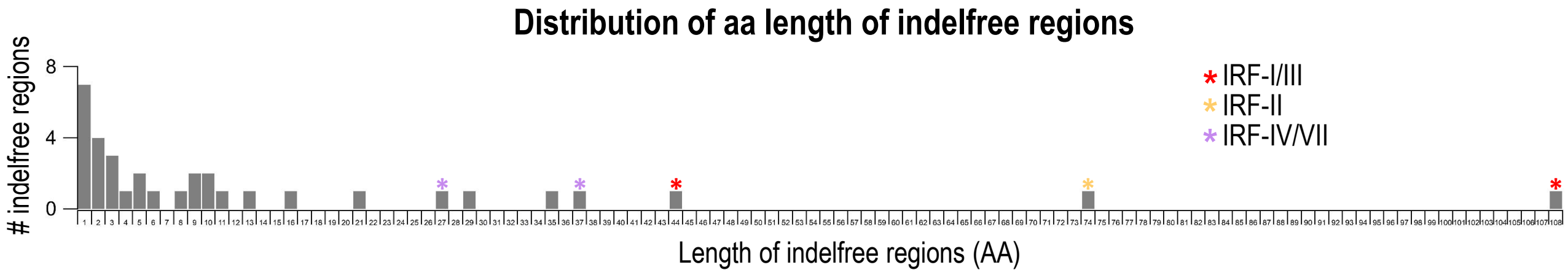
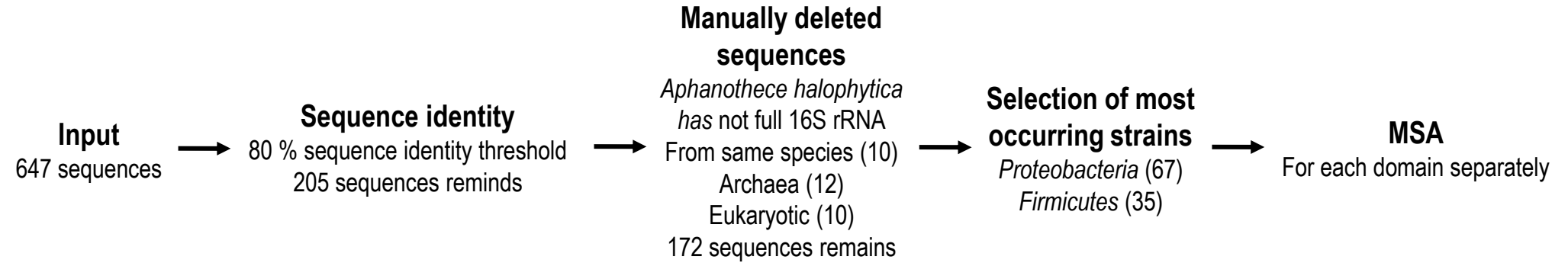


**Figure S1.** Cumulative sum of indels with real number of indels.



**Figure S2.** Various size distribution of indel-free regions.



**Figure S3.** Workflow of sequence selection steps and choosing final taxons.

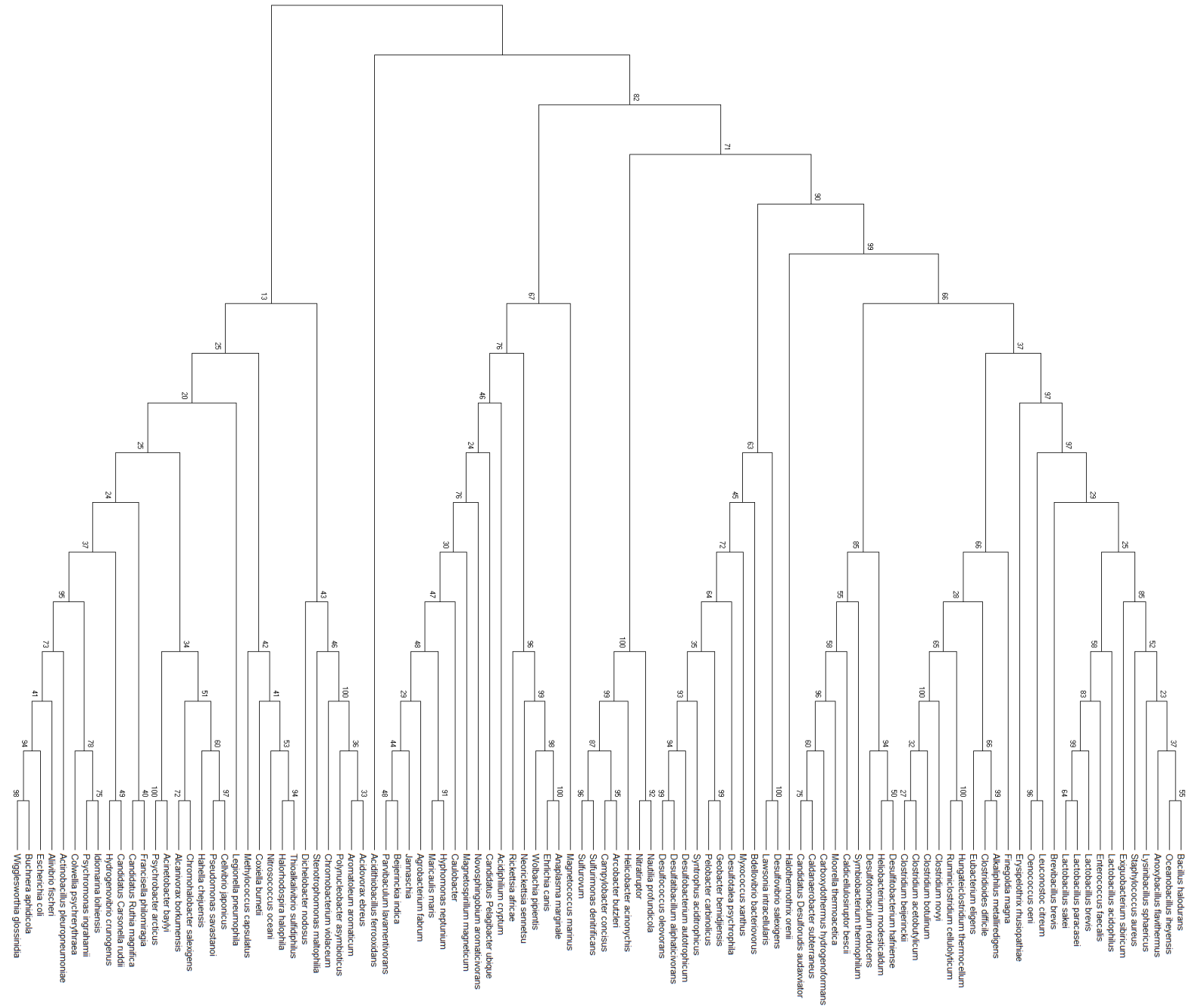
**Table S1.** Average Cα-Cα residue distance in selected Hsp70 proteins.

Strain	Pdb	Form	Method	Average Cα-Cα distance (Å)	References (doi)
<i>Escherichia coli</i>	7KO2	Restraining state	X-RAY DIFFRACTION	36.49	10.1016/j.molcel.2021.07.039
<i>Escherichia coli</i>	7KRT	Restraining state of a truncated DnaK	X-RAY DIFFRACTION	36.65	10.1016/j.molcel.2021.07.039
<i>Escherichia coli</i>	5NRO	J-domain bound	X-RAY DIFFRACTION	36.78	10.1016/j.molcel.2017.12.003
<i>Escherichia coli</i>	4JNE	Allosteric opening	X-RAY DIFFRACTION	36.97	10.1038/nsmb.2583
<i>Escherichia coli</i>	7KRW	Stimulating state fused with a substrate	X-RAY DIFFRACTION	39.71	10.1016/j.molcel.2021.07.039
<i>Escherichia coli</i>	7KRV	Stimulating state of disulfide-bridged	X-RAY DIFFRACTION	34.31	10.1016/j.molcel.2021.07.039
<i>Escherichia coli</i>	7KZI	Intermediate state (QQQ)	X-RAY DIFFRACTION	38.46	10.1107/S2059798321002436
<i>Escherichia coli</i>	7KZU	Quasi-intermediate state (Q) of a truncated Hsp70 DnaK	X-RAY DIFFRACTION	31.99	10.1107/S2059798321002436
<i>Escherichia coli</i>	4JN4	Allosteric opening	X-RAY DIFFRACTION	37.03	10.1038/nsmb.2583
<i>Escherichia coli</i>	7KRU	Stimulating state of a truncated DnaK	X-RAY DIFFRACTION	34.35	10.1074/jbc.M708992200
<i>Geobacillus kaustophilus</i>	2V7Y	Post-ATP hydrolysis state	X-RAY DIFFRACTION	45.92	10.1074/jbc.M708992200
<i>Homo sapiens</i>	5E84	ATP-bound state	X-RAY DIFFRACTION	37.2	10.1016/j.str.2015.10.012
<i>Homo sapiens</i>	6ASY	ATP-bound state	X-RAY DIFFRACTION	36.32	10.1038/s41467-017-01310-z
<i>Homo sapiens</i>	7N1R	ATP hydrolysis to AMP (open conformation)	X-RAY DIFFRACTION	36.29	10.1002/pro.4267
<i>Homo sapiens</i>	3D2F	In complex with Sse1p	X-RAY DIFFRACTION	38.42	10.1016/j.cell.2008.05.022

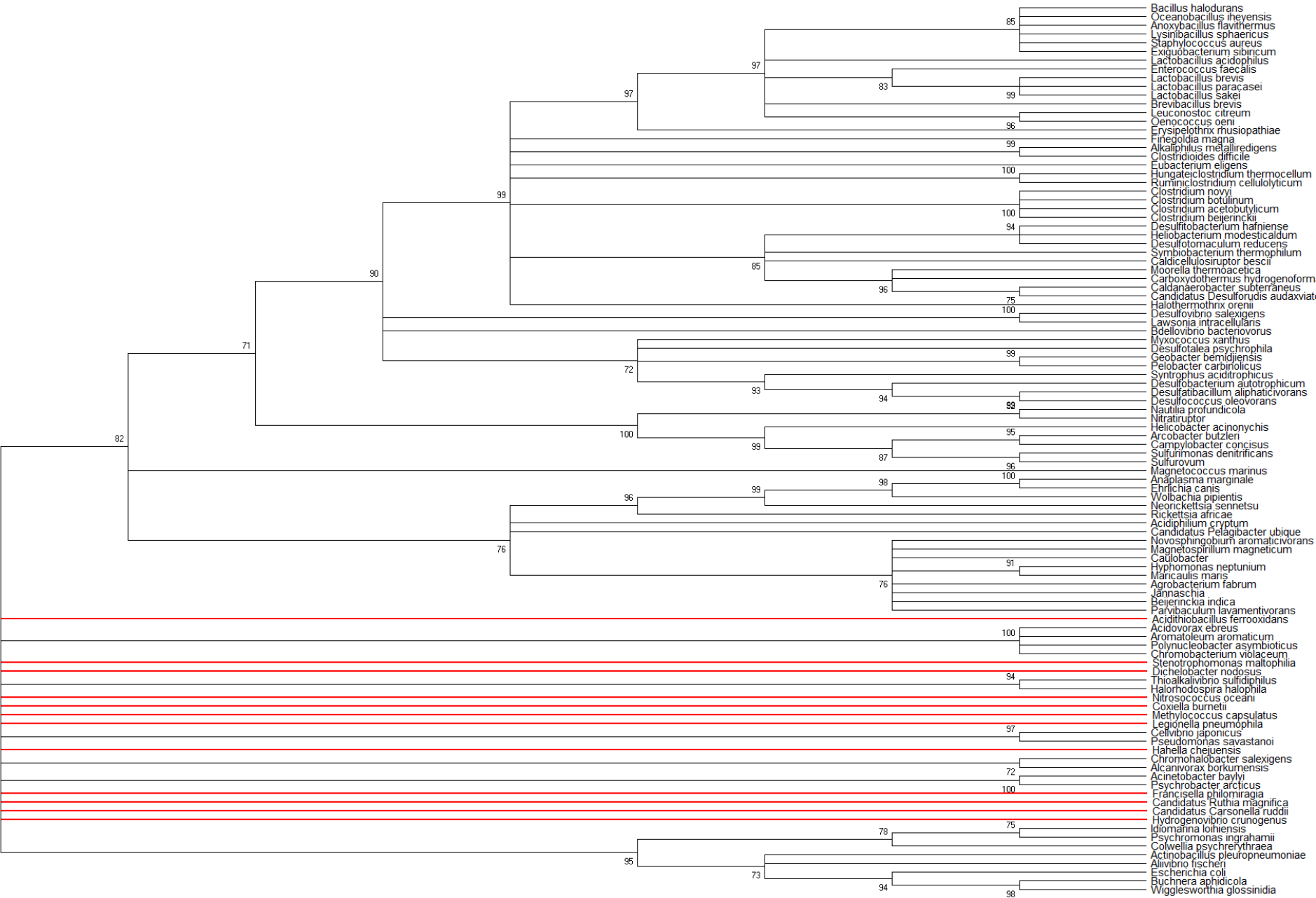
**Table S2.** Phylogenetic distribution of 172 sequences.

Phylum	# bacteria
<i>Acidobacteria</i>	1
<i>Actinobacterila</i>	16
<i>Aquificae</i>	1
<i>Bacteroidetes</i>	11
<i>Chlamydiae</i>	2
<i>Chlorobi</i>	3
<i>Chloroflexi</i>	2
<i>Cyanobacteria</i>	7
<i>Deinococcus-thermus</i>	2
<i>Dictyoglomi</i>	1
<i>Elusimicrobia</i>	2
<b><i>Firmicutes</i></b>	<b>35</b>
<i>Fusobacteria</i>	1
<i>Nitrospirae</i>	1
<i>Planctomycetes</i>	1
<b><i>Proteobacteria</i></b>	<b>67</b>
<i>Spirochaetes</i>	3
<i>Tenericutes</i>	13
<i>Thermotogae</i>	1
<i>Verrucomicrobia</i>	2

**Figure S4.** First ML phylogenetic tree of 102 *Firmicutes* and *Proteobacteria* sequences with bootstrap values of nodes.



**Figure S5.** Condensed version of first ML phylogenetic tree of 102 *Firmicutes* and *Proteobacteria* sequences with bootstrap threshold set to 70%. 12 bacteria did not have any node (red color).

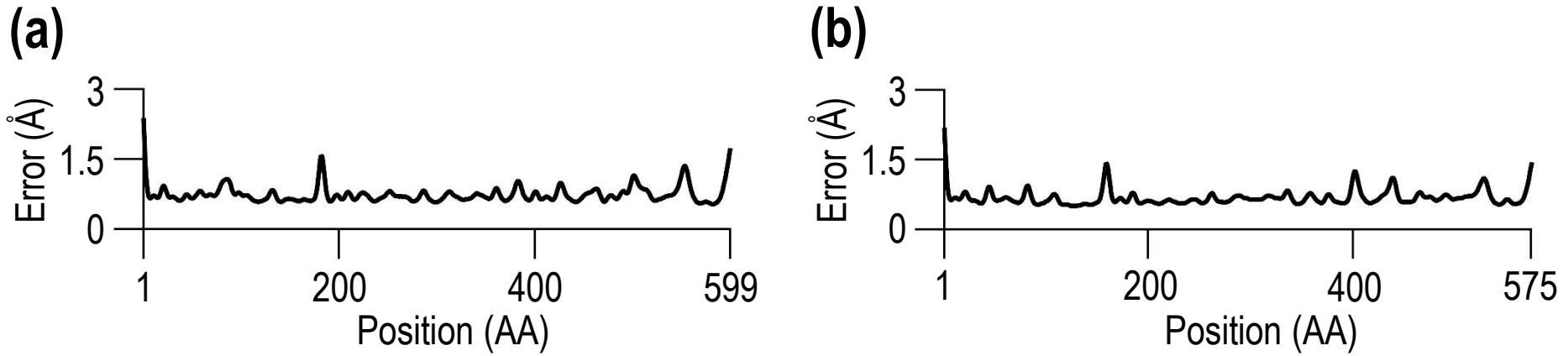


**Table S3.** Final list of 90 *Proteobacteria* and *Firmicutes* strains.

<i>Proteobacteria</i>			<i>Firmicutes</i>	
<i>Buchnera aphidicola</i>	<i>Aromatoleum aromaticum</i>	<i>Nitratiruptor</i>	<i>Halothermothrix orenii</i>	<i>Ruminiclostridium cellulolyticum</i>
<i>Wigglesworthia glossinidia</i>	<i>Magnetococcus marinus</i>	<i>Helicobacter acinonychis</i>	<i>Caldanaerobacter subterraneus</i>	<i>Erysipelothrix rhusiopathiae</i>
<i>Escherichia coli</i>	<i>Anaplasma marginale</i>	<i>Arcobacter butzleri</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Bacillus halodurans</i>
<i>Actinobacillus pleuropneumoniae</i>	<i>Ehrlichia canis</i>	<i>Campylobacter concisus</i>	<i>Candidatus Desulforudis audaxviator</i>	<i>Anoxybacillus flavithermus</i>
<i>Aliivibrio fischeri</i>	<i>Wolbachia pipientis</i>	<i>Sulfurimonas denitrificans</i>	<i>Moorella thermoacetica</i>	<i>Oceanobacillus iheyensis</i>
<i>Colwellia psychrerythraea</i>	<i>Neorickettsia sennetsu</i>	<i>Sulfurovum</i>	<i>Caldicellulosiruptor bescii</i>	<i>Staphylococcus aureus</i>
<i>Idiomarina loihiensis</i>	<i>Rickettsia africae</i>	<i>Desulfovibrio salexigens</i>	<i>Symbiobacterium thermophilum</i>	<i>Lysinibacillus sphaericus</i>
<i>Psychromonas ingrahamii</i>	<i>Candidatus Pelagibacter ubique</i>	<i>Lawsonia intracellularis</i>	<i>Desulfotomaculum reducens</i>	<i>Exiguobacterium sibiricum</i>
<i>Acinetobacter baylyi</i>	<i>Acidiphilium cryptum</i>	<i>Bdellovibrio bacteriovorus</i>	<i>Desulfitobacterium hafniense</i>	<i>Brevibacillus brevis</i>
<i>Psychrobacter arcticus</i>	<i>Novosphingobium aromaticivorans</i>	<i>Myxococcus xanthus</i>	<i>Heliobacterium modesticaldum</i>	<i>Leuconostoc citreum</i>
<i>Chromohalobacter salexigens</i>	<i>Caulobacter</i>	<i>Geobacter bemidjensis</i>	<i>Clostridium acetobutylicum</i>	<i>Oenococcus oeni</i>
<i>Alcanivorax borkumensis</i>	<i>Magnetospirillum magneticum</i>	<i>Pelobacter carbinolicus</i>	<i>Clostridium beijerinckii</i>	<i>Lactobacillus acidophilus</i>
<i>Cellvibrio japonicus</i>	<i>Hyphomonas neptunium</i>	<i>Desulfotalea psychrophila</i>	<i>Clostridium botulinum</i>	<i>Enterococcus faecalis</i>
<i>Pseudomonas savastanoi</i>	<i>Maricaulis maris</i>	<i>Syntrophus aciditrophicus</i>	<i>Clostridium novyi</i>	<i>Lactobacillus brevis</i>
<i>Thioalkalivibrio sulfidiphilus</i>	<i>Jannaschia</i>	<i>Desulfobacterium autotrophicum</i>	<i>Finegoldia magna</i>	<i>Lactobacillus paracasei</i>
<i>Halorhodospira halophila</i>	<i>Parvibaculum lavamentivorans</i>	<i>Desulfatibacillum aliphaticivorans</i>	<i>Eubacterium eligens</i>	<i>Lactobacillus sakei</i>
<i>Chromobacterium violaceum</i>	<i>Agrobacterium fabrum</i>	<i>Desulfococcus oleovorans</i>	<i>Alkaliphilus metalliredigens</i>	
<i>Polynucleobacter asymbioticus</i>	<i>Beijerinckia indica</i>		<i>Clostridioides difficile</i>	
<i>Acidovorax ebreus</i>	<i>Nautilia profundicola</i>		<i>Hungateiclostridium thermocellum</i>	



## Ancestral structures estimated residue error



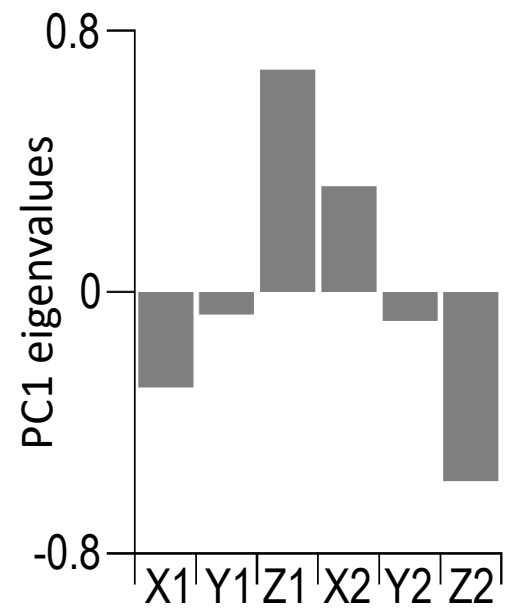
**Figure S6.** Ancestral structures estimated residue error. **(a)** Estimated error of *Proteobacteria* ancestral node 52. **(b)** Estimated error of *Firmicutes* ancestral node 45.

**Table S4.** List of calculation points for *Proteobacteria* divergence time estimation.

1. taxon	2. taxon	Min Time	Max Time	Estimated divergence time (MY)
<i>Escherichia coli</i>	<i>Buchnera aphidicola</i>	850	900	898.88
<i>Colwellia psychrerythraea</i>	<i>Psychromonas ingrahamii</i>	663.1	668.4	667.05
<i>Buchnera aphidicola</i>	<i>Psychromonas ingrahamii</i>	1673	2223	1697.25
<i>Acinetobacter baylyi</i>	<i>Pseudomonas savastanoi</i>	1306	1342	1326.21
<i>Buchnera aphidicola</i>	<i>Pseudomonas savastanoi</i>	1313	1753	1732.38
<i>Chromobacterium violaceum</i>	<i>Aromatoleum aromaticum</i>	1271.4	1834.4	1271.4
<i>Buchnera aphidicola</i>	<i>Aromatoleum aromaticum</i>	2138	2879	2138
<i>Buchnera aphidicola</i>	<i>Beijerinckia indica</i>	1707	2867	2676.9
<i>Magnetospirillum magneticum</i>	<i>Beijerinckia indica</i>	1886	2260	1886
<i>Anaplasma marginale</i>	<i>Beijerinckia indica</i>	1886	2260	2101.73
<i>Buchnera aphidicola</i>	<i>Sulfurovum</i>	2808	2981	2808
<i>Helicobacter acinonychis</i>	<i>Arcobacter butzleri</i>	841	1572	1001.18
<i>Geobacter bemidjensis</i>	<i>Desulfococcus oleovorans</i>	2173	2420	2194.7
<i>Desulfovibrio salexigens</i>	<i>Desulfococcus oleovorans</i>	2309	2508	2508
<i>Geobacter bemidjensis</i>	<i>Pelobacter carbinolicus</i>	1338.8	1412.9	1412.9
<i>Myxococcus xanthus</i>	<i>Geobacter bemidjensis</i>	2173	2420	2237.41

**Table S5.** List of calculation points for *Firmicutes* divergence time estimation.

1. taxon	2. taxon	Min Time	Max Time	Estimated divergence time (MY)
<i>Moorella thermoacetica</i>	<i>Candidatus Desulforudis audaxviator</i>	1997	2381	1997
<i>Desulfotomaculum reducens</i>	<i>Desulfitobacterium hafniense</i>	1682	3181	2025.36
<i>Clostridium novyi</i>	<i>Clostridium botulinum</i>	196	2244	1134.1
<i>Clostridioides difficile</i>	<i>Alkaliphilus metalliredigens</i>	1823	3493	1823
<i>Exiguobacterium sibiricum</i>	<i>Bacillus halodurans</i>	1566	1814	1609.82
<i>Lactobacillus sakei</i>	<i>Haloferoxthermus orenii</i>	2482	3025	3025
<i>Lactobacillus sakei</i>	<i>Bacillus halodurans</i>	1698	2100	1777.1
<i>Lactobacillus sakei</i>	<i>Enterococcus faecalis</i>	805	1401	1243.95
<i>Clostridioides difficile</i>	<i>Eubacterium eligens</i>	1823	3493	2380.1



**Figure S7.** Eigenvalues of PC1. The highest space position variance for both contact residues show Z axis.