLLGVDTPVMTDSGAYQLMVYGRVEVDPLEIVRFQVEIGSDIGVILDIPTKKGVPRGQVLAE
VEETLRRAEASLAVERDGMLLVGPVQGGLYTDIVATAARRLGEMPFDVYAVGGPTQLMEEYDFSELVKLV
MTARLNLPWEAPLHLFGAGHPVMLPLAVAMGVDTFDSASYVLYARDDRVFTSRGTLRLGEVEELPCNCPV
CSKYSARELRELPKQERVVLLARHNLYVIQKELKEIREAIHEGRLWELLEERSMTHPSLRDALRVFARYA
EYIAKRHPVTRSPVQGLFFYSGLSRYRPEVVRHVNRLAQRYKRARDTLVVFEETPQKPFTRAGLVKEILG
ADPMLPVDVDVAVLSGAFSVIPLELDGFYPLSQYEASGEVFREAVDEVLADLAWFVLSKGYRRVVLVYHS
LPKVFLDKLKERLSLEGVLLAYVEVENYDEALLNPSAVAGRIRSMISFLESIA

>gi|15920526|ref|NP_376195.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Sulfolobus tokodaii str. 7]
MVGEFEIKDEDLAGRIGIIETKSGKLETPVFFPVINPFKSEISIKDIENLGFKNLITNAYLIKKITNTKI
QDIHSFLQFNGVIMTDSGAYQILQYGNIEVTNREIVEYERDINTDIAVYLDLPTGDTNSRDEAINSVKIT
LERAKEIEDIVKNDSERIWVHPIQGGRFLDLVEYSAIEADKNEAFKMLALGSPTVVMEKYDYSLLIDMVF
IAKSNVSRGKPFHLFGGGLPHIMPLVIALGVDSFDSASYILYARDNRYITRSRVYRLEELEYFPCSCPIC
LKYTPKELLELPKEERTKLLALHNLYVIKEELNAIKQAIREGRLFEYIQEKAYSHPAVYSAFKKILKYKD
YLEKYDPRVKGNIRGIFLFDLKSLNRPEIVRHYNFLDRINKNNDKAIIICEKRENILKKVKNNENVDIYL
FNPFYGLIPINLIEVYPYFQTEYPEEIDEDVLRYIESKIVEFVRSKGYTKIDFIGCEKYLSHINSIGAFF
S
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>gi|70606466|ref|YP_255336.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Sulfolobus acidocaldarius DSM 639]
MIGDFEIKDEDLAGRIGILETKHGKLETPAFFPVINPVKNEITIQDILSVGFESIITNAFLIKKYIGKEE
DLHSILNYNKILMTDSGAYQILQYGDIDVSNVDIVNYETKLKPDIAVILDIPTGLTEDKKEAEKSVESTI
SRAKEASKFVELSKDEIIWVHPIQGGMYLDLIEYSARIADMNQDYKMLALGSPTVLMQRYEYAPLIDMIY
KSKSNVSRGKPFHLFGGGHPHIFAFAVALGVDTFDSASYILYARDHRYMTRERVYRLEELDYFPCSCPIC
SRYSPKDVMEMPEEQKVRLIALHNLYVIKEEINYIKQSLKEGRLFEYIQQKAYSHPSTFEAFRRILNYSK
YLEKYDPRVKGEVKGVFLFDNSSLHRPEVIRHAYTLSKIKQRSKALVLYCSDSKDNPLKNTEDMKNADVY
IVLPFYGCVPYNVFFTYPYFQSEMPSTIDKDVIYDLKNKLKEFLSQRSYETVSIIGCEKILHVDSIRGAP
VNLLLNKL

>gi|124027358|ref|YP_001012678.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Hyperthermus butylicus DSM 5456]
MKREIGVFEIREKDLAGRIARLYTPHGVLETPALLPVIDPVRQEVPLVEIKRLGFNAVITNAYLLWKRLG
EQAAEKGVHGVLGFEGIVMTDSGAYQLLEYGHVEVEPREIVEYEKKIGSDIAVILDVPTGNTRDRKQAEE
SVRETLRRAEEAQSYIDPDKRIWTLPIQGGPFIDLVRESAEKARKLKDYKLYALGSPTVLLERYEYSTLL
DMVFTARFSLPVTRPLHLFGAGHPMIIPFAVALGVDMFDSASYILYARDNRYMTLTKTYRLENLEYFPCS
CPVCSRYTPQELREMPRDERVKLLALHNLYVLRQAINEVKTAIREGRLWELLEERSRAHPSLATAFAKFR
QYIEKIEQLAPRVKTGFVRGLLLYGSESLYNPRVVAYRRSIRINYKPRIRGELKLIPVSPFEKPFTSSKL
YRSIKKGRRDIHVVGYMLYLGPIPEELAETYPGSQFEVNLTPYWEVVEETARSIAEYVEANKANYTSIEI
YYCQGDGLKWSRPVAEKTTELLRKRGVEVKLAELGEC

>gi|161529044|ref|YP_001582870.1| tRNA-guanine transglycosylase
[Nitrosopumilus maritimus SCM1]
MKIYLFEISKTDLAGRIGTIDTNHGKIETPAYVPVIHPVKQTIPSKKIKEIGFDLVITNAYITRNNYGDK
AVKKGIHKIIDYDRGIMTDSGGYQVLEYGDVPVAPTEMAEFEQGIMTDFAIPLDKPTGFGLPIKKAEAYV
KHTLKVCKQTIEDSKDNGQIWIGPIQGGEHFDLVAKSTKGLIKMGYKMLALGSPVEFMESYEYRLLAQMI
IAAKKQIPHNIPLHLFGAGHPLTIPFAVALGCDTFDSASYMLYAKQNRYITEDGTRDLSDIVVFPCNCEI
CTKYTPDELRQLESIEKINQIAIHNLHAIKLEVDKVKQAIHEGRLWEYVIKKARAHPKLFEMVEVMTENS
EFLKIGTPKFKERAIFLFDKEDQFRPEVQVFHEIVRGFKSKKEKILITNESSTKPGYLSHQFVNLSKKLK
DFEEVQICQYNPQLGLIPIEISDIFPAAHHETSRMNFDPKEFTEFEKTWKIFFENNKFSEIRYNKDDEFL
KYFVKTLPKNIKKKSFL

>gi|41614920|ref|NP_963418.1| hypothetical protein NEQ124 [Nanoarchaeum
equitans Kin4-M]
MFLYMKIKFEIKYKDAAGKVGKIKLNGKTIETPYLFPVINPFKQELPIKEIKKMGFNAIITNAYILFKRK
EEVMEKGIHNFLGFDGIIETDSGAYQLLQYGDIDIENEDIIYFQNEIGVDIGNILDIPSYGKTYEEAKKD
LEITLERLKQAIEMANFAINGPIQGDKYLDLRYKALEEVSKLDIDIYAIGGIVPYMNQYKIESLAKIIGP
LLLDIPRDRPVHLFGLGHPLIMPLFVALGADLFDSASYSLFAKEERILTPFRTFRLEDLTDSYILDYKAS
ELKGMENKTYIIAKHNLLVLRNEINFIRDLIRQNRLWDYVIIKAHAHPSIYFATKYVLENLYEKLKEHEP
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ITKRVGILYQGELTELRSDLRYAIEQLKKLDKSQINDILDYAWPFGQFEIGEKDKKLFFQRFLK
>gi|156937272|ref|YP_001435068.1| tRNA-guanine transglycosylase, various
specificities [Ignicoccus hospitalis KIN4/I]
MIWEPEARDGPARAGKLKVGAYEVETPALLAVVDPDPKKQLVPLDEMRRAGVQVIMTSAFIAKKKVGPTN
LKERLGWEGLLYTDSGTFQAYSRGVRVDPEESVRYQISAGSDIITPVDLFSLPTDSKEVAAKKAEVSFRR
WLRARELKEEVSAPVQGGLYPDVRAAVARRYSEAGARLLAVGGIVPLMEEYKFKELVNAVLPVLASRPPE
AAVHAFGAGHPLAFPLLAFLGVDLFDSAMYAIAAREGRYLTPFGTFRLEELVMMREFPCDCPACSSLSPR
DLLSMSEEERTKFLALHNLYAAIKMVKEIRERIVYGTFHKWAIAFAHSHPRLYEAFAEALGKWRGYFERE
RNALPKSPVPEGCELCDSSPERDPVGEGTYGTRELFNVIAKRAYGKGLSSEEPLEALRPPRASFSPRALR
ELCSVTLNSPPKGRAIRKKDLKEVNCLLRPNHEVLVLWEGGEAKAVSLVSFAELSLASEEAVALLLPPEG
LHGVKLGGLVGRVEAGDHGG
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>gi|292656129|ref|YP_003536026.1| queuine/archaeosine tRNA-ribosyltransferase
[Haloferax volcanii DS2]
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LREEALDVGLHEMLDFDGAIMTDSGSFQLAEYGEIDTTTEEILQFQRDIGTDIATPVDIPTPPDVAREQA
EADLEITRQALADAEAADTGEMLVNAPVQGSTYPDLREEAGRHADATDLDVFPVGAVVPMMNAYRYDDMV
DAVAAAKRGLGVDAPVHLFGAGHPMMLALAVALGCDLFDSAAYALYARDGRYLTVRGTEHLEDLDYLPCT
CPICTEYSPDDLREKGSKRQEQLLAEHNLHVTFAELRRIKQAIRDGDLMELVEERARSHPAMLDGYRALL
AHVDQLEREDPASKGAFFYASNESAHRPEVARHHARMDRLTAEGHVLLTEGGVPSGDDFDATWRVVPPFG
PFPRSLSETYPLTAEVPERLDRDAYEQAARGVSRLVEENPDAAFTLAHDDWPESALARVPESVELESLSA
VSERLGDEASVGGDDGDDGGSASSAE
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>gi|118576825|ref|YP_876568.1| queuine/archaeosine tRNA-ribosyltransferase
[Cenarchaeum symbiosum A]
MTFEIFKCDLGGRIGSIQTSRGTVETPAFVPVIHPVRQGIPAAKIQDMGFNLVITNAYIAMKNHGEEAVR
RGIHGIIGFDGPVMTDSGGYQVLEYGDVDTDPAAMARFEEGIGSDIAVPLDRPTGIGLSRKRAGELVRHT
LRVSKETLENSSGGPLWAGPIQGSEHLDLVRSSAKALTGYGFRMMALGSPVEFMESYEYGPLAGMIAAAR
ESIPDSVPLHLFGAGHPLTIPLAISLGCDTFDSASYILYARQGRYITEDGTRRIKEMGYLSCSCEVCSKY
TAPELAGAKDKERIDGIALHNLHAIKSEVDRVKEAIHEGRLWEYTMKKMRAHPRLFESARILEQNGARFI
RTTPRFKSRAVFLFGPEDQYRPEVISYHNMAREYTTRKKILCITRDAQIKPAYLSPQYSTLKARFIDPGK
VQFCQYNPVLGIIPVEISDIFPAAHYVYGGRAEPGDFAEFAITWDAFLARNKFAEIHYEKTDPFISHFIK
RAKGARRLALKSRKRKNT
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ArcTGT from crenarcheota that don't have ArcS:

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>gi|14601784|ref|NP_148325.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Aeropyrum pernix K1]
MFEIRDVDLAGRIGRIYTQHGVVETPAFFPVIDVYRQEVSVDEVRAAGFGQVITNAYLLWKRFGWEAAEK
GVHRILGFPGVVMTDSGAYQILEYGGVELSQGEVVEYQKRLGSDIAVILDIPTGDVGRREAEESVRETIR
RALEARVMIEGDERIWVYPVQGGRYFDLVEESARVGGRLGFYRMYGIGSPTVFLERYMYHVVVEAVYRAK
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KHLPWGRPVHLFGAGHPLIFPYAVALGVDTFDSASYILYAREGRYITEYGVYRIEDLDYLPCSCPVCSRY
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KGSARGRGVVRGVRAYGLESLSNPRLSRFSSDAARLVEAMAEKWGGGKAVLKPLDPKPEPGQCESMVGGG
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ARAVEALKTAAAGDLPTVEACG

>gi|70606466|ref|YP_255336.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Sulfolobus acidocaldarius DSM 639]
MIGDFEIKDEDLAGRIGILETKHGKLETPAFFPVINPVKNEITIQDILSVGFESIITNAFLIKKYIGKEE
DLHSILNYNKILMTDSGAYQILQYGDIDVSNVDIVNYETKLKPDIAVILDIPTGLTEDKKEAEKSVESTI
SRAKEASKFVELSKDEIIWVHPIQGGMYLDLIEYSARIADMNQDYKMLALGSPTVLMQRYEYAPLIDMIY
KSKSNVSRGKPFHLFGGGHPHIFAFAVALGVDTFDSASYILYARDHRYMTRERVYRLEELDYFPCSCPIC
SRYSPKDVMEMPEEQKVRLIALHNLYVIKEEINYIKQSLKEGRLFEYIQQKAYSHPSTFEAFRRILNYSK
YLEKYDPRVKGEVKGVFLFDNSSLHRPEVIRHAYTLSKIKQRSKALVLYCSDSKDNPLKNTEDMKNADVY
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VNLLLNKL

>gi|227828119|ref|YP_002829899.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Sulfolobus islandicus M.14.25]
MTVFEVKYEDLAGRIGILKTRSGNLETPVFFPVINILKDEISIDEIKNMGFKNFITNSYILYKNKYIKDD
IHKELNSEDMIIMTDSGAYQILEYGDIGITNYEIVNYQLKIKPDIGVILDLPTGNTNDYENAKITVYETL
KRIEEASKIIVKNQDNNIIWVYPIQGGKYLDLVKTSAEGLSKFEDIYNMVALGSPTVLLERYMYDTVIDM
IYTAKSNIKRGIPFHLFGGGLPHIIPFAVALGVDSFDSASYIIYARDNRYITRTRVYKLEDLEYFPCSCP
ICSKYTPKDLLEMNEEERTRALAIHNLYTILEEFKATKQAIKEGRLFEYLQEKAYSHPAVYSAFKRLIKY
KDYLEKYDPRIRGDPKGLFLFDNNSLHRPEIIRHSRFLERYVQKKDKVAIYCYDKAISDTAYDYIKSIKE
RITDHNSSDIFIAIPFFGLIPLEVSDSYPLSQFEIPNEIDEDVIADMKTKIVSFLRCKNYQKVELVNCEK
LGLHIDSISTSS

>gi|126465809|ref|YP_001040918.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Staphylothermus marinus F1]
MEYYDPRDYDLAGRIARLKTRHGVIETPYLFPVVNPLRQQPSIEKLYELGFNAFITNAYLFYRRNKGEIR
NIHEALGWNHVIMTDSGGYQILVYGSVEIDNKTIVEYEKKIGVDIGVILDIPTGTRMSWGEAREAVFETY
KRAVEALPLIMDSDQLWVLPIQGSPYKDLLIYSSIKAWTLPYHIHALGSPTVLLEKYDYEKIVELTAIAR
IHLPPHKPLHVFGVGHPMIIPFLVALGADLFDSASYILYARDNRYMTETGTKRLEELQYLPCNCPVCSRY
SVKELLEMPRYKRIELLALHNLYMLKKELNNTKQAIKEGRLWEYLEYKSKAHPSLRKAFNILKKYLEYIK
KYNPATKGTTLALLLNDSDSLINPRLSLTKENTKEYILKKYRGKQILLLPAIEKPFNQEKTYLRTKKQYK
GYEILFYHPFLGVFPPQLSNTYPFFQHEVGVINENVIENLVNELKKVILEINPEKIVLVKIGMKPYDDII
DELLKDKNLVSTYTLDVLSLSA

>gi|146305018|ref|YP_001192334.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Metallosphaera sedula DSM 5348]
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MIGDFEVKDEDLAGRIGILETKHGKLETPVFFPVINPLKQEVFLEELKAVGFNNFITNSFILKKNNILQG
TIHEKFGDNFVIMTDSGAYQILEYGEIEQTNRDIVSFEAKIRPDIAVFLDIPTGNTDDREEAKFSVEMTL
ERGKEIADIVDQNEDIIWVHPIQGGQFLDLVEYSAREANKRTNYKMLALGSPTVFMEKYKYDTLVDMIYT
AKSSVSRGVPFHLFGGGVPHIIPFAVALGVDSFDSASYAIFARDNRYLTSERTYRLEDLEYFPCSCPVCS
RYDPSELLEMKSEERYKLLAIHNLWKIREEVNRVKQAIKEGRLFEYIQQKAYSHPALYSAFKSILKYSSY
LEKYDPRVKGNVKGLLLFDHNSMNRPELLRHSEFMANLKPKRNKVIIICGDKLGSPFISDPKVKSIQGRN
RDYDTFVALPFYGLVPVMASEAFPLSQFEIPDIIDDTTLNETILKIKETLRNKNYAEIKFMECEKSVLSH
IMSINPTL

>gi|307595970|ref|YP_003902287.1| tRNA-guanine transglycosylase, various
specificities [Vulcanisaeta distributa DSM 14429]
MSFEILDKDLAGRIGRLRTRSGVIETPALFPVINPVKQVVPLSDIADIGFNQVITNAYLLKRHYGDLVRE
VGVHKLLNWDKPIMTDSGAYQLLMYGRVEVSPDEILKYEIDIGTDIGVILDIPTQWGRPKEAVVLEVEET
LRRARAALTKARIWDPEHRMLIVGPVQGGDKLDILSYSATKMSELNFDIYAIGSPTTLLEEYEFSTIMRM
IAVVKEKLPPGKPVHLFGAGHPLILPFAVAMGIDLFDSASYVLYARDDRLILRDRTVRLSEVKVDKIPCN
CPVCRKYTVKELMSMNKAERERLLAIHNLYVLWEEIQEIKARIKEGTLWEYLEEKAGGDARVKSALLALR
RGLRALLKVIPDESGRVRALHITSVESLHRPEVIRHVERLLNNYEPPQGCVVMVLPGELLDRPYIRDSLV
YWLIDQLSTSNLLGKVHVVINNPVLGPIPYEISEIYPLSQHEYPEPTPNHMRSLSHYLLRKYLSKLRNRG
FTDIIIIVKESRGKIMNELVNLGFRGVTVLEAGSRQELYSLMPWVIKEVSGIQCGNQM

>gi|119872581|ref|YP_930588.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Pyrobaculum islandicum DSM 4184]
MSFEIVAKDIAGRIGLLYTKSGVVETPALFPVVDPRRQEVPLEVVEKYFNQVITNAYFLYRLTGGKAVDI
KKLLGWRGVVMTDSGAYQILRYGTVEVDPDEILYFQQKIGSDIGVILDLPFDYEEPYESALLKVEETIRR
AKRAAAVLKDLDMLVVAPIQGALYTDLLIRSAKELSKLGFHIYAIGSPTTLLEEYKFDLILKIVLDVKLN
MMREAPLHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIILRDRTLRLDDVKTEYLPCSTKLCYTP
VKELREMPKQERMMLIAEHNLAVLREELLEIKQRIYEGTLWEYLEIKARAHPALYRFLKNLHRYRRLLEE
YDPETHPDPHGLFFFADTASSRPEPMRHWTRMSHVSALSKKAVVIRVNEKPYNRSWEYIYLKGIIGDRAH
ILFYDPIFGIIPEELAEIYPLSQNESEGEDEAARARAYEWLDRYDVIITYNVDIPLVNKSVIKASSLEEI
ALYV

>gi|18312172|ref|NP_558839.1| 7-cyano-7-deazaguanine tRNA-ribosyltransferase
[Pyrobaculum aerophilum str. IM2]
MSFEIIAKDLAGRVGKLYTKSGVIETPALFPVVDPRKQELPSAVIERYFGQIITNAYFVYRLTGGRAVDI
KKVLSWNAVLMTDSGAYQILRYGSVEVDPDEILQFQARIGSDIGVILDLPFDYEEPYESALLKVEETIRR
AKRASAMLDKLEDMLVVGPIQGGLYLDLLATSAREISKLGFHIFAVGSPTTLLEEYRFDLLLEVILHVKA
NILREAPLHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIMLRDRTLRLEDVKTDYLPCSTKLCHK
PVKELREMPHEERIQLIAEHNLAILREELLEIKQRIHEGTLWEYLEIKARAHPTLYRFLRSLGRYKRLIE
EYDPETHPETHGLFFYQDTAESRPEPHRHWSRTANLYTPSKVAIVIRAGEKPYNKSWEYRYLKSLVGDRA
HVLFYDPVFGLVPEEVAEIYPLSQNEAEGESEAARAFAYEWLNNYDVILLYRVDLPMLSKKVIPLRSLDD
VLHYI
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ribosyltransferase [Thermoproteus neutrophilus V24Sta]
MSFEIVAKDLAGRVGLLHTKSGVVETPALFPVVDPRKQEVPLEAVQRYFGQVITNSYFIYRLTGGRAVDV
KKMLGWGGVVMTDSGAYQILRYGTVEVDPDEILRFQQKIGSDIGVILDLPFDYEEPYGSALLKVEETIRR
AKRAAAYLKEMDMLVVAPIQGALYADLLARSARELSRLGFHIFAIGSPTTLLEEYRFDLILRVVLEVKAN
IAREAPIHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIILRDRTLRLDDVKTDYLPCSTKLCYTP
VKELREMPKQERTLLIAEHNLAVLREELLEIRQRIHEGTLWEYLEIKARAHPTLYRFLKSIDRRLLEEYD
PETHPDPHGLFFYSDTAPRRPEPARHRERLNNVEPLSRRAVVIKVEEKPYNRNWQYLYLKKTLGDRAHIL
FYDPIFGIIPEEIAEVYPLSQNESEGEDEEARAHAYEWLRRYDLVVLYNVDIPLMGKKVVKARSLEEVAL
YIQP
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>gi|126460752|ref|YP_001057030.1| 7-cyano-7-deazaguanine tRNA-
ribosyltransferase [Pyrobaculum calidifontis JCM 11548]
MGVFEIVAKDLGGRIGRLYTKSGVVETPALFPVVDPRKQEVPSEVLKRYFGQVITNSYFIYRLAKGGDVN
VKRLLKWDGVVMTDSGAYQILRYGTVDVDPDEILLYQHKIGSDIGVILDLPFDYEEPYHSALLKVEETLR
RARRATRLLKDLDMLVVAPIQGATYLDLLRRSARELSQMGFPIYAVGSPTTLLEEYRFDTILEIVLEAKM
AMVRDAPLHLFGAGHPLILPFAVALGVDLFDSASYILYARDGRIMLRDRTLRLDDAKSEYLPCSTKLCQT
PVKELREMPREERTLLIAEHNLAVLREELMEIRQRIHEGTLWEYLEAKARAHPSLYRFLKKLSKFRRYIE
ALDPETHPKPHGLFFYHDTSPSRPEPLRHFTRLENVDPPSKKALVVKASTKPYNRSWEYRYLKELVGERV
HIIFYDPVFGAVPEELAEVYPLSQNEAEGESEEARARLYEWLNRYDEIYTYGVDIPLVGKAVRRLSSIED
ARYIA
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>gi|159040616|ref|YP_001539868.1| tRNA-guanine transglycosylase [Caldivirga
maquilingensis IC-167]
MFEVIEKDEIARIGKLYTRHGVIETPALFPVINPSKQYVDLSRIKELGFNQIITNAYIIRKTYGDAAREV
GLHEIIGWDGPLMTDSGAYQILQYGNIEVSPDEALRYQVEIGSDIGVILDIPTSYPRPRELVEVEVNETI
RRARRAILQLKELDPEHRMLIVGPTQGGVYRDLLAYSARMVSELPFDIYAIGSPTTLLQAYNFTGIIKMI
LTVKSIIPPGKPVHLFGVGHPLILPLAVALGIDLFDSASYMLYANDDRIILSSRTVRLSELDKDYVLDGC
GKRAGELMEMSKEERIRLIAQHNLWTLSRELMEIKQRIKEHDIWSYVAQKARQHPSVYRAYLTLSNSSLF
RKLTIKLASGLKVNATQLSILDNSDLARPEVQWARRRLRRLIKGMGVLTNVLIIIGDYEEPFIRTQIANE
VTRLGVRVFTYNEVYGLIPIELSDVYPFSQTVRVNVKPRPIKYEIRDSVILVEDKYRDLVKFIKCSGECF
IIYVDSLKNIKAYEKYVGLALMKRVNEGSGIK
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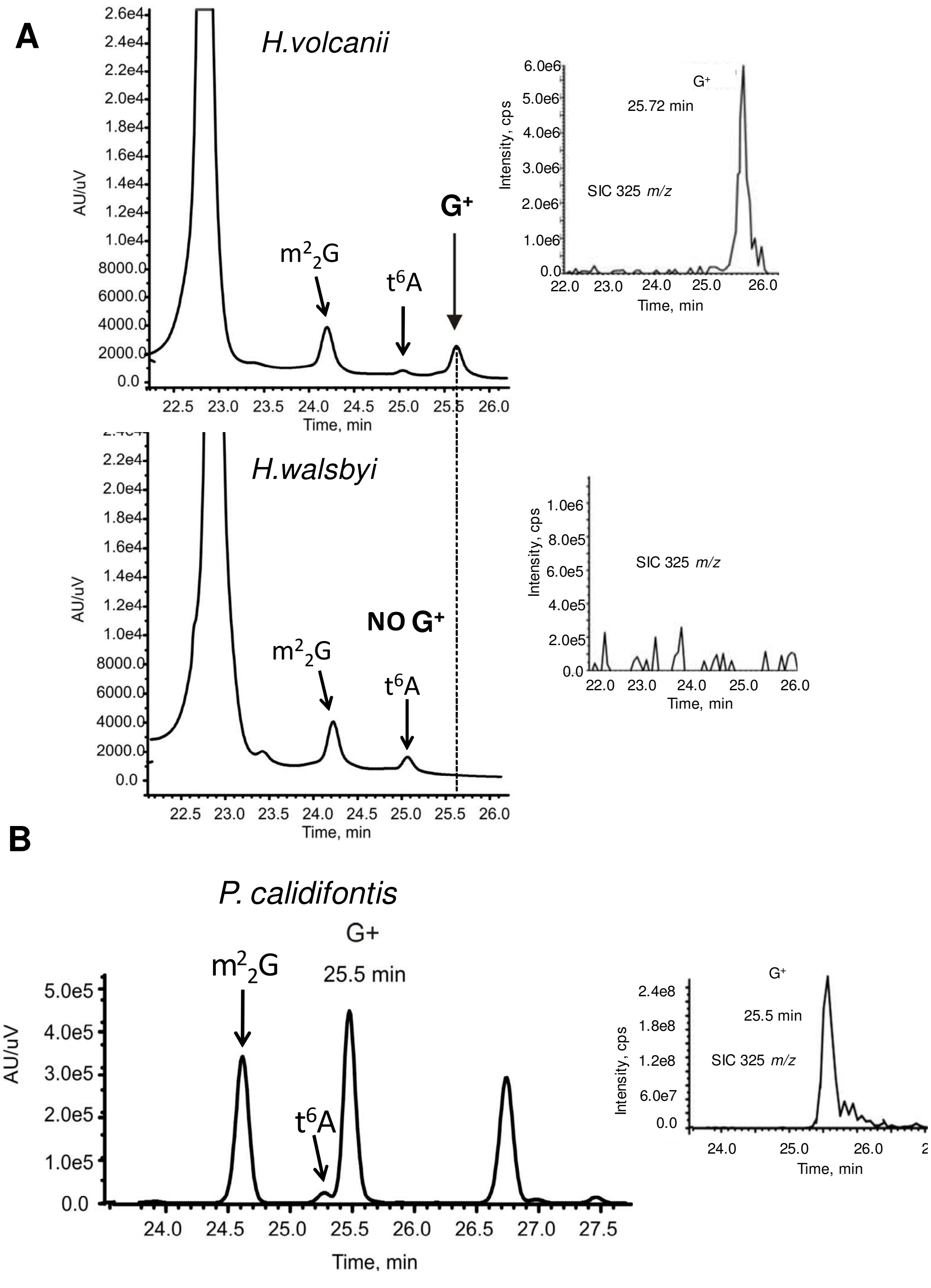
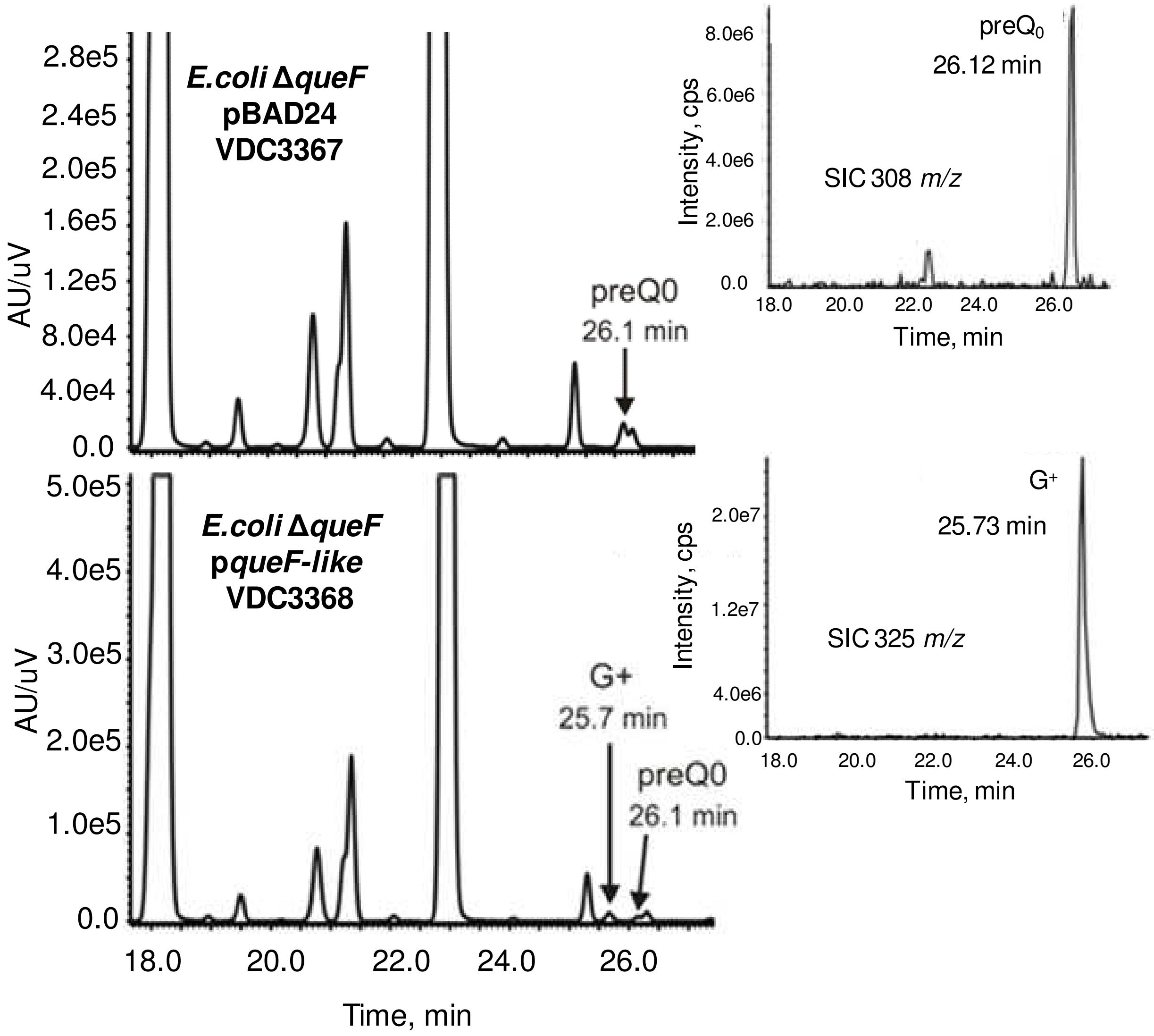
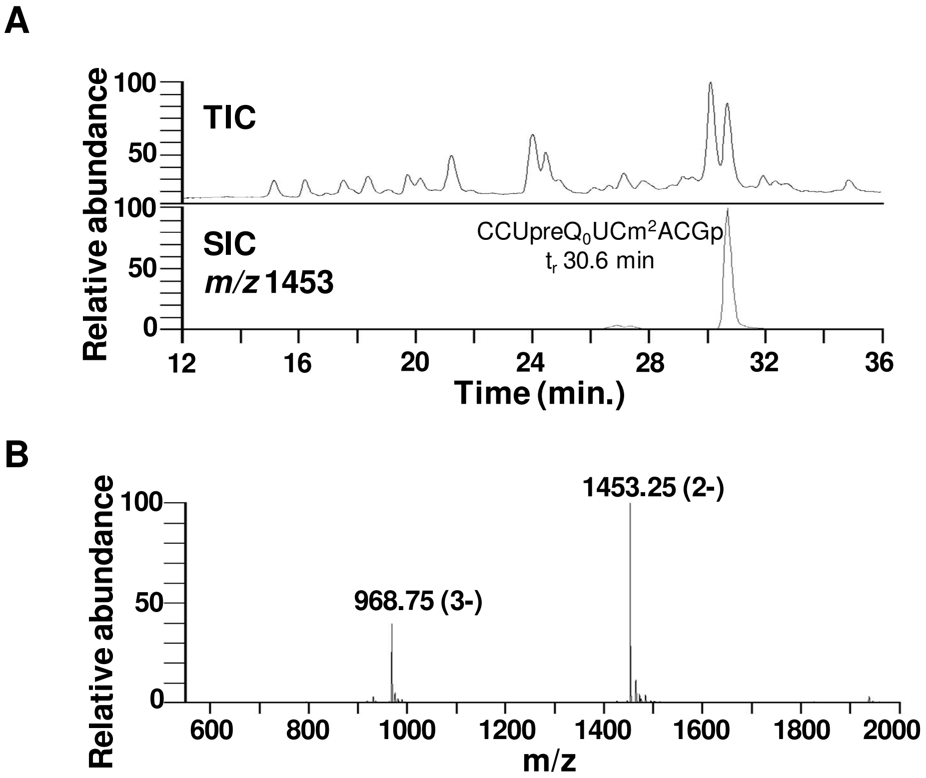
4. Supplemental Figures



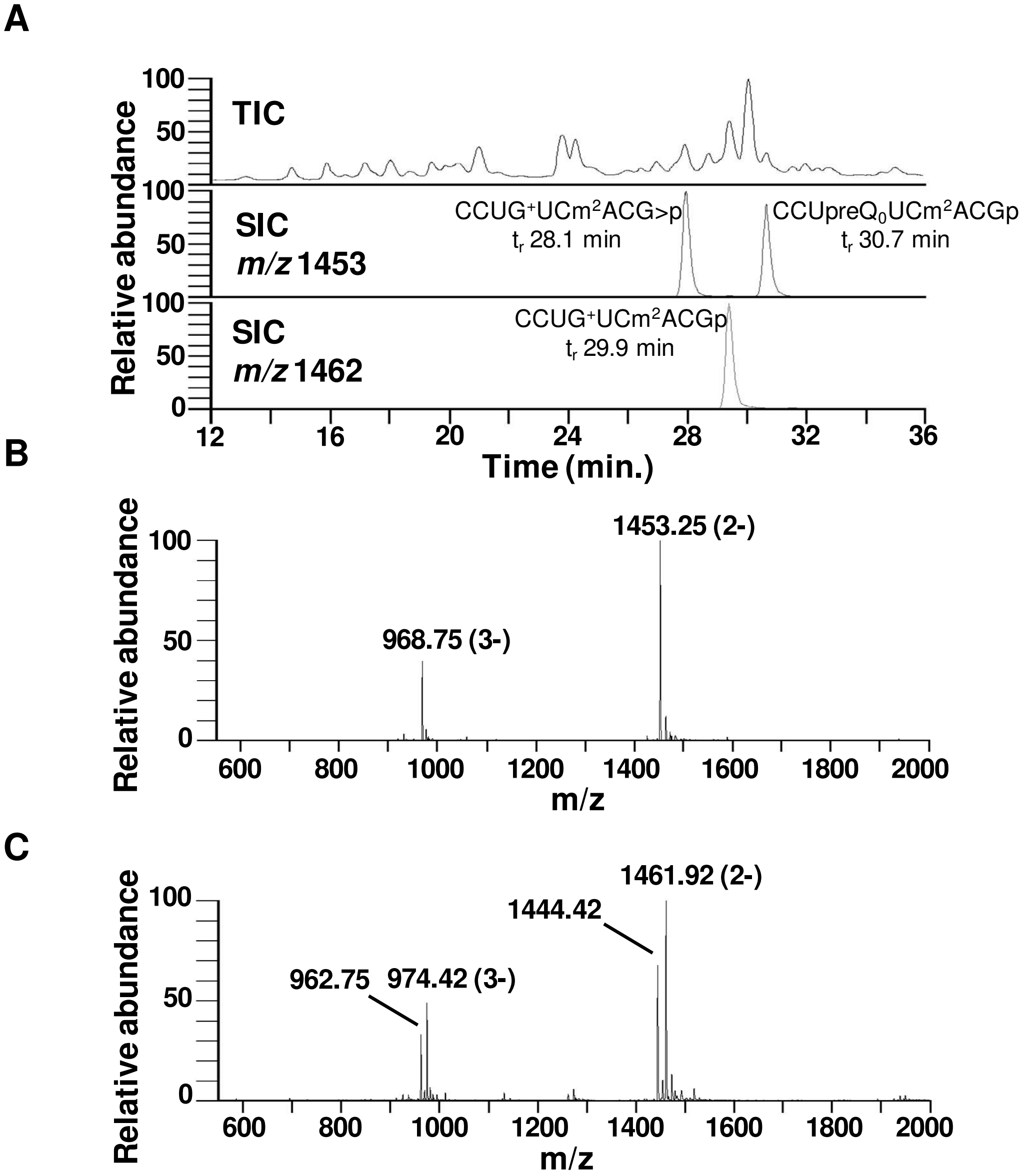
Supplemental Figure 1. LC-MS/MS analysis of bulk tRNA extracted from (A) *H. walsbyi* compared to *H. volcanii;* the G$^+$ peak eluted at 25.7 min in the UV chromatogram (254 nm) of the tRNA extracted from *H. volcanii* was not detected in the UV chromatogram (254 nm) of the bulk tRNA extracted from *H. walsbyi*. The extraction ion chromatograms for 325 *m/z* are shown in the insets. (B) *P. calidifontis*. The UV traces at 254 nm and the extraction ion chromatograms (insets) for 325 *m/z* are shown. The G$^+$ peak elutes at 25.5 minutes. As internal standards, the m$^2_2$G and t^{6}A peaks are shown.



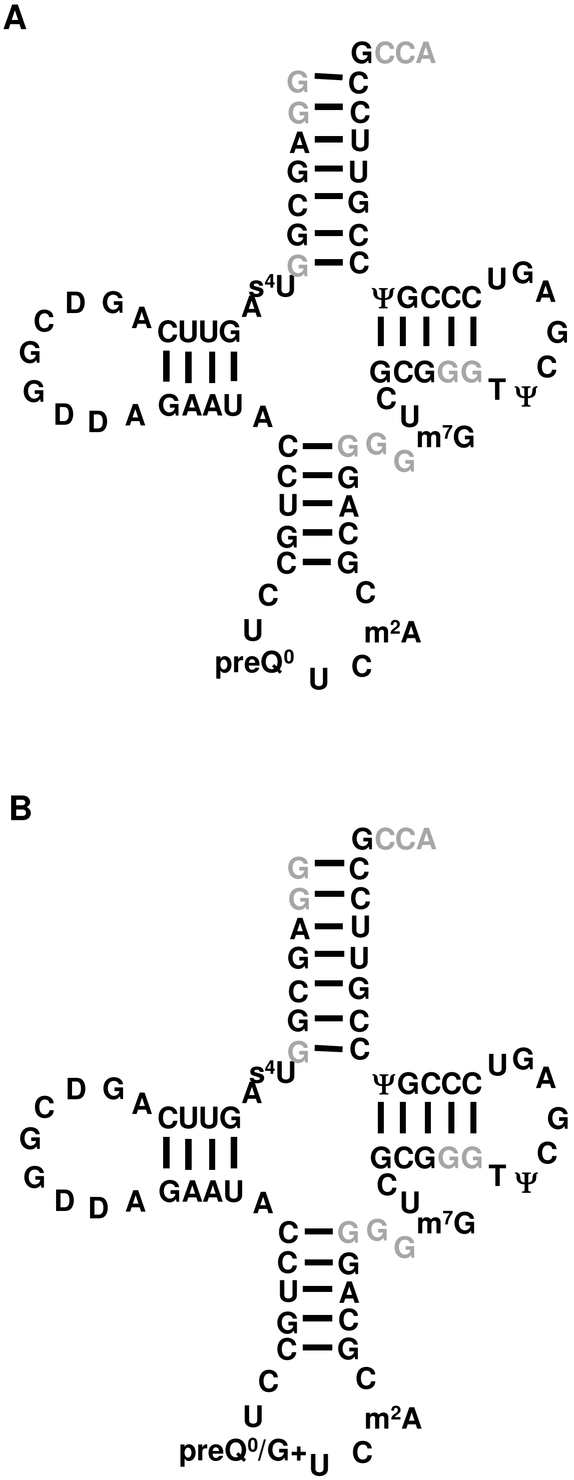
Supplemental Figure 2. LC-MS/MS analysis of bulk tRNA extracted from *E. coli* Δ*queF* derivative strains. The UV traces at 254 nm and the extraction ion chromatograms (insets) for 325 *m/z* are shown. The UV chromatogram of the bulk tRNA extracted from VDC3368 showed the G^+ peak eluted at 25.7 min and $preQ_0$ peak eluted at 26.1 min. The UV chromatogram of the bulk tRNA extracted from VDC3367 (the negative control) showed only the $preQ_0$ peak eluted at 26.1 min.



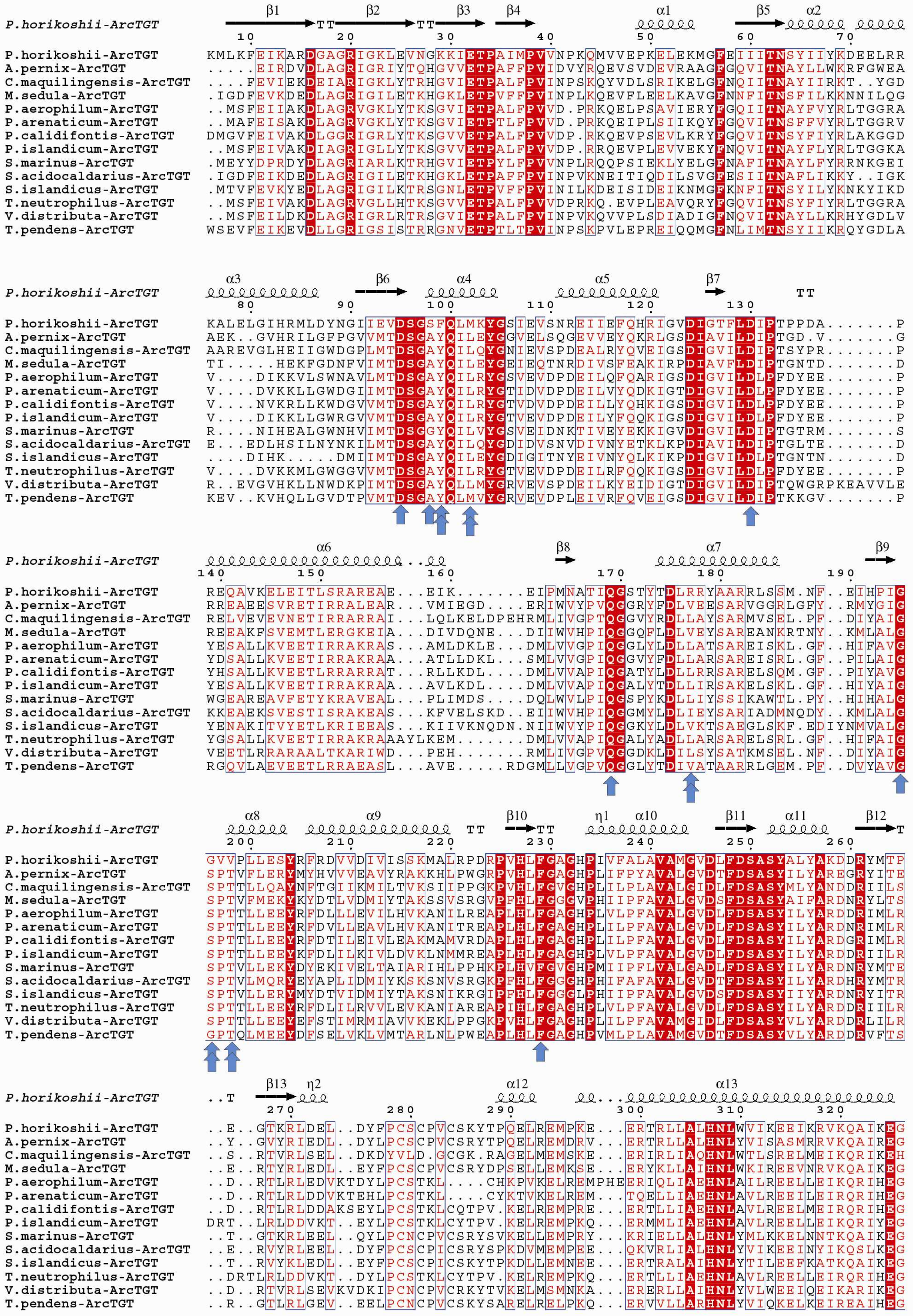
Supplemental Figure 3. LC-MS of strain VDC3367 tRNA^{Asp} resulted in the identification of one RNase T1 digestion product not expected in wild type tRNA^{Asp}. (A) TIC of RNase T1 digested tRNA^{Asp} and the SIC for *m/z* 1453 (2-). (B) Mass spectrum corresponding to the *m/z* 1453 SIC peak eluting at 30.6 min. These two ions, *m/z* 1453.25 (2-) and *m/z* 968.75 (3-), correspond to a molecular ion mass of 2908 Da, which is consistent with an RNase T1 digestion product with the sequence CCUpreQ$_0$UCm^{2}AGp. The sequence of this RNase T1 product was confirmed by collision-induced dissociation (CID) tandem mass spectrometry (Figure 3D, top).



Supplemental Figure 4. LC-MS of strain VDC3368 tRNA^{Asp} resulted in the identification of three RNase T1 digestion products not found in wild type tRNA^{Asp}. (A) TIC of RNase T1 digested tRNA^{Asp} and the SICs for *m/z* 1453 (2-) and 1462 (2-). (B) Mass spectrum corresponding to the *m/z* 1453 SIC peak eluting at 30.7 min. The two ions correspond to a molecular ion mass of 2908 Da, which is consistent with an RNase T1 digestion product with the sequence CCUpreQ$_0$UCm^{2}AGp, as in Supplemental Figure 3. (C) Mass spectrum corresponding to the *m/z* 1462 SIC peak eluting at 29.9 min. The two ions, *m/z* 1461.92 (2-) and *m/z* 974.4 (3-), correspond to a molecular ion mass of 2926 Da, which is consistent with an RNase T1 digestion product with the sequence CCUG$^+$UCm^{2}AGp. The sequence of this RNase T1 product was confirmed by CID tandem mass spectrometry (Figure 3D, bottom). The peak eluting at 28.1 min in the *m/z* 1453 SIC trace in (A) corresponds to the cyclic phosphate RNase T1 digestion product, CCUG$^+$UCm^{2}AG>p.



Supplemental Figure 5. tRNA sequences of tRNA^{Asp} determined by LC-MS/MS from strain (A) VDC3367 and (B) VDC3368. Identified RNase T1 digestion products, including modified nucleosides found in wild type tRNA^{Asp}, are depicted in bold type. Regions of the tRNA that could not be sequenced by LC-MS/MS, primarily single Gp resides, are in gray. In strain VDC3367 only preQ$_0$ was found at the wobble position whereas in VDC3368 digestion products with both preQ$_0$ and G$^+$ at the wobble position were found.



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P.horikoshii-ArcTGT        KMLKFEIKARDGAGRIGKLEVNGKKIETPAIMPVVNPKQMVVEPKELEKMGFEIIITNSYIIYKDEELRR
A.pernix-ArcTGT            .....EIRDVDLAGRIGRIYTQHGVVETPAFFPVIDVYRQEVSVDEVRAAGFGQVITNAYLLWKRFGWEA
C.maquilingensis-ArcTGT    ....FEVIEKDEIARIGKLYTRHGVIETPALFPVINPSKQYVDLSRIKELGFNQIITNAYIIRKT..YGD
M.sedula-ArcTGT            .IGDFEVKDEDLAGRIGILETKHGKLETPVFFPVINPLKQEVFLEELKAVGFNNFITNSFILKKNNILQG
P.aerophilum-ArcTGT        ..MSFEIIAKDLAGRVGKLYTKSGVIETPALFPVVD.PRKQELPSAVIERYFGQIITNAYFVYRLTGGRA
P.arenaticum-ArcTGT        ..MAFEISAKDLAGRVGKLYTKSGVIETPTLFPVVD.PRKQEIPLSIIKQYFGQVITNSFFVYRLTGGRV
P.calidifontis-ArcTGT      DMGVFEIVAKDLGGRIGRLYTKSGVVETPALFPVVDP.RKQEVPSEVLKRYFGQVITNSYFIYRLAKGGD
P.islandicum-ArcTGT        ..MSFEIVAKDIAGRIGLLYTKSGVVETPALFPVVDP.RRQEVPLEVVEKYFNQVITNAYFLYRLTGGKA
S.marinus-ArcTGT           .MEYYDPRDYDLAGRIARLKTRHGVIETPYLFPVVNPLRQQPSIEKLYELGFNAFITNAYLFYRRNKGEI
S.acidocaldarius-ArcTGT    .IGDFEIKDEDLAGRIGILETKHGKLETPAFFPVINPVKNEITIQDILSVGFESIITNAFLIKKY..IGK
S.islandicus-ArcTGT        .MTVFEVKYEDLAGRIGILKTRSGNLETPVFFPVINILKDEISIDEIKNMGFKNFITNSYILYKNKYIKD
T.neutrophilus-ArcTGT      ..MSFEIVAKDLAGRVGLLHTKSGVVETPALFPVVDPRKQ.EVPLEAVQRYFGQVITNSYFIYRLTGGRA
V.distributa-ArcTGT        ..MSFEILDKDLAGRIGRLRTRSGVIETPALFPVINPVKQVVPLSDIADIGFNQVITNAYLLKRHYGDLV
T.pendens-ArcTGT           WSEVFEIKEVDLLGRIGSISTRRGNVETPTLTPVINPSKPVLEPREIQQMGFNLIMTNSYIIKRQYGDLA

P.horikoshii-ArcTGT        KALELGIHRMLDYNGIIEVDSGSFQLMKYGSIEVSNREIIEFQHRIGVDIGTFLDIPTPPDA.......P
A.pernix-ArcTGT            AEK..GVHRILGFPGVVMTDSGAYQILEYGGVELSQGEVVEYQKRLGSDIAVILDIPTGD.V.......G
C.maquilingensis-ArcTGT    AAREVGLHEIIGWDGPLMTDSGAYQILQYGNIEVSPDEALRYQVEIGSDIGVILDIPTSYPR.......P
M.sedula-ArcTGT            TI.....HEKFGDNFVIMTDSGAYQILEYGEIEQTNRDIVSFEAKIRPDIAVFLDIPTGNTD.......D
P.aerophilum-ArcTGT        V....DIKKVLSWNAVLMTDSGAYQILRYGSVEVDPDEILQFQARIGSDIGVILDLPFDYEE.......P
P.arenaticum-ArcTGT        V....DVKKLLGWDGIIMTDSGAYQILRYGTIDVDPDEILVYQDKIGTDIGVILDLPFDYEE.......P
P.calidifontis-ArcTGT      V....NVKRLLKWDGVVMTDSGAYQILRYGTVDVDPDEILLYQHKIGSDIGVILDLPFDYEE.......P
P.islandicum-ArcTGT        V....DIKKLLGWRGVVMTDSGAYQILRYGTVEVDPDEILYFQQKIGSDIGVILDLPFDYEE.......P
S.marinus-ArcTGT           R....NIHEALGWNHVIMTDSGGYQILVYGSVEIDNKTIVEYEKKIGVDIGVILDIPTGTRM.......S
S.acidocaldarius-ArcTGT    E...EDLHSILNYNKILMTDSGAYQILQYGDIDVSNVDIVNYETKLKPDIAVILDIPTGLTE.......D
S.islandicus-ArcTGT        ....DIHK.....DMIIMTDSGAYQILEYGDIGITNYEIVNYQLKIKPDIGVILDLPTGNTN.......D
T.neutrophilus-ArcTGT      V....DVKKMLGWGGVVMTDSGAYQILRYGTVEVDPDEILRFQQKIGSDIGVILDLPFDYEE.......P
V.distributa-ArcTGT        R..EVGVHKLLNWDKPIMTDSGAYQLLMYGRVEVSPDEILKYEIDIGTDIGVILDIPTQWGRPKEAVVLE
T.pendens-ArcTGT           KEV..KVHQLLGVDTPVMTDSGAYQLMVYGRVEVDPLEIVRFQVEIGSDIGVILDIPTKKGV.......P

P.horikoshii-ArcTGT        REQAVKELEITLSRAREAE...EIK.......EIPMNATIQGSTYTDLRRYAARRLSSM.NF..EIHPIG
A.pernix-ArcTGT            RREAEESVRETIRRALEAR...VMIEGD....ERIWVYPVQGGRYFDLVEESARVGGRLGFY..RMYGIG
C.maquilingensis-ArcTGT    RELVEVEVNETIRRARRAI...LQLKELDPEHRMLIVGPTQGGVYRDLLAYSARMVSEL.PF..DIYAIG
M.sedula-ArcTGT            REEAKFSVEMTLERGKEIA...DIVDQNE...DIIWVHPIQGGQFLDLVEYSAREANKRTNY..KMLALG
P.aerophilum-ArcTGT        YESALLKVEETIRRAKRAS...AMLDKLE...DMLVVGPIQGGLYLDLLATSAREISKL.GF..HIFAVG
P.arenaticum-ArcTGT        YDSALLKVEETIRRAKRAA...ATLLDKL...SMLVVGPIQGGVYFDLLARSAREISRL.GF..PILAIG
P.calidifontis-ArcTGT      YHSALLKVEETLRRARRAT...RLLKDL....DMLVVAPIQGATYLDLLRRSARELSQM.GF..PIYAVG
P.islandicum-ArcTGT        YESALLKVEETIRRAKRAA...AVLKDL....DMLVVAPIQGALYTDLLIRSAKELSKL.GF..HIYAIG
S.marinus-ArcTGT           WGEAREAVFETYKRAVEAL...PLIMDS....DQLWVLPIQGSPYKDLLIYSSIKAWTL.PY..HIHALG
S.acidocaldarius-ArcTGT    KKEAEKSVESTISRAKEAS...KFVELSKD..EIIWVHPIQGGMYLDLIEYSARIADMNQDY..KMLALG
S.islandicus-ArcTGT        YENAKITVYETLKRIEEAS...KIIVKNQDN.NIIWVYPIQGGKYLDLVKTSAEGLSKF.EDIYNMVALG
T.neutrophilus-ArcTGT      YGSALLKVEETIRRAKRAAAYLKEM.......DMLVVAPIQGALYADLLARSARELSRL.GF..HIFAIG
V.distributa-ArcTGT        VEETLRRARAALTKARIWD...PEH.......RMLIVGPVQGGDKLDILSYSATKMSEL.NF..DIYAIG
T.pendens-ArcTGT           RGQVLAEVEETLRRAEASL...AVE.....RDGMLLVGPVQGGLYTDIVATAARRLGEM.PF..DVYAVG

P.horikoshii-ArcTGT        GVVPLLESYRFRDVVDIVISSKMALRPDRPVHLFGAGHPIVFALAVAMGVDLFDSASYALYAKDDRYMTP
A.pernix-ArcTGT            SPTVFLERYMYHVVVEAVYRAKKHLPWGRPVHLFGAGHPLIFPYAVALGVDTFDSASYILYAREGRYITE
C.maquilingensis-ArcTGT    SPTTLLQAYNFTGIIKMILTVKSIIPPGKPVHLFGVGHPLILPLAVALGIDLFDSASYMLYANDDRIILS
M.sedula-ArcTGT            SPTVFMEKYKYDTLVDMIYTAKSSVSRGVPFHLFGGGVPHIIPFAVALGVDSFDSASYAIFARDNRYLTS
P.aerophilum-ArcTGT        SPTTLLEEYRFDLLLEVILHVKANILREAPLHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIMLR
P.arenaticum-ArcTGT        SPTTLLEEYRFDVLLEAVLHVKANITREAPLHLFGAGHPLILPFAVALGVDLFDSASYILYARDNRIMLR
P.calidifontis-ArcTGT      SPTTLLEEYRFDTILEIVLEAKMAMVRDAPLHLFGAGHPLILPFAVALGVDLFDSASYILYARDGRIMLR
P.islandicum-ArcTGT        SPTTLLEEYKFDLILKIVLDVKLNMMREAPLHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIILR
S.marinus-ArcTGT           SPTVLLEKYDYEKIVELTAIARIHLPPHKPLHVFGVGHPMIIPFLVALGADLFDSASYILYARDNRYMTE
S.acidocaldarius-ArcTGT    SPTVLMQRYEYAPLIDMIYKSKSNVSRGKPFHLFGGGHPHIFAFAVALGVDTFDSASYILYARDHRYMTR
S.islandicus-ArcTGT        SPTVLLERYMYDTVIDMIYTAKSNIKRGIPFHLFGGGLPHIIPFAVALGVDSFDSASYIIYARDNRYITR
T.neutrophilus-ArcTGT      SPTTLLEEYRFDLILRVVLEVKANIAREAPIHLFGAGHPLVLPFAVALGVDLFDSASYILYARDDRIILR
V.distributa-ArcTGT        SPTTLLEEYEFSTIMRMIAVVKEKLPPGKPVHLFGAGHPLILPFAVAMGIDLFDSASYVLYARDDRLILR
T.pendens-ArcTGT           GPTQLMEEYDFSELVKLVMTARLNLPWEAPLHLFGAGHPVMLPLAVAMGVDTFDSASYVLYARDDRVFTS

P.horikoshii-ArcTGT        ..E..GTKRLDEL..DYFPCSCPVCSKYTPQELREMPKE...ERTRLLALHNLWVIKEEIKRVKQAIKEG
A.pernix-ArcTGT            ..Y..GVYRIEDL..DYLPCSCPVCSRYTPQELREMDRV...ERTRLLALHNLYVISASMRRVKQAIREG
C.maquilingensis-ArcTGT    ..S..RTVRLSEL..DKDYVLD.GCGK.RAGELMEMSKE...ERIRLIAQHNLWTLSRELMEIKQRIKEH
M.sedula-ArcTGT            ..E..RTYRLEDL..EYFPCSCPVCSRYDPSELLEMKSE...ERYKLLAIHNLWKIREEVNRVKQAIKEG
P.aerophilum-ArcTGT        ..D..RTLRLEDVKTDYLPCSTKL....CHKPVKELREMPHEERIQLIAEHNLAILREELLEIKQRIHEG
P.arenaticum-ArcTGT        ..D..RTLRLDDVKTEHLPCSTKL....CYKTVKELREM...TQELLIAEHNLAVLREEILEIRQRIHEG
P.calidifontis-ArcTGT      ..D..RTLRLDDAKSEYLPCSTKLCQTPV.KELREMPRE...ERTLLIAEHNLAVLREELMEIRQRIHEG
P.islandicum-ArcTGT        DRT..LRLDDVKT..EYLPCSTKLCYTPV.KELREMPKQ...ERMMLIAEHNLAVLREELLEIKQRIYEG
S.marinus-ArcTGT           ..T..GTKRLEEL..QYLPCNCPVCSRYSVKELLEMPRY...KRIELLALHNLYMLKKELNNTKQAIKEG
S.acidocaldarius-ArcTGT    ..E..RVYRLEEL..DYFPCSCPICSRYSPKDVMEMPEE...QKVRLIALHNLYVIKEEINYIKQSLKEG
S.islandicus-ArcTGT        ..T..RVYKLEDL..EYFPCSCPICSKYTPKDLLEMNEE...ERTRALAIHNLYTILEEFKATKQAIKEG
T.neutrophilus-ArcTGT      ..DRTLRLDDVKT..DYLPCSTKLCYTPV.KELREMPKQ...ERTLLIAEHNLAVLREELLEIRQRIHEG
V.distributa-ArcTGT        ..D..RTVRLSEVKVDKIPCNCPVCRKYTVKELMSMNKA...ERERLLAIHNLYVLWEEIQEIKARIKEG
T.pendens-ArcTGT           ..R..GTLRLGEV..EELPCNCPVCSKYSARELRELPKQ...ERVVLLARHNLYVIQKELKEIREAIHEG
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Supplemental Figure 6. Differences in the substrate binding pocket between the canonical (euryarchaeal) arcTGT and crenarcheal arcTGT. a) Structure based multisequence alignment of the crenarcheal arcTGT and the canonical arcTGT in the catalytic domain. Of the canonical arcTGTs, only the *Pyrococcus horikoshii* sequence is shown. Secondary structure elements from *P. horikoshii* arcTGT crystal structure (PDB ID 1IQ8, (*10*)) are shown on top. Blue arrows indicate substrate recognition determinants in the canonical arcTGT that are conserved in crenarcheal arcTGT. Double arrows indicate substrate binding determinants that are absent in crenarcheal sequences. The recognition features for the preQ$_0$ cyano group are not conserved in crenarcheal arcTGT. These include Met102 (Leu in all crenarcheal arcTGT), and Val198 (Thr in crenoarcheal arcTGT) located in the preQ$_0$ selection helix that harbors the major antideterminants against recognition of preQ$_1$.

5. Supplemental References

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