

Concerted gene recruitment in early plant evolution

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1. Materials and methods

Data sources: Protein sequences for the red alga *Cyanidioschyzon merolae* were obtained from the *Cyanidioschyzon* Genome Project [41, 73]. EST sequences of several protists were obtained from TBestDB [74]. All other sequences were from the NCBI protein sequence database.

Identification of ancient HGT: Anciently acquired genes in this study include those horizontally acquired prior to the split of red algae and green plants. A list of ancient HGT candidates was first identified based on phylogenomic screening of the *Cyanidioschyzon* genome using PhyloGenie and the NCBI non-redundant protein sequence database. The vast majority of the genes on this list are predominantly identified in bacteria and archaea, and therefore are likely of prokaryotic origin. To reduce the complications arising from potential cases of IGT, we adopted an approach combining sequence comparison, phylogenetic analyses, and statistical tests. Each gene on the list was first used to search the NCBI protein sequence database. Because of the cyanobacterial origin of plastids and the α -proteobacterial origin of mitochondria, genes with cyanobacterial and plastid-containing eukaryotic homologs as top hits were considered as likely plastid-derived; those with proteobacterial and other eukaryotic homologs were considered as likely mitochondrion-derived. These potentially organelle-derived genes were removed from the candidate list and the remaining genes were subject to detailed phylogenetic analyses (see below). Gene tree topologies generated through detailed phylogenetic analyses were subject to careful inspections; any genes that formed a monophyly with cyanobacterial homologs or with proteobacterial and other eukaryotic sequences were also eliminated from further consideration. Additionally, alternative topologies representing various evolutionary scenarios for each gene were statistically evaluated based on AU tests [43] (see below); genes for which a straightforward IGT scenario (versus IGT followed by secondary transfers) could not be rejected (p -value > 0.05) were also removed from the HGT candidate list.

Detailed phylogenetic analyses: Sequences were sampled from representative groups (including major phyla of bacteria and major groups of eukaryotes) within each domain of life (bacteria, archaea, and eukaryotes). Because of the potential for sequence contaminations, eukaryotic EST sequences whose authenticity is suspicious (e.g., high nucleotide sequence percent identity with bacterial homologs and/or absence of homologs in genomes of closely related taxa) were not included in the analyses. Multiple protein sequence alignments were performed using MUSCLE [77] and clustalx [78], followed by cross-comparisons and manual refinement. Only unambiguously aligned sequence portions were used. Phylogenetic analyses were performed with a maximum likelihood method using PHYLML [79], a Bayesian inference method using MrBayes [80], and a distance method using the program *neighbor* of PHYLIP version 3.65 [81] with maximum likelihood distances calculated using TREE-PUZZLE [82]. All maximum likelihood calculations were based on a substitution matrix determined using ProtTest [83] and a mixed model of 4 gamma-distributed rate classes plus invariable sites.

Maximum likelihood distances for bootstrap analyses were calculated using TREE-PUZZLE and PUZZLEBOOT v1.03 (by Michael E. Holder and Andrew J. Roger, available on the web [84]). Branch lengths and topologies of the trees depicted in all figures were calculated with PHYML. For the convenience of presentation, gene trees were rooted using archaeal (or archaeal + eukaryotic) sequences, or paralogous gene copies if ancient gene families were involved, as outgroups; otherwise, trees were rooted in a way that no top hits of the sequence similarity search were used as an outgroup. Nevertheless, all gene trees should be strictly interpreted as unrooted.

AU tests on alternative tree topologies: Following detailed phylogenetic analyses, alternative tree topologies for each remaining HGT candidates were assessed for their statistical confidence using Treefinder [85]. In most cases, multiple constraint trees were generated using Treefinder for each HGT candidate by enforcing **a**) monophyly of all eukaryotic sequences, **b**) monophyly of cyanobacterial, plant and other plastid-containing eukaryotic sequences, and **c**) monophyly of cyanobacterial, plant, and closely related bacterial sequences. These alternative topologies assumed that the subject gene in plants are not HGT-derived; they served as null hypotheses that all eukaryotic sequences have the same eukaryotic or mitochondrial origin or that plants acquired the subject gene from plastids, sometimes followed by secondary HGT to other bacterial groups. AU tests, which have been recommended for general tree tests, were performed on alternative tree topologies (non-HGT hypotheses) and the tree generated from detailed phylogenetic analyses (HGT hypothesis). In this study, topologies with a p-value < 0.05 were rejected.

Prediction of protein localization: Targeting signal of identified protein sequences was predicted using ChloroP [86] and TargetP [87]. Additional information about protein localization in green plants was obtained from The *Arabidopsis* Information Resource (TAIR).

2. Protein sequence alignment used for phylogenetic analyses and resulting phylogenetic trees. Each sequence name includes a GI number from GenBank (or ID number from other databases) followed by species name. Numbers above the branches of the gene tree show bootstrap support values for maximum-likelihood analyses and distance analyses, and posterior probability from Bayesian analyses, respectively. Asterisks indicate support values below 50%. N denotes genes whose homologs are rarely found in cyanobacteria and that likely possessed novel functions; E denotes genes for which plastid-derived homologs already exist in plants; D denotes genes for which a possible replacement of an endogenous homolog cannot be excluded.

1. GCN5-related N-acetyltransferase (E)

CLUSTAL X (1.83.1) multiple sequence alignment

32444545_Rhodopirellula_baltic
21541979_Mycobacterium_leprae
48834901_Thermobifida_fusca
7106696_Streptomyces_coelic
30409279_Cyanidioschyzon_merol

KADTLIEAMGWIRRFRGKTTVIKLGSSLLEDREALQHLLLDVIFMETVGL
KAEVLAAEALPWLKQLHGKVVVVVKYSGNAMTDDMLRRRAFAADMFLRNCGI
KAKTLIEALPWLSALHGKTVVVVKYGGNAMTDPPDLRARFAENIVFLRYAGL
KAQILIEALPWLTTRHHGKTVVIKFGGNAMIDEDLKAFAAQDVFLRHAGL
RVQVLTEALPYIQKWRNEIMVIKYGGAVVKQD---ADIIKDILFLTCCGF

21673937_Chlorobium_tepidu
11498879_Archaeoglobus_fulgid
48841339_Methanosa_rina_barker
45655673_Leptospira_interr
33237476_Prochlorococcus_marin
6706427_Arabidopsis_thalia
50911447_Oryza_sativa
50927665_Oryza_sativa
35214504_Gloeobacter_viola
67920085_Crocospaera_watsonii
48764058_Rhodospirillum_rubrum
28867322_Pseudomonas_syring
23102640_Azotobacter_vinela
46311045_Burkholderia_cepaci
17427041_Ralstonia_solana
52007208_Thiobacillus_denitr
30249008_Nitrosomonas_europa
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RVDVLSEALPFIQRFKGKTVVVVKYGGAAMKSPLEQASVIRDVLVLLSCVGL
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FVRWFREAWPYLWAHRGCTFVVIISGEIIAGSSC-DAILKDIABLHHLG

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FVDWLRAVAPYIHAFRDKTFVIGFGGEVKGML-GALVNDIALLHAMGM
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HIVLVHGSRPQVEEQLALRHVQTQFVGDGIRVTDNAALESAKEA-SGE
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RLVLVHGARPOIERRRRAKLOTKVNGLRVTDEAMEAVKEA-NGAVRV
RLVLVHGVRPQVEARLTENGDIRYVSGLRVTDDAALACVKEA-AGTVRV

ELTEMMERAVNLSPRTTCVLKGKKDDLGFGVEVTEVDRDVIESLAYTDQV
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EIVGLINFAVGMSGEDAHLFTAERVDIGLVEVVDVQPGAVRSLLDDGRI
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QIVNGINSAVGLCGIDGKLIEARPGSYGLVGEVARVNADVIEPLIANGYI
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HLVSLINTAVGLCGKDARLLTARPAALGFVGEVSRVDAVLHPIIASGHI
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DIEATFSAGARISVVSNGNFVTPRVDFOHTGLVRKIDAESIQHSLSNRKI
EIEALLSANAAIRVASGNFVTARPVDLQYTGEVRRINITAILDQLEQGTV
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AVIPSCLCTDDKGQQLYNVNADTAAMAVAQSLGADKLVFLSDNVGVRDPEI
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PIISSIARIASAEggVYNVNADTAALAAVALGAAKLIVLTDVEGLYADYPL
PVVSSIARIQSQDDHVYNVNADTAALAAALGAETLMVLTDVEGLYEDWPV
PVIAVASVAASHDGTYYLNADVVAGELA1KLKAKKLIFLTDKGILADINV
PVIAPIGYDMEGNIYNINADAAAIAVALKAELIYVSDVEGVRVGNRI
PVVSPVATDLAGNTYLNADVVAGDIAAALAKKLIMLTDVPGILENPDL
PVISPIAVDAKGNALNINADIVAGDIAAALHAKKLILMTDVGLLRDMNH
PVISPVAENSEGESLNNADTFAGEIAAGALKAEKLILLTDQGILIDNQL
PVISSVASSVEGINYNINADTVAGEIAAAIGAEKLILLTDTSGILKNSL
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PVISSVAADETQAHNNADTVAGEIAAALGAEKLLILLTDAGILENYQL
PVIAPIGIGEAGETFNINADTAAGALAAQMKAARLLMLTDVGVLVDKDKL
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Note: All top hits of GenBank Searches (using *Cyanidioschyzon* sequence and *Arabidopsis* accession number NP_974701 as queries) are from gamma and beta-proteobacteria. Multiple copies of this gene exist in plants and in bacteria. One of these copies in red algae and green plants forms a group with homologs from gamma and beta-proteobacteria with strong support, whereas the other copy groups with cyanobacterial sequences with modest support (the *Cyanidioschyzon* sequence in that group is encoded in the plastidic genome). In *Arabidopsis*, the protein product of the gamma and beta-proteobacteria-related gene copy is annotated as located in cytoplasm (GenBank accession number NP_974701 and TAIR locus AT4G37670). The EST sequence of glaucophyte *Cyanophora* was obtained from tbESTdb; this sequences groups with homologs of red algae and green plants in preliminary analyses, but was removed from the detailed phylogenetic analyses because of its very short length (only 32 aa)

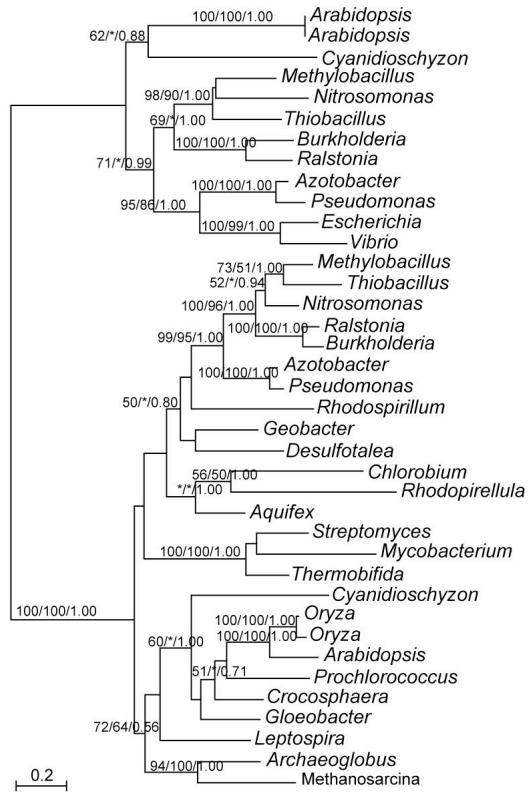


Figure 1. Molecular phylogeny of GCN5-related N-acetyltransferase. P-value = 0.235 from AU test on the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of all red algal and green plant sequences, and (B) monophyly of cyanobacterial and all red algal, green plant sequences. These alternative topologies investigate if both copies of this gene in red algae and green plants have the same plastidic origin. P-values < 0.001 from AU tests on both alternative topologies.

2. Glycyl-tRNA synthetase (D)

Note: GlyRS in living organisms exists in forms of homodimer (α_2) and heterotetramer ($\alpha_2\beta_2$); the former is distributed in eukaryotes, archaea, and many bacteria whereas the latter is only found in bacteria, red algae and green plants. Few bacteria contain both glyRS types. The α - and β -subunits of tetrameric glyRS are usually encoded in separate genes. In a few groups, the two subunits are encoded in a single fused gene; these include actinomycetes, chlamydiae, red algae, and green plants. Not only are sequences from actinomycetes, red algae and green plants similar in gene structure, they also have the highest percent identity and share several conserved amino acid residues. Phylogenetic analyses of each of the subunits strongly suggest a common origin of actinomycete, red algal and plant sequences (Figures 2A-2B). Based on

the gene structure and molecular phylogeny, it is likely that primary photosynthetic eukaryotes acquired this gene from either actinomycetes or chlamydiae (Chlamydiae are the only bacterial group aside from actinomycetes and plants that possess a fused gene in our database searches). The second scenario requires an independent HGT event from photosynthetic eukaryotes or chlamydiae to actinomycetes. The *Arabidopsis* sequence (GenBank accession number NP_190394, TAIR locus AT3G48110) is experimentally determined to be targeted to both chloroplasts and mitochondria.

2A. Alpha subunit

CLUSTAL X (1.83.1) multiple sequence alignment

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67931183_Solibacter_usitat
4980714_Thermotoga_mariti
34484120_Wolinella_succin
15611961_Helicobacter_pylori
50914779_Streptococcus_pyogen
23099404_Oceanobacillus_iheyen
46907687_Listeria_monocy
51891712_Symbiobacterium_therm
15606265_Aquifex_aeolic
34763703_Fusobacterium_nuclea
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68213209_Methylobacillus_flage
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26986806_Pseudomonas_putida
1573948_Haemophilus_influe
46580307_Desulfovibrio_vulgar
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39982448_Geobacter_sulfur
68207211_Desulfitobacterium_ha
72382617_Prochlorococcus_marin
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68230324_Frankia_alpha
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15605530_Chlamydia_tracho
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CM269_Cyanidioschyzon_merolae

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50914779_Streptococcus_pyogen
23099404_Oceanobacillus_iheyen
46907687_Listeria_monocy
51891712_Symbiobacterium_therm
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66855635_Anaeromyxobacter_deha
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26986806_Pseudomonas_putida
1573948_Haemophilus_influe
46580307_Desulfovibrio_vulgar
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39982448_Geobacter_sulfur
68207211_Desulfobacterium_ha
72382617_Prochlorococcus_marin
17131075_Nostoc_sp.
46135082_Anabaena_variab
68230324_Frankia_alpha
68172611_Frankia_sp.
28572421_Tropheryma_whippl
46399968_Parachlamydia_sp.
C_130039_Chlamydomydomonas_reinh
15836479_Chlamydophila_pneumo
15605530_Chlamydia_tracho
55296761_Oryza_sativa
30692978_Arabidopsis_thalia
CM269_Cyanidioschyzon_merolae

67931183_Solibacter_usitat
4980714_Thermotoga_mariti
34484120_Wolinella_succin
15611961_Helicobacter_pylori
50914779_Streptococcus_pyogen
23099404_Oceanobacillus_iheyen
46907687_Listeria_monocy
51891712_Symbiobacterium_therm
15606265_Aquifex_aeolic
34763703_Fusobacterium_nuclea
66855635_Anaeromyxobacter_deha
39934120_Rhodopseudomonas_palu
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23013834_Magnetospirillum_magn
48766000_Rhodospirillum_rubrum
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50914779_Streptococcus_pyogen
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34763703_Fusobacterium_nuclea

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13476181_Mesorhizobium_loti	RQSYILVRNLAKACGEAFLKTQ
23013834_Magnetospirillum_magn	RQAYIGRVRALAKACCEGWLASR
48766000_Rhodospirillum_rubrum	RAAYIGRVRALAKGCCEGWLRAR
68213209_Methylobacillus_flage	RATYIGRIRNLARAVAASYLDSR
56476158_Azoarcus_sp.	RAAVIGRIRNLSRAVAQAYSASR
26986806_Pseudomonas_putida	RQRYILRVRTLARDVAQSYLQAR
1573948_Haemophilus_influe	RQRYILRIRALTKGVAEAYYASR
46580307_Desulfovibrio_vulgar	RTGYIGRVRYLASGVARLYAAQR
68179079_Desulfuromonas_acetox	RAHYIGRVRNLSRLCAEGYVAQR
39982448_Geobacter_sulfur	RASYIGRVNRNVARLCAEGYLKLR
68207211_Desulfitobacterium_ha	RTGYIARVRNLRARLCQAYVEQR
72382617_Prochlorococcus_marin	RTKIIARISSLARKVAEAWLEER
17131075_Nostoc_sp.	RTRYIARIRHLARKVANLYVEQR
46135082_Anabaena_variab	RTRYIARIRHLARKVANLYVEQR
68230324_Frankia_alpha	RATSFAQMRGMSREVAQLWRDRR
68172611_Frankia_sp.	RATSFAQMRGLSREVATLWRDRR
28572421_Tropheryma_whippel	RAKAFSLMRTLTKEISATWVESR
46399968_Parachlamydia_sp.	RTGYIGRIRELAKQVAESYIKSR
C_130039_Chlamydimonas_reinh	RAECFAVLRGLAREITGLWVSRR
15836479_Chlamydophila_pneumo	RTRYIARIRQLTRLVADSYVEWR
15605530_Chlamydia_tracho	RTRYIAKIRQLARAADKYVAWR
55296761_Oryza_sativa	RARYFGRMRSLARQCAQLWKTR
30692978_Arabidopsis_thalia	RARYFGRMRSLARQCAQLWLATR
CM269_Cyanidioschyzon_merolae	RAAYFRRMRSLAREVAALWLERR
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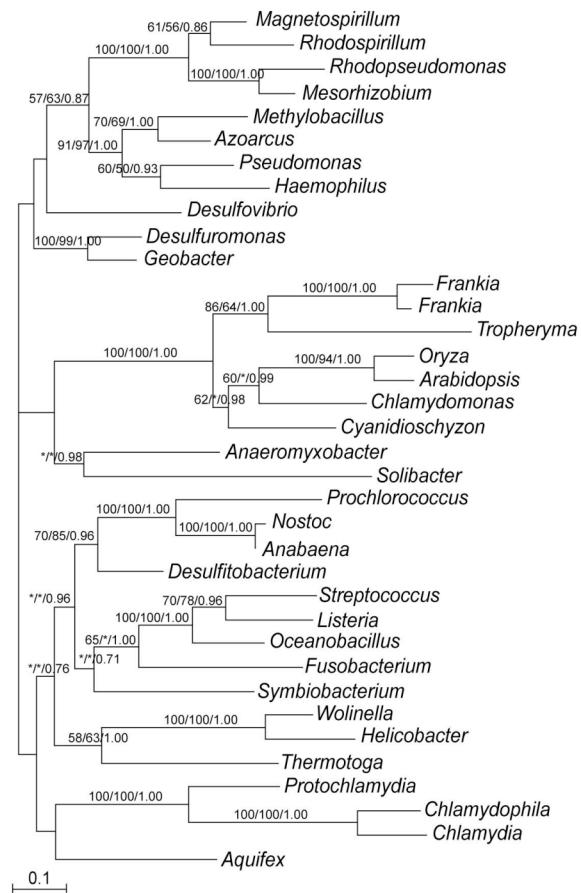


Figure 2A. Molecular phylogeny of alpha subunit of glycyl-tRNA synthetase. P-value = 0.838 from AU test on the presented tree. AU tests were also performed for alternative topologies, including (A) monophyly of cyanobacterial and photosynthetic eukaryotic sequences, (B) monophyly of cyanobacterial, *Frankia*, *Tropheryma*, and photosynthetic eukaryotic sequences, and (C) monophyly of chlamydial, *Frankia*, *Tropheryma*, and photosynthetic eukaryotic sequences. These tests investigate different evolutionary scenarios, including a) plastidic origin of photosynthetic eukaryotic sequences, b) photosynthetic eukaryotes acquired this gene from plastids and subsequently spread to *Frankia* and *Tropheryma* by a secondary HGT event, c) photosynthetic eukaryotes acquired this gene from chlamydiae and subsequently spread to *Frankia* and *Tropheryma*. P-value < 0.001 from AU test for topology A whereas P-values = 0.247 and 0.287 for topologies B and C, respectively. Therefore, the scenario of a plastidic or chlamydial origin of this gene in red algae and green plants, though less parsimonious, cannot be confidently rejected.

2B. Beta subunit

CLUSTAL X (1.83.1) multiple sequence alignment

<pre> 68172611_Frankia_sp. 68230323_Frankia 28572421_Tropheryma_whippl 46399968_Parachlamydia_sp. C_130039_Chlamydomydomonas_reinh 15836479_Chlamydophila_pneumo 15605530_Chlamydia_tracho 55296761_Oryza_sativa 30692978_Arabidopsis_thalia CM269_Cyanidioschyzon_merolae 34482366_Wolinella_succin 15611973_Helicobacter_pygori 15607085_Aquifex_aeolic 66855634_Anaeromyxobacter_deha 46580306_Desulfovibrio_vulgar 4980715_Thermotoga_mariti 68207212_Desulfitobacterium_ha 50914778_Streptococcus_pyogen 17133246_Nostoc_sp. 45508785_Anabaena_variab 72382003_Prochlorococcus_marin 46907686_Listeria_monocy 23099403_Oceanobacillus_iheyen 68179078_Desulfuromonas_acetox 39982449_Geobacter_sulfur 51891713_Symbiobacterium_therm 67931182_Solibacter_usitat 1573946_Haemophilus_influe 26986805_Pseudomonas_putida 13476182_Mesorhizobium_loti 39934122_Rhodopseudomonas_palu 23013833_Magnetospirillum_magn 68213210_Methyllobacillus_flag </pre>	<pre> LFEIGTEELPAAEVTRVTEAVRAGLVERLAATRLTLGTPRRIVAIVDEVA -----MTRQTAAVRAALVERLAATRLTQGTPRRIVAIVDDVA LFEIGTEEMPS--TQEIIITVENTLREKLGRSQLKDATPRRIVIRIYKMH VIEIGSQELPASFVSIGGQNLERAIRALLEKEGISYGTPRRIAVYIYQLS VLEVGGEELPPDDVVSAGHQLRERPVALLAKLRLSEGTPRRLLAVVVEALA LLEIGSEELPATFVPIGIOQQLESARQLVLTDHNVILGSPPRLALLVKNA LLEIGSEELPATFVPTGIQOQLESLAKKLLADHGIALGTPRRLALCIEGLS VLEIGTEELPPHDVIAEATQKLEKSLQILEKRRLSYGTPRRLAVVVENLN IIEIGTEEMPPQDVINASEQLRVLVLELENQRLRFGTTPRRLVLDAMS LIEIGTEELPPADLDAAVEQLREHLLQQLHHSSRLLRATPDRHAILVEGVA LIEIGIELPAIPFLRELPHLSSKWNTLLOQKYRMEMFYTPRRLVLLSQAPP LVEVLVDELPQALLNEYKEMPKKLHALFQKRALEFYTPRRLCLFKVDFP LIEIGTEELPAGVINPAGLQLEDDLAKALADARLAVGTPRRLAVWARDVA LFEIGAEIIPAGFVGALRQLEDDLAKALADARLAVGTPRRLAVWARDVA VLEIGTEELPARFLPALERELAERTRALADAGIEMSTPRRAVVRMDAVN LLEVGLLELPASEFHISLKQLEEKSAELLKAYRVSFVGSRFGVILKNLP LLEIGTEEIPAKFAPGVNLQLREQAQKYCQELRLDYTPRRLAVRVSGLA LIEGLGLEELPAYVVTPESEKQLGERLATFLTNRLSFSTPRRLAVRVSGLA LLEVGTPELPSFLGQIAQWRSRIPQSLAANSLTGTPRRLAVLITGLP LLEIGTEELPPASLGIAQWRSRIPQSLAANSLTGTPRRLAVLITGLP LLEIGTEELPADLAESVISOLELSVNNNDLNSAQIKTTTPRRIALTIEGIA LLEIGLEEMPAKYVTSSVLQLEKRVTDWLKDNDQIEYSTPRRLTVLVEEMA LIEIGLEELPARFIDDAELQLYTKQWLEENRISYSPTPRRLAVFVKNM FLEIGTEEIAPAGMLPVAMRDLERMRKELTNRVAYATPDRRLVLSVADVA FLEIGTEEIAPAGFLPKAMADMEAIVTKELNARLAFATPDRRLALVVKGLP ILEIGTEEIIPARFCAPARQLKENAAKALAEARLDFTGTPRRLVLYVRNLA LLEIGTEEIIPDWMITTALENLRMS----FEKLEITDATPDRRLVLRVEGP LVEIGTEELPPKALKTLATSFADNEAELNQAGLSFAAPRRLAVKVLNLA LVELGTEELPPKALASLGDAFLAGIEKGLQAAGLNYAAPRRLAVLIRQLD LLELRSEEIPARMQRKAAGDLKMMTDGLVEAGLTYWTPRRLTLDIRGLT LLELFSEEIPARMQGKAADDLKRVLTDKLVAEGLVFATPDRRLTLCVHGIP LLEIFSEEIPARMQARAADDLRGMVTDGLAKNGITYVTPRRLVLFIEGLP LVELFVEELPPKALQNIGISFADQIYHSLVTQDLIFASPRRIAVWIRNT : . ** . </pre>	<pre> PREPDVERVVRGPPTKAAGVGARGOGHVALVRTEAGRDAARVLAGVGEV PREPDVERVVRGPPTKAATGFVRGQGHVALVRDTGFRPATEVLAEVGAVL PRQSDSSVRIRGPPTDALTGFLKANGVFITK-NIPGQSAAKLLEQIAEIV KGKPSQTIEKKGPVKAGEGFFRSLGVLFGSVKQEGRATTSILAEPPLI AAQTSEESKVRGGPPTKALEGFCRKNGYVWAVVKDAGRPAVEVLTDEPALV PEVVQKAFEKKGPVSPGQOFFASQGYLFLHPEIRLRTADILMQEPPLI HVTIRPESEKKGPVSPQGEQFFSSHGLYFLVVIPEERKETAAILVNEPQLI </pre>
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55296761_Oryza_sativa
 30692978_Arabidopsis_thalia
 CM269_Cyanidioschyzon_merolae
 34482366_Wolinella_succin
 15611973_Helicobacter_pylori
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 66855634_Anaeromyxobacter_deha
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 1573946_Haemophilus_influe
 26986805_Pseudomonas_putida
 13476182_Mesorhizobium_loti
 39934122_Rhodopseudomonas_palu
 23013833_Magnetospirillum_magn
 68213210_Methylobacillus_flage

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 68230323_Frankia
 28572421_Tropheryma_whippel
 46399968_Parachlamydia_sp.
 C_130039_Chlamydomydomonas_reinh
 15836479_Chlamydophila_pneumo
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RAGPLDVPSSASGYPELLAARILLDPVVRRELVVVLTEVANLVEFPCPILG
 RAGPIEVGAVGYPPELLAENVLLDPAARRELIVLDEVITNLVEFPRPVLG
 NSKIVPVKAADNYEEFLTRNVILSRSKRREIIPFIEITDLVEAPSLVIC
 QLSAIHLHDHAKNYLKSQHQMADPNEREESILVIPQVLNLVEWPYLTIS
 NAATLPVPSAEAYLPAAAISLPAGRAEAIWLLLEEVTNLVEAPSVMG
 QLDKISISSPQDYVETLROQAVVVSQKERRMIIEELIEATFLSEHPFVSCG
 QLDQLTIPSSNMYVDTLRSAVIVSOKERRAIKLIKIDETVFLTEHPFVISA
 NSSNFKVEAAELYLHTLEKAILIDMQRKQRILLVQEVIINLVEAPMPIIG
 NTASLLVQNAESYEDTMRNSINIEIEERKKIILLNEVANLVEAPVPLIG
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34482366_Wolinella_succin
15611973_Helicobacter_pylori
15607085_Aquifex_aeolic
66855634_Anaeromyxobacter_deha
46580306_Desulfovibrio_vulgar
4980715_Thermotoga_mariti
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17133246_Nostoc_sp.
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IDSPQPYEDIASFALLAQVILDPKVRREKILLLEEVVAITEYPTALLG
QEGFIEVDSPKAYFEVLEKNVILDPKREAKILLDEVVAITEYPTALLG
FLSEVTINNPADYEKTKEHVIPDFNERKEIILLVEEVTNLVEYPFPVLG
FLAAITALKGTPDDYLAKLRRVLADPEERRAALLLVEQVLYLVVEEPSAVVG
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LYGKLEINDAKTYFDQLQDVVTDRDRRLSCINLLNELTDLIESPLLVG
FLGTATINQPSDYPNALLEQVVVNASERKQAIVLLEEVTLNVEYPTVLAG
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FHNVLITTPANYVEDLRL-KVLADEAERRELISLDEVFTALVEWPVPLVC
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FMAEFSVRFFDDYFDEAKLFAAVVLDSARRKIDILLLEDEVSGLVEWPVALMG
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KFDEKYLELPPVLIITVAAHHQRFCCFLNYFLGISNNKPIKEGYEVKLR
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 SMCGALLSIADKADTLAGCFGGLGMIPTGAADPYALRRCVGLGIARIIDDFAS
 TVIGSILGIADRIDTIVGNFAIGNVPTSSKDPYGLKSADTIFRIPEFQD

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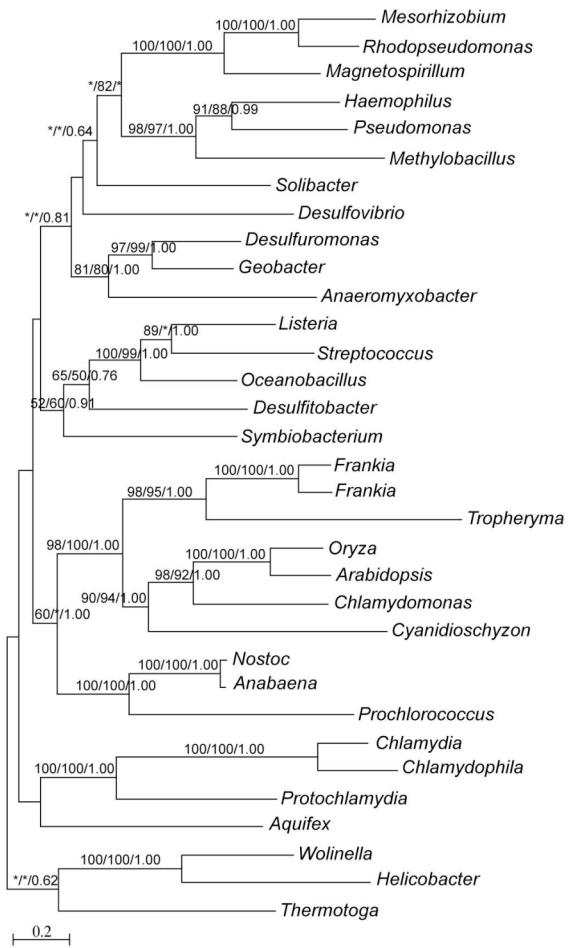


Figure 2B. Molecular phylogeny of beta subunit of glyycl-tRNA synthetase. P-value = 0.731 from AU test for the presented tree. AU tests were also performed on alternative topologies, including (A) monophyly of cyanobacterial and photosynthetic eukaryotic sequences, and (B) monophyly of chlamydial, *Frankia*, *Tropheryma*, and photosynthetic eukaryotic sequences. These tests investigate different scenarios, including a) plastidic origin of photosynthetic eukaryotic sequences, and b) photosynthetic eukaryotes acquired this gene from chlamydiae and subsequently spread to *Frankia* and *Tropheryma*. P-values = 0.085 and 0.446 from AU tests for these alternative topologies respectively. Therefore, the scenario of a plastidic or chlamydial origin of this gene in red algae and green plants cannot be confidently rejected based on these statistical tests. However, given that the two glyycl-tRNA subunits likely acquired from a single event, we have chosen to present this gene here as a likely case of HGT.

3. Dihydridipicolinate synthase (dapA) (D)

CLUSTAL X (1.83.1) multiple sequence alignment

15605085_Chlamydia_tracho
 88858626_Pseudoalteromonas_tun
 119468235_Alteromonadales_bact
 77359151_Pseudoalteromonas_hal
 83765535_Aspergillus_oryzae
 71281575_Colwellia_psychr
 31543060_Homo_sapien
 119720569_Thermofilum_penden
 83770010_Aspergillus_oryzae
 33594549_Bordetella_pertus
 84321353_Pseudomonas_aerugi
 126701253_Clostridium_diffic
 CDL00000863_Cyanophora_paradox
 71279937_Colwellia_psychr
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 30695296_Arabidopsis_thalia
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94969520_Acidobacteria_bacter
32472136_Rhodopirellula_baltic
23501533_Brucella_suis
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24216005_Leptospira_interr
126700845_Clostridium_diffic
GTL00002039_Guillardia_theta
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33592660_Bordetella_pertus
15606400_Aquifex_aeolic
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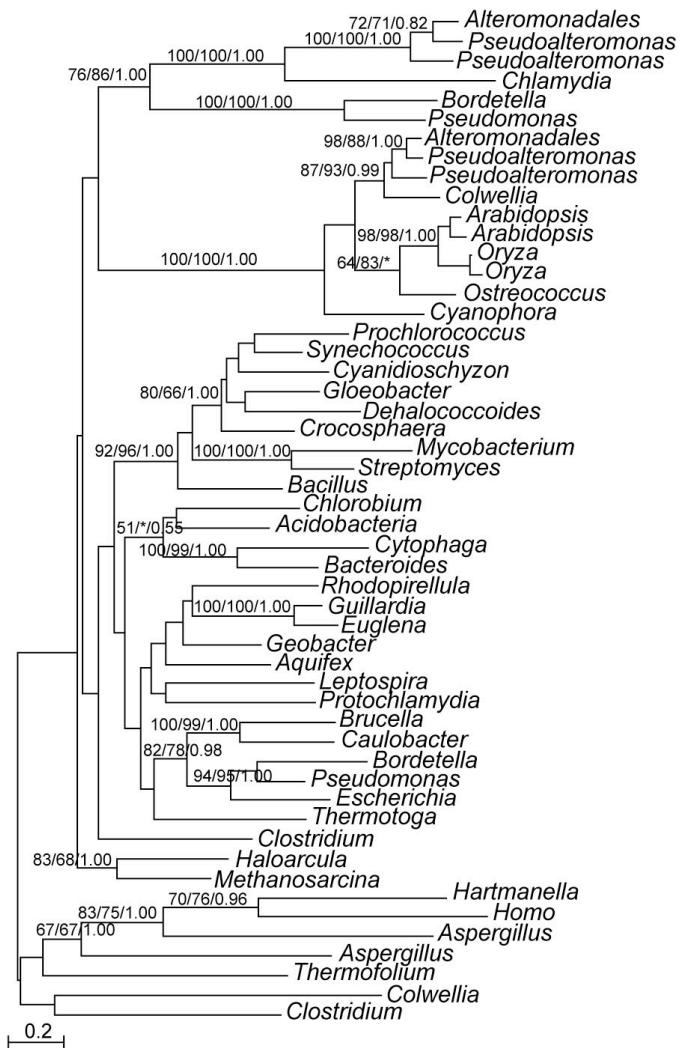


Figure 3. Molecular phylogeny of dihydrodipicolinate synthase (dapA). The red algal *Cyanidioschyzon* sequence forms a group with cyanobacterial and *Dehalococcoides* homologs with strong support whereas green algal and glaucophyte sequences form another strongly supported group with gamma-proteobacterial homologs. This could be explained by an HGT from gamma-proteobacteria and subsequent gene losses among primary photosynthetic eukaryotes. See text for explanations. Sequences of *Cyanophora*, *Guillardia*, *Hartmanella*, and *Euglena* were obtained from TBestDB. P-value = 0.199 from the AU test for the presented tree.

AU tests were also performed on alternative topologies, including (A) monophyly of all eukaryotic sequences except *Cyanidioschyzon* that appears to be plastid-derived, (B) monophyly of *Cyanidioschyzon* and cyanobacterial sequences, and (C) monophyly of all plastid-containing eukaryotic and cyanobacterial sequences. P-values < 0.001 from AU test for topologies A and C, suggesting that green plant and glaucophyte sequences are unlikely of the same origin (mitochondrial or plastidic origin) with other eukaryotic sequences. P-value = 0.801 from AU test for topology B, confirming the likely plastidic origin of the *Cyanidioschyzon* sequence.

4. Thic family protein (D)

CLUSTAL X (1.83.1) multiple sequence alignment

15922177_Sulfolobus_tokoda
18311872_Pyrobaculum_aeroph
15789892_Halobacterium_sp.
34541691_Porphyromonas_gingiv
48764428_Rhodospirillum_rubrum
71836834_Pelobacter_propio
67938921_Chlorobium_phaeob
54027290_Nocardia_farcin
45658515_Leptospira_interr
66797754_Deinococcus_geothe
73539911_Ralstonia_eutrop
149920854_Plesiocystis_pacific
21232748_Xanthomonas_campes
15141415_Sinorhizobium_melilo
17936444_Agrobacterium_tumefa
CM937_Cyanidioschyzon_merolae
26991600_Pseudomonas_putida
26250765_Escherichia_coli
48855688_Cytophaga_hutchi
16077944_Bacillus_subtil
61100363_Erythrobacter_litora
157028_Chlamydomonas
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33241220_Prochlorococcus_marin
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46198627_Thermus_thermo
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20090641_Methanoscincina_acetiv
57158692_Thermococcus_kodaka
18977903_Pyrococcus_furios
71838180_Pelobacter_propio
19712998_Fusobacterium_nuclea
67875148_Clostridium_thermo

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15922177_Sulfolobus_tokoda
18311872_Pyrobaculum_aeroph
15789892_Halobacterium_sp.
34541691_Porphyromonas_gingiv
48764428_Rhodospirillum_rubrum
71836834_Pelobacter_propio
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45658515_Leptospira_interr
 66797754_Deinococcus_geothe
 73539911_Ralstonia_eutrop
 149920854_Plesiocystis_pacific
 21232748_Xanthomonas_campes
 15141415_Sinorhizobium_melilo
 17936444_Agrobacterium_tumefa
 CM937_Cyanidioschyzon_merolae
 26991600_Pseudomonas_putida
 26250765_Escherichia_coli
 48855688_Cytophaga_hutchi
 16077944_Bacillus_subtil
 61100363_Erythrobacter_litora
 157028_Chlamydomonas
 22136156_Arabidopsis_thalia
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 32445237_Rhodopirellula_baltic
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67875148_Clostridium_thermo

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CPO00022869_Cyanophora_paradox
32445237_Rhodopirellula_baltic

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26991600_Pseudomonas_putida	ASMCGKFCSMKITQEV
26250765_Escherichia_coli	GSMCGKFCSMKISQEV
48855688_Cytophaga_hutchisonii	ASMCGHFCSMKITQEV
16077944_Bacillus_subtilis	ASMCGKFCSMRISQDI
61100363_Erythrobacter_litoralis	ASMCGKFCSMKITQEV
157028_Chlamydomonas	ASMCGKFCSMNITQEL
22136156_Arabidopsis_thaliana	ASMCGKFCSMKITEDI
CPO00022869_Cyanophora_paradoxa	-----
32445237_Rhodopirellula_baltica	FSMCGKYCSMKITEDI
67930575_Solibacter_usitatissimum	FSMCCKFCMSMNISAKV
15606443_Aquifex_aeolicus	YSMCGEFCSYKISQKV
33241220_Prochlorococcus_marinus	YSMCGKHCPMQTKITD
16331858_Synechocystis_sp.	YSMCGKFCPMQTKVDA
71675053_Trichodesmium_erythraeum	YSMCGKFCPMQTKVDA
45507375_Anabaena_variabilis	YSMCGKFCPMQTKVDA
4981317_Thermotoga_maritima	GSMCGPFCAIKIAEEF
46198627_Thermus_thermophilus	ASMCGPFCPMNLVEAV
71839731_Pelobacter_propionicus	NTMCDFCASRGAGRL
20093117_Methanosaeca_acetivorans	DTMCGNFCAKIVQNQN
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71838180_Pelobacter_propionicus	GTMCGEFCAVKVMDDA
19712998_Fusobacterium_nucleatum	DTMCGKMCMSRTMKKM
67875148_Clostridium_thermophilum	DTMCGRMACVKNTNKA

Note: All top hits in GenBank searches (using *Cyanidioschyzon* sequence and *Arabidopsis* GI 22136156 as queries) are from proteobacteria, firmicutes and spirochaetes. Primary photosynthetic eukaryotic sequences share many conserved residues with non-cyanobacterial sequences, and likely are not of cyanobacterial origin. The same is also supported by the phylogenetic analyses. The *Arabidopsis* sequence (GenBank accession number NP_180524 and TAIR locus AT2G29630) is annotated as a chloroplast precursor. The glaucophyte *Cyanophora* sequence was obtained from TBestDB.

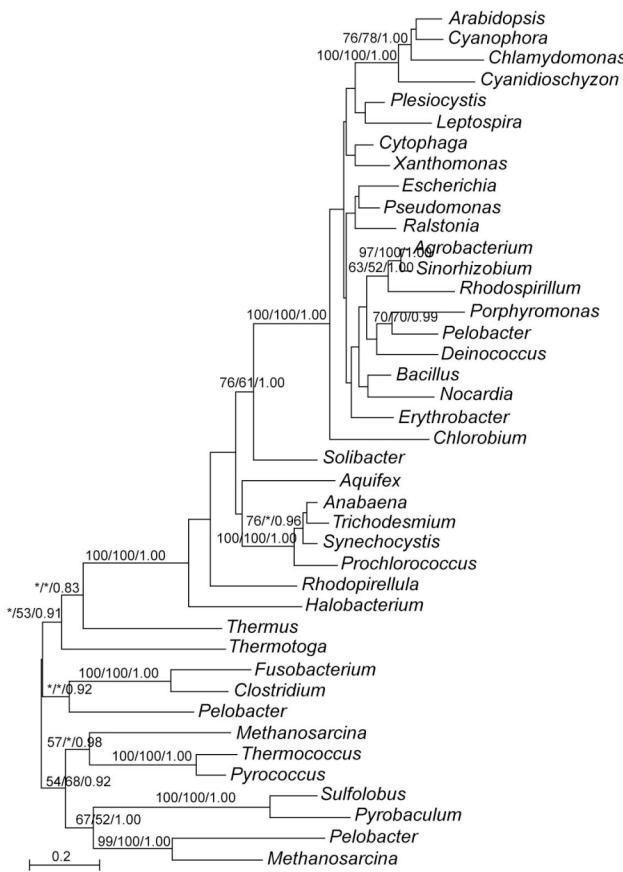


Figure 4. Molecular phylogeny of ThiC. P-value = 0.539 from AU test for the presented tree. AU tests were also performed on alternative topologies, including (A) monophyly of red algal, green plant and cyanobacterial sequences, and (B) monophyly of red alga, green plant and archaeal sequences. These tests investigate if red algal and green plant sequences has a plastidic or an archaeal (or eukaryotic) origin. P-values < 0.001 from AU tests for both alternative topologies.

5. Diaminopimelate decarboxylase (lysA) (D)

CLUSTAL X (1.83.1) multiple sequence alignment

66825991_Dictyostelium_discoides	TPFHIYNGDKIKENGELLMNYFAVKATPNPSILKLLKEIGMGVDCSSLAE
24213747_Leptospira_interr	TPVFVYSRERIEKSCEVALVRYAMKANPNRTVLEIMKRKGIQIDASSEYE
116056027_Ostreococcus_tauri]	TPTTYVYDAPTLEARAAVAKVRYAMKASPNAAILKIFKKAGLHVDAASSGYE
87306324_Blastopirellula_marin	TPTFVYDAKIVERIEDLRIRYAQKACSNLHILRLMREEGVHVDAVSEGE
21224744_Streptomyces_coelic	TPLWLYDAATIRAQIDRLRIRYAQKACSNLHILRLMREEGVKVDSVSLGE
75239202_Escherichia_coli	CPVWVYDAQIIRRQIAALKVRFAQKACSNIHIILRLMREQGVKVDVSLGE
118385090_Tetrahymena_thermo	TPLYVYNSQVIKERQCQLLALYACKANTNIAIIKLMKEQGIGIDAVSIQE
145525334_Paramecium_tetraureum	SPLYVYDEEIIKRCQCTITVLYACKANTNPNVLKIMKSNVGVIDAVSPNE
48477088_Picrophilus_torrid	TPLIVLSEKRINNNYLKIKVHYALKANSNPAVISILRRLGAGADAANPNE
126007985_Ferroplasma_acidar	TPVIVYNMARVNRENRIRRVHFAVKSNYNPVIQSIIKEGTGIDAANYNE
60682355_Bacteroides_fragilis	TPFYYYDTKVLRDTLACVNHYAVKANANPKVLTIIRESGLGADCVSGGE
123416580_Trichomonas_vagina	-----
45358763_Methanococcus_maripalae	TPLYVMSETQTVKNFTRYVISFAYKANTNLAVTRLLSKLGCGADIVSAGE

15644265_Thermotoga_mariti
CM1350_Cyanidioschyzon_merolae
15231844_Arabidopsis_thalia
88601236_Glaucocystis_nostoc
8134392_Archaeeoglobus_fulgi
67919461_Chlorobium_limico
91202264_Candidatus_Kuenen
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16079395_Bacillus_subtil

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21224744_Streptomyces_coelic
75239202_Escherichia_coli

TPTTYVYFEETLRKRSRLVKPTFAVKANNNPVLLKILREEGFGMDDVTKGESP
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66825991_Dictyostelium_discoi
 24213747_Leptospira_interr
 116056027_Ostreococcus_tauri]
 87306324_Blastopirellula_marin
 21224744_Streptomyces_coele
 75239202_Escherichia_coli
 118385090_Tetrahymena_thermo
 145525334_Paramecium_tetrau
 48477088_Picrophilus_torrid
 126007985_Ferroplasma_acidar
 60682355_Bacteroides_fragil
 123416580_Trichomonas_vagina
 45358763_Methanococcus_maripa
 15644265_Thermotoga_mariti
 CM1350_Cyanidioschyzon_merolae
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 8134392_Archaeoglobus_fulgi
 67919461_Chlorobium_limico
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 118443019_Clostridium_novyi
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 16079395_Bacillus_subtil

66825991_Dictyostelium_discoi
 24213747_Leptospira_interr
 116056027_Ostreococcus_tauri]
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 118062084_Roseiflexus_casten
 23128550_Nostoc_puncti
 33240555_Prochlorococcus_marin

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 PSIYNAYHHIPVADIVGPICETGDFLALDREIEVQRGEYLAVLSAGAYG
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 PALYHARYECAVAVTVVGRYCESGDFVLLTDAAVDVAPGDLAVFATGAYN
 PALYQAYEAAAASVIAGKCCESGDLIWIIDLEVKEGDLAVFCTGAYG

* : * *. * . . **:

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 ASMASNYNLLSRPAEVLLEGDREILTKGHGLESMLA
 EIMASGYNLCRELPKGYI-----SEELV-----
 ESMASSYLCRDIPLGYI-----SEEFQDN--
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 FVMSSNYNTRGRRAAEVLVDRGERAHEVRRRETVPELY
 FVMASNYNTRRNKPAAEVMVGDQSHLIRRRETISDQLM
 FTMSSNYNSRRRVPEIMVKGDQVMVIRKRETYDDLIK
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87303422_Synechococcus_sp.	ASMASNYNRIPRPAAVLVHGGQAELVQRRERPDELL
21223714_Streptomyces_coelic	RSMASNYNHALRPPVVAVRDGAARVVVRRETEEDLLR
116671170_Arthrobacter_sp.	WALSSNNYNLARPVGVAVRDGSARLIVRGETEQDLLN
118443019_Clostridium_novyi	YSMSSNNYKIPRPAVVFVNRGQSCLKRQSYEDLVS
51892948_Symbiobacterium_therm	YSMASRYNRFPTPAVVFVRDGRADLVRRETFAADMAA
16079395_Bacillus_subtil	YSMANNYNRIPRPAVVFVENGEAHLVVKRETYEDIVK
	*

Note: All top hits in GenBank searches (using *Cyanidioschyzon* sequence and *Arabidopsis* GI 15231844 as queries) are from various non-cyanobacterial groups. The donor of the acquired gene in primary photosynthetic eukaryotes (upper part of the tree) is difficult to pinpoint because of the lack of sufficient internal support on the gene tree, but it is unlikely from cyanobacteria based on AU tests. The *Arabidopsis* sequence (GenBank GI 15231844 and TAIR locus AT3G14390) is annotated as a chloroplast precursor.

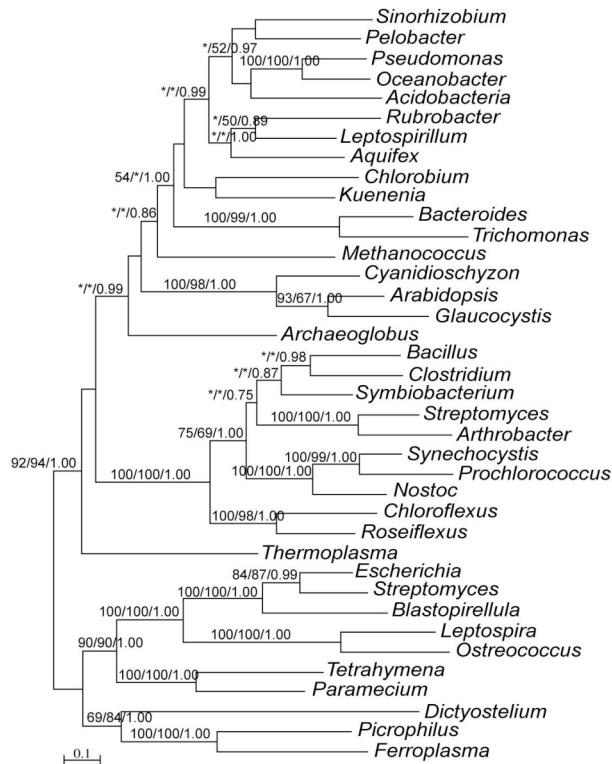


Figure 5. Molecular phylogeny of *lysA*. See text for detailed explanation. P-value = 0.235 from AU tests for the presented tree. AU tests were also performed on alternative topologies, including (A) monophyly of all eukaryotic sequences, (B) monophyly of cyanobacterial and primary photosynthetic eukaryotic sequences (except for the *Ostreococcus* sequence that appears to be distant). These tests investigate if red algal, green plant and glaucophyte sequences have the same origin (mitochondrial or eukaryotic) with other eukaryotic sequences and if red algae, green plants and glaucophytes acquired this gene from plastids. P-values < 0.001 from AU tests for both alternative topologies.

6. MGDG synthase (N)

CLUSTAL X (1.83.1) multiple sequence alignment

125975655_Clostridium_thermo
 89202509_Bacillus_cereus
 49483181_Staphylococcus_aureus
 56478620_Azoarcus_sp.
 116623425_Solibacter_usitat
 125974899_Clostridium_thermo
 76259735_Chloroflexus_aurant
 118063998_Roseiflexus_casten
 118062512_Roseiflexus_casten
 145343579_Ostreococcus_lucima
 18397057_Arabidopsis_thalia
 115475782_Oryza_sativa
 30687800_Arabidopsis_thalia
 115479239_Oryza_sativa
 30689152_Arabidopsis_thalia
 CM1391_Cyanidioschyzon_merolae
 GT000086114_Guillardia_theta
 94985286_Deinococcus_geothe
 51891890_Symbiobacterium_therm
 37522798_Gloeobacter_violac
 76259734_Chloroflexus_aurant
 78061400_Burkholderia_sp.
 118063689_Roseiflexus_casten

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 51891890_Symbiobacterium_therm
 37522798_Gloeobacter_violac
 76259734_Chloroflexus_aurant
 78061400_Burkholderia_sp.

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 51891890_Symbiobacterium_therm
 37522798_Gloeobacter_violac
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 78061400_Burkholderia_sp.
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 LEPDRFTVLLTAGGVGSGLGELVQTLLEQQFLVVTGKRNALYEEELRLPHT
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 FVEGFTKEVPRYMQLADYFIGKPGPGSIVSEALAMKLPVIVNAWTLPQERY
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 RIFGFVTNMEELMAASDIVISKAGPGLMEALVMRRPVIVTQAVGMQERG
 HIYGFVP-LADM MYAADI IATKAGGLSVSEALAVGRPLL IYGSAPGQEA
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 KVRFGETOMEKWMGACDCIITKAGPGTIAESLIRSLPII LN DYI PGQEK
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 118063689_Roseiflexus_casten
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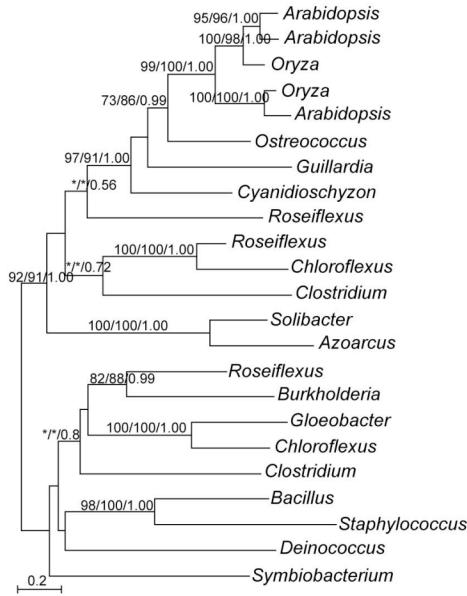


Figure 6. Molecular phylogeny of MGDG synthase. See text for detailed explanation. P-value = 0.235 from AU test on the presented tree. AU tests were also performed an alternative topology enforcing a monophyly of photosynthetic eukaryotic sequences with the rare cyanobacterial homolog (i.e., *Gloeobacter* in this case). P-value < 0.001 from AU test for the alternative topology.

7. Alpha amylase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

CM1405_Cyanidioschyzon_merolae	YGCHPTVNFAIYSTEQRHMSLI1YNRMGKVWHVAVQAGYRYAWRVRKGKG-
33635247_Prochlorococcus_marin	LGSTIGVNFSVAAPTAKRVELLLLFRSGDYWHVEVETGCCGYRIFGPHH
149177467_Planctomyces_maris	LGETWAYNFAIYSKHAERVSSLFFNKTAEIWHCRIPPATYYAYQIEGPSD
32398065_Rhodopirellula_baltic	LGASWSFNFSLYSRHATAVHLLLYNKSGPFWHCRVREAWYYAYRVVDGPAD
33236242_Chlamydophila_pneumo	LGASKRYRFALYASQATEVILALTHTRTGAIWHIEIEDQSSYAFRVHGP-S
42525620_Treponema_dentic	LGAKLGVNFVFSRRNAKEIVLHLFNKTGDFWHVFVSSWAFYLYTADGEFD
19714344_Fusobacterium_nuclea	LGANLGCASFIAYAKNVNSLSLNIFHKLGLDIWSIFLEEGTLYNWEING---
150017328_Clostridium_beijer	NGAVGVNFNTIHQSATSKCLLFRYIRGNVYSMIVLEEFYAYSVDPGYN
48894542_Trichodesmium_erythr	FGATGMVNFSIFSSYATCFLVLFRRIGNVYCMIVFENIEGYRMDGPND
16330244_Synechocystis_sp.	FGATIGVNFSIYSSHSTACTLVLFRIGNVYCMVVFNLEYGYRMEGPND
CM4050_Cyanidioschyzon_merolae	YGVSLGTNFAIFTDEEEHVALIDLYHRTGHVWHVQIAEHAYAWRIDRPTG
22328517_Arabidopsis_thalia	LGVSQGINFALFSQNATSVTLCLSNKTGDTWHICVENNVLYGYRVDGPGD
15225595_Arabidopsis_thalia	FGPTVGVNFSVYSTNSVSATICLINRTGHVWHVFLRKDMLYGYRFDGKFD
46446740_Parachlamydia_sp.	FGANIKVNFAIYAKNIEKISLCLFUNKTGNVWHIAIEPYTLYAFRV----
116626210_Solibacter_usitatus	LGATLGVNFAIYSKQAGVFLLLFARDKLVWHACVKAGQLYGYKVSGEYN
48765636_Rhodospirillum_rubrum	LGAMAGVNIAVFSEAAATQIDLCLFARTGDIWHGFLPPGLRYGLRAHGPYN
24373069_Shewanella_oneide	LGATGVNFALFSAHATGVELCLFEQTQOIWHLYVHAGQLYGYRVYGPYN
1707700_Sulfolobus_solfat	LGSNWGVNFSLFSENAEKVELLLYNKTGDIWHVFVPPGQLYAYRVYGPYN
48784580_Burkholderia_fungor	LGASWGVNFAVFSANAQKIELCLFECTDEVWHGYLPPGTAYGFRAGGPYN
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45916433_Mesorhizobium_sp.
6457937_Deinococcus_radiod
48894700_Trichodesmium_erythr
108803168_Rubrobacter_xyloph
25028566_Corynebacterium_effic
21224411_Streptomyces_coelic
21225613_Streptomyces_coelic
149179477_Planctomyces_maris
32446352_Rhodopirellula_baltic
150020446_Thermosiphon_melanesi
48837599_Methanosaarcina_barker
116622006_Solibacter_usitatus
48893487_Trichodesmium_erythr
16329290_Synechocystis_sp.

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48893487_Trichodesmium_erythr
16329290_Synechocystis_sp.

CM1405_Cyanidioschyzon_merolae
33635247_Prochlorococcus_marin
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46446740_Parachlamydia_sp.
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1707700_Sulfolobus_solfat
48784580_Burkholderia_fungor
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48785916_Burkholderia_fungor
21223814_Streptomyces_celic
45916433_Mesorhizobium_sp.
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16330244_Synechocystis_sp.
CM4050_Cyanidioschyzon_merolae
22328517_Arabidopsis_thalia

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15225595_Arabidopsis_thalia
 46446740_Parachlamydia_sp.
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 48837599_Methanosa**cina**_barker
 116622006_Solibacter_usitatus
 48893487_Trichodesmium_erythr
 16329290_Synechocystis_sp.

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 EDNRDGTENFSYNHVEGETDEPKIICKRKQQVKNPITILMISHGTPMI
 ENNRDGEENNLSWNCGVEGETEDPEVETLRLREROKNFAILLLSIGVPMI
 ESNRDGCNDNRSWNCGVEGSPNDPEIEALRNROIKNFLTITLLSLGVPMI
 EDNRDGANHNFWSNCIEGTTDQEEMRLRLRQIKNFFTILFFSQGTPMI
 EKNRDGTNDNFWSNCVGEGETDDPKINQLRLRQIKNFLTILFFSQGTPML

. ** : : * ** : * : . **.

CM1405_Cyanidioschyzon_merolae
 33635247_Prochlorococcus_marin
 149177467_Planctomyces_maris
 32398065_Rhodopirellula_baltic
 33236242_Chlamydophila_pneumo
 42525620_Treponema_dentic
 19714344_Fusobacterium_nuclea
 150017328_Clostridium_beijer
 48894542_Trichodesmium_erythr
 16330244_Synechocystis_sp.
 CM4050_Cyanidioschyzon_merolae
 22328517_Arabidopsis_thalia
 15225595_Arabidopsis_thalia
 46446740_Parachlamydia_sp.
 116626210_Solibacter_usitatus
 48765636_Rhodospirillum_rubrum
 24373069_Shewanella_oneide
 1707700_Sulfobolus_solfat
 48784580_Burkholderia_fungor
 17548459_Ralstonia_solana
 118048461_Chloroflexus_aggrega
 48763460_Rhodospirillum_rubrum
 58582972_Xanthomonas_oryzae
 58579740_Xanthomonas_oryzae
 26990756_Pseudomonas_putida
 48785916_Burkholderia_fungor
 21223814_Streptomyces_coelic
 45916433_Mesorhizobium_sp.
 6457937_Deinococcus_radiod
 48894700_Trichodesmium_erythr
 108803168_Rubrobacter_xyloph
 25028566_Corynebacterium_effic
 21224411_Streptomyces_coelic
 21225613_Streptomyces_coelic
 149179477_Planctomyces_maris
 32446352_Rhodopirellula_baltic
 150020446_Thermosiphon_melanesi
 48837599_Methanosa**cina**_barker
 116622006_Solibacter_usitatus
 48893487_Trichodesmium_erythr
 16329290_Synechocystis_sp.

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 RMGDEFLOTQRGNNNNPYNQDNETSWLDWIFNFVREVISFRKSHPSISRSR
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 LGGDELSRTQRGNNNNACQDNETIWTFDWLEFVKKMIQFYRAHPAFRRER
 LAGDESRTQHGNNNNAYCQDNELSWLDWMTAFVARVITALRKQHPLLRETR
 LGGDEFGRTOQHGNNNNAYCQDNELSWLDWLFAYTARLIALRKTSPALRWP
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 VMGDEVRTQHGNNNNAYCQDNETSWSWFLHFRVKLLIARRLLRDIDSDRT
 LMGDEVRTQHGNNNNAYCQDNELSWFDWETHFLRGIIALTQSLSLFLEES

** .. *..* : : . *

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48765636_Rhodospirillum_rubrum
24373069_Shewanella_oneide
1707700_Sulfolobus_solfat
48784580_Burkholderia_fungor
17548459_Ralstonia_solana
118048461_Chloroflexus_aggrega
48763460_Rhodospirillum_rubrum
58582972_Xanthomonas_oryzae
58579740_Xanthomonas_oryzae
26990756_Pseudomonas_putida
48785916_Burkholderia_fungor
21223814_Streptomyces_coelic
45916433_Mesorhizobium_sp.
6457937_Deinococcus_radiod
48894700_Trichodesmium_erythr
108803168_Rubrobacter_xylophan
25028566_Corynebacterium_effic
21224411_Streptomyces_coelic
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32446352_Rhodopirellula_baltic
150020446_Thermosiphon_melanesi
48837599_Methanosaarcina_barker
116622006_Solibacter_usitatus
48893487_Trichodesmium_erythr
16329290_Synechocystis_sp.

LLYIAFNASHEACDITAPWAFLNTAHMEPYSAVLMV
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FIYVMINAAANTCCFGIHWKCVVDTADVQARSVVLI
FIYVMINAGRDSVEFGIQWRALAITDSNVNARSICVLV
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FFYVMTNSYNNDITVRLPWHRLIDTSVVLSSRTTVVLI
PFYIAFNNSYTEQLCFELPWYILTDTCVLPKSSVILI
EVYLCINAHHWETQYVRLPWRIAVENTSEIEPRSVMLIV
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HIYVAMNMHYESLWFELPWVHFANTGLIGDRSVVILL
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FLYVAFNASHVVLTVTIPVWWLVNTHKIPSYTSIALK
FLFFILNSDFDSQWVKKLPWFRAIDS TIANARSTVVLL
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FLLVLVNASHTEVVFTLPWETVIDTMALPGRSLQVLR
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FLLLVLNNAHHDVVNFTLPWTCLLDTNQVTARSLLLLA
FFFLAVLHAGGEPAVGFLVLPYEVVVVDTGTVPARTVLLLR
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FFLLLLNAHHEDLSFVLPWEAVVDTAPLKARSMAVLA
FFFLMFNAHHAEIDFTLPWKLLVDTTTVPARSSMLLR
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FFFLLLNNSHWEPAFDRLPWTALVDTATVEQRSLVLLS
FLMILVNSSPDPQAFELPNLTIDTSVLP PRSLCYV
YVILMFNSTGDHREFHPWNLFVDTAEVGRHSMRIYV
FIFVILNOWIEPLQFTLPWYRVV DTSIAQPKSSVVLI
YIHVLNMNMYWEPLEFEIPWYRAVDTFIVQGRSVVVL
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LLHLIFNAYWEPLNFELPWVHRLVDTYWVQPRSSVVL
:

Note: Top hits in GenBank searches (using *Cyanidioschyzon* and *Arabidopsis* sequences) are from chlamydiae and cyanobacteria. The *Arabidopsis* sequences are annotated as chloroplast precursors.

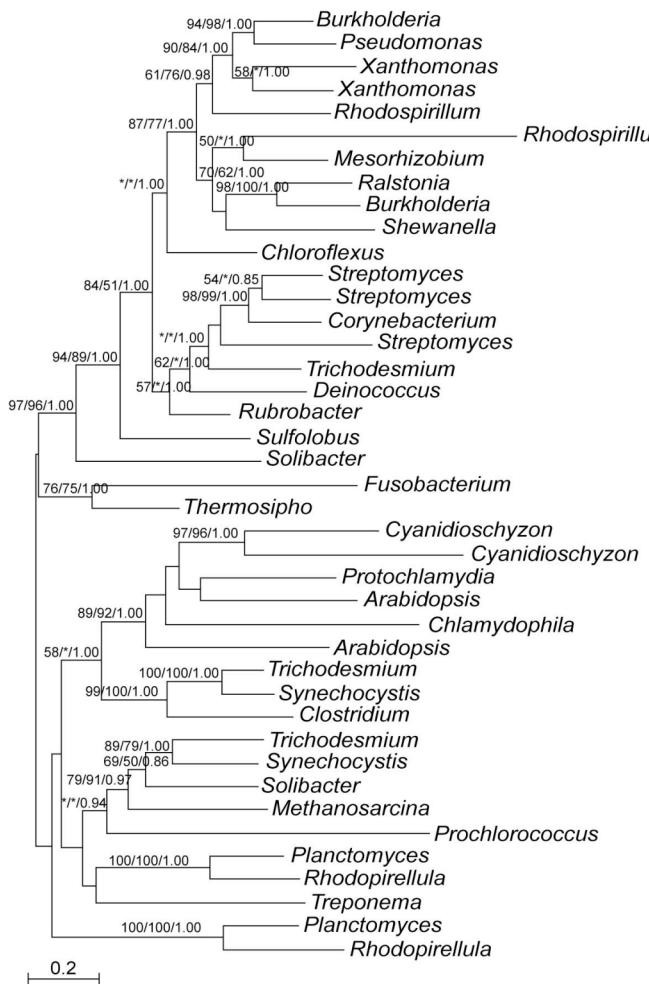


Figure 7. Molecular phylogeny of alpha amylase. P-value = 0.983 from AU test for the presented gene tree. AU tests were also performed on alternative topologies, including (A) monophyly of red algal and green plant sequences with different copies of cyanobacterial homologs, and (B) monophyly of red algal, green plants, cyanobacterial, and chlamydial sequences. These tests investigate if red algal and green plant sequences are plastid-derived and if red algae and green plants acquired the gene from plastids and subsequently spread to chlamydiae. P-values < 0.001 from AU tests for scenarios **A** whereas the P-value = 0.017 for scenario **B**.

8. 3-dehydroquinate synthase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

45508724_Anabaena_variab
KMO00293952_Karlodinium

IELRLFPITITEPDKTIQTFERVIDVFADFKLVRKEPVLVGGGLITDVV
IKFTKLVSGNEADKDIRDVERILVALKEIGLARNEPLLVVGGSVIADIA

34498288_Chromobacterium_viola
ELO000198527_Euglena
74023174_Rhodoferax_ferrir
39937112_Rhodopseudomonas_palu
15836567_Chlamydophila_pneumo
60491665_Bacteroides_fragil
48430319_Picrophilus_torrid
13542147_Thermoplasma_volcan
19714428_Fusobacterium_nuclea
57234788_Dehalococcoides_ethen
18312973_Pyrobaculum_aeroph
65318882_Bacillus_anthra
15922603_Sulfolobus_tokoda
18978063_Pyrococcus_furios
21674227_Chlorobium_tepidu
57234699_Dehalococcoides_ethen
45655651_Leptospira_interr
6458486_Deinococcus_radiod
46399348_Parachlamydia_sp.
67928196_Solibacter_usitat
54025607_Nocardia_farcin
15827180_Mycobacterium_leprae
67916807_Clostridium_thermo
39933580_Rhodopseudomonas_palu
48766569_Rhodospirillum_rubrum
CM1664_Cyanidioschyzon_merolae
74024228_Rhodoferax_ferrir
26249983_Escherichia_coli
22655384_Arabidopsis_thalia
34496282_Chromobacterium_viola
164848_Chlamydomonas_rein
67158019_Azotobacter_vinela
15600231_Pseudomonas_aerugi
68179822_Desulfuromonas_acetox
39984013_Geobacter_sulfur
57223777_Cryptococcus_neofor
68466574_Candida_albica
BN000088117_Bigelowiella
53796836_Chloroflexus_aurant
33240459_Prochlorococcus_marin
46135112_Anabaena_variab
16330660_Synechocystis_sp.

45508724_Anabaena_variab
KMO00293952_Karlodinium
34498288_Chromobacterium_viola
ELO000198527_Euglena
74023174_Rhodoferax_ferrir
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15836567_Chlamydophila_pneumo
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48430319_Picrophilus_torrid
13542147_Thermoplasma_volcan
19714428_Fusobacterium_nuclea
57234788_Dehalococcoides_ethen
18312973_Pyrobaculum_aeroph
65318882_Bacillus_anthra
15922603_Sulfolobus_tokoda
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48766569_Rhodospirillum_rubrum
CM1664_Cyanidioschyzon_merolae
74024228_Rhodoferax_ferrir

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PSVMKYLVPCGDACKSDVMSDLVQWL FASKATKRSV I VTWGGAVLNVG
NHPKVIVIEATEENKSIE RVI PVIARL VANKARRGDTLVAIGGGII QDIT
EAKRRI RIDALEANKSLEYAPHLISELRKAGADRSTH LVAIGGGII QDIT
YQVIVLTFFPGEPNKTWTETFISLQYQLVDQNISP KSSI I GIGGGT VLD
KDAVEITIGAEDVHKTLETLASVWQVLSEKGATRH SLLI NLGGGMV TDLG
FIKNRI I IDDGERAKSMEYLTLI INELLNKRVERGDS II YIGGGTTGDL
NSVKVKISLNDGESLKSLRN YQKIVK VLLERKVDRRSLLVYIGGGTVGDL
DKVFYFAIKDGEYEYK NIESILPVYDFMELNNFSRKSLI ISLGGGVICDMG
TETHLLA FPAGEAS KTLSTLEDIGSKL LDLRATKSSV I VCLGGGVGNL
PNAPALAKGGEGVK SLEALSKVYVFLQEAEADRGSTLV AVGGGALLDL
QKVFSVVP SGEKEKS FENFYAAHTSALENKLDRNSL I ALGGGMIGDL
KSFIEIPIIDGEKA DIQYALKLVKLLFENGFD RGDYVIA LGGGTVTD
FKPQTIVVPDGEKYK DLD TVRYIWEK LLEM GFTRRSLLI GLGGGVITD
FRTIELVVPARETSKSV STAWKLYGQMIA DADVDRS WNL CAGGGVVGDL
IGSLFLELP EGEI HSKL DASHI YPQ LINHFAERNTPI ALGGGVIGDL
LPIKTIYIKGGEKSKH INRTGEVYNQ LIEYGADRKSL LIA FGGGVGD
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IPFEI NL PPGEDQ KRLAP VEALAEEMVORGADRSSM VIAYGGIVTDM
IDAHRV EI PDAEAGKDLA VAGFCW VLGRIGL TRNDVV VSLGGGAATDL
VDAHRI EIPDAEAGKDL SVMDFI WEV LGRIGL TRNDVV VSLGGGAATDL
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IAHDHVVL PAGEATKFSO QLEELL DLLA ARFERSTTLL ALGGGVIGDL
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VTVES VIL LPDGE KYK DMDT LMKVFD KAES RLDRR CTF VALGGGVIGDM
VACSSV VLPDGEQ HKDW QTLN LIF DALLSGNAERKTT LIALGGGVIGDM
LQDV E VLPDGE EYKSMEV LGKVWD KALE SRMDRGVTFL ALGGGVGD
HELTPV VLPDGE AFK WTQ TLK QF DGLLEARH DRR RT LIALGGGVGD
HEVTPV VLPDGE AYK QWET LQ LIF DVL LKERH DRKT T LIALGGGVGD
YDVE QIV VEDGE SSKYNA ETLN T YTR LIE LGCDR HSG I I ALGGGVGD
FTVLRID IPDGE EHK TS VLT SYDEL I KGL TRD SLL V ALGGGVGD
ARFLV YEVAPGEGAKSRKVKG E IEDWMLDNK CTRD T VLA FGGGVIGDL
SRL LT CYSPG ENNKNRATKA AVE DFLL QQG CTRD T VLA VGGGVIGDM

YRVHTT T VPSGEQSKSF GELNR LYDWMIEHG IERR DVV LA LGGGVGD
YDAN L I LEAGEN KKNYES I ALI HNA YE HQLDRGS L I ALGGGVIGDM
FOV ASY CLPAG ERYK TLNS I QKLY DIALEN RLERS STM V ALGGGVIGDM
YE VFQH LIPAGE THK TLAS I NELY DVA FQAN LERN STLL SLGGGVIGDM

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GFATSMY SRNTPYV MLC T SIVAGI D AGPS PRV CNGFDYKNLYGSYHPPV
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13542147_Thermoplasma_volcan

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* : * : * : *

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* : * : *

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21674227_Chlorobium_tepidu
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: : * * * :
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KKLDFV
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Note: All top hits in GenBank searches (using *Cyanidioschyzon* and *Arabidopsis* sequences as queries) are from gamma-proteobacteria. Phylogenetic analyses show that red algal and green plant sequences group with beta and gamma-proteobacterial homologs with strong support. The *Arabidopsis* sequence (GenBank accession number AAM98284 and TAIR locus AT5G66120) is annotated as a chloroplast precursor. Sequences of *Euglena* and *Karlodinium* were obtained from TBESTDB.

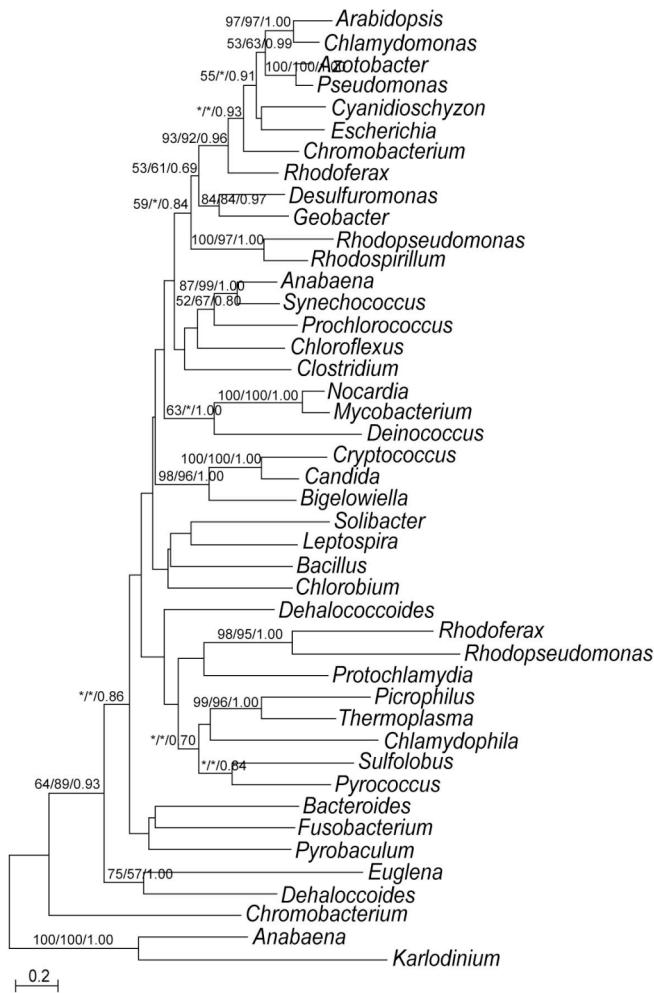


Figure 8. Molecular phylogeny of 3-dehydroquinate synthase. P-value = 0.997 from AU test on the presented tree. AU tests were also performed on alternative topologies, including (A) monophyly of all eukaryotic sequences (except for *Karlodinium*, which appears to be of cyanobacterial origin), (B) monophyly of cyanobacterial, green plant

and red algal sequences. These tests investigate if red algal and green plant sequences have the same origin (mitochondrial or eukaryotic) and if they acquired the gene from plastids. P-values < 0.001 from AU tests for both alternative topologies.

9-10. 2-methylthioadenine synthetase (E, D)

CLUSTAL X (1.83.1) multiple sequence alignment

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111225093_Frankia_alni
15835786_Chlamydophila_pneumo
76258351_Chloroflexus_aurant
116060729_Ostreococcus_tauri
CM3493_Cyanidioschyzon_merolae
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121522286_Methanococcus_maripa
18978284_Pyrococcus_furios
15920732_Sulfolobus_tokoda
119871859_Pyrobaculum_island

KASIITYGCQMNVNESAKIKKIFONLGYDNADAVFLNTCTVREGAAQTQIF
KFFIKTFCGQMNFNDSERIRGLLKTIGYEEADLILNNTCTIREKPQKVL
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RVHIETFGCQMNEHDSEIMYGILAQMGYDDADLLLNFNTCAVRESAVEHAF
KFYIPTYGCQMNEHDTEVMAGIFMALGYDDANVILLNTCAIRENAENKVF
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KIVFEAYGCTLNQGETALYVNKLLENEGKNEADLSIIGTCAVIKKTEDHMM
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RIYVETYGCWLAKADAIELRQRL---GYDEADVILVYTCAVREDGEVRQL

24213838_Leptospira_interr
111225093_Frankia_alni
15835786_Chlamydophila_pneumo
76258351_Chloroflexus_aurant
116060729_Ostreococcus_tauri
CM3493_Cyanidioschyzon_merolae
94417985_Pseudomonas_aerugi
94984483_Deinococcus_geothe
119886282_Thermotoga_petrop
116620691_Solibacter_usitat
118443956_Clostridium_novyi
32476670_Rhodopirellula_baltic
108760706_Myxococcus_xanthu
51892771_Symbiobacterium_therm
33862448_Prochlorococcus_marin
16331757_Synechocystis_sp.
15606200_Aquifex_aeolic
21674219_Chlorobium_tepidu
34540013_Porphyromonas_gingiv
126662084_Flavobacteria_bacter
110637073_Cytophaga_hutchi
119885033_Thermotoga_petrop
116622276_Solibacter_usitat
34580504_Rickettsia_sibiri
15836008_Chlamydophila_pneumo
24213088_Leptospira_interr
66576257_Chlorobium_tepidu
19704549_Fusobacterium_nuclea
34541783_Porphyromonas_gingiv
126663485_Flavobacteria_bacter
110638137_Cytophaga_hutchi
32477342_Rhodopirellula_baltic
16079597_Bacillus_subtil
118444753_Clostridium_novyi
15605955_Aquifex_aeolic
32475869_Rhodopirellula_baltic
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Jakaba_bahamensis
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30690642_Arabidopsis_thalia
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110638221_Cytophaga_hutchi
119885210_Thermotoga_petrop
108759982_Myxococcus_xanthu
34581161_Rickettsia_sibiri

QTILSAQQKLVVVGCFEAERYPVDFLFFGTGKYSQAGKILNYSKPYAYVKV
DALLAADRAVVAVGCLAERYGADAVLGFDAYPSIGEHLITAGPVAALKI
DYLDHLIDAKIIIVTGCMTSNHKIHLLGSGDVENILSAISTPKHYAYLK
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16329745_Synechocystis_sp.
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15605821_Aquifex_aeolic
118443818_Clostridium_novyi
51892897_Symbiobacterium_therm
16078764_Bacillus_subtil
71075619_Giardia_lamblia
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119871859_Pyrobaculum_island

24213838_Leptospira_interr
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 ETEEDFMETIDLVEKAQFERLNLAIIYSPRKGTVAWKVPEEKVRRMQFLM
 ETEEEFEMTMLQYTEQVRYDNQFSFVYSPRPGTGAALVPEHVKIAHLERLQ
 ETDDEFGLDLVRVVKYQCYCSFKYSPRPGTPGATIPEHIKSERLTLQ
 ETEKDFEQTMKLVEEVGFDFSFYIYSARPGTPAAIDLPEEVKKRRLQILQ
 ETEEFQRTLELMEALQLDNVNTAAYSPRPFTPAASVPEAKVMERLHRIN
 ETDAQFQNTLNLLIEEVGFQVNTAAYSPRPNTPAATLPEAVKVERLKQLN
 ETEAQFENTLNLLIEEVGFQVNTAAYSPRPQTPAAFLSEEVKGDRQLRN
 ETEADFAATLDVVRQARFAGAFTQYSPRPGTPAATIDRTVAERYARLV
 ETEEDFLDTIDVVQKVSFNDNSYMFMSIRKGTKAAATIESVKKERLQRLM
 ETEEDFETLVDLKKVRFEQVFSFKSPRPGTPAAEIIPDDVKTTERMRL
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 ETEEEHQETLSLVEEVQYDAAFMFMSYERAGTPAAQSLVPEKKERLQRLM
 ETDEQFETLSLYREVEFDSAYTFIYSPREGTPAAKVPMRVKKERLQRLN
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 ENDSFETKVLQKIEKIRPEIINVTKFSPRELT--ADKPTNILKEQDRMIA
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 ESEFAQRSLELIKRKIPDKVNVSRSYSPRPGTIAAKLPGWWVKERSRIMH
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 ETEEDFOETVKLVEELQFDKIHVAFSPRPTEAAVVPDAEKRRSKILS
 *

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 GVIGERIERRFAEAFGSTLKVLFEGYSEHYLRSRGQVREVIIDGFGEL
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 DLSDLRLAEIFYRSQEGTHRRVLWEDGFTENYIRVAVGKVEEIVLGKTD

126663485_Flavobacteria_bacter
 110638137_Cytophaga_hutchi
 32477342_Rhodopirellula_baltic
 16079597_Bacillus_subtil
 118444753_Clostridium_novyi
 15605955_Aquifex_aeolic
 32475869_Rhodopirellula_baltic
 76261232_Chloroflexus_aurant
 94985469_Deinococcus_geothe
 116625754_Solibacter_usitat
 66807387_Dictyostelium_discoi
 118402077_Tetrahymena_thermo
 116061814_Ostreococcus_tauri
 CM1685_Cyanidioschyzon_merolae
 24213805_Leptospira_interr
 Jakaba_bahamensis
 28872782_Homo_sapien
 21674804_Chlorobium_tepidu
 30690642_Arabidopsis_thalia
 34540755_Porphyromonas_gingiv
 126663275_Flavobacteria_bacter
 110638221_Cytophaga_hutchi
 119885210_Thermotoga_petrop
 108759982_Myxococcus_xanthu
 34581161_Rickettsia_sibiri
 94417083_Pseudomonas_aerugi
 CM4402_Cyanidioschyzon_merolae
 33862596_Prochlorococcus_marin
 16329745_Synechocystis_sp.
 111225071_Frankia_alni
 19703810_Fusobacterium_nuclea
 15605821_Aquifex_aeolic
 118443818_Clostridium_novyi
 51892897_Symbiobacterium_therm
 16078764_Bacillus_subtil
 71075619_Giardia_lamblia
 13542169_Thermoplasma_volcan
 71029160_Theileria_parva
 71661088_Trypanosoma_cruzi
 93277076_Homo_sapien
 118373032_Tetrahymena_thermo
 116056662_Ostreococcus_tauri
 18409989_Arabidopsis_thalia
 121522286_Methanococcus_maripa
 18978284_Pyrococcus_furios
 15920732_Sulfobolus_tokoda
 119871859_Pyrobaculum_island

GLSVKKRRAFYEQIGTKRTVLFENGFTENYVKVKVNTLHEINLTKIDE
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 KLNEINEKKFIDKFIGEDMEVLYEQGYTPNYIKVISGKIVNTKLIETKDE
 ELDQKKRQEYEKNGKELRALVIE-----TENYIDIKREGYKE-
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 QLOEQIATERMARFLGQTVEVLVEGGRTPGNRLVFTGQLVPVKITATSPW
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 DVFKHNQELVNKQEVGRHLVLEGGRTDTNKRVINGDFVLRVDSVSR
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 ILGTTQHVKV
 INTHKW
 AFFN--SYRTLDHMVGEVHSVALLEGHTKAYV
 QVLLGETV
 TVVIMSTTKY
 RVFH--SYS
 PYDHKIGERQQLV
 TEAHNQFYEQVLM
 GKMVEVDIYESGH
 ELFD--TFKRW
 DHLLGTTORI
 WINDGHTKO
 YAVK
 VMLGK
 SVI
 IKV
 TKV
 LWT
 VLFE--SYOPHQH
 LVGKTER
 WVSDA
 HTK
 NYT
 QI
 LMG
 RSAM
 VE
 IYESS
 SRW
 SVFE--AFAPY
 TGMECRE
 ERIW
 ITE
 GH
 TKG
 YV
 QV
 LLG
 TSAM
 RITS
 VGRW
 ELR
 RELS
 YEN
 NTR
 HI
 GET
 FE
 ILV
 TK
 GT
 TD
 NC
 KN
 VI
 IGE
 FRR
 KV
 GAK
 TF
 RVRLQ
 ISYE
 IN
 QRY
 VGR
 KVD
 ILV
 HG
 AV
 TM
 NY
 KH
 II
 KGE
 FAR
 AK
 VNG
 AT
 ST
 EVY
 EVA
 YSV
 HSE
 YLGS
 NAL
 VLT
 TEG
 RT
 IN
 YIP
 VVL
 GE
 WIN
 VK
 I
 TE
 ASFF
 EVAMRIA
 HLRNGA
 QVG
 KRD
 VVL
 IDE
 GRAS
 DYR
 QV
 VLG
 E
 F
 VNV
 RIA
 AASPV

24213838_Leptospira_interr
 111225093_Frankia_alni
 15835786_Chlamydophila_pneumo
 76258351_Chloroflexus_aurant
 116060729_Ostreococcus_tauri
 CM3493_Cyanidioschyzon_merolae
 94417985_Pseudomonas_aerugi
 94984483_Deinococcus_geothe
 119886282_Thermotoga_petrop
 116620691_Solibacter_usitat
 118443956_Clostridium_novyi
 32476670_Rhodopirellula_baltic
 108760706_Myxococcus_xanthu
 51892771_Symbiobacterium_therm
 33862448_Prochlorococcus_marin
 16331757_Synechocystis_sp.
 15606200_Aquifex_aeolic
 21674219_Chlorobium_tepidu
 34540013_Porphyromonas_gingiv
 126662084_Flavobacteria_bacter
 110637073_Cytophaga_hutchi
 119885033_Thermotoga_petrop
 116622276_Solibacter_usitat
 34580504_Rickettsia_sibiri

DMNGT
 DLVAE
 DLVGR
 DLWGE
 DLEAQ
 DLLCV
 DLWAE
 DLYGE
 DMWGS
 DVVGT
 DLIGV
 DLVAA
 DLVAR
 DLLGE
 DLTGQ
 DLYGM
 DLAGR
 ELFGR
 DLYAR
 DLYAE
 DLFGE
 GVISC
 GLREQ
 YMKCM

15836008	<i>Chlamydophila_pneumo</i>	GLIGE
24213088	<i>Leptospira_interr</i>	DKEGT
66576257	<i>Chlorobium_tepidu</i>	QLHGR
19704549	<i>Fusobacterium_nuclea</i>	ILIAE
34541783	<i>Porphyromonas_gingiv</i>	--SGE
126663485	<i>Flavobacteria_bacter</i>	SVRLQ
110638137	<i>Cytophaga_hutchi</i>	LVEIE
32477342	<i>Rhodopirellula_baltic</i>	RLELA
16079597	<i>Bacillus_subtil</i>	YNEGQ
118444753	<i>Clostridium_novyi</i>	YAVGK
15605955	<i>Aquifex_aeolic</i>	--VGK
32475869	<i>Rhodopirellula_baltic</i>	TLIGR
76261232	<i>Chloroflexus_aurant</i>	SLQGV
94985469	<i>Deinococcus_geothe</i>	MLYGR
116625754	<i>Solibacter_usitat</i>	SLVGE
66807387	<i>Dictyostelium_discoi</i>	TLKGK
118402077	<i>Tetrahymena_thermo</i>	SLFCT
116061814	<i>Ostreococcus_tauri</i>	TLVAE
CM1685	<i>Cyanidioschyzon_merolae</i>	RLIGE
24213805	<i>Leptospira_interr</i>	TLKGR
Jakaba_bahamensis		TLRAE
28872782	<i>Homo_sapien</i>	TLRGH
21674804	<i>Chlorobium_tepidu</i>	TLSGE
30690642	<i>Arabidopsis_thalia</i>	SLFGE
34540755	<i>Porphyromonas_gingiv</i>	TLFGE
126663275	<i>Flavobacteria_bacter</i>	TLIGE
110638221	<i>Cytophaga_hutchi</i>	TLFGE
119885210	<i>Thermotoga_petrop</i>	PLYGK
108759982	<i>Myxococcus_xanthu</i>	QLAGK
34581161	<i>Rickettsia_sibiri</i>	SLTGE
94417083	<i>Pseudomonas_aerugi</i>	SLRGD
CM4402	<i>Cyanidioschyzon_merolae</i>	SLTGE
33862596	<i>Prochlorococcus_marin</i>	SLSGT
16329745	<i>Synechocystis_sp.</i>	SLTGE
111225071	<i>Frankia_alni</i>	HLTAD
19703810	<i>Fusobacterium_nuclea</i>	TLYGE
15605821	<i>Aquifex_aeolic</i>	SLEGD
118443818	<i>Clostridium_novyi</i>	SLTGE
51892897	<i>Symbiobacterium_therm</i>	TLEGE
16078764	<i>Bacillus_subtil</i>	SLDGE
71075619	<i>Giardia_lamblia</i>	ALIGS
13542169	<i>Thermoplasma_volcan</i>	SLIGR
71029160	<i>Theileria_parva</i>	HLECE
71661088	<i>Trypanosoma_cruzi</i>	SVVGR
93277076	<i>Homo_sapien</i>	FMKGQ
118373032	<i>Tetrahymena_thermo</i>	HIEGE
116056662	<i>Ostreococcus_tauri</i>	SCKAR
18409989	<i>Arabidopsis_thalia</i>	SVFGE
121522286	<i>Methanococcus_maripa</i>	GLSGK
18978284	<i>Pyrococcus_furios</i>	YLLGE
15920732	<i>Sulfolobus_tokoda</i>	DLRGK
119871859	<i>Pyrobaculum_island</i>	YLYGE

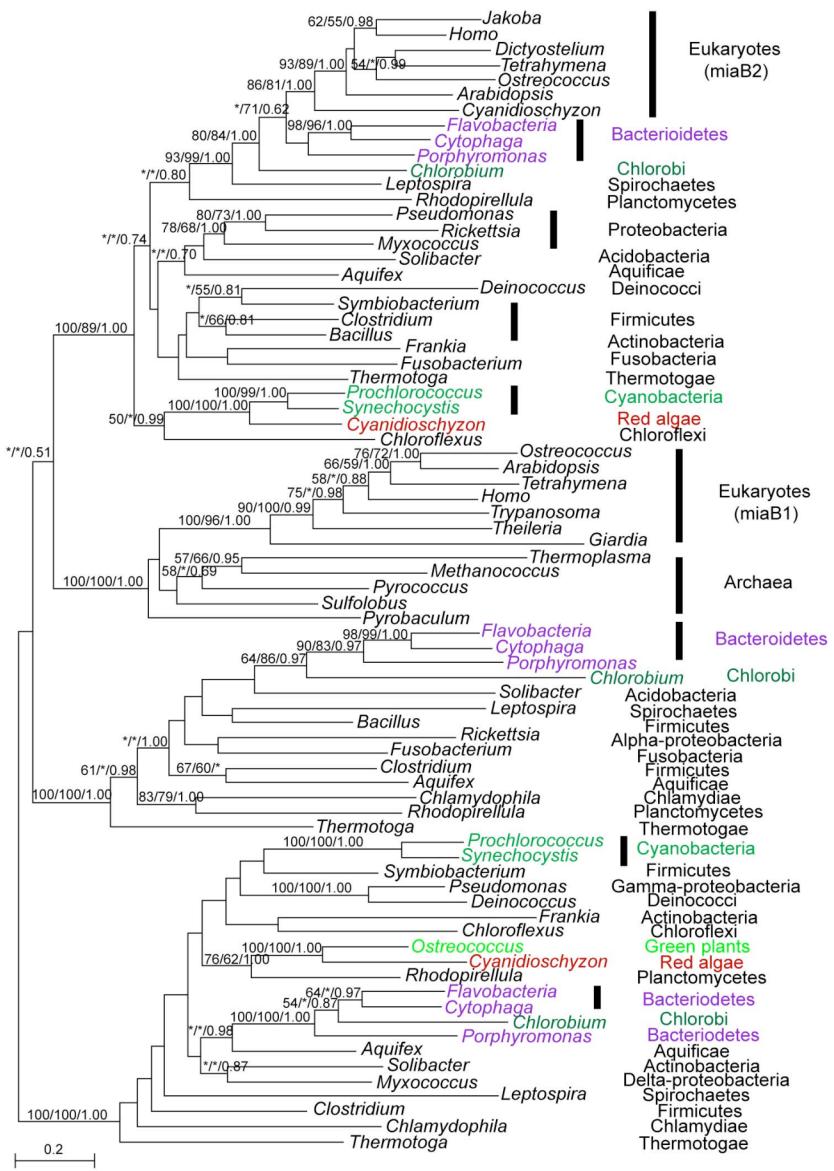


Figure 9. Molecular phylogeny of 2-methylthioadenine synthetase. P-value = 0.983 from AU test for the presented tree. See text for a more detailed discussion. AU tests were also performed on alternative topologies, including (A) *miaB1* and *miaB2* forming a monophly, (B) *miaB1* and *miaB2* forming a monophly that in turn groups with archaeal sequences, and (C) *miaB2* forming a monophly with proteobacterial sequences from the top part of the tree. These tests investigate if (a) *miaB1* and *miaB2* have the same origin, (b) *miaB1* and *miaB2* have a eukaryotic origin and are related to archaeal homologs, and (c) *miaB2* has a mitochondrial origin. P-values < 0.001 from AU tests for these alternative topologies.

11. Uroporphyrinogen III synthase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

15606833_Aquifex_aeolic
 10175667_Bacillus_halodu
 CM1920_Cyanidioschyzon_merolae
 145352552_Ostreococcus_lucima
 20196944_Arabidopsis_thalia
 15807683_Deinococcus_radiod
 6458497_Deinococcus_radiod
 48895608_Trichodesmium_erythr
 45510631_Anabaena_variab
 48845542_Geobacter_metall
 46579147_Desulfovibrio_vulgar
 12655814_Selenomonas_rumina
 108804799_Rubrobacter_xyloph
 153813575_Ruminococcus_obeum
 2127342_Clostridium_josui

15606833_Aquifex_aeolic
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 CM1920_Cyanidioschyzon_merolae
 145352552_Ostreococcus_lucima
 20196944_Arabidopsis_thalia
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 12655814_Selenomonas_rumina
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 20196944_Arabidopsis_thalia
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 45510631_Anabaena_variab
 48845542_Geobacter_metall
 46579147_Desulfovibrio_vulgar
 12655814_Selenomonas_rumina
 108804799_Rubrobacter_xyloph
 153813575_Ruminococcus_obeum
 2127342_Clostridium_josui

15606833_Aquifex_aeolic

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 VVCTRERGKNGALMNLLAARGARCVELPLVEAATFPDVLESWDWVCVTSP
 VVVTRERGKNNQIIKALEKGNGISSLEPLIQFDRLASVLKSFDWIIIITS
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 VAVTRPDGGGRGLELLRAQGAEVLSVPLIRPRALWSGLRGVDWLVTSP
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 ILVTRSSGQSSQFSDRLLTGATVIEMPALEWEELDQAISQFEWLILST
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 VVVTRARAQAGELSRLLEELGAAVVEFPTIEFGPLDEAIDSFDWLVTSP
 VLVTRPKGRSSRTAELRRGAEVLELPSIROSTLVHAFSSYQWIVFTSP
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 EAAGVFLEAYEVRAVAVVAGTGKVLAKAEMDKQFTPSKATAATMAAEEL
 PC EAGSVFLEAWKVGQVQVVGAGTARVFEAMLVAFTPSKATGKVLASELP
 E QAVRALARTLLARIAAVGSGTAWTLREYGLAPDFVPTRSGRHLGAELPA
 QGGRLLGEALATLRAVEGATEARELARGLPVDLPTTATALSLGRKLPA
 NGVDYFFERLAVKIAVVGKTAESLKTGCLVPDFIPPNFVADSLVANFPE
 NGVDYFFERLNVKIAVVGKTAESLKTGCLVPDFIPPNFVADSLVHFPE
 NAVRFFFERLACRVCAVGPKTAALAPFGIRPDILPADYKGEGVVEVFRS
 NGVKHFWNQLARKVAIAGPATADALRDKGIAPDFIPEKYVAEGVVEGMLA
 NGVGRFFARLFAKIAAIGLATAEKLKQYGLVADVIPQEYRAEGVLEALKG
 NGVEAFLERLAARIAAAPIGATAGRVRVREAGLVRDVVPREYRAEALLEEISG
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 NGIQIFFDYLNTKIGTVGSQATAKALKEVGLISDFTPEIFDGRHLALGIAE
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 GSLILY P ASKKAATTLDQGLEA R GATVVR LNTYSTERVERLSPEDVAAAD
 RSSVLYPASLKAGNDIVEGLSKRGFEVVR LNTYTTV PVQSVDT VLLQQAP
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 GQAALHLSQSLSENTLRLGELAARGV TYRRL ELYHTA PAVL NAA E R V A L V S
 GKKILFPRVETGREV L V K E L N S Q G A E V I E V A A Y E S S C P E N I L P E A L L A I D
 NKKILFPRV E S G R E V I L V K E L S A K G A E V I E V A A Y Q S C C P S S I P P A A E L A I D
 GKRIIFPKGDRARDVIPQGLAELGEV TAPVAYRN V P D S L P A G V I A E I T
 GKRVLLPRALEAREVLP EELRKAGATDVLPVYVTVPSAARRDV L V A R H
 HAKLILPRAEEAREVLPDTLREMGAEV E V A P A Y R T I C Q V D G E A L A A E I D
 GRRVLIPRAAREVLP ERLREAGAEVSVPPAYETVPSAEGREELARGVD
 GEKILLPRARRGNQKLVKILKQGQVSDIPTYD TVYEKSSLIHIDREID
 NEKVLICDAAIASDDIVNILRSNNIKFDRVPLYNTNYINENS N KVKKSLK
 : . : : * . * . * . :

FIIFYSPSAVKAFFANLQSLKFVAIGKTTKEELQKHVKNVITSERPSTE
 IIIFTSPSAVDHFVTLHDLLYAYIGPIAA RAKQRGLPVHIVAKTYTGE
 LVTLASP SAVK VWAQ RVG KQPAVCIGKTSADA AKEVGFSEVYAPS DPGLE
 VVTFGSPSAVRAWE LCGOPAYV CIGKTS A E C A N C E L P D V F Y P E L P G I E
 VLSVASPAVRAWLHLIQSNYVACIGETTASAAR R LGLKNVYYPEKPGLE
 VVTLASAVGARALA QVAGSFTA AVIGPQTEL A AREAGFTR L I L A E Q P T L A
 AVT L A S G S A A Q G L A I A G R L P V A A I G E Q T A A A A W T L G F V S V T A P Q P S L E
 VITFAS SKTVKNFCY LIGNICIASIGPQTSQSCHSLLGRVDVEA Q E Y T L D
 IITFAS SKTVQFFHQ L V D G I C I A S I G P Q T S K T C H T L L G R V D V E A E E Y T L D
 CVTF TSS STVENLA I L G G V T I A A I G P I T A R T C R E L G L E V H V E P S K Y T L A
 CVTF GSS STVENFFS L V P E V K L A C I G P V T K K T L E G F G F T C H I Q P D D Y T I P
 LVTFTSS STVENFVAA FG G T R V A C I G P I T A A A R E R G L R V D V E A G E Y T I E
 CVVFTSASTVKG F V E S T G K V T A A C I G K Q T K A A A D D Y G M Q - T Y M S E K A T I D
 YITFTSASTVEGFIASMKSLTAVC I G N T A E A A K K Y N L R - Y V V A E K S T I D
 : . : . * * : .

DIVKKLKE

10175667_Bacillus_halodur	GLVNAICD
CM1920_Cyanidioschyzon_merolae	AWVDTLVR
145352552_Ostreococcus_lucima	GWAETVFL
20196944_Arabidopsis_thalia	GWVESIME
15807683_Deinococcus_radiod	SLSDAVQR
6458497_Deinococcus_radiod	GLVAAAIK
48895608_Trichodesmium_erythr	GLTKAIIIE
45510631_Anabaena_variab	GLTQSIIN
48845542_Geobacter_mettall	AMTDALVD
46579147_Desulfovibrio_vulgar	ALVDELTR
12655814_Selenomonas_rumina	GLVEAICK
108804799_Rubrobacter_xyloph	GLVRVAG
153813575_Ruminococcus_obeum	SLVELVVK
2127342_Clostridium_josui	SMIDKLLE

Note: This sequence has identifiable homologs only in green plants and bacteria (using *Cyanidioschyzon* and *Arabidopsis* GI 20196944 as queries). The top hits in GenBank searches are from firmicute and *Deinococcus* homologs. The *Arabidopsis* sequence (GenBank accession number NP_565625 and TAIR locus AT2G26540) is annotated as a chloroplast precursor, but appears not likely to be plastid-derived. Cyanobacterial sequences have a 1-aa insertion not shared by any other sequences.

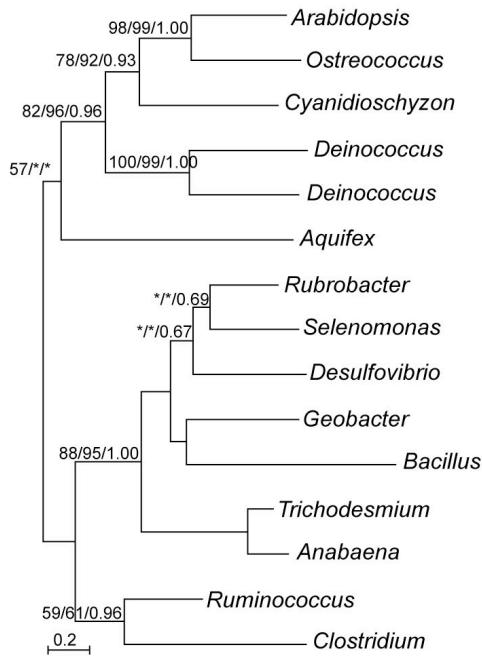


Figure 11. Molecular phylogeny of Uroporphyrinogen-III synthase. P-value = 0.959 from the AU test on the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of red algal, green plant and cyanobacterial sequences, and (B) red algal, green plant and *Deinococcus* sequences forming a monophly that in turn groups with cyanobacterial homologs. These tests investigate if a) red algae and green plants acquired this genes from plastids, and b) red algae and green plants acquired the genes from plastids and subsequently spread to *Deinococcus* by secondary HGT. P-value < 0.001 from AU test for topology A and p-value = 0.04 for topology B.

12. ACT-domain containing protein (N)

CLUSTAL X (1.83.1) multiple sequence alignment

113648684_Oryza_sativa
 9758449_Arabidopsis_thalia
 CM2077_Cyanidioschyzon_merolae
 88857979_Pseudoalteromonas_tun
 46133622_Haemophilus_influe
 46143705_Actinobacillus_pleuro
 71546701_Syntrophobacter_fumar
 46449059_Desulfovibrio_vulgar
 67985038_Kineococcus_radiot
 58002716_Protein-PII_uridyl
 103488264_Sphingopyxis_alaske
 23012593_Magnetospirillum_magn
 78697304_Bradyrhizobium_sp.
 86747159_Rhodopseudomonas_palu

113648684_Oryza_sativa
 9758449_Arabidopsis_thalia
 CM2077_Cyanidioschyzon_merolae
 88857979_Pseudoalteromonas_tun
 46133622_Haemophilus_influe
 46143705_Actinobacillus_pleuro
 71546701_Syntrophobacter_fumar
 46449059_Desulfovibrio_vulgar
 67985038_Kineococcus_radiot
 58002716_Protein-PII_uridyl
 103488264_Sphingopyxis_alaske
 23012593_Magnetospirillum_magn
 78697304_Bradyrhizobium_sp.
 86747159_Rhodopseudomonas_palu

113648684_Oryza_sativa
 9758449_Arabidopsis_thalia
 CM2077_Cyanidioschyzon_merolae
 88857979_Pseudoalteromonas_tun
 46133622_Haemophilus_influe
 46143705_Actinobacillus_pleuro
 71546701_Syntrophobacter_fumar
 46449059_Desulfovibrio_vulgar
 67985038_Kineococcus_radiot
 58002716_Protein-PII_uridyl
 103488264_Sphingopyxis_alaske
 23012593_Magnetospirillum_magn
 78697304_Bradyrhizobium_sp.
 86747159_Rhodopseudomonas_palu

VLIDQDSRDRATIVQLSGFDRLGALLDTMKALKDLGLDVTKGSV-STESA
 VMIDQDADPEATIVQLSGGNRLGALIDTMRALKDGLDVKGTV-STEGS
 -----ATRLVVTCRDRKGLLSLTDALKSIGLQIRRAVA-RTKDG
 IMVSEEPMHGGTQVFVYSQDEINLFARLVNALGSKKAHIHYAQVMSTKD
 VKISNRFLGGTEVFYICODQPHLFNKVVSTIGTKKFSIHDAQIITTQDG
 VLVSNEYARGATEIFIVYCEDQAQLFIRIAQILSQQKKVSIHDAQIITSQNG
 WEVESSGDTA-EITIVSWEKPGLLSRCAGLLTLHSMNILGAQVFTMHNG
 VVMERGRPVHGLWEVTIARDQQGLFATLAGVVALHGLNVYAADAFVWRDG
 -----VVTVVTPDRTGVFADLAGLLAGHRF-LVRSALVRTLDG
 VEAYPIPVERGVTELTVLCADHPGLFSQIAGALAVSGASIVDARIHTLSDG
 IAAVPPDDRGATLVMVLAADHPGLFYRIAGGIHLAGGNIIDARIHTTRDG
 TTYETDPVRGVTELTVYSPDHPRLLAIITGACATTGGNIVDAQIFTTTDG
 INVGFDPARGVTELTFAMDPWLLSIIAGACASAGANIVDAQIYTTTDG
 VNNGFDEARGVTELTLAVDHPWLLSIIAGACASAGANIVDAQIYTTTDG

: : : : : : : : : ..

VTOTKFHIMLEKIRLTVINNLLQYHVVTHVIVEDDGPKRSMLYIETADRP
 IKQTKFSTITLEQIRLTIINNLLKYHIATHIHVKEDGPKRSSLVIETADRP
 IASDEFVTLDLAVEQALQPVMGTSGRGVHVYVDNHASQHTTITVNAPDRP
 YAIDNFVVLINSIRRSIEOAIKEGSIKPKQVIVRPHGRKDTLIEIQAVDIP
 YVFDSFIITRRELEQALTVALQSEKVTQDVRFLHENKKETEMELVALDKP
 LVLDLSFIVTCEQIKQSLEKVLNTSERQTKVRLADSQONTAFELFTLDRE
 LILLIFQCRWNNAVRLDLVERLLLGKMAFSQVLPVDNOSSAMTILEVYTVDRV
 TALDFVFTWGKVRSVQYAMTGKLRPAEVRVNDGLSDFTV1DFFAPDRP
 VAVDSSWWVEAVLLRQGLERIVGGDVAEPRIVILPGASERTVLEVRAADRP
 MALDTFWVQLGRNLNHVEQALSGRLVPVRVIDNTASRTVIEVNGRDRP
 LALDNFLVQIARLTRAIEDALANRQVAPNVFDNKASNRTVIEVNAQDRP
 FALDSIFISAGRIATAIERALKGEIVPPDVSIDNALSSRTVVEITGLDRP
 RALDTIAISATRIGETIEQVLEGKLVEPEVSINNOWSELTVIEVSGLDRP
 RALDTISISATRIGEMIEEVLEGKLVEPEVSINNNWSRTVIEVSGLDRP

: : : : : : : : : .. : : * : : :

GLLLEIVKIITDVNVDESÆIDTEGLVAKDKFHVS-YRGAKLNSSLV
 GLVVEMIKVMADVNIDVESÆIDTEGLVAKDKFHVS-YQGQALNRSSLV
 NLLNEIIIDVLHELELNITFACLSTYADENKDIFHVTMMSGEQVDAVLREI
 GLLTKIAEVFHSMALNIHAARITTVGERAEDFFVVVSNNFLALNDNEQSI
 GLLAQVSQIYTELNLLNNAKITTVEGEKAEDFFILTNQFGQALDSQQRIL
 GLLARVSSVFNQLGLNLNAKITTIGERVEREDFFVTTQHQALDDKAQAL
 GLLYTIGRTLFELOQIRISVAKITTKIDQVADVFYVRTHQGEKVSDPEQEL
 ALLYDVARTLQSLHLDVLFAKVSTLGNRTADTFSVRTAQGOKLTDEEHEV
 GLLHALGRALAEEGIDIRSAHVATYAAQAVDVLYLAEASGERLSPP-RVA
 GLLHDVTALSASSASLQISSAHITTYGMRAVDVFYVRDLLGMKITDPVRRL
 ALLNQLAYALFQSKVTVHSAHVATYGERAVDFTFYVTDLIGDKIDSPARTL
 GLLYELTTAISKLNLIASAHVATFGERARDVFYVTDLGQINAPTRAI
 GLLYQLTAAISKLNLIASAHVATFGERARDVFYVTDLGQITAPTRAI

* : : : : : * : * : * : : :

VNCLRYYL
 VNCLRYFL
 MNTLYFL
 QNALIKRL
 RNVLYRN
 KSALLDEL
 KRALLFWL
 RAALLHAV
 IRVLSDA
 RETLLASL
 EKRLLEAA
 RAAVMEVF
 KSALLHLL
 KRALVHLL
 :

Note: Identifiable homologs of this gene (using *Cyanidioschyzon* sequence and *Arabidopsis* GI 9758449 as queries) are restricted to red algae, green plants, proteobacteria and firmicutes with low sequence similarities (Evalue e-4 and lower). The annotation of the

Arabidopsis sequence (TAIR locus AT5G04740) indicates that the protein is located on the chloroplast thylakoid membrane.

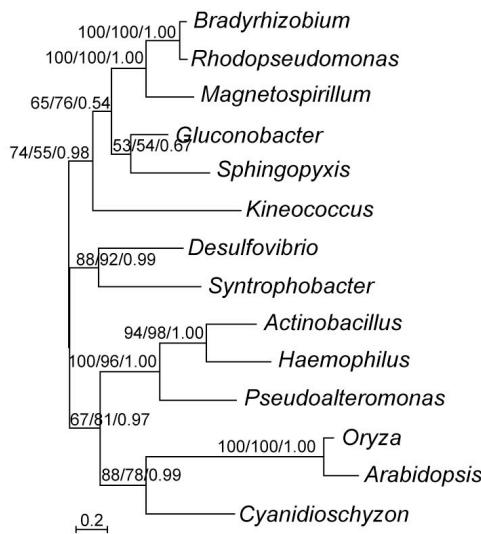


Figure 12. Molecular phylogeny of ACT-domain containing protein. Since no other eukaryotic or cyanobacterial homologs were identified, no statistical test on alternative topologies was given.

13. Queuine tRNA-ribosyltransferase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

42520578_Wolbachia_endosy	SGSARVGTIKTPNGSVETPAFIFCATKAAIKAADIERISGTQIILSNTYH
58613533_Heterocapsa_trique	-----
45656892_Leptospira_interr	LTKARTGILNLNGIELKTPVFMPPVGTRGVVKTLSADDLEEYSLILGNTYH
19068923_Encephalitozoon_cunic	TTNARVSTLELNGTSLELPIFMPVGTYGAMKGIRVSDLE--SMILTNTYH
3881825_Caenorhabditis_elegan	AGFARRGNLHLPHSIVETPVFMPPVGQTGTMKGIVPEQLVDRCRILLCNTH
56473322_Entamoeba_histol	DGRAROCTLTLPHGEVETPVFMPPVGTKASVKSLSLYEVQVKGCQILLANTYH
46229743_Cryptosporidium_parvu	CGRARYGVNLNLPHGVRDTPIFMPVATHGSIKGLSSLQVENVPILLGNAYH
TPO00008041_Trimastix_pyriform	-----
12597314_Homo_sapien	RSRARAGELWLPHGTATPVFMPPVGQATMKGITTEQLDGCRICLGNTYH
60463331_Dictyostelium_discoi	WGKARAALKTLPHHQCSTPMFMPVGTVQGTVKGLTSQQLVNCVVLGNTYH
62360604_Trypanosoma_brucei	-----
50900348_Oryza_sativa	-MPVATQATLKGVTVQEQLEDVEIILGNTYH
JBO00061016_Jakoba_bahamensis	FNRARAARLTLPHFTCQTPLFMPVGTQGTIKGLTTDQLEGQCIIILGNTYH
28850382_Dictyostelium_discoi	-----
116059209_Ostreococcus_tauri	KSNARVTKITTPHGIIMQPNFVPGTVGTIKFLDPISTKSQLMFVNNTYH
CM2163_Cyanidioschyzon_merolae	RSRARVGEIRTPHGVVRTPGFVSGTVNAALKAVVHETLDGLDMFANTYH
46446428_Parachlamydia_sp.	RSSARIGRLHTPHGTVETPAFVAVGTVNAALKMVDRRADGLELLFCNTYH
76788915_Chlamydia_trachomat	KSRARVGRISHGIIDTPNFVAVGTVNGTCKALNNNTMLHGLQLMFCNTYH
29840339_Chlamydophila_caviae	KSRARVGRRIETAHYGIDTPAFVPAVATNGALKGVLDH---NIPLMFNCNTYH
11499080_Archaeoglobus_fulgid	KSRARVGKIETAHGIIIDTPAFVPAVATNGALKGVIDH---NIPLMFNCNTYH
21674218_Chlorobium_tepidu	LDRMRVGVKTRHGSFETPVFIPVATLAAIRGLDNRDLKGVEVILANTYH
48855206_Cytophaga_hutchii	HSAARCGVLSTSHTSHGDIPPTIFMPVGTVRASVKSVEPNELKDAKIIILANTYH
53713592_Bacteroides_fragil	GTSARAGVITTDHGTFETPIFMPVGTAGTVKAMHQRELEDAPIIILGNTYH
76258051_Chloroflexus_auranti	KSNARAGLITTDHGQIQTPIFMPVGTIGSVKGVHQTELKQAQIILGNTYH
15644309_Thermotoga_mariti	ESRARAGLLQTAHGTATPVFMPPVGTRATVKSLSLPHELRGASIILGNTYH
6460406_Deinococcus_radiod	FGKARLGVMKLHHGAETPVFMPPVGTVNASVKLLTPRDLLEGAEIILSNTFH
48891338_Trichodesmus_erythr	DGRARTATFQTPRGAVTTPMFPVGTVQGTVKGISPQELLGSQMILANTYH
56751072_Synechococcus_elonga	YTKARAGIFITPHGSINTPRFMPVGTVLANVKTLPAPAQLEGAQMILANTYH

20455294_Nostoc_sp
46106898_Rubrobacter_xyloano
42523694_Bdellovibrio_bacter
15611336_Helicobacter_pylori
15606515_Aquifex_aeolic
32397050_Rhodopirellula_baltic
39996277_Geobacter_sulfur
23473888_Desulfovibrio_desulf
39997713_Geobacter_sulfur
52006768_Thiobacillus_denitr
53732897_Haemophilus_influe
48730213_Pseudomonas_fluore
19715156_Fusobacterium_nuclea
56963323_Bacillus_clausii
16800633_Listeria_innocu
39935672_Rhodopseudomonas_palu
52011430_Silicibacter_sp.
15604558_Rickettsia_prowaz

42520578_Wolbachia_endosy
58613533_Heterocapsa_trique
45656892_Leptospira_interr
19068923_Encephalitozoon_cunic
3881825_Caenorhabditis_elegan
56473322_Entamoeba_histol
46229743_Cryptosporidium_parvu
TP000008041_Trimastix_pyriform
12597314_Homo_sapien
60463331_Dictyostelium_discoi
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50900348_Oryza_sativa
JB000061016_Jakoba_bahamensis
28850382_Dictyostelium_discoi
116059209_Ostreococcus_tauri
CM2163_Cyanidioschyzon_merolae
46446428_Parachlamydia_sp.
76788915_Chlamydial_trachomat
29840339_Chlamydophila_caviae
11499080_Archaeoglobus_fulgid
21674218_Chlorobium_tepidu
48855206_Cytophaga_hutchi
53713592_Bacteroides_fragil
76258051_Chloroflexus_auranti
15644309_Thermotoga_mariti
6460406_Deinococcus_radiod
48891338_Trichodesmium_erythr
56751072_Synechococcus_elonga
20455294_Nostoc_sp
46106898_Rubrobacter_xyloano
42523694_Bdellovibrio_bacter
15611336_Helicobacter_pylori
15606515_Aquifex_aeolic
32397050_Rhodopirellula_baltic
39996277_Geobacter_sulfur
23473888_Desulfovibrio_desulf
39997713_Geobacter_sulfur
52006768_Thiobacillus_denitr
53732897_Haemophilus_influe
48730213_Pseudomonas_fluore
19715156_Fusobacterium_nuclea
56963323_Bacillus_clausii
16800633_Listeria_innocu
39935672_Rhodopseudomonas_palu
52011430_Silicibacter_sp.
15604558_Rickettsia_prowaz

42520578_Wolbachia_endosy
58613533_Heterocapsa_trique
45656892_Leptospira_interr
19068923_Encephalitozoon_cunic
3881825_Caenorhabditis_elegan

QTKARS GIFTLTPH GIVETPRFMPVGT LANVKTVTPAQLKG A QMVL SNTYH
CGAARAGLLRTPH GAVETPTFMPVGT KAVKGLSPRDLAGAGI VLGN TYH
DGHARRATLMTA HGPVQT P VFM AVG TKA TVKAMTPEELKG TQVVLG NTYH
DDNARAGV LNL AHQS VETPV FMPV GTQGTVKAM LHKL LDGTQ IILG NTYH
DGKARRG RIYTPH GVIETPV FMPV GTQGTVKAM LHKL LDGTQ IILG NTYH
DGGARRGV FHTP RGPV RPTPG FMPV GTL GTVK GLTIDQ VAGAD M ILG NTYH
KTSARRGS LTT P HGTIE TPI FMPV GT HAAMKAM TPSQ VKGA QII LSNTYH
DGKARTGV LQTA HGPV RPTPI FMPV GTVG SVKG VAP DLDG AEI ILG NTYH
DGAARLGSL TT P HGLI T P FMPV GTQATV KAMTPEELD GAR IILANTYH
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DGKARRG RLTF P RGT VETPA FMPV GTY CTGV KGM L PRD IVGAEI ILG NTFH
DGKARAGV ITTLH GEI ET P V FM PG VGT QATV KTM SKEELL GS EII ILG NTYH
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QTGARL GK IHTPH GTFD T P FMPV GTQATV KTM SPEDLK DGA II LSNTYH
DGAARTG RLTT P HGT QV VRT PA FMPV GTY AGAM KGL HW REV RGAD I VLG NTYH
DGKARTGV IHTP RGEIR TPA FMPV GTA ATV KAM MPES VRGAD ILLG NTYH
YKKARSGV ITTA HGEIR TPA FMPV GT RGA V KAM L TEAV V GAD ILLG NTYH

LMLQPGENTVGLRK MIGWNGPMLTD SG YQIFS IN EDGA IFRSY I NGK IY

LYLRPGT SVL GLKKF STWK ALL TD SG YQFS YE QDG VRF QSH IDG SRH
L-----NIKE FMK YNR GM LTD SG FQIG SVT EDG VRF G-----DS
LGHR PG HER VGL HKM MNW NR SILT D SG FQOM VS DENG VNF E SPHT GEMM
MFLHPGV DV LGL H QFA KWD GN ILT D SG FQMV S VNE QGV IF QSI VDH KPI
LGSRP GD EII GLHN FM RW NR NI LT D SG FQMV S IEE GV EFRH PY TNANL

LGLRP GP E IGL HG FM MNW PHN NLL TD SG FQMV S VTE EG VRF RSPY DG NET
LGH RP GP E VM GL H KF MN Y P RAM L TD SG FQMV S ITE EG VRF QST HGG S L
LGLRP G D E I L G I H L QGW KR NI LT D SG FQMV S ITE EG VRF QSPV DG KPM
-----EG VRF QSPH DG SEM
MV VNS DPK L IGL H KF I NY QN PI IT D SG FQV FS I K DSG V KFL SY KNG DT I
LMLQ PG A E T V G VHE MM GRR PT I TD SG FQV FS VSE EG AM FR SY RD GKT M
LLLQ PG P E I V G L H R F M Q R S R P I M T D SG FQV FS VSE NG P V R F S Y RD G R K I
MMLQ PG D I V G L H L S F I Q R K L P I I TD SG FQV FS I TEE G V L F R S Y RD G S K V
LIVHPG AEA IGL H QF I GR N A P I I TD SG FQV FS VND D G V H F K S Y RD G R K L
LLVHPG TE A IGL H K F M R N A P I I TD SG FQV FS V N D E G V W F K S Y RD G H K L
LHLRP G D E I L G I H K F M N F D G V I T D SG FQV FS I QAF S V T D R G V R F K D P K S G R I V
LYLKP GND I L G V H R F M N W D G P L L T D SG Y QV Y S I S E E G V M F K S H L D G S K L
LYLRP GLN I IGL H K F I G W K R S I L T D SG FQV Y S I N E E G V K F K S H I D G S A H
LYLRP GL D V L G L H K F M N F D R P M L T D SG FQV FS L H E E G A E F R S H I D G S K H
LYLQP G H E I L G I H S F M G W N G P I L T D SG FQV FS V T E E A V F K S Y L D G S R H
LMLKP G V E I I G L H N F M G W K R P I L T D SG FQV FS I D D E G V V F R S P I D G S K V
LMLRP G Q E O L V G L P G F T A Y P G P F L T D SG FQV FS I S E E G V V F K N H L D G S R V
LHLQP G E S I V G L H K F M A W N R P I L T D SG FQV FS I S E A G V K F R S P H D G S M I
LHLQP G E E I V G L H R F M N W S G P M L T D SG FQV FS I E E R G V T F R S P R D G A V I
LHLQP G E A I V G L H K F M G W N G P M L T D SG FQV FS I TEE G V T F R S P R D G Q I I
LYLRP G A E V V G L H E F T G W S G P M L T D SG Y QV FS I S E E G V R F T S V Y D G S V H
LHLRP G E K T I I G L H K F M N W H G P I L T D SG FQV FS I M T E E G V E F R S H L D G A K H
MYLRP G E K V V G L H R F A Q F Q G S F L T D SG FQV FS I Q A F S L Q E D G I V F K S H I D G S K H
LYLRP G T E I I G L H R F I S W N K P I L T D SG Y QV FS I S E E G V F Q V S D E G V E F Q D H L Q G D K H
LRLRP G H E T V G L H K F M C G W D G P I L T D SG FQV FS V N E H A A T F R S H I D G A K I
LHLRP G E D L I G L H R F M G W D G P I L T D SG FQV FS I T D E G V F F R E V S G E E V
LYLRP G D E L V G L H E F N A W R K P I L T D SG FQV FS I S E E G V E F R S H L D G S K H
LYI RP G H E I L G I L H R F M H W D R P I L T D SG FQV FS I S E E G V R F Q S H L D G S Y H
LWLRP G L E V I G L H R F M G W D K P I L T D SG FQV FS I TEE G V K F A S P T N G D K L
LWLRP G O E I M D L H D F M Q W H R P I L T D SG FQV FS I TEE G V K F Q N P I N G E R I
LWLRP G T E V I D L H D F M Q W K G P I L T D SG FQV FS I K E E G V T F A S P V D G S K V
LYLRP N D E L I G L H K F M N W D R P I L T D SG FQV FS I K E E G V F S S H I D G S K H
LWLRP G E E I I G L H Q F M N W D K P I L T D SG FQV FS I TEE G V H F R N H L N G D K L
LMLRP G A E R I G L Q R F T T W N G P M L T D SG FQV FS I K E E G V H F R N H L N G D K L
LMLRP T A E R I G L H K F M N W D R P I L T D SG FQV FS I TEE G V F T F K S H I D G S R H
LMLQP S A E R I G L H K F M N W D K P I L T D SG FQV FS I TEE G V S F R S H I N G N K Y

CLTPEKSVQIQQKL GAD LIL V L D E C T P F H V S K E Y T A K S M L M S H R W A E R S L
--SPESSIVI QREL GAD LIL V L D E C T P F H V D K A Y T A R A M R R S H R W A V R S L
YFTPN S VIDI QRS I G S D I M V L D C A F D S G P E R L K Q S L D R T H R W A E M S V
LFT P E D S M N I Q M M L G A D I I M Q L D D V V N P C D T R E K I E I A M R R S I R W M D R C I
ALPPEK SIE I Q Q A L G A D I M M Q L D H V I H V L T T G D I V K E A M H R S I R W L D R C K

56473322_Entamoeba_histol
46229743_Cryptosporidium_parvu
TP000008041_Trimastix_pyriform
12597314_Homo_sapien
60463331_Dictyostelium_discoi
62360604_Trypanosoma_brucei
50900348_Oryza_sativa
JBO00061016_Jakoba_bahamensis
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116059209_Ostreococcus_tauri
CM2163_Cyanidioschyzon_merolae
46446428_Parachlamydia_sp.
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29840339_Chlamydophila_caviae
11499080_Archaeoglobus_fulgid
21674218_Chlorobium_tepidu
48855206_Cytophaga_hutchi
53713592_Bacteroides_fragil
76258051_Chloroflexus_auranti
15644309_Thermotoga_mariti
6460406_Deinococcus_radiod
48891338_Trichodesmium_erythr
56751072_Synechococcus_elonga
20455294_Nostoc_sp
46106898_Rubrobacter_xyano
42523694_Bdellovibrio_bacter
15611336_Helicobacter_pylo
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32397050_Rhodopirellula_baltic
39996277_Geobacter_sulfur
23473888_Desulfovibrio_desulf
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52006768_Thiobacillus_denitr
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56963323_Bacillus_clausi
16800633_Listeria_innocu
39935672_Rhodopseudomonas_palu
52011430_Silicibacter_sp.
15604558_Rickettsia_prowaz

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42520578_Wolbachia_endosy
58613533_Heterocapsa_trique
45656892_Leptospira_interr
19068923_Encephalitozoon_cunic
3881825_Caenorhabditis_elegan
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48891338_Trichodesmium_erythr
56751072_Synechococcus_elonga
20455294_Nostoc_sp
46106898_Rubrobacter_xyano

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42523694_Bdellovibrio_bacter
15611336_Helicobacter_pylori
15606515_Aquifex_aeolic
32397050_Rhodopirellula_baltic
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* : * : * . : . : * * . : . : * . : . : *

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TP000008041_Trimastix_pyriform
12597314_Homo_sapien
60463331_Dictyostelium_discoi
62360604_Trypanosoma_brucei
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23473888_Desulfovibrio_desulf	SIHLNLTYFLDLVRGAREAIAQGTF
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52011430_Silicibacter_sp.	TWHNLHYFQDIMAGMRESIAAGTF
15604558_Rickettsia_prowaz	TWHNLTYFQNLMMSRITYIKLGKD

Note: This is an intriguing case of ancient HGT. The *Dictyostelium* sequence forms a group with homologs of green and red algae as well as chlamydiae. This specific affiliation of *Dictyostelium* and plant sequences has been observed in multiple cases (Huang, unpublished data), likely resulting from plant-*Dictyostelium* transfer. A plausible explanation is that primary photosynthetic eukaryotes acquired this gene from chlamydiae and then further spread to *Dictyostelium* via secondary gene transfer. *Heterocapsa* sequence is a chloroplast precursor based on the original GenBank annotation. *Heterocapsa* and *Wolbachia* sequences also share indels and many conserved residues. The remaining eukaryotic sequences are much more similar to bacterial than to archaeal homologs, and they are likely of bacterial origin. One possible explanation is that they are derived from mitochondria. Nevertheless, most of these eukaryotic sequences lack a N-terminal extension. Sequences of *Trimastix* and *Jakoba* were obtained from TBESTDB.

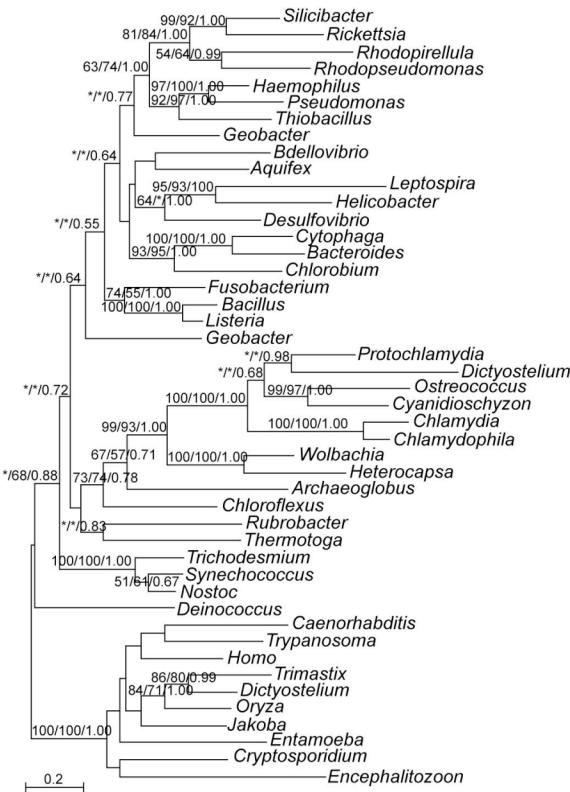


Figure 13. Molecular phylogeny of queuine tRNA-ribosyltransferase. P-value = 0.369 from the AU test for the presented gene tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of red algal, green

plant, *Dictyostelium*, and cyanobacterial sequences. These tests investigate if all eukaryotic sequences have the same origin (mitochondrial or eukaryotic) and if red algal and green plant sequences are likely derived from plastids. P-values < 0.001 from AU tests for both alternative topologies.

14. SAM-dependent methyltransferase (N)

CLUSTAL X (1.83.1) multiple sequence alignment

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23509703_Plasmadium_falcip
16080887_Bacillus_subtil
29375834_Enterococcus_faecal
54294134_Legionella_pneumo
42571865_Arabidopsis_thalia
CM2295_Cyanidioschyzon_merolae
42522494_Bdellovibrio_bacter
14600479_Aeropyrum_perinx
42524664_Bdellovibrio_bacter
39998234_Geobacter_sulfur
34763641_Fusobacterium_nuclea
6457710_Rhodobacter_sphaer
32444786_Rhodopirellula_baltic
21110809_Xanthomonas_axonop
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56679104_Silicibacter_pomero
15605998_Aquifex_aeolic
34556476_Wolinella_succin
46446677_Parachlamydia_sp.
53712322_Bacteroides_fragil
48854326_Cytophaga_hutchi
20094503_Methanopyrus_kandle
14591657_Pyrococcus_horiko
15607037_Aquifex_aeolic
53796614_Chloroflexus_aurant
26246990_Escherichia_coli
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47573089_Rubrivivax_gelati
48770381_Ralstonia_metall
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15644445_Thermotoga_mariti
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48857855_Clostridium_thermo
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21110809_Xanthomonas_axonop
15837009_Xylella_fastid
53797508_Chloroflexus_aurant

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54294134_Legionella_pneumo	RILGVYGPASCHPTLAASFPEGNYLTAVLLA
42571865_Arabidopsis_thalia	TVVREAGAASDHPLDPSYPQGQYLSNLLLR
CM2295_Cyanidioschyzon_merolae	TILAERGAALDHVVSPAAPEGRYLTA
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14600479_Aeropyrum_perinx	RILGDRAASPDMVYRGE-EYLSYLKGA
42524664_Bdellovibrio_bacter	RSVLRGGHAAHDHPTLMQFPEGFYLKMYVH
39998234_Geobacter_sulfur	RVISLAGQPEDFPYPVTFPEGRYLKVVSV
34763641_Fusobacterium_nuclea	SVLGINYQPEDHPWILHIPE
6457710_Deinococcus_radiod	TLYKAVFLQ
32444786_Rhodopirellula_baltic	IVLESRGPSPDHVFAVSCPESDYLKCI
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53797508_Chloroflexus_aurant	QIIHEAGHPLDHPVPAGFPEGRYLKIIAR
22960169_Rhodobacter_sphaer	QLIHTGFAGPDHPILQLPOLAESGYLKSLFFR
56679104_Silicibacter_pomero	QLIHTGFAGPDHPQLPOLAESGYLKAVFFR
15605998_Aquifex_aeolic	IIKSLNLIQPIDHPYLPTFPESLYLKCLHAI
34556476_Wolinella_succin	QILQYHLQDIDHPAILNMPHSLYLRGIVFE
46446677_Parachlamydia_sp.	RLIGRHQLACDHPINLHFPHESDYLKSFL
53712322_Bacteroides_fragil	RILHQLTQPADHPVNHYHPEGEYLKGLVLY
48854326_Cytophaga_hutchi	RIIHQMHOQADHPINIYHPEGEYLKGLVLY
20094503_Methanopyrus_kandle	RMLGPRGQPPCHPIVEGNPDTRYLKAMFCA
14591657_Pyrococcus_horiko	KMLEPRTQAPDHPIILMASKDTEYLKCLFLY
15607037_Aquifex_aeolic	RVIAKTFQDLDHPWILQMPNTLYLKGVWVE
53796614_Chloroflexus_aurant	QILERLTTQADHPVLLSFPEAEYLKGLICR
26246990_Escherichia_coli	QFIEQFRQAADHPVIATYPEGLYLKGFA
29142267_Salmonella_enteri	QFIEQFRQAADHPVIATYPEGLYLKGFA
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21674038_Chlorobium_tepidu	RLISRAAOPPDHPVLLAMPETSYLKFA
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39997720_Geobacter_sulfur	RVVGVRSAQPDHPVLLAVPETEYLYKCFI
46199218_Thermus_thermo	RVVEKRGOQFDHPVLLNHPETHYLKFAVFO
48857855_Clostridium_thermo	RQIEARQQAPDHPILWNVPETDYLKFYIFQ
37523934_Gloeobacter_violac	CLVEQRGQAPDHPILLHIPETEYLYKLLIS
51894272_Symbiobacterium_therm	RLVEERAQAVDHPIVVGYDESHYLKCLIYE

**

Note: This gene has identifiable homologs only in prokaryotes and plastid-containing eukaryotes (red algae, green plants, and apicomplexan *Plasmodium*) in our GenBank and TBESTDB searches (using *Cyanidioschyzon* and *Arabidopsis* sequences as queries). Homologs of the gene are rarely found in cyanobacteria. All top hits are from gamma and beta-proteobacteria.

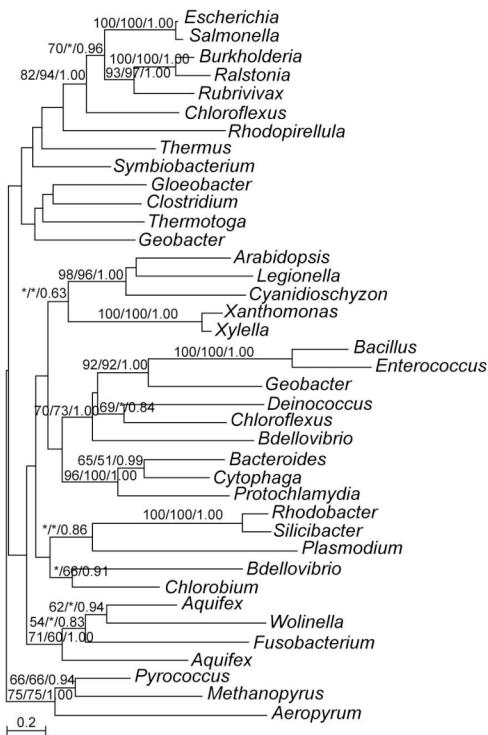


Figure 14. Molecular phylogeny of SAM-dependent methyltransferase. P-value = 0.921 from AU test for the presented gene tree. AU tests were also performed on alternative topologies including (A) monophyly of red algal, green plant and *Plasmodium* sequences, (B) monophyly of red algal, green plant, *Plasmodium* and cyanobacterial sequences, and (C) monophyly of red algal, green plant, *Plasmodium*, and archaeal sequences. Topology A investigates if red algal, green plant, and *Plasmodium* (which also has a relict plastid) sequences have the same origin. Topology B investigates if the three plastid-containing groups acquired the genes from plastids. Topology C investigates if these plastid-containing eukaryotic sequences have an archaeal or eukaryotic origin. P-value = 0.082 from AU test for topology A whereas p-values < 0.001 for topologies B and C.

15. Semialdehyde dehydrogenase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

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32408531_Neurospora_crassa
5155_Saccharomyces_cerevi
24214878_Leptospira_interr
21673935_Chlorobium_tepidu
27360910_Vibrio_vulnif
53711823_Bacteroides_fragil
34558370_Wolinella_succin
47529651_Bacillus_anthra
38233757_Corynebacterium_diph
29610427_Streptomyces_avermi

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 11499653_Archaeoglobus_fulgid
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 48853180_Ferroplasma_acidar
 18313659_Pyrobaculum_aeroph

NU000054019_Capsaspora_owczar
 60466242_Dictyostelium_discoi
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 48853180_Ferroplasma_acidar
 18313659_Pyrobaculum_aeroph

NU000054019_Capsaspora_owczar
 60466242_Dictyostelium_discoi
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 21673935_Chlorobium_tepidu
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 IRIL-VELKKVQHTNFLDI-SLRKRENTLVVVSALDNLVKGAAAGQALQNI
 VRLRVTEVSHVAYTNFCDLAFES-DGSLVIITAIDNLVKGAAAGQAVQNM
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 IRLRPVEIKAVAGTHFCDI-YAMSOQRDLWVNSAIDNLLRGASSQAIANA
 IRIRFPSPKEVRGSNYCDMIAYDERTGRVTIVSVDNMKGAAAGQAIQNA
 VHILQPOTQAVVGSNMCHIVDADDNSKKLTVTSAINLTKTAGAAVQCM
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 IKVCYPOTKWAAGSNLCIIVEVDPRTGRVIVMSAIDNLNIKGQAGQAIQCL
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 IRIILPETRWVKGNSNYCDIIRIDKRTNKIIIVLSAIDNLMKGAASQAVQNM
 VRVLLPALRNVRGTMYCDMLVVDPRTHLIIIVSCIDNLCRGASGQALANA
 VHVLVPQTRYVRGSNHVMVFIDPRLQGRAILVVAIDNLVKGAAAGQAVQNM
 VHVLVPQTRYVRGSNHVMVFIDPRLQGRAILVVAIDNLVKGAAAGQAVQNM
 VRVVSPTHRVGRGSNFVLIVHADRVPGRVIVTCVEDNLVKGASGQAIQDM
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 IRLIYPDPKTIVGSNFADIFAIDKYVKRVSFGAIDNLNIKGAAAGNAVQSM
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: * : . :

NU000054019_Capsaspora_owczar
 60466242_Dictyostelium_discoi
 32408531_Neurospora_crassa
 5155_Saccharomyces_cerevi
 24214878_Leptospira_interr
 21673935_Chlorobium_tepidu
 27360910_Vibrio_vulnif
 53711823_Bacteroides_fragil
 34558370_Wolinella_succin
 47529651_Bacillus_anthra
 38233757_Corynebacterium_diph
 29610427_Streptomyces_avermi
 41407459_Mycobacterium_avium
 15606911_Aquifex_aeolic
 32397402_Rhodopirellula_baltic
 11499653_Archaeoglobus_fulgid
 16331759_Synechocystis_sp.
 46135514_Anabaena_variab
 57168350_Campylobacter_coli
 15895656_Clostridium_acetob
 23473460_Desulfovibrio_desulf
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 22956652_Rhodobacter_sphaer
 CM2432_Cyanidioschyzon_merolae
 48765484_Rhodospirillum_rubrum
 50428699_Oryza_sativa
 19699208_Arabidopsis_thalia
 39997965_Geobacter_sulfur
 7379404_Neisseria_mening
 30249453_Nitrosomonas_europa
 53613186_Azotobacter_vinela
 15595859_Pseudomonas_aerugi
 24211481_Deinococcus_rediod
 55232034_Haloarcula_marism
 48853180_Ferroplasma_acidar

NIALGLRELTDGI
 NICLGLDELASI
 NLALGYAEYEGLI
 NLAMGYGEYAGI
 NLMTGAKETLGL
 NLMLGFGETTAL
 NIHYGFSPLTAL
 NLMFNLNEETVGL
 NLMLGLPEGAGL
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 NVAQGWDERAGL
 NVALGIEETAGL
 NIMNGFNEYEGL

18313659_Pyrobaculum_aeroph

NVAMGFPPEDEGL

*: . ..

Note: This gene is found in green plants, red algae, fungi, *Capsaspora* and prokaryotes. All top hits in GenBank searches are from alpha-proteobacteria. Phylogenetic analyses also support an alpha-proteobacterial origin of the gene in red algae and green plants. Fungal and *Capsaspora* sequences are apparently different (with an about 500 aa N-terminal extension and many conserved residues shared between them) from red algal, green plant and prokaryotic sequences. The *Neurospora* sequence (GenBank accession number P54898) is experimentally determined to be a mitochondrial precursor. Red algal and green plant sequences share many conserved residues with alpha-proteobacterial homologs. The *Arabidopsis* sequences (GenBank accession numbers and TAIR loci NP_565461, AT2G19940 and NP_849993, AT2G19940 respectively) are annotated to be located in cytoplasm. Sequence of *Capsaspora* sequence was obtained from TBESTDB.

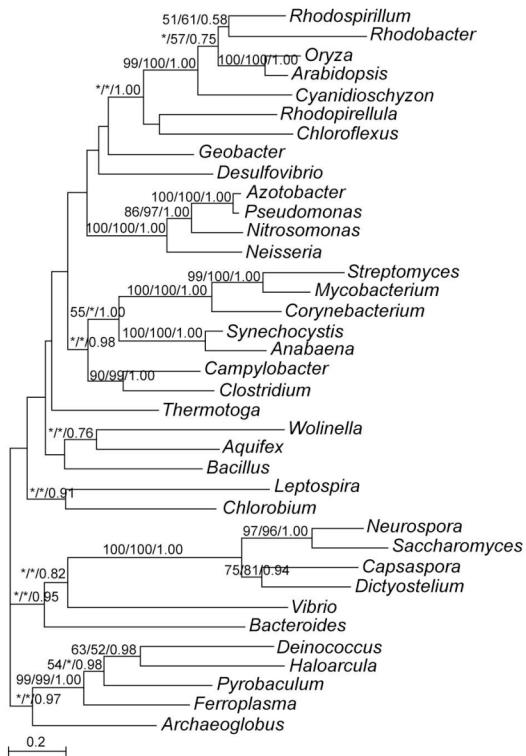


Figure 15. Molecular phylogeny of semialdehyde dehydrogenase. P-value = 0.981 from AU test for the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of cyanobacterial, red algal, green plant, and alpha-proteobacterial sequences. Topology A investigates if all eukaryotic sequences have the same origin (mitochondrial or eukaryotic) whereas topology B investigates if red algae and green plants acquired this gene from plastids and further spread to alpha-proteobacteria. P-value = 0.045 from AU test for topology A and p-value < 0.001 for topology B.

16. Dihydrodipicolinate reductase (dapB) (D)

CLUSTAL X (1.83.1) multiple sequence alignment

116055387_Ostreococcus_tauri]
 18406430_Arabidopsis_thalia
 42572737_Arabidopsis_thalia
 CM2591_Cyanidioschyzon_merolae
 ISO00167059_Isochrysis_galbana
 121542607_Candidatus_Desulf
 123966140_Prochlorococcus_mari
 73748738_Dehalococcoides_sp.
 123216321_Mycobacterium_tuberc
 145593931_Salinispora_tropic
 121535277_Thermosinus_carbox
 29376120_Enterococcus_faecal
 116873342>Listeria_welshi
 125973685_Clostridium_thermo
 20094858_Methanopyrus_kandle
 118070755_Shewanella_woodyi
 89899565_Rhodoferax_ferrir
 120613186_Acidovorax_avenae
 20093259_Methanosarcina_acetiv
 116754136_Methanosaeta_thermo

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 ISIMVNCGSGKMGKAVIKAA-DSAGVNIVPISFEREKVLSVFIVVDYTI
 LALVSNGLPGRMALAEVAQAALRRG-LELVPSLERAQLATKLKICVDFQ
 TRVSLYD--PNARDALAQVVRRETYPV-----DGEAIL---ICVDYTH
 IPIMLNGLPGNVAKMIVSHVLRDSRFLALVPFLVRAARIGDIKISVVDYTH
 IPVLVSGALGKMGREVVNSVLNASDCELVAIDIDLEGTLCSISVLDFTH
 IKVVVHGASGKMGQEVLKTCQENNFPVGVIDLSSILSQTKVMVDFTV
 MRVGVLGAKGKVGATMVRAVAADDLTLSALDAAPLSLLTDGNVVIDFTH
 LRVGVLGARGMGRGIEVKAVDAAPDMELVAVDQDWLFNASDAGVVVDFTT
 IRVMVCGAGKMGREVVNLKAVHRDEQLSIVGVVIDLQTVINETKVMVDFTN
 IKIIVAGFKGRMGSTATOMVLETADFLVGYDPRLEFLA-VKVVWIDFTV
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 : * :

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 18406430_Arabidopsis_thalia
 42572737_Arabidopsis_thalia
 CM2591_Cyanidioschyzon_merolae
 ISO00167059_Isochrysis_galbana
 121542607_Candidatus_Desulf
 123966140_Prochlorococcus_mari
 73748738_Dehalococcoides_sp.
 123216321_Mycobacterium_tuberc
 145593931_Salinispora_tropic
 121535277_Thermosinus_carbox
 29376120_Enterococcus_faecal
 116873342>Listeria_welshi
 125973685_Clostridium_thermo
 20094858_Methanopyrus_kandle
 118070755_Shewanella_woodyi
 89899565_Rhodoferax_ferrir
 120613186_Acidovorax_avenae
 20093259_Methanosarcina_acetiv
 116754136_Methanosaeta_thermo

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 PSAVNGNAEWAANKFFPVFMGTTG---GDRESLLDAVTGVTSVIAPNMAK
 PTAVNGNCFYCAHGLPFVFMGTTG---GDRDRLDPAVRASSIVIAPNMAK
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 PEGTLAHLAVCAERGVKAVIGTTGFTEAQKAEIEAFAQRTAVV MAPNMSV
 AGATIVNAPIAAGCCVNLIIIGTTGTFPEQRKVIDETIQKNKVVISPNYSV
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 : * * * * : * :

116055387_Ostreococcus_tauri]
 18406430_Arabidopsis_thalia
 42572737_Arabidopsis_thalia
 CM2591_Cyanidioschyzon_merolae
 ISO00167059_Isochrysis_galbana
 121542607_Candidatus_Desulf
 123966140_Prochlorococcus_mari
 73748738_Dehalococcoides_sp.
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 145593931_Salinispora_tropic
 121535277_Thermosinus_carbox
 29376120_Enterococcus_faecal
 116873342>Listeria_welshi
 125973685_Clostridium_thermo
 20094858_Methanopyrus_kandle
 118070755_Shewanella_woodyi
 89899565_Rhodoferax_ferrir
 120613186_Acidovorax_avenae
 20093259_Methanosarcina_acetiv
 116754136_Methanosaeta_thermo

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 GVNLLWKLLEVA AAE VMG DIEI EGG HRY KKDAPS GTALK MGEVIAETLGR
 GVNVTFLK LLEMAA KALATIE IIEA HRR KV DAPS GTALK MGEVIAQ ALGR
 GVN VFFK IVREA AKY LSDIE IIEA HHN QKDAPS GTAL RAADV I SE AL GG
 GVN VFWKL VEMA ARS LS DVE VIEA HRR KKDAPS GTAM RT VEIL SRSL GI

116055387_Ostreococcus_tauri]
 18406430_Arabidopsis_thalia
 42572737_Arabidopsis_thalia

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 SYDMDQIQLGV PEEH ISGHAFHLYH-LTSPDET VS FEFQHNVCGRSIYAE
 SYDMDQIQLGV PEEH VSGHAFHLYH-LTSPDKTVS FEFQHNVCGRSIYAE

CM2591_Cyanidioschyzon_merolae ISO00167059_Isochrysis_galbana 121542607_Candidatus_Desulf 123966140_Prochlorococcus_mari 73748738_Dehalococcoides_sp. 123216321_Mycobacterium_tuberc 145593931_Salinispora_tropic 121535277_Thermosinus_carbox 29376120_Enterococcus_faecal 116873342_Listeria_welshi 125973685_Clostridium_thermo 20094858_Methanopyrus_kandle 118070755_Shewanella_woodyi 89899565_Rhodoferax_ferrir 120613186_Acidovorax_avenae 20093259_Methanosarcina_acetiv 116754136_Methanosaeta_thermo	TGFGGEQDIGVPESCLQGHAFHTYR-LTSPDGSRVVFQHNVCGRRVYAE ----- GVHFSEDQIGIPEEHGGHGWHTYT-LTSPDRTVTFEFKHNINGRDIYAD YNEGLVKETGLNIHSIRLPGLLAHQVVMMSGPGETYTRHDTIDRKAYMP AFNKKPKENGIRVHSVRLPGLLAHQEVIFGAAGQTLTIRHDAFSRECYMP LPPNPDATSGIPVHAVRLAGLVAHQEVLFGEGETLTIRHDSLDRTSFVP AGQGPAPDAGVRVHSVRLPGATGETLTIRHDSYDRASFMP MQQHHPAEVGIRIHSVRLPGYVAHQEVIFGGLGQTLTIRHDSISRESFMP KKQGNPQEVLRLIHSVRLPGLVAHQQVQFGSVGEGLTIRHDSYDRRSFMT VKQGADEVGMRHISVRLPGLVAHQEVIFGAEGQGLTIRHDSYDRISFMS AGVSIKEKV-IPINAVRAGGVVGRHEVMIVGEDDKIEISHESFSRRAFAL GEKVFGRGEIGVIALVRGGEVVGDHTVMALGEYERIELTHRALSRAFAK DLDKCAVYGTIGFSTIRAGDIVGEHTALFADIGERIEITHKASSRMTFAN DLKDCAVYASIGFATIRGGDIVGDHTVLFAGIGERIEISHKSSSRATYAQ DLKDCAVYGTIGFAAVRGGDIVGDHTVLFAGTGERIEISHKSSSRSGYAQ KEYVCGREG-IGIHAVRGGDITGDHTVLFVGNSERIEIRHMAHSRQIFAK EDVRHGREG-IGVHAVRAGDIVGDHTVLFAGPGERIEIKHOAHSRASAFAR
116055387_Ostreococcus_tauri] 18406430_Arabidopsis_thalia 42572737_Arabidopsis_thalia CM2591_Cyanidioschyzon_merolae ISO00167059_Isochrysis_galbana 121542607_Candidatus_Desulf 123966140_Prochlorococcus_mari 73748738_Dehalococcoides_sp. 123216321_Mycobacterium_tuberc 145593931_Salinispora_tropic 121535277_Thermosinus_carbox 29376120_Enterococcus_faecal 116873342_Listeria_welshi 125973685_Clostridium_thermo 20094858_Methanopyrus_kandle 118070755_Shewanella_woodyi 89899565_Rhodoferax_ferrir 120613186_Acidovorax_avenae 20093259_Methanosarcina_acetiv 116754136_Methanosaeta_thermo	GTVDAGFLKRKTLYDMIDVL GTVDAVLFLAKQRIYNMIDVL GTVDAVLFLAKKRIYNMIDVL GTIDAVCFLDQKIQYDMIDVL ----- GTLDAAVFLHQGTVFSMIDVL GVLQAIRKIGKSLIYGLEKLI GVLLAIKEIVHGFVFLDKLL GVLLAVRRIAEGLTGLEPLL GVLLAVRKVRTGLTVGLDALL GVLLACKRVLTGLVYGLENIM GVALACRQVVQELLYGLEQML GVALSIRKTKETLIYGLENIL GAIKAIEFIHDVGYEMSDVL GALVAAKFVVEPGIYSMRDVL GAMRAASWLVEAGLYDMQQL GSLRALRFLSQAGLYDMADVL GSLRAARFLAATGLFDMFDVL GAVRAAEWICKPGIYSMDDVL GALRAARWVVKPGIHSMEEVL

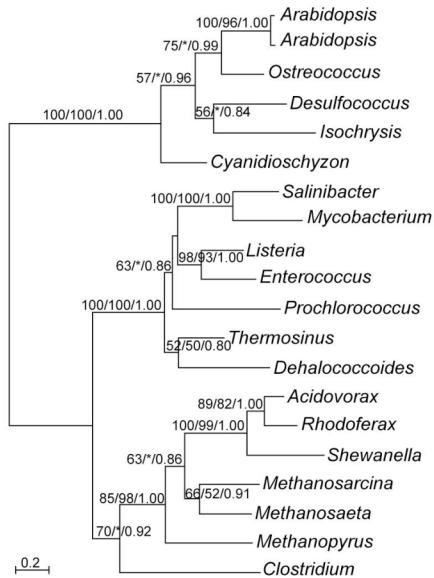


Figure 16. Molecular phylogeny of dihydridipicolinate reductase (dapB). P-value = 0.995 from AU test for the presented tree. AU test was also performed on an alternative topology that enforces a monophyly of

Desulfococcus, cyanobacterial (i.e., *Prochlorococcus* since this gene is very rarely identified in cyanobacteria in our similarity searches), and photosynthetic eukaryotic sequences. P-value < 0.001 from AU test for the alternative topology. See text for more detailed explanation.

17. Leucyl-tRNA synthetase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

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60463472_Dictyostelium_discoi
19683964_Homo_sapien
89306112_Tetrahymena_thermoph
15791045_Halobacterium_sp.
11595633_Neurospora_crassa
53715865_Bacteroides_fragil
15826893_Mycobacterium_leprae
106884629_Clostridium_phytofer
76788931_Chlamydia_trachoma
33235997_Chlamydophila_pneumo
46446681_Parachlamydia_sp.
PLO00010890_Pavlova_lutheri
21674468_Chlorobium_tepidu
42527840_Treponema_dentic
CM3072_Cyanidioschyzon_merolae
87311335_Blastopirellula_marin
83816700_Salinibacter_ruber
150385918_Victivallis_vadensis
10175904_Bacillus_halodu
116058126_Ostreococcus_tauri
7267192_Arabidopsis_thalia
2314727_Helicobacter_pylori
6459971_Deinococcus_radiod
83367833_Rhodobacter_sphaeroi
48766678_Rhodospirillum_rubrum
15889997_Agrobacterium_tumefa
19712730_Fusobacterium_nuclea
106888735_Roseiflexus_sp
46130187_Synechococcus_elonga
53763953_Anabaena_variab
23129999_Nostoc_puncti
16128625_Escherichia_coli
30249126_Nitrosomonas_europa
53762629_Ralstonia_eutrop
126358263_Pseudomonas_putida
7227154_Neisseria_mening
15642942_Thermotoga_mariti
48846878_Geobacter_metallic

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60463472_Dictyostelium_discoi
19683964_Homo_sapien
89306112_Tetrahymena_thermoph
15791045_Halobacterium_sp.
11595633_Neurospora_crassa
53715865_Bacteroides_fragil
15826893_Mycobacterium_leprae
106884629_Clostridium_phytofer
76788931_Chlamydia_trachoma
33235997_Chlamydophila_pneumo
46446681_Parachlamydia_sp.
PLO00010890_Pavlova_lutheri
21674468_Chlorobium_tepidu
42527840_Treponema_dentic
CM3072_Cyanidioschyzon_merolae
87311335_Blastopirellula_marin
83816700_Salinibacter_ruber
150385918_Victivallis_vadensis
10175904_Bacillus_halodu

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FYVLDMFPPYPSGSGLHVGHPEGYTATDIVARYKRKQGFNVLHPMGWDAFG
YYVLDMFPPYPSGAGLHVGHPEGYTATDIICRYRRMKGDNVLHPMGWDAFG
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 46446681_Parachlamydia_sp.
 PLO00010890_Pavlova_lutheri
 21674468_Chlorobium_tepidu
 42527840_Treponema_dentic
 CM3072_Cyanidioschyzon_merolae
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48766678_Rhodospirillum_rubrum 15889997_Agrobacterium_tumefaciens 19712730_Fusobacterium_nucleatum 106888735_Roseiflexus_sp 46130187_Synechococcus_elongata 53763953_Anabaena_variabilis 23129999_Nostoc_punctiforme 16128625_Escherichia_coli 30249126_Nitrosomonas_europaea 53762629_Ralstonia_eutropha 126358263_Pseudomonas_putida 7227154_Neisseria_meningitidis 15642942_Thermotoga_maritima 48846878_Geobacter_metalireducens	LVRHVHKAVAGVTDDLEFNAAVARLRELTAARLAAPMIPHIAEE ASKAAHKTLKAVQEDLFNKAIARIYELVNALVEILIRIIAPMTPHLAEEL LLIKLNQTIKKVTDIAEFNTAIAANMELINEVLKKIIIMLSPFVPHFCDE LYRKHLHETIRKVTLDFNTAIAALMELLNEAAATFARLLSPFAPHIAEE LRRAVHTAIQAVTEDLELNTAIAELMKLTNALVQLVLLAPFAPHIAEE LRRAIHSQSVTEDLEFNTAISELMKLSNALIHTLVVLLAPFAPHIAEE LRRATHTAIQAVTEDLEFNTAISELMKLSNALIRTIVLIIAPFAPHIAADE LRRDVHKTIAKVTDIGFTNTAIAAIMELMNKLLAVVRMLNPTPHICFT LRCQLHQTIKVVTDDLEFNTAIAAIMELMNELLENIVLLLSPIVPHICHV LRRETHGVLKQANYDQYNTVVSATMMLNQFGILLRVLYPVVPHITHG IRRATIHAIAIKQASTDVGFTNTAIAQVMTVMNVILLEAVTLLAPITPHISHE LRHKLHATTAKVSDDYGFNTAIAAVMELLNQYLETAVRLWPIVPHICET LRRKLHSIIKKKEDIEFNTAISGLMELVNHLVEKLTIALSPFAPHIAEE LRRAVHKTIRKVTDIDFNTAIAAIMELVNAAIESVVIMLSPFVPHVTEE
	* * *
60463472_Dictyostelium_discoides 19683964_Homo_sapiens 89306112_Tetrahymena_thermophila 15791045_Halobacterium_sp. 11595633_Neurospora_crassa 53715865_Bacteroides_fragilis 15826893_Mycobacterium_leprae 106884629_Clostridium_phytolyticum 76788931_Chlamydia_trachoma 33235997_Chlamydophila_pneumoniae 46446681_Parachlamydia_sp. PLO00010890_Pavlova_lutheri 21674468_Chlorobium_tepidum 42527840_Treponema_denticola CM3072_Cyanidioschyzon_merolae 87311335_Blastopirellula_marinorum 83816700_Salinibacter_ruber 150385918_Victivallis_vadensis 10175904_Bacillus_halodurans 116058126_Ostreococcus_tauri 7267192_Arabidopsis_thaliana 2314727_Helicobacter_pylorensis 6459971_Deinococcus_radiodurans 83367833_Rhodobacter_sphaeroides 48766678_Rhodospirillum_rubrum 15889997_Agrobacterium_tumefaciens 19712730_Fusobacterium_nucleatum 106888735_Roseiflexus_sp 46130187_Synechococcus_elongata 53763953_Anabaena_variabilis 23129999_Nostoc_punctiforme 16128625_Escherichia_coli 30249126_Nitrosomonas_europaea 53762629_Ralstonia_eutropha 126358263_Pseudomonas_putida 7227154_Neisseria_meningitidis 15642942_Thermotoga_maritima 48846878_Geobacter_metalireducens	HWKSLVFNQRWPKPPTPSALVRDFNSLVIQ IWAGLVLQQAWPAVDPEFLQQEVVQMAVL IAENLEYQFVWPDL-----LAIK CWTALVAEAAWPTPDRDVSDHDRATSLIE CWHVLAARFPVPDGTEGLLKSRSRKQTCAVQ LWDTLVCDAQWPTFNEQYLVEDTVNYTIS LWLRLLLANGPFPQADPAYLVDDTVEYPVQ MWNVLLLANESWPTYDEDAIKEDTTIGVQ LWVLLLVQKSGWPSVSLPEYLEGQTVTIVVQ LWVILIDQAAWPQIDESYLVQAQTIVTFVQQ VWEHLLSFTPYPQVEEKYLQENVITYVVQ MWQRLLAYEPWPQYDEA----- LWQALISGAVWPVFDAKLATDDVLTIAVQ LWQKMIAYSHWPMFVEKFCVDQTCVVVQ MWQEILAYEPWPVDYDQYLVQETVVYAVQ LWAVLLAYHPWPQFDEKFTKDDEIEIPVQ LWARLLAYADWPAYDDELIRREVVEMPVQ LWSTLLAYEPWPVWDEKVLEVNEVEILVQ LWSKLIAYEPWPPTYDEAFLVEDEVEIVVQ MWERLNSKTSWPVADDSSLVVEDTVDIGVQ LWSRLLAYESFPKANPDYLKNTTIVLPVQ LSERLRENFKPIAIDEDALMEDFMTLGLT IWHERVHTAQWPQVDEAAAVRDTVTIGVQ VWSMLVQAQPWPKADEPALLVDDMVTLPIQ MWSHLLAETPWPTWDPLMLIDDQVTIAVQ CWSALVAETPWPTFVASLVEENDVVMPVQ IWEELLFNEKWPEYDEKMLSSDETTIAVQ VHSWCVYDAGWPEWDESALVLDEVEIVLQ LWQQLVHLEGWPVLDESALIVDEIPLVQ LWQLLVTQTPAFDPAALVADEITLVIQ LWHLIHTQTPSFDPAAALVADEITLVIQ LWQELIDNAPWPVADEKAMVEDSTLVVVQ LWRELLLDQWPWPQADDQALIQDEVEIVVQ LWDALLLDAPWPQVDEGALVRTEIEMVQ LWQQLVIDAAPPSVDEQALVQDTITLVVQ LWSELLWEAGWPTVDEAALVKSEIEVMVQ FWHDLVVQOSWPSYDKALEVEEVEIAIQ LWEALVAAAGWPSFDPSAAVDEEFLIVVQ

Note: All top hits in GenBank searches (using the *Cyanidioschyzon* sequence and *Arabidopsis* NP_192344 as queries) are from firmicutes, CFB bacteria and lantispheerae. These sequences also differ from others in sharing several unique residues and a 1-bp insertion. Sequences of *Neurospora* and *Homo* are mitochondrial precursors according to GenBank annotations. In *Arabidopsis* (GenBank accession number NP_192344 and TAIR locus AT4G04350), the protein product is targeted to both chloroplasts and mitochondria. The plant sequences are distantly related to other eukaryotic homologs based on both sequence similarity and phylogenetic analyses. Sequence of *Pavlova* was obtained from TBestDB.

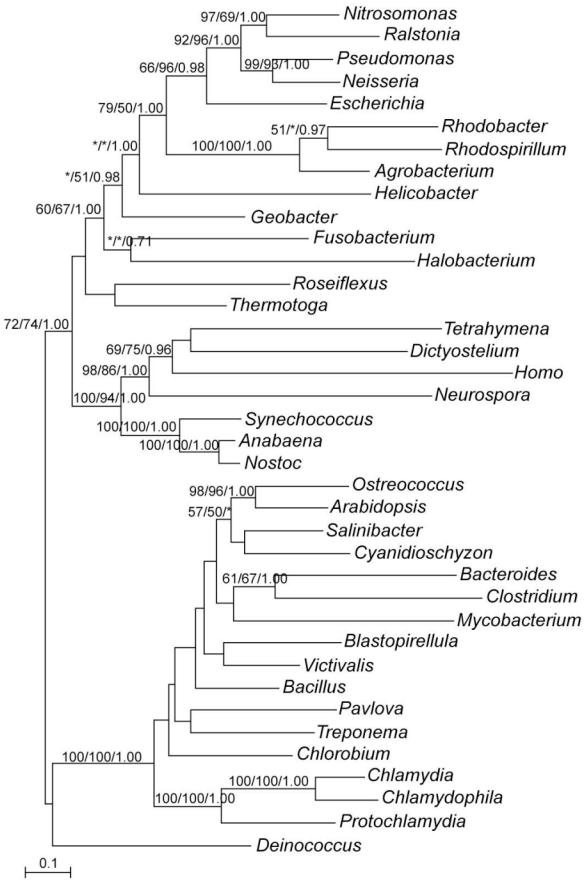


Figure 17. Molecular phylogeny of leucyl-tRNA synthetase. P-value = 0.235 from AU test for the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of cyanobacterial and photosynthetic eukaryotic sequences. Topology A investigates if all eukaryotic sequences have the same origin (e.g. mitochondrial) whereas topology B investigates if photosynthetic eukaryotes acquired this gene from plastids. P-values < 0.001 from AU tests for both alternative topologies.

18. Ribosomal protein L11 methyltransferase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

24214091_Leptospira_interr	EYEEAYKEFYKPFIIISYRVIPTWFPPLLNVNPGLAGFTGHHE
91206171_Rickettsia_bellii	TRLVLGRMGDWVALYQNQLVPIQTRFFICTTLLILIEASRAFGTGT
67940209_Chlorobium_phaeob	THETSGCIEALENNNAEWEANLKPVEIRIMIVQQNLILIEINPKMS
34540441_Porphyromonas_gingiv	FGTGHHETTSLMMSYLLNWNEOWEKNFQPINVKLIRAEFEIIIQPK
108866182_Aedes_aegypti	MQMMNWNEEWEKNFPQPINVKLIRAEFEIIIQPKMSFGTGH
RAO00324465_Reclinomonas_ameri	HATTYLMIQMMNWNALWESSFEPVIVFAVRAHFQEIIITPRMS
78777868_Thiomicrospira_denitr	FGTGHHATTWMMIRSMEDWVKVYQDSIEPLRIKFYIHPTWINIAID
33240884_Prochlorococcus_marin	DPALAFGTGHHPTTASALRAIDWSSSWKKFWKADPVKILIL
77464128_Rhodobacter_sphaer	PSWIVIKLDPGSAGFTGSHPTTRLCLLEDLEDWVAKVRREL

113875614_Sinorhizobium_medica
 34762532_Fusobacterium_nuclea
 37521705_Gloeobacter_violac
 38605120_Thermotoga_neapo
 116057515_Ostreococcus_tauri
 15238878_Arabidopsis_thalia
 CM3418_Cyanidioschyzon_merolae
 33151297_Haemophilus_ducrey
 75828283_Vibrio_choler
 15677697_Neisseria_mening
 67546225_Burkholderia_vietna
 83746797_Ralstonia_solana
 15607057_Aquifex_aeolic
 46198605_Thermus_thermo
 94984946_Deinococcus_geothe
 15924568_Staphylococcus_aureus
 67873260_Clostridium_thermo
 30022391_Bacillus_cereus
 76258363_Chloroflexus_aurant
 88936160_Geobacter_uraniu

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 108866182_Aedes_aegypti
 RAO00324465_Reclinomonas_ameri
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 33240884_Prochlorococcus_marin
 77464128_Rhodobacter_sphaer
 113875614_Sinorhizobium_medica
 34762532_Fusobacterium_nuclea
 37521705_Gloeobacter_violac
 38605120_Thermotoga_neapo
 116057515_Ostreococcus_tauri
 15238878_Arabidopsis_thalia
 CM3418_Cyanidioschyzon_merolae
 33151297_Haemophilus_ducrey
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 DWITKNQDLFQPVEIRLWIVPEWVNIILNPGFAFGTGEHPTTKCLLQQ
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 DMDVLDLDMCGCTSVLAIYAKKKGLGIDIDEWAVENSRENAERNNTIILANI
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 KYVVIDVGTGSGTGISIIASKLGTAVDIDEVAVKVAKENLELNKVIVV
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94984946_Deinococcus_geothe	YAEELHDLLAGEYAAHIVPGGALILTGTILTVKLPLVHAAL
15924568_Staphylococcus_aureus	LAHIIDEMIEDAYNTLNEGGYFITSGIIKEKYEGIQSHM
67873260_Clostridium_thermo	IANVIMDISSRVVPYYLKKDGLFIASGIIKERKQEVLDEC
30022391_Bacillus_cereus	LAEIILLFPEDAARVVKSGGLFITSGLIAAKEKVISEAL
76258363_Chloroflexus_aurant	IAKVLVLLAPDLATALKPGGGLLISSGIIDVKEAEVVAAF
88936160_Geobacter_uraniu	LAEEELVRLAPELVDRTAPGGYLVLSGILTEKEEFVINGF

Note: The identifiable homologs of this gene (using *Cyanidioschyzon* and *Arabidopsis* sequences as queries) are found only in bacteria and eukaryotes, with all top hits being from beta and gamma-proteobacteria. This disjunct distribution suggests that the eukaryotic sequences are likely of bacterial origin. Phylogenetic analyses show a common ancestry of sequences from red algae, green plants and gamma, beta-proteobacteria. The gene was likely transferred independently to two groups of eukaryotes, one to the ancestor of red algae and green plants, another to the bacteriolytic *Reclinomonas*. *Reclinomonas* and *Aedes* share several conserved residues and their grouping together likely resulted from eukaryote-to-eukaryote gene transfer. The *Arabidopsis* gene product is localized in cytoplasm according to GenBank annotation. Sequence of *Reclinomonas* was obtained from TBESTDB.

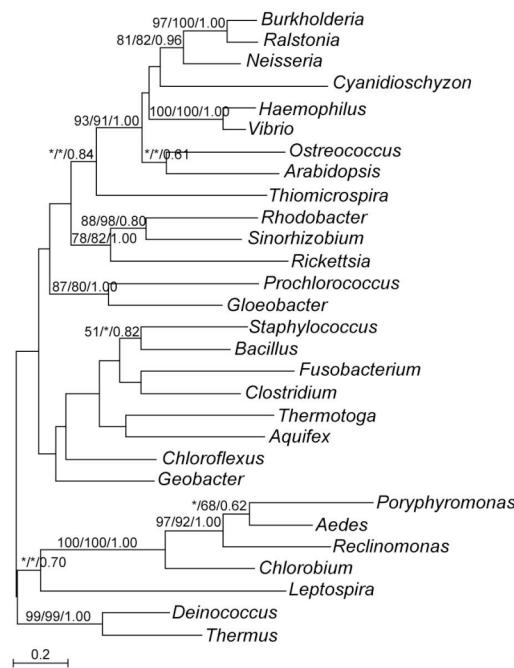


Figure 18. Molecular phylogeny of ribosomal protein L11 methyltransferase. P-value = 0.959 from AU test for the presented gene tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of red algal, green plant, and cyanobacterial sequences. P-value < 0.001 from AU tests for topology A and p-value = 0.041 for topology B.

19. GTP binding protein typA (D)

CLUSTAL X (1.83.1) multiple sequence alignment

34762648_Fusobacterium_nuclea
28210762_Clostridium_tetani
6458935_Deinococcus_radiod
51892172_Symbiobacterium_therm
67920868_Crocospaera_watsonii
16329356_Synechocystis_sp.
33861319_Prochlorococcus_marin
69247248_Enterococcus_faecium
16078541_Bacillus_subtil
21674245_Chlorobium_tepidu
57169035_Campylobacter_coli
15611499_Helicobacter_pylori
45658203_Leptospira_interr
39995607_Geobacter_sulfur
24375888_Shewanella_oneide
16272804_Haemophilus_influe
59801217_Neisseria_gonorr
30250473_Nitrosomonas_europa
32474280_Rhodopirellula_baltic
53715742_Bacteroides_fragil
48854524_Cytophaga_hutchi
91201932_Candidatus_Kuenenia
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30684509_Arabidopsis_thalia
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53795725_Chloroflexus_aurant
113940020_Herpetosiphon_aurant
15608305_Mycobacterium_tuberc
94971430_Acidobacteria_bacteri
46446750_Protochlamydia_amoebo
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42733852_Dictyostelium_discoi
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79323586_Arabidopsis_thalia
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*: *:*****: : * : : * *: * .

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46446750_Protochlamydia_amoeba
 89284086_Tetrahymena_thermoph
 42733852_Dictyostelium_discoi
 34909598_Oryza_sativa
 79323586_Arabidopsis_thalia
 15889919_Agrobacterium_tumefaciens
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51473460_Rickettsia_typhi
56696900_Silicibacter_pomeroy
23612528_Plasmodium_falcip

34762648_Fusobacterium_nuclea
28210762_Clostridium_tetani
6458935_Deinococcus_radiod
51892172_Symbiobacterium_therm
67920868_Crocospaera_watsonii
16329356_Synechocystis_sp.
33861319_Prochlorococcus_marin
69247248_Enterococcus_faecium
16078541_Bacillus_subtil

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94971430_Acidobacteria_bacteri
46446750_Proteobacteria_amoebophil
89284086_Tetrahymena_thermoph
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51473460_Rickettsia_typhi
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23612528_Plasmadium_falcip

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113940020_Herpetosiphon_aurant

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AEMKTM A P T Q T R L E F E I P A R G L I G F R S Q F L T D T K G E G V M N H S F L E F R P F S
AEMKAMNP M Y T R L E F E I P A R G L I G Y R S E F L T D T K G E G V M N H S F L E F R P F S
GELQLM DAHM TR V E F V I P T R G F G I S E T R G E G V M S S F L R F D V Y K
GEMTS L Q P M T V R L E F V V P A R G L G I L R G E L L T E T R G T A V M T H T F H D Y A P Y K
AEMK D M Q L D R V R V D F V I P S R G L I G F Q T E F L T A T S G T G L I Y H S F D H Y G P H K
GEVRDMLPDRVRLEYI I P S R G L I G F R G D F M T M S T G T G L L Y S S F S H Y D E I K
GELTNM E S D R T R L E Y H I P A R G L I G F Q G E F M T L T R G V G L M S H V F D D Y A P V K
GDLLDMV T D R V R L D Y R I P A R G L I G F Q S E F M T L T R G T G I M S H V F D E Y A P M R
GQLEEMK Q R Y S L L R F I V P S R G L I G L R T R L L N A T R G T A I I H H R F E S Y R M V E
GEMTM M E N T R I N L E F D M P S R G I I G L R T N V L T A S A G E A I M A H R F K E Y Q P F K
GELLI M E P K L Q H L E F K I P S R G I I G L R T N V L T A T F G E A I M T H R F D G Y E P Y K
GEI V S M K T E Y T H F E F K A P S R G L I G L R N R L L S S T R G E A V M H H N F D Y E P Y K
GQMFD M Q G V T T F L R Y K I P T R G L L G I L R N A I L T A S R G T A I L N T V F D S Y G P W A
GQMFD M Q G V T T F L R Y K I P T R G L L G I L R N A I L T A S R G T A I L N T V F D S Y G P W A
GQMVD M Q P S T T L L K Y K I P T R G L L G I L R N A I L T A S R G T A I L N T V F D S Y G P W A
GEMLN L T H S Y S I V E Y I V P T R G L L G I L R N A I L T A S R G T A I L N T V F D S Y G P W A
GQM RDMS I R L V H M I F V P T R G L L G F R Q T F L T A T R G T G I M H S I F F G Y E P Y A
GQM RD M R I S S V H V Y V I L V P T R G L L G F R Q L F L T A T R G T G I M H S I F F G Y E P Y A
GTM RDMS V S S V Q F V Y H V P T R G L L G F R Q Q L L T A T R G T G I M N S L F A G Y Q P Y A

15608305_Mycobacterium_tuberc
94971430_Acidobacteria_bacteri
46446750_Protochlamydia_amoeba
89284086_Tetrahymena_thermoph
42733852_Dictyostelium_discoi
34909598_Oryza_sativa
79323586_Arabidopsis_thalia
15889919_Agrobacterium_tumefac
51473460_Rickettsia_typhi
56696900_Silicibacter_pomeroy
23612528_Plasmadium_falciparum

34762648_Fusobacterium_nucleatum
28210762_Clostridium_tetani
6458935_Deinococcus_radiodurans
51892172_Symbiobacterium_thermophilum
67920868_Crocospaera_watsonii
16329356_Synechocystis_sp.
33861319_Prochlorococcus_marinus
69247248_Enterococcus_faecium
16078541_Bacillus_subtilis
21674245_Chlorobium_tepidum
57169035_Campylobacter_coli
15611499_Helicobacter_pylo*r*
45658203_Leptospira_interrae
39995607_Geobacter_sulfuraceus
24375888_Shewanella_oneidensis
16272804_Haemophilus_influe*n*
59801217_Neisseria_gonorrhoeae
30250473_Nitrosomonas_europa
32474280_Rhodopirellula_baltica
53715742_Bacteroides_fragilis
48854524_Cytophaga_hutchinsonii
91201932_Candidatus_kuenenia
30684514_Arabidopsis_thalia
30684509_Arabidopsis_thalia
50906979_Oryza_sativa
CM3614_Cyanidioschyzon_merolae
106888453_Roseiflexus_sp.
53795725_Chloroflexus_aurantiacus
113940020_Herpetosiphon_aurantiacus
15608305_Mycobacterium_tuberculosis
94971430_Acidobacteria_bacteri
46446750_Protochlamydia_amoeba
89284086_Tetrahymena_thermoph
42733852_Dictyostelium_discoi
34909598_Oryza_sativa
79323586_Arabidopsis_thalia
15889919_Agrobacterium_tumefaciens
51473460_Rickettsia_typhi
56696900_Silicibacter_pomeroy
23612528_Plasmodium_falciparum

34762648_Fusobacterium_nucleatum
28210762_Clostridium_tetani
6458935_Deinococcus_radiodurans
51892172_Symbiobacterium_thermophilum
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33861319_Prochlorococcus_marinus
69247248_Enterococcus_faecium
16078541_Bacillus_subtilis
21674245_Chlorobium_tepidum
57169035_Campylobacter_coli
15611499_Helicobacter_pylo*r*
45658203_Leptospira_interrae
39995607_Geobacter_sulfuraceus
24375888_Shewanella_oneidensis
16272804_Haemophilus_influe*n*
59801217_Neisseria_gonorrhoeae
30250473_Nitrosomonas_europa

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AEMTNMRPSKSRSITFIGPSRGLIGYQNLTETRGTGIINRIFYGYTDYK
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* : * : * . : * . : * . : * . : *

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GEIPTQRQGSLLVASETGTATQYALYQI0ERGTLFIEPGTEVYVGMVGEN
GELETTRYNGVIIAEEGTSTFYAMKNAEDRGVFIIPGTVKVKGMIIGEN
GDLTRYNGVMVAEEGVATFYAMKNAEDRGVFIITPGTVKVKGMIIGEN
GDFETRNRNGLISFEEGVATFYALKNAEDRGVFIKPGVVKVKGMIIGEN
GQIIGRRHQGLVISDTGKATTYSIMSIEERGTVFVEPGTEVYEGMIIGEN
GQVGRGROGVLVSMENGKATSQYQI0EDRGVIFVEPGTEVYEGMIVGEH
GKLPSRETGALVSAETGVAVAYAISSLEDRGTFFIGPNAKVEGMMVGES
GAVEKRNNALISMENGVALGYSLFNLQERGVLFIEPQTKVTGMIIGEN
GSVESRKNGALISMNGEATAFSLFNIQERGALFINPOTKVVVGMVIGEH
GDIPIGRKNGALISMNGESTAYALWKIQERGELVIGPNTSVPPGMIIIGH
GDIPIGRKNGVLIALEMGETTAYALDALQPRGILFIGPGVEVYGGMIIGQH
GDIQRANGVLISNATGKALTFLGQLDRGRLFIGHAAEVYEGQVVGIIH
GEIGQRKNGVLISNATGKALGYALFGLQERGKLMIDANIEVYEGQIIGH
PDMPGRHNGVLVSQEQQGEAVAYALWNLEDGRMFVSPNDKIYEGMIIIGH
PDMASRKNGVLISAEMGEAVAYALWLQERGRMFVSPGEPLYEGMIVIGH
GDVPRRANGVLISMVGKTMFPALFALQDRAELFVPPSTEVYEGMIVGEN
GDIERRNTGSIAMESGTAFAIDKLQDRGKFFIFPQEEVYAGQVVGIIH
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GDISTRDLGSLVAFEDGTSTSALASAQERGOMFVGSGVDVYKGQIVGIH
GDISTRDLGSLVAFEDGTSTSALASAQERGOMFVGSGVDVYKGQIVGIH
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GTIPGRTRGVLLSNPGKTSQYACFNLDQDRGVMFVAPGDEVYEGMVVGEN
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* : * : * . : * . : * . : * . : * . : * . : * . : *
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SRGQDMDVNCVCKTKHLTNIRAAGADEKLLLDPPRKLTLEEALQIADDEL
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NRSQDLELNICKTKQLTNMRSAGAEEELDTLQSPVDITLERALEYIIGPDEM
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SRPNLDVNPPIKGKNLTVNRASGSSDAIKLTPPPRTMVLEALEWIEEDEL
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ARSNDLTVNCLKGKQLTNMRASGTDQAQVLTTPITLTLQALEFIDDDDEL
SRSNDLTVNCQGKLTNMRASGKDDAIVLTTTPVFKFSLLEQAIIEFIDDDDEL
SRDNDLTVNPPLKGKLTNIRASGTDEAVRLTTTPIKLTLEGAVEFIDDDDEL
SRENLDLVNPPIKGKQLTNIRASGHDEAVVLTPPIQLTLESAIEFIADDEL

32474280_Rhodopirellula_baltic 53715742_Bacteroides_fragil 48854524_Cytophaga_hutchi 91201932_Candidatus_Kuenenia 30684514_Arabidopsis_thalia 30684509_Arabidopsis_thalia 50906979_Oryza_sativa CM3614_Cyanidioschyzon_merolae 106888453_Roseiflexus_sp. 53795725_Chloroflexus_aurant 113940020_Herpetosiphon_aurant 15608305_Mycobacterium_tuberc 94971430_Acidobacteria_bacteri 46446750_Protochlamydia_amoebo 89284086_Tetrahymena_thermoph 42733852_Dictyostelium_discoi 34909598_Oryza_sativa 79323586_Arabidopsis_thalia 15889919_Agrobacterium_tumefa 51473460_Rickettsia_typhi 56696900_Silicibacter_pomeroy 23612528_Plasmadium_falcip	ARENDEMTVNPCTREKKLTNMRASGSDENVLKPPRDMSEAALYEYIEDDEL AHEKDLVNVNTKSLLTNMRASGSDEKARLIPPVQFSLEEALEYIKEDEY NRQNDITVNVRQREKKLTNMRASGTTDDNAKIAPKINFSLLEMMEYIQLDEY CEQNIDIVNVIREKKATNIRQATGEKSVKLSPHREFSLEMALEYIEEDDEL QRPGDGLNLICKKKAAATNIRSN-KDVTVIDTPTLYSLDDCIEYIEEDEL QRPGDGLNLICKKKAAATNIRSN-KDVTVIDTPTLYSLDDCIEYIEEDEL QRPGDLAINVCKKKAAATNVRSN-KETTVVLDEALSYSLDDCIEFIQEDEL KYPEDLRVNVCRVKQLTNHRSATKEVVKTLOGIRTTLQDDALEYISENEF IRERDLEVNVCRRKHLTNIRSSTAEEGIRLETPRILSLLDAIEYISDDEL IRDNDLEVNVCKEKKHLTNMRNNRAETIRLDAPRQLSLDDAIEYISDDEL IRDEDLEVNAACKKKQLTNMRSSGADDALRLDPRNMSLDDCIEYLADEL PRPEDLDINVTRKKTLMRNSSTADVIETLAKPLQLDLERAMELCAPDEC SRDNNDLVNCVREKKLTNMRASTSDAIRLVPFKNLNLEQAIIDFIADDDEL SRDNDLVNVTKGQLTNVRASGSDENIILIPARRFTLEQAIIDYIQDDEL QKESEVELNPTKKELNNIRTKSHEEKIVLQPHRVSIEEAICYIRDDEI SKLTLDVNPVKSQLTNIRTTRVKEEGIRLSPPKLLKLEEAIACVKEDDEL SRDSLDLNPVRTKELTNIRAPGKDENVRLSPPLMSLEAIGYVADEL SRETDLNPKAKELTNIRSAAGKDEAVKLTPPIRMTLDRALSWIQEDEL TRDNNDLEVNLKGKQLTNIRAAGKDEAVKLTPPIRMTLDRALSWIQEDEL NRDNDLVNPPLAKQLSNVRAVGKDEAIRLTPPMLLTLEQAIISYIQDDEL SRDNNDLVNPPLAKQLSNVRAVGKDEAIRLTPPMLLTLEQAIISYIQDDEL FLSNDIEMNAIKVKPVQHRLRNKGHEDTIRIN-HKNITIEYALSFIQDDEE : : * : * : * : : : : : *
34762648_Fusobacterium_nuclea 28210762_Clostridium_tetani 6458935_Deinococcus_radiod 51892172_Symbiobacterium_therm 67920868_Crocospaera_watsonii 16329356_Synechocystis_sp. 33861319_Prochlorococcus_marin 69247248_Enterococcus_faecium 16078541_Bacillus_subtil 21674245_Chlorobium_tepidu 57169035_Campylobacter_coli 15611499_Helicobacter_pylori 45658203_Leptospira_interr 39995607_Geobacter_sulfur 24375888_Shewanella_oneide 16272804_Haemophilus_influe 59801217_Neisseria_gonorr 30250473_Nitrosomonas_europa 32474280_Rhodopirellula_baltic 53715742_Bacteroides_fragil 48854524_Cytophaga_hutchi 91201932_Candidatus_Kuenenia 30684514_Arabidopsis_thalia 30684509_Arabidopsis_thalia 50906979_Oryza_sativa CM3614_Cyanidioschyzon_merolae 106888453_Roseiflexus_sp. 53795725_Chloroflexus_aurant 113940020_Herpetosiphon_aurant 15608305_Mycobacterium_tuberc 94971430_Acidobacteria_bacteri 46446750_Protochlamydia_amoebo 89284086_Tetrahymena_thermoph 42733852_Dictyostelium_discoi 34909598_Oryza_sativa 79323586_Arabidopsis_thalia 15889919_Agrobacterium_tumefa 51473460_Rickettsia_typhi 56696900_Silicibacter_pomeroy 23612528_Plasmodium_falcip	VEVTPTNIRLRK VEVTPISIRMK VELTPQSIRLRK VEITPKSIRLRK VEVTPESIRLRK VEITPESIRLRK LEVTPDSIRMRK CEVTPESIRLRK CEVTPESIRLRK LEVTPENIRRK VEVTPQNVVRK LEVTPLNLRIRK LEVTPTSIRLRK VEVTPQSIRLRK VEVTPKSIRVRK VEVTPESIRRK VEVTPESIRRK VEVTPQSIIRRK VEITPKSIRRK VEVTPESIRLRK VEVTPKAMRMK IEVTPKSIRMRK IEVTPKTFRLRK VEVTPSSIRMCK VEVTPSSIRMCK VEVTPASIRMCK VEVTPQSIRMFK VEVTPKSIRLRK VEVTPKGWRIRK LEVTPLFRLRK VEVTPEIVRIRK CEVTPKTLRLRK IEVTPDSIRMRK VEVTPKEIRIRK IEITPKSIRLRK IEASFYLLLM IEVTPKTIIRLRK MEVTPKSIRLRK VEVTPKSIRLRK VEVTPNTIRLRK IEVTPKRIVMRK * : :

Note: This gene is distributed in bacteria and various eukaryotes, including several protists. Sequences from TBESTDB are too short for phylogenetic analyses. There are multiple versions of this gene in photosynthetic eukaryotes, one of which forms a monophyletic group with homologs GNS bacterial sequences with strong support (middle part of the tree). The other eukaryotic sequences group with alpha-proteobacterial homologs, likely resulting from intracellular gene transfer from mitochondria to the nucleus. Protein products of GNS bacteria-related genes (GIs: 30684514 and 30684509) are targeted to chloroplasts and intracellular components in *Arabidopsis*.

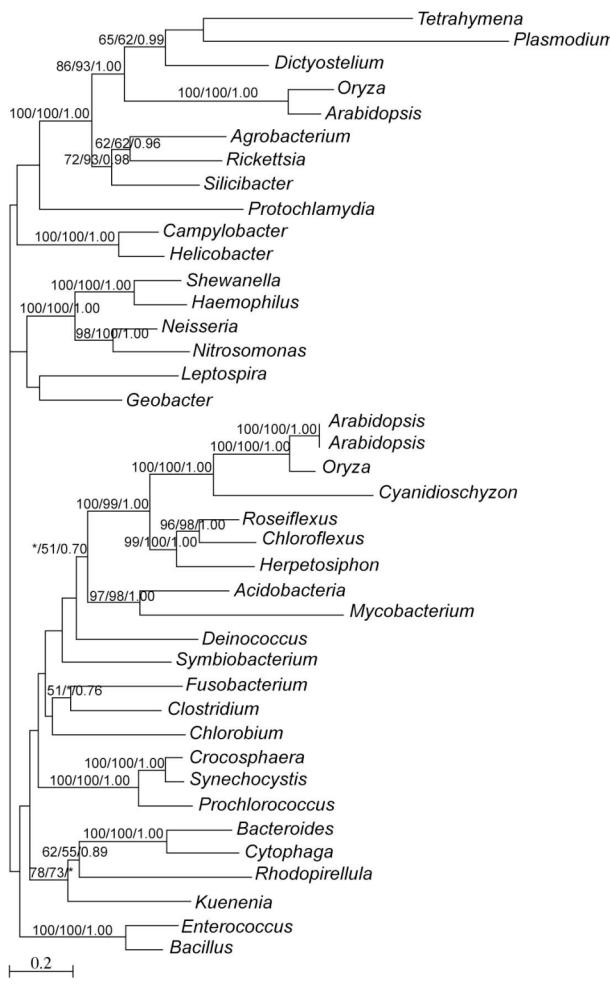


Figure 19. Molecular phylogeny of GTP binding protein, typA. P-value = 0.811 from AU test for the presented gene tree. AU tests were performed on alternative topologies including **(A)** monophyly of all eukaryotic sequences, **(B)** monophyly of red algal, green plant and cyanobacterial sequences, and **(C)** monophyly of red algal, green plant, cyanobacterial, and chloroflexi sequences. Topologies **A** and **B** investigate if red algae and green plants acquired the gene from mitochondria or plastids respectively, without invoking scenarios of secondary HGT. Topology **C** investigates if red algae and green plants acquired their genes from plastids and subsequently spread to chloroflexi through secondary HGT events. P-values < 0.001 from AU tests for topologies **A** and **B** whereas p-value = 0.195 for topology **C**. Therefore, the scenario of a plastidic origin in red algae and green plants and secondary HGT, although less parsimonious, cannot be confidently rejected.

20. Histidinol-phosphate transaminase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

14601796_Aeropyrum_pernix
37522992_Gloeobacter_violac
16330049_Synechocystis_sp.
45507495_Anabaena_variab
45655703_Leptospira_interr
34762988_Fusobacterium_nuclea
118725567_Clostridium_cellulo
13474816_Mesorhizobium_loti
57234598_Dehalococcoides_ethen
15643798_Thermotoga_mariti
15807448_Deinococcus_radiod
53764865_Anabaena_variab
16330453_Synechocystis_sp.
27381336_Bradyrhizobium_japoni
15599643_Pseudomonas_aerugi
27376508_Bradyrhizobium_japoni
45358779_Methanococcus_maripa
118727390_Clostridium_cellulo
29609816_Streptomyces_avermi
21674079_Chlorobium_tepidu
37520892_Gloeobacter_violac
45508256_Anabaena_variab
15607044_Aquifex_aeolic
48767779_Ralstonia_metall
57234394_Dehalococcoides_ethen
CM4361_Cyanidioschyzon_merolae
CP000006602_Cyanophora_paradox
118046429_Chloroflexus_aggreg
145342157_Ostreococcus_lucima
15217609_Arabidopsis_thalia
18416245_Arabidopsis_thalia
79327596_Arabidopsis_thalia
145334365_Arabidopsis_thalia
48855138_Cytophaga_hutchii
53714475_Bacteroides_fragil
32411469_Neurospora_crassa
57223533_Cryptococcus_neofor
57506033_Campylobacter_upsali
45658350_Leptospira_interr
29607917_Streptomyces_avermi
13475919_Mesorhizobium_loti
42522616_Bdellovibrio_bacter
16079319_Bacillus_subtil
118046536_Chloroflexus_aggreg
15598361_Pseudomonas_aerugi
48771086_Ralstonia_metall

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LDRLDTNESPCDLPRYPDGGHEELKDAIAQYITAANISVGNGSDELIRSL
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IIKLDANEENPYGCVCIYPDPESRELREALAAFVPAASRLLVGHGADELIDLI

IVKLDANEENPYGPAIYDPEQTALESALASYRPIEEILCGAGADELIDL
IVKLDANEENPYGPPPIYDPESRRLAALAAEVPTEENLLVGCGADELIDL
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IVKLDANEENPYGPPVYDPQSRRRLDQAQDLESEYILVGCGADELIDL
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GILLDANEENAIGPSRYPSPTHDELKREIAKLPDENVFLGVGSDEVIDML
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IVKLASNENPLGASRYPDGNGFELKKLRAERVDAAQVTLGNGSNDILD
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14601796_Aeropyrum_pernix
37522992_Gloeobacter_violac
16330049_Synechocystis_sp.
45507495_Anabaena_variab
45655703_Leptospira_interr
34762988_Fusobacterium_nuclea
118725567_Clostridium_cellulo
13474816_Mesorhizobium_loti
57234598_Dehalococcoides_ethen
15643798_Thermotoga_mariti
15807448_Deinococcus_radiod
53764865_Anabaena_variab
16330453_Synechocystis_sp.
27381336_Bradyrhizobium_japoni
15599643_Pseudomonas_aerugi
27376508_Bradyrhizobium_japoni
45358779_Methanococcus_maripa
118727390_Clostridium_cellulo
29609816_Streptomyces_avermi
21674079_Chlorobium_tepidu
37520892_Gloeobacter_violac
45508256_Anabaena_variab
15607044_Aquifex_aeolic
48767779_Ralstonia_metall
57234394_Dehalococcoides_ethen

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GTVLPIPAFQFDYRRAAFLDAPYISVFSLPGDAVMLANPHNPSGYLYPT
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GTVLITPAFQFDYRTLRAYHARVLEFRVLGNYALLNNPHNPTGKLFTR
PALIAVPCYSGYKEAISLLKIPCIEVFRLDKALVFLGHPNPTGVTLDKI
IVLILAPCFAEYERALKSVSAKIEFFYPNYDLLLFCNPNNPTGQFIKE
ATLLTAPTFSEYEEAVKFDSNITYHFNDIRLMLCNPNNTGILTEKE
YVAMMSPGFSFNRLKATLQAGAQFVEIHSPLAKFILLANPNNPTGTFVPVA
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MSVFFPPTYSCYRIFAKAVGAKFLEVRLPGDVVFIPNPNNTGHFVFERE
TVLTTDPTFSVYTLAALMGAELVLTFSLPPGVFYVTOPHAPTHGSDRPE
IVVYPMPTVYVYRTLTMQAADILEINVPLGAVTFIASPNPSGHHIVPND
VVAFLDLTYSLYETIASVHGAKVQKIFDLTAKLIFLASPNNPKGKHNLRE
FLLFPDVTVSFYVCPVCRYLGVAHEEVMRIESSAIIICPNPNPTGIALPRD
FLLFPDVTVSFYVCPVYCGLYGIAHEKIFRIRGGIIF-PNPNAFTGCLLPLD
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AVLYPTPSFGMYKDSCVIAGGRAVDYFSYSPKVIYICNPNNTGPNLMPQD
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FVLIPEPSFSLYDKLARLQAGVVEVLSFDVDFIVLSTPNNTGSKLSHD
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LILVANPTFSMYGILAQTGLGIPVVTVFEIDIRVVFVVPNSPTANPL
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ISKE

CM4361_Cyanidioschyzon_merolae
CPO00006602_Cyanophora_paradox
118046429_Chloroflexus_aggreg
145342157_Ostreococcus_lucima
15217609_Arabidopsis_thalia
18416245_Arabidopsis_thalia
79327596_Arabidopsis_thalia
145334365_Arabidopsis_thalia
48855138_Cytophaga_hutchi
53714475_Bacteroides_fragil
32411469_Neurospora_crassa
57223533_Cryptococcus_neofor
57506033_Campylobacter_upsali
45658350_Leptospira_interr
29607917_Streptomyces_avermi
13475919_Mesorhizobium_loti
42522616_Bdellovibrio_bacter
16079319_Bacillus_subtil
118046536_Chloroflexus_aggreg
15598361_Pseudomonas_aerugi
48771086_Ralstonia_metall

14601796_Aeropyrum_perinx
37522992_Gloeobacter_violac
16330049_Synechocystis_sp.
45507495_Anabaena_variab
45655703_Leptospira_interr
34762988_Fusobacterium_nuclea
118725567_Clostridium_cellulo
13474816_Mesorhizobium_loti
57234598_Dehalococcoides_ethen
15643798_Thermotoga_mariti
15807448_Deinococcus_radiod
53764865_Anabaena_variab
16330453_Synechocystis_sp.
27381336_Bradyrhizobium_japoni
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57223533_Cryptococcus_neofor
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29607917_Streptomyces_avermi
13475919_Mesorhizobium_loti
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16079319_Bacillus_subtil
118046536_Chloroflexus_aggreg
15598361_Pseudomonas_aerugi
48771086_Ralstonia_metall

14601796_Aeropyrum_perinx
37522992_Gloeobacter_violac

FVVNAPPTFGMYQFDADLNAAKVVSVFRFPPRIVFVTCPPNNPDGSRISDS

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CVLINRITFAMYKIYALOCGAKIYSTAFLCDPDKIIFLCTPSNPAGDALKS
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DLREL GALAVVVDES FADLS--GVIVARS LTKTLALQGLRIGFAYDDA AL
ALA ALRF KLLIFDEA FIDFLHPGLIV RSLTKFYSLPGLRLGYAVLPVAM
EILALHYQWVLLDEAFMDFLHPNL IILRSLTKFYRLPGLRLGYALAH PDL
A ILPLQF ALVVVDEAFMDFVQPNL VRLT KFYSLPGLRLGYAIAH PDR
EVLK IONS VFV IDES FPI HCFCTENMILI QSM T KI ALP GLRIG I CYASPLI
DIKEIK NTRI FIDE A FIE FI -KNIFIMRAFTKFAI PGL RLGYGV F DDEI
MVL RITNT I L VV D ECF M EFL YD NII V LKAFT KI YAMAGL RLGY G ISDENI
DIE RLS DR L I L V D E A Y V D F A Y S N L L V L R T F S K S Y A A A G V R V G F G H P E I
DIE KV TD L L V L D E A Y I A F A -G N I I V R S M T K D C A L A G L R L G Y G M A S A E I
E I E R I T G A F V A L D E A Y Y E F H Y E N L A V I R T F S K A F S L A A Q R V G Y V V A S E K F
D V R V L G W V T V D I E A Y S Q Y A G K H V L S L R T F S K A W G L A V R A G Y L L T N P E L
D L R K L L S G V L V D I E A Y V D F A Y E N V I L I R T L S K G Y S L A G L R L G F G V G N P K L
F L W Q T A E G V V V D I E A Y G D F S F D N V I I S R T L S K S Y S L A G M R V G L A I A A P A L
A V A A P L D P R L V V V D E A Y V D F G H D N L L V I Q T F S K S R A L A G L R V G F A I G Q R P L
A I E A M P D S V V V D E A Y V D F G Y P N L V I T Q T L S K S R S L A G L R V G L A V G H A D L
E V K R L S H V L L V L D A Y C D Y T E N T V V T H T F S K I H G L A A R I G W M F G P E H I
D I K K I T D A L V M D H A Y I E Y S D N V L V L R T F S K V F G L A Q R V G Y G V T S K K V
E I L R V T K S V V V V D E A Y A E F S Y E N L L I L R T F S K A F G L A G I R C G Y S I A S E K L
T V L A L K P S M V V V D E A Y I E F S R P H L V V S R T M S K A F G A A G L R L G Y L A A H P A V
E I E R I A D A I V L V D E A V V E F S Y P N L V I L R T M S K A L A L A G M R I G F A I A N P E L
I I E R L L P V L V V L D E A Y Y E F S R P N L V I L R T F S K A F R L A N F R V G Y A L A N P E I
E L R W L E Q I V V V D E A Y Y E F F S R P N W V I L R T F S K A F R L A A M R V G Y C V A H P E A
K I E I R G V F C V I D E A Y Y E F F S R P N W V I L R T L S K I -G M A S L R V G I L I G K G E I
D M A A I C Q S L V V V D E A Y Q P F A F G N L L V M R T V S K -L G L A G I R L G Y V A G A P E W
D I R Q I L G V P T V D I E A Y Y E F T Y P N L M I L R T F S K W A G L A G L R V G Y G L F P P V I
D L R R L L P T L V V L D E A Y I E F A Y Q N L I V L R T F S K W A A L A G L R V G Y G A F P E A L

H E N L V L L R T F S K M A G L A G M R V G Y G A F P L H V
D L L R I L P I L V V V D E A Y V E F A Y P N L V I L R T F S K W A G L A G L R V G Y G L F P R W L
D L L R L P P I L V V L D E A Y I E F A R K N L I V L R T F S K R A A L A G L R V G Y G A F P S D L
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D L L K I M P I L V V L D E A Y I E F S Y E N L I V L R T F S K R A G L A G L R V G Y G A F P L S I
D L L K I M P I L V V L D E A Y I E F S Y E N L I V L R T F S K R A G L A G L R V G Y G A F P L S I
D L L K I M P I L V V L D E A Y I E F S Y E N L I V L R T F S K R A G L A G L R V G Y G A F P L S I
K V K R I F G S I V V V D E A Y I D F A Y P N M V L L Q T F S K A W G M A A L R L G T A F A S K E I
E I I K V F E G L V I L D E A Y N D F S Y P N L I I L Q T F S K A F G C A A I R L G M A F A S E G I
D I Q K V W N G V V V V D E A Y I D F A W P N L V V M O T L S K A F G L A G I R L G A A F T S P P I
V I K R I F G V V V V D E A Y D F S Y A N I C V S Q T L S K S F G L A A I R L G Y L L A P P L
S V E E F E C L I V I D A A Y N E F A P P N V L Y L G T F S K L Y G L G G L R V G Y G M A D E E L
D V Y E F L D T L V V I D A A Y M E F G F P N V Y T G T F S K V Y G L G G M R I G Y G I G N Q E L
E L E R F S D V L V V L D E A Y R E F I R P N V C V L R T F S K A Y G L A G L R V G F A I A H E P V
D L S K V D E T L I V V D E A Y A E Y A D Q P W I V L R T F S K A F G L A G L R I G F G I G D P E L
E V E A F D D V M I I F D E A Y N E F V Y K N L I V L R T F S K I Y G L A G F R L G A M I A P P E V
E L L A F S R V L V V L D E A Y Y E V Y S N L M I L R T F S K A Y G L A A L R V G Y G I A D E N L
E M A A F D D V L V V V D E A Y I E F V R D N L L L R T F A K I H G L A G L R L G Y A F G A P D L
A L E R F A E V L V V L D E A Y I E Y A H P N L L V S R T F S K A Y G L A S L R V G Y A L S S K A V
E I E A F P H V V V V L D E A Y N E Y L Y P N L M V S R T F S K A Y G L A G L R V G Y G V A Q P Q L

: : * * *

A A R L D M A R Q P W N I A A L L V E R L L G D M E V K G H I A R A I E A I E G L K P R L Y S A V A
A A R W Q A W R D P W P V N A L A Q H L G V A A L A D I L D F E R R T R A W L G P A R S R L A G L L

16330049_Synechocystis_sp.
45507495_Anabaena_variab
45655703_Leptospira_interr
34762988_Fusobacterium_nuclea
118725567_Clostridium_cellulo
13474816_Mesorhizobium_loti
57234598_Dehalococcoides_ethen
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CP000006602_Cyanophora_paradox
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145342157_Ostreococcus_lucima
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32411469_Neurospora_crassa
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42522616_Bdellovibrio_bacter
16079319_Bacillus_subtil
118046536_Chloroflexus_aggreg
15598361_Pseudomonas_aerugi
48771086_Ralstonia_metallic

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14601796_Aeropyrum_perinx
37522992_Gloeobacter_violac
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27381336_Bradyrhizobium_japoni
15599643_Pseudomonas_aerugi
27376508_Bradyrhizobium_japoni
45358779_Methanococcus_maripa
118727390_Clostridium_cellulo
29609816_Streptomyces_avermi
21674079_Chlorobium_tepidu
37520892_Gloeobacter_violac
45508256_Anabaena_variab
15607044_Aquifex_aeolic
48767779_Ralstonia_metallic
57234394_Dehalococcoides_ethen
CM4361_Cyanidioschyzon_merolae
CPO00006602_Cyanophora_paradox

KPYSSQAPFILVRHRGVYIRLTPYHSRISVAPGERLERLQEAILD
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118046429	<i>Chloroflexus aggreg</i>	TPFPFSVANFILCRVNGVLVRLLDGYIRISVGTPDQTDLLATIAE
145342157	<i>Ostreococcus lucima</i>	EPFTSESNFILCRVKGVVIREIADCIRISVGRPEDDAVVSALAS
15217609	<i>Arabidopsis thalia</i>	NPYPYSNFI CEVTGVMRELKGYVRRSAGKPEHTDVLMECLKQ
18416245	<i>Arabidopsis thalia</i>	NPYPYSNFI CEVTGVMRELKGYVRRSAGKPEHTDVLMECLKQ
79327596	<i>Arabidopsis thalia</i>	NPYPYSNFI CEVTGVMRELKGYVRRSAGKPEHTDVLMECLKQ
145334365	<i>Arabidopsis thalia</i>	NPYPYSNFI CEVTGVMRELKGYVRRSAGKPEHTDVLMECLKQ
48855138	<i>Cytophaga hutchi</i>	ELYPSDANFILMRTAIVTRLCEGCRITVGTEAENKRLLDVLT
53714475	<i>Bacteroides fragil</i>	QVFPSANFFLARVTGIIIVRLCGNCLRVTGTRAENAKLIGALKK
32411469	<i>Neurospora crassa</i>	KLAGESNFI LLYEMLGVVVRGCEGCLRITVGTEEEVTRCLGALRE
57223533	<i>Cryptococcus neoform</i>	GLLGGNNANFVLCQIVGVVVVRGCEGCLRITVGTKEECKQATEHIAA
57506033	<i>Campylobacter upsali</i>	DFIPSYTNFITYFFKGIIIVRDLNA-IRISIGKPYENTRFFEEFES
45658350	<i>Leptospira interr</i>	EFDISYANFITFFARGIIILRDLNA-LRITIGRPEQNDLVLEALEK
29607917	<i>Streptomyces avermi</i>	TVPETQANFVWLRLGGVVVRGV----RTVGENEANDIFLKVTTER
13475919	<i>Mesorhizobium loti</i>	DVAPSQRGNFLFFFNCRGVIVKGFDSYVRVSIGSPAENDHFMAALSQ
42522616	<i>Bdellovibrio bacter</i>	PYIPSQGNFVMFDTLGIIMRGFKTHLRLSGVRDHNEAAAMVALAE
16079319	<i>Bacillus subtil</i>	KCYPQTNFVLIDFKGYIVRGFPTSLRITIGTKEQNEEILAILAE
118046536	<i>Chloroflexus aggreg</i>	TVIPGETNFIAVAVHGVLVTGLPGWIRISFGTEEENDACIAALQA
15598361	<i>Pseudomonas aerugi</i>	SWIPSKGNFIAVDLAGIVVRGMPTFLRVSIGLPEENDRFLQALGK
48771086	<i>Ralstonia metall</i>	EYVKSSGFVMVRVGGIVVRGLPQWLRVTIGLPEENDAFIAALEQ

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Note: Identifiable homologs of this gene are only found in bacteria, primary photosynthetic eukaryotes and fungi. Protein sequences of primary photosynthetic eukaryotes and chloroflexi have the highest percent identity and they also share many conserved residues. As expected, these sequences also form a well-supported group in phylogenetic analyses. Fungal sequences have the highest percent identity with those of CFB bacteria and form another group with them. It is likely that primary photosynthetic eukaryotes and fungi acquired this gene from chloroflexi and CFB bacteria, respectively. The *Arabidopsis* sequence (GenBank accession number NP_568226 and TAIR locus AT5G10330) is annotated to be a chloroplast precursor. Sequence of *Cyanophora paradoxa* was obtained from TBESTDB.

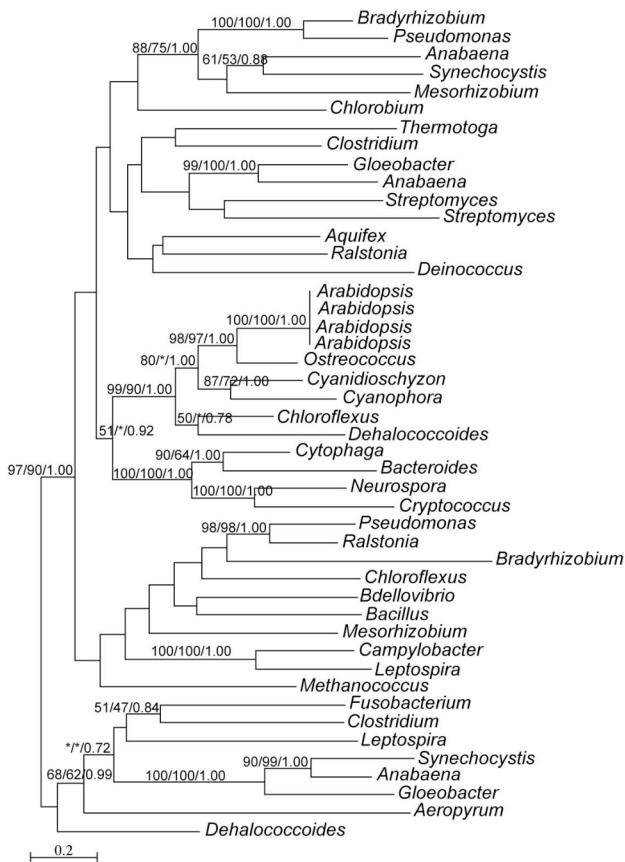


Figure 20. Molecular phylogeny of histidinol-phosphate transaminase. P-value = 0.999 from AU test for the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of red algal, green plant, glaucophyte, and different versions of cyanobacterial sequences. P-values < 0.001 from AU tests for both alternative topologies.

21. tRNA methyltransferase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

71030526_Theileria_parva 34540110_Porphyromonas_gingiva 53712700_Bacteroides_fragil CM4720_Cyanidioschyzon_merolae 116060467_Ostreococcus_tauri 15223816_Arabidopsis_thalia 149197386_Lentisphaera_araneos 2688619_Borrelia_burgdo 60464825_Dictyostelium_discoi 116054971_Ostreococcus_tauri 15612319_Helicobacter_pylori 15643286_Thermotoga_marit 53711506_Bacteroides_fragil 53715285_Bacteroides_fragil 15606458_Aquifex_aeolic 24214187_Leptospira_interr 34763143_Fusobacterium_nuclea 42526204_Treponema_dentic 6459532_Deinococcus_radiod 56752503_Synechococcus_elong 67922387_Crocospaera_watsonii 21674668_Chlorobium_tepidu 68230476_Frankia_sp 15827913_Mycobacterium_leprae 15604175_Rickettsia_prowaz 39934381_Rhodopseudomonas_pal 53795994_Chloroflexus_aurant 94969297_Acidobacteria_bacteri 39997664_Geobacter_sulfur 87306814_Blastopirellula_marin 149175981_Planctomyces_maris 34762114_Fusobacterium_nuclea 49651723_Yarrowia_lipoly 8134740_Homo_sapiens 21357477_Drosophila_melano RA000086770_Reclinomonas 62184976_Chlamydophila_abortu HVO00042429_Hartmanella_vermic 46447434_Protochlamydia_amoebo 106895797_Clostridium_sp 52141111_Bacillus_cereus 15597822_Pseudomonas_aerugi 50121369_Erwinia_carotovora 38703944_Escherichia_coli	RVAVLVSGGVDSLLAIWLMKFDVHAFYLKVVTLHVLPFSKAYSEHILSGV DVAALVSGGVDSVVHRLCYKPAIFIYIRICPFEVVDLHKEYWERVVSYT DIAALLSGGVDSVVHLLCYKPTLFYIKILALEVVVLHREYWDNVAAYA EVAVLLSGGVDSAVAMASLLFRVQPFYLKIVQLETLSLQEEYWSRVVQYT RVAVLLSGGVDSVALALLRHECRAFTYLFQIVELETVPPLTDAYWNLVVSDS RVAVLLSGGVDSVALRLHHSCATAFLKIVPLEVVLHTDEYWERVVSYI KIAMLLSGGVDSVALLLKHEITAFLKLIPLQVPLQKEYMDRVVSYV KIAVLLSGGVDSVALYRIISNIKCYLKIVPVEIINFOKEYYNKVVSYT KVCIGMSGGVDSTITAKLLKFDVTGFVIKSIPMYKADFVKDYWNRFVDF SVVVLGSGGVDSAAIAAWALKADVTAAATTKNIPLTEIDFTREYWNDVFEPY KIAVLLSGGVDSSSYASLKHVELGYLKLIPLEVLDFQKDFKSAVYDEF KVGVALSGGVDSAVAVALYLLHVEFKAHMKTVEIEIVDVKEIFREKVIIEPF RVVLMGSGGTDSVVAALLLYEVTVGTVFRRIGHITYDARKVFQEIIDYF RVLVGMGGIDSTATCLMLQYEIVGVTMRVIEHYVADERVPFKDTIVKNF RVAVGMGGVDSSVTALLKHDVIRVTLRFIPHVLFSWEEIFKSKVIDYF KIIAVMGGVDSAVTAGLLMYEVIGVNLRTIPFYVIKMEKFQEKVIDRF KVGVMAMGGVDSSTVAYLLKYDIFGVTMKTIEHYVLDVRNEFKEKVMDF KVLVGLSGGVDSAVAACKLILYDVTGVTMQLIKHIVYDMRETFKTEIIDYF RVLVGMGGVDSVTAALLKYQVIGAMMRFVFPYLLDYREPQRHIVGPF RVVVLGSGGVDSVAAALLHYAVEGLTL--PVYHVVDSDRDFQEAIVDVY KVVVLGSGGVDSVAAASLQYDVGTLT--IAHHIVDTRDLFTENIIDYF HVIIGLSGGVDSAVAACLLIYHTVGLNIRVFPVFTLNLSAKFARDVVGYF RVLVAMGGVDSVAAARAHVHDVTVGVLALIPFYVWDLAERFETEVIDEF KVLVAMGGVDSVAAARMVHDVVGVLALIPFYVWDFAEKFQEDVIDNF TIVVAMGGVDSVAAAMLHHNVIGITLQLIPHYYLDYENKFKEVIDNF RVVVMAMGGVDSVAAALLKYDVGTLTQLIPHYYLDYESRFRESVIDSF ----MSGGDSSLAAALLHQVTGVTMHLIPFYVFNQREFRRHVIDYF TIAVAMGGVDSSTVAAMLRYNLVGLTMQLIPYYVNVQEDRFERDVVKPF ----MSGGDSSVAAALLKHEVIGISMQVIPFYVVFEEFFRLVIDDF ----MSGGDSSVAAHLLHEVIGVFMRHIPFYALNLQREFG-QIIDYF RVVLMAMGGVDSVAAAHLLEVIGVFMRSIPFHANFKDAFG-RIKDYF KVVIGMSGGVDSSVASYLLKYEVIGVTLNQIIHKVVNIRKDFENIVKF HVYVAMSAGVDSSVTAALMAGRVTGVFMNRIPAVNLSLEKEYWLFEPM HVVCALSGGVDSAVAALLRYQVTGVFMKNIPFHQVSYVKEYWNDVFSDF NVVVGSGGVDSAVSAHLLAFKVLGVFMNRVELRQVNVRREYWTAVFSQF SVAVGMGGVDSAVSAVYLLKYRVEGVFMNRNIECRQVDFVREYNNVFSF TVVVAMGGVDSVVAVYLLKYRVLGIMKNIPYYTVSFAREYRERVFSRF SVVVLGSGGVDSVSSVASYLLQYKVRGLYMRNIPYDIVSLEKEYWNLVFEPA TVVVGMSGGVDSSVSSVALLMKYNVIGLFMKNIPYYAVNFVENYRKQVFTQF KVVIGMSGGVDSSVAAALLKYDVGIFMKNIPYYSVNFEKEYWDRVFIFY RVVIGMSGGVDSSVAAALLKYDVGIFMKNIPYYAVNFKEQYWDKVFTYF RVVIGMSGGVDSSVSSVALLLYQVEGLFMKNIKLHTANFAAEYWDNVFEHF KVVIGMSGGVDSSVASYLLQYHVEGLFMKNIELHTVNFAAEYWDNVFELF KVVIGMSGGVDSSVSSVALLQYQVEGLFMKNIELHTVNFAAEYWDNVFELF : *.* ***: : : :	LDDYRKGEVPNPDPVLNCNSRIKGGEFFDYVASGHYATLLCLSNTLKDQTY VETVRRGLTPNPDMMCNKLIKFGCFDRIATGHYATTLSTA KDPVKDQTD IEKIRKGQTNPDPVMCNKLIKFGCFDLTATGHYATTLSTA KDPVKDQTD IDEARRGRTPNPDIMCNNSRIKGAFYALVASGHYARVLFCSPDIVKDQTY VEEIRRGRTPNPDPVLNCNSRVKFGAFFDRVASGHYAAVLVMSGDAFKDQTY IEEYRCGRTPNPDVLNCNTRIKFGAFYDYGSGHYAKVLELSQDMVKDQTY LAELKQGRTPSPDILCNERIKFGAFYDKVATGHYAQVLLRAPDPVKDQTY
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 60464825_Dictyostelium_discoi
 116054971_Ostreococcus_tauri
 15612319_Helicobacter_pylori
 15643286_Thermotoga_mariti
 53711506_Bacteroides_fragil
 53715285_Bacteroides_fragil
 15606458_Aquifex_aeolic
 24214187_Leptospira_interr
 34763143_Fusobacterium_nuclea
 42526204_Treponema_dentic
 6459532_Deinococcus_radiod
 56752503_Synechococcus_elong
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 21674668_Chlorobium_tepidu
 68230476_Frankia_sp
 15827913_Mycobacterium_leprae
 15604175_Rickettsia_prowaz
 39934381_Rhodopseudomonas_palu
 53795994_Chloroflexus_aurant
 94969297_Acidobacteria_bacteri
 39997664_Geobacter_sulfur
 87306814_Blastopirellula_marin
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 34762114_Fusobacterium_nuclea
 49651723_Yarrowia_lipoly
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 52141111_Bacillus_cereus
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 50121369_Erwinia_carotovora
 38703944_Escherichia_coli

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 34540110_Porphyrimonas_gingiva
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REFLGRYGIITVDGDEIGEHQGLMYHTLQORKGLGEEPWVVDKDVENN

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IIYVSFKIRHTPEFIKGIIISSEKLQGIAPIGQFGVIYCFGSGEI
IIVLVSVKIRHGPRSVAIALDEPESGTAPIQGQFAVFYCLGGVII
VYVVTVKVRHGENIYSCVTINQDDQGLAPGQYAVFYCLGGCGKI
VVFVSKVRHGPFGYSCVHLDEDDQGLAAGQFAFYCIGSGVII
IVYISVCLRHGPQLVEAIKLAEKDKTAPIQGQSCIFYCLGRGVII
IIYISIKIRHGEKKYCSISLNKKDQGQISPQFAIFYCLGGAKI
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VYVAAKARYASPLTPVATFDAPERAVTPGQALVLYCLGGAVI
ELIVGIKARYRSVPTKADEVFKEPYVAKGQALVYLLGGGVII
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EVVLAVKIRYRKQENHICRLHEPLSIAEGQAAFYLLGGGI
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24214187_Leptospira_interr
 34763143_Fusobacterium_nuclea
 42526204_Treponema_dentic
 6459532_Deinococcus_radiod
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 15604175_Rickettsia_prowaz
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 94969297_Acidobacteria_bacteri
 39997664_Geobacter_sulfur
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 HVO00042429_Hartmanella_vermif
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 106895797_Clostridium_sp
 52141111_Bacillus_cereus
 15597822_Pseudomonas_aerugi
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 38703944_Escherichia_coli

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 QVIVGCKIRYRHQPVCVRFRQPEKS VTPGQAVVFYVLGGGI
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 EIVLGARPRFSSTGFYGFKYNEENTHNAKGQHIVFFVVGGEI
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 DVFVAFRFRHQMALVPCVTAVQAVRALATGQFAVFYCLGSGKI
 TIYVAFRFQHTKPLVDCVELDAPLRAITPGQYAVFYCLGSARI

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 ILVYDAKFRYRQEDN KVL CDEPIRAITPGQAVVFYCLGGATTI
 VLLVGAKVRYRQSDQDCAVFDEPQRAVTPGQSVVFYCLGGGI
 ILYVAVKTRYRQADISCVRFDEPFAAVTPGQS A VFYCLGGGI
 ILIVAVKTRYRQTDIPCVIFDEPVAAVTPGQS A VFYCLGGGI

Note: This gene appears to be restricted to bacteria and eukaryotes. All top hits of GenBank searches are from *Borrelia*, *Lentisphaeria*, and CFB bacteria. Protein product of the *Arabidopsis* sequence (GenBank accession number NP_175542 and TAIR locus AT1G51310) is localized in both chloroplasts and cytoplasm. The major eukaryotic sequence group contains some mitochondrial precursors (e.g. *Homo* sequence) and is likely of mitochondrial origin, although it is not particularly related to alpha-proteobacterial homologs. Please also note that the green alga *Ostreococcus* contains two versions of this gene, one of which groups with the eukaryotic mitochondrial clade while the other version with sequences of red algae, *Theileria*, CFB bacteria and spirochaetes. Sequences of *Reclinomonas* and *Hartmanella* were obtained from TBestDB; these two sequences formed a group with *Homo*, *Dictyostelium*, and other eukaryotic sequences in preliminary phylogenetic analyses, but were excluded from detailed phylogenetic analyses because of their short length. *Theileria* is an apicomplexan parasite containing a plastid derived from an algal endosymbiont.

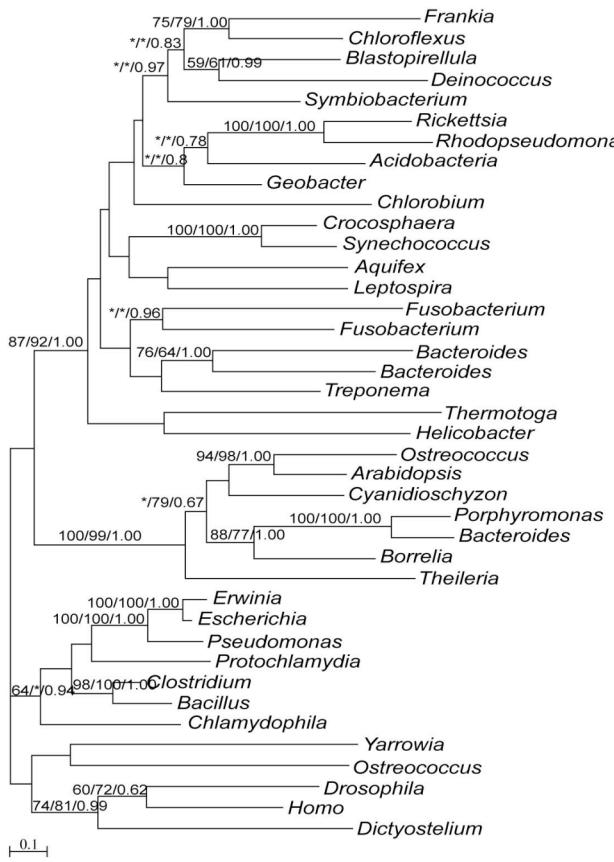


Figure 21. Molecular phylogeny of tRNA methyltransferase. P-value = 0.977 from AU test for the presented tree. AU tests were also performed on alternative topologies including (A) monophyly of all eukaryotic sequences, and (B) monophyly of cyanobacterial, red algal, and green plant sequences. These alternative topologies investigate if red algae and green plants acquired the genes from mitochondria or plastids respectively. P-values < 0.001 from AU tests for both alternative topologies.

22. Isoleucyl-tRNA synthetase (D)

CLUSTAL X (1.83.1) multiple sequence alignment

118193839_Cenarchaeum_symbio	VRSHLEGADL-DRMIVMFIEGPPTMNGPPHAGHLRGRVIKDLWYRYNTLR
6325217_Saccharomyces_mt	LVYKEQLRDFEEFSPILHDGPPYANGELHLGHALNKILKDIINRYQLSQ
66800699_Dictyostelium_mt	LLK--DPYKLYKWQWLWVFHDGPPYANGDLHMGHALNKILKDIVNRYKVLK
118751535_Marinomonas_sp.	MLKRWQDMMDLYQKVRFILHDGPPYANGSIHIGHAVNKILKDIIVKSKTVS
46129096_Haemophilus_influe	MLKNWYEKNLYQKIRFILHDGPPYANGNIHIGHAVNKILKDIIIKSKTAL
75237181_Escherichia_coli	MLARWTDDDLGYIIRFILHDGPPYANGSIHIGHSVNKILKDIIIKSKGGLS
47572014_Rubrivivax_gelati	WVKQWEQQGTYQRLRFVLHDGPPYANGQLHIGHALNKVLKDMIVKARQLA
1174519_Pseudomonas_fluorescen	ILQRWNSIGLYGKLRFVLHDGPPYANGTIHIGHALNKILKDMILRSKTLS
83594296_Rhodospirillum_rubrum	ILARWEKLDIYARLRFVLHDGPPYANGHLHNGHALNKILKDVTIRSQQLM
94968888_Acidobacteria_bacter	MLARWEQRIYELIRYILHDGPPYANGPIHLGHALNKCLKDFVVKSKTMA
15644113_Thermotoga_maritima	FLEEWEMKMDLYNYVLFVLHDGPPYANGHIHGTALNKILKDIVVKYKTMR
15605834_Aquifex_aeolic	IIEKWK--GLYEKIQFVLHDGPPYANGHIHVGHALNKILKDMIVKSQQMS
116747573_Syntrophobacter_fuma	IIEKWEAMGLYDMILRYILHDGPPYANGHIHLGTALNKILKDMIVKSQQMS
116057018_Ostreococcus_tauri	MQEWWRERGVYETLRFVLHDGPPYANGDLHIGHALNKILKDFVNRWEMMN
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56751678_Synechococcus_elonga
113477079_Trichodesmium_erythr
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47570310_Bacillus_cereus
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24665024_Drosophila_melano
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14600670_Aeropyrum_perinx
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14521127_Pyrococcus_abyssi
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54023744_Nocardia_farcin
19074910_Encephalitozoon_cunic
51599084_Borrelia_garini
42528163_Treponema_dentic
46446128_Protochlamydia_amoeba
89898154_Chlamydophila_felis
118749311_Marinomonas_sp.
68130224_Leishmania_major
CM4722_Cyanidioschyzon_merolae
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71076452_Giardia_lamblia
42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
66816517_Dictyostelium_discoi
24668543_Drosophila_melano
94721239_Homo_sapien
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47570085_Bacillus_cereus
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94985204_Deinococcus_geothe
76261183_Chloroflexus_aurant

118193839_Cenarchaeum_symbio
6325217_Saccharomyces_mt
66800699_Dictyostelium_mt
118751535_Marinomonas_sp.
46129096_Haemophilus_influe
75237181_Escherichia_coli
47572014_Rubrivivax_gelati
1174519_Pseudomonas_fluorescen
83594296_Rhodospirillum_rubrum
94968888_Acidobacteria_bacter
15644113_Thermotoga_maritima
15605834_Aquifex_aeolic
116747573_Syntrophobacter_fuma
116057018_Ostreococcus_tauri
CM546_Cyanidioschyzon_merolae
33239720_Prochlorococcus_marin
22327660_Arabidopsis_cp
56751678_Synechococcus_elonga
113477079_Trichodesmium_erythr

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** :

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 GYRIIRREGWDTHGLPVEIEIEKKLGFQGIAEFNRLCRESVWEYIQEWEKA

* : * : * : *

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 18312756_Pyrobaculum_aeroph
 70606414_Sulfolobus_acidoc

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 SEM VGGSALAAEAEVEYQDKTSLSLDVRYAPVVIWTTTPWTLPASQAVSI
 HPEF DCGSSLAAEAEVEYEDKVSPIYVRFPAIIWTTTPWTMPSNRAIA
 VNADL DCRSALAAEAEVEYYDKTPSIDVAFQALVWTTTPWTLPANRA
 ISIAPDF DCGSSLAAEAEFEIYEENKSPTVDVAFIAVIWTTTPWTLP
 ANQALNLP
 DCGSALAAEAEVEYEDKKSSTIDVAFPIIVIWTTTPWTLPANQAL
 NVHPEF VEKTALADEAEVEYQDHSTTIWIRFPVVVIWTTTPWTMPGN
 RAVAYGPGM HDKTALADEAEVEYEHTSPSVYVRYMAIIWTTTPWTLP
 PASMAIAFSPNA RCRTALADEAEYEHDHSKPSIYVFKRSIVI
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 LEYPEHFSQSIVAFPVLAIWTTTPWTMPANA
 AVAVNAKL SSRTALADEAELEYE
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 AVAVNP
 NL SSRTALADEAELEYE
 PHSRSIYAFKLLA
 VWTTPWTLPANMA
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 AKL SSRTALADEAELEYE
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 VAVN
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 QAVL
 IWT
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 PAN
 VAI
 CLN
 ENF TSESALADEAEIEYQDKKSASIYV
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 PV
 II
 WT
 TTPWTLP
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 LG
 ISV
 HPEL SSRTALADEAELEYE
 PHSRSIYAFKPLI
 LV
 WT
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 A
 V
 C
 YM
 PES SSRTALADEAELEYE
 DAHIS
 PSVYV
 RFA
 LAL
 V
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 PAN
 SNO
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 CY
 NASL RGGSAYSQMEVGRKLSVHKS
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 LI
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 L
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 A
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 L RCETSLSDHEVGYDEREDPSIYV
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 KDF

14600670_Aeropyrum_pernix
84490249_Methanospaera_stadtma
11498241_Archaeoglobus_fulgid
14521127_Pyrococcus_abyssi
75401346_Pseudomonas_fluoresce
102191610_Rickettsia_canade
116670944_Arthrobacter_sp.
15827608_Mycobacterium_leprae
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51599084_Borrelia_garini
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46446128_Protochlamydia_amoeba
89898154_Chlamydophila_felis
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CM4722_Cyanidioschyzon_merolae
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23619270_Plasmodium_falcip
71076452_Giardia_lamblia
42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
66816517_Dictyostelium_discoi
24668543_Drosophila_melano
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30681405_Arabidopsis_thalia
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46852147_Homo_mt
24665024_Drosophila_melano
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18312756_Pyrobaculum_aeroph
70606414_Sulfolobus_acidoc
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84490249_Methanospaera_stadtma
11498241_Archaeoglobus_fulgid
14521127_Pyrococcus_abyssi

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ELETVLSNFE ANYKEVQDP AVT VLFK LFAA WT TTPWTLP SNL ALC VG PDI
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SCQTSL S S HEV GY KTV KDL S A T V K F V K F L G WTTTPWTLP SN V AL A V H P N M
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RISTTSL RAEL S YRM VDDPS VY VRF PV L V V WTTTPWTLP SN T L A A V N P D L
RCGTSLS DHEV GARD V D D P S V Y I K F RL V A WTTTPWTLP SN V AL A V K H D A

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PYSLVVAQDLV ET LNKKV I V P G S E L V G M T Y R H P L Y R V V G G D Y I T T E S G T G L
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EYVLILAKARLDHVV L V K G S Q L E G L R Y R H P L K V V M A P E A V T P H E G S G L
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15827608_Mycobacterium_leprae
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51599084_Borrelia_garini
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46446128_Protochlamydia_amoeba
89898154_Chlamydophila_felis
118749311_Marinomonas_sp.
68130224_Leishmania_major
CM4722_Cyanidioschyzon_merolae
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23619270_Plasmodium_falcip
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42523714_Bdellovibrio_bacter
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48477483_Picrophilus_torrid
84029573_Thermoplasma_acidophi
60681847_Bacteroides_fragil
83815822_Salinibacter_ruber
21673150_Chlorobium_tepidu
111221641_Frankia_alni
28210025_Clostridium_tetani
47570085_Bacillus_cereus
87311784_Blastopirellula_marin
94985204_Deinococcus_geothe
76261183_Chloroflexus_aurant

118193839_Cenarchaeum_symbio
6325217_Saccharomyces_mt
66800699_Dictyostelium_mt
118751535_Marinomonas_sp.
46129096_Haemophilus_influe
75237181_Escherichia_coli
47572014_Rubrivivax_gelati
1174519_Pseudomonas_fluorescen
83594296_Rhodospirillum_rubrum
94968888_Acidobacteria_bacter
15644113_Thermotoga_maritima
15605834_Aquifex_aeolic
116747573_Syntrophobacter_fuma
116057018_Ostreococcus_tauri
CM546_Cyanidioschyzon_merolae
33239720_Prochlorococcus_marin
22327660_Arabidopsis_cp
56751678_Synechococcus_elonga
113477079_Trichodesmium_erythr
67921387_Crocospaera_watson
34763705_Fusobacterium_nuclea
47570310_Bacillus_cereus
46852147_Homo_mt
24665024_Drosophila_melano
149179295_Planctomyces_maris
18312756_Pyrobaculum_aeroph
70606414_Sulfolobus_acidoc
14600670_Aeropyrum_perinx
84490249_Methanospaera_stadt
11498241_Archaeoglobus_fulgid
14521127_Pyrococcus_abysse
75401346_Pseudomonas_fluoresce
102191610_Rickettsia_canade
116670944_Arthrobacter_sp.
15827608_Mycobacterium_leprae

NYVLWLAEGCLGGILERCTGAELAGLRYLPVVHRRVVTADFVQMGDGSIGI
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EHPY

54023744_Nocardia_farcin
19074910_Encephalitozoon_cunic
51599084_Borrelia_garini
42528163_Treponema_dentic
46446128_Protochlamydia_amoeba
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71076452_Giardia_lambl
42523714_Bdellovibrio_bacter
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66816517_Dictyostelium_discoi
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94721239_Homo_sapien
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30681405_Arabidopsis_thalia
67481173_Entamoeba_histol
57157197_Trichomonas_vagina
48477483_Picrophilus_torrid
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LEGAR

46446128_Protochlamydia_amoeba
89898154_Chlamydophila_felis
118749311_Marinomonas_sp.
68130224_Leishmania_major
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68130224_Leishmania_major

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DWCISRQRSGWGVPIPVYETAHVQKIDAwwEMSVEELL PYRKGTDTMDV
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DWCISRQRAGWGVPIPVYETINHVADLNWFEREAKDLLPFRKETDIMDV
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YWCISRQRAGWGVPIPVYALIEHLCNLDFWWVKSVEEVLVPLVKGTDILD
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DNWISRSRVWGTPLPVWRLVIGSLELLVHVRPWIDQVKISWVREPYVMDV
DWVISRQRFWGTPLPPIWIVVGGVDELDLHVRPWIDSVVRARRISDVADV
DWVISRQRYWGPNIPLPVWVHVVGSVEELNLHVRPWVDRVELKMKRPDVLDV
DWTISRORYWGPIPLPIWTVVIGSKELFVHPRHDVNTIPMHRVPDFLDV
DWCISRQRYWGIPIPVWIKVVGGSINEIDLHRPKIDAVTFSMRVPDFDV
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CM4722_Cyanidioschyzon_merolae
118380025_Tetrahymena_thermo
23619270_Plasmadium_falcip
71076452_Giardia_lambl
42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
66816517_Dictyostelium_discoi
24668543_Drosophila_melano
94721239_Homo_sapien
116061521_Ostreococcus_tauri
30681405_Arabidopsis_thalia
67481173_Entamoeba_histol
57157197_Trichomonas_vagina
48477483_Picrophilus_torrid
84029573_Thermoplasma_acidophi
60681847_Bacteroides_fragil
83815822_Salinibacter_ruber
21673150_Chlorobium_tepidu
111221641_Frankia_alni
28210025_Clostridium_tetani
47570085_Bacillus_cereus
87311784_Blastopirellula_marin
94985204_Deinococcus_geothe
76261183_Chloroflexus_aurant

118193839_Cenarchaeum_symbio
6325217_Saccharomyces_mt
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CM546_Cyanidioschyzon_merolae
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11498241_Archaeoglobus_fulgid
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102191610_Rickettsia_canade
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15827608_Mycobacterium_leprae
54023744_Nocardia_farcin
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46446128_Protochlamydia amoeba
89898154_Chlamydophila_felis
118749311_Marinomonas_sp.
68130224_Leishmania_major
CM4722_Cyanidioschyzon_merolae
118380025_Tetrahymena_thermo
23619270_Plasmodium_falcip
71076452_Giardia_lambl
42523714_Bdellovibrio_bacter
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94985204_Deinococcus_geothe
76261183_Chloroflexus_aurant

DWNVSRNRYWGTPPLPVWTTIVVGSI
AELDLHRHNIDSIEIRLRRVEEVFDC
DWCFSRSRFWGPNPIPIWVV
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42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
66816517_Dictyostelium_discoi
24668543_Drosophila_melano
94721239_Homo_sapien
116061521_Ostreococcus_tauri
30681405_Arabidopsis_thalia
67481173_Entamoeba_histol
57157197_Trichomonas_vagina
48477483_Picrophilus_torrid
84029573_Thermoplasma_acidophi
60681847_Bacteroides_fragil
83815822_Salinibacter_ruber
21673150_Chlorobium_tepidu
111221641_Frankia_alni
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47570085_Bacillus_cereus
87311784_Blastopirellula_marin
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76261183_Chloroflexus_aurant

118193839_Cenarchaeum_symbio
6325217_Saccharomyces_mt
66800699_Dictyostelium_mt
118751535_Marinomonas_sp.
46129096_Haemophilus_influe
75237181_Escherichia_coli
47572014_Rubrivivax_gelati
1174519_Pseudomonas_fluorescen
83594296_Rhodospirillum_rubrum
94968888_Acidobacteria_bacter
15644113_Thermotoga_maritima
15605834_Aquifex_aeolic
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116057018_Ostreococcus_tauri
CM546_Cyanidioschyzon_merolae
33239720_Prochlorococcus_marin
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47570310_Bacillus_cereus
46852147_Homo_mt
24665024_Drosophila_melano
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18312756_Pyrobaculum_aeroph
70606414_Sulfolobus_acidoc
14600670_Aeropyrum_perinx
84490249_Methanospaera_stadt
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51599084_Borrelia_garini
42528163_Treponema_dentic
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89898154_Chlamydophila_felis
118749311_Marinomonas_sp.
68130224_Leishmania_major
CM4722_Cyanidioschyzon_merolae
118380025_Tetrahymena_thermo
23619270_Plasmadium_falcip
71076452_Giardia_lamblia
42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
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24668543_Drosophila_melano

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94721239_Homo_sapien
 116061521_Ostreococcus_tauri
 30681405_Arabidopsis_thalia
 67481173_Entamoeba_histol
 57157197_Trichomonas_vagina
 48477483_Picrophilus_torrid
 84029573_Thermoplasma_acidophili
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 116061521_Ostreococcus_tauri
 30681405_Arabidopsis_thalia
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 EARNINRMLNVVWNNAVRFAHLKIEDRWLWISLRESFIKEANEAMEGYQVHR
 -VEQVRMLNWLNVVVLASFREEDKWLISRVNLSLISEVENGIESFYLT
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 IREGVSHALRPLWNAWTFLQTHVLDRLYILAKLAQTRDGMTEALEVYDIAG
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 VRDVLKGILIPFWNSYSFYINNPLDLWISLVTESLKVADVTEALDKYDLSQ
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 VESILKOILLPLTNVLNPKYSEIDKWLISLTDLYTVVGVKRESMNSYNLNT
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 VKGVVKDILLPLFNAKFFITNEMDRWILASCQSLRKYKAEMRLYHLYN
 VKDIVRKILLRWWNSYFFAPNILDQWVLSRLNGLIANTHKEMDAYRLY
 VKEVVSKVLLPWNSFKFLDDNVMDRWLASMOSLVQFIHEEMGQYKLYT
 VQDMIKDVFLPWFNAYRFFVENVMDKWLASCQSLIAFVRAEMAAYRLY
 VRDIIKDVFLPWFNAYRFLIPNITDRWLISFMQSLIGFFTEMAYRLY
 VFGVLKDVFPLPWNAYRFLVTNVLIDIWITSSTNSLKVFTTEMQDYKLYT
 VLGVVKDVFPLPWNAYRFLVANILDQWIHSATQSLVRFVREEMDAYRLY
 LKELISTIFLPWFNTLRLYKMNMDRWLSSMSLVKKVREEMKMFRLY

57151797_Trichomonas_vagina
48477483_Picrophilus_torrid
84029573_Thermoplasma_acidophili
60681847_Bacteroides_fragil
83815822_Salinibacter_ruber
21673150_Chlorobium_tepidu
111221641_Frankia_alni
28210025_Clostridium_tetani
47570085_Bacillus_cereus
87311784_Blastopirellula_marin
94985204_Deinococcus_geothe
76261183_Chloroflexus_aurant

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RLDLRRTFGTLENVYRFFARPELDOWIISRLHTTTOAVEALDEYDPTT
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VAEAQRNYVNTLWNVYAFFVRPEMDRWLALARLEETVRDVITSLDAYDARG
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118193839_Cenarchaeum_symbio
6325217_Saccharomyces_mt
66800699_Dictyostelium_mt
118751535_Marinomonas_sp.
46129096_Haemophilus_influe
75237181_Escherichia_coli
47572014_Rubrivivax_gelati
1174519_Pseudomonas_fluorescen
83594296_Rhodospirillum_rubrum
94968888_Acidobacteria_bacter
15644113_Thermotoga_maritima
15605834_Aquifex_aeolic
116747573_Syntrophobacter_fuma
116057018_Ostreococcus_tauri
CM546_Cyanidioschyzon_merolae
33239720_Prochlorococcus_marin
22327660_Arabidopsis_cp
56751678_Synechococcus_elonga
113477079_Trichodesmium_erythr
67921387_Crocospaera_watson
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47570310_Bacillus_cereus
46852147_Homo_mt
24665024_Drosophila_melano
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14600670_Aeropyrum_perinx
84490249_Methanospaera_stadt
11498241_Archaeoglobus_fulgid
14521127_Pyroccoccus_abyssi
75401346_Pseudomonas_fluoresce
102191610_Rickettsia_canade
116670944_Arthrobacter_sp.
15827608_Mycobacterium_leprae
54023744_Nocardia_farcin
19074910_Encephalitozoon_cunic
51599084_Borrelia_garini
42528163_Treponema_dentic
46446128_Protochlamydia_amoeba
89898154_Chlamydophila_felis
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68130224_Leishmania_major
CM4722_Cyanidioschyzon_merolae
118380025_Tetrahymena_thermo
23619270_Plasmadium_falcip
71076452_Giardia_lamblia
42523714_Bdellovibrio_bacter
6319395_Saccharomyces_cerevi
66816517_Dictyostelium_discoi
24668543_Drosophila_melano
94721239_Homo_sapien
116061521_Ostreococcus_tauri
30681405_Arabidopsis_thalia
67481173_Entamoeba_histol
57157197_Trichomonas_vagina
48477483_Picrophilus_torrid
84029573_Thermoplasma_acidophi
60681847_Bacteroides_fragil

83815822_Salinibacter_ruber	AARAVEDFVEELSNWLRRSRPRFWYQTIHECLAATAKLMSPIAPIFFGEW
21673150_Chlorobium_tepidu	ACRLIGDFVDDLSNWYIIRRSRKRFWYQTLSTVLETLAKLMPFPFIAEK
111221641_Frankia_alni	AGRRIARFIDDLNSNWYVRRSRRRFW-TTLYTCLDALTRVMAPFTPFLTDW
28210025_Clostridium_tetani	AALATEDFVDELSNWYVRRNRSRFWVFTLYKVLTTSLIAAPFVPFITEE
47570085_Bacillus_cereus	AAREIAALVDEVSNWYVRRSRRNRFWYETLHEVLVTISKLIAPFAPFVAED
87311784_Blastopirellula_marin	ACGALTNFVDALSNWYVRRSRDRFWYWTLYECLITTCKLIAPFTPFLAEG
94985204_Deinococcus_geothe	GGRALERFVHDLSNWYVRRNRSRFWYATLHEALLTVSQLTAPFTPFLAEA
76261183_Chloroflexus_aurant	AANAIEHFVDELSNWYVRRNRRRFWYQTLYTCLTVAKLAAPFIPFVSEE
.	
118193839_Cenarchaeum_symbio	LYSILLEGW
6325217_Saccharomyces_mt	VWNPMRGKWW
66800699_Dictyostelium_mt	VFSVFAHGW
118751535_Marinomonas_sp.	IWSVFLETW
46129096_Haemophilus_influe	IWFVFTEEF
75237181_Escherichia_coli	VWVFTGEW
47572014_Rubrivivax_gelati	AWSIFTQTY
1174519_Pseudomonas_fluorescen	LWSVMLNTW
83594296_Rhodospirillum_rubrum	AWSVHFQSF
94968888_Acidobacteria_bacter	LWTVHAAWF
15644113_Thermotoga_maritima	VYTVAQEYW
15605834_Aquifex_aeolic	LWSVFLYEM
116747573_Syntrophobacter_fuma	AWTVHLEEF
116057018_Ostreococcus_tauri	AFSVFIAGW
CM546_Cyanidioschyzon_merolae	LWSIFQNGW
33239720_Prochlorococcus_marin	IWSVFQRYW
22327660_Arabidopsis_cp	VWFVFEKLW
56751678_Synechococcus_elonga	IWSVFOAGW
113477079_Trichodesmium_erythr	IWSVFESGW
67921387_Crocospaera_watson	IWSVFEAGW
34763705_Fusobacterium_nuclea	IWSIFLADW
47570310_Bacillus_cereus	VWSVQLTDM
46852147_Homo_mt	VFSVFRGTW
24665024_Drosophila_melano	SWGAFHEQI
149179295_Planctomyces_maris	MYSVHLCDF
18312756_Pyrobaculum_aeroph	LWSVHLAQY
70606414_Sulfolobus_acidoc	IYSISMEKI
14600670_Aeropyrum_perinx	LYSIHMLDM
84490249_Methanospaera_stadt	IYSVHMLDW
11498241_Archaeoglobus_fulgid	FYSIFMEY
14521127_Pyrococcus_abyssi	IYSVHMLDW
75401346_Pseudomonas_fluoresce	VYSVHLQDW
102191610_Rickettsia_canade	IYSVHLCNY
116670944_Arthrobacter_sp.	IWSVHLADW
15827608_Mycobacterium_leprae	IWSVHLTDW
54023744_Nocardia_farcin	IWSVHLADW
19074910_Encephalitozoon_cunic	SYSVHFQEY
51599084_Borrelia_garini	IYSIHLNDY
42528163_Treponema_dentic	IWSIHLADY
46446128_Protochlamydia_amoeba	IYSVHLCDF
89898154_Chlamydophila_felis	IYSVHLCDF
118749311_Marinomonas_sp.	LYSVHLCDY
68130224_Leishmania_major	LYSVHYLMI
CM4722_Cyanidioschyzon_merolae	TYSVHFLPL
118380025_Tetrahymena_thermo	FYSIHFLRI
23619270_Plasmodium_falcip	IYSVHFIML
71076452_Giardia_lamblia	MYSIHFWAV
42523714_Bdellovibrio_bacter	TYSVHLESF
6319395_Saccharomyces_cerevi	IYSVHFLSY
66816517_Dictyostelium_discoi	MYSVHYVMF
24668543_Drosophila_melano	IFSVHYQMM
94721239_Homo_sapien	MYSIHYLML
116061521_Ostreococcus_tauri	IYSIHFCF
30681405_Arabidopsis_thalia	LYSVHYCSI
67481173_Entamoeba_histol	CYSVHFVOI
57157197_Trichomonas_vagina	-----
48477483_Picrophilus_torrid	LYSVHMESY
84029573_Thermoplasma_acidophi	IYSVHLEAF
60681847_Bacteroides_fragil	LYSVHLAKF
83815822_Salinibacter_ruber	LYSVHLASF
21673150_Chlorobium_tepidu	IWSVHLADW
111221641_Frankia_alni	LWSVHLAAW
28210025_Clostridium_tetani	IYSIHLCNW

47570085_Bacillus_cereus	IHSVHLLEDY
87311784_Blastopirellula_marin	LWSVHLCDF
94985204_Deinococcus_geothe	LYSVHLTRW
76261183_Chloroflexus_aurant	IYSVHLAQW

Note: There are two eukaryotic sequence clades for this gene, each of which clusters within bacterial homologs with strong support. The minor eukaryotic sequence clade (lower part of the tree) contains chloroplast precursors from plants and mitochondrial precursors from opistokonts. Sequences in the major eukaryotic sequence clade are cytosolic. It is likely that the major eukaryotic sequence clade resulted from an ancient HGT event prior to the split of most eukaryotic super groups. An alternative explanation is that the common ancestor of cellular organisms contained two copies of this gene, which were differentially retained among lineages.

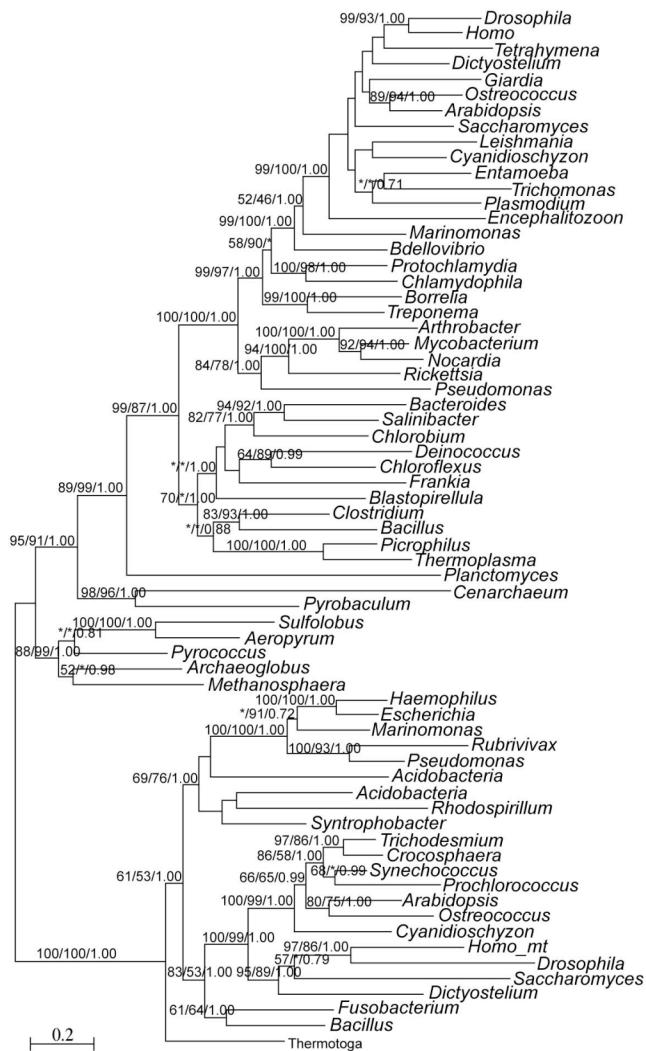


Figure 22. Molecular phylogeny of isoleucyl-tRNA synthetase. P-value = 0.235 from AU test for the presented tree. AU test was also performed on an alternative topology enforcing a sequence monophly of archaea and the major eukaryotic group. Such an alternative topology is based on the common belief that archaea and eukaryotes are more closely related than each is to bacteria. P-value < 0.001 from AU test for the alternative topology.

23. IspD (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

24. Polynucleotide phosphorylase (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

25. ATP/ADP translocase (N)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

26. Glycero-3-phosphate acyltransferase (N)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

27. Sodium:hydrogen antiporter (N)

See Huang and Gogarten 20007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

28. GcpE (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

29. Beta-ketoacyl-ACP synthase (fabF) (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

30. Aspartate aminotransferase (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

31. tyrosyl-tRNA synthetase (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

32. Cu-ATPase (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

33. IspE (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

34. Enoyl-ACP reductase (fabI) (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

35. Florfenicol resistance protein (E)

See Huang and Gogarten 2006. Ancient horizontal gene transfer can benefit phylogenetic reconstruction. *Trends in Genetics* 22:361–366.

36. 23S rRNA (Uracil-5-)methyltransferase (D)

See Huang and Gogarten 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* 8:R99.

37. Topoisomerase 6 subunit B (TOP6B) (N)

See Huang and Gogarten 2006. Ancient horizontal gene transfer can benefit phylogenetic reconstruction. *Trends in Genetics* 22:361–366.