Supplementary Information

The Metabolic Network of the Last Bacterial Common Ancestor

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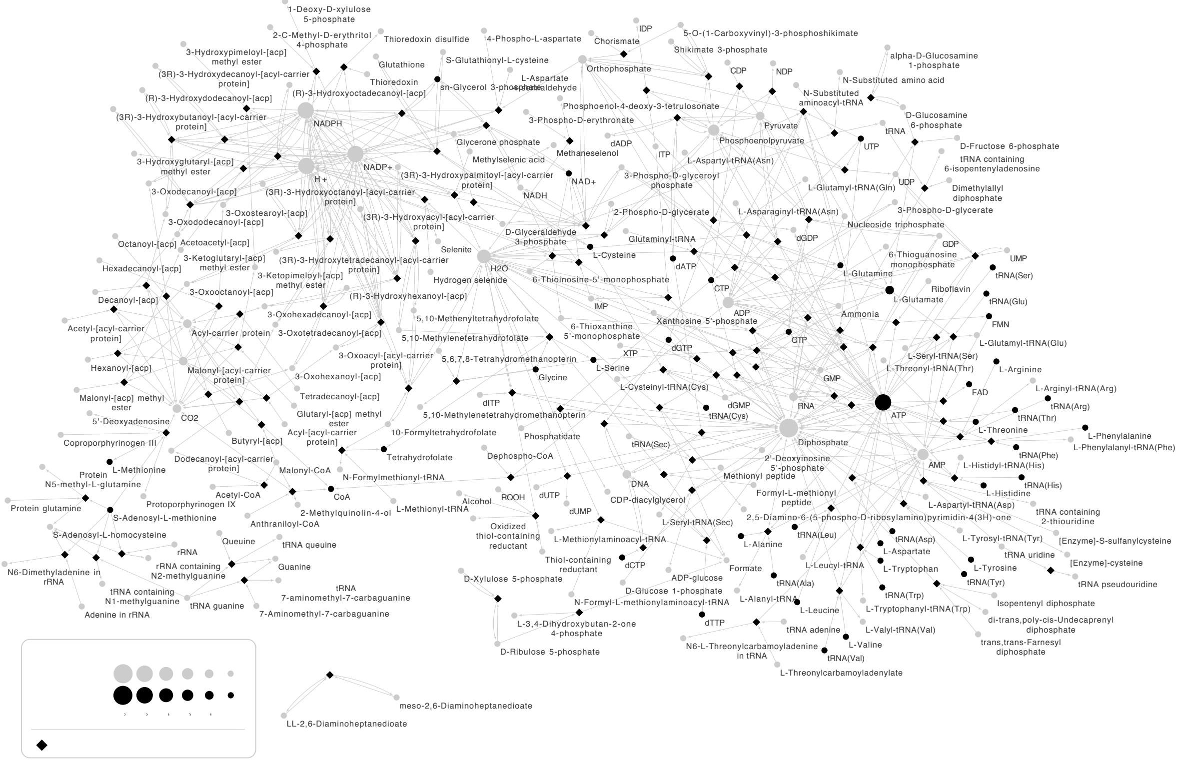
Supplementary Table 1│Bacterial genomes per taxonomic group. For each taxonomic group, the number of genomes present in the dataset with no filters, after filtering out small genomes (<1000 sequences) and after filtering out the aerobic genomes are shown.

Taxonomic group	Unfiltered	No small genomes	No small genomes and no aerobes
Gammaproteobacteria	1,565	1,513	123
Bacilli	1,012	1,012	416
Actinobacteria	593	591	110
Betaproteobacteria	493	484	47
Alphaproteobacteria	460	415	9
Epsilonproteobacteria	253	253	3
Bacteroidetes	185	174	47
Clostridia	169	169	165
Tenericutes	141	18	18
Chlamydiae	113	14	10
Cyanobacteria	94	94	2
Deltaproteobacteria	74	73	23
Spirochaetes	66	50	34
Thermotogae	32	32	32
Other bacteria	29	28	18
Deinococcus-Thermus	26	26	0
Chloroflexi	26	26	17
Fusobacteria	20	20	20
Aquificae	16	16	3
Chlorobi	12	12	7
Negativicutes	12	12	12
Verrucomicrobia	10	9	3
Acidobacteria	8	8	1
Planctomycetes	8	8	0
Erysipelotrichia	8	8	8
Nitrospirae	8	8	1
Acidithiobacillia	5	5	0
Synergistetes	5	5	5
Total	**5443**	**5083**	**1089**

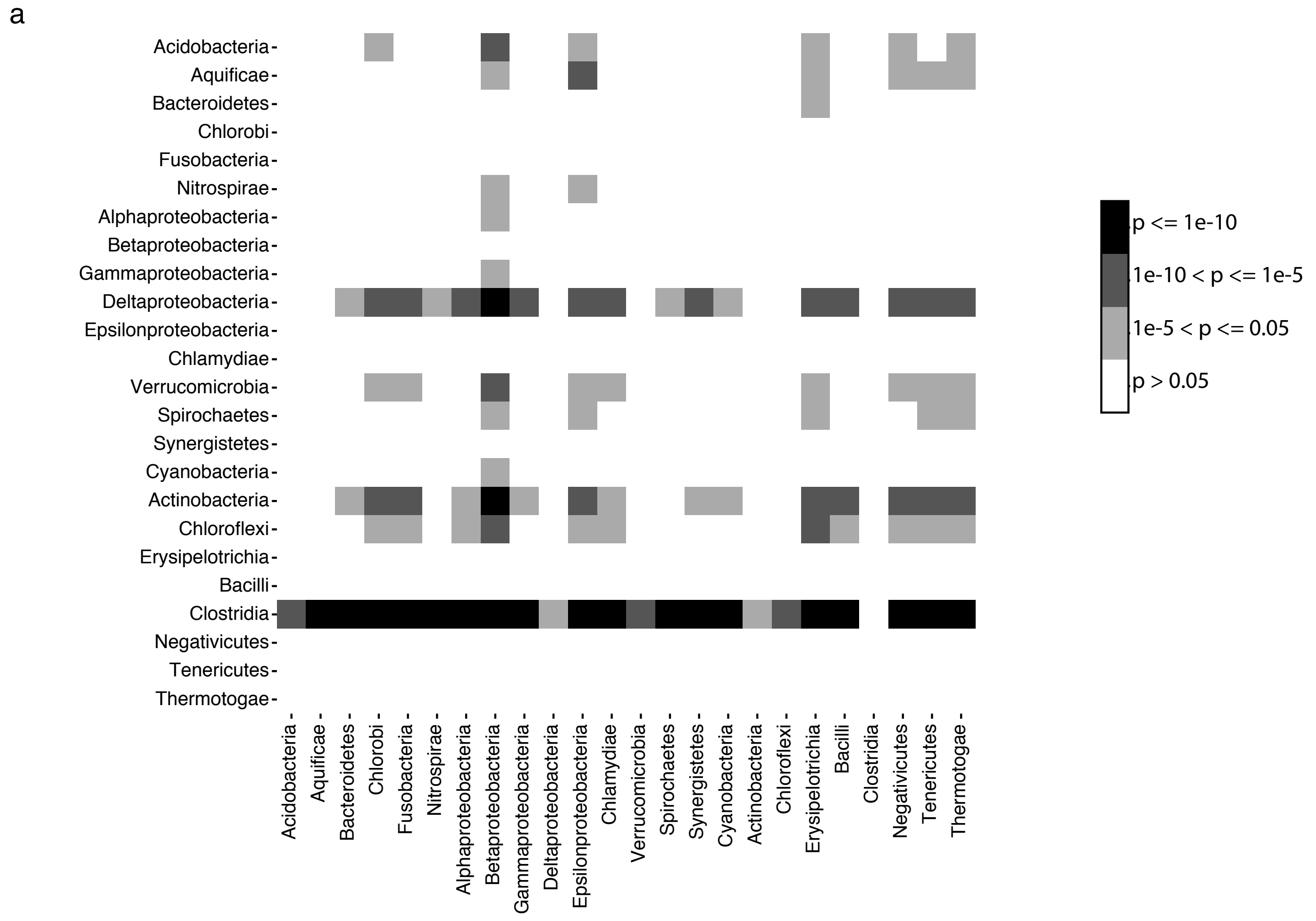
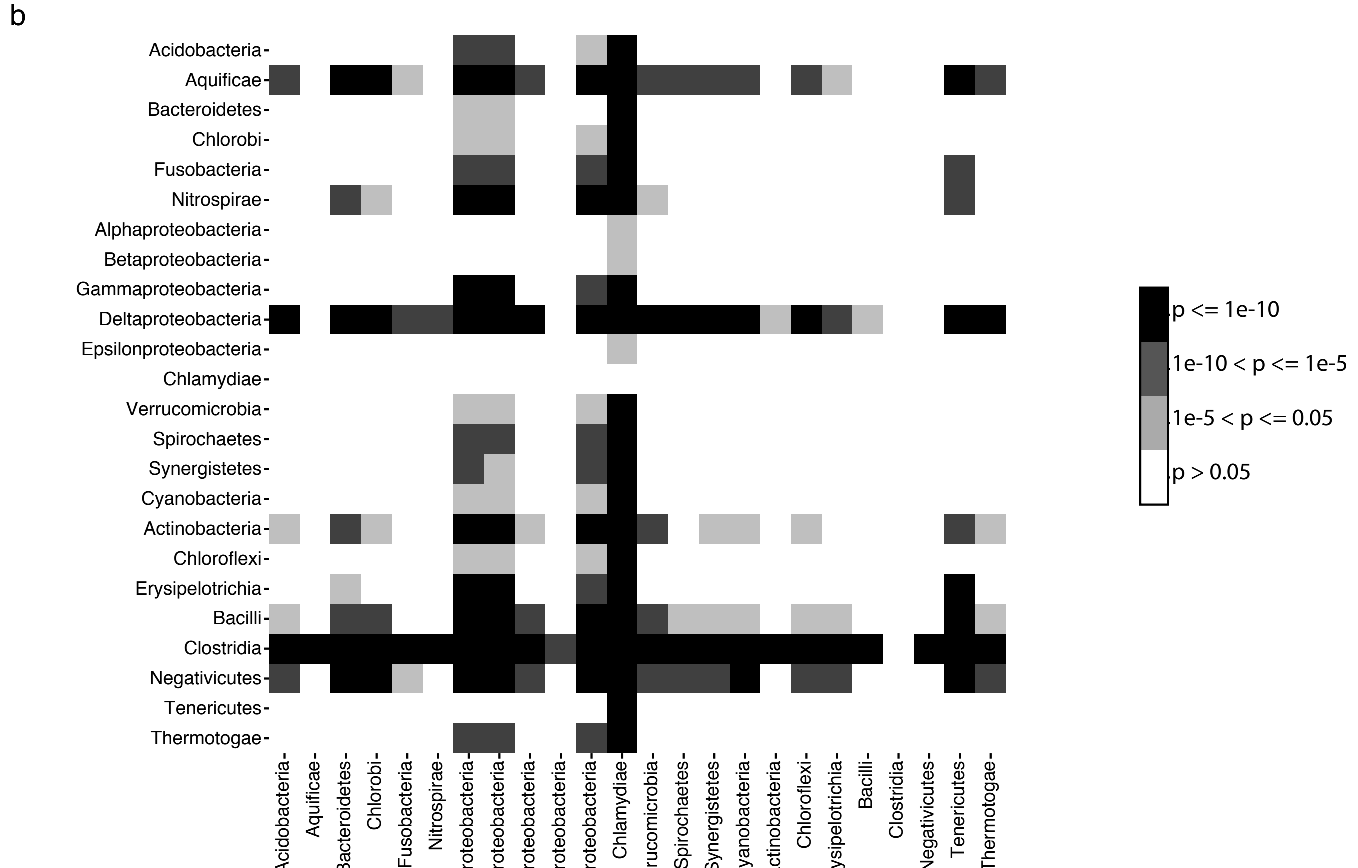
Supplementary Table 2 | List of 40 genomes closest to the bacterial root by sequence divergence (branch length) for all trees.

Genome ID	Average smallest distance (branch length)	Taxonomic group	Species name
GCF_000175295.2	0,4853	Clostridia	'Thermoanaerobacter brockii subsp. finnii Ako-1'
GCF_000019085.1	0,4856	Clostridia	'Thermoanaerobacter pseudethanolicus ATCC 33223'
GCF_000148425.1	0,4918	Clostridia	'Thermoanaerobacter sp. X513'
GCF_000019065.1	0,4918	Clostridia	'Thermoanaerobacter sp. X514'
GCF_000092965.1	0,4919	Clostridia	'Thermoanaerobacter mathranii subsp. mathranii str. A3'
GCF_000147695.2	0,4950	Clostridia	'Thermoanaerobacter wiegelii Rt8.B1'
GCF_000763575.1	0,4957	Clostridia	'Thermoanaerobacter kivui strain=LKT-1'
GCF_000025645.1	0,4972	Clostridia	'Thermoanaerobacter italicus Ab9'
GCF_000144645.1	0,5039	Clostridia	'Thermosediminibacter oceani DSM 16646'
GCF_000092945.1	0,5062	Clostridia	'Thermincola potens JR'
GCF_000145615.1	0,5096	Clostridia	'Thermoanaerobacterium thermosaccharolyticum DSM 571'
GCF_000144695.1	0,5099	Clostridia	'Acetohalobium arabaticum DSM 5501'
GCF_000189775.2	0,5150	Clostridia	'Thermoanaerobacterium xylanolyticum LX-11'
GCF_000328545.1	0,5154	Clostridia	'Thermoanaerobacterium thermosaccharolyticum M0795'
GCF_000007085.1	0,5214	Clostridia	'Caldanaerobacter subterraneus subsp. tengcongensis MB4'
GCF_000328625.1	0,5265	Clostridia	'Halobacteroides halobius DSM 5150'
GCF_000020485.1	0,5280	Clostridia	'Halothermothrix orenii H 168'
GCF_000184925.1	0,5280	Clostridia	'Ruminiclostridium thermocellum DSM 1313'
GCF_001692755.1	0,5287	Clostridia	'Ruminiclostridium thermocellum DSM 2360'
GCF_000213235.1	0,5290	Clostridia	'Tepidanaerobacter acetatoxydans Re1'
GCF_000255615.2	0,5291	Clostridia	'Ruminiclostridium thermocellum AD2'
GCF_000328765.2	0,5292	Clostridia	'Tepidanaerobacter acetatoxydans Re1 [redundant no. 2]'
GCF_000015865.1	0,5300	Clostridia	'Ruminiclostridium thermocellum ATCC 27405'
GCF_000299355.1	0,5303	Clostridia	'[Clostridium] acidurici 9a'
GCF_000214435.1	0,5306	Clostridia	'Desulfotomaculum nigrificans CO-1-SRB'
GCF_000014125.1	0,5309	Clostridia	'Clostridium novyi NT'
GCF_000092345.1	0,5325	Clostridia	'Clostridium botulinum F str. 230613'
GCF_000017045.1	0,5332	Clostridia	'Clostridium botulinum A str. Hall'
GCF_000017025.1	0,5342	Clostridia	'Clostridium botulinum A str. ATCC 19397'
GCF_001483965.1	0,5348	Bacilli	'Carnobacterium sp. CP1'
GCF_000063585.1	0,5354	Clostridia	'Clostridium botulinum A str. ATCC 3502'
GCF_000253195.1	0,5358	Clostridia	'Clostridium botulinum H04402 065'
GCF_000017065.1	0,5363	Clostridia	'Clostridium botulinum F str. Langeland'
GCF_000172575.2	0,5365	Bacilli	'Enterococcus faecalis OG1RF'
GCF_000012865.1	0,5366	Clostridia	'Carboxydothermus hydrogenoformans Z-2901'
GCF_000317975.2	0,5366	Bacilli	'Carnobacterium maltaromaticum LMA28'
GCF_000829015.1	0,5371	Clostridia	'Clostridium botulinum strain=111'
GCF_000007625.1	0,5373	Clostridia	'Clostridium tetani E88'
GCF_000018325.1	0,5376	Clostridia	'Alkaliphilus oremlandii OhILAs'
GCF_000022765.1	0,5381	Clostridia	'Clostridium botulinum A2 str. Kyoto'

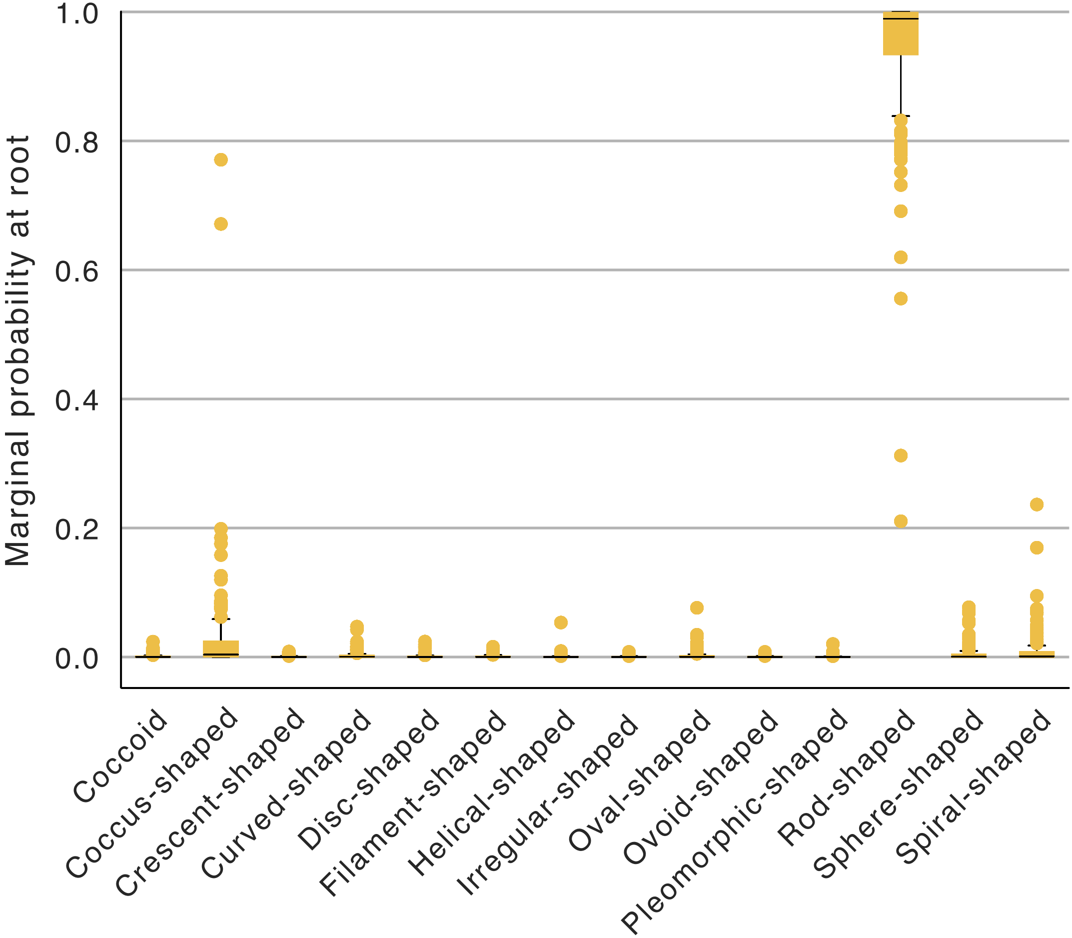
Supplementary Figure 1 | Taxonomic distribution of LBCA protein families. 146 protein families (rows) in all taxonomic groups (columns). The color in each cell indicates the proportion of the group (%) where the protein is present according to the scale bar.



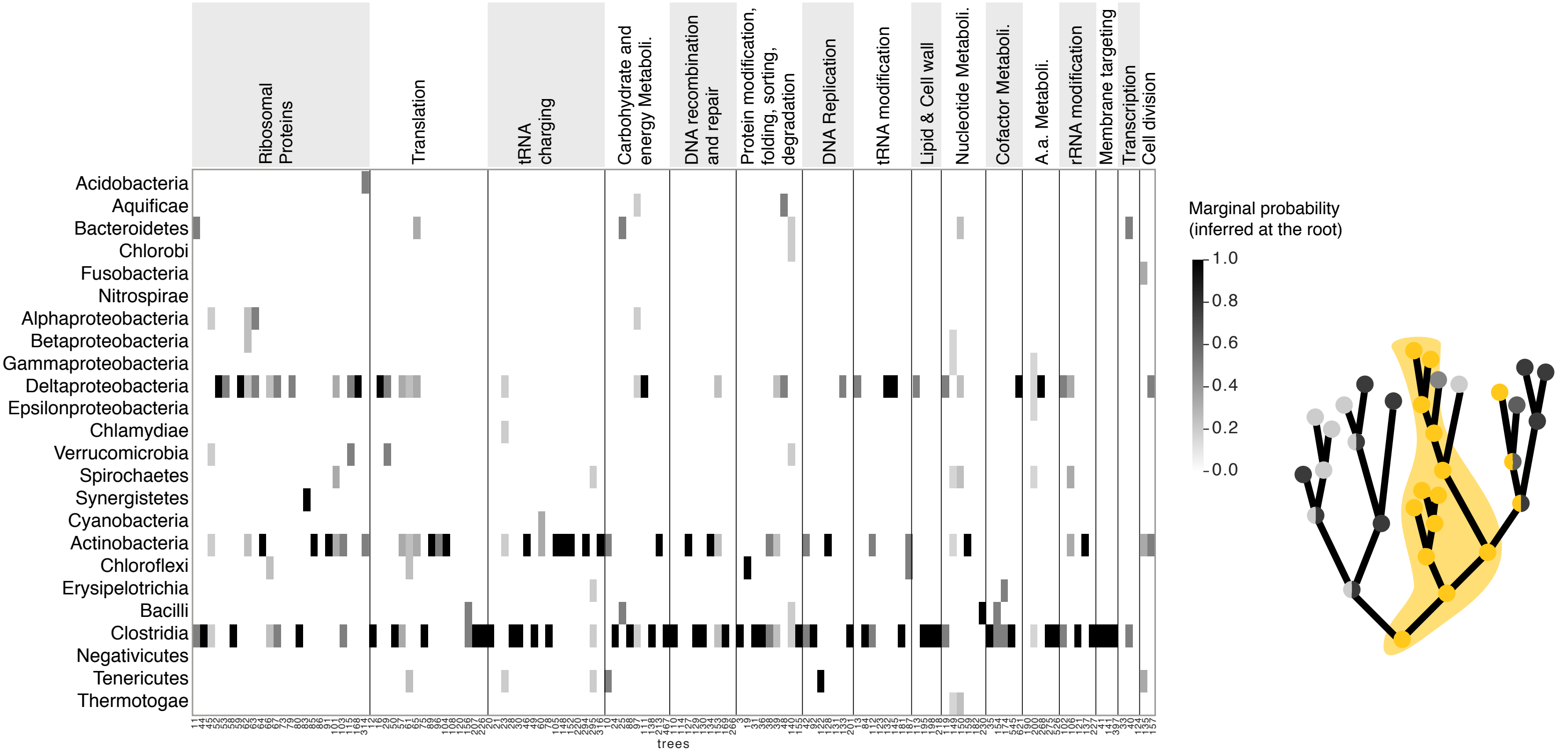
Supplementary Figure 2 – LBCA's metabolic network before expansion. Metabolic interconversions encoded by 146 LBCA genes are shown in a bipartite graph, with 224 metabolites (circular nodes) and 121 reactions (diamond nodes). Black circles are the 57 universal target metabolites and grey circles are the remaining metabolites (note that some of these are also universal (e.g. NADH) directly connected to the chosen targets (e.g. in that case NAD+)). Node sizes grow according to degree.



Supplementary Figure 3 – Statistical tests on divergence analysis for all groups and 131 universal trees. Metrics were a) node depth and b) branch length (see Methods section).



Supplementary Figure 4 – Marginal probabilities for each cell-shape at the root of 131 universal trees. Reconstructions by Maximum Likelihood (see Methods).



Supplementary Figure 5 – Marginal probabilities for each taxonomic group at the root of 131 universal trees. Presence-absence pattern shows marginal probabilities for each group (rows) and each tree (columns). Reconstructions by Maximum Likelihood (see Methods). An illustrative tree portrays the question asked in the analysis, where the yellow group is the one inferred at the root.