

Supplemental Data 1 *Recently acquired B. anthracis genes*

The genes that were recruited by *B. anthracis* in the time leading up to the acquisition of pXO1 and pXO2 by HGT may be related in some way to the enhanced virulence of other clade 1 strains. Using the whole-genome phylogeny, we mapped the likely acquisition of genes by HGT in the *B. anthracis* lineage (Supplemental Data 1). The proteome of *B. anthracis* Ames ancestor was screened against the entire genome set using translated BLAST (Altschul et al. 1990) with an E-value cut-off for detection of 10^{-5} . In addition to finding hits to full-length genes, this alignment method finds gene remnants in other strains that may have underlying sequence errors or recently picked up null mutations and was used to track the acquisition of new genes in the *B. anthracis* genome. A Perl script based on the bioperl Bio::Tree module was used to track possible gene acquisition points to the node of the tree based on recursively moving down nodes of the tree from the *B. anthracis* leaf and testing homoplasy of gene homologs on the child branches. 57 *B. anthracis*-specific genes did not have homologs in any of the other *B. cereus* genomes using TBLASTN alignment. These genes were all either orthologs of known phage genes, hypothetical functions, or were found in prophage regions. There were 20 *B. anthracis* genes with homologs only in the five most closely related *B. cereus* genomes, and these fell into the same functional groups as the *B. anthracis* unique set. The pattern was repeated moving back stepwise on the phylogeny with only rare exceptions, such as a small cluster that contained the S-layer outer surface protein and methyl-dependent restriction enzyme (GBAA_2315-GBAA_2317) found only in clade 1 *B. cereus s.l.* strains. None of these genes have a known role in

virulence; therefore it is probable that they simply reflect random gains and losses of genes that occur in terminal branches of every *B. cereus* s.l. lineage. Similar patterns of accessory gene acquisition were also seen in the history of G9241 and BACI.

Each panel is a list of *B. anthracis* genes unique to a group of closely related strains. Each list consists of the NCBI unique protein and gene ID for a *B. anthracis* Ames locus (Ravel et al. 2009), an annotation and the locus tag. Each list of genes are unique with the genomes analyzed in this study to the cluster of strains (identified by locus tag) and all other larger clusters of strains. For instance all genes in list 1 (*B. anthracis* specific) are by inheritance also only found in the clusters of strains for lists 2-5. Matches were generated by aligning all *B. anthracis* proteins to the DNA genome sequences using TBLASTN.

1. Unique to *B. anthracis*

gi|47525707|ref|YP_017056.1| hypothetical protein GBAA_0431
gi|47525720|ref|YP_017069.1| prophage lambdaba04 transactivating regulatory domain-containi
gi|47777815|ref|YP_022530.1| hypothetical protein GBAA_0447
gi|47777816|ref|YP_022531.1| hypothetical protein GBAA_0448
gi|47525723|ref|YP_017072.1| hypothetical protein GBAA_0450
gi|47525725|ref|YP_017074.1| hypothetical protein GBAA_0452
gi|47525727|ref|YP_017076.1| hypothetical protein GBAA_0454
gi|47525728|ref|YP_017077.1| hypothetical protein GBAA_0455
gi|47777817|ref|YP_022532.1| hypothetical protein GBAA_0459
gi|47525735|ref|YP_017084.1| hypothetical protein GBAA_0463
gi|47777820|ref|YP_022535.1| hypothetical protein GBAA_0473
gi|47525747|ref|YP_017096.1| prophage LambdaBa04, tape measure protein
gi|47525749|ref|YP_017098.1| hypothetical protein GBAA_0479
gi|47525750|ref|YP_017099.1| hypothetical protein GBAA_0480

gi|47777822|ref|YP_017105.2| hypothetical protein GBAA_0486
gi|47526959|ref|YP_018308.1| flagellar capping protein
gi|47526982|ref|YP_018331.1| group 2 family glycosyl transferase
gi|47526983|ref|YP_018332.1| hypothetical protein GBAA_1694
gi|47526985|ref|YP_018334.1| hypothetical protein GBAA_1695
gi|47526986|ref|YP_018335.1| hypothetical protein GBAA_1696
gi|47526987|ref|YP_018336.1| hypothetical protein GBAA_1697
gi|47526988|ref|YP_018337.1| TPR/glycosyl transferase domain-containing protein
gi|47526990|ref|YP_018339.1| hypothetical protein GBAA_1701
gi|47526994|ref|YP_018343.1| hypothetical protein GBAA_1704
gi|47526996|ref|YP_018345.1| flagellin
gi|47527444|ref|YP_018793.1| hypothetical protein GBAA_2149
gi|47527615|ref|YP_018964.1| hypothetical protein GBAA_2316
gi|47528061|ref|YP_019410.1| hypothetical protein GBAA_2770
gi|47528512|ref|YP_019861.1| hypothetical protein GBAA_3222
gi|47528658|ref|YP_020007.1| hypothetical protein GBAA_3374
gi|47528739|ref|YP_020088.1| hypothetical protein GBAA_3455
gi|47528814|ref|YP_020163.1| hypothetical protein GBAA_3529
gi|47528887|ref|YP_020236.1| hypothetical protein GBAA_3601
gi|47529049|ref|YP_020398.1| hypothetical protein GBAA_3759
gi|47529050|ref|YP_020399.1| prophage LambdaBa01 TPR domain-containing protein
gi|47778208|ref|YP_022655.1| hypothetical protein GBAA_3762
gi|47778216|ref|YP_020430.2| hypothetical protein GBAA_3798
gi|47529086|ref|YP_020435.1| hypothetical protein GBAA_3802
gi|47529088|ref|YP_020437.1| hypothetical protein GBAA_3804
gi|47529089|ref|YP_020438.1| prophage LambdaBa01, acyltransferase
gi|47529092|ref|YP_020440.1| hypothetical protein GBAA_3807
gi|47529094|ref|YP_020443.1| hypothetical protein GBAA_3809
gi|47529095|ref|YP_020444.1| hypothetical protein GBAA_3810
gi|47529111|ref|YP_020460.1| hypothetical protein GBAA_3827
gi|47529112|ref|YP_020461.1| hypothetical protein GBAA_3828
gi|47529113|ref|YP_020462.1| hypothetical protein GBAA_3829
gi|47529371|ref|YP_020720.1| hypothetical protein GBAA_4077
gi|47529393|ref|YP_020742.1| hypothetical protein GBAA_4097
gi|47529394|ref|YP_020743.1| hypothetical protein GBAA_4098
gi|47529401|ref|YP_020750.1| hypothetical protein GBAA_4104
gi|47778249|ref|YP_020754.2| hypothetical protein GBAA_4110
gi|47778250|ref|YP_020755.2| prophage LambdaBa02, membrane protein
gi|47529423|ref|YP_020772.1| hypothetical protein GBAA_4127
gi|47530532|ref|YP_021881.1| hypothetical protein GBAA_5227
gi|47778378|ref|YP_022725.1| hypothetical protein GBAA_5232
gi|47530649|ref|YP_021998.1| hypothetical protein GBAA_5339
gi|47530650|ref|YP_021999.1| hypothetical protein GBAA_5340
gi|47778389|ref|YP_022000.2| hypothetical protein GBAA_5342
gi|47530652|ref|YP_022001.1| hypothetical protein GBAA_5343
gi|47530653|ref|YP_022002.1| prophage LambdaBa03, HNH endonuclease family protein

gi|47530654|ref|YP_022003.1| hypothetical protein GBAA_5345
 gi|47530655|ref|YP_022004.1| hypothetical protein GBAA_5346
 gi|47530656|ref|YP_022005.1| hypothetical protein GBAA_5347
 gi|47778392|ref|YP_022008.2| hypothetical protein GBAA_5350
 gi|47530661|ref|YP_022010.1| hypothetical protein GBAA_5352
 gi|47530663|ref|YP_022012.1| hypothetical protein GBAA_5354
 gi|47530664|ref|YP_022013.1| hypothetical protein GBAA_5355
 gi|47530665|ref|YP_022014.1| prophage LambdaBa03, terminase, large subunit
 gi|47778393|ref|YP_022015.2| hypothetical protein GBAA_5357
 gi|47530667|ref|YP_022016.1| hypothetical protein GBAA_5358
 gi|47530668|ref|YP_022017.1| prophage LambdaBa03, prohead protease
 gi|47530669|ref|YP_022018.1| HK97 family phage major capsid protein
 gi|47530670|ref|YP_022019.1| hypothetical protein GBAA_5361
 gi|47530672|ref|YP_022021.1| hypothetical protein GBAA_5362
 gi|47778394|ref|YP_022022.2| prophage lambdaba03, site-specific recombinase phage integrase
 gi|47566384|ref|YP_016394.2| hypothetical protein GBAA_pX01_0063
 gi|47566441|ref|YP_016452.2| hypothetical protein GBAA_pX01_0121
 gi|47566466|ref|YP_016485.2| transposase x
 gi|47566479|ref|YP_016498.2| hypothetical protein GBAA_pX01_0167
 gi|47566488|ref|YP_016507.2| hypothetical protein GBAA_pX01_0176
 gi|47566722|ref|YP_016561.2| hypothetical protein GBAA_pX02_0051

2. Unique to B. anthracis, BCAH8 ,bce16 ,bth10 ,bth7, bth12

gi|47525726|ref|YP_017075.1| hypothetical protein GBAA_0453
 gi|47777869|ref|YP_022549.1| hypothetical protein GBAA_0920
 gi|47777893|ref|YP_022556.1| hypothetical protein GBAA_1076
 gi|47526369|ref|YP_017718.1| hypothetical protein GBAA_1095
 gi|47777900|ref|YP_017719.2| hypothetical protein GBAA_1096
 gi|47526371|ref|YP_017720.1| hypothetical protein GBAA_1097
 gi|47526543|ref|YP_017892.1| hypothetical protein GBAA_1276
 gi|47527781|ref|YP_019130.1| hypothetical protein GBAA_2492
 gi|47527786|ref|YP_019135.1| hypothetical protein GBAA_2499
 gi|47528261|ref|YP_019610.1| hypothetical protein GBAA_2968
 gi|47529367|ref|YP_020716.1| prophage lambdaba02, n-acetylmuramoyl-l-alanine amidase famil
 gi|47529402|ref|YP_020751.1| hypothetical protein GBAA_4106
 gi|47778391|ref|YP_022728.1| hypothetical protein GBAA_5349
 gi|47530662|ref|YP_022011.1| prophage LambdaBa03 transcriptional regulator domain-containin
 gi|47530831|ref|YP_022180.1| hypothetical protein GBAA_5513
 gi|47778408|ref|YP_022181.2| group 1 family glycosyl transferase
 gi|47530833|ref|YP_022182.1| hypothetical protein GBAA_5515
 gi|47530834|ref|YP_022183.1| hypothetical protein GBAA_5516
 gi|47530835|ref|YP_022184.1| hypothetical protein GBAA_5517
 gi|47530840|ref|YP_022189.1| putative lipoprotein

3. Unique to *B. anthracis*, BCAH8 ,bce16 ,bth10 ,bth7 , bth12 ,bth9
gi|47777804|ref|YP_016951.2| hypothetical protein GBAA_0335
gi|47527826|ref|YP_019175.1| spore coat protein
gi|47528583|ref|YP_019932.1| hypothetical protein GBAA_3298

4. Unique to *B. anthracis*, BCAH8 ,bce16 ,bth10 ,bth7 , bth12 ,bth9 ,BT
gi|47526405|ref|YP_017754.1| hypothetical protein GBAA_1136
gi|47778248|ref|YP_022673.1| hypothetical protein GBAA_4107

5. Unique to *B. anthracis*, BALH,bce4 ,bce21 ,BCAH8 ,bce16 ,bth10 ,bth7 , bth12 ,bth9 ,BT
gi|47527614|ref|YP_018963.1| S-layer protein
gi|47527616|ref|YP_018965.1| Mrr restriction protein-related
gi|47527722|ref|YP_019071.1| hypothetical protein GBAA_2428
gi|47778045|ref|YP_022606.1| hypothetical protein GBAA_2432
gi|47528091|ref|YP_019440.1| hypothetical protein GBAA_2798

Even though *B. anthracis* had a genome size and gene density similar to other *B. cereus* strains, we looked for the possibility of aberrant patterns of either gene loss or gain in the genome. We could find no evidence of unusual levels of core gene loss in *B. anthracis* (and G9241 compared with other clade 1 genomes. Moreover, other studies have shown the numbers of pseudogenes in *B. anthracis* (45) (Read et al. 2003) were similar to those in other *Bacillus* (Rasko et al. 2005) and much lower than in the other recently evolved high-consequence pathogens *Yersinia pestis* and *Salmonella typhi* (Parkhill et al. 2001; Holt et al. 2009). Therefore, macroscopic gene loss was also not a feature of *B. anthracis* genomes.

Supplemental data 2 - *The B. thuringiensis “species” is defined by the presence of a single, horizontally transferred gene cluster*

In addition to mapping character genes onto phylogenic groups, we used a hypergeometric test (Tavazoie et al. 1999) to find character genes associated with the presence of δ -endotoxin genes. Delta endotoxin genes are borne on plasmids that have been acquired independently on multiple lineages of the *B. cereus* species (Figure 1). Production of crystalline δ -endotoxins multimers is necessary for insect killing (Aronson and Shai 2001), and strains expressing this phenotype are named *B. thuringiensis*. We found that several strains in this study labeled as *B. thuringiensis* lacked δ -endotoxins genes, either because plasmids carrying the genes were lost during culture or because the strain was misnamed on isolation (for instance, there is no evidence that crystal toxin production was tested for *B. thuringiensis* konkukian (Hernandez et al. 1998)). No other gene cluster was found to be associated only with all 11 strains containing the delta-endotoxin gene. Thus, membership in the *B. thuringiensis* “species” may be defined by the presence of only one gene acquired by HGT on a plasmid. This was further evidence that *B. thuringiensis* is not a monophyletic group (Helgason et al. 2000) and also exemplified how as little as a single gene can be sufficient to convert a non-pathogenic *B. cereus* into a pathogen (in this case , for insects).

Apart from the thuringiensis and anthracis biotypes, at a false-discovery rate of less than 0.05, we found no strong association between the presence of other gene

clusters and known biotypes (pathogens, food-associated strains) or the 10 *B. cereus* s.l. bacteria isolated on the same day in Rockville, Maryland.

Supplemental Data 3 Underlying assembly quality of annotated genes

Shotgun contigs were screened for regions more likely to contain sequencing artifacts using the scripts developed for the *amosvalidate* package (Phillippy et al. 2008). Areas flagged as suspect had one or more of the following characteristics: (a) less than 6-fold sequence read redundancy; (b) localized regions with more than 3 times the average sequence read coverage (indicative of a possible tandem repeat (Phillippy et al. 2008)); (c) a 500-bp window with more than 2 22-mer oligonucleotides with more than 3 times average redundancy; (d) more than 2 significant non-consensus base calls in the assembly pile-up within in a 500-bp window. This class of features was found almost always to be caused by polymorphisms in homopolymer number typical for 454 sequencing chemistry. “Significant non-consensus bases” were cases in the assembly where there were a minimum of at least 4 variant reads that were at least 40% of the total reads covering the base. The *clusterSNPs* script from *amosvalidate* was used to mark regions with at least 2 such SNPs within 500 bp.

All the features identified were matched for overlap against the genes called from each project by DIYA (see above). If there was as little as one base within the gene that overlapped a suspect feature, the gene was tagged as suspect. Of the 324,864 genes called in this project, only 19,701 (6.1%) were classified in this manner.

As a quality control measure, we identified 256 gene clusters (see “Core Cluster Alignments” below) containing exactly one gene from each of the 58 genomes,

where every gene from the 46 genomes sequenced in this study exceeded the stringent quality criteria described in the paragraph above. These were termed the “high quality aligned core.” We found that the phylogenetic tree constructed with these genes concatenated had identical topology to the one constructed using all the core genes, and also that the pairwise dN/dS ratios of these were almost identical. This was an indication that even core clusters that fell below these stringent quality standards contained a very small number of sequence errors, and these did not significantly alter the results of the analyses.

Supplemental Figure Legends

Figure S1 Phylogeny based on parsimony analysis of MAUVE aligned genome fragments.

The input data was the whole genome MAUVE alignment (see materials and Methods). A cutoff of 1,500 bp was set as the minimum LCB (Locally co-linear block) length. LCBs for each genome were extracted from the output of the program and concatenated. PHYLIP (Felsenstein 1989) programs were used to build a consensus tree of the MAUVE alignment with bootstrapping of 1,000 replicates. The underlying model for each replicate was Fitch-Margoliash. The final phylogeny was resolved according to the majority consensus rule.

Figure S2 PhyML neighbor-joining phylogeny.

The tree was generated from the same dataset of aligned core proteins as Figure 1. PhyML 3.0 (Guindon et al. 2009) was used with default settings.

*Figure S3 ClonalFrame phylogeny of *B. cereus* s.l.*

The figure shows the phylogeny constructed by ClonalFrame (Vos and Didelot 2009). Each branch is labeled with the number of substitutions assigned as mutations (left of the dash) and recombination events (right of dash).

Figure S4 Gene Ontology Categories enriched in the character gene set

The figure was generated using the GOEAST program (Zheng and Wang 2008). GO terms are illustrated that were enriched using a hypergeometric test with a false discovery rate of < 0.05 . The more intense the color the lower the p value.

Figure S5. Estimation of character gene gains and losses using Badirate.

We used BadiRate (Librado et al. 2012) to estimate the gene gain and loss by the GD-FR-ML turnoverRates-BranchModel-Estimation procedure along with -anc option, where GD model estimates the turnover rates (gene gain and death rates), FR (Free Rates) model assumes each branch has its own turnover rate and Maximum likelihood (ML) to estimate the turnover rate. -anc option reports the number of members at internal phylogenetic nodes for each family. The phylogeny is based on the concatenated protein sequences (Figure 1). Figures in red are the estimated number of gene gains and losses on each branch.

Figure S6. Conservation of transposon sequences in B. cereus s.l. genomes

Genomes that shared at least one nucleotide sequence annotated by the ACLAME (Leplae et al. 2010) database as a transposon at 100% length and 99.5% sequence by BLASTN were represented as a spring-embedded network using the Cytoscape (Kohl et al. 2011). Blue: Clade 1, Red: Clade 2, Green: outlying clades.

Figure S7 Pairwise dN/dS against number of synonymous sites for the B. cereus s.l. pan-genome

The inverse of median dN/dS of conserved genes for each pairwise combination of genomes is plotted against the genetic distance estimated by the number of synonymous sites. The inverse is used because it makes the plot easier to visualize (Kuo et al. 2009; Hershberg and Petrov 2010). Best fit curve were fitted to the clade 1 and 2 values (A) and clade 3 values (B).

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Table S1. Breakdown of Cluster Distribution across Clades 1-3

Category	Number of Clusters
Extended core (found in more than 51 genomes)	3,902
Clade 1 only, all 22 genomes	0
Clade 1 only, 14-21 genomes	6 (6C)
Clade 1 only, less than 14 genomes	4,033 (72C)
Clade 2 only, all 20 genomes	1 (1C)
Clade 2 only, 13-19 genomes	12 (12C)
Clade 2 only, less than 13 genomes	4,427 (120C)
Clade 3 only, all 16 genomes	0
Clade 3 only, 11-15 genomes	25 (25C)
Clade 3 only, less than 11 genomes	4,258 (87C)
Distributed across clades 1-3	6,311 (3,631C)
Total	22,975

For each clade the number of gene families found only in that clade and in less than half, three quarters, and all the genomes is shown. Genes that are not core and are

found in more than one clade are grouped into the final row. C = number of character genes.

Table S2. Number of genes present in at least 75% (16) of clade 1 genomes missing in each genome

bce28	191
BCE	249
bth9	90
bce13	152
bce21	115
bth1	106
G924	283
bth12	52
bce10	98
BACI	107
BALH	440
BCAH1	112
GBAA	240
BCZK	170
bth10	59
bce6	195
BCAH8	34
BT	173
bth7	54
bce16	62
bce1	143
bce4	91

Supplemental Table 1. Assembly statistics of the 45 Whole Genome Shotgun Projects

Key: N50Contig Bases = median size of assembled contigs. “Big Contigs” are contigs > 1000 bp. BigContigLength = total size of all big contigs. SmallContigLength = total size of all contigs <1001. BigContigsPercentBases = BigContigLength / (BigContigLength + smallContigLength). ReadsInContigs are the total number of reads that fall into contigs. Other terms are self explanatory.

Genom	TotalContig	TotalBasesInContig	MeanContigSize	MaxContigSize	N50ContigBase	TotalBigContig	BigContigLength	BigContigsPercentBase	TotalSmallContig	SmallContigLength	TotalRead	ReadsInContig	AvSizeTotalGenes	TotalGeneNum
bcere0001	1680	6535637	3890	220516	66101	123	6167762	94	1557	367875	2350820	2350797	816.2	
bcere0002	1421	6952794	4892	247967	80441	119	6663733	95	1302	289061	2259000	2258989	808.48	
bcere0004	1354	6709270	4955	275062	73095	111	6384865	95	1243	324405	1848670	1848670	810.71	
bcere0005	1250	6848797	5479	350469	122208	88	6632564	96	1162	216233	1929356	1929356	816.3	
bcere0006	1088	6553560	6023	337867	91057	102	6294632	96	986	258928	1888585	1888585	814.6	
bcere0007	424	5868931	13841	285493	69889	113	5664313	96	311	204618	488497	488497	801.35	
bcere0009	1746	7018974	4020	340990	89981	103	6698899	95	1643	320075	2512032	2512032	814.58	
bcere0010	158	5392765	34131	262484	136384	66	5311724	98	92	81041	411803	411803	814.01	
bcere0011	1267	6207324	4899	446073	140233	76	6040618	97	1191	166706	1739516	1739516	822.2	
bcere0012	954	6439641	6750	278072	87834	97	6189118	96	857	250523	1964579	1964579	811.1	
bcere0013	311	5781134	18588	319411	68291	96	5520393	95	215	260741	561434	561434	777.65	
bcere0014	1908	6841865	3585	231137	56889	147	6354984	92	1761	486881	2253668	2253672	804.28	
bcere0015	178	5534731	31093	263846	134844	68	5419180	97	110	115551	384947	384947	819.18	
bcere0016	342	5769469	16869	364059	115722	72	5413826	93	270	355643	491929	491929	801.16	
bcere0017	379	6058178	15984	260059	88293	94	5854103	96	285	204075	431468	431468	806.83	
bcere0018	486	6273447	12908	211148	80915	101	6041261	96	385	232186	952379	952379	813.31	
bcere0019	3259	6542628	2007	254635	58316	121	5610956	85	3138	931672	871952	871952	793.37	
bcere0020	509	6409647	12592	327188	105766	92	6136420	95	417	273227	1114132	1114132	802.81	
bcere0021	370	5491474	14841	183330	61207	116	5242886	95	254	248588	480336	480336	800.32	
bcere0022	2599	5381832	2070	128424	34087	130	4421485	82	2469	960347	828553	828553	794.06	
bcere0023	514	6219486	12100	494774	74295	111	5698648	91	403	520838	907512	907512	797.56	
bcere0025	710	6427079	9052	330133	66225	126	6065259	94	584	361820	552085	552085	807.63	
bcere0026	695	6281480	9038	200190	62804	137	5949800	94	558	331680	971467	971467	805.67	
bcere0027	566	5936075	10487	177231	41804	154	5334249	89	412	601826	704704	704704	800.25	
bcere0028	788	6078590	7713	192749	39872	165	5560980	91	623	517610	869301	869301	805.48	
bcere0030	849	6251930	7363	105978	28757	192	5391016	86	657	860914	1250847	1250848	785.71	
bmyco0001	419	5764282	13757	239361	87432	102	5532482	95	317	231800	440542	440542	790.73	
bmyco0002	1197	6197368	5177	199519	43950	135	5152865	83	1062	1044503	827316	827316	749.48	
bmyco0003	750	5930906	7907	223876	60849	119	5411181	91	631	519725	531861	531861	760.74	
bpmx0001	519	5979404	11521	483273	74527	100	5441003	91	419	538401	482004	482004	767.29	
bthur0001	1375	6797732	4943	157778	76578	125	6498268	95	1250	299464	1844300	1844301	792.99	
bthur0002	272	6377419	23446	551249	67719	104	6116403	95	168	261016	794474	794474	776.17	
bthur0003	419	6545187	15620	261856	63467	139	6202125	94	280	343062	564698	564698	800.76	
bthur0004	2780	7351603	2644	232363	44917	174	6514171	88	2606	837432	1971585	1971585	731.33	
bthur0005	2684	7356499	2740	164935	43236	161	6251486	84	2523	1105013	2795253	2795253	774.72	
bthur0006	6135	6435885	3014	167299	66996	120	6866944	91	2015	568941	2069293	2069293	797.45	
bthur0007	2626	6796780	10857	283986	65266	126	6456895	95	500	339885	895762	895762	795.56	
bthur0008	314	6507666	20725	262793	60803	139	6163410	94	175	344256	727100	727100	805.91	
bthur0009	697	5852279	8396	277739	85650	93	5531198	94	604	321081	974920	974920	798.76	
bthur0010	515	6428970	12483	255654	100077	98	6221786	96	417	207184	1009420	1009423	804.99	
bthur0011	699	6656021	9522	254759	81034	112	6238521	93	587	417500	1252795	1252795	820.36	
bthur0012	685	6493391	9479	352969	77385	113	6210495	95	572	282896	1152333	1152333	804.26	
bthur0013	535	7069669	13214	301599	70606	122	6708425	94	413	361244	679320	679320	797.43	
bthur0014	750	6814111	9085	391352	45475	161	6242065	91	589	572046	560985	560985	785.26	

Table S4 Genes under positive selection

All Clusters

cluster#	internal	accession	locus_tag	geneID	proteinID	Description	Pfam
CDD_ID?	COF_Superfamily						
535	GBAA_00640	GBAA_0685	47525965	YP_017314.1	hypothetical protein		
	GBAA_0685	[Bacillus anthracis str. 'Ames Ancestor']					
466	GBAA_00465	GBAA_0501	47525770	YP_017119.1	PTS system N-acetylglucosamine-specific transporter subunit IIBC	[Bacillus anthracis str. 'Ames Ancestor']	
	prk10255	430528	Carbohydrate transport and metabolism				
1775	GBAA_04682	GBAA_5166	47530472	YP_021821.1	hypothetical protein		
	GBAA_5166	[Bacillus anthracis str. 'Ames Ancestor']					
1216	GBAA_03509	GBAA_3926	47529219	YP_020568.1	sugar ABC transporter ATP-binding protein	[Bacillus anthracis str. 'Ames Ancestor']	
	prk13549	859025	Carbohydrate transport and metabolism				
1866	GBAA_05006	GBAA_5520	47530838	YP_022187.1	rod shape-determining protein Mbl	[Bacillus anthracis str. 'Ames Ancestor']	
	prk13928	2411799	""Cell cycle control, mitosis and meiosis""				
1221	GBAA_03518	GBAA_3936	47529227	YP_020576.1	aspartate kinase I	[Bacillus anthracis str. 'Ames Ancestor']	
	prk08210	418855	Amino acid transport and metabolism				
1655	GBAA_04412	GBAA_4879	47530172	YP_021521.1	argininosuccinate lyase	[Bacillus anthracis str. 'Ames Ancestor']	
	prk00855	429571	Amino acid transport and metabolism				
906	GBAA_01801	GBAA_1980	47777986	YP_018624.2	hypothetical protein		
	GBAA_1980	[Bacillus anthracis str. 'Ames Ancestor']					
394	GBAA_00265	GBAA_0292	47525555	YP_016904.1	phosphoribosylformylglycinamide synthase subunit PurS	[Bacillus anthracis str. 'Ames Ancestor']	
	prk05974	209818	Nucleotide transport and metabolism				
835	GBAA_01443	GBAA_1567	47526840	YP_018189.1	hypothetical protein		
	GBAA_1567	[Bacillus anthracis str. 'Ames Ancestor']					
1665	GBAA_04429	GBAA_4898	47530192	YP_021541.1	small, acid-soluble spore protein B	[Bacillus anthracis str. 'Ames Ancestor']	
1838	GBAA_04867	GBAA_5364	47530674	YP_022023.1	phosphopyruvate hydratase	[Bacillus anthracis str. 'Ames Ancestor']	
	prk00077	413983	Carbohydrate transport and metabolism				
1798	GBAA_04728	GBAA_5216	47530522	YP_021871.1	hypothetical protein		
	GBAA_5216	[Bacillus anthracis str. 'Ames Ancestor']					
	prk10948	431177	""Posttranslational modification, protein turnover, chaperones""				
926	GBAA_01865	GBAA_2048	47527338	YP_018687.1	DedA family protein	[Bacillus anthracis str. 'Ames Ancestor']	
	prk10847	431081	Function unknown				
1061	GBAA_02593	GBAA_2864	291612217	YP_003541031.1	GNAT family acetyltransferase	[Bacillus anthracis str. 'Ames Ancestor']	
1280	GBAA_03601	GBAA_4021	47529314	YP_020663.1	orotate phosphoribosyltransferase	[Bacillus anthracis str. 'Ames Ancestor']	

prk00455 414123 Nucleotide transport and metabolism
 1904 GBAA_05071 GBAA_5588 47530909 YP_022258.1 3-hydroxybutyryl-CoA
 dehydrogenase [Bacillus anthracis str. 'Ames Ancestor'] prk05808 417264
 Lipid transport and metabolism
 574 GBAA_00797 GBAA_0855 47526143 YP_017492.1 amino acid ABC transporter
 amino acid-binding protein [Bacillus anthracis str. 'Ames Ancestor']
 prk11260 431418 Multifunctional
 335 GBAA_00095 GBAA_0099 47525354 YP_016703.1 50S ribosomal protein L10
 [Bacillus anthracis str. 'Ames Ancestor'] prk00099 414002 Translation
 1024 GBAA_02316 GBAA_2550 47527839 YP_019188.1 hydroxymethylglutaryl-CoA
 lyase [Bacillus anthracis str. 'Ames Ancestor'] prk05692 209537 Amino acid
 transport and metabolism
 1882 GBAA_05038 GBAA_5553 47530873 YP_022222.1 F0F1 ATP synthase subunit A
 [Bacillus anthracis str. 'Ames Ancestor'] prk05815 417271 Energy production
 and conversion
 980 GBAA_02054 GBAA_2258 47527554 YP_018903.1 putative lipoprotein
 [Bacillus anthracis str. 'Ames Ancestor']
 1427 GBAA_03915 GBAA_4342 47529639 YP_020988.1 hypothetical protein
 GBAA_4342 [Bacillus anthracis str. 'Ames Ancestor']
 1797 GBAA_04727 GBAA_5215 47530521 YP_021870.1 aminotransferase, class V
 [Bacillus anthracis str. 'Ames Ancestor'] prk09295 213122 Amino acid
 transport and metabolism
 878 GBAA_01672 GBAA_1835 47527126 YP_018475.1 sodium-dependent transporter
 [Bacillus anthracis str. 'Ames Ancestor']
 1714 GBAA_04519 GBAA_4992 47530294 YP_021643.1 permease [Bacillus
 anthracis str. 'Ames Ancestor']
 1878 GBAA_05032 GBAA_5547 47530867 YP_022216.1 F0F1 ATP synthase subunit
 beta [Bacillus anthracis str. 'Ames Ancestor'] prk09280 213107 Energy
 production and conversion
 392 GBAA_00263 GBAA_0290 47525553 YP_016902.1 adenylosuccinate lyase
 [Bacillus anthracis str. 'Ames Ancestor'] prk07492 418407 Nucleotide
 transport and metabolism
 751 GBAA_01301 GBAA_1419 47526691 YP_018040.1 ketol-acid reductoisomerase
 [Bacillus anthracis str. 'Ames Ancestor'] prk05479 209349 Multifunctional
 1330 GBAA_03733 GBAA_4155 47529453 YP_020802.1 protoheme IX
 farnesyltransferase [Bacillus anthracis str. 'Ames Ancestor'] prk04375
 416529 """"Posttranslational modification, protein turnover, chaperones""""
 1333 GBAA_03736 GBAA_4158 47529456 YP_020805.1 cell cycle protein FtsW
 [Bacillus anthracis str. 'Ames Ancestor'] prk10774 431012 """"Cell cycle
 control, mitosis and meiosis""""
 524 GBAA_00610 GBAA_0653 47777841 YP_017282.2 sulfate permease family
 protein [Bacillus anthracis str. 'Ames Ancestor'] prk11660 431758 Inorganic
 ion transport and metabolism
 450 GBAA_00371 GBAA_0402 47525673 YP_017022.1 tellurium resistance protein
 [Bacillus anthracis str. 'Ames Ancestor']
 1685 GBAA_04474 GBAA_4945 47530241 YP_021590.1 thioredoxin family protein

[Bacillus anthracis str. 'Ames Ancestor'] prk09381 213197
 ""Posttranslational modification, protein turnover, chaperones""
 645 GBAA_01085 GBAA_1180 47526444 YP_017793.1 hypothetical protein
 GBAA_1180 [Bacillus anthracis str. 'Ames Ancestor']
 1736 GBAA_04574 GBAA_5052 47778357 YP_022714.1 spore germination protein
 gerpf-like protein [Bacillus anthracis str. 'Ames Ancestor']
 1728 GBAA_04551 GBAA_5025 47530325 YP_021674.1 hypothetical protein
 GBAA_5025 [Bacillus anthracis str. 'Ames Ancestor']
 644 GBAA_01083 GBAA_1177 47526441 YP_017790.1 ATP-dependent Clp protease
 ATP-binding subunit ClpB [Bacillus anthracis str. 'Ames Ancestor'] prk10865
 431099 ""Posttranslational modification, protein turnover, chaperones""
 1669 GBAA_04438 GBAA_4908 47530203 YP_021552.1 30S ribosomal protein S4
 [Bacillus anthracis str. 'Ames Ancestor'] prk05327 209203 Translation
 879 GBAA_01674 GBAA_1837 47527129 YP_018478.1 hypothetical protein
 GBAA_1837 [Bacillus anthracis str. 'Ames Ancestor']
 1563 GBAA_04166 GBAA_4613 47529911 YP_021260.1 hypothetical protein
 GBAA_4613 [Bacillus anthracis str. 'Ames Ancestor'] prk13678 989501
 Function unknown
 1282 GBAA_03603 GBAA_4023 47529316 YP_020665.1 dihydroorotate
 dehydrogenase 1B [Bacillus anthracis str. 'Ames Ancestor'] prk07259 418302
 Nucleotide transport and metabolism
 1680 GBAA_04469 GBAA_4939 47530236 YP_021585.1 nicotinate
 phosphoribosyltransferase [Bacillus anthracis str. 'Ames Ancestor']
 prk07188 418260 Coenzyme transport and metabolism
 1175 GBAA_03343 GBAA_3738 47778203 YP_020376.2 hypothetical protein
 GBAA_3738 [Bacillus anthracis str. 'Ames Ancestor']
 1600 GBAA_04221 GBAA_4674 47529973 YP_021322.1 50S ribosomal protein L27
 [Bacillus anthracis str. 'Ames Ancestor'] prk05435 209305 Translation
 1316 GBAA_03643 GBAA_4063 47529358 YP_020707.1 hypothetical protein
 GBAA_4063 [Bacillus anthracis str. 'Ames Ancestor']
 1470 GBAA_03989 GBAA_4421 47529714 YP_021063.1 elongation factor P
 [Bacillus anthracis str. 'Ames Ancestor'] prk00529 414170 Translation

Clade 1

cluster#	internal	accession	locus_tag	geneID	proteinID	Description	Pfam
509	GBAA_00572	GBAA_0614	47525891	YP_017240.1	proton/peptide symporter family protein	[Bacillus anthracis str. 'Ames Ancestor']	prk15462 2912572
Amino acid transport and metabolism							
1608	GBAA_04230	GBAA_4683	47529982	YP_021331.1	rod shape-determining protein MreC	[Bacillus anthracis str. 'Ames Ancestor']	prk13922 2411794
Cell wall/membrane biogenesis							
1216	GBAA_03509	GBAA_3926	47529219	YP_020568.1	sugar ABC transporter ATP- binding protein	[Bacillus anthracis str. 'Ames Ancestor']	prk13549 859025

Carbohydrate transport and metabolism

1027 GBAA_02348 GBAA_2583 47527873 YP_019222.1 hypothetical protein
GBAA_2583 [Bacillus anthracis str. 'Ames Ancestor']

435 GBAA_00340 GBAA_0371 47525642 YP_016991.1 glycosyl hydrolase family
protein [Bacillus anthracis str. 'Ames Ancestor'] prk10933 431164

Carbohydrate transport and metabolism

863 GBAA_01584 GBAA_1731 47527022 YP_018371.1 permease [Bacillus anthracis
str. 'Ames Ancestor'] prk10489 430743 Carbohydrate transport and metabolism

1682 GBAA_04471 GBAA_4941 47530238 YP_021587.1 hypothetical protein
GBAA_4941 [Bacillus anthracis str. 'Ames Ancestor']

1939 GBAA05144 GBAA_5670 47778425 YP_022734.1 group 1 family glycosyl
transferase [Bacillus anthracis str. 'Ames Ancestor'] prk15427 2912537 Cell
wall/membrane biogenesis

1176 GBAA_03344 GBAA_3739 47778204 YP_020377.2 hypothetical protein
GBAA_3739 [Bacillus anthracis str. 'Ames Ancestor']

1866 GBAA05006 GBAA_5520 47530838 YP_022187.1 rod shape-determining
protein Mbl [Bacillus anthracis str. 'Ames Ancestor'] prk13928 2411799

""""Cell cycle control, mitosis and meiosis""""

524 GBAA_00610 GBAA_0653 47777841 YP_017282.2 sulfate permease family
protein [Bacillus anthracis str. 'Ames Ancestor'] prk11660 431758 Inorganic
ion transport and metabolism

1728 GBAA_04551 GBAA_5025 47530325 YP_021674.1 hypothetical protein
GBAA_5025 [Bacillus anthracis str. 'Ames Ancestor']

733 GBAA_01260 GBAA_1377 47777924 YP_017999.2 ABC transporter permease
[Bacillus anthracis str. 'Ames Ancestor']

1402 GBAA03872 GBAA_4299 47529594 YP_020943.1 ABC transporter ATP-binding
protein [Bacillus anthracis str. 'Ames Ancestor'] prk13537 859013 Defense
mechanisms

1407 GBAA_03883 GBAA_4310 47529606 YP_020955.1 hypothetical protein
GBAA_4310 [Bacillus anthracis str. 'Ames Ancestor']

1719 GBAA04532 GBAA_5005 47530307 YP_021656.1 aspartate racemase family
protein [Bacillus anthracis str. 'Ames Ancestor'] prk10200 430479 Cell
wall/membrane biogenesis

835 GBAA_01443 GBAA_1567 47526840 YP_018189.1 hypothetical protein
GBAA_1567 [Bacillus anthracis str. 'Ames Ancestor']

394 GBAA_00265 GBAA_0292 47525555 YP_016904.1

phosphoribosylformylglycinamide synthase subunit PurS [Bacillus anthracis
str. 'Ames Ancestor'] prk05974 209818 Nucleotide transport and metabolism

1175 GBAA_03343 GBAA_3738 47778203 YP_020376.2 hypothetical protein
GBAA_3738 [Bacillus anthracis str. 'Ames Ancestor']

935 GBAA_01880 GBAA_2065 47527357 YP_018706.1 hypothetical protein
GBAA_2065 [Bacillus anthracis str. 'Ames Ancestor']

1693 GBAA04484 GBAA_4955 47530251 YP_021600.1 2'-5' RNA ligase family
protein [Bacillus anthracis str. 'Ames Ancestor'] prk15124 2846239

Translation

1868 GBAA_05011 GBAA_5525 47530843 YP_022192.1 ABC transporter permease

[*Bacillus anthracis* str. 'Ames Ancestor']
1249 GBAA03554 GBAA_3973 47529263 YP_020612.1 succinyl-CoA synthetase subunit alpha [*Bacillus anthracis* str. 'Ames Ancestor'] prk05678 209523 Energy production and conversion
419 GBAA_00302 GBAA_0332 47525599 YP_016948.1 NupC family nucleoside transporter [*Bacillus anthracis* str. 'Ames Ancestor']
1581 GBAA04190 GBAA_4640 47529938 YP_021287.1 cation efflux family protein [*Bacillus anthracis* str. 'Ames Ancestor'] prk09509 419620 Inorganic ion transport and metabolism
1896 GBAA_05057 GBAA_5573 47530892 YP_022241.1 thymidine kinase [*Bacillus anthracis* str. 'Ames Ancestor'] prk04296 416475 Nucleotide transport and metabolism
1111 GBAA_02993 GBAA_3343 47528628 YP_019977.1 alpha/beta fold family hydrolase [*Bacillus anthracis* str. 'Ames Ancestor'] prk14875 2845990 Multifunctional
387 GBAA_00236 GBAA_0258 47525517 YP_016866.1 hypothetical protein GBAA_0258 [*Bacillus anthracis* str. 'Ames Ancestor'] prk10646 430888 General function prediction only
607 GBAA_00991 GBAA_1069 47526345 YP_017694.1 penicillin-binding protein 1 [*Bacillus anthracis* str. 'Ames Ancestor'] prk14850 2845965 Cell wall/membrane biogenesis
1149 GBAA_03260 GBAA_3641 47528926 YP_020275.1 hypothetical protein GBAA_3641 [*Bacillus anthracis* str. 'Ames Ancestor'] prk00216 123056 Coenzyme transport and metabolism
406 GBAA_00279 GBAA_0307 47525570 YP_016919.1 putative lipoprotein [*Bacillus anthracis* str. 'Ames Ancestor']
1870 GBAA05013 GBAA_5528 47530847 YP_022196.1 stage II sporulation protein D [*Bacillus anthracis* str. 'Ames Ancestor']
1701 GBAA04499 GBAA_4971 47530269 YP_021618.1 molybdopterin converting factor subunit 1 [*Bacillus anthracis* str. 'Ames Ancestor'] prk11130 431330 Coenzyme transport and metabolism
531 GBAA_00629 GBAA_0674 47525954 YP_017303.1 multidrug resistance protein [*Bacillus anthracis* str. 'Ames Ancestor'] prk09874 430178 Carbohydrate transport and metabolism
1411 GBAA_03891 GBAA_4318 47529615 YP_020964.1 oxidoreductase [*Bacillus anthracis* str. 'Ames Ancestor'] prk10625 430867 Energy production and conversion
1665 GBAA_04429 GBAA_4898 47530192 YP_021541.1 small, acid-soluble spore protein B [*Bacillus anthracis* str. 'Ames Ancestor']
1538 GBAA04111 GBAA_4553 47529850 YP_021199.1 ComE operon protein 1 [*Bacillus anthracis* str. 'Ames Ancestor']
1797 GBAA04727 GBAA_5215 47530521 YP_021870.1 aminotransferase, class V [*Bacillus anthracis* str. 'Ames Ancestor'] prk09295 213122 Amino acid transport and metabolism
611 GBAA_00998 GBAA_1077 47777894 YP_022557.1 hypothetical protein GBAA_1077 [*Bacillus anthracis* str. 'Ames Ancestor']

924 GBAA_01863 GBAA_2046 47527336 YP_018685.1 spore coat protein H
 [Bacillus anthracis str. 'Ames Ancestor']
 856 GBAA_01503 GBAA_1632 47526918 YP_018267.1 hypothetical protein
 GBAA_1632 [Bacillus anthracis str. 'Ames Ancestor']
 1839 GBAA04868 GBAA_5365 47530675 YP_022024.1 phosphoglyceromutase
 [Bacillus anthracis str. 'Ames Ancestor'] prk05434 209304 Carbohydrate
 transport and metabolism
 501 GBAA_00560 GBAA_0600 47525879 YP_017228.1 hypothetical protein
 GBAA_0600 [Bacillus anthracis str. 'Ames Ancestor']
 1641 GBAA04381 GBAA_4846 47530141 YP_021490.1 acetyl-CoA carboxylase
 subunit beta [Bacillus anthracis str. 'Ames Ancestor'] prk05654 209499
 Lipid transport and metabolism
 928 GBAA_01867 GBAA_2050 47777995 YP_018689.2 magnesium transporter cora
 family protein [Bacillus anthracis str. 'Ames Ancestor']
 1714 GBAA04519 GBAA_4992 47530294 YP_021643.1 permease [Bacillus anthracis
 str. 'Ames Ancestor']
 1282 GBAA03603 GBAA_4023 47529316 YP_020665.1 dihydroorotate dehydrogenase
 1B [Bacillus anthracis str. 'Ames Ancestor'] prk07259 418302 Nucleotide
 transport and metabolism
 1680 GBAA_04469 GBAA_4939 47530236 YP_021585.1 nicotinate
 phosphoribosyltransferase [Bacillus anthracis str. 'Ames Ancestor']
 prk07188 418260 Coenzyme transport and metabolism
 1430 GBAA_03920 GBAA_4348 47529644 YP_020993.1 MarR family transcriptional
 regulator [Bacillus anthracis str. 'Ames Ancestor'] prk03573 207533
 Transcription
 1056 GBAA_02574 GBAA_2840 47528134 YP_019483.1 hypothetical protein
 GBAA_2840 [Bacillus anthracis str. 'Ames Ancestor']
 1484 GBAA04022 GBAA_4457 47529752 YP_021101.1 shikimate kinase [Bacillus
 anthracis str. 'Ames Ancestor'] prk00131 414030 Amino acid transport and
 metabolism
 772 GBAA_01329 GBAA_1446 47526721 YP_018070.1 CbiX domain-containing
 protein [Bacillus anthracis str. 'Ames Ancestor'] prk00923 123742 Function
 unknown
 897 GBAA_01778 GBAA_1952 47777984 YP_018596.2 NLP/P60 family protein
 [Bacillus anthracis str. 'Ames Ancestor'] prk13914 2411786 Cell wall/
 membrane biogenesis
 726 GBAA_01250 GBAA_1366 47526636 YP_017985.1 acetyltransferase [Bacillus
 anthracis str. 'Ames Ancestor'] prk03624 207581 General function prediction
 only
 771 GBAA_01327 GBAA_1444 47526719 YP_018068.1 hypothetical protein
 GBAA_1444 [Bacillus anthracis str. 'Ames Ancestor']
 1136 GBAA03210 GBAA_3589 47778179 YP_020224.2 hypothetical protein
 GBAA_3589 [Bacillus anthracis str. 'Ames Ancestor']
 598 GBAA_00956 GBAA_1027 47526306 YP_017655.1 glycerol-3-phosphate
 dehydrogenase, aerobic [Bacillus anthracis str. 'Ames Ancestor'] prk12266
 501361 Energy production and conversion

918 GBAA_01841 GBAA_2022 47527320 YP_018669.1 spermidine acetyltransferase [Bacillus anthracis str. 'Ames Ancestor'] prk09491 419608 General function prediction only
 1416 GBAA_03899 GBAA_4326 47778279 YP_020973.2 LacI family sugar-binding transcriptional regulator [Bacillus anthracis str. 'Ames Ancestor'] prk10703 430944 Transcription
 1123 GBAA_03069 GBAA_3423 47528708 YP_020057.1 transcriptional regulator [Bacillus anthracis str. 'Ames Ancestor']
 1773 GBAA04673 GBAA_5156 47778369 YP_021810.2 hypothetical protein GBAA_5156 [Bacillus anthracis str. 'Ames Ancestor']
 1635 GBAA04374 GBAA_4839 47530134 YP_021483.1 citrate synthase [Bacillus anthracis str. 'Ames Ancestor'] prk14034 2502113 Energy production and conversion
 1135 GBAA_03197 GBAA_3575 47528860 YP_020209.1 glycolate oxidase subunit GlcD [Bacillus anthracis str. 'Ames Ancestor'] prk11230 431395 Energy production and conversion
 1060 GBAA_02592 GBAA_2863 47778091 YP_019507.2 hypothetical protein GBAA_2863 [Bacillus anthracis str. 'Ames Ancestor']
 1695 GBAA04487 GBAA_4959 47530256 YP_021605.1 polysaccharide biosynthesis family protein [Bacillus anthracis str. 'Ames Ancestor']
 1710 GBAA04512 GBAA_4985 47530284 YP_021633.1 spore germination protein GerHB [Bacillus anthracis str. 'Ames Ancestor']
 763 GBAA_01314 GBAA_5744 291612212 YP_003541026.1 phosphoribosyl-ATP pyrophosphohydrolase [Bacillus anthracis str. 'Ames Ancestor'] prk00400 123237 Amino acid transport and metabolism
 923 GBAA_01858 GBAA_2041 47527331 YP_018680.1 oligopeptide ABC transporter oligopeptide-binding protein [Bacillus anthracis str. 'Ames Ancestor'] prk15104 2846219 Amino acid transport and metabolism
 1384 GBAA_03844 GBAA_4270 47529563 YP_020912.1 transcription antiterminator GlcT [Bacillus anthracis str. 'Ames Ancestor'] prk09772 430083 Transcription
 646 GBAA_01086 GBAA_1181 47526445 YP_017794.1 UDP-glucose 4-epimerase-like protein [Bacillus anthracis str. 'Ames Ancestor']
 510 GBAA_00579 GBAA_0621 47525899 YP_017248.1 hypothetical protein GBAA_0621 [Bacillus anthracis str. 'Ames Ancestor'] prk15181 2912291 Multifunctional
 1800 GBAA_04733 GBAA_5222 47530528 YP_021877.1 ABC transporter ATP-binding protein [Bacillus anthracis str. 'Ames Ancestor'] prk11153 431349 Inorganic ion transport and metabolism
 760 GBAA_01311 GBAA_1429 47526701 YP_018050.1 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase [Bacillus anthracis str. 'Ames Ancestor'] prk00748 123573 Amino acid transport and metabolism
 1951 GBAA_05184 GBAA_5713 47531050 YP_022399.1 hypothetical protein GBAA_5713 [Bacillus anthracis str. 'Ames Ancestor']
 944 GBAA_01903 GBAA_2089 47527383 YP_018732.1 acetyltransferase [Bacillus

anthracis str. 'Ames Ancestor'] prk15130 2846245 Translation
732 GBAA_01259 GBAA_1376 47526649 YP_017998.1 ABC transporter ATP-binding
protein [Bacillus anthracis str. 'Ames Ancestor'] prk13537 859013 Defense
mechanisms
761 GBAA_01312 GBAA_1430 47526703 YP_018052.1 imidazole glycerol phosphate
synthase subunit HisF [Bacillus anthracis str. 'Ames Ancestor'] prk02083
501235 Amino acid transport and metabolism
751 GBAA_01301 GBAA_1419 47526691 YP_018040.1 ketol-acid reductoisomerase
[Bacillus anthracis str. 'Ames Ancestor'] prk05479 209349 Multifunctional
599 GBAA_00969 GBAA_1041 47526319 YP_017668.1 peptidylprolyl isomerase
[Bacillus anthracis str. 'Ames Ancestor'] prk03095 415670
""Posttranslational modification, protein turnover, chaperones""
1857 GBAA_04967 GBAA_5479 47530797 YP_022146.1 glycine betaine transporter
[Bacillus anthracis str. 'Ames Ancestor'] prk09928 430223 Cell wall/
membrane biogenesis
644 GBAA_01083 GBAA_1177 47526441 YP_017790.1 ATP-dependent Clp protease
ATP-binding subunit ClpB [Bacillus anthracis str. 'Ames Ancestor'] prk10865
431099 ""Posttranslational modification, protein turnover, chaperones""
1559 GBAA_04162 GBAA_4609 47529906 YP_021255.1 U32 family peptidase
[Bacillus anthracis str. 'Ames Ancestor'] prk15452 2912562
""Posttranslational modification, protein turnover, chaperones""
1703 GBAA_04502 GBAA_4974 47778349 YP_021621.2 molybdopterin biosynthesis
protein moea [Bacillus anthracis str. 'Ames Ancestor'] prk14498 2516318
Coenzyme transport and metabolism
615 GBAA_01010 GBAA_1089 47526363 YP_017712.1 lipoate-protein ligase A
[Bacillus anthracis str. 'Ames Ancestor'] prk03822 207765 Coenzyme
transport and metabolism
1463 GBAA_03976 GBAA_4408 47529702 YP_021051.1 acetyl-CoA carboxylase
biotin carboxylase subunit [Bacillus anthracis str. 'Ames Ancestor']
prk08591 419139 Lipid transport and metabolism
755 GBAA_01305 GBAA_1423 47526695 YP_018044.1 isopropylmalate isomerase
small subunit [Bacillus anthracis str. 'Ames Ancestor'] prk01641 414943
Amino acid transport and metabolism

Clade 2

cluster#	internal	accession	locus_tag	geneID	proteinID	Description	Pfam
535	GBAA_00640	GBAA_0685	47525965	YP_017314.1	hypothetical protein		
	GBAA_0685	[Bacillus anthracis str. 'Ames Ancestor']					
509	GBAA_00572	GBAA_0614	47525891	YP_017240.1	proton/peptide symporter		
		family protein [Bacillus anthracis str. 'Ames Ancestor'] prk15462 2912572					
		Amino acid transport and metabolism					
733	GBAA_01260	GBAA_1377	47777924	YP_017999.2	ABC transporter permease		
		[Bacillus anthracis str. 'Ames Ancestor']					
744	GBAA_01279	GBAA_1397	47526669	YP_018018.1	aminoglycoside		

phosphotransferase family protein [Bacillus anthracis str. 'Ames Ancestor']
1563 GBAA_04166 GBAA_4613 47529911 YP_021260.1 hypothetical protein
GBAA_4613 [Bacillus anthracis str. 'Ames Ancestor'] prk13678 989501
Function unknown
863 GBAA_01584 GBAA_1731 47527022 YP_018371.1 permease [Bacillus anthracis
str. 'Ames Ancestor'] prk10489 430743 Carbohydrate transport and metabolism
1545 GBAA_04120 GBAA_4562 47778309 YP_021207.2 GTP-binding protein YqeH
[Bacillus anthracis str. 'Ames Ancestor'] prk13796 989618 General function
prediction only
1141 GBAA_03227 GBAA_3606 47778184 YP_020241.2 inosine-uridine preferring
nucleoside hydrolase family protein [Bacillus anthracis str. 'Ames
Ancestor'] prk10443 430703 Nucleotide transport and metabolism
926 GBAA_01865 GBAA_2048 47527338 YP_018687.1 DedA family protein
[Bacillus anthracis str. 'Ames Ancestor'] prk10847 431081 Function unknown
1868 GBAA_05011 GBAA_5525 47530843 YP_022192.1 ABC transporter permease
[Bacillus anthracis str. 'Ames Ancestor']
1147 GBAA_03257 GBAA_3638 47778191 YP_022649.1 hypothetical protein
GBAA_3638 [Bacillus anthracis str. 'Ames Ancestor']
1775 GBAA_04682 GBAA_5166 47530472 YP_021821.1 hypothetical protein
GBAA_5166 [Bacillus anthracis str. 'Ames Ancestor']
1176 GBAA_03344 GBAA_3739 47778204 YP_020377.2 hypothetical protein
GBAA_3739 [Bacillus anthracis str. 'Ames Ancestor']
1835 GBAA_04838 GBAA_5335 47530644 YP_021993.1 carboxylesterase [Bacillus
anthracis str. 'Ames Ancestor'] prk14875 2845990 Multifunctional
854 GBAA_01497 GBAA_1626 161611190 YP_018260.3 hypothetical protein
GBAA_1626 [Bacillus anthracis str. 'Ames Ancestor'] prk07708 429879
""Replication, recombination and repair""
899 GBAA_01785 GBAA_1960 47527257 YP_018606.1 aminoglycoside 6-
adenylyltransferase [Bacillus anthracis str. 'Ames Ancestor']
1721 GBAA_04536 GBAA_5009 47530312 YP_021661.1 hypothetical protein
GBAA_5009 [Bacillus anthracis str. 'Ames Ancestor'] prk10749 430989 Lipid
transport and metabolism
1785 GBAA_04708 GBAA_5194 47530501 YP_021850.1 hypothetical protein
GBAA_5194 [Bacillus anthracis str. 'Ames Ancestor']
574 GBAA_00797 GBAA_0855 47526143 YP_017492.1 amino acid ABC transporter
amino acid-binding protein [Bacillus anthracis str. 'Ames Ancestor']
prk11260 431418 Multifunctional
1527 GBAA_04095 GBAA_4536 229220844 YP_021182.2 16S ribosomal RNA
methyltransferase RsmE [Bacillus anthracis str. 'Ames Ancestor'] prk11713
431786 Function unknown
501 GBAA_00560 GBAA_0600 47525879 YP_017228.1 hypothetical protein
GBAA_0600 [Bacillus anthracis str. 'Ames Ancestor']
1185 GBAA_03434 GBAA_3843 47778220 YP_020479.2 tRNA delta(2)-
isopentenylpyrophosphate transferase [Bacillus anthracis str. 'Ames
Ancestor'] prk00091 413996 Translation
878 GBAA_01672 GBAA_1835 47527126 YP_018475.1 sodium-dependent transporter

[*Bacillus anthracis* str. 'Ames Ancestor']

1434 GBAA_03926 GBAA_4354 47529650 YP_020999.1 bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein [*Bacillus anthracis* str. 'Ames Ancestor'] prk05388 209260 Amino acid transport and metabolism

1162 GBAA_03311 GBAA_3699 47528983 YP_020332.1 hypothetical protein GBAA_3699 [*Bacillus anthracis* str. 'Ames Ancestor']

1134 GBAA_03191 GBAA_3567 47528851 YP_020200.1 hypothetical protein GBAA_3567 [*Bacillus anthracis* str. 'Ames Ancestor'] prk00029 413941 Function unknown

1736 GBAA_04574 GBAA_5052 47778357 YP_022714.1 spore germination protein gerpf-like protein [*Bacillus anthracis* str. 'Ames Ancestor']

1210 GBAA_03503 GBAA_3918 47529210 YP_020559.1 hypothetical protein GBAA_3918 [*Bacillus anthracis* str. 'Ames Ancestor']

1941 GBAA_05148 GBAA_5674 47531007 YP_022356.1 hypothetical protein GBAA_5674 [*Bacillus anthracis* str. 'Ames Ancestor']

1192 GBAA_03453 GBAA_3863 47529152 YP_020501.1 hypothetical protein GBAA_3863 [*Bacillus anthracis* str. 'Ames Ancestor']

1375 GBAA_03818 GBAA_4243 47529536 YP_020885.1 hypothetical protein GBAA_4243 [*Bacillus anthracis* str. 'Ames Ancestor']

871 GBAA_01646 GBAA_1804 47527092 YP_018441.1 helicase [*Bacillus anthracis* str. 'Ames Ancestor'] prk04537 416667 Multifunctional

1825 GBAA_04793 GBAA_5284 47530592 YP_021941.1 MarR family transcriptional regulator [*Bacillus anthracis* str. 'Ames Ancestor']

616 GBAA_01012 GBAA_1091 47777898 YP_017714.2 acyl-CoA synthetase [*Bacillus anthracis* str. 'Ames Ancestor'] prk07656 418483 Multifunctional

1669 GBAA_04438 GBAA_4908 47530203 YP_021552.1 30S ribosomal protein S4 [*Bacillus anthracis* str. 'Ames Ancestor'] prk05327 209203 Translation

1097 GBAA_02798 GBAA_3099 47528392 YP_019741.1 Signal peptidase I U [*Bacillus anthracis* str. 'Ames Ancestor'] prk10861 431095 Intracellular trafficking and secretion

1433 GBAA_03925 GBAA_4353 47529649 YP_020998.1 acetylglutamate kinase [*Bacillus anthracis* str. 'Ames Ancestor'] prk00942 414447 Amino acid transport and metabolism

923 GBAA_01858 GBAA_2041 47527331 YP_018680.1 oligopeptide ABC transporter oligopeptide-binding protein [*Bacillus anthracis* str. 'Ames Ancestor'] prk15104 2846219 Amino acid transport and metabolism

1451 GBAA_03958 GBAA_4388 47529683 YP_021032.1 phosphate butyryltransferase [*Bacillus anthracis* str. 'Ames Ancestor'] prk07742 418559 Energy production and conversion

1198 GBAA_03468 GBAA_3881 47529171 YP_020520.1 hypothetical protein GBAA_3881 [*Bacillus anthracis* str. 'Ames Ancestor']

1526 GBAA_04093 GBAA_4534 47529831 YP_021180.1 30S ribosomal protein S21 [*Bacillus anthracis* str. 'Ames Ancestor'] prk00270 123110 Translation

962 GBAA_02018 GBAA_2219 47527512 YP_018861.1 hypothetical protein GBAA_2219 [*Bacillus anthracis* str. 'Ames Ancestor']

563 GBAA_00715 GBAA_0763 47526047 YP_017396.1 spore germination protein

GerYA [Bacillus anthracis str. 'Ames Ancestor']

1839 GBAA_04868 GBAA_5365 47530675 YP_022024.1 phosphoglyceromutase
[Bacillus anthracis str. 'Ames Ancestor'] prk05434 209304 Carbohydrate
transport and metabolism

928 GBAA_01867 GBAA_2050 47777995 YP_018689.2 magnesium transporter cora
family protein [Bacillus anthracis str. 'Ames Ancestor']

767 GBAA_01323 GBAA_1440 47526715 YP_018064.1 phosphoadenosine
phosphosulfate reductase [Bacillus anthracis str. 'Ames Ancestor'] prk02090
124849 Multifunctional

1049 GBAA_02530 GBAA_2789 47528082 YP_019431.1 RNA polymerase factor
sigma-70 [Bacillus anthracis str. 'Ames Ancestor'] prk06704 429817
Transcription

1135 GBAA_03197 GBAA_3575 47528860 YP_020209.1 glycolate oxidase subunit
GlcD [Bacillus anthracis str. 'Ames Ancestor'] prk11230 431395 Energy
production and conversion

1116 GBAA_03033 GBAA_3383 47528667 YP_020016.1 putative lipoprotein
[Bacillus anthracis str. 'Ames Ancestor']

1297 GBAA_03620 GBAA_4040 47529333 YP_020682.1 hypothetical protein
GBAA_4040 [Bacillus anthracis str. 'Ames Ancestor'] prk10723 430963
Function unknown

587 GBAA_00868 GBAA_0933 47526218 YP_017567.1 DnaD domain-containing
protein [Bacillus anthracis str. 'Ames Ancestor']

1187 GBAA_03438 GBAA_3847 47529134 YP_020483.1 1-phosphofructokinase
[Bacillus anthracis str. 'Ames Ancestor'] prk13508 858984 Carbohydrate
transport and metabolism

696 GBAA_01204 GBAA_1309 47526574 YP_017923.1 glycolate oxidase subunit
GlcD [Bacillus anthracis str. 'Ames Ancestor'] prk11230 431395 Energy
production and conversion

897 GBAA_01778 GBAA_1952 47777984 YP_018596.2 NLP/P60 family protein
[Bacillus anthracis str. 'Ames Ancestor'] prk13914 2411786 Cell wall/
membrane biogenesis

1432 GBAA_03924 GBAA_4352 47778283 YP_022688.1 acetylornithine
aminotransferase [Bacillus anthracis str. 'Ames Ancestor'] prk02936 501265
Amino acid transport and metabolism

1006 GBAA_02175 GBAA_2395 47527689 YP_019038.1 zinc transporter family
protein [Bacillus anthracis str. 'Ames Ancestor'] prk04201 416408 Inorganic
ion transport and metabolism

1358 GBAA_03777 GBAA_4201 47529496 YP_020845.1 glutaredoxin family protein
[Bacillus anthracis str. 'Ames Ancestor'] prk10638 430880
""Posttranslational modification, protein turnover, chaperones""

463 GBAA_00392 GBAA_0424 47525700 YP_017049.1 polysaccharide deacetylase
[Bacillus anthracis str. 'Ames Ancestor']

1119 GBAA_03046 GBAA_3396 47528679 YP_020028.1 hypothetical protein
GBAA_3396 [Bacillus anthracis str. 'Ames Ancestor']

772 GBAA_01329 GBAA_1446 47526721 YP_018070.1 CbiX domain-containing
protein [Bacillus anthracis str. 'Ames Ancestor'] prk00923 123742 Function

unknown

783 GBAA_01342 GBAA_1459 47526734 YP_018083.1 branched chain amino acid ABC transporter carrier protein [Bacillus anthracis str. 'Ames Ancestor'] prk15433 2912543 Amino acid transport and metabolism

491 GBAA_00541 GBAA_0580 47525855 YP_017204.1 putative lipoprotein [Bacillus anthracis str. 'Ames Ancestor']

1827 GBAA_04803 GBAA_5295 47530603 YP_021952.1 hypothetical protein GBAA_5295 [Bacillus anthracis str. 'Ames Ancestor']

1372 GBAA_03808 GBAA_4233 47529526 YP_020875.1 hypothetical protein GBAA_4233 [Bacillus anthracis str. 'Ames Ancestor'] prk06720 429821 Multifunctional

851 GBAA_01494 GBAA_1623 47526908 YP_018257.1 ribonuclease H [Bacillus anthracis str. 'Ames Ancestor'] prk13907 2411779 ""Replication, recombination and repair""

1123 GBAA_03069 GBAA_3423 47528708 YP_020057.1 transcriptional regulator [Bacillus anthracis str. 'Ames Ancestor']

1055 GBAA_02573 GBAA_2839 47528133 YP_019482.1 hypothetical protein GBAA_2839 [Bacillus anthracis str. 'Ames Ancestor']

1098 GBAA_02799 GBAA_3100 47528393 YP_019742.1 copper homeostasis protein CutC [Bacillus anthracis str. 'Ames Ancestor'] prk11572 431678 Inorganic ion transport and metabolism

1689 GBAA_04478 GBAA_4949 47530245 YP_021594.1 metallo-beta-lactamase family protein [Bacillus anthracis str. 'Ames Ancestor']

1628 GBAA_04365 GBAA_4830 47530126 YP_021475.1 formamidopyrimidine-DNA glycosylase [Bacillus anthracis str. 'Ames Ancestor'] prk01103 414568 ""Replication, recombination and repair""

697 GBAA_01205 GBAA_1310 47526575 YP_017924.1 transcriptional regulator [Bacillus anthracis str. 'Ames Ancestor'] prk11477 431593 Multifunctional

776 GBAA_01334 GBAA_1451 47526726 YP_018075.1 hypothetical protein GBAA_1451 [Bacillus anthracis str. 'Ames Ancestor']

1831 GBAA_04819 GBAA_5316 47530625 YP_021974.1 set domain-containing protein [Bacillus anthracis str. 'Ames Ancestor']

1666 GBAA_04431 GBAA_4900 47530194 YP_021543.1 aminotransferase, class V [Bacillus anthracis str. 'Ames Ancestor'] prk14012 2502093 Amino acid transport and metabolism

1682 GBAA_04471 GBAA_4941 47530238 YP_021587.1 hypothetical protein GBAA_4941 [Bacillus anthracis str. 'Ames Ancestor']

824 GBAA_01428 GBAA_1552 47526825 YP_018174.1 uracil-DNA glycosylase family protein [Bacillus anthracis str. 'Ames Ancestor']

680 GBAA_01166 GBAA_1267 47526534 YP_017883.1 hypothetical protein GBAA_1267 [Bacillus anthracis str. 'Ames Ancestor']

1855 GBAA_04964 GBAA_5472 47778407 YP_022729.1 D-amino acid aminotransferase [Bacillus anthracis str. 'Ames Ancestor'] prk12400 535956 Multifunctional

1000 GBAA_02140 GBAA_2355 47527650 YP_018999.1 homoserine/threonine efflux protein [Bacillus anthracis str. 'Ames Ancestor'] prk10520 430769 Amino

acid transport and metabolism

1490 GBAA_04038 GBAA_4476 47778298 YP_021119.2 hypothetical protein
GBAA_4476 [Bacillus anthracis str. 'Ames Ancestor']
1583 GBAA_04192 GBAA_4642 47529940 YP_021289.1 hypothetical protein
GBAA_4642 [Bacillus anthracis str. 'Ames Ancestor']
1553 GBAA_04141 GBAA_4587 47529882 YP_021231.1 pterin-4-alpha-
carbinolamine dehydratase [Bacillus anthracis str. 'Ames Ancestor']
prk00823 414376 Coenzyme transport and metabolism
659 GBAA_01109 GBAA_1206 47526469 YP_017818.1 oligoendopeptidase F
[Bacillus anthracis str. 'Ames Ancestor']
1091 GBAA_02790 GBAA_3090 50196941 YP_052632.1 pyrrolidone-carboxylate
peptidase [Bacillus anthracis str. 'Ames Ancestor'] prk13197 858325
""Posttranslational modification, protein turnover, chaperones""
1865 GBAA_04991 GBAA_5504 47530822 YP_022171.1 DNA-binding response
regulator [Bacillus anthracis str. 'Ames Ancestor'] prk11083 431290
Multifunctional
419 GBAA_00302 GBAA_0332 47525599 YP_016948.1 NupC family nucleoside
transporter [Bacillus anthracis str. 'Ames Ancestor']
606 GBAA_00990 GBAA_1068 47526344 YP_017693.1 hypothetical protein
GBAA_1068 [Bacillus anthracis str. 'Ames Ancestor'] prk13313 858720 General
function prediction only
1743 GBAA_04588 GBAA_5066 47530366 YP_021715.1 hypothetical protein
GBAA_5066 [Bacillus anthracis str. 'Ames Ancestor']
1186 GBAA_03437 GBAA_3846 47529133 YP_020482.1 PTS system fructose-
specific transporter subunit IIABC [Bacillus anthracis str. 'Ames
Ancestor'] prk09765 430079 Carbohydrate transport and metabolism
1496 GBAA_04045 GBAA_4484 47529778 YP_021127.1 hypothetical protein
GBAA_4484 [Bacillus anthracis str. 'Ames Ancestor']
1860 GBAA_04978 GBAA_5491 291612227 YP_003541041.1 hypothetical protein
GBAA_5491 [Bacillus anthracis str. 'Ames Ancestor']
1464 GBAA_03980 GBAA_4412 47529706 YP_021055.1 stage III sporulation
protein AF [Bacillus anthracis str. 'Ames Ancestor']
640 GBAA_01076 GBAA_1169 47526434 YP_017783.1 peptidylprolyl isomerase
[Bacillus anthracis str. 'Ames Ancestor'] prk03002 415616
""Posttranslational modification, protein turnover, chaperones""
943 GBAA_01901 GBAA_2086 47527379 YP_018728.1 acetyltransferase [Bacillus
anthracis str. 'Ames Ancestor'] prk10146 430427 Multifunctional
916 GBAA_01838 GBAA_2019 47527317 YP_018666.1 acetyltransferase [Bacillus
anthracis str. 'Ames Ancestor'] prk10151 430432 Translation
339 GBAA_00102 GBAA_0106 47525362 YP_016711.1 30S ribosomal protein S7
[Bacillus anthracis str. 'Ames Ancestor'] prk05302 209178 Translation
940 GBAA_01890 GBAA_2075 47527367 YP_018716.1 SPFH domain-containing
protein/band 7 family protein [Bacillus anthracis str. 'Ames Ancestor']
prk10930 431161 ""Posttranslational modification, protein turnover,
chaperones""
645 GBAA_01085 GBAA_1180 47526444 YP_017793.1 hypothetical protein

GBAA_1180 [Bacillus anthracis str. 'Ames Ancestor']
 293 GBAA_00026 GBAA_0028 47777765 YP_016633.2 DNA polymerase III subunit
 delta' [Bacillus anthracis str. 'Ames Ancestor'] prk08058 211894
 ""Replication, recombination and repair""
 1059 GBAA_02583 GBAA_2853 47528146 YP_019495.1 cell division protein DivIC
 [Bacillus anthracis str. 'Ames Ancestor']
 1508 GBAA_04060 GBAA_4499 47529794 YP_021143.1 superoxide dismutase, Mn
 [Bacillus anthracis str. 'Ames Ancestor'] prk10925 431157 Inorganic ion
 transport and metabolism
 1770 GBAA_04669 GBAA_5152 47530456 YP_021805.1 UTP-glucose-1-phosphate
 uridylyltransferase [Bacillus anthracis str. 'Ames Ancestor'] prk13389
 858868 Cell wall/membrane biogenesis
 1863 GBAA_04989 GBAA_5502 47530820 YP_022169.1 hypothetical protein
 GBAA_5502 [Bacillus anthracis str. 'Ames Ancestor']
 1145 GBAA_03234 GBAA_3613 47778186 YP_020248.2 hypothetical protein
 GBAA_3613 [Bacillus anthracis str. 'Ames Ancestor']
 450 GBAA_00371 GBAA_0402 47525673 YP_017022.1 tellurium resistance protein
 [Bacillus anthracis str. 'Ames Ancestor']
 534 GBAA_00634 GBAA_0679 47525959 YP_017308.1 hypothetical protein
 GBAA_0679 [Bacillus anthracis str. 'Ames Ancestor']
 699 GBAA_01207 GBAA_1312 47526577 YP_017926.1 DNA-binding response
 regulator [Bacillus anthracis str. 'Ames Ancestor'] prk09468 419593
 Multifunctional

Figure S1

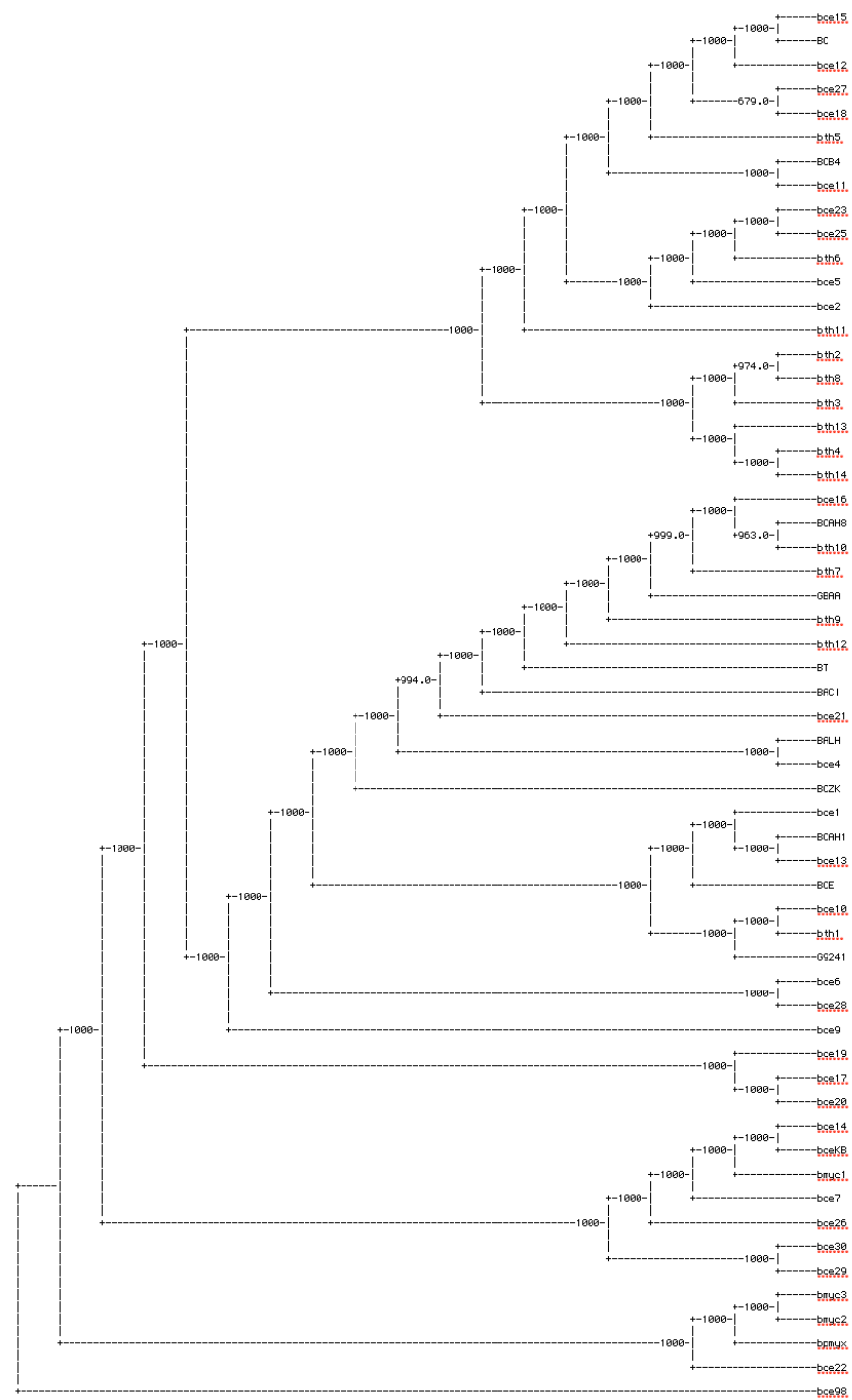


Figure S2

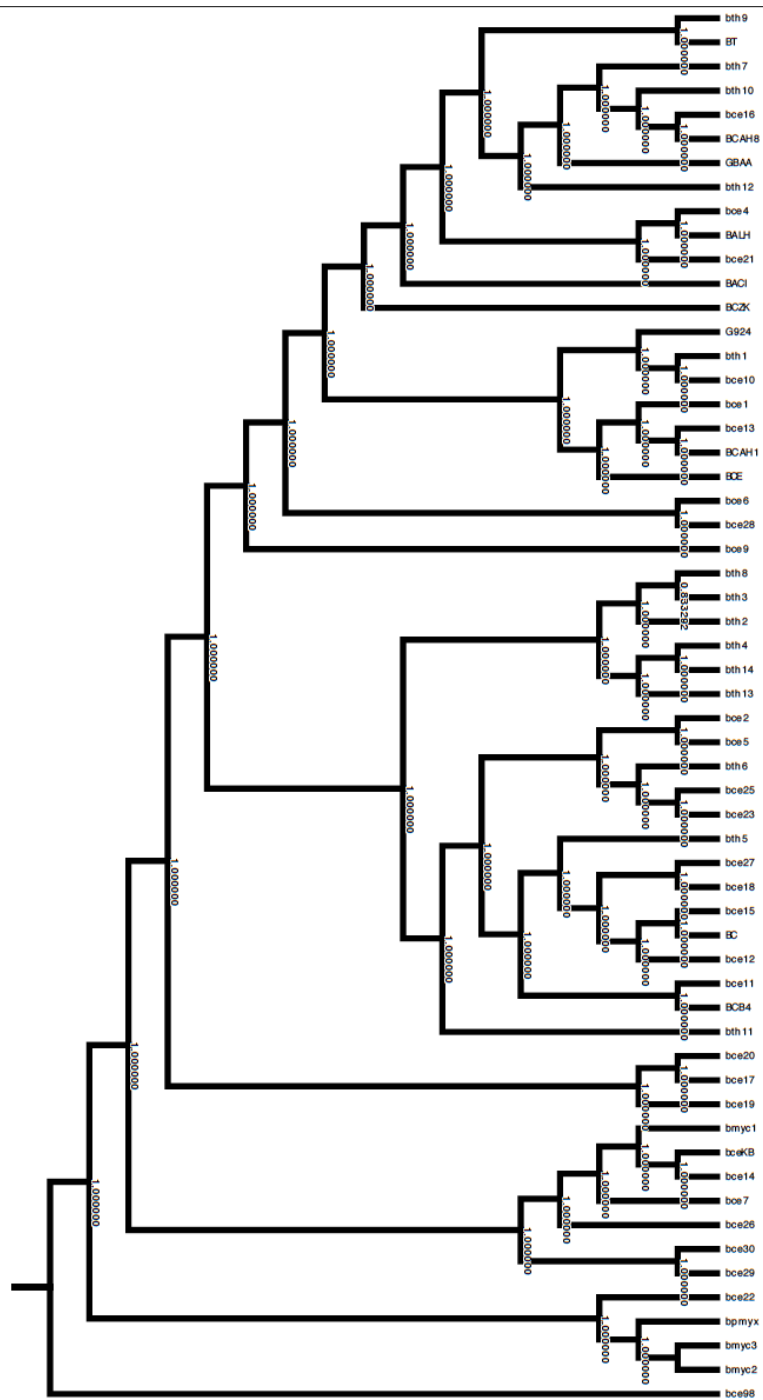


Figure S3



Figure S4

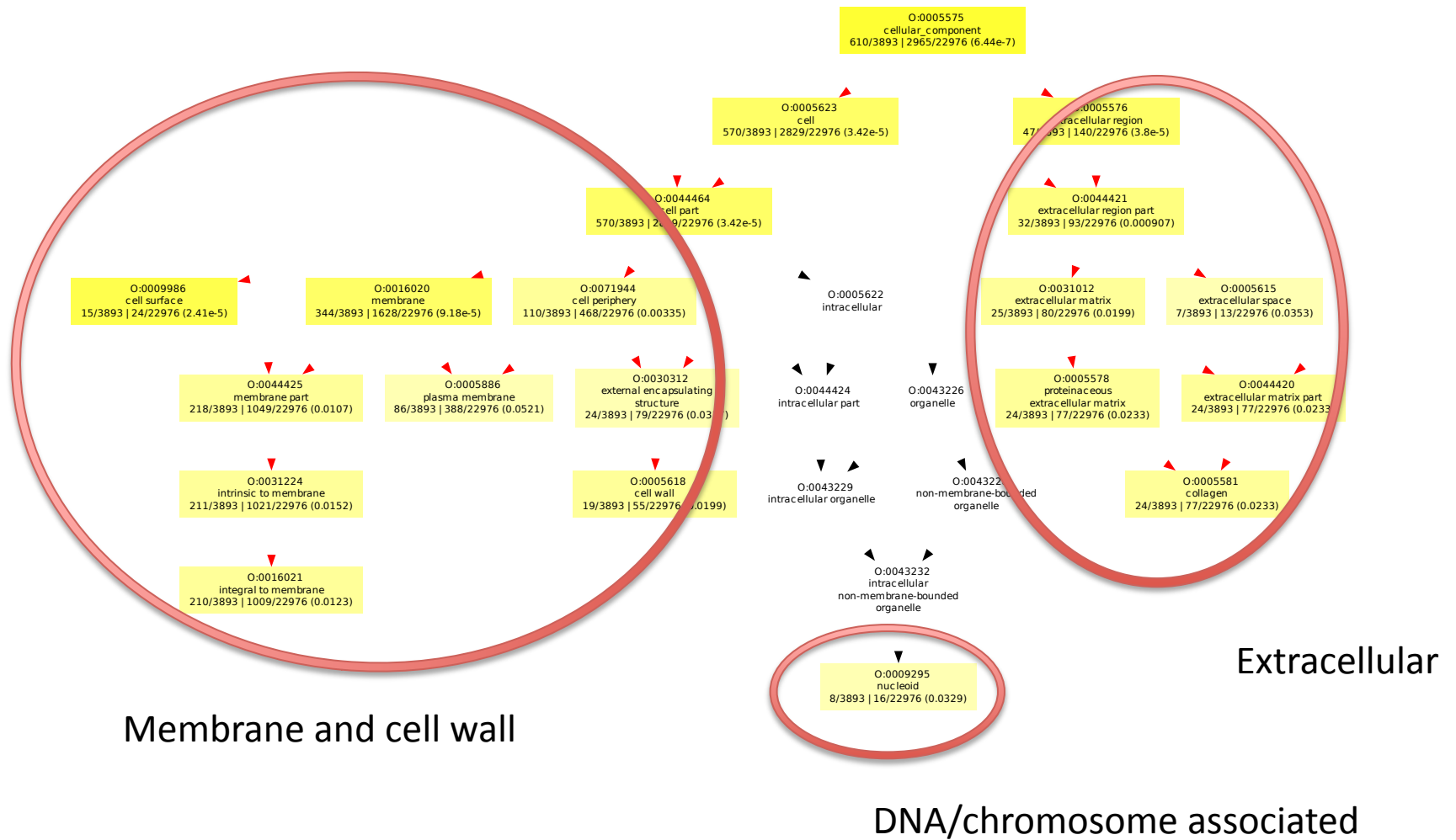


Figure S5

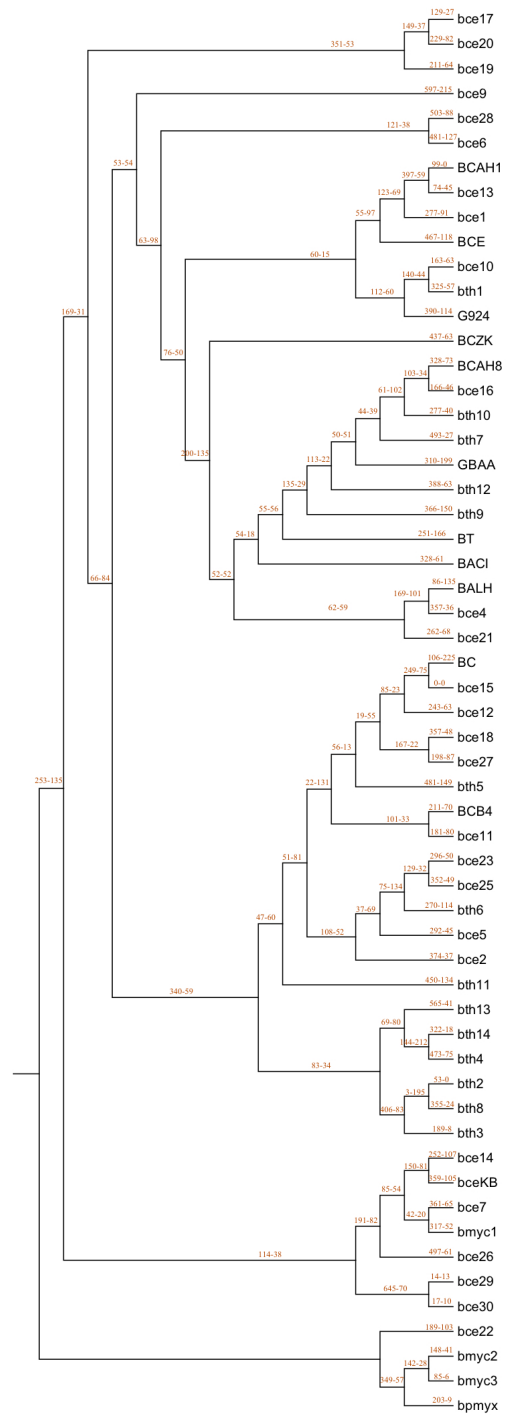


Figure S6

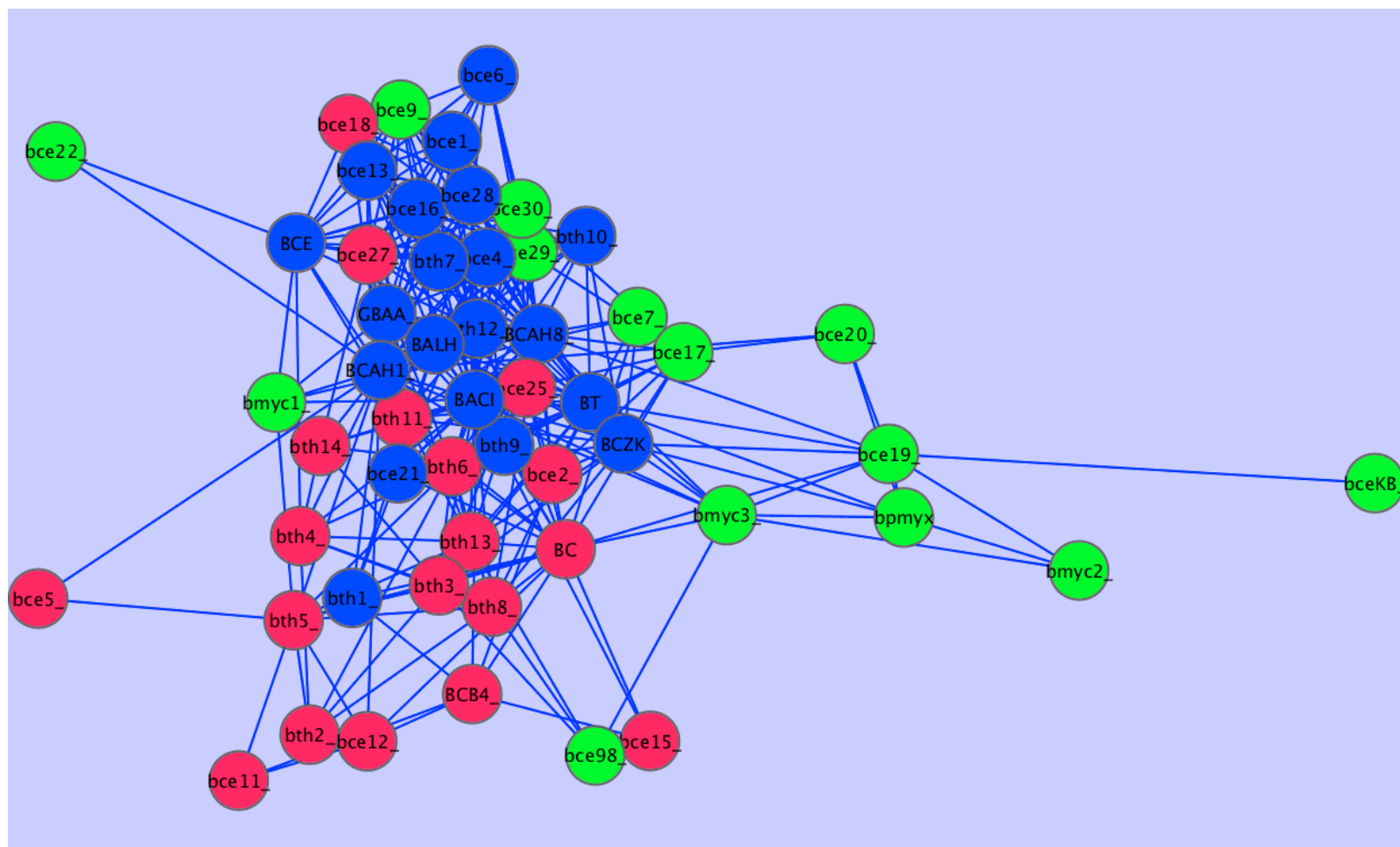
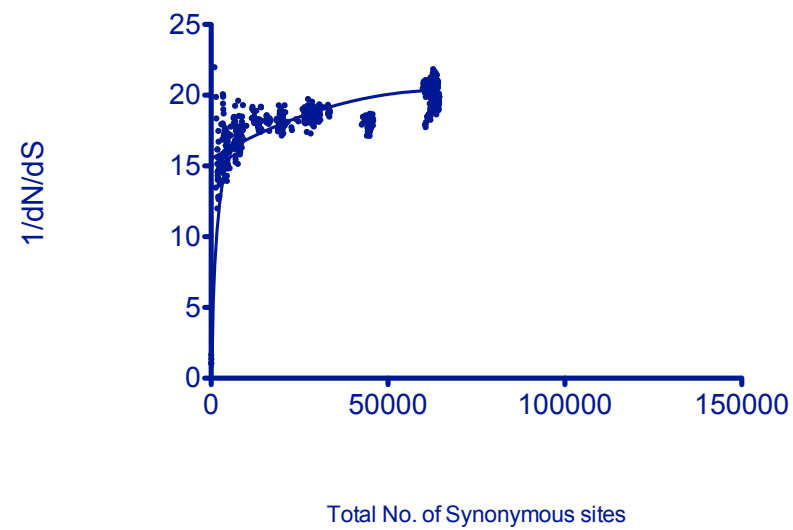


Figure S7

A



B

