

Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*

Claire M. Fraser*, Sherwood Casjens†, Wai Mun Huang‡, Granger G. Sutton*, Rebecca Clayton*, Raju Lathigra‡, Owen White*, Karen A. Ketchum*, Robert Dodson*, Erin K. Hickey*, Michelle Gwinn*, Brian Dougherty*, Jean-Francois Tomb*, Robert D. Fleischmann*, Delwood Richardson*, Jeremy Peterson*, Anthony R. Kerlavage*, John Quackenbush*, Steven Salzberg*, Mark Hanson‡, Rene van Vugt†, Nanette Palmer†, Mark D. Adams*, Jeannine Gocayne*, Janice Weidman*, Teresa Utterback*, Larry Watthey*, Lisa McDonald*, Patricia Artiach*, Cheryl Bowman*, Stacey Garland*, Claire Fujii*, Matthew D. Cotton*, Kurt Horst*, Kevin Roberts*, Bonnie Hatch*, Hamilton O. Smith* & J. Craig Venter*

* The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, Maryland 20850, USA

† Division of Molecular Biology and Genetics, Department of Oncological Sciences, University of Utah, Salt Lake City, Utah 84132, USA

‡ MedImmune, Inc., 35 West Watkins Mill Road, Gaithersburg, Maryland 20878, USA

The genome of the bacterium *Borrelia burgdorferi* B31, the aetiologic agent of Lyme disease, contains a linear chromosome of 910,725 base pairs and at least 17 linear and circular plasmids with a combined size of more than 533,000 base pairs. The chromosome contains 853 genes encoding a basic set of proteins for DNA replication, transcription, translation, solute transport and energy metabolism, but, like *Mycoplasma genitalium*, it contains no genes for cellular biosynthetic reactions. Because *B. burgdorferi* and *M. genitalium* are distantly related eubacteria, we suggest that their limited metabolic capacities reflect convergent evolution by gene loss from more metabolically competent progenitors. Of 430 genes on 11 plasmids, most have no known biological function; 39% of plasmid genes are paralogues that form 47 gene families. The biological significance of the multiple plasmid-encoded genes is not clear, although they may be involved in antigenic variation or immune evasion.

In the mid-1970s, a geographic clustering of an unusual rheumatoid arthritis-like condition was reported in Connecticut¹. That cluster of cases focused attention on the syndrome that is now called Lyme disease. It was subsequently realized that a similar disorder had been known in Europe since the beginning of this century. Lyme disease is characterized by some or all of the following manifestations: an initial erythematous annular rash, flu-like symptoms, neurological complications, and arthritis in about 50% of untreated patients². In the United States, the disease occurs primarily in northeastern and midwestern states, and in western parts of California and Oregon. These regions coincide with the ranges of various species of *Ixodes* ticks, the primary vector of Lyme disease. Lyme disease is now the most common tick-transmitted illness in the United States, and has been reported in many temperate parts of the Northern Hemisphere.

It was not until the early 1980s that a new spirochaete, *Borrelia burgdorferi*³, was isolated and cultured from the midgut of *Ixodes* ticks, and subsequently from patients with Lyme disease^{4,5}. Analysis of genetic diversity among individual *Borrelia* isolates has defined a closely related cluster containing at least 10 tick-borne species of Lyme disease agents, called '*B. burgdorferi* (*sensu lato*)'. *B. burgdorferi* resembles most other spirochaetes in that it is a highly specialized, motile, two-membrane, spiral-shaped bacterium that lives primarily as an extracellular pathogen. *Borrelia* is fastidious and difficult to culture *in vitro*, requiring a specially enriched media and low oxygen tension⁶.

One of the most striking features of *B. burgdorferi* is its unusual genome, which includes a linear chromosome approximately one megabase in size^{7–10} and numerous linear and circular plasmids^{11–13}, with some isolates containing up to 20 different plasmids. The plasmids have a copy number of approximately one per chromosome^{10,14}, and different plasmids often appear to share regions of homologous DNA^{13,15,16}. Long-term culture of *B. burgdorferi* results in the loss of some plasmids, changes in protein expression profiles,

and a loss in the ability of the organism to infect laboratory animals, suggesting that the plasmids encode important proteins involved in virulence^{17–19}.

Because of its importance as a pathogen of humans and animals, and the value of complete genome sequence information for understanding its life cycle and advancing drug and vaccine development, we sequenced the genome of *B. burgdorferi* type strain (B31), using the random sequencing method previously described^{20–24}. Here we summarize the results from sequencing, assembly and analysis of the linear chromosome and 11 plasmids.

Chromosome analysis

The linear chromosome of *B. burgdorferi* has 910,725 base pairs (bp) and an average G+C content of 28.6%. Base pair one represents the first double-stranded base pair that we observed at the left telomere. Previous genome characterizations agree with the nucleotide sequence of the large chromosome^{10,25–28}. The 853 predicted coding sequences (open reading frames; ORFs) have an average size of 992 bp, similar to that observed in other prokaryotic genomes, with 93% of the *B. burgdorferi* genome representing

Figure 1 Linear representations of the *B. burgdorferi* B31 chromosome and plasmids. The location of predicted coding regions colour-coded by biological role, RNA genes, and tRNAs is indicated. Arrows represent the direction of transcription for each predicted coding region. Numbers associated with tRNA symbols represent the number of tRNAs at a locus. Numbers associated with GES represent the number of membrane-spanning domains according to the Goldman, Engelman and Steitz scale as calculated by TopPred⁴⁹. Only proteins with five or more GES are indicated. Members of paralogous gene families are identified by family number. Transporter abbreviations: mal, maltose; P, gly and bet, proline, glycine, betaine; glyc, glycerol; aa, amino acid; E, glutamate; fru, fructose; glu, glucose; s/p, spermidine/putrescine; pan, pantothenate; Pi, phosphate; lac, lactate; rib, ribose; ?, unknown.

coding sequence. Biological roles were assigned to 59% of the 853 ORFs using the classification scheme adapted from Riley²⁹ (Fig. 1), 12% of ORFs matched hypothetical coding sequences of unknown function from other organisms, and 29% were new genes. The average relative molecular mass (M_r) of the chromosome-encoded proteins in *B. burgdorferi* is 37,529 ranging from 3,369 to 254,242, values similar to those observed in other bacteria including *Haemophilus influenzae*²⁰ and *Mycoplasma genitalium*²¹. The median isoelectric point (pI) for all predicted proteins is 9.7.

Analysis of codon usage in *B. burgdorferi* reveals that all 61 triplet codons are used. When both AU- and GC-containing codons specify a single amino acid, there is a marked bias (from 2-fold to more than 20-fold, depending on the amino acid) in the use of AU-rich codons. The most frequently used codons are AAA (Lys, 8.1%), AAU (Asn, 5.9%), AUU (Ile, 5.9%), UUU (Phe, 5.7%), GAA (Glu, 5.0%), GAU (Asp, 4.2%) and UUA (Leu, 4.2%). The most common amino acids are Ile (10.6%), Leu (10.3%), Lys (10.2%), Ser (7.8%) and Asn (7.2%). The high value for Lys is in agreement with the median calculated isoelectric point of 9.7.

Plasmid analysis

Analysis of the nucleotide sequence and Southern analyses on *B. burgdorferi* DNA indicate that, in addition to the large linear chromosome, isolate B31 contains linear plasmids of the following approximate sizes: 56 kilobase pairs (kbp) (lp56), 54 kbp (lp54), four plasmids of 28 kbp (lp28-1, lp28-2, lp28-3 and lp28-4), 38 kbp (lp38), 36 kbp (lp36), 25 kbp (lp25) and 17 kbp (lp17); and circular plasmids of the following sizes: 9 kbp (cp9), 26 kbp (cp26) and five or six homologous plasmids of 32 kbp (cp32). These include all of the plasmids previously identified in this strain, but comparisons with other B31 cultures suggest that this isolate may have lost one 21 kbp linear and one or two 32 kbp circular plasmids during growth in culture since its original isolation^{11–14,19,30}. The sequences of all plasmids were assembled as part of this project. However, the assembled sequences of the cp32 and related lp56 plasmids could not be determined with a high degree of confidence because of DNA sequence similarity among them ($\geq 99\%$ in several regions of

3,000–5,000 bp per plasmid)^{13,16} (Table 1). Improved assembly strategies are being tested to achieve closure on these plasmids (G. Sutton, unpublished). Plasmid lp17 is identical to that of lp16.9 from Barbour *et al.*¹⁵.

The 11 plasmids we have described contain a total of 430 putative ORFs with an average size of 507 bp; plasmid G+C content ranges from 23.1% to 32.3%. Only 71% of plasmid DNA represents predicted coding sequences, a value significantly lower than that on the chromosome. This indicates that average intergenic distances are greater in the plasmids than in the chromosome, and that many potential ORFs contain authentic frameshifts or stops (see E29, for example), suggesting that they are decaying genes not encoding functional proteins. Of the 430 plasmid ORFs, only 70 (16%) could be identified and these include membrane proteins such as OspA-D, decorin-binding proteins, the VlsE lipoprotein recombination cassette, and the purine ribonucleotide biosynthetic enzymes GuaA and GuaB. We found that 100 ORFs (23%) match other hypothetical proteins from plasmids in this and related strains of *B. burgdorferi*^{15,16,31}; 10 ORFs (2.3%) match hypothetical proteins from species other than *Borrelia*; and 250 ORFs (58%) have no database match.

We found that 47 paralogous gene families containing from 2 to 12 members account for 39% (169 ORFs) of the plasmid-encoded genes with no known biological role (Fig. 1). Parologue families 32 and 50, typified by previously identified *B. burgdorferi* plasmid genes cp32 orfC and cp8.3 orf2, respectively, have some similarities to proteins involved in replication, segregation and control of copy number in other bacterial systems^{16,31}. Previous studies have reported examples of plasmid gene duplication, but the extent of

Table 1 Genome features in *Borrelia burgdorferi*

Chromosome	910,725 bp (28.6% G+C)
Coding sequences (93%)	
RNAs (0.7%)	
Intergenic sequence (6.3%)	
853 coding sequences	
500 (59%) with identified database match	
104 (12%) match hypothetical proteins	
249 (29%) with no database match	
Plasmids	
cp9	9,386 bp (23.6% GC)
cp26	26,497 bp (26.3% GC)
lp17	16,828 bp (23.1% GC)
lp25	24,182 bp (23.3% GC)
lp28-1	26,926 bp (32.3% GC)
lp28-2	29,771 bp (31.5% GC)
lp28-3	28,605 bp (25.1% GC)
lp28-4	27,329 bp (24.4% GC)
lp36	36,834 bp (26.8% GC)
lp38	38,853 bp (26.1% GC)
lp54	53,590 bp (28.1% GC)
Coding sequences (71%)	
Intergenic sequence (29%)	
430 coding sequences	
70 (16%) with identified database match	
110 (26%) match hypothetical proteins	
250 (58%) with no database match	
Ribosomal RNA	Chromosome coordinates
16S	444581–446118
23S	438590–441508
5S	438446–438557
23S	435334–438267
5S	435201–435312
Stable RNA	
tmRNA	46973–47335
mpB	750816–751175
Transfer RNA	
34 species (8 clusters, 14 single genes)	

*The telomeric sequences of the nine linear plasmids assembled as part of this study were not determined; estimation of the number of missing terminal nucleotides by restriction analysis suggests that less than 1,200 bp is missing in all cases. Comparisons with previously determined sequences of lp 16.9 and one terminus of lp28-1 indicate that 25, 60 and 1,200 bp are missing, respectively.

Table 2 Gene identification numbers are listed with the prefix BB as in Fig. 2. Each gene identified is listed in its functional role category (adapted from Riley²⁹). The percentage of similarity and a two-letter abbreviation for genus and species for the best match are also shown. An expanded version of this table with additional information is available on the World-Wide Web at <http://www.tigr.org/tdb/mdb/bbdb/bbdb.htm>. Abbreviations of gene names are: Ac, acetyl; BP, binding protein; biosyn, biosynthesis; cello, cellobiose; CPDase, carboxypeptidase; Dcase, decarboxylase; DHase, dehydrogenase; flgr, flagellar/flagellum; fru, fructose; GBP, glycine, betaine, L-proline; glu, glucose; Kase, kinase; mal, maltose; MC-methyl-accepting chemotaxis; MTase, methyltransferase; NAG, N-acetylglucosamine; OH, hydroxy; OP, oligopeptide; P, phosphate; PPTase, phosphotransferase; PPase, phosphatase; prt, protein; put, putative; RDase, reductase; RG, ribose/galactose; SAM, S-adenosyl-methionine; Sase, synthetase/synthase; SP, spermidine/putrescine; ss, single-stranded; sub, subunit; Tase, transferase.

Abbreviation of genus and species are: Ah, *Aeromonas hydrophila*; Ar, *Agrobacterium radiobacter*; Al, *Alteromonas* sp.; Ab, *Anabaena* sp.; An, *Anacystis nidulans*; At, *Arabidopsis thaliana*; Av, *Azotobacter vinelandii*; Bf, *Bacillus firmus*; Bl, *Cacillus licheniformis*; Bm, *Bacillus megaterium*; Bs, *Bacillus stearothermophilus*; Bc, *Bacillus subtilis*; Bb, *Borrelia burgdorferi*; Bc, *Borrelia coriaceae*; Bh, *Borrelia hermsii*; Ba, *Buchnera aphidicola*; Ca, *Clostridium acetobutylicum*; Cl, *Clostridium longisporum*; Cp, *Clostridium perfringens*; Cg, *Corynebacterium glutamicum*; Cb, *Coxiella burnetii*; Cp, *Cyanophora paradoxa*; Dd, *Dictyostelium discoideum*; Ec, *Escherichia coli*; Eh, *Entamoeba histolytica*; Ee, *Enterobacter cloacae*; El, *Enterococcus faecalis*; Eh, *Enterococcus hirae*; Ha, *Haemophilus aegyptius*; Hi, *Haemophilus influenzae*; Hp, *Helicobacter pylori*; Hs, *Homo sapiens*; La, *Lactobacillus acidophilus*; Ll, *Lactococcus lactis*; Li, *Leptospira interrogans* serovar lai; Mj, *Methanococcus jannaschii*; Mb, *Methanosarcina barkeri*; Ml, *Mycobacterium leprae*; Mt, *Mycobacterium tuberculosis*; Mc, *Mycoplasma capricolum*; Mg, *Mycoplasma genitalium*; Mh, *Mycoplasma hominis*; Mh, *Mycoplasma hyorhinis*; Mm, *Mycoplasma mycoides*; Mp, *Mycoplasma pneumoniae*; Mx, *Mycoplasma xanthus*; Ng, *Neisseria gonorrhoeae*; Nm, *Neisseria meningitidis*; Os, *Odontella sinensis*; Pt, *Paramecium tetraurelia*; Pa, *Pediococcus acidilactici*; Pf, *Plasmodium falciparum*; Pg, *Porphyromonas gingivalis*; Pv, *Proteus vulgaris*; Pa, *Pseudomonas aeruginosa*; Pm, *Pseudomonas mevalonii*; Pp, *Pseudomonas putida*; Rm, *Rhizobium meliloti*; Rc, *Rhodobacter capsulatus*; Rs, *Rhodobacter sphaeroides*; Rp, *Rickettsia prowazekii*; Sc, *Saccharomyces cerevisiae*; Sc, *Salmonella choleraesuis*; St, *Salmonella typhimurium*; Sh, *Serpulina hyodysenteriae*; Sd, *Shigella dysenteriae*; So, *Spinacia oleracea*; Sc, *Staphylococcus carnosus*; Se, *Staphylococcus epidermidis*; Sp, *Streptococcus pyogenes*; Sc, *Streptomyces coelicolor*; Ss, *Sulfolobus solfataricus*; Syn, *Synechococcus* sp.; Sp, *Synechocystis* PCC6803; Tt, *Thermoplasma aerophilum*; Tm, *Thermoplasma acidophilum*; Tb, *Thermophilic bacterium* RT8.B4; Tv, *Thermoproteus tenax* virus; Tm, *Thermotoga maritima*; Tat, *Thermus aquaticus thermophilus*; Ta, *Thermus aquaticus*; Td, *Treponema denticola*; Tp, *Treponema pallidum*; Ta, *Triticum aestivum*; Tb, *Trypanosoma brucei* mitochondrion; Vc, *Vibrio cholerae*; Vp, *Vibrio parahaemolyticus*; Zm, *Zymomonas mobilis*.

this redundancy has become even more apparent with the complete sequence of these 11 plasmids from isolate B31. Moreover, a preliminary search of 221 putative ORFs from the cp32s and lp56 indicates that at least 50% display $\geq 70\%$ amino-acid similarity to ORFs from the other 11 plasmids presented here (data not shown). Although plasmid-encoded genes have been implicated in infectivity and virulence^{17–19}, the biological roles of most of these genes are not known. The significance of the large number of paralogous plasmid-encoded genes is not understood. These proteins may be expressed differentially in tick and mammalian hosts, or may undergo homologous recombination to generate antigenic variation in surface proteins. This hypothesis is supported by the identification of 63 plasmid-encoded putative membrane lipoproteins (Fig. 1).

Several copies of a putative recombinase/transposase similar to IS891-like transposases were identified in the *B. burgdorferi* plasmids. Linear plasmid 28-2 contains one full-length copy of this gene. Although no inverted repeats were found on either side of the transposase, there is a putative ribosome-binding site several nucleotides upstream of the apparent start codon, and a stem-loop structure ($-27 \text{ kcal mol}^{-1}$) 195 bp downstream of the stop codon in an area with no ORFs. This transposase might represent a functional gene important for the frequent DNA rearrangements that presumably occur in *Borrelia* plasmids. There are other partial or nearly complete copies of the transposase gene that contain frame-destroying mutations elsewhere in the genome: two copies on lp17, one on lp36, one on lp38, one on lp28-3, two on lp28-1, and one near the right end of the large linear chromosome.

Origin of replication

The replication mechanism for the linear chromosome and plasmids in *B. burgdorferi* is not yet known. Replication possibly begins at the termini, as has been proposed for the poxvirus hairpin telomeres³², or may begin from a single origin somewhere along the length of the linear replicon. Of the genes on the linear chromosome, 66% are transcribed away from the centre of the chromosome (Fig. 1), similar to the transcriptional bias observed for the genomes of *M. genitalium*²¹ and *M. pneumoniae*³³. It has been suggested that bacterial genes are optimally transcribed in the same direction as that in which replication forks pass over them, particularly for highly transcribed genes^{34,35}.

Given the transcriptional bias observed in *B. burgdorferi*, it seems likely that the origin of replication is near the centre of the chromosome. Because bacterial chromosomal replication origins are usually near *dnaA*³⁶, it is intriguing to note that this gene (BB437) lies almost exactly at the centre of the linear *B. burgdorferi* chromosome^{10,27}. A centrally initiated, bi-directional replication fork would be equidistant from the two chromosome ends, and replication would traverse the rRNA genes in the same direction as transcription.

An analysis of GC skew, $(G - C)/(G + C)$ calculated in 10-kilobase (kb) windows across the chromosome, shows a clear break at

the putative origin of replication. The GC-skew values are uniformly negative from 0 to 450 kb (minus strand), and uniformly positive (plus strand) from 450 kb to the end of the chromosome (Fig. 2). Additional evidence for the location of the origin of replication comes from our discovery of an octamer, TTGTTTTT, whose skewed distribution in the plus versus the minus strand of the chromosome matches the GC skew (Fig. 2). The biological significance of this octamer has not yet been determined, although it may be analogous to the Chi site in *Escherichia coli* that is implicated in *recBCD* mediated recombination. No GC skew was observed in any of the plasmids, although the heptamer ATTTTTT displays a skewed distribution in the plus versus the minus strand of lp28-4 that changes at the approximate midpoint of the plasmid (not shown).

Transcription and translation

Genes encoding the three subunits (α , β , β') of the core RNA polymerase were identified in *B. burgdorferi* along with σ^{70} and two alternative σ factors, σ^{54} and *rpoS*. The role and specificity of each of these σ factors in transcription regulation in *B. burgdorferi* are not known. The *nusA*, *nusB* and *rho* genes, which are involved in transcription elongation and termination, were also identified.

A region of the genome with a significantly higher G + C content (43%), located between nucleotides 434,000 and 447,000, contains the rRNA operon. As previously reported, the rRNA operon in *B. burgdorferi* contains a 16S rRNA–Ala-tRNA–Ile-tRNA–23S rRNA–5S rRNA–23S rRNA–5S rRNA^{37,38}. All of the genes are present in the same orientation, except for that encoding Ile tRNA. Four unrelated genes, encoding 3-methyladenine glycosylase, hydrolyase and two with no database match, are also present in the rRNA operon. Three of these genes are transcribed in the same direction as the rRNAs.

We identified in the chromosome 31 tRNAs with specificity for all 20 amino acids (Fig. 1). These are organized into 7 clusters plus 13 single genes. All tRNA synthetases are present except glutamyl tRNA-synthetase. A single glutamyl tRNA synthetase probably aminoacylates both tRNA^{Glu} and tRNA^{Gln} with glutamate followed by transamidation by Glu-tRNA amidotransferase, a heterotrimeric enzyme present in *B. burgdorferi* and several Gram-positive bacteria and archaea³⁰. The lysyl-tRNA synthetase (LysS) in *B. burgdorferi* is a class I type that has no resemblance to any known bacterial or eukaryotic LysS, but is most similar to LysS from the archaea⁴⁰.

Replication, repair and recombination

The complement of genes in *B. burgdorferi* involved in DNA replication is smaller than in *E. coli*, but similar to that in *M. genitalium*²¹. Three ORFs have been identified with high homology to four of the ten polypeptides in the *E. coli* DNA polymerase III: α , β and γ , and τ . In *E. coli*, the γ and τ proteins are produced by programmed ribosomal frameshifting. This observation suggests that DNA replication in *B. burgdorferi*, like that in *M. genitalium*, is accomplished with a restricted set of genes. *B. burgdorferi* has one

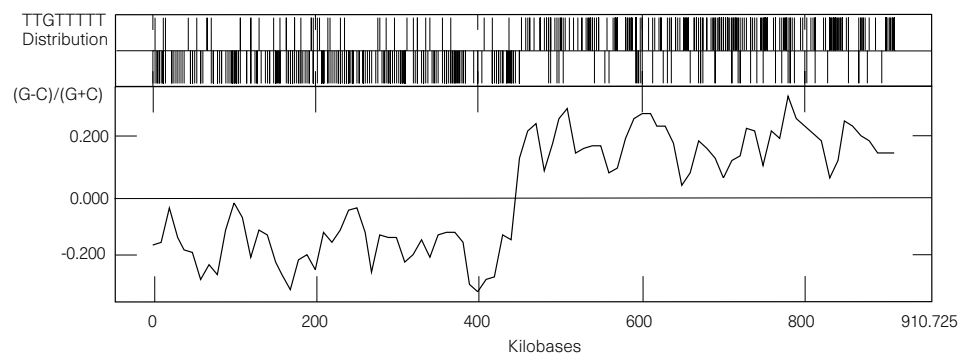


Figure 2 Distribution of TTGTTTTT and GC skew in the *B. burgdorferi* chromosome. Top, distribution of the octamer TTGTTTTT. The lines in the top panel represent the location of this octamer in the plus strand of the sequence, and those in the second panel represent the location of this oligomer in the minus strand of the sequence. Bottom, GC skew.

type I topoisomerase (*topA*) and two type II topoisomerases (gyrase and topoisomerase IV) for DNA topology management and chromosome segregation, despite its linear chromosomal structure. This suggests that topoisomerase IV may be required for more than the separation of circular DNAs during segregation.

The DNA repair mechanisms in *B. burgdorferi* are similar to those in *M. genitalium*. DNA excision repair can presumably occur by a pathway involving endonuclease III, PolI and DNA ligase. The genes for two of three DNA mismatch repair enzyme (*mutS*, *mutL*) are

present. The apparent absence of *mutH* is consistent with the lack of GATC (*dam*) methylation in strain B31 (S. Casjens, unpublished). Also present are genes for the repair of ultraviolet-induced DNA damage (*uvrA*, *uvrB*, *uvrC* and *uvrD*) (Table 2).

B. burgdorferi has a complete set of genes to perform homologous recombination, including *recA*, *recBCD*, *sbcC*, *sbcD*, *recG*, *ruvAB* and *recJ*. 3'-Exonuclease activity associated with *sbcB* in *E. coli* may be encoded by *exoA* (exodeoxynuclease III). Although *recA* is present, we found no evidence for *lexA*, which encodes the repressor that

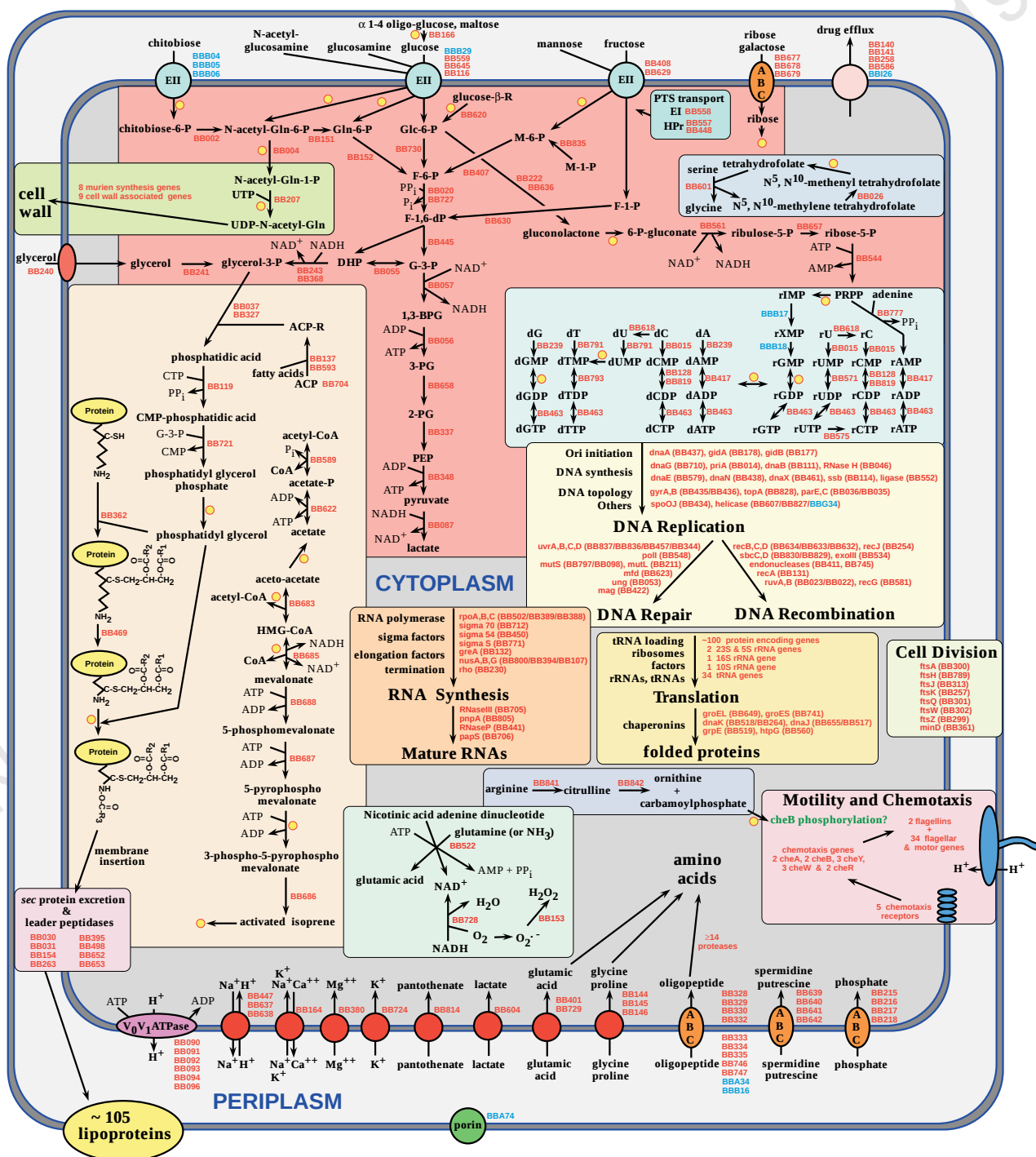


Figure 3 Solute transport and metabolic pathways in *B. burgdorferi*. A schematic diagram of a *B. burgdorferi* cell providing an integrated view of the transporters and the main components of the metabolism of this organism, as deduced from the genes identified in the genome. The ORF numbers correspond to those listed

in Table 2 (red indicates chromosomal and blue indicates plasmid ORFs). Presumed transporter specificity is indicated. Yellow circles indicate: places where particular uncertainties exist as to the substrate specificity, subcellular location or direction of catalysis: or expected activities that were not found.

regulates SOS genes in *E. coli*. No genes encoding DNA restriction or modification enzymes are present.

Biosynthetic pathways

The small genome size of *B. burgdorferi* is associated with an apparent absence of genes for the synthesis of amino acids, fatty acids, enzyme cofactors, and nucleotides, similar to that observed with *M. genitalium*²¹ (Fig. 3, Table 2). The lack of biosynthetic pathways explains why growth of *B. burgdorferi* *in vitro* requires serum-supplemented mammalian tissue-culture medium. This is also consistent with previous biochemical data indicating that *Borrelia* lack the ability to elongate long-chain fatty acids, such that the fatty-acid composition of *Borrelia* cells reflects that present in the growth medium⁶.

Transport

The linear chromosome of *B. burgdorferi* contains 46 ORFs and the plasmids contain 6 ORFs that encode transport and binding proteins (Fig. 3, Table 2). These gene products contribute to 16 distinct membrane transporters for amino acids, carbohydrates, anions and cations. The distribution of transporters between the four categories of functions in this section is similar to that observed in other heterotrophs (such as *Haemophilus influenzae*, *M. genitalium* and *H. pylori*), with most being dedicated to the import of organic compounds.

There are marked similarities between the transport capacity of *B.*

burgdorferi and *M. genitalium*. Both genomes have a limited number of recognizable transporters, so it is not clear how they can sustain diverse physiological reactions. Several of the identified transporters in both genomes exhibit broad substrate specificity, exemplified by the oligopeptide ABC transporter (*opp* operon) or the glycine, betaine, L-proline transport system (*proVWX*). Therefore, these organisms probably compensate for their restricted coding potential by producing proteins that can import a wide variety of solutes. This is important because *B. burgdorferi* is unable to synthesize any amino acids *de novo*. We were unable to identify any transport systems for nucleosides, nucleotides, NAD/NADH or fatty acids, although they are likely to be present.

Glucose, fructose, maltose and disaccharides seem to be acquired by the phosphoenolpyruvate:phosphotransferase system (PTS). The two nonspecific components, enzyme 1 (*ptsI*) and Hpr (*ptsH*), are associated in one operon with an apparently glucose-specific, phosphohistidine-sugar phosphotransferase enzyme IIA (*crr*). Separate from this operon are four permeases (enzyme IIBC), *fruA* in two copies (fructose), *ptsG* (glucose) and *malX* (glucose/maltose) (Fig. 3, Table 2). The fructose-specific enzyme IIA is induced in the ORF with IIBC (*fruA*), as has been observed in *M. genitalium*⁴¹. Ribose may be imported by an ATP-binding cassette transporter (*rbsAC*). The *rbsAC* genes are transcribed in an operon with a methyl-accepting chemotaxis protein that may respond to β -galactosides, suggesting that movement of the organisms towards sugars may be coupled to the transport process.

Energy metabolism

The limited metabolic capacity of *B. burgdorferi* is similar to that found in *M. genitalium* (Fig. 3, Table 2). Genes encoding all of the enzymes of the glycolytic pathway were identified. Analysis of the metabolic pathway suggests that *B. burgdorferi* uses glucose as a primary energy source, although other carbohydrates, including glycerol, glucosamine, fructose and maltose, may be used in glycolysis. Pyruvate produced by glycolysis is converted to lactate, consistent with the microaerophilic nature of *B. burgdorferi*. Generation of reducing power occurs through the oxidative branch of the pentose pathway. None of the genes encoding proteins of the tricarboxylic acid cycle or oxidative phosphorylation were identified. The similarity in metabolic strategies of two distantly related, obligate parasites, *M. genitalium* and *B. burgdorferi*, suggests convergent evolutionary gene loss from more metabolically competent, distant progenitors.

Addition of N-acetylglucosamine (NAG) to culture medium is required for growth of *B. burgdorferi*⁶. NAG is incorporated into the cell wall, and may also serve as an energy source. The cp26 plasmid encodes a PTS cellobiose transporter homologue that could have specificity for the structurally similar compound chitobiose (di-N-acetyl-D-glucosamine). A gene product on the chromosome with sequence similarity to chitobiose (BB2) may convert chitobiose to NAG. *B. burgdorferi* can metabolize NAG to fructose-6-phosphate, which then can enter the glycolytic cycle through the action of N-acetylglucosamine-6-phosphate deacetylase and glucosamine-6-phosphate isomerase. NAG is the primary constituent of chitin, which makes up the tick cuticle⁶, and may be a source of carbohydrate for *B. burgdorferi* when it is associated with its tick host.

The parallels between *B. burgdorferi* and *M. genitalium* appear to extend to other aspects of their metabolism. Both organisms lack a respiratory electron transport chain, so ATP production must be accomplished by substrate-level phosphorylation. Consequently, membrane potential is established by the reverse reaction of the V_1V_0 -type ATP synthase, here functioning as an ATPase to expel protons from the cytoplasm (Fig. 3, Table 2). The ATP synthase genes in *B. burgdorferi* appear to be transcribed as part of a seven-gene operon. They are not typical of those usually found in eubacteria, more closely resembling the eukaryotic vacuolar (V-type) and archaeal (A-type) H^+ -translocating ATPases⁴², both in size

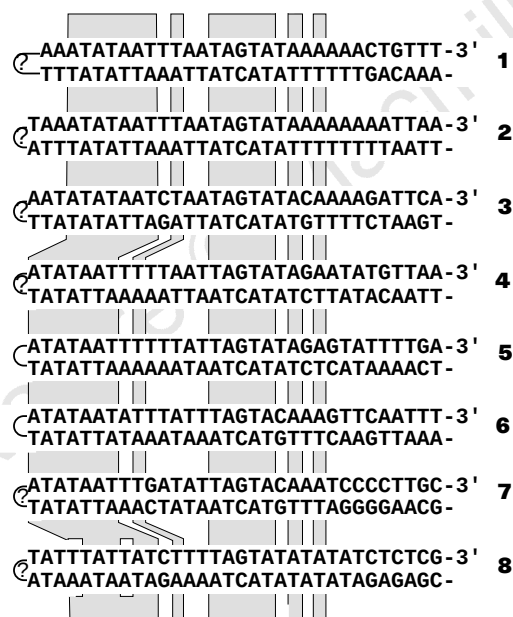


Figure 4 Telomere nucleotide sequences from *Borrelia* species. Nucleotide sequences are shown for known *Borrelia* telomeres as indicated: 1, *B. burgdorferi* Sh-2-82 chromosome left end; 2, *B. burgdorferi* B31 chromosome left end; 3, *B. afzelii* R-IP3 chromosome right end; 4, *B. burgdorferi* B31 chromosome right end; 5, *B. burgdorferi* B31 plasmid Ip17 left end; 6, *B. burgdorferi* B31 plasmid Ip17 right end; 7, *B. hermsii* plasmids bp7E and pb21E right ends; 8, *B. burgdorferi* B31 plasmid Ip28-1 right end. In each case the telomere is at the left. Question marks (?) indicate locations where S1 nuclease was used to open terminal hairpins during the sequence determinations. Stippled areas highlight regions that appear to have been most highly conserved among these telomeres; no strong sequence conservation has been found near the right of the terminal 26 bp among the different sequences listed, except between the chromosomal left ends from strains B31 and Sh-2-82 (see text). The telomeric sequences of the strain B31 chromosome were determined in this report; the others are from references 14, 28, 30, 45, 46.

and sequence similarity, than the bacterial F_1F_0 ATPases. Genome analysis of *Treponema pallidum*, the pathogenic spirochaete that causes syphilis, has also revealed the presence of a V_1V_0 -type ATP synthase (C. M. F. *et al.*, manuscript in preparation), suggesting that this may be a feature of spirochaetes.

Regulatory systems

Although the expression of *Borrelia* genes varies according to the current host species, temperature, host body location and other local factors, control of gene expression appears to differ from more well studied eubacteria. A typical set of homologues of heat-shock response genes is present (*groES*, *groEL*, *grpE*, *dnaJ*, *hslU*, *hslV*, *dnaK* and *htpG*), and *B. burgdorferi* is known to have such a response; however, it lacks the σ -32 that controls their transcription in *E. coli*. Only a few homologues to other eubacterial regulatory proteins are present, including only two response-regulator two-component systems.

Motility and chemotaxis

Like other spirochaetes, *B. burgdorferi* has periplasmic flagella that are inserted at each end of the cell and extend towards the middle of the cell body. The unique flagella allow the organism to move through viscous solutions, an ability that is presumed to be important in its migration to distant tissues following deposition in the skin layers⁴³. Proteins involved in motility and chemotaxis are encoded by 54 genes, more than 6% of the *B. burgdorferi* chromosome, most of which are arranged in eight operons containing between 2 and 25 genes.

B. burgdorferi contains several copies of the chemotaxis genes (*cheR*, *cheW*, *cheA*, *cheY* and *cheB*) downstream of the methyl-accepting chemotaxis proteins. Other eubacteria also have duplications of some *che* genes, but those genes in *B. burgdorferi* are the most redundant set yet found. *B. burgdorferi* lacks recognizable virulence factors; thus, its ability to migrate to distant sites in the tick and mammalian host is probably dependent on a robust chemotaxis response. Multiple chemotaxis genes may provide redundancy in this system in order to meet such challenges or, alternatively, these genes may be differentially expressed under varied physiological conditions. Another speculative possibility is that the flagellar motors at the two ends of the *B. burgdorferi* cell are different and require different *che* systems. In support of this idea is the observation that one of the motor switch genes, *fliG*, is also present in two copies.

Membrane protein analysis

Much of the previous work on *B. burgdorferi* has focused on outer-surface membrane genes because of their potential importance in bacterial detection and vaccination. Nearly all *Borrelia* membrane proteins have been found to be typical bacterial lipoproteins. A search of *B. burgdorferi* ORFs for a consensus lipobox in the first 30 amino acids identified 105 putative lipoproteins, representing more than 8% of coding sequences. This contrasts with a total of only 20 putative lipoproteins in the 1.67-million base pair *H. pylori* genome (1.3% of coding sequences)²³. The periplasmic binding proteins involved in transport of amino acids/peptides and phosphate in *B. burgdorferi* are candidate lipoproteins, suggesting that they may be anchored to the outer surface of the cytoplasmic membrane as in Gram-positive bacteria, rather than localized in the periplasmic space.

In better-characterized eubacteria, prolipoprotein diacylglycerol transferase (*lgt*), prolipoprotein signal peptidase (*lsp*), and apolipoprotein:phospholipid *N*-acyl transferase (*lnt*) are required for post-translational processing and addition of lipids to the amino-terminal cysteine. Genes for the first two of the enzymes (*lgt* and *lsp*) are present in the *B. burgdorferi* genome, but the gene for *lnt* was not identified, although biochemical evidence argues for all three activities in *B. burgdorferi*⁴⁴. The sequence similarity of an *lnt*

homologue in *B. burgdorferi* may be too low to be identified using our search methods, or its activity may be present in a new enzyme. In *E. coli* the Sec protein export system moves lipoproteins through the inner membrane, and *Borrelia* carries a complete set of these protein-secretion gene homologues (*secA/D/E/F/Y* and *tth*; only the non-essential *secB* is missing).

Analysis of telomeres

The two chromosomal telomeres of strain B31 have similar 26-bp inverted terminal sequences (Fig. 4). We found no other similarity between the two ends, and these 26-bp sequences are very similar to the previously characterized *Borrelia* telomeres. Terminal restriction fragments from both B31 chromosomal termini were shown to exhibit snapback kinetics (data not shown), strongly indicating that both terminate in covalently closed hairpins, like previously characterized *Borrelia* telomeres^{28,45,46}.

The left chromosomal telomere of strain B31 is identical to the previously characterized left telomere of strain Sh-2-82 (ref. 28), except for a 31 bp insertion in B31 26 bp from the end. The right-most 7,454 bp contains surprisingly few ORFs, given the ORF density elsewhere on the chromosome. The function of this region is unknown, but it contains several unusual features. The right terminal 900 bp contains considerable homology to the left ends of lp17 and lp28-3. The region between 3,600 bp and 8,000 bp from the right end also contains several areas with similarity to plasmid sequences, including a portion of the transposase-like gene approximately 4,500 bp from the right end. The spacing between the two conserved motifs (ATATAAT and TAGTATA) in the right 26-bp terminal repeat is the same as most previously known plasmid telomeres but different from the previously known chromosomal telomeres. These findings support the idea that the right end of the *Borrelia* chromosome has historically exchanged telomeres with the linear plasmids²⁸.

Conclusions

The *B. burgdorferi* genome sequence will provide a new starting point for the study of the pathogenesis, prevention and treatment of Lyme disease. With the exception of a small number of putative virulence genes (haemolysins and drug-efflux proteins), this organism contains few, if any, recognizable genes involved in virulence or host-parasite interactions, suggesting that *B. burgdorferi* differs from better-studied eubacteria in this regard. It will be interesting to determine the role of the multi-copy plasmid-encoded genes, as previous work has implicated plasmid genes in infectivity and virulence. The completion of the genome sequence from a second spirochaete, *Treponema pallidum* (C.M.F. *et al.*, manuscript in preparation) will allow for the identification of genes specific to each species and to this bacterial phylum, and will provide further insight into prokaryotic diversity. □

Methods

Cell lines. A portion of a low-passage subculture of the original Lyme-disease spirochaete tick isolate⁴ was obtained from A. Barbour. The type strain of *B. burgdorferi* (ATCC 35210)³, B31, was derived from this isolate by limiting dilution cloning⁵. Cells were grown in Barbour-Stoenner-Kelly medium II (BSKII)⁶, omitting the additions of antibiotics and gelatin, in tightly closed containers at 33–34 °C. Cells were subcultured three or fewer times *in vitro* between successive rounds of infection in C3H/HeJ mice to minimize loss of infectivity and plasmid content^{17,18}. After four successive transfers of infection in mice, a primary culture of B31, established from infected ear tissue, was expanded to 2.5 l by four successive subcultures. All available evidence indicates that the B31 line used for preparation of genomic DNA was probably clonal, as genetic heterogeneity was undetectable by several criteria including macrorestriction analysis (S. Casjens, unpublished data) and plasmid analysis of clonal derivatives of the B31 line¹³.

Sequencing. The *B. burgdorferi* genome was sequenced by a whole-genome random sequencing method previously applied to other microbial genomes^{20–24}.

An approximately 7.5-fold genome coverage was achieved by generating 19,078 sequences from a small insert plasmid library with an average edited length of 505 bases. The ends of 69 large insert lambda clones were sequenced to obtain a genome scaffold; 50% of the genome was covered by at least one lambda clone. Sequences were assembled using TIGR Assembler as described^{20–24}, resulting in a total of 524 assemblies containing at least two sequences, which were clustered into 85 groups based on linking information from forward and reverse sequence reads. All *Borrelia* sequences that had been mapped were searched against the assemblies in an attempt to delineate which were derived from the various elements of the *B. burgdorferi* genome. Some contigs were also located on the existing physical map by Southern analysis. Sequence and physical gaps for the chromosome were closed as described^{20–24}. At the completion of the project, less than 3% of the chromosome had single-fold coverage. The linear chromosome of *B. burgdorferi* has covalently closed hairpin structures at its termini that are similar to those reported for linear plasmids in this organism¹¹. The telomeric sequences (106 and 72 bp, respectively, from the left and right ends) were obtained after nicking the terminal loop with S1 nuclease and amplifying terminal sequences by ligation-mediated polymerase chain reaction (PCR) as described²⁸. The unknown terminal sequence was determined in both directions on four independent plasmid clones of the amplified DNA from each telomere. A minimum amount of S1 nuclease was used and, because of their sequence similarity to other *Borrelia* telomeres, it is likely that few, if any, nucleotides were lost from the B31 chromosomal telomeres in this process.

Identification of ORFs. Coding regions (ORFs) were identified using compositional analysis using an interpolated Markov model based on variable-length oligomers⁴⁷. ORFs of >600 bp were used to train the Markov model, as well as *B. burgdorferi* ORFs from GenBank. Once trained, the model was applied to the complete *B. burgdorferi* genome sequence and identified 953 candidate ORFs. ORFs that overlapped were visually inspected, and in some cases removed. Non-overlapping ORFs that were found between predicted coding regions and >30 amino acids in length were retained and included in the final annotation. All putative ORFs were searched against a non-redundant amino-acid database as described^{20–24}. ORFs were also analysed using 527 hidden Markov models constructed for several conserved protein families (PFAM v2.0) using HMMER⁴⁸. Families of paralogous genes were constructed by pairwise searches of proteins using FASTA. Matches that spanned at least 60% of the smaller of the protein pair were retained and visually inspected. A total of 94 paralogous gene families containing 293 genes were identified (Fig. 1).

Identification of membrane-spanning domains (MSDs). TopPred⁴⁹ was used to identify potential MSDs in proteins. A total of 526 proteins containing at least one putative MSD were identified, of which 183 were predicted to have more than one MSD. The presence of signal peptides and the probable position of a cleavage site in secreted proteins were detected using Signal-P as described²³; 189 proteins were predicted to have a signal peptide. Lipoproteins were identified by scanning for a lipobox in the first 30 amino acids of every protein. A consensus sequence relaxed from that used for *H. pylori*²³ was defined for the purpose of this search based on known or putative *B. burgdorferi* lipoprotein consensus sequences.

Received 3 November; accepted 18 November 1997.

- Steere, A. C. et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum.* **20**, 7 (1977).
- Steere, A. C. Lyme disease. *New Engl. J. Med.* **321**, 586–596 (1989).
- Johnson, R. C., Schmidt, G. P., Hyde, F. W., Steigerwalt, A. G. & Brenner, D. J. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int. J. Syst. Bacteriol.* **34**, 496–497 (1984).
- Burgdorfer, W. et al. Lyme disease—a tick-borne spirochetosis? *Science* **216**, 1317–1319 (1982).
- Barbour, A. G., Burgdorfer, W., Hayes, S. F., Peter, O. & Aeschlimann, A. Isolation of a cultivable spirochete from *Ixodes ricinus* ticks of Switzerland. *Curr. Microbiol.* **8**, 123–126 (1983).
- Barbour, A. & Hayes, S. F. Biology of *Borrelia* species. *Microbiol. Rev.* **50**, 381–400 (1986).
- Baril, C., Richaud, C., Baranton, G. & Saint Girons, I. Linear chromosome of *Borrelia burgdorferi*. *Res. Microbiol.* **140**, 507–516 (1989).
- Ferdows, M. S. & Barbour, A. G. Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. *Proc. Natl Acad. Sci. USA* **86**, 5969–5973 (1989).
- Davidson, B., MacDougall, J. & Saint Girons, I. Physical map of the linear chromosome of the bacterium *Borrelia burgdorferi* 212, a causative agent of Lyme disease and localization of rRNA genes. *J. Bacteriol.* **174**, 3766–3774 (1992).
- Casjens, S. & Huang, W. M. Linear chromosome physical and genetic map of *Borrelia burgdorferi*, the Lyme disease agent. *Mol. Microbiol.* **8**, 967–980 (1993).
- Barbour, A. G. & Garon, C. F. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. *Science* **237**, 409–411 (1987).
- Barbour, A. G. Plasmid analysis of *Borrelia burgdorferi*, the Lyme disease agent. *J. Clin. Microbiol.* **26**, 475–478 (1988).

- Casjens, S., van Vugt, R., Stevenson, B., Tilly, K. & Rosa, P. Homology throughout the multiple 32-kilobase circular plasmids present in Lyme disease spirochetes. *J. Bacteriol.* **171**, 217–227 (1997).
- Hinnebusch, J. & Barbour, A. G. Linear- and circular-plasmid copy numbers in *Borrelia burgdorferi*. *J. Bacteriol.* **174**, 5251–5257 (1992).
- Barbour, A. G., Carter, C. J., Bundoc, V. & Hinnebusch, J. The nucleotide sequence of a linear plasmid of *Borrelia burgdorferi* reveals similarities to those of circular plasmids of other prokaryotes. *J. Bacteriol.* **178**, 6625–6639 (1996).
- Zuckert, W. R. & Meyer, J. Circular and linear plasmids of Lyme disease spirochetes share extensive homology: characterization of a repeated DNA element. *J. Bacteriol.* **178**, 2287–2298 (1996).
- Schwan, T. G., Burgdorfer, W. & Garon, C. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of *in vitro* cultivation. *Infect. Immun.* **56**, 1831–1836 (1988).
- Xu, Y., Kodner, C., Coleman, L. & Johnson, R. C. Correlation of plasmids with infectivity of *Borrelia burgdorferi sensu stricto* type strain B31. *Infect. Immun.* **64**, 3870–3876 (1996).
- Norris, S. J., Howell, J. K., Garza, S. A., Ferdows, M. S. & Barbour, A. G. High- and low-infectivity phenotypes of clonal populations of *in vitro*-cultured *Borrelia burgdorferi*. *Infect. Immun.* **63**, 2206–2212 (1995).
- Fleischmann, R. D. et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**, 496–512 (1995).
- Fraser, C. M. et al. The minimal gene complement of *Mycoplasma genitalium*. *Science* **270**, 397–403 (1995).
- Bult, C. J. et al. Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273**, 1058–1072 (1996).
- Tomb, J.-F. et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**, 539–547 (1997).
- Klenk, H.-P. et al. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* **390**, 364–370 (1997).
- Casjens, S., Ley, H., DeLange, M., Rosa, P. & Huang, W. Linear chromosomes of Lyme disease agent spirochetes: genetic diversity and conservation of gene order. *J. Bacteriol.* **177**, 2769–2780 (1995).
- Casjens, S. & Huang, W. In *Bacterial Genomes: Physical Structure and Analysis* (eds de Bruijn, F., Lupski, J. & Weinstein, G. J.) (Chapman and Hall, New York, in the press).
- Old, I., MacDougall, J., Saint Girons, I. & Davidson, B. Mapping of genes on the linear chromosome of the bacterium *Borrelia burgdorferi*: possible locations for its origin of replication. *FEMS Microbiol. Lett.* **99**, 245–250 (1992).
- Casjens, S. et al. Telomeres of the linear chromosomes of Lyme disease spirochetes: nucleotide sequence and possible exchange with linear plasmid telomers. *Mol. Microbiol.* **26**, 581–596 (1997).
- Riley, M. Functions of gene products of *Escherichia coli*. *Microbiol. Rev.* **57**, 862–952 (1993).
- Zhang, J., Hardham, J., Barbour, A. & Norris, S. Antigenic variation in Lyme disease *Borreliae* by promiscuous recombination of VMP-like sequence cassettes. *Cell* **87**, 275–285 (1997).
- Dunn, J. J. et al. Complete nucleotide sequence of a circular plasmid from the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Bacteriol.* **176**, 2706–2717 (1994).
- Traktman, P. In *DNA Replication in Eukaryotic Cells* (ed. DePamphilis, M.) 775–798 (Cold Spring Harbor Laboratory Press, NY, 1996).
- Himmelreich, R. et al. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res.* **24**, 4420–4449 (1996).
- Brewer, B. When polymerases collide: replication and the transcriptional organization of the *E. coli* chromosome. *Cell* **53**, 679–686 (1988).
- French, S. Consequences of replication fork movement through transcription units *in vivo*. *Science* **258**, 1362–1365 (1992).
- Ogasawara, N. & Yoshikawa, H. Genes and their organization in the replication origin region of the bacterial chromosome. *Mol. Microbiol.* **6**, 629–634 (1992).
- Gazumyan, A., Schwartz, J. J., Liveris, D. & Schwartz, I. Sequence analysis of the ribosomal RNA operon of the Lyme disease spirochete, *Borrelia burgdorferi*. *Gene* **146**, 57–65 (1994).
- Ojaimi, C., Davidson, B., Saint Girons, I. & Old, I. Conservation of gene arrangement and an unusual organization of rRNA genes in the linear chromosomes of Lyme disease spirochaetes *Borrelia burgdorferi*, *B. garinii* and *B. afzelii*. *Microbiology* **140**, 2931–2940 (1994).
- Cumow, A. W. et al. Glu-tRNA^{Gln} amidotransferase: a novel heterotrimeric enzyme required for correct decoding of glutamine codons during translation. *Proc. Natl Acad. Sci. USA* **94**, 11819–11826 (1997).
- Ibba, M., Bobo, J. L., Rosa, P. A. & Soll, D. Archaeal-type lysyl-tRNA synthetase in the Lyme disease spirochete *Borrelia burgdorferi*. *Proc. Natl Acad. Sci. USA* (in the press).
- Reizer, J., Paulsen, I. T., Reizer, A., Titzmeyer, F. & Saier, M. H. Jr Novel phosphotransferase system genes revealed by bacterial genome analysis: The complete complement of *pts* genes in *Mycoplasma genitalium*. *Microb. Comp. Genom.* **1**, 151–164 (1996).
- Takase, K. et al. Sequencing and characterization of the *ntp* gene cluster for vacuolar-type Na⁺-translocating ATPase of *Enterococcus hirae*. *J. Biol. Chem.* **269**, 11037–11044 (1994).
- Sadziane, A., Rosa, P. A., Thompson, P. A., Hogan, D. M. & Barbour, A. G. Antibody-resistant mutants of *Borrelia burgdorferi*: *in vitro* selection and characterization. *J. Exp. Med.* **176**, 799–809 (1992).
- Brandt, M. E., Riley, B. S., Radolf, J. D. & Norgard, M. V. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. *Infect. Immun.* **58**, 983–991 (1990).
- Hinnebusch, J., Bergstrom, S. & Barbour, A. Cloning and sequence analysis of linear plasmid telomeres of the bacterium *Borrelia burgdorferi*. *Mol. Microbiol.* **4**, 811–820 (1990).
- Kitten, T. & Barbour, A. Juxtaposition of expressed variable antigen genes with a conserved telomere in the bacterium *Borrelia hermsii*. *Proc. Natl Acad. Sci. USA* **87**, 6077–6081 (1990).
- Salzberg, S., Delcher, A., Kasif, S. & White, O. Microbial gene identification using interpolated Markov models. *Nucleic Acids Res.* (in the press).
- Sonnhammer, E. L. L., Eddy, S. R. & Durbin, R. Pfam: a comprehensive database of protein families based on seed alignments. *Proteins* **28**, 405–420 (1997).
- Claros, M. G. & von Heijne, G. TopPred II: an improved software for membrane proteins structure predictions. *Comput. Appl. Biosci.* **10**, 685–686 (1994).

Acknowledgements. We thank A. G. Barbour for isolation of the *Borrelia burgdorferi* strain; A. Barbour, P. Rosa, K. Tilly, J. Riberio, B. Stevenson and D. Soll for discussions; N. K. Patel for technical assistance; M. Heaney, J. Scott and A. Saeed for software and database support; and V. Sapito, B. Vincent and D. Maas for computer system support. This work was supported by a grant to J.C.V. and C.M.F. from the G. Harold and Leila Y. Mathers Charitable Foundation.

Correspondence and requests for materials should be sent to C.M.F. (e-mail: gbb@tigr.org). The annotated genome sequence and gene family alignments are available on the World-Wide Web at <http://www.tigr.org/tldb/mdb/bbdb/bbdb.html>. Sequences have been deposited with GenBank under the following accession numbers: AE00783 (chromosome); AE00784 (lp28-3); AE00785 (lp25); AE00786 (lp28-2); AE00787 (lp38); AE00788 (lp36); AE00789 (lp28-4); AE00790 (lp54); AE00791 (cp9); AE00792 (cp26); AE00793 (lp17); and AE00794 (lp28-1).



Table 2. Identification of *E. burgdorferi* genes

BB#	Identification (Species)	Genes	Accession	Function	BB#	Identification (Species)	Genes	Accession	Function
Animal and Microbial									
Swine family									
BB01	swine CHMTase (glyA) (Eo)	73	BBH20	outer membrane porin (omae%) prt (Eb)	74	BB291	tig basal-body rod prt (flF) (Eb)	100	
Biosynthesis of cofactors, prosthetic groups, and cofactors									
Folate									
BB02	methyltetrahydrofolate DHase (fdD) (Es)	61	BBE09	prt p23 (Eb)	93	BB292	tig basal-body rod prt (flG) (Eb)	100	
Heme and porphyrin									
BB197	protoporphyrinogen oxidase prt (Es)	58	BBE06	exported prt A (appA) (Eb)	100	BB293	tig basal-body rod prt (flH) (Eb)	100	
BB658	oxygen-independent coproporphyrinogen II oxidase prt (Es)	51	BBE07	outer surface prt C (ospC) (Eb)	100	BB294	tig basal-body rod prt (flI) (Eb)	100	
Menadiquinone and ubiquinone									
BB314	coenzyme Q9 synthase (cpB) (Eo)	57	BBE08	outer surface prt D (ospD) (Eb)	46	BB295	tig basal-body rod prt (flJ) (Eb)	100	
Pantoic acid									
BB312	pantoic acid methyltransferase prt (Eb)	53	BBE09	outer surface prt E (ospE) (Eb)	46	BB296	tig basal-body rod prt (flK) (Eb)	100	
Pyridoxine									
BB768	pyridoxal kinase (pdxK) (Sc)	47	BBE10	outer surface prt F (ospF) (Eb)	46	BB297	tig basal-body rod prt (flL) (Eb)	100	
Thiamine									
BB21	4-methyl-5-(b-oxoethyl)-thiazole monophosphate prt (Hs)	53	BBE11	outer surface prt G (ospG) (Eb)	46	BB298	tig basal-body rod prt (flM) (Eb)	100	
Pyridine nucleotides									
BB522	NH ₄ ⁺ -dependent NAD ⁺ synthase (Fol)	65	BBE12	outer surface prt H (ospH) (Eb)	46	BB299	tig basal-body rod prt (flN) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE13	outer surface prt I (ospI) (Eb)	46	BB300	tig basal-body rod prt (flO) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE14	outer surface prt J (ospJ) (Eb)	46	BB301	tig basal-body rod prt (flP) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE15	outer surface prt K (ospK) (Eb)	46	BB302	tig basal-body rod prt (flQ) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE16	outer surface prt L (ospL) (Eb)	46	BB303	tig basal-body rod prt (flR) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE17	outer surface prt M (ospM) (Eb)	46	BB304	tig basal-body rod prt (flS) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE18	outer surface prt N (ospN) (Eb)	46	BB305	tig basal-body rod prt (flT) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE19	outer surface prt O (ospO) (Eb)	46	BB306	tig basal-body rod prt (flU) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE20	outer surface prt P (ospP) (Eb)	46	BB307	tig basal-body rod prt (flV) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE21	outer surface prt Q (ospQ) (Eb)	46	BB308	tig basal-body rod prt (flW) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE22	outer surface prt R (ospR) (Eb)	46	BB309	tig basal-body rod prt (flX) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE23	outer surface prt S (ospS) (Eb)	46	BB310	tig basal-body rod prt (flY) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE24	outer surface prt T (ospT) (Eb)	46	BB311	tig basal-body rod prt (flZ) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE25	outer surface prt U (ospU) (Eb)	46	BB312	tig basal-body rod prt (flA) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE26	outer surface prt V (ospV) (Eb)	46	BB313	tig basal-body rod prt (flB) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE27	outer surface prt W (ospW) (Eb)	46	BB314	tig basal-body rod prt (flC) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE28	outer surface prt X (ospX) (Eb)	46	BB315	tig basal-body rod prt (flD) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE29	outer surface prt Y (ospY) (Eb)	46	BB316	tig basal-body rod prt (flE) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE30	outer surface prt Z (ospZ) (Eb)	46	BB317	tig basal-body rod prt (flF) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE31	outer surface prt A (ospA) (Eb)	46	BB318	tig basal-body rod prt (flG) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE32	outer surface prt B (ospB) (Eb)	46	BB319	tig basal-body rod prt (flH) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE33	outer surface prt C (ospC) (Eb)	46	BB320	tig basal-body rod prt (flI) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE34	outer surface prt D (ospD) (Eb)	46	BB321	tig basal-body rod prt (flJ) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE35	outer surface prt E (ospE) (Eb)	46	BB322	tig basal-body rod prt (flK) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE36	outer surface prt F (ospF) (Eb)	46	BB323	tig basal-body rod prt (flL) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE37	outer surface prt G (ospG) (Eb)	46	BB324	tig basal-body rod prt (flM) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE38	outer surface prt H (ospH) (Eb)	46	BB325	tig basal-body rod prt (flN) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE39	outer surface prt I (ospI) (Eb)	46	BB326	tig basal-body rod prt (flO) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE40	outer surface prt J (ospJ) (Eb)	46	BB327	tig basal-body rod prt (flP) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE41	outer surface prt K (ospK) (Eb)	46	BB328	tig basal-body rod prt (flQ) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE42	outer surface prt L (ospL) (Eb)	46	BB329	tig basal-body rod prt (flR) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE43	outer surface prt M (ospM) (Eb)	46	BB330	tig basal-body rod prt (flS) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE44	outer surface prt N (ospN) (Eb)	46	BB331	tig basal-body rod prt (flT) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE45	outer surface prt O (ospO) (Eb)	46	BB332	tig basal-body rod prt (flU) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE46	outer surface prt P (ospP) (Eb)	46	BB333	tig basal-body rod prt (flV) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE47	outer surface prt Q (ospQ) (Eb)	46	BB334	tig basal-body rod prt (flW) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE48	outer surface prt R (ospR) (Eb)	46	BB335	tig basal-body rod prt (flX) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE49	outer surface prt S (ospS) (Eb)	46	BB336	tig basal-body rod prt (flY) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE50	outer surface prt T (ospT) (Eb)	46	BB337	tig basal-body rod prt (flZ) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE51	outer surface prt U (ospU) (Eb)	46	BB338	tig basal-body rod prt (flA) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE52	outer surface prt V (ospV) (Eb)	46	BB339	tig basal-body rod prt (flB) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE53	outer surface prt W (ospW) (Eb)	46	BB340	tig basal-body rod prt (flC) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE54	outer surface prt X (ospX) (Eb)	46	BB341	tig basal-body rod prt (flD) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE55	outer surface prt Y (ospY) (Eb)	46	BB342	tig basal-body rod prt (flE) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE56	outer surface prt Z (ospZ) (Eb)	46	BB343	tig basal-body rod prt (flF) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE57	outer surface prt A (ospA) (Eb)	46	BB344	tig basal-body rod prt (flG) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE58	outer surface prt B (ospB) (Eb)	46	BB345	tig basal-body rod prt (flH) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE59	outer surface prt C (ospC) (Eb)	46	BB346	tig basal-body rod prt (flI) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE60	outer surface prt D (ospD) (Eb)	46	BB347	tig basal-body rod prt (flJ) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE61	outer surface prt E (ospE) (Eb)	46	BB348	tig basal-body rod prt (flK) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE62	outer surface prt F (ospF) (Eb)	46	BB349	tig basal-body rod prt (flL) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE63	outer surface prt G (ospG) (Eb)	46	BB350	tig basal-body rod prt (flM) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE64	outer surface prt H (ospH) (Eb)	46	BB351	tig basal-body rod prt (flN) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE65	outer surface prt I (ospI) (Eb)	46	BB352	tig basal-body rod prt (flO) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE66	outer surface prt J (ospJ) (Eb)	46	BB353	tig basal-body rod prt (flP) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE67	outer surface prt K (ospK) (Eb)	46	BB354	tig basal-body rod prt (flQ) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE68	outer surface prt L (ospL) (Eb)	46	BB355	tig basal-body rod prt (flR) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE69	outer surface prt M (ospM) (Eb)	46	BB356	tig basal-body rod prt (flS) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE70	outer surface prt N (ospN) (Eb)	46	BB357	tig basal-body rod prt (flT) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE71	outer surface prt O (ospO) (Eb)	46	BB358	tig basal-body rod prt (flU) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE72	outer surface prt P (ospP) (Eb)	46	BB359	tig basal-body rod prt (flV) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE73	outer surface prt Q (ospQ) (Eb)	46	BB360	tig basal-body rod prt (flW) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE74	outer surface prt R (ospR) (Eb)	46	BB361	tig basal-body rod prt (flX) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE75	outer surface prt S (ospS) (Eb)	46	BB362	tig basal-body rod prt (flY) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE76	outer surface prt T (ospT) (Eb)	46	BB363	tig basal-body rod prt (flZ) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE77	outer surface prt U (ospU) (Eb)	46	BB364	tig basal-body rod prt (flA) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE78	outer surface prt V (ospV) (Eb)	46	BB365	tig basal-body rod prt (flB) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE79	outer surface prt W (ospW) (Eb)	46	BB366	tig basal-body rod prt (flC) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE80	outer surface prt X (ospX) (Eb)	46	BB367	tig basal-body rod prt (flD) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE81	outer surface prt Y (ospY) (Eb)	46	BB368	tig basal-body rod prt (flE) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE82	outer surface prt Z (ospZ) (Eb)	46	BB369	tig basal-body rod prt (flF) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE83	outer surface prt A (ospA) (Eb)	46	BB370	tig basal-body rod prt (flG) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE84	outer surface prt B (ospB) (Eb)	46	BB371	tig basal-body rod prt (flH) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE85	outer surface prt C (ospC) (Eb)	46	BB372	tig basal-body rod prt (flI) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE86	outer surface prt D (ospD) (Eb)	46	BB373	tig basal-body rod prt (flJ) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE87	outer surface prt E (ospE) (Eb)	46	BB374	tig basal-body rod prt (flK) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE88	outer surface prt F (ospF) (Eb)	46	BB375	tig basal-body rod prt (flL) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE89	outer surface prt G (ospG) (Eb)	46	BB376	tig basal-body rod prt (flM) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE90	outer surface prt H (ospH) (Eb)	46	BB377	tig basal-body rod prt (flN) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE91	outer surface prt I (ospI) (Eb)	46	BB378	tig basal-body rod prt (flO) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE92	outer surface prt J (ospJ) (Eb)	46	BB379	tig basal-body rod prt (flP) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE93	outer surface prt K (ospK) (Eb)	46	BB380	tig basal-body rod prt (flQ) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE94	outer surface prt L (ospL) (Eb)	46	BB381	tig basal-body rod prt (flR) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE95	outer surface prt M (ospM) (Eb)	46	BB382	tig basal-body rod prt (flS) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE96	outer surface prt N (ospN) (Eb)	46	BB383	tig basal-body rod prt (flT) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE97	outer surface prt O (ospO) (Eb)	46	BB384	tig basal-body rod prt (flU) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE98	outer surface prt P (ospP) (Eb)	46	BB385	tig basal-body rod prt (flV) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE99	outer surface prt Q (ospQ) (Eb)	46	BB386	tig basal-body rod prt (flW) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE100	outer surface prt R (ospR) (Eb)	46	BB387	tig basal-body rod prt (flX) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE101	outer surface prt S (ospS) (Eb)	46	BB388	tig basal-body rod prt (flY) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE102	outer surface prt T (ospT) (Eb)	46	BB389	tig basal-body rod prt (flZ) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE103	outer surface prt U (ospU) (Eb)	46	BB390	tig basal-body rod prt (flA) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE104	outer surface prt V (ospV) (Eb)	46	BB391	tig basal-body rod prt (flB) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE105	outer surface prt W (ospW) (Eb)	46	BB392	tig basal-body rod prt (flC) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE106	outer surface prt X (ospX) (Eb)	46	BB393	tig basal-body rod prt (flD) (Eb)	100	
BB10	surface-associated membrane prt 1 (bnp1) (Hi)	46	BBE107	outer surface prt Y (ospY) (Eb)	46	BB394	tig basal-body rod prt (flE) (Eb)	100	
BB153	S2 prt (Eb)	60	BBE108	outer surface prt Z (ospZ) (Eb)	46	BB395	tig basal-body rod prt (flF) (Eb)	100	
Cell envelope									
Membranes, lipoproteins, and porins									
BB332	basal membrane prt B (bnpB) (Eb)	100	BBE109	outer surface prt A (ospA) (Eb)	46	BB396	tig basal-body rod prt (flG) (Eb)	100	
BB333	basal membrane prt A (bnpA) (Eb)	100	BBE110	outer surface prt B (ospB) (Eb)	46	BB397	tig basal-body rod prt (flH) (Eb)	100	
BB334	basal membrane prt C (bnpC) (Eb)	100	BBE111	outer surface prt C (ospC) (Eb)	46	BB398	tig basal-body rod prt (flI) (Eb)	100	
BB335	basal membrane prt D (bnpD) (Eb)	100	BBE112	outer surface prt D (ospD) (Eb)	46	BB399	tig basal-body rod prt (flJ) (Eb)	100	
BB108	basal membrane prt (Tp)	50	BBE113	outer surface prt E (ospE) (Eb)	46	BB400	tig basal-body rod prt (flK) (Eb)	100	
BB337	transmembrane prt (Tp)	50	BBE114	outer surface prt F (ospF) (Eb)	46	BB401	tig basal-body rod prt (flL) (Eb)	100	
BB347	transmembrane prt (Tp)	53	BBE115	outer surface prt G (ospG) (Eb)	46	BB402	tig basal-body rod prt (flM) (Eb)	100	
BB442	inner membrane prt (Hi)	63	BBE116	outer surface prt H (ospH) (Eb)	46	BB403	tig basal-body rod prt (flN) (Eb)	100	
BB345	lipoprotein Lx7 (Eb)	100	BBE117	outer surface prt I (ospI) (Eb)	46	BB404	tig basal-body rod prt (flO) (Eb)	100	
BB603	membrane-associated prt p23 (Eb)	100	BBE118	outer surface prt J (ospJ) (Eb)	46	BB405	tig basal-body rod prt (flP) (Eb)	100	
BB753	membrane opening prt prt (Sc)	40	BBE119	outer surface prt K (ospK) (Eb)	46	BB406	tig basal-body rod prt (flQ) (Eb)	100	
BB755	outer membrane prt (Ng)	48	BBE120	outer surface prt L (ospL) (Eb)	46	BB407	tig basal-body rod prt (flR) (Eb)	100	
BB167	outer membrane prt (Tp)	48	BBE121	outer surface prt M (ospM) (Eb)	46	BB408	tig basal-body rod prt (flS) (Eb)	100	
BB756	rare lipoprotein A (flA) (Hi)	53	BBE122	outer surface prt N (ospN) (Eb)	46	BB409	tig basal-body rod prt (flT) (Eb)	100	
BB10	surface								

lp28-2			ATP-proton motive force interconversion	BB575	CTP Sase (pyrG) [Mj]	71
BBG08	stage 0 sporulation prt J (spoOJ) [Bb]	66	BB094 V-type ATPase, sub A (atpA) [Mb]	64	<i>Salvage of nucleosides and nucleotides</i>	
			BB093 V-type ATPase, sub B (atpB) [Mb]	62	adenine phosphoribosylTase (apt) [Ta]	63
<i>Cell killing</i>			BB092 V-type ATPase, sub D (atpD) [Mj]	51	BB618 cytidine deaminase (cdd) [Mp]	61
BB143	-hemolysin (hlyA) [Ah]	62	BB096 V-type ATPase, sub E (atpE) [Mj]	54	BB239 deoxyguanosine/deoxyadenosine kinase(I) sub 2 (dck) [La]	59
BB117	hemolysin III (yplQ) [Bs]	61	BB091 V-type ATPase, sub I (atpI) [Eh]	53	BB375 pfs prt (pfs-1) [Ec]	64
BB506	hemolysin (tlyA) [Sh]	59	BB090 V-type ATPase, sub K (atpK) [Mj]	54	BB588 pfs prt (pfs-2) [Hi]	59
BB059	hemolysin (tlyC) [Sh]	65			BB791 thymidine kinase (tdk) [Bs]	47
BB202	hemolysin, put [Syn]	54	<i>Electron transport</i>		BB015 uridine kinase (udk) [Bb]	100
			BB061 thioredoxin (trxA) [Ec]	59		
<i>Chaperones</i>			BB515 thioredoxin RDase (trxB) [Bb]	99		
BB741	chaperonin (groES) [Pg]	77	<i>Fermentation</i>			
BB602	chaperonin, put [Cb]	72	BB622 acetate kinase (ackA) [Ec]	63	lp36	
BB519	grpE prt (grpE) [Bb]	100	BB589 P AcTase (pta) [Tt]	65	BBK17	adenine deaminase (adeC) [Bs]
BB295	heat shock prt (hslU) [Bb]	100				
BB296	heat shock prt (hslV) [Bb]	100	<i>Glycolysis</i>		Regulatory functions	
BB649	heat shock prt (groEL) [Bb]	100	BB337 enolase (eno) [Bs]	79	<i>General</i>	
BB517	heat shock prt (dnaI-1) [Bb]	100	BB445 fructose-bisP aldolase (fba) [Ec]	80	BB184	carbon storage regulator (csrA) [Hi]
BB655	heat shock prt (dnaI-2) [Ca]	59	BB730 glucose-6-P isomerase (pgi) [Pf]	62		63
BB264	heat shock prt 70 (dnaK-1) [Bb]	61	BB057 glyceraldehyde 3-P DHase (gap) [Bb]	99	BB647	ferric uptake regulation prt (fur) [Sp]
BB518	heat shock prt 70 (dnaK-2) [Bb]	100	BB630 1-phosphofructoKase (fruK) [Hi]	52		48
BB560	heat shock prt 90 (htpG) [Bb]	100	BB056 phosphoglycerate Kase (pgk) [Bb]	99	BB198	guanosine-3',5'-bis(diP) 3'-pyrophosphohydrolase (spoT) [Ec]
			BB658 phosphoglycerate mutase (gpmA) [Ec]	79	BB737	histidine phosphoKase/PPase, put [Ml]
<i>Detoxification</i>			BB348 pyruvate Kase (pyk) [Bs]	62		49
BB153	superoxide dismutase (sodA) [Hi]	68	BB727 pyroP-fructose 6-P 1-PPTase (pfk) [Eh]	65	BB176	methanol DHase regulator (moxR) [Bb]
BB690	neutrophil activating prt (napA) [Hi]	57	BB020 pyroP-fructose 6-P 1-PPTase, sub (pfpB) [Bb]	100		99
BB179	thiophene and furan oxidation prt (thdF) [Bb]	100	BB055 trioseP isomerase [Bb]	100	BB416	pheromone shutdown prt (traB) [Ef]
						61
<i>Protein and peptide secretion</i>					BB042	P transport system regulatory prt (phoU) [Pa]
BB154	preprt translocase sub (secA) [Bb]	100				57
BB395	preprt translocase sub (secE) [Bl]	62	<i>Pentose phosphate pathway</i>		BB379	prt Kase C1 inhibitor (pkcl) [Bb]
BB498	preprt translocase sub (secY) [Sc]	64	BB222 glucose-6-P 1-DHase, put [As]	48		100
BB362	prolipopt diacylglycerol Tase (lgt) [Ec]	56	BB636 glucose-6-P 1-DHase (zwf) [Hi]	64	BB419	response regulatory prt (rrp-1) [Syn]
			BB561 phosphogluconate DHase (gnd) [Sd]	71		57
BB652	prt-export membrane prt (secD) [Ec]	63	BB657 ribose 5-P isomerase (rpi) [Mj]	61	BB763	response regulatory prt (rrp-2) [Ec]
						67
BB653	prt-export membrane prt (secF) [Hi]	63	<i>Sugars</i>		BB764	sensory transduction histidine Kase, put [Bs]
BB030	signal peptidase I (lepB-1) [Bs]	51	BB407 mannose-6-P isomerase (manA) [Ec]	54		60
BB031	signal peptidase I (lepB-2) [Syn]	57	BB444 nucleotide sugar epimerase [Vc]	69	BB420	sensory transduction histidine Kase, put [Syn]
BB263	signal peptidase I (lepB-3) [St]	57	BB676 phosphoglycolate PPase (gph) [Hi]	50		61
BB469	signal peptidase II (lsp) [Sc]	60	BB207 UTP-glucose-1-P uridylylTase (gtaB) [Bs]	63	BB693	xylose operon regulatory prt (xylR-1) [Th.]
BB694	signal recognition particle prt (ffh) [Bs]	70	BB545 xylulokinase (xylB) [Bs]	43		48
BB610	trigger factor (tig) [Hi]	50			BB831	xylose operon regulatory prt (xylR-2) [Syn]
						51
<i>Transformation</i>			Fatty acid and phospholipid metabolism		lp54	
BB591	competence locus E, put [Bs]	54	<i>General</i>		BBA07	chpAl prt, put [Ec]
BB798	competence prt F, put [Hi]	52	BB037 1-acyl-sn-glycerol-3-P AcTase (plsC) [Bb]	100		55
			BB685 3-OH-3-methylglutaryl-CoA RDase (mvaA) [Pm]	52	Replication	
Central intermediary metabolism			BB683 3-OH-3-methylglutaryl-CoA Sase (At)	53	<i>Degradation of DNA</i>	
<i>General</i>			BB109 Ac-CoA C-AcTase (fadA) [Hi]	67	BB411	endonuclease precursor (nucA) [As]
BB241	glycerol kinase (glpK) [Ec]	74	BB704 acyl carrier prt [Syn]	65		53
BB243	glycerol-3-P DHase, anaerobic (glpA) [Hi]	52	BB721 CDP-diacylglycerol-glycerol-3-P 3-phosphatidylTase [Bs]	55	<i>DNA replication, restriction, modification, recombination, and repair</i>	
BB376	SAM Sase (metK) [Bs]	72	BB327 glycerol-3-P O-acylTase, put [So]	50	BB422	3-methyladenine DNA glycosylase (mag) [At]
<i>Amino sugars</i>			BB368 glycerol-3-P DHase, NAD(P) ⁺ (gpsA) [Bs]	54		56
BB152	glucosamine-6-P isomerase (nagB) [Hi]	79	BB137 long-chain-fatty-acid CoA ligase (Syn)	54	BB827	ATP-dep helicase (hrpA) [Ec]
BB151	N-Acglucosamine-6-P deAcase (nagA) [Hi]	54	BB593 long-chain-fatty-acid CoA ligase [Syn]	56	BB437	chromosomal replication init prt (dnaA) [Bb]
			BB688 mevalonate Kase [Mj]	51		100
<i>Degradation of polysaccharides</i>			BB686 mevalonate pyroP DCase [Sc]	52	BB435	DNA gyrase, sub A (gyrA) [Bs]
BB620	-glucosidase, put [Syn]	58	BB119 phosphatidate cytidylTase (cdsA), AFS[Ec]	61	BB436	DNA gyrase, sub B (gyrB) [Bb]
BB002	-N-Achexosaminidase, put [As]	54	BB249 phosphatidylTase [Hp]	52	BB344	DNA helicase (uvrD) [Ec]
			BB687 phosphomevalonate Kase, put [Sc]	53	BB552	DNA ligase (lig) [Ta]
<i>Phosphorus compounds</i>					BB211	DNA mismatch repair prt (mutL) [Hi]
BB533	phnP prt (phnP) [Ec]	48				55
<i>Polysaccharides - (cytoplasmic)</i>			Purines, pyrimidines, nucleosides, nucleotides		BB797	DNA mismatch repair prt (mutS) [Hi]
BB166	4- -glucanoTase (malG) [Syn]	55	<i>Nucleotide and nucleoside interconversion</i>			57
BB004	phosphoglucomutase (femD) [Mj]	52	BB417 adenylate kinase (adk) [Bs]	64	BB098	DNA mismatch repair prt, put [Syn]
BB835	phosphomannomutase (cpsG) [Hi]	57	BB128 cytidylate kinase (cmk-1) [Bs]	58		51
			BB819 cytidylate kinase (cmk-2) [Mj]	57	BB548	DNA polymerase I (polA) [Hi]
Energy metabolism			BB463 nucleoside-diP kinase (ndk) [Bs]	70	BB579	DNA polymerase III, sub (dnaE) [Ec]
<i>Aerobic</i>			BB793 thymidylate kinase (tmk) [Mj]	59		62
BB728	NADH oxidase, water-forming (nox) [Sh]	59	BB571 uridylate kinase (smbA) [Mj]	54	BB438	DNA polymerase III, sub (dnaN) [Bb]
						100
<i>Amino acids and amines</i>			<i>Purine ribonucleotide biosynthesis</i>		BB461	DNA polymerase III, sub / (dnaX) [Bs]
BB841	arginine deiminase (arcA) [Cp]	75	BB544 phosphoribosyl pyroP Sase (prs) [Mp]	59		61
BB842	ornithine carbamoylTase (arcB) [Ng]	74			BB710	DNA primase (dnaG) [Bs]
			cp26			56
<i>Anaerobic</i>			BBB18 GMP Sase (guaA) [Bb]	100	BB581	DNA recombinase (recG) [Syn]
BB016	glpE prt (glpE) [Hi]	53	BBB17 IMP DHase (guaB) [Bb]	100		60
BB087	L-lactate DHase (ldh) [Bs]	72			BB828	DNA topoisomerase I (topA) [Syn]
			<i>Pyrimidine ribonucleotide biosynthesis</i>		BB035	DNA topoisomerase IV (parC) [Bb]
					BB036	DNA topoisomerase IV (parE) [Bb]
					BB745	endonuclease III (nth) [Syn]
					BB837	excinuclease ABC, sub A (uvrA) [Ec]
						4
					BB836	excinuclease ABC, sub B (uvrB) [Ec]
						71
					BB457	excinuclease ABC, sub C (uvrC) [Syn]
						57
					BB534	exodeoxyribonuclease III (exoA) [Bs]
						67
					BB632	exodeoxyribonuclease V, chain

BB633	(recD) {Ec}	54	BB833	isoleucyl-tRNA Sase (ileS) {Sc}	66	BB703	ribosomal prt L32 (rpmF) {Bs}	62
	exodeoxyribonuclease V, chain (recB) {Hi}	51	BB251	leucyl-tRNA Sase (leuS) {Bs}	70	BB396	ribosomal prt L33 (rpmG) {Bs}	76
BB634	exodeoxyribonuclease V, chain (recC) {Hi}	51	BB659	lysyl-tRNA Sase {Mj}	54	BB440	ribosomal prt L34 (rpmH) {Bb}	100
BB829	exonuclease SbcD (sbcD) {Ec}	55	BB587	methionyl-tRNA Sase (metG) {Sc}	67	BB189	ribosomal prt L35 (rpmI) {Ba}	74
BB830	exonuclease SbcC (sbcC) {Ec}	52	BB514	phenylalanyl-tRNA Sase, sub (pheT) {Bb}	100	BB499	ribosomal prt L36 (rpmJ) {Bs}	89
BB177	glucose-inhibited div prt B (gidB) {Bb}	99	BB513	phenylalanyl-tRNA Sase, sub (pheS) {Bb}	100	BB127	ribosomal prt S1 (rpsA) {Ec}	55
BB178	glucose-inhibited div prt A (gidA) {Bb}	100	BB402	prolyl-tRNA Sase (proS) {Sc}	65	BB123	ribosomal prt S2 (rpsB) {Pa}	79
BB022	Holliday junction DNA helicase (ruvB) {Bb}	100	BB226	seryl-tRNA Sase (serS) {Bs}	62	BB484	ribosomal prt S3 (rpsC) {Hi}	71
BB023	Holliday junction DNA helicase (ruvA) {Bb}	100	BB720	threonyl-tRNA Sase (thrZ) {Bs}	67	BB615	ribosomal prt S4 (rpsD) {Hi}	63
BB014	primosomal prt N (priA) {Bb}	100	BB005	tryptophanyl-tRNA Sase (trsA) {Cl}	65	BB495	ribosomal prt S5 (rpsE) {Bs}	77
BB131	recA prt (recA) {Bb}	100	BB370	tyrosyl-tRNA Sase (tyrS) {Bs}	62	BB115	ribosomal prt S6 (rpsF) {Os}	50
BB607	rep helicase, ss DNA-dep ATPase (rep) {Hi}	61	BB738	valyl-tRNA Sase (valS) {Bs}	67	BB386	ribosomal prt S7 (rpsG) {Sc}	75
BB111	replicative DNA helicase (dnaB) {Ec}	58	<i>Degradation of proteins, peptides, and glycopeptides</i>			BB492	ribosomal prt S8 (rpsH) {Syn}	66
BB114	ss DNA-BP (ssb) {Syn}	62	BB608	aminoacyl-histidine dipeptidase (pepD) {Hi}	55	BB338	ribosomal prt S9 (rpsI) {Hi}	71
BB254	ss-DNA-specific exonuclease (recJ) {Hi}	52	BB366	aminopeptidase I (yscI) {Bb}	100	BB477	ribosomal prt S10 (rpsJ) {Bb}	100
BB623	transcription-repair coupling factor (mfd) {Hi}	60	BB069	aminopeptidase II {Bs}	57	BB501	ribosomal prt S11 (rpsK) {Hi}	77
BB053	uracil DNA glycosylase (ung) {Hi}	68	BB611	ATP-dep Clp protease proteolytic component (clpP-1) {Hi}	79	BB387	ribosomal prt S12 (rpsL) {An}	89
lp28-2			BB757	ATP-dep Clp protease proteolytic component (clpP-2) {Hi}	67	BB500	ribosomal prt S13 (rpsM) {Op}	76
BBG32	replicative DNA helicase, put {Bs}	59	BB369	ATP-dep Clp protease, sub A (clpA) {Ec}	56	BB491	ribosomal prt S14 (rpsN) {Bs}	72
lp25			BB612	ATP-dep Clp protease, sub X (clpX) {Ec}	75	BB804	ribosomal prt S15 (rpsO) {Ti}	77
BBE29	adenine specific DNA MTase, put {Hp}	57	BB834	ATP-dep Clp protease, sub C (clpC) {Pp}	67	BB695	ribosomal prt S16 (rpsP) {Bs}	70
Transcription			BB253	ATP-dep protease LA (lon-1) {Bb}	100	BB487	ribosomal prt S17 (rpsQ) {Mc}	76
<i>General</i>			BB613	ATP-dep protease LA (lon-2) {Hi}	65	BB113	ribosomal prt S18 (rpsR) {Bs}	78
BB052	spoU prt (spoU) {Ec}	54	BB359	carboxyl-terminal protease (ctp) {Syn}	65	BB482	ribosomal prt S19 (rpsS) {Bb}	99
<i>Degradation of RNA</i>			BB203	Lambda CII stability-governing prt (hflK) {Ec}	56	BB233	ribosomal prt S20 (rpsT) {Bb}	100
BB805	polyribonucleotide nucleotidylTase (pnpA) {Bs}	68	BB204	Lambda CII stability-governing prt (hflC) {Ec}	56	BB256	ribosomal prt S21 (rpsU) {Mx}	68
BB046	ribonuclease H (rnhB) {Hi}	66	BB248	oligoendopeptidase F (pepF) {Li}	58	BB516	rRNA methylase (yacO) {Mc}	66
BB705	ribonuclease III (rnc) {Bs}	62	BB067	peptidase, put {Sc}	56	<i>tRNA modification</i>		
BB441	ribonuclease P prt component (rnpA) {Bb}	100	BB104	periplasmic serine protease DO (htrA) {Hi}	60	BB821	2-methylthio-N6-isopentyladenosine tRNA modification enzyme (miaA) {Ec}	53
<i>DNA-dependent RNA polymerase</i>			BB430	proline dipeptidase (pepQ) {Hi}	49	BB084	AT (nifS) {Syn}	61
BB502	DNA-directed RNA polymerase (rpoA) {Bs}	64	BB769	sialoglycoprotease (gcp) {Hi}	60	BB343	glu-tRNA amidoTase, sub C (gatC) {Bs}	56
BB389	DNA-directed RNA polymerase (rpoB) {Bb}	97	BB627	vacuolar X-prolyl dipeptidyl aminopeptidase I (pepX) {Mi}	55	BB341	glu-tRNA amidoTase, sub B (gatB) {Bs}	63
BB388	DNA-directed RNA polymerase (rpoC) {Ec}	71	BB118	zinc protease, put {Hi}	54	BB342	glu-tRNA amidoTase, sub A (gatA) {Bs}	61
BB771	RNA polymerase sigma factor (rpoS) {Pa}	61	BB536	zinc protease, put {Hi}	52	BB064	methionyl-tRNA formylTase (fmt) {Ec}	56
BB712	RNA polymerase sigma-70 factor (rpoD) {Bb}	100	<i>Nucleoproteins</i>			BB787	peptidyl-tRNA hydrolase (pth) {Bb}	100
BB450	RNA polymerase sigma-54 factor (ntrA) {Av}	57	BB232	hbbU prt {Bb}	100	BB012	pseudouridylylase I (hisT) {Bb}	100
<i>Transcription factors</i>			<i>Protein modification</i>			BB021	SAM: tRNA ribosylTase-isomerase {Bb}	96
BB107	N utilization substance prt B (nusB) {Ec}	62	BB105	methionine aminopeptidase (map) {Bs}	68	BB809	tRNA-guanine transglycosylase (tgt) {Zm}	60
BB800	N-utilization substance prt A (nusA) {Bs}	62	BB065	polypeptide deformylase (def) {Syn}	67	BB698	tRNA (guanine-N1)-MTase (trmD) {Mg}	68
BB394	transcription antitermination factor (nusG) {Ec}	64	BB648	serine/threonine kinase, put {Pf}	51	BB803	tRNA pseudouridine 55 Sase (truB) {Ec}	57
BB132	transcription elongation factor (greA) {Ec}	56	<i>Ribosomal proteins: synthesis and modification</i>			<i>Translation factors</i>		
BB355	transcription factor, put {Mx}	47	BB392	ribosomal prt L1 (rplA) {Bs}	71	BB088	GTP-B membrane prt (lepA) {Hi}	76
BB230	transcription termination factor Rho (rho) {Bb}	100	BB481	ribosomal prt L2 (rplB) {Bb}	99	BB196	peptide chain release factor 1 (prfA) {Hi}	73
<i>RNA processing</i>			BB478	ribosomal prt L3 (rplC) {Bb}	99	BB074	peptide chain release factor 2 (prfB) {Sc}	70
BB706	polynucleotide adenylylTase (papS) {Bs}	57	BB479	ribosomal prt L4 (rplD) {Bb}	100	BB121	ribosome releasing factor (frr) {Mt}	68
Translation			BB490	ribosomal prt L5 (rplE) {Hi}	80	BB169	translation initiation factor 1 (infA) {Ec}	87
<i>General</i>			BB493	ribosomal prt L6 (rplF) {Sc}	72	BB801	translation initiation factor 2 (infB) {Bs}	73
BB590	dimethyladenosine Tase (ksgA) {Bs}	61	BB390	ribosomal prt L7/L12 (rplL) {Sc}	75	BB190	translation initiation factor 3 (infC) {Pv}	72
BB802	ribosome-B factor A (rbfA) {Bs}	62	BB112	ribosomal prt L9 (rplI) {Ec}	57	BB691	translation elongation factor G (fus-2) {Tm}	67
<i>Amino acyl tRNA synthetases</i>			BB391	ribosomal prt L10 (rplJ) {Bs}	61	BB214	translation elongation factor P (efp) {Ec}	56
BB220	alanyl-tRNA Sase (alaS) {Ec}	62	BB393	ribosomal prt L11 (rplK) {Tm}	73	BB476	translation elongation factor TU (tuf) {Bb}	100
BB594	arginyl-tRNA Sase (argS) {Mj}	55	BB339	ribosomal prt L13 (rplM) {Hi}	72	BB122	translation elongation factor TS (tsf) {Hi}	57
BB101	asparaginyl-tRNA Sase (asnS) {Ec}	73	BB488	ribosomal prt L14 (rplN) {Tm}	79	BB540	translation elongation factor G (fus-1) {Tm}	68
BB446	aspartyl-tRNA Sase (aspS) {Ec}	66	BB497	ribosomal prt L15 (rplO) {Bs}	68	Transport and binding proteins		
BB599	cysteinyl-tRNA Sase (cysS) {Hi}	58	BB485	ribosomal prt L16 (rplP) {Syn}	81	<i>General</i>		
BB372	glutamyl-tRNA Sase (gluX) {Rm}	63	BB503	ribosomal prt L17 (rplQ) {Ec}	63	BB573	ABC transporter, ATP-BP {Bs}	53
BB371	glycyl-tRNA Sase (glyS) {Ta}	68	BB494	ribosomal prt L18 (rplR) {Bs}	69	BB742	ABC transporter, ATP-BP {Syn}	57
BB135	histidyl-tRNA Sase (hisS) {Mj}	59	BB699	ribosomal prt L19 (rplS) {Ec}	74	BB466	ABC transporter, ATP-BP {Hi}	74
			BB188	ribosomal prt L20 (rplT) {Ec}	70	BB754	ABC transporter, ATP-BP {Bi}	60
			BB778	ribosomal prt L21 (rplU) {Ec}	58	BB080	ABC transporter, ATP-BP {Mj}	63
			BB483	ribosomal prt L22 (rplV) {Bb}	100	BB269	ATP-BP (yixH-1) {Bb}	100
			BB480	ribosomal prt L23 (rplW) {Bb}	100	BB726	ATP-BP (yixH-2) {Bb}	54
			BB489	ribosomal prt L24 (rplX) {Ec}	64	<i>Amino acids, peptide, and amines</i>		
			BB780	ribosomal prt L27 (rpmA) {Hi}	82	BB729	glutamate transporter (gluTP) {Bs}	55
			BB350	ribosomal prt L28 (rpmB) {Ec}	62	BB401	glutamate transporter, put {Bs}	53
			BB486	ribosomal prt L29 (rpmC) {Bs}	65	BB146	GBP ABC transporter, ATP-BP	
			BB496	ribosomal prt L30 (rpmD) {Bs}	60			
			BB229	ribosomal prt L31 (rpmE) {Bs}	69			

