

<b>Species</b>	<b>Median expected MFE</b>	<b>Median observed MFE</b>	<b>p-value</b>
<i>Anolis carolinensis</i>	-9.8	-7.6	2.150e-38
<i>Bos taurus</i>	-9.4	-7.3	2.755e-48
<i>Caenorhabditis elegans</i>	-8.4	-6.1	1.622e-72
<i>Callithrix jacchus</i>	-9.4	-7.4	6.827e-40
<i>Canis familiaris</i>	-10.5	-8.2	7.198e-41
<i>Cavia porcellus</i>	-9.7	-7.7	5.008e-32
<i>Danio rerio</i>	-9.1	-6.9	1.324e-42
<i>Drosophila melanogaster</i>	-8.8	-7.3	2.928e-45
<i>Equus caballus</i>	-9.6	-7.7	7.464e-37
<i>Felis catus</i>	-9.9	-8.0	2.241e-38
<i>Gallus gallus</i>	-9.7	-7.7	5.803e-44
<i>Gasterosteus aculeatus</i>	-10.2	-8.8	4.017e-11
<i>Gorilla gorilla</i>	-10.1	-7.7	3.359e-48
<i>Homo sapiens</i>	-9.8	-8.4	1.500e-18
<i>Loxodonta africana</i>	-9.4	-7.8	1.895e-24
<i>Macaca mulatta</i>	-9.5	-7.6	3.644e-41
<i>Meleagris gallopavo</i>	-10.0	-8.8	2.748e-12
<i>Microcebus murinus</i>	-9.6	-7.7	4.077e-43

<b><i>Monodelphis domestica</i></b>	-9.4	-7.1	3.484e-46
<b><i>Mus musculus</i></b>	-9.8	-8.0	1.783e-21
<b><i>Nomascus leucogenys</i></b>	-9.9	-7.7	3.664e-39
<b><i>Ornithorhynchus anatinus</i></b>	-9.3	-7.5	3.728e-24
<b><i>Oryctolagus cuniculus</i></b>	-11.0	-9.5	5.332e-21
<b><i>Oryzias latipes</i></b>	-9.8	-8.6	1.177e-24
<b><i>Ovis aries</i></b>	-9.9	-7.6	2.909e-47
<b><i>Pan troglodytes</i></b>	-10.0	-8.0	1.045e-32
<b><i>Petromyzon marinus</i></b>	-10.1	-8.9	3.406e-13
<b><i>Rattus norvegicus</i></b>	-9.7	-7.6	8.945e-52
<b><i>Saccharomyces cerevisiae</i></b>	-7.9	-5.3	1.212e-83
<b><i>Sus scrofa</i></b>	-9.4	-7.3	1.952e-33
<b><i>Taeniopygia guttata</i></b>	-9.9	-8.2	2.798e-19
<b><i>Takifugu rubripes</i></b>	-10.1	-8.5	2.567e-22
<b><i>Tetraodon nigroviridis</i></b>	-10.1	-8.3	6.676e-44
<b><i>Xenopus tropicalis</i></b>	-8.9	-6.5	1.533e-56
<b><i>Acinetobacter baumannii</i></b>	-8.5	-5.9	2.989e-81
<b><i>Actinobacillus</i></b>	-8.8	-6.1	2.674e-94

<i>pleuropneumoniae</i>			
<i>Aeromonas hydrophila</i>	-12.2	-11.3	3.487e-21
<i>Aeropyrum pernix</i>	-11.6	-9.6	4.811e-66
<i>Anaplasma phagocytophilum</i>	-8.6	-6.6	6.184e-64
<i>Burkholderia pseudomallei</i>	-14.8	-14.0	4.810e-10
<i>Campylobacter jejuni</i>	-7.2	-4.1	2.281e-106
<i>Candidatus korarchaeum</i>	-10.7	-8.3	1.040e-81
<i>Chlamydia trachomatis</i>	-8.8	-6.1	1.640e-68
<i>Chlorobium tepidum</i>	-10.4	-8.8	1.211e-54
<i>Clostridium botulinum</i>	-6.9	-3.6	2.173e-103
<i>Corynebacterium glutamicum</i>	-10.4	-8.8	1.449e-50
<i>Coxiella burnetii</i>	-8.7	-6.1	1.676e-81
<i>Desulfovibrio vulgaris</i>	-12.8	-11.2	1.465e-37
<i>Enterococcus faecalis</i>	-8.2	-4.9	1.545e-108
<i>Escherichia coli</i>	-10.3	-8.4	6.433e-69
<i>Flavobacterium psychrophilum</i>	-6.7	-3.5	8.408e-111

<i>Francisella tularensis</i>	-7.2	-4.5	1.006e-99
<i>Haemophilus influenzae</i>	-8.6	-5.7	1.258e-91
<i>Haloarcula marismortui</i>	-11.6	-9.8	1.051e-48
<i>Halobacterium salinarum</i>	-12.1	-10.6	7.539e-31
<i>Haloferax volcanii</i>	-12.9	-11.8	1.195e-19
<i>Helicobacter pylori</i>	-7.9	-5.1	2.902e-91
<i>Klebsiella pneumoniae</i>	-11.1	-9.1	2.711e-67
<i>Lactococcus lactis</i>	-7.8	-4.8	1.545e-108
<i>Legionella pneumophila</i>	-8.2	-5.4	4.305e-101
<i>Leuconostoc mesenteroides</i>	-7.7	-5.1	1.044e-81
<i>Listeria monocytogenes</i>	-7.8	-4.6	9.034e-106
<i>Lysinibacillus sphaericus</i>	-8.1	-5.0	1.117e-111
<i>Mesoplasma florum</i>	-6.9	-3.5	4.396e-80
<i>Methanobrevibacter smithii</i>	-7.4	-4.3	1.888e-106
<i>Methanococcus maripaludis</i>	-7.4	-4.0	4.694e-115

<b><i>Microcystis aeruginosa</i></b>	-8.6	-5.9	1.444e-84
<b><i>Moorella thermoacetica</i></b>	-11.1	-9.2	5.245e-78
<b><i>Mycobacterium tuberculosis</i></b>	-13.5	-12.5	8.686e-31
<b><i>Mycoplasma pneumoniae</i></b>	-7.8	-5.2	8.711e-67
<b><i>Myxococcus xanthus</i></b>	-14.5	-13.9	6.959e-05
<b><i>Neisseria meningitidis</i></b>	-10.3	-8.0	4.961e-77
<b><i>Nitrosopumilus maritimus</i></b>	-7.7	-4.5	8.049e-107
<b><i>Paracoccus denitrificans</i></b>	-14.0	-12.9	3.271e-31
<b><i>Porphyromonas gingivalis</i></b>	-9.2	-6.9	7.527e-87
<b><i>Prochlorococcus marinus</i></b>	-8.2	-5.2	1.847e-101
<b><i>Propionibacterium acnes</i></b>	-11.9	-10.6	1.852e-25
<b><i>Pseudomonas aeruginosa</i></b>	-13.9	-12.9	2.532e-20
<b><i>Rhizobium leguminosarum</i></b>	-12.5	-10.9	1.791e-40
<b><i>Rhodobacter</i></b>	-14.4	-13.6	4.976e-14

<i>sphaeroides</i>			
<i>Rhodospirillum rubrum</i>	-13.5	-12.6	3.375e-25
<i>Salinibacter ruber</i>	-13.1	-12.4	2.054e-22
<i>Salmonella enterica</i>	-10.4	-8.6	2.345e-60
<i>Staphylococcus aureus</i>	-7.3	-4.1	1.450e-107
<i>Streptococcus pneumoniae</i>	-8.4	-5.5	8.344e-86
<i>Thermus thermophilus</i>	-15.0	-14.3	4.446e-29
<i>Ureaplasma parvum</i>	-6.4	-3.3	1.415e-65
<i>Vibrio cholerae</i>	-9.5	-7.4	1.062e-62
<i>Vibrio fischeri</i>	-8.4	-5.8	7.876e-85
<i>Xanthomonas campestris</i>	-13.7	-13.0	2.095e-18
<i>Yersinia pestis</i>	-9.9	-7.7	3.353e-62

**Table S10.** Median minimum free energy (MFE) at the 3'-end (observed MFE), median MFE expected by the global (not spatial) codon bias (expected MFE), and p-value for testing whether the median difference between pairs of expected and observed values equals zero (FDR-corrected two-sided sign test).