Supplementary Materials: Coevolution Theory of the Genetic Code at Age Forty: Pathway to Translation and Synthetic Life

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(A)

Figure S1. Cont.



(B)

Figure S1. Cont.



(C)

Figure S1. Gene trees for tRNAs bearing GNN and UNN anticodons. (**A**) UUN codon box for Phe/Leu; (**B**) CAN codon box for His/Gln; and (**C**) AGN codon box for Ser/Arg.

Archaea :	Average Δ = 13.2	
AfuAspGAC AfuGluGAA Δ=12	GCCCGGGIGGIGIGICCGCCCATCATGCGGGCCCGCGCGCGCGCGCGCGCGCGCG	75 75
ApeAspGAC ApeGluGAA Δ=6	GCCGCGGTAGTATAGCCTGACTGCAGGCCTGTCAAGCCCGGGTTCAAATCCCGGCGCGGGGGGGCGGCGCGCGGTAGTATAGCCCGGCCGCGGCGGGCCTTTCCAGCCCGGCGCGGCCGGC	75 75
DkaAspGAC DkaGluGAA ∆=4	GCCGCCGTAGTATAGCCCGGCCTGTGTGTGGGCCCGTGAGCCCGGGTTGAAAACCCGGCGCGCGC	75 75
FacAspGAC FacGluGAA $\Delta = 16$	************************************	73 75
HalAspGAC HalGluGAA $\Delta = 25$	ccccsatisgteragr - gecccatcatacatectgtcacgetgtcaaatcccccccccc	75 75
HbuAspGAC HbuGluGAA $\Delta = 4$	GCCGCGGTAGTAGCCCGCCCAGTATGCGGGCCTGTCAAGCCCGGGTTCAAATCCCGGCCGCGGCG GCCGCGGTAGTATGCCGGCCAGTAGCCGGCCAGGCCGCGGCG GCCGCGGTAGTATGCCGGCCAGGCCAGGCCTTCAAGCCCGTGACCCGGGTTCAAATCCCGGCCGCGGCG 10 20 30 40 50 60 70	75 75
MacAspGAC MacGluGAA $\Delta = 21$	GCCCGGATAGTCTAGAGCCCTATCATGGAGCCCGACTCGGAGCTCCGACTCGGGTCGGATCCGGCCGG	75 75
MbaAspGAC MbaGluGAA ∆=21	* * * ********************************	75 75
MjaAspGAC MjaGluGAA ∆=15	GCCCTGGTGGTGTGGCCGGCCTATCATACGGGCTGCGCCGGCGTGGCGGGCTGGGCGTGGCGGCGATCCAATCCCGGCCGG	75 75
MkaAspGAC MkaGluGAA ∆=9	GCCCCGGCGGTGTAGCCCGGTTTATCATCCGGGCCTGTCGAGCCCGGGGCGGCGGGCG	75 73
MmaAspGAC MmaGluGAA Δ=22	* * *********************************	75 75
$\begin{array}{l} \texttt{MthAspGAC}\\ \texttt{MthGluGAA}\\ \texttt{\Delta}=9 \end{array}$	ccccrgcrgcrgcrgcrgcrgcrgcrgcrgcrgcrgcrgc	75 75
PabAspGAC PabGluGAA ∆=12	GCCCGGGTGTGTGCCCGGCCTACCATGCGGGCCTCCCGGCCGG	75 75
PaeAspGAC PaeGluGAA $\Delta = 7$	GCCCCGCTAGTATAGCCCGGACTAGTATGCGGGCCTGTCGAGCCCGTGACCCGGGTTCAAATCCCGGCCGG	75 75
PfuAspGAC PfuGluGAA $\Delta = 20$	GCCCGGGTGGTGTGGCCGGCCTATCATGCGGGACTGCGCCGCGCCGGGCCGGGCGGG	75 75
PhoAspGAC PhoGluGAA ∆=11	GCCCGGGTGGTGGCCGGCCAACCAAACCACGGCCTTCCAACCCGGCCCGGGCGGCGGCCGCCGGCGGGCG	75 75
SmaAspGAC SmaGluGAA ∆=5	GCCCCCGTAGTATAGCCCGGCCTAGTATGCGGGCCTGTCGAG-CCCGTGCCCGGGTTCAAATCCCGGCGGGGGGG GCCGCCGTAGTATAGCCCGGCCTAGTATGCGGGCCTTTCGAG-CCCGTGCCCGGGTTCAAATCCCGGCGCGCGCG 110203040506070	74 74
SsoAspGAC SsoGluGAA ∆=11	GCCGCGGTGTTTGTGGTCTAGGTGGGGGCCTGTCGACCCGGGTTCAAATCCCGGCGCGGGGGGGCGCCGCGGTGGATGAGATCCCGGCCCTTTCGACCCCGGCGCGCGC	75 75
StoAspGAC StoGluGAA $\Delta = 7$	GCCGCGCTAGTCTAGCCCGGTCTAGGATGGGGGCCTGTCGAGCCCTGACCCGGGGTTCAAATCCCGGCCGCGGGG GCCGCGGTAGTATAGCCCGGTCAGTATGCGGGCCTTTCGAGCCCGGGTTCAAATCCCGGCCGCGCG 110203040506070	75 75
TacAspGAC TacGluGAA ∆=18	GCCCCGGTAGTGTAGTGCCTATCATACAGGCCTGTCCAGCCTGTGACATGGGTTCAAATCCCGGCGGCG GCTCCGGTGTGTAGTCCGGACAAAGCATATCGCGCCTTTCCAGCCGGGCCGACCTGGGCCGAAATCCCGGCGGCGA 11020	75 75
TonAspGAC TonGluGAA ∆=17	GCCCGGGTGGTGTGGCCGGCCGTCATCATCGGGGCTGGACTCGGGCTCGAATCCCGGCCGG	75 75
TvoAspGAC TvoGluGAA ∆=18	cccccccccccccccccccccccccccccccccccc	75 75

(A)

Figure S2. Cont.

Bacteria : Average Δ = 30.8								
AaeAspGAC AaeGluGAA ∆=21	GCACCCCTCACCCCCCCCCCCCCCCCCCCCCCCCCCCCC	74 75						
AnaAspGAC AnaGluGAA ∆=35	* * * * * * * * * * * * * * * * * * *	73 73						
AtuAspGAC AtuGluGAA Δ=34	*** * * * * * * * * * * * * * * * * * * *	73 72						
BapAspGAC BapGluGAA Δ=28	GGTGCGGTAGTTCAGTCGGTTAGAATACCGGCCTGTCACGCCGGGGCGCGGGGTTCGAATCCCGTCGGCACG GTCCCCTTCGTCTAGA-GGTTAGAATACCGGCCTTTCACGGCGGGCACAGGGGTTCGAATCCCCTAGGGCACG 1102030405060	73 73						
BbuAspGAC BbuGluGAA ∆=35	* * * * * * * * * * * * * * * * * * *	73 72						
BloAspGAC BloGluGAA ∆=37	GCCCCCTAGCTCAGTTGGTTAGAGCGCCCCCCCTGTCAGGGGGGGG	73 72						
BsuAspGAC BsuGluGAA ∆=25	GCTCCGCT>TCAGTTGGTTAGAATGCCTGCCTGTCACGCAGGGGGGGG	73 72						
CacAspGAC CacGluGAA ∆=31	GCTTGGTAGCTCAGCTGGTTAGAGTGCTGGCTGTCACGCCAGAGGGCAGGGTTCGATCCCCTTCCAAGTCG GGCCCGTTGG-TCAAGCGGTTAAGACACCCCCCTTCACGGTGGTAACATGGGTTCGATTCCCGTACGGGTCA 1	73 72						
CcrAspGAC CcrGluGAA ∆=35	CGGR TGT ACCCCGT GGT AGGACGCCCGCAGACCCCGCAGGGCGCGGGCTCGACCCCCTCATCGGC -GGTCCCTTCGTCTATCGGT AGGACGCCCGCAGATTTTCATCTGGAGAGGGGGGCTCGACCCCCTAGGGACTG 110	73 72						
CjeAspGAC CjeGluGAA ∆=27	CCAGCGGTAGTTCGGTGGTTAGAATGCCGCCCTGTCACGGCGGGGGCGGGGTTCGAGCCCGTCCGT	73 72						
CtrAspGAC CtrGluGAA ∆=40	** * * * **** ** **** ** *** *** *** *	73 75						
DraAspGAC DraGluGAA ∆=28	text	72 72						
EcoAspGAC EcoGluGAA Δ=32	GCAGCGCTAGTCAGTCGGTTAGATACCTGCCTGTCACGCAGGGGGCGCGGGTTCGAGTCCCGTCCG CTCCCCTTCGTCTAGAGGCCCAGGACACCGCCCTTTCACGGGGGTAACAGGGGTTCGAATCCCCTAGGGGACG 1	73 73						
HinAspGAC HinGluGAA ∆=35	GCAGTGCTRGTTCAGGTGGTTAGAATACCTGCCTGTCACGCAGGGGGCGCGGGGTTCGAGTCCCCGTCCATCCG GTCCCCATCGTCTAGAGGCCTAGGACATCGCCCTTTCACGGCGGTAACCGGGGTTCGAATCCCCGTGGGACG 110	73 73						
HpyAspGAC HpyGluGAA ∆=38	GCAGCGCTACTCAGCTGGTTAGAATATCTGCCTGTCACGCAGGGGGCGCGGGGTTCGAGTCCGCCGCCGCGCGGAGCGCGGGGAGCGCGGGAGCGCGGGAACGGAAGCGGAGCGCGGAGCGCGGAGCGCGGAACGGAACGGAAGCGGAGCGCGGAGCGCGGAACGAACG	73 73						
LinAspGAC LinGluGAA ∆=24	GCTTCCCTGCTACGGGTTAACATGCCTGCCTGCCACGCAGGCGCGGGTCCAAATCCCGTCGGGACG GCCCCTTGCTCAAGCGCTTAAGACACCGCCCTTTCACGCGGTAACACGGGTTCGAATCCCCTACGGGTCA 110	72 72						
LlaAspGAC LlaGluGAA ∆=23	GGTTCTGT>GT&GCGGTTATCACGTCGCCCTGTCACGGCGAAGACGCGGGTTCGATTCCCGTCACAACCG GGTCCCTTGGTCAAGGGGTTAGACACGCCCTTTTCACGCGGGTACACGGGGTTCGAATCCCCTACGGACTA 110	72 72						
MpnAspGAC MpnGluGAA ∆=28	GCTTCCATGGTGTAGTGATAACATATCTCCCTGTCACGGGGGGGTGCGGGGTTGCAGGGTTGCATCCCGTGGAACCG GCGCCTTGGTGAAGACCTTACACACACGCCTTTCACGCGTCCATCACGGGTTTGAATCCCGTACGCGTCA 110	71 73						
MtuAspGAC MtuGluGAA ∆=24	GCCCCTCTGGCGCAGTTGGT-TAGCGCGCCGCCCTCTCAGGGCGCGGGGGGGGGG	74 74						
NmeAspGAC NmeGluGAA $\Delta = 30$	GCGGTGGTAGTTAGTTAGATACCGGCCTGTCACGCCGGGGGCGCGGGTTCGAGCCCGTCGGCCGG GCGCCCATCGTCTAGC-GGTTAGGACATCGCCCTTTCACGGCGGTAACCGGGGTTCGATTCCCCGTGGGCGTG 1	73 72						
PsaAspGAC PsaGluGAA ∆=27	GCAGCGGTAGTTCAGTCGGTTAGAATACCGGCCTGTCACGCGGGGGCGCGGGGTTCGAGTCCCGTCCGGTCG GTCCCCTTCGTCTAGTGGCCTAGGACACCGGCCTTTCACGGCGGTAACAGGGGTTCGAGTCCCCTAGGGACG 1	73 73						
RprAspGAC PprGluGAA ∆=32	GGGGTGTAGCTCAGTTGGTTAGAGCGCTGCCGCTGCACGCAGAGAGCGCGGGGTCGAGCCCGCGCACGCGCTCGGGTCAGGCCCGCGCACGCGCTCGAGCCCGGCTCAGGCCCGGGTCAGGCCCGCGCGCACGCGCCCGCGCGCG	73 72						

(A)

Figure S2. Cont.

RsoAspGAC RsoGluGAA ∆=34	* ** ** ** * *** * *** * *** ***** ** *	73 73
ScoAspGAC ScoGluGAA A=30	GCTCCTCTGCACCCTTGCACTGCCCCCCCCCCCCC	G 74 A 73
SpnAspGAC SpnGluGAA Δ=21	GGTCCGTTGGTCAAGGGGTTATCACGTCGCCCTTTTCACGGCGAAGACGCGGGTTCGATCCGCGTCGGGACCCGGGTTCGATCCCGCTTGGCGCCTTTCACGCGGCTAACCGGGGTTCGAATCCCCTTCGCGCGTAACCGGGGTTCGAATCCCCTTCACGGCGTAACCGGGGTTCGAATCCCGTACGCGTTCGAATCCCGTACGCGGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGCGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTCGGGTTCGAACCGGTTCGAATCCGGTTCGAATCCGGTTACTCGGTTCGGGTTCGAATCCGGTTCGGTTCGAATCCGGTTTCGACTCGGTTCGAATCCGGTTTCGAATCCGGTTTCGAATCCGGTTCGAATCCGGTTTCGAATCCGGTTTCGACTCGGTTTCGACTCGGTTCGAATCCGGTTCGAATCCGGTTCGAATCCGGTTTCGAATCCGGTTTCGAATCCGGTTTCGAATCCGGTTTCCGACTTTCCGGTTTCGACTCGGTTTCGAATCCGGTTTCCGACTTTCCGGTTTCCGACTTTCCGGTTTCGACTCGGTTTCGAATCCGGTTTCCGACTTTCCGACTTTCCGGCGGTTTCGAATCCGCGGTTTCGAATCCGACTTTCCGGCGGTTTCGAATCCGCGTTTCGAATCCGCGGTTCCGACTTTCGACTTTCCGCGGTTTCGAATCCGCGGTTTCGAATCCGC	72 72
StyAspGAC StyGluGAA ∆=32	* * * * * * * * * * * * * * * * * * *	73 73
SynAspGAC SynGluGAA ∆=36	* * * * * * * * * * * * * * * * * * *	73 73
TelAspGAC TelGluGAA ∆=35	* *	73 73
TmaAspGAC TmaGluGAA ∆=34	GGGGCGTGGGGGCGGGGGGGGGGGGGGGGGGGGGGGGG	74 72
TpaAspGAC TpaGluGAA ∆=36	** * * * * * * ****** * *** * *** * * *	73 72
TteAspGAC TteGluGAA Δ=24	***** *** **** **** *** *** *** *** **	73 72
VchAspGAC VchGluGAA Δ=29	************************************	73 73
XcaAspGAC XcaGluGAA Δ=33	* * * * * * * * * * * * * * * * * * *	73 73
XfaAspGAC XfaGluGAA Δ=34	* * * * * * * * * * * * * * * * * * *	73 73
Eukarya :	Average $\Delta = 24.3$	
AthAspGAC AthGluGAA Δ=20	GTCGTTGTAGTAGTGGTAAGTATTCCCGCCTGTCACGCGGGTGGACCGGGTTCGATCCCGCCACGGCG TCCGATGTCGTCCACGGGTTAGGATATCTGGCTTCACCCAGGGGACCCGGGTTCGATTCCCGGCATCGGAG 110	72 72
CelAspGAC CelGluGAA ∆=21	TCCTCGTCTTGTGTGTGTGTGTGTCGCGTCGTCGTCGGCCGGGGTCGATTCCCGGCGGGGGGGG	72 72
DmeAspGAC DmeGluGAA $\Delta = 22$	TCCCGATAGTAGTGGTTAGTGGTTGCCCGCCCCCCGCGGGACGGCCGGGGTTCAATTCCCCGCTGCGGGGAG TCCCATAGTGTCTAGTGGCTAGGATATCCGCCTTCACCCAGAGGCCCGGGTTCGATTCCCCGCTATGGGAA 11020	72 72
EcuAspGAC EcuGluGAA Δ=28	* ************************************	73 72
HsaAspGAC HsaGluGAA Δ=24	TCCTCGTTAGTAGTGGTGAGTATCCCCGCCTGTCACGCGGGACGGGGTTCGATTCCCCGACGGGGAG TCCCTGGTGTGTGGTGAGGTTAGGTT	72 72
SceAspGAC SceGluGAA ∆=23	TCCGTGATAGTTTAATGGTCAGAATGGGCGCTGTCGCGCGGCGCGCGGGGTCGAATTCCCCGTGCGGGG TCCGTATAGTCTAACGGCTACACATCACGCTTTCACCGTGAGACCGGGGTTCGACTCCCCCTATCGCG 110203040506070.	72 72
SpoAspGAC SpoGluGAA ∆=32	TCTCCTTTAGTATAGGGG TAGTACACAAGCCTGCACGCCTTGCAGCCCGGGTTCGATCCCGAGGGAGG	71 72



Figure S2. Cont.



Figure S2. Sequence alignments of AspGAC and GluGAA genes from different species. (A) Sequence alignments from different species. The archaeal, bacterial and eukaryotic species aligned comprise species from Figure 4 and reference [83]. (B) Sequence alignments between ApeAspGAC and HbuAspGAC, and between ApeGluGAA and HbuGluGAA. Δ represents number of base differences between a pair of aligned genes. Three-letter names for species additional to those included in Figure 4 are: ARCHAEA. Mba Methanosarcina barkeri, Pab Pyrococcus abyssi, Pho Pyrococcus horikoshii, Sto Sulfolobus tokodaii, Tac Thermoplasma acidophilum. BACTERIA. Atu Agrobacterium tumefaciens, Bap Buchnera aphidicola, Bbu Borrelia burgdorferi, Cac Clostridium acetobutylicum, Ccr Caulobacter crescentus, Hin Haemophilus influenzae, Lin Listeria innocua, Lla Lactococcus lactis, Mpn Mycoplasma pneumoniae, Mtu Mycobacterium tuberculosis, Psa Pseudomonas aeruginosa, Rso Ralstonia solanacearum, Sco Streptomyces coelicolor, Spn Streptococcus pneumoniae, Sty Salmonella typhi, Syn Synechocystis 6803, Tel Thermosynechococcus elongatus, Vch Vibrio cholerae, Xca Xanthomonas campestris, Xfa Xylella fastidios. EUKARYA. Ath Arabidopsis thaliana, Cel Caenorhabditis elegans, Ecu Encephalitozoon cuniculi, Hsa Homo sapiens, Spo Schizosaccharomyces pombe.

Archaea : Average $\Delta = 17.7$



Figure S3. Cont.

av. <u>A</u> = 27.0 MbaSerTCC MbaSerTCA	* * ** *** ** ** ** ** **** ** ** ** **	71 71
MbaSerAGC Δ(C) = 23 Δ(A) = 34 av.Δ = 28.5	GACGAGATAGCCAAGCCCGGTATGGCGCGGGATTGCTAATCCCGTGACGGGGGTTCGAGTCCCCCTCTCGTCG 110203040506070 ** * *** *** *** *** * * **** ****	73
MjaSerTCC MjaSerTCA MjaSerAGC Δ (c) = 17 Δ (A) = 20	GCGCAGGTAGCCTAGCCCGG-CCAAGGCCTGGACTGGACATCCCATGGCGGGGGTTCAAATCCCCCCCC	74 75 73
$\begin{array}{l} \text{MmaSerTCC} \\ \text{MmaSerTCA} \\ \text{MmaSerAGC} \\ \Delta (C) = 25 \\ \Delta (A) = 26 \\ av \Delta = 25.5 \end{array}$	* **** *** *** ** *******************	73 73 73
MthSerTCC MthSerTCA MthSerAGC Δ (C) = 22 Δ (A) = 22 av. Δ = 22.0	GCCGGGATAGCCTAGCCAGGTAAGGCGCAAGACTGGAAATCTTGTGGCCTGGGTTCAAATCCCAGTCCCGGCG GCCGGGATAGCCTAGCC	73 73 73
PabSerTCC PabSerTCA PabSerAGC Δ (C) = 10 Δ (A) = 10 $av\Delta$ = 10.0	GCCGGGGTAGCCTAGCCGGGGAGGGCGGGGCCTGGAGAGCCCGTGGCCGGGGTTCAAATCCCCGCCCG	73 73 73
PaeSerTCC PaeSerTCA PaeSerAGC Δ (C) = 12 Δ (A) = 9 av. Δ = 10.5	GCCGGGGTGGCCGAGG-GGCCCAAGGCGCGGGACTGGAGATCCCGTCCCCGGGTTCAAATCCCGGCCCGGGC GCCGGGGTGGCCGAGG-GGCCCAAGGCGCGGGACTTGAGATCCCGTCCGTGGGTTCAAATCCCACCCCGGCG GCCGGGGTGGCCGAGTTGGTCCAAGGCGCGGGCCTGCTAAGCCCGTCCGT	74 74 74
PfuSerTCC PfuSerTCA PfuSerAGC Δ (C) = 10 Δ (A) = 10 av. Δ = 10.0	GCCGGGGTAGCCTAGCCAGGGAAGGCGCGGGGCCTGGAGAGCCCGTGGCCGGGGTTCAAATCCCCGCCCG	73 73 73
PhoSerTCC PhoSerTCA PhoSerAGC Δ (C) = 9 Δ (A) = 10 $av\Delta$ = 9.5	GCCGGGGTAGCCTAGCCTGGGAAGGCGCGGGCCTGGAGAGCCCGTGGCCGGGGTTCAAATCCCCGCCCG	73 73 73
SmaSerTCC SmaSerTCA SmaSerAGC Δ (C) = 18 Δ (A) = 17 av Δ = 17.5	GCCGGGGTGCCCGAGCGGACTAAGGGGCTGGCCTGGAGAGCCAGTGCCGCGGGGTTCGAATCCCGCCCG	75 75 75
SSOSETTCC SSOSETTCA SSOSETAGC Δ (C) = 18 Δ (A) = 22 av Δ = 20.0	GCCGGGGTGCCCGAGCGGACTAAGGGGCTGGCCTGGAGAGCCAGTGGCGCGGGGTTCAAATCCCGCCCCGGCG GCCGGGTGCCCGAGTGGCCTAAGGGCTGGCCTTGACAGCCAGTAGCGGGGTTCAAATCCCGCCCCGGCG GCCGAGTCGCCTAGCCAGGTAGGGCGGCCTGCTAAGCCGCTGGCGGGGGTTCAAATCCCGCCCTGGCG 1102030405060	73 73 73
StoSerTCC StoSerTCA StoSerAGC Δ (C) = 15 Δ (A) = 19 av Δ = 17.0	GCCGGGGT GCCCGGGGTCTAAGGGGGCGGCCTGGAGAGCCGTGGCGGGGTCAAGTCCCGCCCCGGCG GCCGGGGT GCCCGAGTGGACTAAGGGGCTGGCCTGAGAGCCAGTGGCGCGGGTTCAAATCCCGCCCCGGCG GCCGGGGTCGCCTAGCCCGGTTAGGGCGGCGCGCCTGCTAAGCCGCGGGGTCAAATCCCGCCCCGGCG GCCGGGGTCGCCTAGCCCGGTTAGGGCGGCGGCCTGCTAAGCCGCGGGGTCAAATCCCGCCCCGGCG 10	74 74 74
TacSerTCC TacSerTCA TacSerAGC Δ (C) = 23 Δ (A) = 18 av. Δ = 20.5	GCTGGGATAGCCCAGCTAAGGCGCAGGTCTGGAAAACCTGTGCCGGGAGTTCAAATCTCCCTCC	73 71 71
TonSerTCC TonSerTCA TonSerAGC Δ (C) = 14 Δ (A) = 18 av Δ = 16.0	GCCGGGGTAGCCTAGCCTGGGA - AGCCGCGGGCCTGGAGAGTCCGTGGCCCGGGTTCAAATCCCTGCCCGGCG GCCGGGATCGCCTAGCCTGGGAT-GGCGGGGCCTGAGGCCGGGCCCGGGGTTCAAATCCCCGTCCGGGG GCCGCGGTAGCCTGGCA-GGCGCGGCCTGCTAGCCGGTGGGC-GGGGTTCAAATCCCCGCCGCG 110	74 74 73
TvoSerTCC TvoSerTCA TvoSerAGC Δ (C) = 23 Δ (A) = 20 av Δ = 21.5	GCTGGGATAGCCTGGTAAGGCGCAGGTCTGGAAAACCTGTGCCGGGAGTTCAAATCTCCCTCC	73 73 73

Figure S3. Cont.

Bacteria : Average Δ = 22.3

AaeSerTCC AaeSerTCA AaeSerAGC Δ (C) = 14 Δ (A) = 18 av. Δ = 16.0	GCAAGGGTGGCCGAGCGAGCGCGCGCGCGCGCGCGCGCGC	73 73 73
AnaSerTCC AnaSerTCA AnaSerAGC Δ (C) = 24 Δ (A) = 19 av A = 21 5	GARGAGATGGCCAGTGGTTTAAGGCGCAGACCTGGAAAGTCTGTAACTAGGGTTCAAATCCCTATCTCCCG GGAGAGGTGGCAGAGTGGTCGAATGCACTGGACTTGAAATCGAGCGACGGGAGTTCGAATCTCCCCCTCTCCG GGAGAGTGGCTGACTGGTCGAAAGCGCCAGATTGCTAATCTTTGTCGAGGGTTCGAATCCCTCCC	73 73 73
AtuSerTCC AtuSerTCA AtuSerAGC Δ (C) = 13 Δ (A) = 24 av. Δ = 18.5	cccccccccccccccccccccccccccccccccccc	73 73 73
BapSerTCC BapSerTCA BapSerAGC Δ (C) = 14 Δ (A) = 23 av. Δ = 18.0	GOTGAGATGTCCGAGAGGCTTAAGGAACACGCCTGGAAAGCGTGTATCAAGGGTTCGAATCCCTTTCTCACCA GGAGAGGTGGCCGAGTGGTTTAAGGCAGCGGTCTGAAAACCGCCGACGAGAGTTCGAATCTCTCTC	73 73 73
BbuSerTCC BbuSerTCA BbuSerAGC Δ (C) = 18 Δ (A) = 22 av. Δ = 20.0	GCAGAGATGGCCGAGTGGCTTAAGGC-GCACGCTTGGAAAGCGTGTATCATGGGTTCGAATCCCATTCTCTCG GCAGAGTGGCACAGTGGTTTAATGCTACGGTCTTGAAAACCGT-TGTCGTGAGTTCGAATCTCACCCTCTCG GGAGAGATGCCAGAGTGGCCGAATGGGGCTTCCTGCTAAGAAGT-TGTCATGGGTTCGAATCCCATTCTCCCG 1	74 74 74
BloSerTCC BloSerTCA BloSerAGC Δ (C) = 28 Δ (A) = 29 av. Δ = 28.5	GGAGGATCGCCTGAGCGCCCTAAGGGCACCCTGGAAAGCGTGTGCACGAGTTCGAATCCCGTGCCCG GGACGGGTGTCTGAGCGCCCTAAGGAGCGCGCCTGGAAAACCGT-TGACGCGGGTCCGAATCCCCCTGCCG GGAGTCATGCCTGAGCGCCCTAAGGGCACCCTGCTAAGGTGT-TGACGCGGGTTCGAATCCCCTGCCCG 110203040	73 73 73
BsuSerTCC BsuSerTCA BsuSerAGC Δ (C) = 23 Δ (A) = 25 av. Δ = 24.0	GARGAGCTGTCCAAGTGGTCCAAGGAGCACGATTGGAAATCGTGTAGCAAGGGTTCGAATCCCTTGCTCTCCG GGAGGAATACCCAAGTCCGGCTGAAGGGATCGGTCTTGAAAACCGACAGCGGGGGTTCGAATCCCTCTTCCTCCG GGAGAAGTACTCAAGTGGCTGAAGAGGGCGCCCCTGCTAAGGGTGTAGCGAGGGTTCAAATCCCTCCTTCCCCG 11020	73 75 73
CacSerTCC CacSerTCA CacSerAGC Δ (C) = 29 Δ (A) = 33 av. Δ = 31.0	GARGAGATGTCCGAGT-GGTTTAAGGAGCACGCCTGGAACGCCT-GTGTCGAGGGTTCGAATCCCTCTTTCTCCG GGAAAGATGGTCGAGTTGGTTTAAGGCACCGGTCTTGAAAACCG-GCGTCTAGGGTTCGAATCCCTATCTTTCCG GGAGAAGTACTCAAGTGGTTGAAGAGGGTGCCCTTGCTAAGGGTATAGCGAGAGTTCGAAACTCTCCTTCTCCG 11020	73 74 73
CcrSerTCC CcrSerTCA CcrSerAGC Δ (C) = 21 Δ (A) = 18 av. Δ = 19.5	GGTGAGGTGGCCGAGTGGTTGAAGGCGCACGCCTGGAACGCGTGTGCGAGTCGAATCCCTCTCTCCGCG GGACAGGTGGCCGAGTGGTTTAAGGCAGCGGTCTGAAAACCGCCGTCGTGGGTTCGAATCCCACCTGTCCG GGACACGTGGCCGAGAGGCTGAAGGCACCGCTTTGCTAAAGCGGCATCGAGGGTTCGAATCCCTCCGTCTCCG 11020	73 73 73
CjeSerTCC CjeSerTCA CjeSerAGC Δ (C) = 23 Δ (A) = 31 av. Δ = 27.0	ACACAGOTGTCCCAGCGGTTGAAGGAGCACGCCTGGAACCGTGTAACCAGGGTTCCAATCCCTTCTCTGTCTG	73 73 73
CtrSerTCC CtrSerTCA CtrSerAGC Δ (C) = 20 Δ (A) = 23 av. Δ = 21.5	GAGAGATGTCCGAGTGGCTTAAGGAGCCGCGTGGAAAGCGTGTGTGGAGGGTTCGAATCCCTCTCTCCG GGAGGATGGCAGAGCGGTTTAATGCACCTGTCTGAAAACAGGAGACGGGGGTTCGAATCCCTCTTCTTCCG GGAAAGATGACTGAGTGGTCGAAAGTACGTCCCTGCTAAGGACGCGTCGAGGGTTCGAATCCCTCTCTTCCG 11020	73 73 73
DraSerTCC DraSerTCA DraSerAGC Δ (C) = 28 Δ (A) = 25 av. Δ = 26.5	GGAACGGTGTCCGAGTGGTTGAAGGAGCGCCTGGAAAGCGTGTATCGAGGGTTCGAATCCCTCCGTCCG	73 73 73
EcoSerTCC EcoSerTCA EcoSerAGC Δ (C) = 11 Δ (A) = 29 av. Δ = 20.0	GGTGAGGTGGCCGAGCGCTGAAGGCGCCGCTGGAAAGTGTGTATCGGGCGGTCGAACCCCCCCC	73 73 73
HinSerTCC HinSerTCA HinSerAGC Δ (C) = 14 Δ (A) = 27 av. Δ = 20.5	GGTGAGATGTCCGAGT - GGTTGAAGGAGCACGCCTGGAAAGCGTGTATCAAGGGTTCAAATCCCTTTCTCACG GGAGGAATGGTCGAGT - GGTTGAAGGCACCGGTCTTGAAAACCGGCGACTAGGGTTCGAATCCCTATTCCTCCG GGTGAGATGGCCGAGCAGGCTGAAGGCGCCCCCTGCTAAGGGAGTATCGAGGGTTCGAATCCCTCTCTCACCG 110203040506070	73 73 74

Figure S3. Cont.

av.Δ = 20.5	* ** ** **** ** ** ** ** ** ** ** ** **	
HpySerTCC HpySerTCA HpySerAGC Δ (C) = 24 Δ (A) = 29 cr A = 26 5	GGGGAGATGTCCAGT - GGTTGAAGGACGACGCCTGGAACGCCTGTAACGAGGGTTCGAATCCCTCTCTCCG CGGGAGATGGCTGAGT - GGTTGAAAGCGGCGTCTTGAAAACCGTTGACGGGGGTTCGAATCCCTCCTCCCGG GGACAGGTGGGTGAGTTGGCTGAAACCACACCCCGCTAAGGATGCGTCGAGGGTCGAATCCCTCCC	74 74 74
LinSerTCC LinSerTCA LinSerAGC Δ (C) = 25 Δ (A) = 24 av. Δ = 24.5	*** * *** *** **** ************************************	73 75 73
LlaSerTCC LlaSerTCA LlaSerAGC Δ (C) = 30 Δ (A) = 19 av. Δ = 24.5	GGAGAAGTGTCCGAGT GGCCGAAGGAGCACGCCTGGAAAGTGTGTATCGGGGGTTCGAATCCCCTCTTCTCCT GGAGGATTACCCAAGTCCGGCTGAAGGGAACGGTCTTGAAAACCGTCAGCGTGGGTTCGAATCCCACATCCTCCT GGGGGGTTACTCAAGA GGCTGAAGAGGACGGTTTGCTAAATCGTTAGCGAGGGTTCGAATCCCTTACCCCCT 110203040506070	73 75 73
MpnSerTCC MpnSerTCA MpnSerAGC Δ (C) = 22 Δ (A) = 27 av. Δ = 24.5	GGATACTTACCCAAGTGGCTGAAGGGGTAGGCTTGGAAAGCTTATAGCGAGGGTTCGAATCCCTCAGTATCCG GGAGACTTACCCAAGCGGCTGAAGGGTTCGGATCGGTCTGAAAACCGAGAGGGTCGAATCCCTCAGTCTCCG GGGAGCTACCAAGTGGTT-AAGAGGACACCCTGCTAAGGTGTTAGCGGGGTCGAATCCCACCGCTTCCG 110203040506070 ** ** * ** ** ** ** ** ** ** ** ** ** *	73 73 73
MtuSerTCC MtuSerTCA MtuSerAGC Δ (C) = 32 Δ (A) = 25 av. Δ = 28.5	GGAGGATTCGCCTAGTGGCCTATGGCGCTCGCCTGGAACGCGGGTTGCGCGGGTTCAAATCCCGCATCCTCCG GGTGGCGTGGC	73 73 73
NmeSerTCC NmeSerTCA NmeSerAGC Δ (C) = 24 Δ (A) = 32 av. Δ = 28.0	AGAGAGGTGGATGAGTGGTTTAAGTCGCACGCCTGGAAAGCGTGTATCGAGGGTTCGAATCCCTTCCTG GGAAGCGTGGCAGAGTGGTTTAATGCAACGGTCTTGAAAACCGTCGACGTGAGTTCGAATCTCACCGCTTCCG GGAGTAATGGCTGACAGGCTGAAGGCACTTCCCTGCTAAGGAAGCATCGAGGGTTCGAATCCCTCTTACTCCG 11020304050	73 73 73
PsaSerTCC PsaSerTCA PsaSerAGC Δ (C) = 17 Δ (A) = 28	GGTGAGGTGTCCGAGTGGTTGAAGGAGCACGCTGGAAAGTGTGTATCGAGGGTTCGAATCCCTCACCG GGAGGTGTGGCCGAGTGGTTTAAGGCAACGGTCTGGAAAACCGTCGCGCGTCGAGTTCGAATCTCACCGCCTCCG GGAGAGGTGGCCGAGTGGCCGAGGGCGCCCCCCGCTAAGGGAGTACCGGGGTTCGAATCCCCCCCTCTCCG 110203040506070	73 73 73
RprSerTCC RprSerTCA RprSerAGC Δ (C) = 18 Δ (A) = 22 av Δ = 20.0	************************************	73 73 73
RsoSerTCC RsoSerTCA RsoSerAGC Δ (C) = 20 Δ (A) = 26 av Δ = 23.0	GAGAGATGGATGGATTAAGTCGCACGCCTGGAAGCGTGTATCGGGGGTTCGAATCCCCCCTCTCCCG GGAGCCTGGCCGAGTGGTTTAAGGCAGCAGTCTTGAAAACTGCCGACGTGAGTTCGAATCTCACCGTTCCG GGAGACGTGGCCGAGAGGCACGCGACTCCCCTGCTAAGGGAGCATCGAGGGTTCGAATCCCCCGTCTCCG 11020304050	73 73 73
ScoSerTCC ScoSerTCA ScoSerAGC Δ (C) = 24 Δ (A) = 30 av. Δ = 27.0	GGAGGATTCGCCTAGT GGCCTAGGGCCG-ACGCTTGGAAAGCGTGTTGCACGAGTTCGAATCTCGTATCCCTCG -GGTGGGTTGCCCGAG-GGCCTAAGGGAACGGTCTTGAAAACCG-TCGTCGTGGGTTCAAATCCCACACCCACGGAGGCGCGCGC	73 73 75
SpnSerTCC SpnSerTCA SpnSerAGC Δ (C) = 17 Δ (A) = 19 av. Δ = 18.0	GGAAGATTACTCAAGA GGCTTAAGAGGCCGTGTTGGAAACGCGGTAGCGTGGGTTCGAATCCCATGTCTTCCG GGAGGATTACCCAAGTCCGGCTGAAGGGAACGGTCTTGAAAACCGTCAGGGTGGGT	73 75 73
StySerTCC StySerTCA StySerAGC Δ (C) = 11 Δ (A) = 29 av. Δ = 20.0	GGTGAGGTGTCCGAGTGGCTGAAGGAGCACGCCTGGAAAGTGTGTATCGGGGGTTCGAATCCCCCCCTCACCG GGAAGTGTGGCCGAGCGGTTGAAGGCACCGGTCTTGAAAACCGGCGACCAGAGTTCGAATCTCTGCGCTTCCG GGTGAGGTGGCCGAGAGGCTGAAGGCGCTCCCCTGCTAAGGGAGTATCCGGGGGTTCGAATCCCCGCCTCACCG 110203040	73 73 73
SynSerTCC SynSerTCA SynSerAGC Δ (C) = 15 Δ (A) = 20 av Δ = 17 5	GGAGAGGTGGCCGAGCGGTTGAAGGCGCAGCACTGGAATGCTGTTTCGAGGGTTCGAATCCCTCCC	73 73 73
TelserTCC TelserTCA TelserAGC Δ (C) = 16 Δ (A) = 15 av Δ = 15 5	GAGAGATGGCCGAGTGGTCGAAGGCCAGCACTGGAAATGCTGTGTCGAGGGTTCGAATCCCTCTCTCCG GGAGAGAGGCGAGAGTGGTCGAATGCGCTCGACTGGAAATGCGCGGGGTCGAGTCGAATCCCTCTCTCCG GGAGAGGCGGCGCGGC	73 73 73
TmaSerTCC TmaSerTCA TmaSerAGC Δ (C) = 11 Δ (A) = 21 $av\Delta$ = 16.0	GAGAGGTGGCCGAGCGAGCGCTTGAGGCGCGCGCGGGTGGGGGGGG	73 73 73

Figure S3. Cont.

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TpaSerTCC TpaSerTCA TpaSerAGC Δ (C) = 20 Δ (A) = 30 av. Δ = 25.0	GGAGAG GGAG <mark>T</mark> G GGAG <mark>C</mark> G 1	ATGTC GTGGC GTGCG 10. * *	CGAGI CGAGI AGAGI	GGCC GGTT GGTC 20	GAAGG GAAGG GAATC	TACACO CGACGO GGGCTO 30	GATTGO GTCTTO CCCTGC	AAGTC AAAAC TAAGG 40.	GTGTG' CGCTG' AGTTG' 	TCGAG TCGGG CCGGG 50.	GGTTC GGTTC GGTTC	GAATCO GAATCO GAATCO 60	CCTCTC CCCCCT CCCCCCT	TCTCCG GCTCCG .70	73 73 73
TteSerTCC TteSerTCA TteSerAGC Δ (C) = 21 Δ (A) = 20 av. Δ = 20.5	GGAGAG GGAGAG GGAGAA 1	ATGAC GTGTC GTACC 10. ** *	CGAGT CGAGT CAAGT	GGTC GGCT GGT- 20	GAAGG TAAGG GAAGG · · · · · ·	TGCACC AGCTGC GGCGGC 30	GACTGG GTCTTG ICCTGC	AAATCO AAAACO TAAGAO 40.	GTGTG CAGTG CTGTA · · · · · ·	TCGAG ACGTG GCGTG 50.	GGTTC GGTTC GGTTC	GAATCO GAATCO GAATCO 60	CCTCTC CCACCC CCACCCI	TCTCCG TCTCCG TCTCCG .70	73 73 73
VchSerTCC VchSerTCA VchSerAGC Δ (C) = 14 Δ (A) = 27 av. Δ = 20.5	GG <mark>T</mark> GAG GGAGAG GG <mark>T</mark> GAG 1	TTGTC ATGGC GTGGC 10.	CGAGI TGAGI CGAGA	GGCT GGTT GGCT 20	GAAGG GAAAG GAAGG	AGCAC CACCGO CGCTCO 30	GCCTGC GTCTTC CCCTGC	AAAGT AAAAC TAAGG 40.	GTGTA CGGCG GAGTA · · · · · ·	TCGAG ACTAG TCGAG 50.	GGTTC GGTTC GGTTC	GAATC AAATCO GAATCO 60.	CCTATCA CCTATCCI	TCACCO	73 73 73
XcaSerTCC XcaSerTCA XcaSerAGC Δ (C) = 16 Δ (A) = 23 av. Δ = 19.5	GGAGAG GGAGAG GGAGC 1	GTGTC ATGGC ATGCC 10. ** *	CGAGT CGAGC CGAGC	GGTT GGTT GGCT 20	GAAGG TAAGG GAAGG · · · · · ·	AGCACC CACCGC GGCTCC 30	GCCTGG GTCTTG CCCTGC	AAAGTO AAAACO TAAGGO 40.	GTGTA GGCCG GAGTA · · · · · ·	ACGGG ACGTG TCGAG 50.	GGTTC GGTTC GGTTC	GAATCO GAATCO GAATCO 60	CCCCTC CCTCTC * *	TCTCCG TCTCCG GCTCCG .70	73 73 73
XfaSerTCC XfaSerTCA XfaSerAGC Δ (C) = 17 Δ (A) = 33 av Δ = 25.0	GGAGAG GGAAG <mark>C</mark> GGAG <mark>T</mark> G 1	GTGTC GTGGC ATGCC 10.	CGAGT AGAGT CGAGA	GGTT GGTC GGCC 20	GAAGG GATTG G <mark>AA</mark> GG	AGCACC CACCGO GGCTCO 30	GCCTGG GTCTTG CCCTGC	AAAGT AAAA <mark>CC</mark> TAAGG 40.	GTGTAI CGGCAI GAGTA	ACGGG ACGTG TCGAG 50.	GGTTC AGTTC GGTTC	GAATCO GAATCO GAATCO 60	CCCCTC CACCG CCTCTC	TCTCCG CTTCCG ACTCCG .70	73 73 73

Eukarya : Average Δ = 22.9



Figure S3. Sequence alignments of tRNA genes for Ser from different species. Δ (C) represents number of base differences between SerAGC and SerTCC, and Δ (A) represents number of base differences between SerAGC and SerTCA, within the same species. Because the eukaryotic species examined lack SerTCC but contain SerTCT, the base difference between SerAGC and SerTCT is estimated instead and shown as Δ (T).