

Supplementary Materials: The *Escherichia coli* COG1738 Member YhhQ Is Involved in 7-Cyanodeazaguanine (preQ₀) Transport

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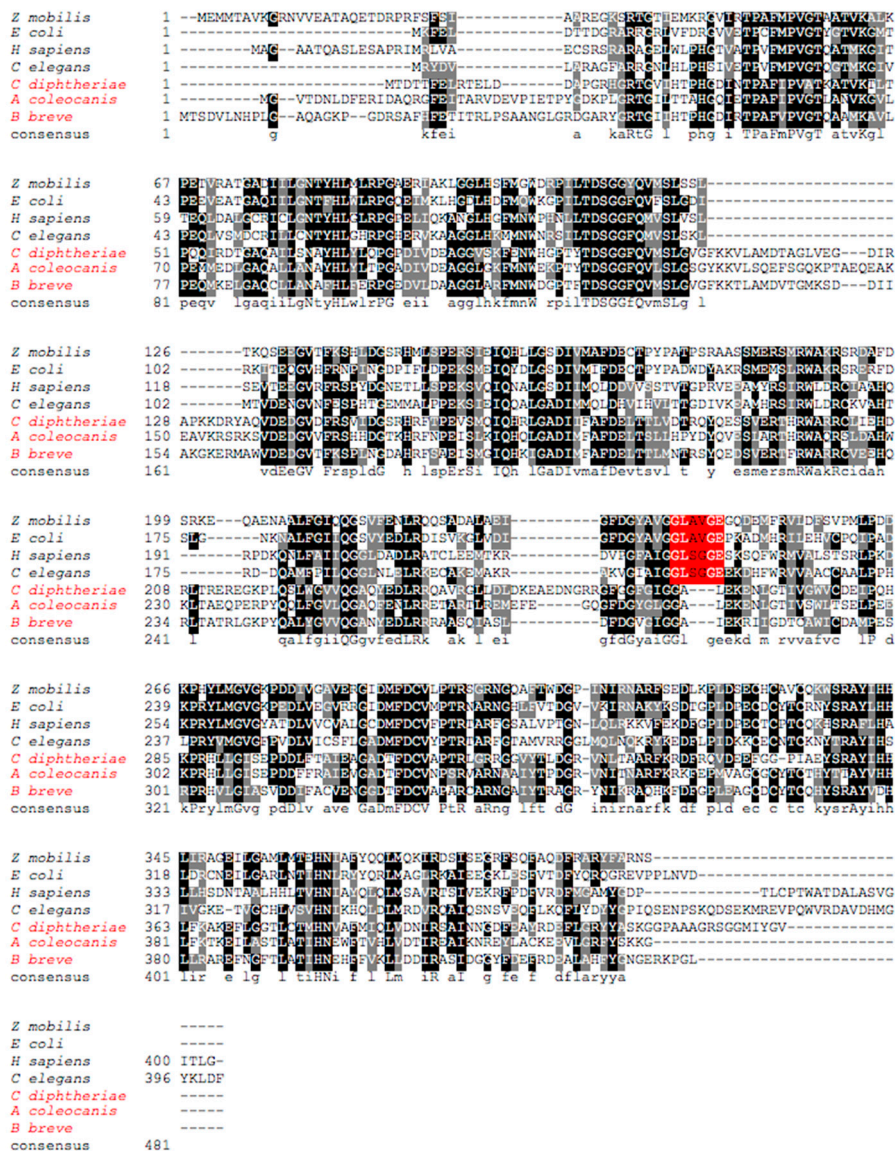


Figure S1. Sequence alignment of tRNA guanine⁽³⁴⁾ transglycosylase (TGT) proteins. Bacteria that do not have queuosine biosynthesis capability, but have TGT, and not QueA (tRNA preQ₁₍₃₄₎ S-adenosylmethionine ribosyltransferase-isomerase (EC 2.4.99.17)), QueG or QueH (tRNA epoxyqueuosine⁽³⁴⁾ reductase (EC 1.17.99.6)) (with species names in red), have non-canonical substrate binding residues in their TGT sequence. For reference, the substrate binding pockets of the different TGT families that have been well characterized are presented in red boxes [32]. Alignment realized with Clustal Omega [50]. Abbreviations: *Z mobilis*: *Zymomonas mobilis*; *E coli*: *Escherichia coli*; *H sapiens*: *Homo sapiens*; *C elegans*: *Caenorhabditis elegans*; *C diphtheriae*: *Corynebacterium diphtheriae*; *A coleoecanis*: *Actinomyces coleoecanis*; *B breve*: *Bifidobacterium breve*.

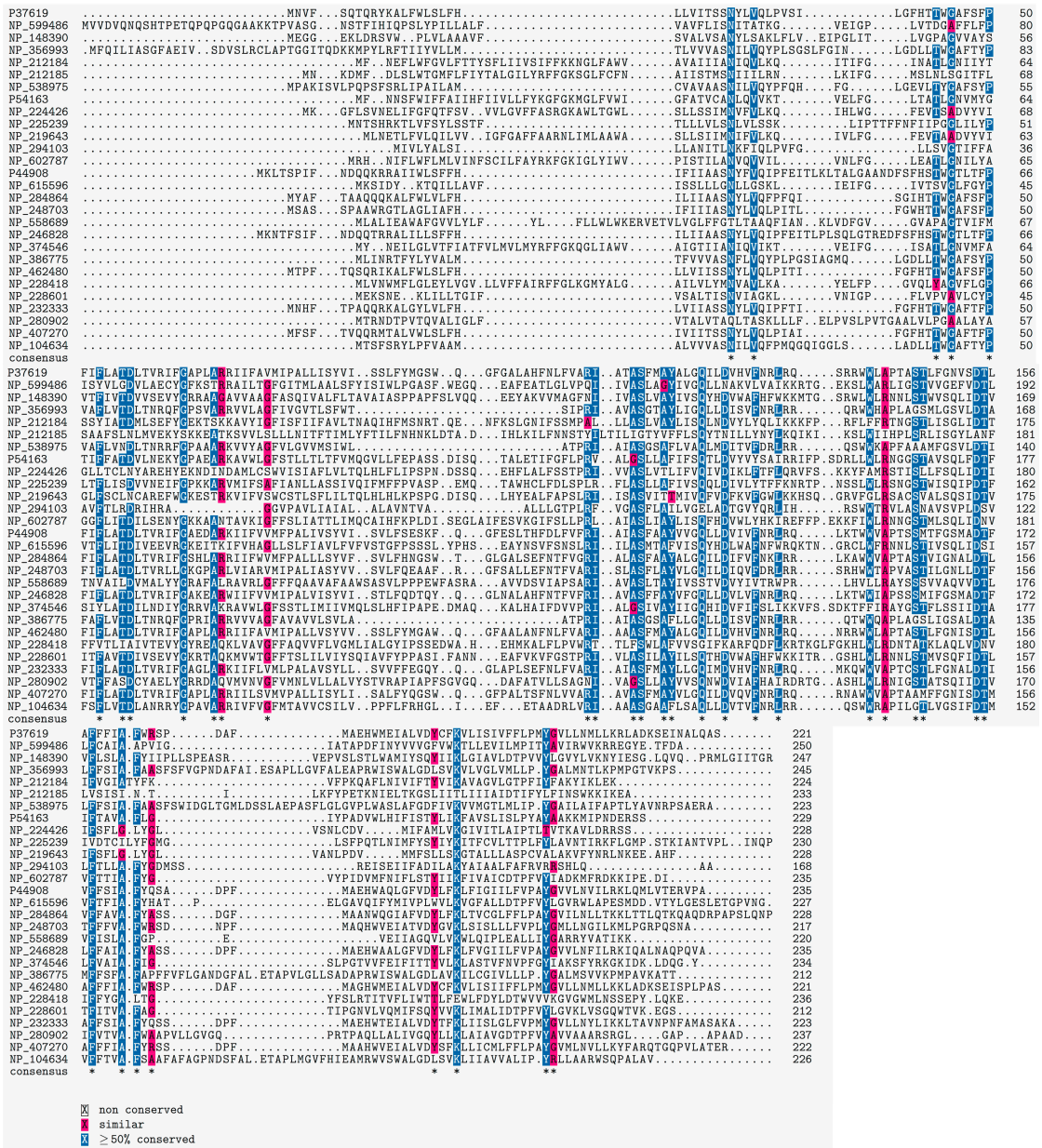


Figure S2. Sequence alignment of COG1738 protein. Alignment of members of the COG1738 group realized with Clustal Omega [50], and shading with TexShade [52]. P37619: *Escherichia coli* K12; NP 599486: *Corynebacterium glutamicum* ATCC 13032; NP 148390: *Aeropyrum pernix* K1; NP 356993: *Agrobacterium tumefaciens* C58; NP 212184: *Borrelia burgdorferi* B31; NP 212185: *Borrelia burgdorferi* B31; NP 538975: *Brucella melitensis* 1 16M; P54163: *Bacillus subtilis* 168; NP 224426: *Chlamydomphila pneumoniae* CWL029; NP 225239: *Chlamydomphila pneumoniae* CWL029; NP 219643: *Chlamydia trachomatis* D/UW-3/CX; NP 294103: *Deinococcus radiodurans* R1; NP 602787: *Fusobacterium nucleatum* ATCC 25586; P44908: *Haemophilus influenzae*; NP 615596: *Methanosarcina acetivorans* 2C; NP 284864: *Neisseria meningitidis* Z2491; NP 248703, *Pseudomonas aeruginosa* PAO1; NP 558689, *Pyrobaculum aerophilum* IM2; NP 246828: *Pasteurella multocida* Pm70; NP 374546: *Staphylococcus aureus* N315; NP 386775: *Sinorhizobium meliloti* 1021; NP 462480: *Salmonella enterica* LT2; NP 228418: *Thermotoga maritima* MSB8; NP 228601: *Thermotoga maritima* MSB8; NP 232333: *Vibrio cholerae* O1 N16961; NP 280902: *Halobacterium* NRC-1; NP 407270: *Yersinia pestis* CO92; NP 104634: *Mesorhizobium loti* MAFF303099.

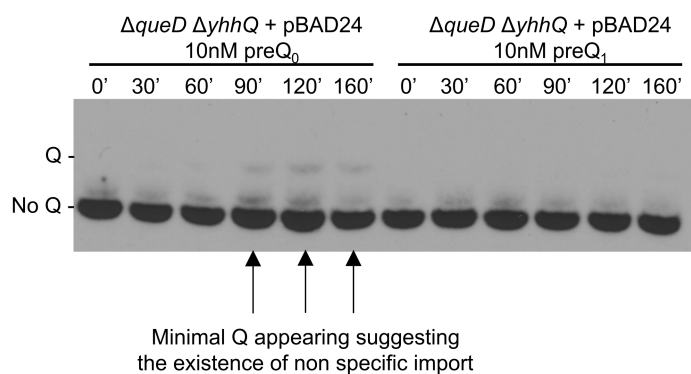


Figure 3. Non-specific transport of 7-cyano-7-deazaguanine (preQ₀). Detection of Queuosine in tRNA^{AspGUC} as a representative of the salvage of the Q precursors preQ₀ and 7-aminomethyl-7-deazaguanine (preQ₁). *E. coli* bulk tRNA was separated on an 8 M urea, 8% polyacrylamide gel containing 0.5% 3-(acrylamido)phenylboronic acid and transferred to a nylon membrane. The transferred tRNA was probed with a biotinylated primer, and detected by Northern blot. At long incubation times, Q is detected in tRNAs extracted from the $\Delta queD \Delta yhhQ$ strain (carrying pBAD24), when fed with 10 nM preQ₀, suggesting the presence of low-affinity transporters.