INVESTIGATION OF THE LIPOPROTEOME OF THE LYME DISEASE BACTERIUM *BORRELIA BURGDORFERI*

BY

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Abstract

The spirochete bacterium *Borrelia burgdorferi* is the causative agent of Lyme borreliosis, the top vector-borne disease in the United States. *B. burgdorferi* is transmitted by hard-bodied *Ixodes* ticks in an enzootic tick/vertebrate cycle, with human infection occurring in an accidental, "dead-end" fashion. Despite the estimated 300,000 cases that occur each year, no FDA-approved vaccine is available for the prevention of Lyme borreliosis in humans. Development of new prophylaxes is constrained by the limited understanding of the pathobiology of *B. burgdorferi*, as past investigations have focused intensely on just a handful of identified proteins that play key roles in the tick/vertebrate infection cycle. As such, identification of novel *B. burgdorferi* virulence factors is needed in order to expedite the discovery of new anti-Lyme therapeutics. The multitude of lipoproteins expressed by the spirochete fall into one such category of virulence factor that merits further study. These lipoproteins play diverse roles in the organism and localize to different membrane-peripheral cellular compartments.

The overarching goal of the current study was to further define the structure-function of the *B. burgdorferi* cell envelope by comprehensively and conclusively defining the localization of the bacterium's predicted lipoproteome, using an epitope-tagged lipoprotein expression library. The data show that the majority of *B. burgdorferi* lipoproteins are surface-exposed, and that the plasmids of *B. burgdorferi* are enriched in surface lipoprotein genes relative to the chromosome. The study also establishes and validates a high-throughput proteomics approach that can be used in future studies to assess localization of endogenously expressed untagged lipoproteins. Finally, an analysis of the lipoproteome using data mining and *in silico* fold recognition algorithms

demonstrates potential roles for several uncharacterized lipoproteins and identifies new targets for vaccine development.

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Chapter 1: Background

1.1 History of Lyme borreliosis (Lyme Disease)

Although Lyme borreliosis has only recently been described and the etiologic agent determined to be the spirochete *Borrelia burgdorferi*, evidence exists that humanity has been afflicted with this disease for much longer. European researchers had long known that the bite of certain hard-bodied ticks could results in a disease with dermatological and neurological complications (1). One of the first documented presentations of possible Lyme borreliosis came from Arvid Afzelius, a founder of the Swedish Society of Dermatology, in 1909. He presented the case of an elderly woman with the now-characteristic expanding annular erythema, which he termed "erythema migrans", at the site of an *Ixodes reduvius* tick bite. Subsequent clinical observations of tick-bitten patients found similar dermatological manifestations and occasionally observed pathology of the central nervous system (lymphocytic meningitis). The 1922 findings of Garin & Bujadoux are believed to be the first recorded description of Lyme neuroborreliosis, although a retrospective diagnosis of their patient has led to speculation that he may have been co-infected with other tick-borne pathogens (2, 3). Researchers in Europe eventually concluded that a causal link existed between the disease and ticks, with speculation that a tick salivary gland toxin or tick-associated pathogen was ultimately responsible (4). Evidence exists that human infection by *B. burgdorferi* may even predate these published accounts. PCR analysis of DNA present in the Tyrolean Iceman "Ötzi", a 5,000-year-old preserved mummy from the Italian Alps, showed that he had been infected with *B. burgdorferi* and is the oldest known human to have had Lyme borreliosis (5).

In 1975, the Connecticut State Health Department was contacted concerning an epidemic of juvenile rheumatoid arthritis in Lyme, Connecticut, with an unknown etiology. Dr. Allen

Steere, working on behalf of a joint investigation between the Yale University School of Medicine and Connecticut State Health Department, published his observations of oligoarticular arthritis in both children and adults (6). Investigation showed that juvenile rheumatic arthritis was not the cause of the observed symptoms, as the incidence of reported arthritis was 100 times the expected prevalence, and consequently the new affliction was termed "Lyme arthritis". Many patients also developed expanding erythematous lesions reminiscent of those observed in Europe, and a further subset of patients also developed cardiological and/or neurological complications (7). Although the causative agent of Lyme arthritis was still known, it was demonstrated that early administration of antibiotics could ameliorate the symptoms of the disease (8). Epidemiological studies implicated *Ixodes* ticks with the transmission of disease, similar to the previous findings in Europe (9).

The identification of the spirochete *B. burgdorferi* came later, during 1978 and 1979 investigations into outbreaks of the tickborne Apicomplexan parasite babesiosis in the Northeast. Serosurveys conducted in Shelter Island, New York, identified a number of individuals who did not have babesiosis but instead reported symptoms resembling patients from Lyme, Connecticut. Sera from these patients were reactive to spirochetes grown in Kelly's Medium from ticks collected on Shelter Island, demonstrating a connection between these spirochetes and the disease from Lyme (10). These spirochetes were also later isolated directly from patients with Lyme disease, further strengthening the evidence that this bacterium was the causative agent (11, 12). The organism was named *Borrelia burgdorferi* to honor Dr. Willy Burgdorfer's efforts in the organism's discovery, with its accompanying disease renamed "Lyme borreliosis" (13). Today, Lyme borreliosis is the most common vector-borne disease in the United States, and an estimated 300,000 cases occur each year (14). There is no FDA-approved vaccine currently

available for the prevention of Lyme borreliosis. Lyme borreliosis is considered an emerging disease and research into the pathogenesis of *B. burgdorferi*, as well as prophylaxis and treatments, is ongoing (15).

1.2 The Enzootic Cycle of *B. burgdorferi*

In nature, *B. burgdorferi* exists in a complex relationship with its tick and vertebrate hosts, transitioning between the two at different stages of the tick life cycle. Only certain hard-bodied ticks of the genus *Ixodes* can become infected with *B. burgdorferi*, and the molecular specificity of the tick-spirochete interaction suggests that *B. burgdorferi* has evolved to utilize this genus of tick as the means by which it spreads between vertebrates. *B. burgdorferi* is not transmitted transovarially in ticks, and newly hatched larvae acquire the bacterium from a previously infected host (16, 17). The transmission cycle typically begins with an uninfected tick larva feeding on an infected vertebrate host, typically a small bird or mammal. The white-footed mouse, *Peromyscus leucopus*, is especially notable as an important natural reservoir of *B. burgdorferi* in North America. Interestingly, laboratory infected adult *Peromyscus* mice can become persistently infected with *B. burgdorferi* but do not develop many of the pathologies associated with human infection, suggesting that host factors may modulate the severity of *B. burgdorferi* infection (18). Spirochetes can be detected in the feeding tick within 12 hours from the start of feeding, and bacterial colonization of the tick continues for the full extent of the feeding period (typically 72-96 hours) (16, 17). *B. burgdorferi* takes up residence in the tick midgut, wherein it can persist for several months while the larval tick molts into a nymph. During this time, the tick does not feed and displays limited metabolic activity. The molecular mechanisms by which *B. burgdorferi* senses host feeding and transits to the tick, as well as how

it survives in the nutrient-depleted midgut for months, are unclear at this time. Microarray studies examining the transcriptome of *B. burgdorferi* in larvae, nymphs, and dialysis membrane chambers have shed light on which genes are important for certain stages of the tick/vertebrate cycle; however, many of these genes encode proteins without identifiable functions or homologs in non-spirochetes, thus preventing full understanding of the molecular mechanism behind the *B. burgdorferi* tick/vertebrate life cycle (19).

After molting, the unfed nymph then proceeds to feed on a second host, usually of the same variety chosen by the unfed larvae (16, 17). Transmission of *B. burgdorferi* to the uninfected vertebrate host occurs at this stage and replenishes the *B. burgdorferi* reservoir in small animals. Accidental transmission to humans, dogs, and other "accidental" hosts also occur at this stage. The steps leading to transmission of *B. burgdorferi* have been well-characterized. During the first 36 hours of tick feeding, *B. burgdorferi* in the tick midgut is immersed in undigested blood from the host and begins a period of rapid cell division and dissemination. The bacteria penetrate the tick gut and migrate to the salivary gland, wherein they pass into the vertebrate host and begin further dissemination. Interestingly, this process of transmission can take up to 72 hours for *B. burgdorferi* but can occur in less than an hour for relapsing fever *Borrelia* spirochetes, which colonize fast-feeding soft-bodied ticks (20). Tick salivary gland proteins are thought to play a beneficial role in *B. burgdorferi* transmission based on gene expression and bacterial surface binding studies (21, 22). The fed nymphs then detach from their hosts and, after several months, molt into male and female adult ticks. Adult ticks seem to play little role in the transmission of *B. burgdorferi* to humans, although they play an important role in nature through perpetuation of the tick population.

1.3 The Fundamental Role of *B. burgdorferi* Lipoproteins

Lipid-modified proteins, or "lipoproteins", are ubiquitous in both Gram-positive and Gram-negative bacteria. *B. burgdorferi* is noteworthy in that it dedicates a large percentage of its genome to the expression of lipoproteins, with one estimate placing the number of lipoprotein encoding-genes at about 8% of all reading frames (23). This is especially significant considering that the *B. burgdorferi* genome is highly reduced at approximately 1.314 MBp (24). Lipoproteins are peripheral membrane proteins, anchored to either the inner or outer membrane through a conserved, acylated cysteine residue at the N terminus (also see 2**. Lipoprotein Biosynthesis in *B. burgdorferi***) (25). Following the acylated cysteine is a span of disordered residues (the "tether") which, in Proteobacteria, contains the necessary information for lipoprotein sorting at the inner membrane (26). Prelimiary evidence shows that, although this region can modulate surface exposure of outer membrane lipoproteins, it does not seem to play a role in the release of outer membrane lipoproteins at the inner membrane (27-30). In *B. burgdorferi*, tether regions are highly variable in both length and amino acid content, with no obvious correlation existing between a lipoprotein's localization and its tether length/biochemical properties (**Table 3**) (27). However, the length of surface lipoprotein tethers may act to position the lipoprotein in a distinct spatial setting relative to other membrane proteins, with its positioning possibly tied to the biological role of the lipoprotein (25). Following the tether region is a more constrained region of the protein that is believed to be responsible for the lipoprotein's biological function.

The lipoproteins of *B. burgdorferi* play diverse roles in the organism, with identified functions ranging from housekeeping duties to host interactions, and consequentially these lipoproteins are necessary for both *in vitro* growth using artificial culture medium as well as the

more complex tick/vertebrate transmission cycle found in nature. Many lipoproteins also have no identifiable function or homology, yet are indispensable to *B. burgdorferi* at a specific stage in its life cycle. Here, the various archetypes of lipoproteins will be discussed using well-researched, prototypical examples.

Certain *B. burgdorferi* lipoprotein play important housekeeping functions to the organism and are among those that show the most homology to proteins in other bacteria (31, 32). These lipoproteins often act as nutrient uptake factors and are frequently found as part of inner membrane ATP-binding cassette (ABC) transporters. These lipoproteins, called substrate-binding proteins, are responsible for coordinating the binding and ATP hydrolysis-driven uptake of specific solutes from the periplasm into the cytoplasm (33). Of these substrate-binding lipoproteins, some of the most prominent belong to the Oligopeptide Permease (OppA) family of substrate-binding proteins. These proteins facilitate the transport of small peptides from the periplasm into the cytoplasm through interaction with the ABC transport subunits OppB/C/D/F. *B. burgdorferi*, interestingly, expresses five copies of *oppA*: three on the linear chromosome, one on the mini-chromosome cp26, and one on the dispensable linear plasmid lp54 (34, 35). These copies seem to have arisen from paralogous gene duplication, as they are homologous in amino acid sequence (≥44% pair-wise identity) but show different peptide binding affinities (34, 36). Transposon mutagenesis screens suggest that these genes are not individually essential, implying that there may be some overlap or redundancy in their substrate specificity that permits growth of *B. burgdorferi in vitro* (37). The authors of this study also came to the conclusion that the OppA homologs in *B. burgdorferi* could complement each other *in vitro*. The *B. burgdorferi* gene *oppA-1* (BB0328) was found to be able to complement an *E. coli* Δ*oppA* strain, further supporting the conservation of these proteins between disparate bacterial lineages (38). The

OppA proteins also show individual transcriptional regulation patterns based on environmental cues and host species, indicating that their expression may correlate with different nutritional demands at various phases of the tick/vertebrate cycle through their dissimilar peptide binding. Further research of their transcription showed that *oppA-1* (BB0328), *oppA-2* (BB0329), and *oppA-3* (BB0330) can be transcribed tricistronically, bicistronically, or individually, and that these genes were not regulated by temperature (34). In contrast, the gene *oppAV* (BBA34) is under control of the alternative sigma factors RpoN (BB0450) and RpoS (BB0771), implicating the expression of this gene in the vertebrate stage of infection and supporting previous findings that this OppA gene is the only one regulated by temperature (34, 39, 40). Similarly, the gene *oppAIV* (BBB16) showed regulation by the *B. burgdorferi* BosR/Fur homolog (BB0647), a transcriptional regulator that has been associated with expression of virulence genes in vertebrates (41). These data suggest that the genes *oppAIV* and *oppAV* may be upregulated during transmission of *B. burgdorferi* from the tick to the vertebrate host. Despite this evidence implicating *oppAIV* and *oppAV* in the infection cycle, transposon mutagenesis studies and gene deletion experiments have not conclusively demonstrated that either gene is required for infection in the mouse model (37, 42). In addition to OppA homologs, other substrate-binding proteins have been identified in *B. burgdorferi*. The protein ProX (BB0144) is a predicted L-proline/glycine betaine substrate-binding protein and is situated in a likely operon with putative ABC transporter components (31, 32). Based on homology, it likely functions in transport of osmoregulatory molecules into the cell; however, this has not been demonstrated experimentally. The protein PstS (BB0215) is also predicted to be a substrate-binding protein, with homology to characterized phosphate-binding proteins. Similar to ProX, PstS exists in a possible operon with predicted ABC transporter proteins. Surprisingly, binding assays performed with soluble,

recombinant PstS show that the protein binds to sulfate anions with at least 20-fold greater affinity than for phosphate, suggesting that PstS may be involved in the transport of both anions into the cytoplasm (43). Transposon mutagenesis assays suggest that both ProX and PstS are essential for *in vitro* cultivation of *B. burgdorferi*, consistent with their predicted role in substrate transport (37). One interesting observation of *B. burgdorferi* substrate-binding proteins is that they appear to be lipoproteins, based on conserved cysteine residues and a putative lipobox motif (23). The structure of these proteins in *B. burgdorferi* contrasts with that of substrate-binding proteins in *E. coli* and other diderms, in which they are soluble periplasmic proteins rather than peripheral, membrane anchored proteins (33). In this regard, the substrate-binding proteins of *B. burgdorferi* are more similar to those of Gram-positive bacteria, which also express substrate-binding proteins as lipoproteins. However, the presence of outer membrane biogenesis machinery, such as the Bam and Lol complexes, suggests that the most recent common ancestor of *B. burgdorferi* with non-spirochetes was Gram-negative, a conclusion supported by 16S rRNA sequencing and alignment (44). Although the evolution of *B. burgdorferi* is unclear based on the early divergence of the phylum Spirochaetes, it is most likely that convergent evolution is responsible for the observation that the substrate-binding proteins of both *B. burgdorferi* and Gram-positive organisms are lipoproteins.

In addition to such metabolic roles, certain *B. burgdorferi* lipoproteins are also mediators of pathogenicity. These lipoproteins have been shown to be important in various stages of the tick/vertebrate infectious cycle, with data showing that their loss interrupts the cycle at a point that corresponds to the biological function of the protein. One such way that lipoproteins mediate pathogenicity is through acting as surface adhesion proteins. Expression of such lipoproteins facilitates survival in the tick vector and infection of the vertebrate host. OspA

(BBA15), an abundant surface lipoprotein, was one of the first *B. burgdorferi* adhesins to be discovered. OspA was first reported as a shared antigen on both human- and tick-isolated *B. burgdorferi* based on affinity of a monoclonal antibody H5332, and it was later determined through [3H]-palmitate metabolic labeling and radioimmunoprecipitation that OspA was lipidated in *B. burgdorferi* (45, 46). Lipidated OspA was also found to be highly immunogenic in mice, and inoculation with lipidated OspA could result in a protective immune response against subsequent intradermal challenges with live *B. burgdorferi* (47). This study and others were the foundation for the first FDA-approved anti-Lyme vaccine, LYMErix, which was based on recombinant OspA as the immunogen (48). The discovery of OspA as an adhesin was preceded by findings that OspA was selectively expressed in the tick vector and downregulated upon transmission to the vertebrate host, leading to speculation that OspA plays a role in tick colonization (49). Further work using tick gut extracts and yeast two-hybrid experiments determined that OspA binds to a tick gut epithelium protein called TROSPA (Tick Receptor for OspA) (50, 51). Studies using infectious *B. burgdorferi* isolates found that OspA is not required for infection or persistence in the mouse model, but the loss of OspA prevents re-acquisition of *B. burgdorferi* by uninfected feeding ticks (52). These results show that OspA acts to adhere *B. burgdorferi* to the midgut epithelium during tick colonization and that it is essential for completion of the natural tick/vertebrate infection cycle. OspA exists in a bicistronic operon with OspB, a lipoprotein with similar amino acid sequence and structure to OspA (53-55). Tick acquisition assays suggest that OspA and OspB are functionally and that spirochetes expressing solely OspB can colonize and survive in larval ticks (56). Likewise, expression of OspA alone has been shown to permit survival of *B. burgdorferi* in nymphal ticks after microinjection (52). Expression of both OspA and OspB, though, has been shown to permit a greater extent of tick

colonization that expression of just OspA, suggesting that these two proteins play complementary, but distinct, functions *in vivo* (52). The current working hypothesis is that OspA and OspB arose from a gene duplication event and have diverged to some extent; however, their precise biological function in relation to each other still has yet to be fully understood. Besides OspA and OspB, other lipoproteins have been shown to act as *B. burgdorferi* adhesins in the vertebrate host. These lipoproteins presumably facilitate the dissemination of *B. burgdorferi* throughout various tissues. One prominent surface adhesin is the fibronectin binding protein P47 (FBP; BBK32). Fibronectin, a large mammalian glycoprotein, is a component of the extracellular matrix and binds a variety of biological polymers such as heparin and collagen. *B. burgdorferi* cell lysates were shown to bind fibronectin after transfer to nitrocellulose membranes, resulting in a single unique band corresponding to FBP (57). Adhesion of *B. burgdorferi* onto fibronectin-coated glass slides was also competitively inhibited by pre-incubation with purified FBP, showing that fibronectin binding is solely due to FBP. Despite these results, disruption of FBP was shown to have no effect on *B. burgdorferi* pathogenicity (58). These results suggest that fibronectin binding is not essential to infectivity, likely due to the actions of other *B. burgdorferi* adhesins. These other adhesins include the decorin-binding proteins A and B (DbpA/B, BBA24/25). Decorin, a proteoglycan that associates closely with collagen, was shown to facilitate the adhesion of *B. burgdorferi* to the wells of microtiter plates and, through radiolabeling, was also shown to be specifically bound by the spirochetes (59). *B. burgdorferi* that had their *dbpAB* genes disrupted were nevertheless able to fully complete the tick/vertebrate infection cycle, suggesting that, like FBP, other surface adhesins may be able to compensate for the lack of decorin binding *in vivo* (60). Finally, it has been established that the putative lipoprotein ErpX (BBQ47) binds laminin, a family of large glycoproteins that are

components of the mammalian extracellular matrix (61). ErpX belongs to a family of paralogous, plasmid-encoded lipoproteins (Erp) that are up-regulated during transmission to the vertebrate host and are immunogenic (62). As the authors demonstrated, *B. burgdorferi* selectively bound to laminin-coated slides and that this binding could be diminished through addition of soluble, recombinant ErpX. Although the role of ErpX in the infection cycle of *B. burgdorferi* has not been investigated, it is highly probable that it, like the other *B. burgdorferi* adhesins, is singularly dispensable for infection in the mouse model due to compensation effects.

Other lipoproteins are associated with transmission of *B. burgdorferi* and the establishment in the vertebrate host. These lipoproteins play a critical role in the natural tick/vertebrate enzootic cycle of *B. burgdorferi*, and their absence results in a noticeable impediment to infection. The surface lipoprotein OspC is the prototypical example of such a protein. It is selectively up-regulated by an increase in temperature or pH, that is, a shift to conditions that mimic those of the vertebrate host (49, 63). Examination of the temporal regulation of OspC *in vivo* has revealed that it is rapidly induced during tick feeding, with immunofluorescence assays showing that 75% of spirochetes expressed the lipoprotein after 48 hours of tick feeding (64). Interestingly, the expression of OspA appeared to be somewhat reciprocal to that of OspC, in which OspA-expressing cells decreased to 40% of the total population by 96 hours (49, 64). The increase in temperature and pH flux appears to be the driving factor of OspC expression, as spriochetes that are continually maintained at the temperature and pH of a vertebrate host slowly down-regulate expression of OspC (64). OspC has been shown to bind the tick salivary gland protein Salp15, an immunomodulatory factor that is believed to allow *B. burgdorferi* to establish itself in the host. Salp15 has been shown to inhibit the activation of $CD4^+$ T-cells through binding of the CD4 receptor and has also been

demonstrated to modulate the action of dendritic cells through binding to the C-type lectin DC-SIGN, ultimately resulting in lower dendritic cell activation and lower levels of cytokine release (65-67). Salp15 has also been implicated in resistance to serum complement through inhibiting the deposition of membrane attack complex factors (as have other lipoproteins, see next section) (68). In addition to Salp15, OspC has been shown to the bind serum plasminogen, which is subsequently converted to enzymatically active plasmin (69). This plasmin, still bound to the surface of *B. burgdorferi*, has been demonstrated to enhance penetration of the endothelium by *B. burgdorferi* and allow it to disseminate beyond the site of tick feeding (70). Corresponding to these identified biological roles, inactivation of OspC in *B. burgdorferi* abrogated infectivity in the mouse model either through needle inoculation or through tick feeding, demonstrating the necessity of this lipoprotein for the transmission of *B. burgdorferi* (71).

In order for *B. burgdorferi* to establish a persistent infection in the vertebrate host, it must tirelessly evade the host's immune defenses. These defenses compose a variety of distinct anti-bacterial responses that interact with specialized *B. burgdorferi* immune evasion proteins. One way that *B. burgdorferi* evades the immune response so is by avoiding destruction by the host's complement system. The surface lipoprotein CRASP-1 (CspA; BBA68) is noteworthy in this regard as it binds the complement regulatory proteins Factor H and Factor H-like protein 1 (FHL-1), soluble plasma proteins that are implicated in endogenous regulation of the alternative complement pathway (72, 73). Factor H and FHL-1 bound in this way were found to be biologically active, and expression of CRASP-1 correlated with resistance to serum complement *in vitro* (73, 74). CRASP-1 was also found to be upregulated during infection of the vertebrate host and downregulated during tick acquisition, further supporting the role of this lipoprotein in host complement evasion (75). In addition to CRASP-1, *B. burgdorferi* has been shown to

expresses one additional Factor H/FHL-1 binding protein CRASP-2 (CspZ; BBH06) and three additional Factor H binding proteins CRASP-3 (ErpP; BBN38), CRASP-4 (ErpC, no locus tag available), and CRASP-5 (ErpA; BBL39) (76). In addition to binding Factor H, CRASP-3 and CRASP-5 have been shown to bind CFHR-2 and CFHR-5, two additional complement regulatory proteins (77). A recent study also found that recombinant, soluble CRASP-1 and CRASP-3, but not other CRASPs, could inhibit hemolysis of rabbit erythrocytes by the alternative complement pathway (78). CRASP-1 was further shown to inhibit all three complement pathways through binding of the terminal complement factors C7 and C9, thereby directly preventing assembly of the membrane attack complex and avoiding complement-mediated lysis. These functional differences are possibly one reason why CRASP-2-deficient *B. burgdorferi* remained resistant to complement and retained full infectivity in mice (79). Interestingly, *B. burgdorferi* could still infect mice deficient in Factor H, further supporting the hypothesis that certain CRASPs may bind complement regulatory proteins somewhat promiscuously (80). Taken together, these results suggest that the interplay between CRASP proteins and the complement system is complex and dynamic, and it can be surmised that these lipoproteins evolved to have complementary, but distinct, functions in preventing complement-mediated lysis of *B. burgdorferi*.

Besides neutralization of the host's complement system, *B. burgdorferi* is also able to evade the humoral immune response so that it can persistently infect its host. Although various anti-*B. burgdorferi* antibodies can be detected in Lyme borreliosis patients, the bacterium is nevertheless able to survive in the face of a targeted humoral response. One important way that *B. burgdorferi* evades the host's humoral defense is through expression of the surface lipoprotein Vmp-like sequence E (VlsE; BBF0041). VlsE undergoes homologous recombination *in vivo*

with 15 neighboring, silent *vls* cassettes that results in antigenic variation of the lipoprotein (81). Lipidation of VlsE was confirmed through [^{3}H]-palmitate metabolic labeling of *B. burgdorferi* and radioimmunoprecipitation. This antigenic variation allows for evasion of previously generated anti-VlsE antibodies, keeping *B. burgdorferi* one step ahead of the host and forcing adaptation of the antibody response against the new VlsE antigen. Deletion studies of the *vls* genetic locus showed that not only is expression of the VlsE lipoprotein necessary for persistence of infection in the mouse model, but recombination of VlsE with the silent *vls* cassettes is indispensable as well (82). It was also observed that recombination of VlsE occurred continuously throughout the infection of mice by *B. burgdorferi*, and the study's authors hypothesized that VlsE recombination does not cease while *B. burgdorferi* persists in the vertebrate host and would result in an immense number of VlsE serotypes (83). A subsequent study found that VlsE does not undergo recombination in the tick vector, conclusively demonstrating that VlsE recombination is a vertebrate-specific phenomenon (84). The importance of VlsE antigenic variation was further shown in experiments conducted with severe combined immunodeficient (SCID) mice, which lack an adaptive immune response, versus immunocompetent controls (85). *B. burgdorferi* recovered from immunocompetent mice showed VlsE recombination at a higher frequency and with a greater amount of complexity as compared with spirochetes from SCID mice, suggesting that the adaptive immune response places selection pressure directly on VlsE to maintain an antigenically novel façade. These results, in total, show that the lipoprotein VlsE is a novel means by which *B. burgdorferi* evades the host immune system in order to establish a persistent infection.

Finally, certain uncharacterized proteins are important for the tick/vertebrate enzootic cycle. These proteins have been shown to be crucial for certain steps of infection, yet lack

characterization of their molecular function and have no identifiable non-spirochete homologs. One such lipoprotein is the small, abundant protein Lp6.6 (BBA62). Lp6.6 was first identified as mitogenic, low molecular weight protein with pronounced abundancy in *B. burgdorferi*, accounting for about 2% of the cell dry weight (86). [3H]-palmitate labeling of *B. burgdorferi* and subsequent radioimmunoprecipitation with the monoclonal anti-Lp6.6 antibody 270.7 demonstrated that Lp6.6 is indeed a lipoprotein. Localization assays revealed that Lp6.6 localized to the inner leaflet of the outer membrane, and is one of only a few lipoproteins to localize in such a way (27, 86, 87). Lp6.6 was found to be up-regulated during tick acquisition and down-regulated during transmission to the vertebrate host (88). Interestingly, *B. burgdorferi* cells lacking Lp6.6 retained full infectivity in mice by needle inoculation, and uninfected ticks that fed on these mice acquired the bacteria equally to those expressing Lp6.6. However, the absence of Lp6.6 led to reduced spirochete load in mouse tissues when transmission occurred by tick feeding, and qRT-PCR analysis of tick tissues showed that bacterial populations in the gut and salivary glands were reduced 5- to 10-fold as compared with wild-type cells. It was also found that Lp6.6 exists in outer membrane complexes with other pathogenesis-associated lipoproteins, such as OspA (88, 89). Structural analysis of Lp6.6 showed that it exists primarily in aggregation-prone α-helices and disordered stretches, and it is hypothesized that its unconstrained tertiary structure may be important for its biological role (90). Another similarly uncharacterized factor is the lipoprotein (Ip)LA7 (P22; BB0365). LA7 was first identified based on reactivity with Lyme patient sera: it appeared as an approximately 22 kilodalton protein with an acidic pI (5.7) that was chromosomally encoded and lipidated, based on [^{3}H]-palmitate assays (91, 92). Further studies determined that it localized primarily to the inner membrane of the periplasm and that its expression could be induced by a temperature shift, pH shift, or through

addition of the autoinducer-2 precursor 4,5-dihydroxy-2,3-pentanedione (27, 93). This regulation may be due to a putative binding site 5' of *la7* for the DNA-binding protein EbfC (BB0462), which has been implicated in the vertebrate host-induced expression of other plasmid-encoded lipoproteins (94, 95). Correspondingly, expression of LA7 in tick midguts declined significantly as compared with expression in the mouse model, suggesting that LA7 is specifically expressed in the vertebrate host (93). Recent studies also found that LA7 expression spiked during tick feeding, and deficiency of LA7 in *B. burgdorferi* resulted in pronounced inability to both transmit to mice and re-colonize uninfected ticks (96, 97). LA7-deficient spirochetes were also significantly impaired in their ability to migrate to tick salivary glands following the initiation of feeding. The combined results of LA7 expression and inactivation data suggest that LA7 may play a role in chemotaxis of the organism, allowing it to recognize the need to disseminate to a new host. The absence of LA7, in turn, would impair the spirochete's recognition of chemotaxis or dissemination signals. This hypothesis is supported by data showing that inactivation of LA7 does not impair *B. burgdorferi*'s ability to maintain itself within the tick or to establish infection in the mouse after needle inoculation (96, 97). Further support is found in that inactivation of a *B. burgdorferi luxS* homolog also does not interfere with infection of mice by needle inoculation, which then suggests that chemotaxis proteins in general may not be essential for establishing a persistent infection in vertebrates (98).

1.4 Development of Anti-Lyme borreliosis Vaccines

There is currently no FDA-approved vaccine to prevent Lyme borreliosis, despite the 300,000+ cases of the disease that occur each year (14). The lack of preventative measures against *B. burgdorferi* infection has numerous implications, the economic burden of which has been

estimated at over $16,000 for a single late-stage/chronic Lyme patient (14). A vaccine would be highly beneficial to areas where *B. burgdorferi* is endemic, as 95% of 2015 Lyme borreliosis cases occurred in just two geographic foci comprising 14 U.S. states (14). Past research has demonstrated that numerous *B. burgdorferi* proteins are naturally immunogenic; however, the majority of natural *B. burgdorferi* immunogens occur on the spirochete's plasmids, which are naturally less stable and more dynamic than the chromosome (99, 100). However, it has also been observed that the plasmids cp26 and lp54 are highly conserved in all clinical *B. burgdorferi* isolates, and therefore it is no surprise that the immunogenic proteins from these genetic elements were among the first to be examined for potential as vaccine candidates (101, 102). OspA, as noted in the previous section, plays an indispensable role in the tick/vertebrate transmission cycle and has been thoroughly investigated for potential as a vaccinogen. It has been observed that pre- or co-administration of an OspA-based antibody to mice inhibited the transmission of *B. burgdorferi* from feeding ticks (103). Specifically, migration to tick salivary glands and spirochete multiplication in the feeding tick were disrupted due to passive immunization with anti-OspA antibodies. These results suggest that a pre-established immune response against OspA may prevent the transmission of *B. burgdorferi* from feeding ticks. Further experiments also found that a high titer of passive OspA immunization was necessary to expunge the spirochetes from a feeding tick, but a significantly lower titer was nevertheless protective against infection in the mouse model (104). These studies eventually led to the development of the first FDA-approved vaccine against *B. burgdorferi*, LYMErix, which used recombinant, lipidated OspA as its immunogen (105, 106). To compensate for the noted heterogeneity of the OspA protein, the most common North American OspA sequences were used as the basis for the vaccine (107). Initally, the outlook for LYMErix was promising.

However, some reports of neurological and rheumatological adverse effects were reported by users. Although it was determined that these adverse events of OspA vaccination were below background based on a comparison with the clinical trials, the subsequent unpopularity of the vaccine and the need for yearly booster vaccines led to its voluntary withdrawl in 2002 (108, 109). Investigation of the OspA epitope has revealed that the potential for cross-reactivity exists between it and the immune cell surface molecule LFA-1, which could promote the development of autoimmunity (110). The correlation of *B. burgdorferi* infection and autoimmune disorders has been previously observed in individuals with certain HLA-DR alleles (e.g. HLA-DR4) (111). These results suggest that certain genetic backgrounds are more susceptible to OspA-promoted autoimmunity, and this hypothesis is being addressed in the future generation of OspA-based vaccines through the engineering of OspA sequences to lack the molecular mimicry of the LFA-1 epitope (112). In addition, investigations into chimeric OspA sequences are being conducted so as to improve protection against multiple *B. burgdorferi* serotypes (113). Still other proteins are currently being investigated as potential vaccinogens. OspC, a lipoprotein essential for the transmission of *B. burgdorferi* to vertebrates, elicits an early, type-specific antibody response *in vivo*, suggesting potential role as a human immunogen (114). In contrast to OspA, however, is the diversity of OspC serotypes (115, 116). This remarkable diversity impedes development of OspC-based vaccines by preventing broad coverage based on immunization with a single serotype of the lipoprotein. To overcome this, chimeric OspC lipoproteins have been constructed based on conserved regions between different serotypes and are undergoing investigation for potential as vaccinogens (117, 118). These have the potential to convey broad coverage against *B. burgdorferi* without the pitfalls of OspA vaccines, although further testing is still needed to assess their efficacy in human subjects. Finally, other researchers are

investigating the novel avenues of tick salivary gland proteins or Δ*ospAB B. burgdorferi* cell preparations to induce protective immune responses (119, 120). In any case, it is undeniable that an anti-Lyme vaccine is needed and that future research should focus on development of prophylaxes against this disease.

Chapter 2: Lipoprotein Biosynthesis in *B. burgdorferi*

The understanding of lipoprotein biosynthesis in *B. burgdorferi* is primarily based on that of *E. coli*. Like *B. burgdorferi*, *E. coli* is a diderm bacterium that localizes lipoproteins to both its outer and inner membranes (25, 26, 121). Although *E. coli* does not express the multitude of surface lipoproteins characteristic of *B. burgdorferi*, recent studies have shown that some lipoproteins are indeed surface-exposed in this organism (122-126). The primary biochemical research in which the function of various biosynthetic enzymes was also conducted, for the most part, in *E. coli*. Therefore, the lipoprotein biosynthetic pathway will be described using *E. coli* as a model and, where appropriate, clarifications will be made for differences observed in *B. burgdorferi*. The lipoprotein biosynthesis genes in *B. burgdorferi* are given using the standard nomenclature in parentheses following the accepted *E. coli* common name (31, 32, 127).

Lipoproteins are first translated in the cytoplasm without lipid modification. The translated protein contains an N-terminal signal sequence, a characteristic region of amino acids that directs the unfolded protein to the Sec translocase for export into the periplasm. In some bacteria, certain lipoproteins are translocated in a folded conformation via the Tat (Twin arginine translocation) machinery; however, this system appears to be absent from *B. burgdorferi* and will not be discussed further (31, 32, 128). The signal sequence region typically contains three sub-regions: an N-terminal region containing positively charged amino acids, a central hydrophobic region with uncharged amino acids, and a C-terminal region containing a characteristic consensus sequence termed the "lipobox" (25, 26). In *E. coli*, the lipobox consensus sequence is typically Leu-[Ala/Ser]-[Gly/Ala]-Cys, with the final cysteine absolutely conserved in all lipoproteins and acting as the site of lipid modification (25, 26). *B. burgdorferi*, however, has been found to contain a much more degenerate lipobox (23). Based on comparisons of

experimentally verified lipoproteins, the *B. burgdoreri* lipobox likely includes one additional amino acid at the N-terminal side and tolerates a larger variety of residues at each position with the exception of the final cysteine residue, which is absolutely conserved in all lipoproteins. This degeneracy of the *B. burgdorferi* lipobox has complicated *in silico* calculation of the lipoprotein proteome (lipoproteome), and the identity of the *B. burgdorferi* has yet to be conclusively established.

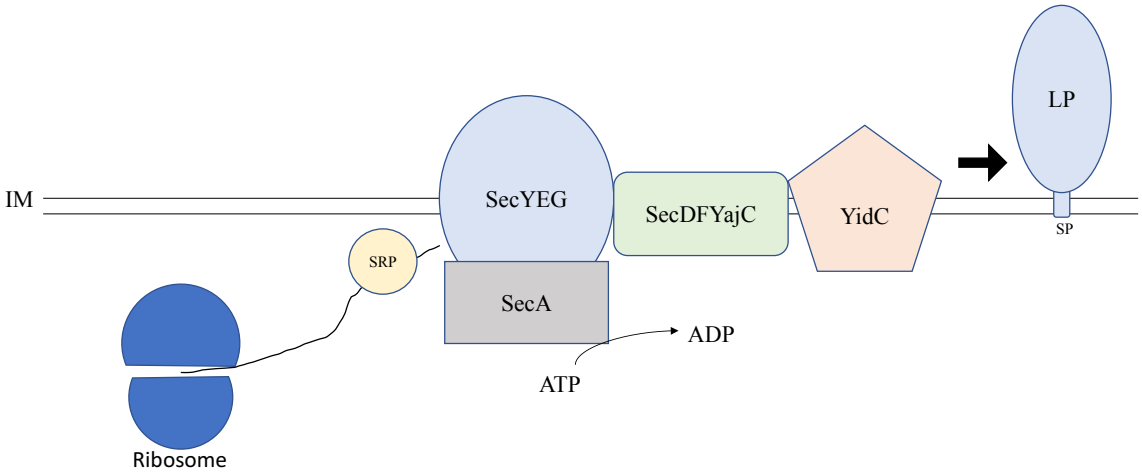
After translation, the unmodified lipoprotein peptide ("preprolipoprotein") is then directed to the Sec translocase for secretion to the periplasm (**Figure 1)** (25, 26, 121). Evidence exists that, in *E. coli*, lipoproteins are exported by Sec in a co-translational manner mediated by signal recognition particle (SRP; BB0694) and the inner membrane SRP receptor FtsY (BB0076) (129). The Sec translocase is composed of a primary heterotrimer of the channel-forming, integral inner membrane proteins SecY (BB0498), SecE (BB0395), and SecG (BB0054). The ATPase SecA (BB0154) provides the driving force for protein translocation through ATP hydrolysis. In addition, the integral membrane protein YidC (BB0442) assists with the lateral transfer of the hydrophobic signal sequence region into the inner membrane, anchoring the preprolipoprotein to the outer leaflet of the inner membrane (129). The accessory factors SecD (BB0652), SecF (BB0653), and YajC (BB0651) form an inner membrane complex that serves to assist protein translocation by coordingating YidC and SecYEG (130). These accessory proteins, unlike the other members of the Sec translocase, are nonessential (121). In addition, certain other bacteria (primarily the α/β/γ-proteobacteria) express the soluble cytosolic chaperone SecB (no homolog in *B. burgdorferi*) that presumably serves to prevent premature folding in proteins that are secreted post-translationally (131). Its absence from *B. burgdorferi*, and indeed many other bacterial classes, suggests that other means of preventing premature folding of secreted

proteins have evolved. The necessity of these genes in *B. burgdorferi* is suggested indirectly through transposon mutagenesis assays; no cells were recovered containing disruption of any of the above listed components of the Sec translocase (37). This suggests that, in contrast to *E. coli*, the SecDFYajC complex is essential in this organism.

After translocation, the preprolipoprotein is anchored at the outer leaflet of the inner membrane through the hydrophobic region of its N-terminal signal sequence. The preprolipoprotein is then modified through the addition of diacylglycerol to the thiol group of the absolutely conserved cysteine by a thioether linkage. This reaction is catalyzed by the inner membrane protein prolipoprotein diacylglyceryl transferase (Lgt; BB0362), with the diacylglycerol group derived from phosphatidylglycerol, an abundant membrane phospholipid (**Figure 2**) (132). The prolipoprotein is then liberated of its signal peptide through the action of lipoprotein signal peptidase (Lsp; Signal Peptidase II; BB0469). Lsp cleaves the prolipoprotein on the N-terminal side of the acylated cysteine, leaving the lipoprotein still embedded in the membrane through its diacylglycerol modification. It has been shown that cleavage of the signal sequence peptide is dependent on protein modification by Lgt, possibly due to the action of the diacylglycerol group in coordinating the Lsp-prolipoprotein interaction (133). *E. coli* Lsp is reversibly and noncompetitively inhibited by the cylic peptide antibiotic globomycin, preventing signal peptide processing and subsequent export of outer membrane lipoproteins (134, 135). Although data are limited, there is evidence that globomycin similarly inhibits Lsp homologs in the genus *Borrelia*, lending support to the notion that lipoprotein biogenesis proteins are highly conserved in diderm bacteria (136).

Cleaved signal peptides are then digested by an inner membrane protease, though the identity of this protease is currently unclear. Initally, the atypical serine protease SppA (no

Figure 1. Sec-dependent translocation of lipoproteins. Secretion of lipoproteins occurs co-translationally through the binding of signal recognition particle (SRP) to the N-terminal hydrophobic signal peptide (121, 129, 130). Translocation occurs through the SecYEG channel and is driven by SecA-catalyzed ATP hydrolysis. Insertion of the lipoprotein's signal peptide into the inner membrane is mediated by the integral membrane protein YidC, which associates with SecYEG through the SecDFYajC complex. IM = "inner membrane." LP = "lipoprotein." SP = "signal peptide."


IM
SecYEG
SecDFYajC
YidC
LP
SP
SRP
SecA
ADP
ATP
Ribosome

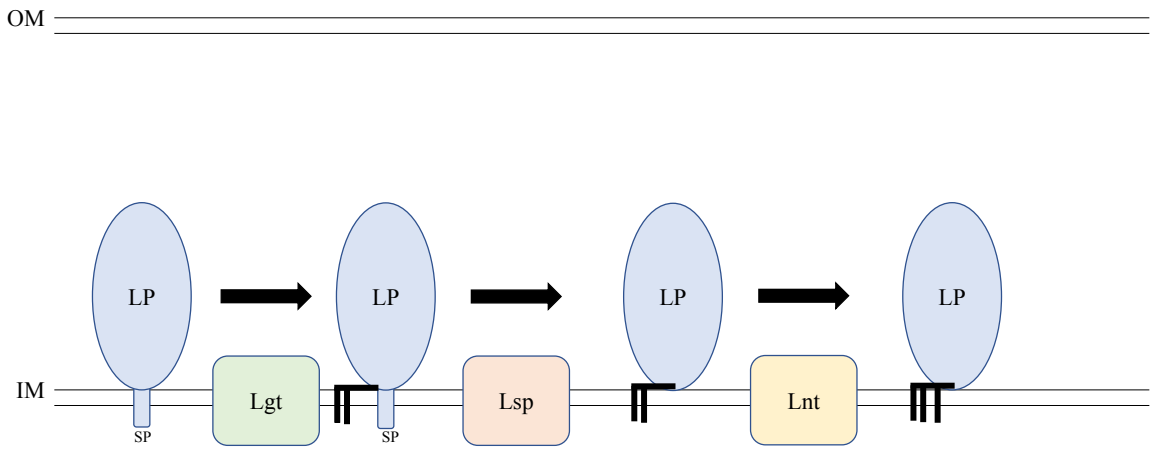
homolog in *B. burgdorferi*) was thought to be responsible for this digestion, as it was shown that SppA, also called Protease IV, could digest signal peptides in detergent extracts (137). Later studies instead pointed to RseP (BB0118), a member of the site-2 metalloprotease family I-CLiP, as the protein responsible for signal peptide degradation (138). RseP also plays a role in the regulation of the extracytoplasmic stress response through targeted cleavage of RseA (no homolog in *B. burgdorferi*), the anti-sigma factor for *E. coli* σ^E (139). While *sppA* is not an essential gene, *rseP* (*yaeL/ecfE*) is only dispensable when *rseA* is also deleted (140, 141). These results suggest that the accumulation of cleaved signal peptides may be tied to the bacterial extracytoplasmic stress response, and that multiple proteins may ultimately be responsible for degradation of cleaved signal peptides. The *rseP* gene could be disrupted by transposon mutagenesis in *B. burgdorferi*, suggesting that this gene is nonessential for *in vitro* growth (37). This is not surprising, as the *B. burgdorferi* genome does not contain RseP's cognate RseA nor does it contain a σ^E homolog, with only three (*rpoD* (BB0712)*, rpoN* (BB0450)*, rpoS* (BB0771)) sigma factor genes present in the genome (31, 32, 142). The dispensability of *rseP* in *B. burgdorferi* suggests that this organism possesses other, undiscovered means of degrading cleaved signal peptides.

The final step of lipoprotein biosynthesis is acylation of the newly liberated amino group on the N-terminal *S*-acylated cysteine through an amine bond (143). This is performed by the inner membrane protein apolipoprotein *N*-acyltransferase (Lnt; BB0237) using phosphatidylglycerol as the preferred acyl donor, although other phospholipids can be utilized (144). This final acylation step is primarily only found in Gram-negative bacteria, although exceptions do exist (notably, *Mycobacterium*) (145). Acylation by Lnt is a prerequisite for release of lipoproteins from the inner membrane, as in depletion of the otherwise-essential Lnt

protein in *E. coli* results in lethal retention of Lpp (no homolog in *B. burgdorferi*) in the inner membrane (146). Subsequent studies found that deletion of *lnt* could be tolerated by *E. coli* only when both the lipoprotein localization complex LolCDE was overexpressed and either Lpp or Lpp-peptidoglycan transpeptidases were absent (147). The other genes of lipoprotein biosynthesis, *lgt* and *lsp*, have likewise been found to be essential in *E. coli* (140). Interestingly, the gene for *lnt* is not essential in the Proteobacteria *Francisella tularensis* and *Neisseria gonorrhoeae* (148). The deletion of *lnt* in these bacteria had no major effects on cell physiology, suggesting that lipoprotein transport to the outer membrane was not dependent on *N*-acylation. The authors of this study suggest that the dispensability of *lnt* in these bacteria is linked to expression of a homodimeric, hybrid LolC/LolE protein (termed "LolF", see next paragraph) and speculate that bacteria expressing this hybrid protein may not require *N*-acylation of their lipoproteins. Regulation of lipoprotein processing could also be a means by which pathogenic bacteria regulate interactions with their host, a hypothesis which merits further inquiry in future studies (149). *B. burgdorferi* contains two distinct genes for LolC and LolE; therefore, it is believed that all lipoproteins are *N*-acylated and that this step is required for release of lipoproteins to the outer membrane. Supporting this are transposon mutagenesis experiments showing that no mutants could be recovered when *lgt, lsp,* or *lnt* were disrupted (37).

Lipoproteins are initially all situated at the outer leaflet of the inner membrane following the final processing step by Lnt. In order for these proteins to perform their proper biological function, they must then be sorted to an appropriate destination. In *E. coli*, most lipoproteins are either sorted to either the inner leaflet of the outer membrane or retained in the outer leaflet of the inner membrane, although some exceptions have recently been found (126). Proper sorting of lipoproteins is key to their biological role in the cell and, indeed, lipoprotein mislocalization

Figure 2. Post-translational modification of lipoproteins. After Sec-mediated secretion, lipoproteins undergo a sequential series of modifications in which the absolutely conserved N-terminal cysteine residue is lipidated and the N-terminal signal peptide is removed (25, 26, 143, 147). OM = "outer membrane." IM = "inner membrane." LP = "lipoprotein." SP = "signal peptide."


OM
LP
LP
LP
LP
IM
Lgt
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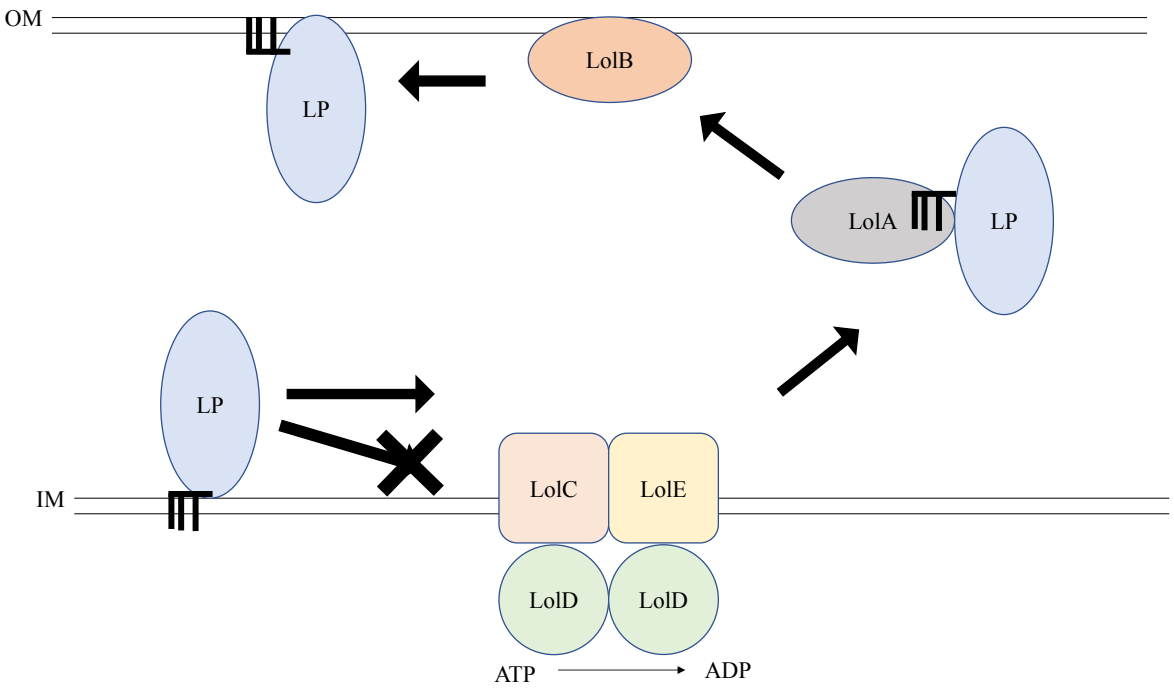
Lnt
SP
SP

can often prove detrimental to bacterial viability. In *E. coli*, for example, mislocalization of the major outer membrane lipoprotein Lpp to the inner membrane as a result of *lnt* deletion is toxic to the cell due to aberrant peptidoglycan crosslinking (147). Sorting of lipoproteins to the outer membrane is accomplished by the Lol machinery (25, 26, 121). The Lol sorting machinery is composed of an inner membrane protein complex of LolCDE, the periplasmic lipoprotein chaperone LolA, and the outer membrane lipoprotein acceptor LolB. Initially, lipoproteins either associate with the inner membrane LolCDE complex or "avoid" association with it based on whether the lipoprotein is destined to sort to the outer membrane or remain in the inner membrane. In *E. coli*, discrimination between inner and outer membrane lipoproteins is achieved through recognition of amino acid residues immediately following the acylated cysteine. Initially, it was found that an aspartic acid at the "+2" position, where the acylated cysteine represents "+1", causes lipoprotein retention in the inner membrane (150). It was later discovered that the amino acid identity at +3 can also affect inner membrane retention of lipoproteins in *E. coli* (151, 152). The mechanism behind this retention has been shown to be possibly the result of steric and electrostatic interactions of the +2 and +3 amino acids with the abundant membrane phospholipid phosphatidylethanolamine (153). Similar studies in the related bacterium *Pseudomonas aeruginosa* showed that the amino acids at +2/+3/+4 could all influence lipoprotein sorting in this organism (154, 155). However, in *B. burgdorferi* results have shown that retention of inner membrane lipoproteins is not strictly dependent on the amino acid identities at +2/+3/+4, and the exact logic by which *B. burgdorferi* retains lipoproteins in the inner membrane remains unknown (27-30, 156). In *E. coli,* the complex LolCDE is composed of the integral inner membrane proteins LolC (BB0078/0079, region originally mis-sequenced in B31) and LolE (BB0081), as well as a homodimer of the ATPase LolD (BB0080). The

homodimer of LolD binds two ATP molecules on the cytoplasmic face of the inner membrane, utilizing ATP hydrolysis to drive the transfer of lipoproteins from the inner membrane to the periplasmic chaperone LolA through the actions of LolC and LolE (**Figure 3**) (157). The proteins LolC and LolE show remarkable similarity to each other; however, despite their similarity they seem to play distinct roles in the cell (158). Site-directed crosslinking studies have shown that LolE is responsible for binding lipoproteins at the inner membrane whereas LolC binds LolA in the periplasm, with ATP hydrolysis driving a conformational change in the proteins that results in "mouth-to-mouth" transfer of lipoproteins from LolE to LolC and, finally, to LolA in the periplasm (159, 160). Studies in *E. coli* have shown that expression of the LolCDE complex is essential for cell viability, and that disruption of the complex results in the lethal accumulation of lipoproteins at the inner membrane (161). Likewise, transposon mutagenesis studies in *B. burgdorferi* have supported the hypothesis that expression of LolCDE is necessary for cell survival (37).

The periplasmic chaperone LolA (BB0346) is responsible for transport of lipoproteins between the inner and outer membranes. Originally termed "p20", it was first discovered in *E. coli* as the factor responsible for the release of soluble Lpp from spheroplasts (162). The solved crystal structure of LolA revealed that the protein contains a distinct hydrophobic cavity formed by an 11-stranded, antiparallel β-sheet in an unclosed β-barrel configuration with an α-helical "lid" (163). This hydrophobic cavity was proposed to contain the insoluble lipid moiety of the cargo lipoproteins, although to this date to LolA-lipoprotein co-crystals have been achieved. A subsequent crystallization of the *P. aeruginosa* revealed that it contains additional hydrophobic surface patches that, when mutated, reduce or eliminate lipoprotein transport function of LolA (164). The authors speculate that the surface hydrophobic patches of *P. aeruginosa* may

Figure 3. The sorting of lipoproteins via Lol machinery. Lipoproteins, after lipidation of the conserved cysteine residue and cleavage of the signal peptide, are sorted through the inner membrane LolCDE complex, the periplasmic chaperone LolA, and the outer membrane receptor LolB (25, 26, 157, 162, 165). Lipoproteins either interact with the LolCDE complex or avoid the complex based on bacterial species-specific sorting signals as described in the text.


OM
LP
LolB
LolA
LP
LP
IM
LolC
LolE
LolD
LolD
ATP
ADP

accommodate lipoprotein acyl chains, and that the entire lipid moiety may not be contained within the central hydrophobic cavity. In *E. coli*, LolA is essential for the survival of the bacterium and its depletion results in the toxic buildup of lipoproteins at the inner membrane (166). In *B. burgdorferi*, the homolog of LolA appears to be likewise essential for cell viability (37).

Integration of lipoproteins into the outer membrane is achieved by transfer from LolA to the outer membrane lipoprotein acceptor LolB (no homolog in *B. burgdorferi*), which is itself a lipoprotein (165). Depletion of LolB was lethal for cells and prevented the incorporation of outer membrane lipoproteins into *E. coli* total membrane fractions. Further, a soluble form of LolB was found to be able to accept lipoproteins from LolA *in vitro*. These results also show that lipidation of LolB is disensable for transfer of lipoprotein from LolA *in vitro*. Further experimentation revealed that soluble periplasmic LolB could compensate for loss of lipidated LolB *in vivo*, with the corresponding localization of lipoproteins to the outer membrane (167). Transient mislocalization of outer membrane lipoproteins to the inner membrane was noted, though, and showed that soluble LolB incorporated LolA-delivered lipoproteins into cell membranes indiscriminately. These results show that lipidation of LolB is dispensable for its function and primarily serves to assist LolB in properly integrating lipoproteins into the outer membrane. Site-directed crosslinking studies have shown that LolA transfers lipoproteins to LolB in a "mouth-to-mouth" fashion, wherein the lipid moiety moves from one hydrophobic cavity to the other while being constantly shielded from the hydrophilic periplasm (160). LolB has been crystallized in a soluble form and shows distinct structural similarity to LolA, despite a notable lack of primary sequence homology (163). It too has an unclosed β-barrel structure composed of 11 antiparallel β-strands and an α-helical lid, with a distinct hydrophobic cavity that

accepts the lipid group of transported lipoproteins from LolA. However, structural comparison of crystallized LolA and (soluble) LolB proteins shows that the central cavity of LolB is more hydrophobic than that of LolA (163). This difference in hydrophobicity results in an irreversible transfer of lipoproteins from LolA to LolB due to the difference in affinity of the lipid moiety for the two hydrophobic cavities (168). This allows for an energy-independent transfer of the lipoprotein cargo, a necessity in the ATP-devoid periplasm. Other structural differences between LolA and LolB also contribute to their different functions (169). The mechanism by which LolB releases bound lipoproteins and integrates their lipid moiety into the outer membrane, however, is currently unknown.

Unlike the other members of the Lol machinery, which are widely conserved in Gram-negative bacteria, LolB seems to be found only in the β- and γ-proteobacteria (170). This raises the interesting question of how other bacteria integrate lipoproteins into their outer membrane, as the insolubility of the lipid modification of lipoproteins implies that some outer membrane receptor must accept the lipid moiety from LolA. In *B. burgdorferi*, the abundance of outer membrane lipoproteins suggests that a functional homolog of LolB exists (27). The identity of this outer membrane lipoprotein receptor in *B. burgdorferi*, and whether it can be found in other bacteria that lack an identifiable *lolB* gene, is currently under investigation. Studies of soluble periplasmic LolB in *E. coli* have shown that it could at least somewhat compensate for the absence of lipidated, outer membrane LolB *in vivo* (167). These results suggest that soluble periplasmic factors can receive lipoproteins from LolA and that future searches for the receptor of LolA-lipoprotein complexes should not be limited solely to outer membrane proteins.

The majority of lipoproteins in *E. coli* are localized to the periplasmic face of the outer membrane or retained at the inner membrane. However, recent evidence suggests that surface

Figure 4. Alignment of the *B. burgdorferi* OppA homolog tether sequences. The tether sequences of the five OppA homologs were aligned using the Clustal Omega program using the default settings (173). The OppAIV (BB_B16) tether was defined based on its crystal structure, PDB# 4GL8. Other OppA tethers were extrapolated based on the OppAIV tethers. OppA homologs are annotated using the standard *B. burgdorferi* genetic locus notation (31, 32).

```
BBA34       CSAMSKPKDDI
BBB16       CVNESNRNK-L
BB0329      CNNKERKEG-V
BB0328      CISNAKKEK-I
BB0330      CNNNSEKEK-L
            *    . :  :
```

exposure of lipoproteins is a more common phenomenon than once thought. The major outer membrane lipoprotein Lpp has been known for some time to exist in both a peptidoglycan-bound form and an unbound form, and it was recently shown that the unbound form spans the periplasm and is exposed on the bacterial surface (122). This was the first recorded example of a membrane lipoprotein possessing two distinct cellular localizations. Subsequent studies also found that the membrane lipoprotein RcsF (no homolog in *B. burgdorferi*), the sensor protein of the Rcs envelope stress response system, was also found to adopt a unique bi-localization phenotype (123, 171). RcsF, after localization to the inner leaflet of the outer membrane, is "threaded" through the lumen of several β-barrel outer membrane proteins during assembly by the Bam Complex. This results in RcsF becoming localized on the bacterial surface at its N terminus, while the C-terminal signaling domain remains in the periplasm. RcsF seems to be able to utilize different outer membrane proteins, with interactions detected between RcsF and OmpA, OmpC, and OmpF. Finally, the pathogen *Neisseria meningitidis* expresses two noteworthy outer membrane proteins termed surface lipoprotein assembly modulators (Slam; no homolog in *B. burgdorferi*) (172). Slam proteins were shown to be responsible for export of the outer membrane lipoprotein TbpB to the bacterial surface and, when expressed in *E. coli*, Slam proteins were sufficient to reconstitute surface lipoprotein secretion. Conservation of Slam proteins in Proteobacteria genomes was also reported by the authors. Preliminary analysis of the *B. burgdorferi* genome shows that it does not contain an identifiable homolog to Slam. Because of the abundance of surface lipoproteins in *B. burgdorferi*, it would not be surprising for another membrane protein to be responsible for export of lipoproteins to the bacterial surface.

Chapter 3: Previous Research of *B. burgdorferi* Lipoproteins

Although the *E. coli* proteins involved in the biosynthesis have identifiable homologs in *B. burgdorferi* (with the notable exception of LolB), lipoprotein sorting appears to obey a completely different set of rules in this spirochete than in Proteobacteria (27-30, 156). Notably, *B. burgdorferi* does not follow the above described +2/+3/+4 rule that is present in other bacteria. A review of previously localized lipoproteins shows that residues in these positions are highly variable and residues seem to be common between inner membrane, outer membrane sub-surface, and surface lipoproteins (27-30). It has also been shown that, in contrast to the functional regions of the protein, the N-terminal region can be highly variable even in homologous lipoproteins. For example, an alignment of the five OppA homologs in *B. burgdorferi* shows significant variability in the +2 through +9 positions, in contrast to the conservation seen in the C-terminal regions of the proteins (**Figure 4**). As this N-terminal region was previously thought to contain the information necessary for lipoprotein sorting, this variability suggests that other, unknown factors may dictate sorting. Finally, mutagenesis experiments of the prototypical surface lipoproteins OspA and OspC have shown that, although deletion or mutagenesis of tether residues C-terminal from the +2/+3/+4 positions can modulate surface localization of these lipoproteins, their export to the outer membrane remains largely unaffected through tether mutations (28-30). Currently, it is not well understood why inner membrane lipoproteins are retained and not exported to the outer membrane, although interesting, testable hypotheses can be formulated based on existing data.

In contrast to sorting at the inner membrane, surface exposure of outer membrane lipoproteins has been characterized to a fuller extent. Mutagenesis assays have shown that alteration of key residues in OspA and OspC, residues in so-called “essential tether” motifs, can

cause sub-surface retention of an otherwise wild-type protein. In addition, deletion of OspC tether regions C-terminal of the essential tether can likewise cause subsurface retention of the lipoprotein. These results suggest that the N terminus of a mature lipoprotein plays a role in surface localization and that alteration of this sequence can modulate a lipoprotein's surface exposure (28-30). In addition, experiments involving fusion of OspA to calmodulin, a protein whose folding is dependent on the binding of Ca^{2+}, revealed that OspA containing "sub-surface" tether mutations could be exported to the surface in the context of an unstructured C terminus (174). In addition, a separate study found that a sub-surface mutant of OspA could be restored to the bacterial surface through fold-destabilizing mutations at the C terminus, indicating that lipoprotein secretion through the outer membrane may initiate at the C terminus (29). These results, taken together, suggest that lipoprotein surface exposure is an interplay between recognition of an N-terminal export signal and sufficient disorder at the C terminus. It has also been proposed that the driving force for export of lipoproteins to the surface is the potential energy gradient of protein folding outside of the cell, and that the N-terminal region is crucial in binding holding chaperones that prevent premature folding in the periplasm (25). This model would explain how lipoproteins are exported out of the periplasm in the absence of molecular energy sources such as ATP, as well as accounting for the retention of surface lipoprotein tether mutant that show sub-surface, premature folding (29, 174). However, the identity of the putative holding chaperone, the precise structure-function of the *B. burgdorferi* Lol homologs, the identity of a potential outer membrane LolA-lipoprotein complex receptor, and the outer membrane factors responsible for lipoprotein surface exposure all remain a mystery. Although evidence exists in *E. coli* that the outer membrane β-barrel assembly complex Bam can cause surface localization of lipoproteins by folding outer membrane proteins around them (see RcsF

in 1.3 The Fundamental Role of *B. burgdorferi* Lipoproteins, above), there is currently no evidence to support this function of the Bam complex in *B. burgdorferi*.

The development of a powerful "toolbox" of genomic and proteomic techniques, however, has put the field of *B. burgdorferi* research in a place to finally answer these questions. Although *B. burgdorferi* is not nearly as experimentally tractable as the model organism *E. coli*, endeavoring investigators have discovered ways to manipulate the bacterium in order to overcome many of the limitations inherent to the spirochete. *B. burgdorferi* can be cultured *in vitro* to high densities using artificial culture medium, in contrast to certain other spirochetes such as *Treponema pallidum* (175-177). *B. burgdorferi* can also be transformed with foreign DNA through electroporation, and the development of *E. coli-B. burgdorferi* shuttle vectors has facilitated cloning of spirochetal genes (178, 179). Techniques to localize proteins in *B. burgdorferi* are well-established and include surface "shaving" using a membrane-impermeable protease and fractionation of the outer membrane of the cell from the protoplasmic cylinder (180, 181). Analysis of the products of these assays is accomplished by Western Blot using antisera specific to characteristic proteins (27-30, 156). Finally, it was recently shown that *B. burgdorferi* detergent extracts of surface shaving assays are amenable to proteomics analysis by Multidimensional Protein Identification Technology (MudPIT) (27). This demonstrates that existing protocols can be applied to emerging, high-throughput technologies to expedite characterization of the *B. burgdorferi* proteome.

Chapter 4: Localization of *B. burgdorferi* Lipoproteome

(Note: An abridged version of this chapter was published as: Dowdell AS, Murphy MD, Azodi C, Swanson SK, Florens L, Chen S, Zückert WR. Comprehensive Spatial Analysis of the Borrelia burgdorferi Lipoproteome Reveals a Compartmentalization Bias toward the Bacterial Surface. J Bacteriol. 2017 Feb 28;199(6). pii: e00658-16. doi: 10.1128/JB.00658-16. PubMed PMID: 28069820)

4.1 Rationale for Study

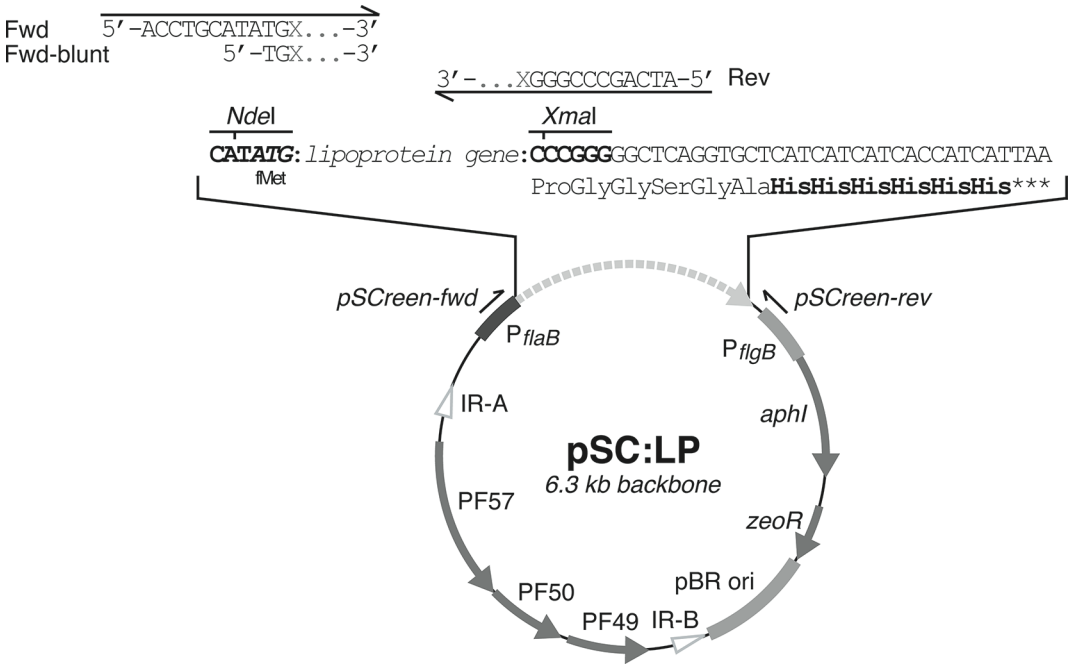
It has been known for some time that certain proteins are indispensable for infection of vertebrates by *B. burgdorferi* (182). For example, the protein OspC is required to establish an infection in the mouse model of Lyme borreliosis, and the genetic locus containing the antigenic variation protein VlsE is necessary for persistence in immunocompetent mice (71, 82). It was also discovered early on that many of these critical proteins are lipoproteins and are immunogenic in humans (46, 99). Therefore, a significant portion of the past few decades of research has been focused on the clinical importance of lipoproteins in the prevention and treatment of Lyme borreliosis (112-114, 117, 118). These efforts yielded the first FDA-approved, anti-Lyme vaccine (LYMErix) at the end of 1998 (101, 105, 106). This vaccine was based on immunization against the *B. burgdorferi* lipoprotein OspA, which is expressed in the unfed tick prior to influx of the blood meal. However, the vaccine's perception by the public was tainted by reports of vaccine-related arthritis and other complications (108, 109). While the CDC and FDA evaluated all available data and concluded that no correlation exists between the vaccine and the reported adverse events, the vaccine was nevertheless perceived negatively by the public and sales declined. In addition, it was found that high anti-OspA titers are required in

order to prevent *B. burgdorferi* transmission, further complicating adoption of LYMErix. For these reasons, the vaccine was voluntarily removed from the market in 2002, just a few years after its introduction.

Previous research has focused on just a limited subset of lipoproteins, with the focus on those that have a clear role in pathogenesis or application as a possible vaccinogen. This has left many lipoproteins under-studied, aside from cursory *in silico* homology analysis. One complication to the further study of *B. burgdorferi* lipoproteins is that, due to their comparatively degenerate lipobox region, it is difficult to predict lipoproteins based from primary sequence alone. The "gold standard" for identifying protein lipid modification, the detection of incorporated [^{3}H]-palmitate into an immunoprecipitated protein, is low-throughput and ideally requires antisera to the protein of interest (46). As such, study of *B. burgdorferi* lipoproteins focused on a handful of "usual suspects" for some time and neglected a potentially large fraction of the remaining *B. burgdorferi* proteome.

In 2006, an algorithm was developed specifically for prediction of lipoproteins in spirochetal genomes. Utilizing experimentally verified lipoproteins as a training set, the SpLip algorithm identified the putative "lipoproteome" of the *B. burgdorferi* type strain B31 (23). Many of the proteins identified as lipoproteins in this study were "under-studied" and lacked the in-depth characterization that had been performed for other lipoproteins such as OspA. Using this *in silico* prediction, our group hypothesized that we could perform an initial characterization of this putative lipoproteome through localization of each protein to a specific cellular location. Based on our previous observation that lipoproteins seem to be secreted to the surface by default, we hypothesized that the majority of lipoproteins would be surface-localized (30). We also hypothesized that homologous lipoproteins would localize similarly, such as those encoded by

Figure 5. Plasmid map of the *B. burgdorferi* lipoprotein expression library vector backbone pSC-LP. pSC-LP is a derivative of the *B. burgdorferi-E. coli* shuttle vector pBSV2 (29, 179) that drives expression of cloned genes using the *B. burgdorferi flaB* promoter (P_{flaB}) and provides a C-terminal hexa-histidine tag preceded by a flexible 6-amino-acid linker peptide. Restriction enzyme sites and primer sequences used for amplification and cloning of lipoprotein genes are indicated (see also **Table 1** and text).


Fwd 5′-ACCTGCATATGX...-3′
Fwd-blunt 5′-TGX...-3′
3′-...XGGGCCCGACTA-5′ Rev
NdeI
XmaI
CATATG: lipoprotein gene:CCCGGGGGCTCAGGTGCTCATCATCATCACCATCATTAA
fMet
ProGlyGlySerGlyAlaHisHisHisHisHisHis***
pSCreen-fwd
pSCreen-rev
PflaB
PflgB
IR-A
aphI
pSC:LP
6.3 kb backbone
PF57
zeoR
PF50
pBR ori
PF49
IR-B

the evolutionarily related cp32 family of plasmids (100, 183). Finally, we hypothesized that, after localizing the entire lipoproteome, sorting rules for *B. burgdorferi* could be established similar to those in Proteobacteria (150, 154).

4.2 Experimental Design

(Note: An unabridged version of experimental protocols can be found in **Chapter 5**)

4.2.1 Bacterial Strains and Culture Conditions

E. coli strains TOP10 and DH5α (Invitrogen) were used for plasmid cloning and propagation. *E. coli* was grown in LB-Miller broth or on LB-Miller agar plates (both from BD Difco) at 37°C, supplemented with 50 μg/mL kanamycin (Sigma-Aldrich) as necessary. *B. burgdorferi* strain B31-e2, a high-passage noninfectious clone of the type strain B31 (95) (provided by Dr. Brian Stevenson, Univ. of Kentucky, Lexington, KY), was chosen due to its amenability to transformation and established use in lipoprotein localization assays (28-30). Additionally, low-passage infectious *B. burgdorferi* B31-A3 (184) (provided by Dr. Patti Rosa, NIH/NIAID Rocky Mountain Laboratories, Hamilton, MT) was used for proteomic analysis of cellular protein fractions by Multidimensional Protein Identification Technology (MudPIT) mass spectrometry. B31-A3 was confirmed to contain all linear and circular plasmids characteristic of strain B31 with the exception of lp5, using a set of multiplex-PCR-compatible oligonucleotide primers (185) (data not shown). *B. burgdorferi* B31-e2 and B31-A3 were maintained in BSK-II complete medium at 34°C, containing 300 μg/mL kanamycin as necessary (175, 177). Recovery of *B. burgdorferi* transformants was performed using semi-solid BSK-II as described, with plates incubated at 34°C in a humidified 5% CO_2 atmosphere until the development of colonies (175, 177).

4.2.2 Construction of an epitope-tagged B. burgdorferi lipoprotein expression library

A total of 127 *B. burgdorferi* type strain B31 lipoproteins were selected for the study based on the cumulative list of "probable", "possible" and "false negative" lipoprotein genes identified by the SpLip algorithm (23) (**Table 2**); of note, this algorithm-based list omits some lipoproteins such the variable surface lipoprotein VlsE (81). Genomic DNA from cultured low-passage infectious *B. burgdorferi* B31-A3 was isolated (Promega Wizard gDNA Kit), and lipoprotein genes were amplified by PCR using Phusion HF enzyme (New England Biolabs) with gene-specific oligonucleotide primers (Integrated DNA Technologies) containing 5' NdeI and XmaI restriction site extensions, respectively (**Table 1**). The resulting PCR amplicons were digested with NdeI and XmaI and ligated into pSC:LP (**Figure 5**), a vector backbone derived from recombinant plasmid pSC1000 (29) by digestion with NdeI and XmaI. pSC1000, a derivative of the *B. burgdorferi-E. coli* shuttle vector pBSV2 (179), expresses the lipoprotein OspA (BBA15) under the constitutive flagellin promoter (P_{flaB}) with a C-terminal his-tag; the NdeI (CA'TATG) and XmaI (C'CCGGG) sites are within the start codon and the his-tag linker, respectively. Due to *Borrelia* DNA being about 70% AT, this approach allowed for the direct amplification by PCR and in-frame cloning of 115 *Borrelia* lipoprotein genes. 10 lipoprotein genes containing internal NdeI sequences were amplified and cloned using a modified forward PCR primer that produced an amplicon with a 5' blunt-end compatible with the pSC:LP NdeI site filled in using Klenow (**Figure 5**). BB0352 and BBB16 (*oppA4*) were cloned under their native promoters, taken to be within about 150 bp upstream of their respective start codons. Recombinant plasmids were recovered from cultured *E. coli* transformants using the QIAprep Spin Kit according to the manufacturer's instructions (QIAGEN) and screened for the expected

Table 1. List of oligonucleotide primers used for *B. burgdorferi* lipoprotein amplification.
Oligonucleotide sequences used to amplify and clone lipoprotein genes as described in the text.

ORF	Cloning Primer Fwd	Cloning Primer Rev
BB_A15	N/A - this is pSC1000	N/A - this is pSC1000
BB_0365	ACCTGCATATGTATAAAAATGGTTTTTTTAAAAAC	ATCAGCCCGGGATTCGTTAACATAGGTGAAATTTT
BB_B19	ACCTGCATATGAAAAAGAATACATTAAGTGC	ATCAGCCCGGGAGGTTTTTTTGGACTTTCTGC
BB_B16	CATGGAGGAATGACATATGAAAATATTGATAAAAAAGTTAAAAG	ATCAGCCCGGGTTTAATTGGTTTTATTTCAGATAAATT
BB_0328	ACCTGCATATGAAAAAGGAAAATCCCATGAAATAT	ATCAGCCCGGGTTTTTTAGTTTTAATATCTTCATATAAAT
BB_0329	ACCTGCATATGAAATTACAAAGGTCATTATTTTTA	ATCAGCCCGGGTTTATTTTTTAATTTTAGCTGAGATA
BB_0330	ACCTGCATATGAGCTTTAATAAAACTAAAAAAATC	ATCAGCCCGGGATTATGTTTTGCATTTTTAATTGG
BB_A24	ACCTGCATATGATTAAATGTAATAATAAAACTTTT	ATCAGCCCGGGGTTATTTTTGCATTTTTCATCAG
BB_A16	GGAGGAATGACATATGAGATTATT	CCCCGGGTTTTAAAGCGTT
BB_P38	TGAATAAGAAAATGAAAATGTTTAT	ATCAGCCCGGGTTTTAAATTTCTTTTAAGCTCTTC
BB_J09	ACCTGCATATGAAAAAATTAATAAAAATACTACTG	ATCAGCCCGGGAGTATTTAACAAGGCCACAAC
BB_R42	ACCTGCATATGAATAAAAAAATAAAAATGTTTATT	ATCAGCCCGGGTTCTTTTTTACCTTCTACAGTTTC
BB_S41	ACCTGCATATGAATAAGAAAATGAAAAATTTAAT	ATCAGCCCGGGTTTTTTATCTTCTATATTTTGAGG
BB_B08	ACCTGCATATGAAAAAAAAGTTTAATTTTATTTTTC	ATCAGCCCGGGTTGTAAAAATTTTTCAATTGC
BB_0028	ACCTGCATATGAAACAAAAATACGAAAAC	ATCAGCCCGGGTTCTTTAGTTAATTTTCTGTTTTC
BB_0689	ACCTGCATATGAAAAAATTGATTATAATTTTTAC	ATCAGCCCGGGATTCTTATATTTTCTTTTTCCAA
BB_0840	ACCTGCATATGAAGAATATTAATAGATTAATATTATT	ATCAGCCCGGGACTTCCTGAGCACCAG
BB_B27	ACCTGCATATGAAGAAGTTTTTAATATCCG	ATCAGCCCGGGAGTTAAAAACTTTTTGAGTATATAT
BB_0324	ACCTGCATATGAAAAAGCTAATATTACTAAAC	ATCAGCCCGGGTTTTTTATTATTTTCTATTTTATTTAAT
BB_0832	ACCTGCATATGTTAAAAACATTAACAAAAATA	ATCAGCCCGGGGGTTTGAATATTTGAAGCTAG
BB_0144	ACCTGCATATGTATAAATTATTTTTATTTTTTATTAT	ATCAGCCCGGGATCAAATAAGGTCTTATATTTTTC
BB_0382	ACCTGCATATGAGAATTGTAATTTTTATATTCG	ATCAGCCCGGGTAACTTTAATATTTGTTTTATAAAAAT
BB_0628	ACCTGCATATGAGAAAGTGTTTTGTTAG	ATCAGCCCGGGATAAACTAATCTAAGTTTTATTGG
BB_0141	ACCTGCATATGAATTTGATTTTTAATATTAATTTAT	ATCAGCCCGGGAATATTGCTTTCGGCTG
BB_B25	ACCTGCATATGAAATACTGTTTTTCTTTG	ATCAGCCCGGGTAAAATTTTGTTTTTAAATGCAT
BB_0542	ACCTGCATATGGATAATTTAAATAGTATAAATAGT	ATCAGCCCGGGACTCTTAAGAATAGAAGAAAC
BB_0227	ACCTGCATATGCTAAATCCAAGAACAA	ATCAGCCCGGGTTTTACAAATTGTGCTATTTC

BB_0460	ACCTGCATATGAAAACTAGAATAATCATTTTTC	ATCAGCCCGGGAGACTGAGAGTGAAGG
BB_0298	ACCTGCATATGAAAATTTTGTGGTTAATAA	ATCAGCCCGGGCTTCAAATTACTCATGATCC
BB_0215	ACCTGCATATGAAAAAAGTTATTATCTTAATTTT	ATCAGCCCGGGTGTTTTTATCCCTAAAAAGC
BB_0385	ACCTGCATATGTATTATGAGATCTATTTTTTG	ATCAGCCCGGGATTTTCCATTTGCAAAACA
BB_0323	TGAATATAAAGAATAAATTAATATCGC	ATCAGCCCGGGTTTGGCAGGAATTATTATCTTC
BB_0664	ATAATCATATGTTACATAATACAATGC	ATCAGCCCGGGTTGGGCTTTGATTTCTTG
BB_0398	ACCTGCATATGAATCTATTGGTCAAAATTG	ATCAGCCCGGGTTTTTTACCTAAATACACGC
BB_0155	ACCTGCATATGAAAAAACACTATAAAGCTC	ATCAGCCCGGGTAATTTTAATAATGCATTTAAATTCC
BB_0384	ACCTGCATATGTTTAAAAGATTTATTTTTATTAC	ATCAGCCCGGGTAACTTTGATTTAAATAAATCAAATG
BB_0158	ACCTGCATATGGTATTTAGAACATATAAAC	ATCAGCCCGGGTATATCTTCAAATAAATCTCCAG
BB_0806	TGAAATTTGTTTTGAATAATTTATTTAAAG	ATCAGCCCGGGTAACTGTTTTTTAATATTTTCATC
BB_0758	ACCTGCATATGCTGCAAAAAAGTATGG	ATCAGCCCGGGATTGATAACTATTTTTTGAGG
BB_0844	ACCTGCATATGAAAAAAAAAAATTTATCAATTTAC	ATCAGCCCGGGTTTACTCGTCTCTAAAAAATC
BB_0193	ACCTGCATATGTCTGGCCCTAAAAAAC	ATCAGCCCGGGTTGTTTATAAATGTTAAATTCATATTG
BB_0823	ACCTGCATATGAATACAAAAACATTATATTTAATA	ATCAGCCCGGGTTTAGAATGATCAATTATTAAATTG
BB_0652	ACCTGCATATGAAAAAAGGATCTAAGC	ATCAGCCCGGGATTACTCTTTGCATATTTTGAAC
BB_0536	GGAGGAATGACATATGAATTATCAAAG	TGAGCCCCCGGGTTCAGGTATTAG
BB_0224	ACCTGCATATGCCTGGAAGAAAGG	ATCAGCCCGGGATTGTATTGGTAATTAAAGCA
BB_0475	ACCTGCATATGATGAAAGCTCAAAAATTTA	ATCAGCCCGGGTTGTTTTTCAAAAATGTATAATG
BB_0213	ACCTGCATATGCAGAGCGGATTAAA	ATCAGCCCGGGGCCAACTTTAAAAAAAAGATT
BB_0171	ACCTGCATATGGGACGAAACTTTTTAG	ATCAGCCCGGGCCAATCTTCTTTTGGAACAG
BB_0456	ACCTGCATATGAAGCAAAAATTAAGTTG	ATCAGCCCGGGAATTCCAGGCGATAAAAC
BB_0352	ACCTGCATATGAATAATTTTATGAGAATAAAAAAT	ATCAGCCCGGGATTTTTATTTTTTTTTAGATTATTAGC
BB_B09	ACCTGCATATGAAATACCTTAAAAACATTTC	ATCAGCCCGGGAAATTTATGCCTACTTGATTG
BB_A62	ACCTGCATATGACAAAATTAATGTACGC	ATCAGCCCGGGCTTTTTCATTGACTTTGTC
BB_A57	ACCTGCATATGAACGGCAAGCTTAG	ATCAGCCCGGGTTGATAATTTTTTTCTACCAATAA
BB_A33	ACCTGCATATGAAGAGATATATTTATGTATATATC	ATCAGCCCGGGTTTTTGAATTAGTGCTAAAGC
BB_A34	ACCTGCATATGATAATAAAAAAAAGAGGAC	ATCAGCCCGGGTTCTTCTATAGGTTTTATTTCTG

BB_A05	ACCTGCATATGAATAAAATAGGAATTGCAT	ATCAGCCCGGGACGCCTGCTAATCTCTTTTA
BB_A59	ACCTGCATATGGTTAAAAAAATAATATTTATTTC	ATCAGCCCGGGATTGCTCTGCTCACTCTC
BB_A69	ACCTGCATATGAAAAAAGCCAAACTAAATAT	ATCAGCCCGGGATAAAAGGCAGATTGTAAAG
BB_A04	ACCTGCATATGAAAAGAGTCATTGTATCC	ATCAGCCCGGGGTTAATTAACGAATTAAATGC
BB_A64	ACCTGCATATGAAGGATAACATTTTGAAA	ATCAGCCCGGGCTGAATTGGAGCAAGAATA
BB_A07	ACCTGCATATGTGTGGGAGACGTATG	ATCAGCCCGGGAAACGAAGCAGATGCATC
BB_A68	ACCTGCATATGAAAAAAGCCAAACTAAATAT	ATCAGCCCGGGGTAAAAGGCAGGTTTTAAAG
BB_A36	ACCTGCATATGATGCAAAGGATAAGTATT	ATCAGCCCGGGAACATTTCCATAATTTTTCAAA
BB_A65	ACCTGCATATGAATAAAATAAAATTATCAATATTAAT	ATCAGCCCGGGATTATTATTAAATTTAAATAATGTGTC
BB_A32	ACCTGCATATGAGAATTACCGGTCTTC	ATCAGCCCGGGAAGCTTTCTGTTTCTACG
BB_A03	ACCTGCATATGAAAAAAACGATTATTGTATT	ATCAGCCCGGGTATAGTGTCTTTAAGTTTATTAAGT
BB_A72	ACCTGCATATGCATAAGGAGAGTGTT	ATCAGCCCGGGTTCATTTTTCTGATCTTTTAAC
BB_A14	ACCTGCATATGCAAATTAAAAATTTCCCC	ATCAGCCCGGGAGGTATATTTTTTGAGTATTTTTTG
BB_P28	ACCTGCATATGAAAATCATCAACATATTATT	ATCAGCCCGGGGGACCCATTGCCGCAG
BB_P39	ACCTGCATATGAATAAAAAAACAATTATTATTTG	ATCAGCCCGGGATCTTCTTCATCATAATTATCC
BB_P27	TGAGAAATAAAAACATATTTAAATT	ATCAGCCCGGGATTAGTGCCCTCTTCGAG
BB_S30	ACCTGCATATGAAAATCATCAACATATTATTT	ATCAGCCCGGGGCCACCATTATTGCAGTT
BB_R40	ACCTGCATATGAATAAGAAAATGAAAAATTTA	ATCAGCCCGGGGCCAATACTTTGCTCATC
BB_R28	ACCTGCATATGAAAATTATCAACATATTATTTTG	ATCAGCCCGGGTGAATTTTTGCACGTACTAC
BB_M38	ACCTGCATATGGAGCAACTTATGAATAAG	ATCAGCCCGGGTTCTTTTTTATTAGAATCTTTAGAT
BB_M28	ACCTGCATATGAAAATCATCAACATATTATT	ATCAGCCCGGGGGAACCACCATTGTTGC
BB_M27	TGAGAAATAAAAACATATTTAAATT	ATCAGCCCGGGATTAGTGCCCTCTTCGAG
BB_O39	ACCTGCATATGAATAAGAAAATGAAAATGTT	ATCAGCCCGGGTTCTTTTTTATCTTCTTCTATTC
BB_O40	ACCTGCATATGAATAAAAAAATATTGATTATTTTTG	ATCAGCCCGGGATATGAATTACTATCCTCAATG
BB_O28	ACCTGCATATGAAAATTATCAACATATTATTTTG	ATCAGCCCGGGATTGCAGGTAGCAGTTGC
BB_L39	TGGAGAAATTTATGAATAAGAA	ATCAGCCCGGGTTTTAAATTTCTTTTAAGCTCTTC
BB_L40	ACCTGCATATGAATAAAAAAACAATTATTATTTG	ATCAGCCCGGGATCTTCTTCATCATAATTATCC

BB_L28	ACCTGCATATGAAAATCATCAACATATTATT	ATCAGCCCGGGGGAACCGTTGCATGTAG
BB_N38	TGAATAAGAAAATGAAAATGTT	ATCAGCCCGGGTTTTAAATTTTTTTTAAGCACTTC
BB_N39	ACCTGCATATGAATAAAAAAACATTGATTATT	ATCAGCCCGGGCTGACTGTCACTGATGTATC
BB_N28	ACCTGCATATGAAAATTATCAACATATTATTTTG	ATCAGCCCGGGTTGCTGAGCTTGGCAG
BB_C10	ACCTGCATATGCAAAAAATAAACATAGC	ATCAGCCCGGGATCTTCTTCAAGATATTTTATTATAC
BB_D10	ACCTGCATATGAATTCAAAATTTATTTTAAAG	ATCAGCCCGGGACTGCCACCAAGTAATTTAAC
BB_E08	ACCTGCATATGCAAAAAAATGTTTATTG	ATCAGCCCGGGTAAAACAAAATTTGATAAGCA
BB_E31	TGAAATACCATATAATCGTAAG	ATCAGCCCGGGAAGGTTGCTTATTGACAA
BB_E04	ACCTGCATATGAAAAACCCCAAATCAA	ATCAGCCCGGGATTTTGGTTATTTTCTGGA
BB_F20	ACCTGCATATGAACAAAAAATTTTCTATTTC	ATCAGCCCGGGGTTGCTTTTGCAATATGAA
BB_F01	ACCTGCATATGAAATTATTAAAAATATTTATGTGTG	ATCAGCCCGGGACTTAAACCCTTTACACTT
BB_G25	ACCTGCATATGAAAAATTTAAAGACAAAAATT	ATCAGCCCGGGTCTTCTGCTGTATTTTTTG
BB_G01	ACCTGCATATGAGAAAAAGTTTGTTTTTAT	ATCAGCCCGGGTTTTTTATTGAGACTTTCTAAAAC
BB_H18	GAGGAATGACATATGAAAATGAAGG	TGAGCCCCCGGGTAGGCTAATACC
BB_H32	ACCTGCATATGAAATATAATACGATTATAAGC	ATCAGCCCGGGAAGGGTATATATTAAAATTATCTCG
BB_H06	ACCTGCATATGAAAAAAAGTTTTTTATCAAT	ATCAGCCCGGGTAATAAAGTTTGCTTAATAGCT
BB_H01	ACCTGCATATGAACAAATTATTGATATTCAT	ATCAGCCCGGGGTAATAATGGTTGAACGTG
BB_H37	ACCTGCATATGATTAAGGGAAAGGAGAG	ATCAGCCCGGGAGACTTACTTAAAGCATTTTTTG
BB_I28	ACCTGCATATGAAATGCCATATAATTGC	ATCAGCCCGGGAATCCGACAGATCTGG
BB_I16	ACCTGCATATGAAATACCACATAATTACA	ATCAGCCCGGGAAGTTTATATTTTGACACTATAAG
BB_I42	ACCTGCATATGAGGATTTTGGTTGG	ATCAGCCCGGGTGTAGGTAAAATAGGAACTG
BB_I36	ACCTGCATATGAAAAACTTTAAATTAAATACTATT	ATCAGCCCGGGATCAGCTTGATCTAGTAGAT
BB_I38	ACCTGCATATGAAAAACTTTAAATTAAATACTATT	ATCAGCCCGGGATCAGCTTGATCTAGTAGAT
BB_I39	ACCTGCATATGAAAAACTTTAAATTAAATATTATTAA	ATCAGCCCGGGATCAGCTTGGTTTAGTAG
BB_I14	ACCTGCATATGAAAAACAAAATAATTTTATG	ATCAGCCCGGGGTTTTTTATTTTATCAGCATG
BB_I29	ACCTGCATATGAAAAACAACATAATTTTATG	ATCAGCCCGGGATTCGGCAGTGATAATATG
BB_K50	ACCTGCATATGAATTTAATAATTAAAGTGATGTTG	ATCAGCCCGGGTCTAGAGTCCATATCTTGC
BB_K07	ACCTGCATATGAGTAAACTAATATTGGC	ATCAGCCCGGGATTATTAAAGCACAAATGTATG

BB_K12	ACCTGCATATGAGTAAACTAATATTGGC	ATCAGCCCGGGGCTTAAAGTTGTCAATGTT
BB_K48	ACCTGCATATGAATTTAATTAATAAATTATTTATTCTA	ATCAGCCCGGGTCTAGAGTCCATATCTTGC
BB_K19	ACCTGCATATGAAAAAATATATTATCAATTTAAGT	ATCAGCCCGGGATTGTTAGGTTTTTCTTTTCC
BB_K53	ACCTGCATATGAGGATTTTGGTTGG	ATCAGCCCGGGTGTAGGTAAAATAGAAACTGG
BB_K52	ACCTGCATATGAAAAAGAACATATATATTTTG	ATCAGCCCGGGTTCATCAGTAAAAAGTTTTAATT
BB_K32	ACCTGCATATGAAAAAAGTTAAAAGTAAATATTTG	ATCAGCCCGGGGTACCAAACGCCATTCTTG
BB_K01	ACCTGCATATGAGAAAAAGTTTGTTTTTAT	ATCAGCCCGGGTTTTTTATTGAGACTTTCTAAAAC
BB_J36	ACCTGCATATGAAAAGGAAAAGCAATATATG	ATCAGCCCGGGAAGCGCAACTTTTTTATAAAC
BB_J34	ACCTGCATATGATAAAAGGCAATACGT	ATCAGCCCGGGTTTATCTTTATTTTTAGGCTTAAC
BB_J41	ACCTGCATATGAAAAACCTTAAATTAAATATTATT	ATCAGCCCGGGATCAGCTTGGTTTAGTAGAT
BB_J01	ACCTGCATATGAAATACCATATAATCGTAAG	ATCAGCCCGGGTCCTGCACTAGCTGTTTC
BB_J47	TGAGGAATATTAGCAATTGTATC	ATCAGCCCGGGGTAAGAACTATTCTCCTTTTC
BB_Q46	ACCTGCATATGATTAAAAATGTAATTTATATTTTAC	ATCAGCCCGGGTCCTACAATCCAAATTTTG
BB_Q05	TGAAATACTATATATGTGTGT	ATCAGCCCGGGAAGGTTACTTATTGAAAATATCTG
BB_Q03	ACCTGCATATGAGGATTTTGGTTGG	ATCAGCCCGGGTAAAATTTTTCCATTAATTGTATTT
BB_Q47	ACCTGCATATGAATAAAAAAATGAAAATATTTATT	ATCAGCCCGGGCTGACTGTAACTGATGTATC
BB_Q35	ACCTGCATATGAAAATCATCAACATATTATT	ATCAGCCCGGGGTTTTGCCAATTAGCTG
BB_Q89	ACCTGCATATGAACAAATTATTGATATTCAT	ATCAGCCCGGGGTAATAATGGTTGAACGTG
N/A	CCCAGGCTTTACACTTTATGC	GGCCTCTTCGCTATTACGC

insert by PCR using Taq polymerase and a primer pair complementary to pSC:LP sequences 5' of the NdeI site and 3' of the XmaI site, respectively (pSCreen-fwd, 5'-AAGGATTTGCCAAAGTCAG-3'; pSCreen-rev, 5'-GTAAAACGACGGCCAG-3')(**Figure 5** and **Table 1**). PCR-positive clones were sequenced to verify the expected insert sequence (ACGT; Eton Bioscience). *E. coli* clones harboring the verified recombinant plasmids were stored in LB containing 15% (v/v) glycerol at -80°C.

Next, *B. burgdorferi* B31-e2 were transformed with the verified plasmids by electroporation (178), cloned by plating in semi-solid BSK-II and expanded in liquid BSK-II as described (30). Cultures of putative transformants were screened by both PCR using gene-specific primers (see **Table 1**) and by Western Blot of whole cell lysates with the HisProbe-HRP reagent (Thermo Fisher) according to the manufacturer's instructions. Clones that expressed epitope-tagged protein were then expanded in BSK-II and used in subsequent assays. Verified *B. burgdorferi* clones were stored in BSK-II containing 10% (v/v) DMSO at -80°C (177).

4.2.3 Surface proteolysis of intact B. burgdorferi spirochetes

Proteolytic shaving of intact spirochetes with proteinase K was performed as described (28-30, 180), with minor modifications. Treatment of cells with Pronase (Roche) followed previously published protocols (186, 187) but used the manufacturer's currently recommended reaction conditions (Roche# 10165921001, Version 07). Briefly, cells were grown at 34˚C to late-logarithmic phase in BSK-II and harvested by centrifugation at room temperature using a centrifugal force not exceeding 3,000 x *g* using a Sorvall Legend RT centrifuge. Cells were then washed once by resuspension in sterile, room temperature Dulbecco's phosphate buffered saline (dPBS) containing 5mM $MgCl_2$ (dPBS+Mg) and re-pelleted. Cells were then resuspended in

dPBS+Mg with either dH_2O (mock), proteinase K (Invitrogen, 200 µg/mL final concentration) or Pronase (Roche Life Sciences, 1 mg/mL final concentration). Proteinase K-containing samples and respective controls were incubated for 1 hour at room temperature, and reactions were stopped after 1 hour by addition of PMSF to a final concentration of 5mM (188, 189). Pronase-containing samples and respective controls were incubated for two hours at 37°C, and reactions were stopped by addition of EDTA, PMSF, and Pefabloc SC to 1mM, 0.2mM, and 0.8mM final concentrations, respectively. Subsequently, cells were pelleted by microcentrifugation, resuspended in SDS-PAGE sample buffer containing 50mM dithiothreitol (DTT; final concentration), boiled for 5 minutes and stored at -20°C until analysis by SDS-PAGE (190).

4.2.4 Isolation of B. burgdorferi OM Vesicles (OMVs)

OMVs from spirochetes were isolated as previously described (28-30, 181). Briefly, cells were grown to early exponential phase, harvested, and washed with dPBS containing 0.1% (w/v) bovine serum albumin. Cells were then resuspended in 25mM sodium citrate, pH 3.2 containing 0.1% (w/v) bovine serum albumin (BSA) (citrate buffer+BSA). Cell suspensions were shaken for two hours at room temperature in a New Brunswick C24 incubator at 250 rpm to release OMVs, at which point the cell suspension was harvested, resuspended in citrate buffer+BSA, and loaded onto a discontinuous 56/42/25% (w/w) sucrose gradient in citrate buffer without BSA. Cell suspensions were centrifuged at 100,000 x g for 18 hours at 4°C in a Beckman-Coulter XPN-80 ultracentrifuge using a SW 32 Ti rotor and Beckman UltraClear tubes, and the resulting upper (OMV) bands and lower (protoplasmic cylinder, PC) bands were separated by needle aspiration. Fractions were diluted in cold dPBS, re-pelleted separately, and then resuspended in dPBS containing 1mM PMSF. A portion of the resuspended fractions was used to prepare SDS-

Table 2. B. burgdorferi lipoproteome localization data. Localization data from the current study were reconciled with previously published data and genome-wide studies of in vivo gene expression, protein immunogenicity and requirement for in vitro growth. Column 1 lists the assayed lipoproteins by ORF (open reading frame) nomenclature (31, 32); ORFs that were identical in mature sequence to other analyzed ORFs are marked with an asterisk (*)(see **Fig. 6** and text). Column 2 lists common protein names used in the literature. Column 3 states the determined consensus localization of the assayed lipoproteins, as described in the text. S, surface; P-OM, periplasmic-outer membrane; P-IM, periplasmic-inner membrane; nd, not detected. Column 4 lists the determined localization of the C-terminally His-tagged proteins (**Figs. 6, 7 and 8**). Localizations followed with a dot (•) indicate that the His-tagged protein was resistant to proteinase K (**Fig. 6**), but not pronase (**Fig. 8**). Column 5 lists the dNSAF ratio (dNSAF pK–/dNSAF pK+) determined by MudPIT analysis (see text). nd, not detected. ∞, infinite value due to lack of detection of any peptides after pK treatment, i.e., division by 0.

Column 6 lists the previously determined and published lipoprotein localization along with the PubMed ID (PMID) of the associated publication. Columns 7 and 8 list the predicted and observed molecular masses (in kilodalton, kDa) of each protein. nd, not detected. Column 9 lists the paralogous family with key member and number according to Casjens et al. (31). Column 10 lists the observed in vivo expression pattern according to Iyer et al (19). Transcripts that showed significant elevation in the fed larval stage relative to at least one other stage were classified as important for tick acquisition (TA) or tick persistence (TP), as these genes were up-regulated in the transition from infected mice to naïve larvae. Transcripts that showed significant elevation in the fed nymph stage relative to at least one other stage were associated with vertebrate transmission (VT), based on their apparent importance for the spirochete's passage from the feeding nymph to the naïve mouse. Finally, transcripts that were significantly elevated in dialysis membrane chambers (DMCs) relative to at least one other stage were considered necessary for vertebrate persistence(VP), given their induction in a quasi-steady state mammalian environment. Column 11 lists protein immunogenicity as determined by Barbour et al. (99). A Microsoft Excel version of this table is available upon request.

ORF	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF ratio	Prev. Loc. (ref)	Pred. MW [kDa]	Obs. MW [kDa]	Paralogous Family		*in vivo* Differential Expression				Immuno-genicity
BB0028		P-OM	S•	0.70	P-OM (56)	40	38							
BB0141	BesA	P-IM	P-IM	0.59		35	39							
BB0144	ProX	P-IM	P-IM	0.79		33	32							
BB0155		P-IM	P-IM	nd		44	41							
BB0158	S2	S	S	10.34		27	27	S2	44					
BB0171		S	S	nd		23	20							
BB0193		P-IM	P-IM	2.22		29	28							
BB0213		S	S	1.21		26	26							
BB0215	PstS	P-IM	P-IM	1.04		31	30							+
BB0224		P-IM	P-IM	nd		11	12							
BB0227		P-IM	P-IM	0.57	P-OM (57)	27	26							
BB0298		P-IM	P-IM	0.37	P (58)	26	26							
BB0323		P-OM	P-IM	0.47	P-OM (59)	44	14							+
BB0324		P-OM	P-OM	0.69	P-OM (56)	14	16							
BB0328	OppA1	P-IM	P-IM	0.57	S (60)	60	59	OppA	37					+
BB0329	OppA2	P-IM	P-IM	0.94	S (61)	61	60	OppA	37					+
BB0330	OppA3	P-IM	P-IM	0.92		62	57	OppA	37					
BB0352		S	S	nd		44	39							
BB0365	IpLA7	P-IM	P-IM	0.57	P-IM (62)	22	21			TA	TP			+
BB0382	BmpB	P-IM	P-IM	1.16	S (63)	38	34	Bmp	36					
BB0384	BmpC	P-IM	P-IM	0.45		40	39	Bmp	36					
BB0385	BmpD	P-IM	P-IM	0.89		37	38	Bmp	36					+
BB0398		P-IM	P-IM	0.00		41	36							
BB0456		P-IM	P-IM	0.12		24	23							
BB0460		P-OM	P-OM	2.02		28	29					VT	VP	
BB0475		P-OM	P-OM	nd		15	13							
BB0536		nd	nd	0.67		108	nd							
BB0542		P-IM	P-IM	1.26		22	19					VT		
BB0628		P-IM	P-IM	0.00		27	26							
BB0652	SecD	P-IM	nd	0.18		65	nd							+
BB0664		P-IM	P-IM	0.70		26	28							
BB0689		S	S	19.87	S (58)	18	17					VT		
BB0758		S	S	nd		25	26						VP	
BB0806		P-IM	P-IM	1.22		58	53							
BB0823		S	S	nd		14	17						VP	
BB0832		P-IM	P-IM	0.73		31	27							
BB0844		P-IM	P-IM	nd		37	38	BB0844	12				VP	+
BBA03		P-IM	P-IM	0.70	S (64)	19	17							+
BBA04	S2	S	S	∞		32	33	S2	44			VT		+

BBA05	S1	P-IM	P-IM	1.83	P	(65)	49	52						
BBA07	ChpAI	S	S	∞	S	(66)	18	21				VT		+
BBA14		S	S•	0.63			14	14	OrfD	143			VP	
BBA15	OspA	S	S	65.42	S	(67)	29	31	OspAB	53	TA	TP		+
BBA16	OspB	S	S	185.84	S	(68)	32	34	OspAB	53	TA	TP	VT	+
BBA24	DbpA	S	S	5.95	S	(69)	21	21				VT	VP	
BBA32		S	S	nd			8	13						
BBA33		S	S	nd	S	(70)	21	19					VP	
BBA34	OppA5	P-IM	P-IM	0.74	P	(71)	61	59	OppA	37			VP	+
BBA36		S	S	nd	S	(58)	24	23					VP	+
BBA57		S	S	0.62	S	(72)	47	56					VP	+
BBA59		S	S	2.93			9	18			TA	TP	VT	
BBA62	Lp6.6	P-OM	P-OM	0.59	P-OM	(73)	8	13			TA	TP	VT	
BBA64	P35	S	S	6.23	S	(58)	34	32	P35	54				+
BBA65		S	S•	nd	S	(74)	32	26	P35	54				
BBA68	CspA	S	S	24.36	S	(75)	29	27	P35	54				
BBA69		S	S	∞	S	(58)	30	31	P35	54			VP	
BBA72		P-IM	P-IM	nd			9	13						
BBB08		S	S	0.61			25	27						
BBB09		P-OM	P-OM	nd			41	36				VT		+
BBB16	OppA4	P-IM	P-IM	0.61	P-IM	(76)	61	58	OppA	37				+
BBB19	OspC	S	S	7.86	S	(77)	22	22				VT		+
BBB25		S	S•	0.52			19	18					VP	
BBB27		P-IM	P-IM	1.25	P-OM	(57)	22	21						
BBC10	RevB	S	S	nd			20	19	Rev	63				+
BBD10		S	S	1.83			23	21						
BBE04		S	S	nd			5	13		54				
BBE08		S	S	nd			6	8						
BBE31	P35	S	S	∞	S	(78)	28	27	P35	60		VT		
BBF01	ErpD	S	S	nd			37	50	ErpB	163				
BBF20		S	S	1.84			11	14						
BBG01		S	S	73.49			35	31	BB0844	12			VP	
BBG25		P-OM	P-OM	nd			15	15	OrfD	143			VP	
BBH01		S	S	nd			8	13	BBH01	166			VP	
BBH06	CspZ	S	•S	0.74	S	(79)	27	26	CRASP-2				VP	+
BBH18		S	S	5.94			43	43						
BBH32	P35	S	S	8.36			29	22	P35	60				
BBH37		S	S	62.20			33	37	BB0844	12			VP	
BBI14		S	S	nd			4	8		60			VP	
BBI16	VraA	S	S	∞	S	(80)	54	75	P35	60				

BBI28		S	S	nd			22	21	P35	60			VP	
BBI29		S	S	8.94			26	27	P35	60	TA	TP	VT	
BBI36	P35	S	S	85.93			32	37	P35	54				
BBI38		S	S	0.00			32	38	P35	54			VP	
BBI39		S	S	56.21			33	37	P35	54			VP	
BBI42		S	S	nd	S	(58)	21	20	BBI42	52			VP	+
BBJ01		S	S	nd			7	11	P35	60				
BBJ09	OspD	S	S	22.89	S	(81)	28	29					VP	
BBJ34		S	S	142.70			40	41	CRASP-2	92			VP	
BBJ36		S	S	∞			40	35	CRASP-2	92				
BBJ41	P35	S	S	nd			33	37	P35	54			VP	
BBJ47		P-IM	P-IM	nd			27	26						
BBK01		S	S	97.29			34	38	BB0844	12			VP	
BBK07		S	S	∞	S	(82)	28	31	BBK07	59				+
BBK12		S	S	nd			26	31	BBK07	59				+
BBK19		S	S	39.92			24	30						+
BBK32	Fbp	S	S	nd	S	(83)	41	48						+
BBK48	P37	S	S	nd			33	40	P37	75				
BBK50	P37	S	S	10.42			37	46	P37	75				
BBK52	P23	S	S	nd			33	30	S2	44				+
BBK53		S	S	nd			21	20	BBI42	52			VT	+
BBL28	MlpH	S	S	∞			17	19	Mlp	113				
BBL39*	ErpN	S	S	10.87	S	(84)	20	19	ErpA	162				+
BBL40*	ErpO	S	S	1.03	S	(84)	44	nd	ErpB	163			VT	+
BBM27*	RevA1	S	S	0.93	S	(85)	18	nd	Rev	63			VP	+
BBM28	MlpF	S	S	nd			17	15	Mlp	113				
BBM38	ErpK	S	S	nd	S	(84)	29	37	ErpG	164				
BBN28	MlpI	S	S	nd			16	18	Mlp	113			VP	+
BBN38	ErpP	S	S	38.48	S	(84)	21	20	ErpA	162				+
BBN39	ErpQ	S	S	∞	S	(84)	39	55	ErpB	163				+
BBO28	MlpG	S	S	nd			16	16	Mlp	113				
BBO39	ErpL	S	S	nd	S	(84)	26	29	ErpG	164				+
BBO40	ErpM	S	S	0.61	S	(84)	42	40	ErpB	163				+
BBP27	RevA2	S	S	0.93	S	(85)	18	18	Rev	63			VP	
BBP28	MlpA	S	S	3.16			16	19	Mlp	113			VP	
BBP38	ErpA	S	S	10.87	S	(84)	20	18	ErpA	162				
BBP39	ErpB	S	S	1.03	S	(84)	44	61	ErpB	163			VP	+
BBQ03		S	S	nd			21	19	BBI42	52			VP	+
BBQ05	P35	S	S	1.22			29	30	P35	60			VP	
BBQ35	MlpJ	S	S	1.83			24	21	Mlp	113				+

BBQ46		nd	nd	nd			4	nd					
BBQ47	ErpX	S	S•	nd	S	(84)	40	28	ErpB	163		VP	
BBQ89*		S	S	nd			8	11	BBH01	166			
BBR28	MlpD	S	S	1.06			16	16	Mlp	113		VP	
BBR40	ErpH	S	S	nd			4	9		162		VP	
BBR42	ErpY	S	S	∞	S	(84)	25	27	ErpG	164		VP	+
BBS30	MlpC	S	S	6.55			17	16	Mlp	113			+
BBS41	ErpG	S	S	∞	S	(86)	22	23	ErpG	164		VP	+

PAGE samples and stored at -20°C after boiling. The remainder of the sample was stored at -80°C for later analysis.

4.2.5 SDS-PAGE analysis and immunoblotting

SDS-PAGE analysis and immunoblotting was performed as described (28-30). Protein samples were prepared as described above and separated using a 12% SDS-polyacrylamide gel (Bio-Rad). After transfer, gels were either Coomassie-stained for total protein (EZ-RUN Protein Gel Staining Solution, Fisher) or were transferred to nitrocellulose membranes (Amersham, GE Healthcare) using a Trans Blot SD apparatus (Bio-Rad). Membranes were then blocked post-transfer with either 5% (w/v) nonfat dry milk or 2.5% (w/v) BSA and probed with mouse anti-FlaB (H9724 (191); 1:300 dilution), mouse anti-OspA (H5332 (45); 1:1,000), rabbit polyclonal anti-OppAIV ((34); 1:1,500) , or the HisProbe-HRP reagent according to the manufacturer's instructions. H9724 and H5332 antibodies were a gift from Dr. Alan Barbour (Univ. of California at Irvine, CA), and anti-OppAIV antibody was generously provided by Dr. Patricia Rosa (NIH/NIAID Rocky Mountain Laboratories, Hamilton, MT). Blots were treated with corresponding AP-conjugated secondary antibodies (Sigma-Aldrich Cat. No's A3562, A3687, 1:30,000) and developed using LumiPhos (Thermo Fisher, now discontinued) or Immun-Star AP (Bio-Rad). Blots probed with HisProbe-HRP reagent were developed with SuperSignal West Dura reagent (Thermo Fisher). Signals were detected and captured using a Fujifilm LAS-4000 CCD imager and further processed with Adobe Photoshop CS6.

4.2.6 Analysis of protein fractions by Multidimensional Protein Identification Technology (MudPIT)

Localization of lipoproteins endogenously expressed under standard culture conditions was determined using multidimensional protein identification technology (MudPIT) mass spectrometry (192). *B. burgdorferi* B31-A3 cells were subjected to surface proteolysis with proteinase K as described above. Membrane-associated proteins were then enriched by overnight extraction of the mock and proteinase K-treated samples with Triton X-114 as described (186). The washed detergent extracts were then precipitated overnight using acetone (80% v/v), resuspended in 0.1M Tris-HCl, pH 8.5, and precipitated again overnight using TCA (20% v/v). The addition of acetone precipitation was in the protocol was necessary to effectively remove detergent prior to analysis by MudPIT. Two biological replicates of the resulting desiccated, frozen protein samples were then submitted for MudPIT analysis (Proteomics Center, Stowers Institute for Medical Research; Kansas City, MO). Resuspended protein samples were digested with endoproteinases Lys-C (Roche) and Trypsin (Promega) at 0.1 μg/μL final, each. The protease-digested samples were then analyzed by MudPIT on an LTQ linear ion trap (Thermo Scientific) coupled to a Quarternary Agilent 1100 series HPLC (193). Protein content in mock control vs. proteinase K-treated whole cell protein preparations were analyzed by comparison of the average dNSAF (distributed normalized spectral abundance factor) for each unique protein, which correlates directly with the relative abundance of a particular protein in the sample (194). A dNSAF ratio of control vs. protease-treated sample ($dNSAF_{-pK}/dNSAF_{+pK}$) was calculated for each detected protein. Theoretically, a ratio of 1 indicates that the protein is as abundant after surface proteolysis as before, i.e., not susceptible to proteinase K due to either periplasmic localization or intrinsic resistance to protease. Conversely, a ratio greater than 1 indicates that a protein is less abundant after proteolytic shaving, i.e., surface exposed.

4.3 Experimental Results

4.3.1 Generation of an epitope-tagged lipoprotein expression library in B. burgdorferi

Our long-standing interest in understanding the biogenesis of spirochetal envelopes, particularly the sorting mechanisms for the numerous and abundant *B. burgdorferi* lipoproteins (25, 28-30, 156, 174, 195, 196), has been continually thwarted by the quite limited and biased data set of characterized lipoproteins. As shown in **Table 2**, 49 *B. burgdorferi* lipoproteins have been localized independently to date, with over two thirds of them found on the bacterial surface. We therefore set out to comprehensively localize the published list of proteins that are predicted to make up the *B. burgdorferi* lipoproteome (23). This list was compiled by training a computer algorithm (SpLip) on a set of spirochete proteins that had been experimentally verified to be lipidated, and then using that trained algorithm to scan the published genome of *B. burgdorferi* B31 (31, 32). A total of 127 putative open reading frames (ORFs) were annotated as "probable," "possible," or "false negative" lipoproteins (23). These 127 ORFs were used as our "working lipoproteome" for the creation of a C-terminally histidine-tagged expression library. This approach allowed us to use a single commercial blotting reagent (HisProbe-HRP) to probe the entire lipoprotein library, bypassing the need to generate individual and validated lipoprotein-specific antibodies.

B. burgdorferi B31-e2 clones expressing each individual epitope-tagged lipoprotein were obtained as described above. Of the 127 lipoproteins that were to be cloned, three (BB0536, BB0652 (SecD), and BBQ46.) showed no expression of his-tagged protein in multiple *B. burgdorferi* clones; all clones contained the respective recombinant plasmid when assayed by PCR and DNA sequencing, indicating that the lack of expression was not due to absence of plasmid or mutation of the promoter or coding sequence. These three ORFs were not pursued

Figure 6. Surface proteolysis of *B. burgdorferi* strains expressing a his-tagged lipoprotein library using Proteinase K. Intact cells expressing the his-tagged lipoprotein were treated with Proteinase K or mock-treated as described in the text. Cell lysates were then separated by SDS-PAGE, transferred to nitrocellulose, and analyzed by Western Blotting using anti-OspA or anti-FlaB mouse MAbs, or HisProbe-HRP reagent. Lipoproteins are organized according to open reading frame (ORF) nomenclature (31, 32), with the common name of the protein listed if applicable (**Table 2**). pK–, untreated mock control; pK+: proteinase K-treated sample. Red parentheses flanking the ORF designation indicate the determined lipoprotein localization as follows: **))**ORF, surface; **(**ORF**)**, periplasmic. A red dot (•) indicates proteins where the consensus localization was ultimately changed to the periplasm [•**))**] or surface [**))**•] due to independent data or follow-up pronase digestion (**Figure 8A**). Determined molecular weights of the his-tagged proteins are indicated in **Table 2**.


BesA/AcrA
ProX
S2
PstS
BB0028
BB0141
BB0144
BB0155
BB0158
BB0171
BB0193
BB0213
BB0215
BB0224
BB0227
BB0298
BB0323
pK
HisTag
OspA
FlaB
OppA1
OppA2
OppA3
IpLA7
BmpB
BmpC
BmpD
BB0324
BB0328
BB0329
BB0330
BB0352
BB0365
BB0382
BB0384
BB0385
BB0398
BB0456
BB0460
BB0475
SecD
BB0536
BB0542
BB0628
BB0652
BB0664
BB0689
BB0758
BB0806
BB0823
BB0832
BB0844
nd
S2
S1
OspA
OspB
DbpA
OppA5
BBA03
BBA04
BBA05
BBA07
BBA14
BBA15
BBA16
BBA24
BBA32
BBA33
BBA34
BBA36
BBA57
Lp6.6
P35
CspA
OppA4
OspC
BBA59
BBA62
BBA64
BBA65
BBA68
BBA69
BBA72
BBB08
BBB09
BBB16
BBB19
BBB25
BBB27
RevB
P35
ErpD/Arp
CspZ
BBC10
BBD10
BBE04
BBE08
BBE31
BBF01
BBF20
BBG01
BBG25
BBH01
BBH06
BBH18
BBH32
OspD
BBH37
BBI14
BBI16
BBI28
BBI29
BBI36
BBI38
BBI39
BBI42
BBJ01
BBJ09
BBJ34
BBJ36
P35
Fbp
P37
P37
P23
MlpH
ErpN
BBJ41
BBJ47
BBK01
BBK07
BBK12
BBK19
BBK32
BBK48
BBK50
BBK52
BBK53
BBL28
BBL39
see ErpA BBP38
ErpO
RevA1
MlpF
ErpK
MlpI
ErpP
ErpQ
MlpG
ErpL
ErpM
RevA2
MlpA
ErpA
BBL40
BBM27
BBM28
BBM38
BBN28
BBN38
BBN39
BBO28
BBO39
BBO40
BBP27
BBP28
BBP38
see ErpB BBP39
see RevA2 BBP27
ErpB
P35
MlpJ
ErpX
MlpD
ErpH
ErpY/OspF
MlpC
OspG/ErpG
BBP39
BBQ03
BBQ05
BBQ35
BBQ46
BBQ47
BBQ89
BBR28
BBR40
BBR42
BBS30
BBS41
see BBH01

further. In addition, four pairs of lipoproteins were found to be 100% identical in their mature, processed sequences when analyzed by sequence alignment (T-COFFEE; http://www.tcoffee.org/) (197): BBP38 (ErpA) and BBL39 (ErpN), BBP39 (ErpB) and BBL40 (ErpO), BBP27 (RevA) and BBM27 (RevA), as well as BBH01 and BBQ89. As it is all but certain that proteins with identical primary sequences localize identically (25), each of these pairs is represented by a single member (the first ORF listed of the pair). This resulted in an assayed dataset of 120 unique lipoproteins covering 124 members (or 98%) of the predicted *B. burgdorferi* lipoproteome.

4.3.2 Initial assignment of lipoproteins to the bacterial surface based on proteinase K accessibility

We used an established and validated stepwise experimental protocol to individually localize each epitope-tagged lipoprotein within the spirochetal cell envelope. As described, we first subjected each recombinant *B. burgdorferi* clone to *in situ* surface proteolysis (or "proteolytic shaving") with proteinase K, a membrane impermeable, nonspecific protease that selectively digests surface lipoproteins in the context of an intact OM (28-30). Surface lipoprotein OspA was used as a positive control as it is readily degraded by proteinase K under the assay conditions. Conversely, the periplasmic flagellar protein FlaB was used as a negative control to ascertain OM integrity of the assayed cells. The His-tag epitope was used to assess the sensitivity of the tagged lipoprotein to Proteinase K, assuming that the localization of the C-terminal his-tag mirrors that of the lipoprotein itself. Of note, C-terminal processing of secreted proteins in *B. burgdorferi* is rather specific and limited to a small set of proteins (198, 199), and C-terminal tags are not known to alter lipoprotein localization (29, 200). Lipoproteins that lost

the his-tag signal upon proteolysis were considered to be surface-exposed (S), whereas lipoproteins that showed no loss in signal relative to the controls were considered to localize to the periplasmic (P) face of the OM (OM) or inner membrane (IM). Compiled Western blot results for each of the assayed lipoproteins are shown in **Figure 6**, organized by ascending ORF nomenclature. Of the 124 lipoproteins covered by the analysis, were classified as surface-exposed, while 41 were considered to localize to the periplasm. Interestingly, a subset of lipoproteins exemplified by the Mlp protein family showed only partial degradation of the his-tag after proteinase K treatment (**Figure 6**). This could indicate that only fractions of these lipoproteins are exported to the surface. Alternatively, it could reflect the lipoproteins' native folding, which may render their C termini less accessible to protease in the context of the spirochetal envelope.

4.3.3 Initial assignment of periplasmic lipoproteins to the outer or inner membrane by membrane fractionation

Recombinant *B. burgdorferi* clones that expressed epitope-tagged lipoproteins protected from surface proteolysis with proteinase K were subjected to membrane fractionation. OM vesicles (OMVs) and protoplasmic cylinder (PCs) fractions were obtained as described above by incubating harvested cells in a hypotonic citrate buffer followed by loading on a step-wise sucrose gradient. Note that due to the not entirely efficient separation of the OM during the process (181), the PC fraction should be interpreted as a partially OM-depleted whole cell protein fraction. Thus, the surface/OM control OspA is abundant in the OMV fractions, but also detected in the PC fractions (29, 30). In contrast, the inner membrane lipoprotein OppAIV can be used as a control to assess the purity of the OMV preparation, as it should be absent from an

ideal OMV preparation. A lipoprotein was scored as an OM component if the His-tag was detected in the OMV fraction in a ratio similar to the OspA control. Absence or only traces of a His-tag signal from the OMV fraction indicated that the lipoprotein was retained in the inner membrane. As shown in the Western immunoblots in **Fig. 7**, 31 of the 40 lipoproteins assayed showed an OppAIV-like fractionation pattern, i.e., they were retained in the IM. Conversely, 9 lipoproteins were detected in appreciable amounts in the OMV fraction, indicating that they were released to the OM.

4.3.4 Reassessment of select OM lipoproteins for potential intrinsic proteinase K resistance

The lipoprotein BBQ47 (ErpX) was previously established as a surface-exposed but intrinsically proteinase K resistant protein (187). Consequently, the ErpX-expressing *B. burgdorferi* clone was not subjected to membrane fractionation despite the protein's apparent resistance to proteinase K. Yet, this example raised the specter that other surface lipoproteins may have been erroneously scored as OM periplasmic lipoproteins due to their resistance to proteinase K. We therefore re-evaluated the 9 lipoproteins in our initial P-OM lipoprotein data set using pronase, a mixture of nonspecific proteases that has been shown to digest *B. burgdorferi* proteins that are otherwise protease-resistant (186, 187). ErpX was used as a control.

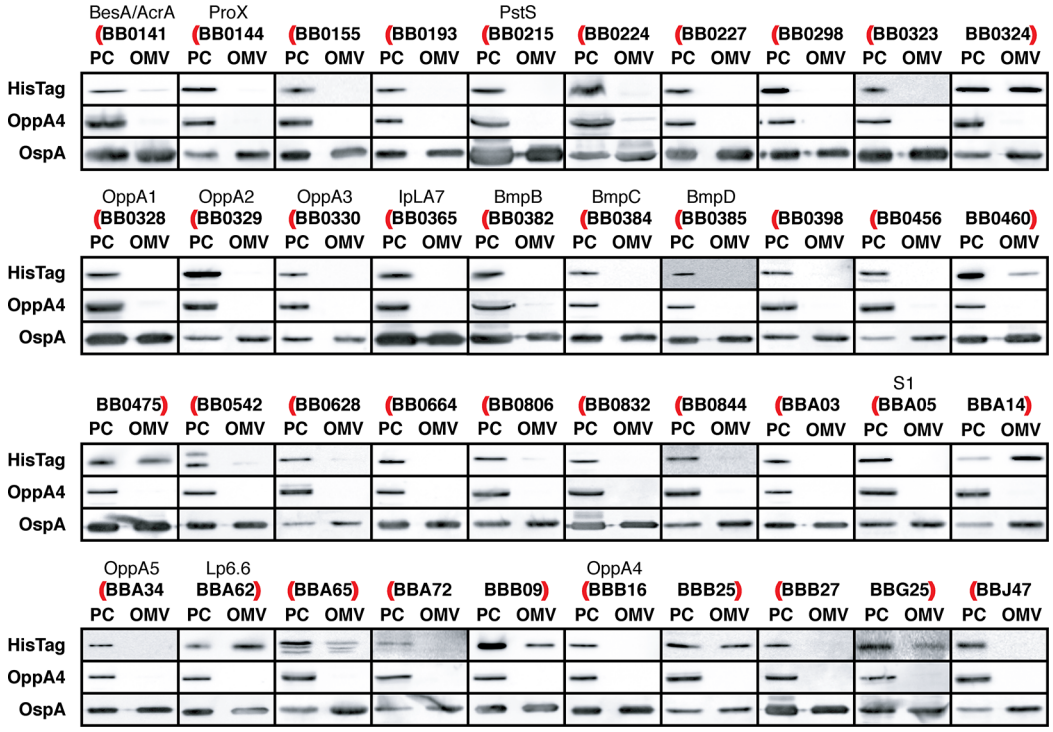
As shown in **Figure 8A**, pronase treatment led to complete degradation of OspA, while FlaB remained intact, indicating that assay conditions lead to selective removal of surface-exposed proteins. Parallel treatment of *B. burgdorferi* B31-A3 cells and staining of SDS-PAGE-separated protein samples by Coomassie (**Figure 8B**) indicated almost indistinguishable overall proteolysis patterns between pK and pronase; the only appreciable difference in the pronase-

treated sample was the absence of a 51 kDa band that has been attributed to a proteinase K-resistant fragment of the OM porin P66 (201). As expected, the control protein BBQ47 (ErpX) was largely susceptible to degradation by pronase. Three additional lipoproteins, BBA14, BBA65, and BBB25, showed selective degradation by pronase as well. The remaining 6 lipoproteins were found to be pronase-resistant in the context of intact cells (**Fig. 8A**), but readily digested and undetectable when the cells were permeabilized (not shown). This indicated that these 6 proteins are not intrinsically protease-resistant, but indeed localize to the periplasmic leaflet of the OM. In summary, this set of experiments localized BBA14 and BBB25 to the surface *de novo*. BBA65 had been localized previously (202). In that study, the authors found that BBA65 was susceptible to both pronase and proteinase K; the latter could be due to the higher concentration of proteinase K used (400 μg/mL). It also established BB0460, BB0475, BBB09, and BBG25 as additional *bona fide* P-OM lipoproteins and confirmed the previous localization results for both BB0324 and Lp6.6 (87, 203).

4.3.5 Localization of endogenously expressed lipoproteins by quantitative mass spectrometry

To validate our lipoproteome expression library data with the localization of lipoproteins endogenously expressed by *B. burgdorferi*, we employed quantitative MudPIT mass spectrometry to analyse the lipoproteome of *B. burgdorferi* B31-A3 (184, 193). Our stock of B31-A3 was shown by multiplex PCR (185) to contain all linear and circular plasmids except for lp5, which is not predicted to encode for any lipoproteins (23) (data not shown). Cells were cultured and treated with proteinase K as above. To reduce the complexity of the samples, the mock control and proteinase K-treated samples were enriched for membrane-associated proteins by extraction with Triton X-114 as described (186). Peptide abundance, expressed as distributed

Figure 7. Membrane fractionation of *B. burgdorferi* strains expressing a his-tagged lipoprotein library. Strains that were found to express proteinase K-resistant recombinant lipoproteins were subjected to membrane fractionation using a hypotonic acidic citrate buffer and sucrose gradient to obtain OMVs. Lipoproteins were then localized based on presence or absence in OMV fraction relative to control proteins. OMVs and PCs were separated by SDS-PAGE, transferred to nitrocellulose, and analyzed by Western Blot using anti-OspA mouse MAb, anti-OppAIV rabbit polyclonal antiserum, or HisProbe-HRP reagent. Lipoproteins are organized by open reading frame with the common name listed, if applicable. OMV, outer membrane vesicle fraction; PC, protoplasmic cylinder fraction. Note that the PC fraction is equivalent of a whole cell protein preparation partially depleted of OM proteins (see text). Red parenthesis flanking the ORF designation indicate the determined lipoprotein localization as follows: **(**ORF, inner membrane; ORF**)**, outer membrane. The localization of BBA65 remains undetermined [(ORF)] due to multiple isoforms with variable distributions. Determined molecular weights of the his-tagged proteins are indicated in **Table 2**.


BesA/AcrA
ProX
PstS
(BB0141
(BB0144
(BB0155
(BB0193
(BB0215
(BB0224
(BB0227
(BB0298
(BB0323
BB0324)
PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV
HisTag
OppA4
OspA
OppA1
OppA2
OppA3
IpLA7
BmpB
BmpC
BmpD
(BB0328
(BB0329
(BB0330
(BB0365
(BB0382
(BB0384
(BB0385
(BB0398
(BB0456
BB0460)
PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV
HisTag
OppA4
OspA
S1
BB0475)
(BB0542
(BB0628
(BB0664
(BB0806
(BB0832
(BB0844
(BBA03
(BBA05
BBA14)
PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV
HisTag
OppA4
OspA
OppA5
Lp6.6
OppA4
(BBA34
BBA62)
(BBA65)
(BBA72
BBB09)
(BBB16
BBB25)
(BBB27
BBG25)
(BBJ47
PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV PC OMV
HisTag
OppA4
OspA

Normalized Spectral Abundance Factor (dNSAF) (194), was captured from two biological replicates and averaged. A comparison of the MudPIT results with the results from our his-tagged lipoprotein assays can be seen in **Table 2** and **Fig. 9**. 86 of the predicted 127 lipoproteins were detectable in *B. burgdorferi* B31-A3 after growth at 34°C in BSKII-C. Among them were 2 of the 3 lipoproteins that were not detectable as His-tagged proteins, BB0536 and BB652 (SecD). The remaining 40 lipoproteins are most likely expressed below the levels detectable by MudPIT under the culture conditions used.

The relative abundance of proteins before and after proteolytic shaving, expressed as a ratio of dNSAF values in the mock control vs. the treated samples ($dNSAF_{-pK}/dNSAF_{+pK}$), was calculated, ranging from 0.00 (BB0628) to 185.84 (BBA16/OspB). 11 lipoproteins were undetectable after proteolysis, resulting in an infinite (∞) ratio; for analysis purposes, the values of these proteins were capped at the highest calculated value (185.84). Plotting the dNSAF ratios for the surface and periplasmic lipoprotein cohorts identified in the expression library showed a clear separation (**Fig. 9**). The mean dNSAF ratio for periplasmic lipoproteins was 0.80(range: 0.00-2.22), below but close to the expected ratio of 1.0. One explanation is that surface proteolysis yields a significantly less complex protein sample (see **Fig. 8B**), which may lead to the "unmasking" of previously undetectable/unassignable peptides. Consequently, the ratio's numerator may be depressed relative to the denominator. The mean dNSAF ratio for surface proteins was 60.00 (range: 0.00-185.84 [capped; see above]). 12 surface-assigned lipoproteins had low dNSAF ratios around or below 1.0. Most of the proteins in this cohort were members of the paralogous Mlp, Rev, and Erp protein families that had shown at least partial resistance to proteinase K in our experiments (**Figure 6**) or prior studies (**Table 2**). Also, 4 of these proteins were subsequently shown to be accessible to *in situ* pronase digestion (**Fig. 8A**).

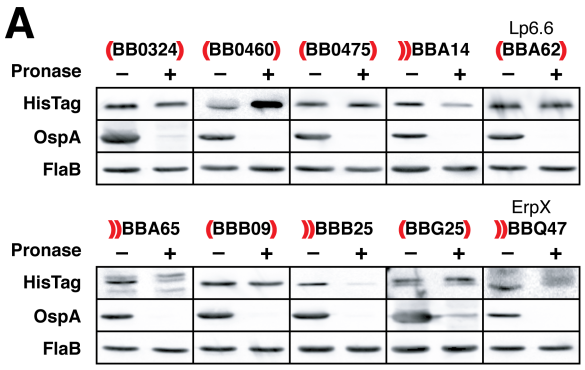
As shown in **Fig. 9**, a dNSAF ratio of 2 or above is a high-confidence predictor for surface exposure. This cutoff was statistically confirmed by a Mann-Whitney non-parametric U test using the OriginPro 9.1 software suite. Parallel analysis of sequence coverage, i.e., the % of protein sequence covered by detected peptides, before and after pK treatment also showed that a reduction of 10% or higher was a statistically valid predictor of surface exposure (see **Fig. S1** and **Table S1**).

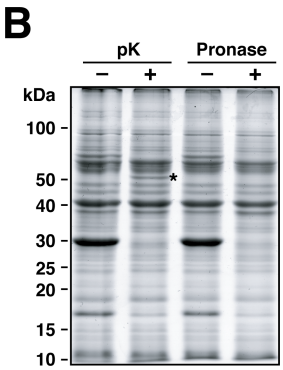
All mass spectrometry data are available from the ProteomeXchange repository under accession number PXD005617. The complete MudPIT mass spectrometry dataset (raw files, peak files, search files, as well as DTASelect result files) can be obtained from the MassIVE database via ftp://MSV000080434@massive.ucsd.edu (password ASD06166).

4.3.6 Reconciliation of independent localization data produces a consensus localization catalog for the B. burgdorferi lipoproteome

Our lipoproteome expression library contained 49 lipoproteins that had been independently localized prior to this study (**Table 2**). The main rationale for their inclusion in the present study was to use them as internal validation controls. For 41 lipoproteins, the current localization data were in unequivocal agreement with published data. Three lipoproteins, BB0298, BBA05/S1 antigen and BBA34/OppA5, were more precisely localized within the periplasmic compartment to the IM. Discrepancies with previously published localization data were found for 8 lipoproteins, but all disagreements could be reconciled: (i) A first set of 4 proteins was previously described as surface-exposed but was found to be restricted to the periplasm in our assays (see **Table 2**). BmpB (BB0382), like the other homologs of this protein family, was localized by us to the IM. An earlier study had detected BmpB on the surface by

Figure 8. Surface proteolysis of *B. burgdorferi* strains expressing a his-tagged lipoprotein library using Pronase. (A) Intact cells expressing his-tagged lipoproteins that were determined to be Proteinase K resistant but enriched in the OM were subjected to surface proteolysis with Pronase. Cell lysates were then separated by SDS-PAGE and analyzed by Western Blot as in **Fig. 6**. Pronase –, untreated mock control; Pronase +: Pronase-treated sample. Red parentheses flanking the ORF designation indicate the determined lipoprotein localization as in **Fig. 6**: **))**ORF, surface; **(**ORF**)**, periplasmic. **(B)** Coomassie-stained SDS-PAGE gel of *B. burgdorferi* strain B31-A3 whole cell protein preparations obtained before (–) or after (+) incubation with proteinase K or Pronase. Protein MWs in kDa, indicated to the left, were derived from a protein molecular weight marker (BioRad). An asterisk (*) indicates a known proteinase K-resistant, but apparently Pronase-sensitive fragment of integral OM protein P66 (201).


A
(BB0324)
(BB0460)
(BB0475)
)BBA14
Lp6.6
(BBA62)
Pronase
– + – + – + – + – +
HisTag
OspA
FlaB
)BBA65
(BBB09)
)BBB25
(BBG25)
ErpX
)BBQ47
Pronase
– + – + – + – + – +
HisTag
OspA
FlaB


B
pK
Pronase
– + – +
kDa
100
50
40
30
25
20
15
10
*

immunofluorescence, albeit without controlling for accidental permeabilization of the fragile spirochetal OM (204). OppA1 and OppA2 were also localized to the IM, as were the other homologs of that oligopeptide-binding lipoprotein family. Again, prior studies had solely relied on immunofluorescence data without controlling for the integrity of the bacterial envelope (205, 206). The fourth protein in this set, BBA03, was also found solely in the IM in the present study. A prior well-controlled localization study remained equivocal in that BBA03 was found by immunofluorescence to be partially surface-exposed, but at the same time was protected from proteinase K in intact, but not permeabilized cells (207). (ii) Two IM lipoproteins, BB0227 and BBB27, were localized to the inner leaflet of the OM in one of our earlier studies (29); we now believe that these localization results were erroneous due to contamination of the OMV fraction and a less stringent interpretation of the data. (iii) BB0323 was localized by us to the IM but was identified by others as an OM periplasmic lipoprotein (208). BB0323 was shown to undergo multistep proteolytic processing in the periplasm (209). Consequently, upon protein maturation, any C-terminal epitope tag will partition together with a C-terminal soluble fragment. Accordingly, we detected only a 14-kDa fragment of the 44-kDa full-length protein in the PC fraction (**Table 2**). We therefore defer to the previous work on BB0323 as a more accurate reflection of this lipoprotein's localization. (iv) Our final, but probably most intriguing disagreement with a previous finding was BB0028. This lipoprotein was found to be at least partially surface-exposed in our work but has been previously described as a periplasmic OM associated with the OM Beta-barrel Assembly Machinery (BAM) complex (203). In other bacterial systems, BAM lipoprotein modules were shown to assume topologies that expose their C termini on the bacterial surface under certain experimental conditions (125, 210). Thus, we hypothesize that BB0028 transiently and partially (i.e., predominantly via its C terminus)

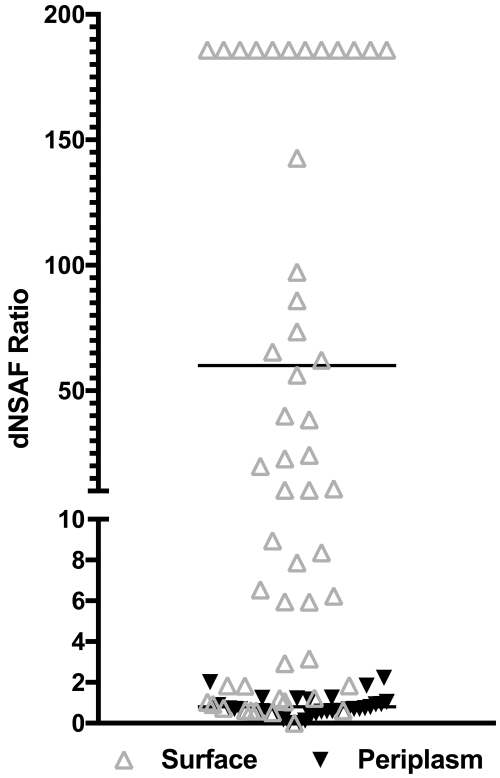
localizes to the surface. Any excess of BB0028 in the *B. burgdorferi* BAM complex may detectably shift the protein's topological equilibrium towards the cell surface.

The aggregation of the present and past data produces a comprehensive and internally validated consensus catalog of lipoprotein localization within the *B. burgdorferi* envelope (**Table 2**). Of the spirochete's 127 predicted lipoproteins within this set, 125 localize conclusively to either the surface (86), periplasmic leaflet of the OM (8), or the IM (31). The remaining two predicted lipoproteins remained undetectable as epitope-tagged proteins and could not be conclusively localized, but one (BB0536) was detected by mass spectrometry with a dNSAF ratio consistent with periplasmic localization.

4.3.7 Reassessment of potential B. burgdorferi lipoprotein sorting motifs within the N-terminal tethers

Our earlier studies indicated that lipoprotein tether peptides are structurally similar in that they are intrinsically disordered. Yet, they lack any significant peptide sequence homology beyond the N-terminal cysteine residue. Together with our extensive mutational analyses, this led us to conclude that there are no specific canonical peptide motifs that direct lipoproteins to the different envelope compartments of *B. burgdorferi* (29). With the lipoproteome localization data in hand, we decided to reopen this inquiry. Tether peptide sequences from the cohorts of surface, periplasmic OM and periplasmic IM lipoproteins were aligned and compared. Again, no compartment-specific peptide motifs emerged from this analysis (**Fig. 6**). A recent study of lipoprotein secretion in the Gram-negative pathogen *Capnocytophaga canimorsus* showed that N-terminal patches of negatively charged Asp and Glu residues in the proper sequential and positional N-terminal context can drive lipoprotein surface localization, and that this surface

Figure 9. Scatter plot of MudPIT-derived dNSAF ratios of surface and periplasmic lipoproteins. dNSAF ratios before and after proteinase K treatment ($dNSAF_{-pK}/dNSAF_{+pK}$) were calculated for 87 lipoproteins detected by MudPIT and plotted using GraphPad Prism. Horizontal lines indicate the mean dNSAF ratios for surface and subsurface proteins. Note that (i) infinite dNSAF values due to undetectable protein after pK treatment were capped at the highest calculated value of 185.84 for BBA16/OspB, and (ii) surface-assigned proteins that are partially resistant to pK (see **Fig. 6** and text) cluster with subsurface proteins (see **Table 2** for specific dNSAF values).


200
150
100
50
10
8
6
4
2
0
dNSAF Ratio
Surface
Periplasm

lipoprotein secretion signal is conserved and recognized in other members of the phylum *Bacteroidetes* (212, 213). While we cannot fully exclude a similar charge-based sorting mechanism in *Borrelia* barring additional experimentation, we did not detect any positional conservation of negative charges in the 86 *B. burgdorferi* surface lipoproteins (**Fig. 10**).

4.4 Discussion of Experimental Results

4.4.1 Overview of results

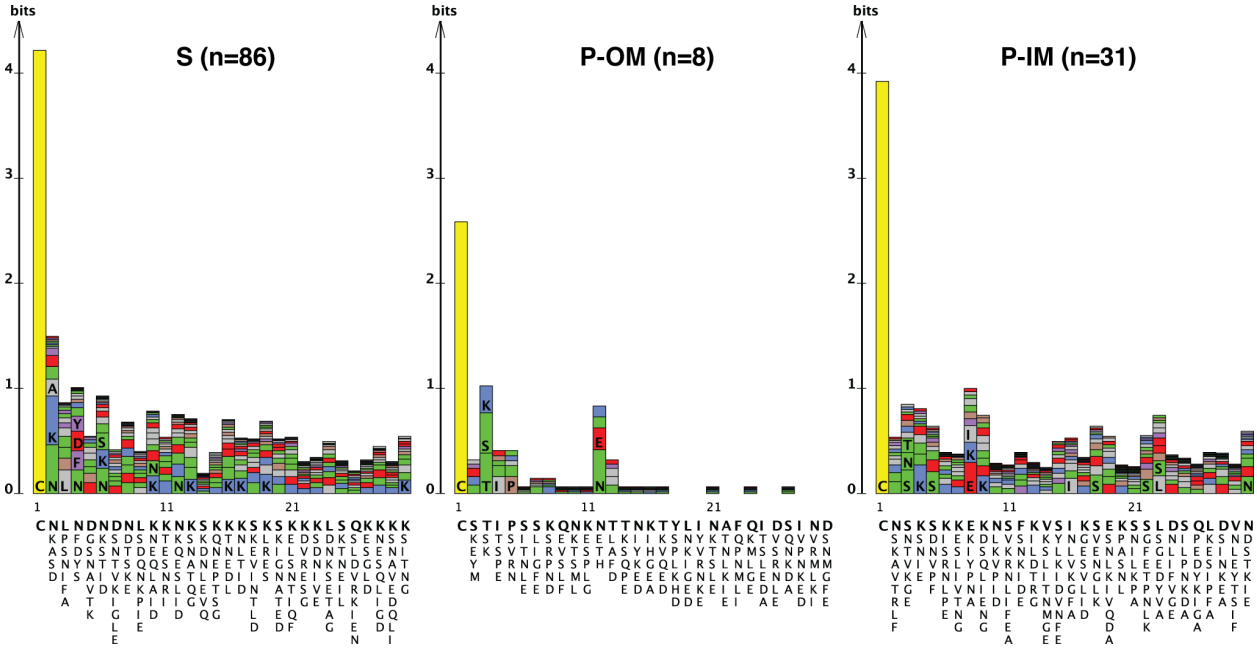
The lipoprotein repertoire of *B. burgdorferi* is exceptionally large, especially when taking into account the spirochete's small and fragmented genome. Compared to *E. coli's* 4-Mb circular genome with about 90 lipoprotein genes (214, 215), *B. burgdorferi*'s 1.6 Mb genome spread across a linear chromosome and a collection of linear and circular plasmids encodes for over 120 lipoproteins. Since the initial identification of OspA and OspB as a common surface antigens of Lyme disease spirochetes (45, 216) three decades ago, numerous studies have shown that lipoproteins play an outsized and multifunctional role as virulence factors in the transmission, colonization, dissemination and persistence of *Borrelia burgdorferi* and the resulting pathology of Lyme disease. Following OspA's and OspB's precedent, much of the research focus has been on identifying additional surface lipoproteins that contribute to *B. burgdorferi's* interface with the tick vector or host and could serve as vaccine targets. Thus, it may be not surprising that 36 of the 47 additional lipoproteins that were subsequently studied localized to the surface. Together, these studies yielded a rather limited and potentially biased data set, covering less than 40% of the predicted *B. burgdorferi* lipoproteome. In the present

study, we have closed this gap in our understanding of the complex cell envelope of *B. burgdorferi* by providing spatial information on 98% of its predicted lipoproteome.

4.4.2 Correlation of gene and protein localization

It has been noted earlier that *B. burgdorferi's* megabase linear chromosome, and to some degree the cp26 "minichromosome", tend to encode for essential genes, while the remainder of the circular and linear plasmids appear non-essential for growth in culture (31, 37, 217-219). While this generalization at least partially extends to the lipoproteins encoded by these replicons, there is a remarkable correlation of gene localization and protein localization: 79 of the 90 plasmid-encoded lipoproteins (88%) are surface-exposed, while only 7 out of the 37 chromosomally encoded lipoproteins (19%) are found on the surface (**Table 2**). This dichotomy is understandable when considering the biological function and primary protein sequence. Many of the plasmid-encoded lipoproteins share significant homology, which has led to their organization into "paralogous gene families" (31). Instructive examples are the Erp, Mlp, and "Pfam54" lipoproteins encoded on the multiple prophage-related *B. burgdorferi* cp32/lp56 plasmids (31, 100, 183, 220). Overall, 62 of the 86 surface lipoproteins (72%) belong to a paralogous group. By contrast, only 14 of the 39 periplasmic lipoproteins (36%) have other paralogs (31) (**Table 2**). This well-titrated variability and redundancy in surface lipoproteins may stem from the need of *B. burgdorferi* to adapt to a multitude of environments during its enzootic cycle. Chromosomally encoded but surface-localized BB0352 and BB0689 were identified to possess putative sugar- and lipid-binding domains by the HHPRED algorithm (221). This suggests that surface lipoproteins encoded by essential genetic elements play housekeeping or metabolic roles. Similarly, sub-surface lipoproteins located on non-essential plasmids seem to

Figure 10. Sequence alignment of *B. burgdorferi* surface, periplasmic OM or IM lipoprotein tether peptides. A LogoBar (211) representation of the N-terminal sequence of known or predicted mature *B. burgdorferi* lipoproteins (23) illustrates the maintained complexity of surface (S), periplasmic OM (P-OM) and IM (P-IM) lipoprotein tether peptides. The height of each column, measured in bits, is proportional to the lack of complexity at a given position. The columns are stacked from the bottom starting with the most frequently occurring residue at that position and continuing upward. Below each column are the six most frequently occurring residues at each position, in order of frequency from top (bold) to bottom. Colors represent residues with similar characteristics (e.g., red for negatively charged Asp and Glu residues).


bits
S (n=86)
P-OM (n=8)
P-IM (n=31)

have a role in transmission and virulence. BBJ47, which we localized to the IM, was identified by HHPRED to belong to Pfam17044. This groups BBJ47 with the *B. burgdorferi* BptA proteins and suggests a role in tick persistence (222).

4.4.3 Correlation of lipoprotein localization to in vivo expression and immunogenicity

To gain further insight into the biological significance of our data, we explored potential correlations of lipoprotein spatial compartmentalization with temporal expression and immunogenicity (**Table 2**). Our working lipoproteome data were first cross-referenced with a previous study that examined the reactivity of Lyme borreliosis patient vs. control sera to various *B. burgdorferi* proteins (99). 43 of the 127 lipoproteins were found in that study to be immunogenic (**Table 2**). Next, we referenced our data set against a study that looked at the transcription of *B. burgdorferi* genes at various stages of the spirochete's enzootic cycle, using an RNA-hybridized microarray assay that was validated through qRT-PCR (19). 53 lipoproteins, mostly plasmid-encoded and surface-localized, were found to have some variable role in the infectious cycle based on significantly different levels of transcripts during tick acquisition, tick persistence, vertebrate transmission and vertebrate persistence (**Table 2**). Of note, this data mining approach neglects any proteins that may be essential to the cell but are not differentially regulated between environments. Immunogenic surface lipoproteins expressed during tick acquisition, tick persistence or vertebrate transmission may work in a preventive setting, similarly to the FDA-approved but no longer available OspA vaccine (109). Within this group are BBA04 (S2 antigen), BBA07, BBA16 (OspB), BBB19 (OspC), BBK53, and BBL40 (ErpO). Both OspB and OspC have previously been shown to elicit protection when used to immunize laboratory mice (223, 224). Of the remaining four lipoproteins (BBA04, BBA07, BBK53, and

BBL40), BBA07 has been implicated in transmission from the tick to vertebrate host and BBL40 is part of the Erp protein family, a group of lipoproteins implicated in Factor H binding and complement resistance (72, 225).

4.4.4 Mechanistic insights into tether peptide-mediated lipoprotein sorting in diderm bacteria

Our results show that two thirds of the lipoproteins expressed by *B. burgdorferi* are exported to the cell surface, while the remaining periplasmic lipoproteins are mostly retained in the inner membrane. Whereas the periplasmic OM lipoprotein Lp6.6 is among the most abundant envelope proteins (88), the relative simplicity of the *B. burgdorferi* lipoproteome in the inner leaflet of the OM is unexpected. In *E. coli*, an organism with a diderm membrane architecture similar to that of *B. burgdorferi*, most of the lipoproteins are exported to the OM as well (226), but the majority are not surface exposed (126). A generalization that lipoprotein surface exposure is rare and limited appears to hold for most diderm bacteria, with the emerging exception of the Gram-negative *Bacteroidetes* such as *Bacteroides fragilis* and *C. capnocytophaga* (212, 213, 227). Yet, the identified charge-based *C. capnocytophaga* lipoprotein secretion signals appear to be restricted to that phylum (212, 213), and the *B. burgdorferi* surface localization determinants identified in our own studies appear to extend only to other members of that expanding genus (196). This hints at significant mechanistic differences in lipoprotein secretion between Gram-negative bacteria and spirochetes. Our current data remain compatible with a lipoprotein secretion model that includes two separate secretion checkpoints: The first checkpoint is set up the IM where some lipoproteins are retained and others are released to the OM after completion of N-terminal processing. Whether lipoprotein retention and release is generally mediated by the partial *B. burgdorferi* Lol pathway

Table 3. Comparison of lipoprotein tether length based on localization. Lipoprotein tethers for each localization phenotype were either predicted *in silico* using PSSPRED (228) or using crystal structure PDB data, if available. PSSPRED predictions were performed using the mature lipoprotein sequences from the residue immediately following the acylated cysteine. Lipoproteins are annotated using the standard *B. burgdorferi* genetic locus notation (31, 32), with the PDB structure given in parentheses if available. Lipoprotein localizations were taken from consensus localization data (see Table 2). Representative lipoproteins were chosen so that only a single member of each paralogous family (Table 2) was represented. Ellipses and numbers indicate the number of tether residues omitted for clarity.

	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10
Surface										
BBA15 (3AUM)	C	K	Q	N	V	S	S	L	D	…(3)
BBA24 (4ONR)	C	G	L	T						
BBA68 (5A2U)	C	A	P	F	S	K	I	D	P	…(40)
OM-PERI										
BBA62	C	E	T	T	R	I				
BB0028	C	S	S							
BB0324	C	Y	T	I	N	L	E	K	L	…(5)
IM										
BBB16 (4GL8)	C	V	N	E	S	N	R	N	K	…(1)
BB0215 (4N13)	C	K	N	Q	D	N	E			
BB0141 (4KKT)	C	V	G	D	N	K	L	D	D	…(12)

(25) and dependent on specific N-terminal tether peptide residues is under current investigation. Alternatively, release from the IM may be hindered by functional interactions of folded IM lipoproteins with other IM protein complexes. Multiple lines of evidence point to a similar "exclusion" mechanism at the second checkpoint in the OM, where periplasmic lipoproteins are blocked from "flipping" through the OM due to assumption of their final tertiary structure (28-30, 174, 195). The *B. burgdorferi* OM β-barrel assembly machinery (BAM) protein BamA was shown to be at least indirectly involved in this process (229), and it remains to be determined if/how the other identified *B. burgdorferi* BAM complex proteins (203, 210), including the IM protein TamB (230), play a role in lipoprotein secretion. Recent work has shown that some *Neisseria* surface lipoproteins are secreted to the surface through the lumen of β-barrel integral OM proteins (172), but homologs are missing from *Borrelia* genomes. Together, this supports our earlier notions that lipoprotein secretion pathways in *Borrelia* spirochetes are unique.

4.4.5 Final Conclusions Regarding Localization of the B. burgdorferi Lipoproteome

We have comprehensively localized the *B. burgdorferi* lipoproteome using an epitope-tagged expression library and validated our findings by proteomics and by reconciling our data with prior independent protein localization data. The used approaches, such as the initial reliance on C-terminal epitope tags for protein detection, are not without limitations that will only be eliminated with the generation of protein-specific antibodies and further in-depth studies of the newly localized proteins. Yet, we are confident that the consensus *B. burgdorferi* lipoproteome localization catalog presented here reflects the proteins' native partition in the spirochetal cell envelope. Lyme borreliosis remains the most common vector-borne illness in the United States and is common throughout temperate climates in the Northern hemisphere, and

there are continuing efforts to improve diagnostics and preventive measures. Placing our lipoproteome localization data into the context of genome- and proteome-wide studies may facilitate the identification of additional diagnostic targets and vaccine candidates. The proteomic localization data presented here also provide a predictive framework for proteomic studies of host-pathogen interfaces and envelope structures of other members of the ever expanding *Borrelia* genus, including the relapsing fever spirochete *Borrelia miyamotoi* (231, 232) and the recently described North American Lyme disease spirochete *Borrelia mayonii* (233, 234).

Chapter 5: Detailed Experimental Protocols

5.1 Preparation of basal Barbour-Stoenner-Kelly II (BSK-II) Medium (175)

1. Pre-rinse all glassware to be used thoroughly with Milli-Q ultra-pure dH_2O.
2. Prepare 1L of 1X CMRL-1066 w/o l-glutamine (U.S. Biologicals# C5900-01) in a 2L glass beaker by adding 9.7g of powder to approximately 1L of Milli-Q dH_2O. Gently mix using a magnetic stir bar.
3. Dissolve 5g of neopeptone (BD Bacto# 211681) into solution.
4. Dissolve 50g of Probumin bovine serum albumin, universal grade (EMD Millipore# 810037) **slowly** into solution using a magnetic stir bar. This process should take at least an hour at room temperature.
5. Dissolve the following reagents into solution in order, making sure that each has dissolved before adding the next. Where no manufacturer is indicated, use ACS grade reagent.
    a. 2g of TC Yeastolate (BD Bacto# 255772)
    b. 6g of HEPES, free acid
    c. 5g of dextrose (D-glucose)
    d. 0.7g of sodium citrate (tribasic dihydrate)
    e. 0.8g of sodium pyruvate
    f. 0.4g of *N*-acetyl-D-glucosamine
    g. 2.2g of sodium bicarbonate
6. Adjust the pH of the medium to 7.6 using 1M NaOH. The NaOH solution should be no more than one week old.

7. Filter sterilize the solution using two 0.22μm PES filter units (Corning# 431097) using fiberglass prefilters (Corning# 431412). Prefilters should be pre-wetted using Milli-Q dH_2O and applied to the top of the filter unit under vacuum before applying medium.
8. Under a biological safety hood, aseptically remove the vacuum unit from the bottles and cap. Distribute the medium evenly between the two bottles so that each contains approximately one-half of the total preparation of medium.
9. Store up to six months in the dark at 4°C.

5.2 Preparation of complete BSK-II Medium (175)

1. Equilibrate one bottle of basal medium (see Section 5.1) to 37°C in a water bath.
2. Prepare a 100mL solution of 7% (w/v) gelatin:
    I. Pre-rinse a 250mL bottle thoroughly with Milli-Q dH_2O.
    II. Measure 7g of gelatin (BD Difco# 214340) into the bottle.
    III. Add approximately 95mL of Milli-Q dH_2O to the bottle, swirl gently to mix, and incubate at room temperature 10 minutes to hydrate the gelatin.
    IV. Solubilize the gelatin, either using a microwave or by autoclaving.
    V. Equilibrate to 37°C in a water bath.
3. Equilibrate a 40mL aliquot of sterile, trace-hemolyzed rabbit serum (Pel-Freez# 31126-5) to 37°C in a water bath.
4. Thoroughly rinse a 1L bottle with Milli-Q dH_2O. Combine the basal BSK-II, gelatin, and rabbit serum in the 1L bottle and swirl to mix. (*Note: At this stage, a portion of medium can be measured out using a pre-rinsed glass graduated cylinder for the addition of additives, e.g. antibiotics*).

5. Filter sterilize the medium using 0.22μm PES filter units (Corning# 431096 & 431097). This step must be completed before the medium has cooled to below room temperature; otherwise, the gelatin will begin to solidify and impede filtration of the medium.
6. Aseptically cap the bottles under a biological safety hood. Store the completed medium at 4°C in the dark and should be used within one month of preparation for optimal results. Before use, medium must be equilibrated to 34-37°C due to solidification of gelatin at lower temperatures.

5.3 Preparation of Competent *B. burgdorferi* (177, 178)

1. Inoculate 500mL of complete BSK-II with 1mL of the desired culture in late-logarithmic growth phase.
2. Incubate culture at 34°C until the cell density reaches approximately $5x10^7$ cells/mL.
3. Divide the culture evenly between two sterile 500mL centrifuge bottles. Manipulation of *B. burgdorferi* in this step, as well as in all following steps, must be performed aseptically under a biological safety hood.
4. Pellet cells at 4,000 x *g* for 20min at room temperature. Carefully decant the supernatant.
5. Resuspend each cell pellet in 30mL of sterile, ice-cold phosphate buffered saline (pH 7.4) + 5mM $MgCl_2$. Transfer cell suspensions to two sterile, conical 50mL tubes (Corning# 352098) that had been pre-chilled on ice. Keep cells cold or on ice from this point forward.
6. Re-pellet cells at 3,000 x *g* for 15min at 4°C. Carefully remove the supernatant with a serological pipette.

7. Resuspend each cell pellet in 10mL of sterile, ice-cold 272mM sucrose. Transfer cell suspensions to two sterile, conical 15mL tubes (Corning# 352097) that had been pre-chilled on ice.
8. Re-pellet cells at 2,000 x *g* for 10min at 4°C. Carefully remove the supernatant using a serological pipette.
9. Repeat Steps 7 and 8, but without transferring the cell suspensions to new tubes.
10. Pool the cell pellets by resuspension in 1mL of sterile, ice-cold 272mM sucrose. Cells should be resuspended completely – no particulates should be visible when pipetted. Divide cell suspension into 50µL aliquots into sterile, pre-chilled 1.5mL microcentrifuge tubes on ice for electroporation. Alternatively, cells can be resuspended in 1mL of sterile, ice-cold 272mM sucrose + 15% (v/v) glycerol, divided into aliquots, and flash-frozen at -80°C for long-term storage.

5.4 Electroporation of *B. burgdorferi* (177, 178)

1. Thaw a 50µL aliquot of competent cells on ice for 5min, if using a pre-made stock.
2. Add 1-5µL of DNA in dH_2O to competent cells and flick gently to mix. The amount of DNA needed varies depending on the genetic background of the competent cells and on the nature of the DNA to be transformed. High-passage *B. burgdorferi* B31 can be transformed with as little as a few hundred nanograms of the shuttle vector pBSV2, whereas low-passage (infectious) *B. burgdorferi* strains require approximately 30µg total DNA. Preparations of DNA should be free from salts to minimize risk of arcing during electroporation. Manipulation of cells in this step, as well in all following steps, must be performed aseptically.

3. Incubate cells and DNA on ice for 1min.
4. Transfer cells/DNA to a sterile 0.2cm electroporation cuvette (Bio-Rad# 1652086) that had been pre-chilled to 4°C. Tap the cuvette gently to bring the cells/DNA liquid to the bottom of the chamber, so that the liquid spans the void between the two electrodes.
5. Electroporate the cells using a single exponential decay pulse on a Bio-Rad GenePulser at 2.5kV, 25μF, 200Ω. Alternatively, a Bio-Rad MicroPulser can be used on the "Ec2" setting and should optimally result in a time constant of approximately 5.8ms.
6. **Immediately** (within 1 minute of electroporation), add approximately 1mL of pre-warmed (34°C) complete BSK-II to the cells in the cuvette using a sterile Pasteur pipette (Fisher# 13-711-20). Pipette to mix, then transfer the cells to a 15mL Falcon tube containing an additional 11mL of pre-warmed complete BSK-II. Cap the tube and invert several times to mix. At this stage, antibiotics should not be used unless the genetic background of the competent cells requires it.
7. Incubate at 34°C for 18-24 hours to allow for outgrowth of transformants.

5.5 Plating and Selection of *B. burgdorferi* Transformants (177, 178)

1. Prepare 2X basal BSK-II medium using the protocol as described in Section 5.1, except that the initial volume of dH_2O is reduced by half.
2. Measure 250mL of 2X basal BSK-II using a pre-rinsed glass graduated cylinder. Dissolve necessary antibiotics at 2X necessary concentration.
3. Filter-sterilize 250mL of 2X basal BSK-II+antibiotics and 30mL of trace-hemolyzed rabbit serum using a 500mL 0.22μm filter unit. Aspetically cap bottle and equilibrate to 50°C in a water bath.

4. Prepare a 220mL solution of 1.52% (w/v) agarose:
    i. Pre-rinse a 500mL bottle thoroughly with Milli-Q dH_2O.
    ii. Measure 3.33g of SeaKem LE agarose into bottle.
    iii. Add approximately 220mL of Milli-Q dH_2O. Microwave until agarose has been completely dissolved.
    iv. Autoclave solution and equilibrate to 50°C.
5. Aseptically add the agarose solution to the BSKII+antibiotics+rabbit serum. Cap and mix well by swirling the solution. All of the following steps where cells and/or medium is manipulated must be performed aseptically under a biological safety hood.
6. Pipette 15mL of the molten medium into three sterile 100x15mm Petri dishes (Fisher# FB0875712) for each transformation performed. Re-cap bottle of BSKII/agarose and equilibrate to 42°C in a water bath.
7. While the plates are solidifying and BSKII/agarose is equilibrating, pellet transformed cells at 3,000 x *g* for 30min at room temperature. Cells can be examined beforehand under a microscope to examine health after outgrowth.
8. Remove supernatant from transformed cells using a serological pipette except for approximately 1mL of liquid. Resuspend the cells completely in the 1mL of remaining supernatant. Ensure that the plates have solidified and that the BSKII/agarose is at 42°C before proceeding.
9. Label each plate on the outside rim with the date, the cell background, the DNA used for transformation, and one of the following percentages: 1%, 10%, 89%.
10. Label three fresh 15mL Falcon tubes with 1%, 10%, and 89%. To each, add 10µL, 100µL, or 890µL of the resuspended transformed cells (respectively).

11. Add 12mL of BSKII/agarose to one tube of cells, mix by pipetting, and layer cells/BSKII/agarose over the pre-poured plate that corresponds to the cell background, transformation and percentage. Avoid bubbles, if possible.
12. Repeat Step 11 for each tube and each transformation, using a fresh serological pipette for each plate. Allow plates to solidify completely under the hood (≤30min).
13. Incubate plates lid-side-up at 34°C in a humidified 5% CO_2 atmosphere. The time until colonies appear depends on the genetic background of the cells and the nature of the DNA introduced, with 1-3 weeks being commonplace.

5.6 Surface Proteolysis of *B. burgdorferi* Using Proteinase K (180)

1. Inoculate 45mL of complete BSK-II (with antibiotics, if necessary) in a 50mL sterile tube at 1:500 with the desired strain and incubate at 34°C until the culture reaches late-logarithmic growth phase.
2. Transfer 6mL of the culture to a fresh 15mL tube and harvest at 3,000 x *g* for 25min, room temperature. Remove the supernatant using a pipette.
3. Wash the cells once by resuspending gently in 6mL of sterile, room temperature phosphate buffered saline (pH 7.4) + 5mM $MgCl_2$ (PBS/Mg).
4. Re-pellet the cells at 3,000 x *g* for 20min. Remove the supernatant using a pipette.
5. Resuspend the cell pellet gently but completely in 300µL of PBS/Mg. Divide the cell suspension into two 144µL aliquots using fresh 1.5mL microcentrifuge tubes. To one, add 6µL of Milli-Q dH_2O as a control. To the other, add 6µL of 5mg/mL Proteinase K (Invitrogen# AM2544) in Milli-Q dH_2O for 200µg/mL final concentration. Mix gently by flicking the sides of the tubes.

6. Incubate the cells at room temperature for one hour.
7. Add 8µL of 100mM PMSF in DMSO (Sigma-Aldrich# 78830-5G, D2650-5X10ML) to each sample for approximately 5mM final concentration. Mix thoroughly by flicking tubes.
8. Pellet cells at 8,000 x *g* for 10 minutes, room temperature. Completely remove supernatant using a 200µL pipette.
9. Resuspend each cell pellet in approximately 20µL final volume of Milli-Q dH_2O. Add 5µL of 1M DTT (Fisher# BP172-5, freshly made in Milli-Q dH_2O) and 25µL of 2X SDS-PAGE Sample Buffer (Formulated as per Sigma-Aldrich# S3401 but without β-mercaptoethanol). Mix by flicking tube and cap tightly.
10. Boil samples for 5 minutes and store at -20°C until needed.

5.7 Surface Proteolysis of *B. burgdorferi* Using Proteinase K (187, 235)

1. Inoculate 45mL of complete BSK-II (with antibiotics, if necessary) in a 50mL sterile tube at 1:500 with the desired strain and incubate at 34°C until the culture reaches late-logarithmic growth phase.
2. Transfer 6mL of the culture to a fresh 15mL tube and harvest at 3,000 x *g* for 25min, room temperature. Remove the supernatant using a pipette.
3. Wash the cells once by resuspending gently in 6mL of sterile, room temperature phosphate buffered saline (pH 7.4) + 5mM $MgCl_2$ (PBS/Mg).
4. Re-pellet the cells at 3,000 x *g* for 20min. Remove the supernatant using a pipette.
5. Resuspend the cell pellet gently but completely in 300µL of PBS/Mg. Divide the cell suspension into two 142.5µL aliquots using fresh 1.5mL microcentrifuge tubes. To

one, add 7.5µL of Milli-Q dH_2O as a control. To the other, add 7.5µL of 20mg/mL Pronase (Roche# 10165921001) in Milli-Q dH_2O for 1mg/mL final concentration. Mix gently by flicking the sides of the tubes.

6. Incubate the cells at 37°C for two hours.
7. Add the following to each sample. Mix by flicking the tubes gently.
    i. 48.8µL of PBS/Mg
    ii. 0.4µL of 0.5M EDTA (Invitrogen# 15575020)
    iii. 0.4µL of 100mM PMSF in DMSO (Sigma-Aldrich# 78830, D2650)
    iv. 0.4µL of 400mM Pefabloc SC (Sigma-Aldrich# 76307)
8. Pellet cells at 8,000 x *g* for 10 minutes, room temperature. Completely remove supernatant using a 200µL pipette.
9. Resuspend each cell pellet in approximately 20µL final volume of Milli-Q dH_2O. Add 5µL of 1M DTT (Fisher# BP172-5, freshly made in Milli-Q dH_2O) and 25µL of 2X SDS-PAGE Sample Buffer (Formulated as per Sigma-Aldrich# S3401 but without β-mercaptoethanol). Mix by flicking tube and cap tightly.
10. Boil samples for 5 minutes and store at -20°C until needed.

5.8 Membrane Fractionation of *B. burgdorferi* (30, 181)

1. Inoculate two 250mL cultures of complete BSK-II (with antibiotics, if necessary) using 250µL of a late-logarithmic growth phase culture of each desired strain.
2. Incubate at 34°C until the culture reaches early exponential phase, about $5x10^6$ cells/mL. At this point, the culture medium should still have its original color and there should not be visible clumps of cells at the bottom of the bottle. Do not let the culture overgrow.

3. Prepare solutions for experiment:
    a. Prepare 1L of 25mM citrate buffer by dissolving 4.8g of anhydrous citric acid into 800mL of Milli-Q dH_2O, adjusting the pH to 3.2 using freshly made 1M NaOH, and adjusting the volume to 1L using Milli-Q dH_2O. Pre-rinse all glassware to be used thoroughly with Milli-Q dH_2O. Divide the preparation of citrate buffer into two 500mL aliquots.
    b. Prepare 500mL of citrate buffer + 0.1% BSA by adding 0.5g of Probumin BSA(EMD Millipore# 810037) and mixing until dissolved.
    c. Prepare the following sucrose solutions in 50mL conical tubes. Each solution will eventually fully dissolve at room temperature with periodic mixing. Chill on ice when dissolved.
        i. 32g of 25% (w/w) sucrose in citrate buffer
        ii. 40g of 42% (w/w) sucrose in citrate buffer
        iii. 14g of 56% (w/w) sucrose in citrate buffer
4. Transfer each culture into pre-rinsed 500mL centrifuge bottles and harvest at 3,000 x *g* for 25min at room temperature. Decant supernatant.
5. Wash each pellet by resuspension in 250mL of phosphate buffered saline (PBS) + 0.1% (w/v) Probumin BSA.
6. Re-pellet cells at 3,000 x *g* for 20min at room temperature. Decant supernatant.
7. Resuspend each pellet **completely** (no visible particulates) in 45mL of ice-cold 25mM citrate buffer + 0.1% BSA and transfer to fresh 50mL conical tubes. Seal with Parafilm.

8. Incubate at room temperature for two hours on a rocker. Every 30 minutes, vortex each tube for 30 seconds. During this time, pre-chill the XPN-80 ultracentrifuge and SW32Ti rotor to 4°C.
9. Divide each cell suspension evenly between two pre-rinsed SS-34 tubes and pellet at 20,000 x *g* for 30min, room temperature.
10. While the cell suspensions are pelleting, pour the discontinuous sucrose gradients in Beckman UltraClear tubes (#344058). Sucrose solutions must be **ice-cold**. UltraClear tubes are not wettable; therefore, the lightest (25%) layer must be poured first and can be added using a serological pipette. The 42% and 56% layers must then be added in that order by pipetting underneath the previous layer, displacing the lighter layer upwards. Use disposable syringes (BD# 302832, 309604) and a blunt-tip syringe needle (Sigma-Aldrich# Z261351-1EA) to underlay each solution, ensuring crisp interfaces between each layer. Label the outside of each gradient with a Sharpie and store at 4°C until the previous step's centrifugation is finished.
    i. 12.5mL of 25% (w/w) sucrose
    ii. 15.5mL of 42% (w/w) sucrose
    iii. 5mL of 56% (w/w) sucrose
11. Decant the tubes and place on ice. Pool cell pellets from matching samples by resuspending **completely** (no visible particulates) in 5mL of ice-cold citrate buffer + 0.1% BSA.
12. Using a 10mL syringe and an 18½G needle, slowly and carefully overlay each cell suspension over the labeled gradients. Insert each gradient into opposing SW32Ti swinging buckets (e.g. buckets #'s 1 and 4) using tweezers to slowly drop the gradient

into place. Add the caps to the buckets and balance the opposing rotors to within 0.01g, using ice-cold citrate buffer + 0.1% BSA to make up any difference.

13. Place all six buckets into the pre-chilled SW32Ti rotor in their designated places. Run the ultracentrifuge at 100,000 x *g* for 20 hours, 4°C.
14. Immediately remove the gradients from the ultracentrifuge without disturbing the samples. Leave the ultracentrifuge on and cooled for the following steps.
15. Visualize each gradient. There should be a faint "haze" about 2/3rd of the way up from the bottom, at the interface between the 25% and 42% solutions. This is the outer membrane vesicle (OMV) fraction. Towards the bottom of the tube there should be an obvious, flat disc at the interface between the 42% and 56% solutions. This is the protoplasmic cylinder (PC) fraction.
16. Using a fresh 10mL syringe + 18½G needle for each layer, needle-aspirate the OMV and PC fractions from each sample into fresh 50mL conical tubes on ice.
17. Dilute each fraction to approximately 36mL using ice-cold PBS (pH 7.4), which should result in at least a five-fold dilution of the aspirated sample. Vortex each sample thoroughly.
18. Prepare the two fractions as follows:
    a. Pellet the OMV fractions by transferring the diluted samples to fresh UltraClear tubes and balancing using ice-cold PBS (pH 7.4). Run in the ultra-centrifuge at 150,000 x *g* for 4 hours, 4°C. Immediately remove the sample at the end of the run and carefully decant. The pelleted OMV samples should be visible as whitish, faint, glassy pellets at the bottom of the tubes. Resuspend each OMV pellet in 100µL of PBS (pH 7.4) + 1mM PMSF and transfer to fresh 1.5mL

microcentrifuge tubes. Use a portion of each to prepare SDS-PAGE samples as was done in Section 5.6. Store the boiled SDS-PAGE samples at -20°C and the unprocessed OMV suspension at -80°C.

b. Pellet the PC fraction by transferring each to a fresh SS-34 tube and centrifuging at 10,000 x *g* for 20min, 4°C. Decant supernatant. Resuspend each cell completely in 1mL of PBS (pH 7.4) + 1mM PMSF and transfer to fresh 1.5mL microcentrifuge tubes. Use a portion of each to prepare SDS-PAGE samples as was done in Section 5.6. Store the boiled SDS-PAGE samples at -20°C and the unprocessed PC suspension at -80°C.

19. Use equal volumes of each sample for SDS-PAGE analysis.

5.9 Triton X-114 Extraction of Hydrophobic *B. burgdorferi* Proteins (186)

1. Inoculate two 45mL volumes of complete BSK-II (with antibiotics, if necessary) in sterile 50mL conical tubes using 90µL each of the desired strain. Incubate at 34°C until cultures have reached late-logarithmic growth phase. Alternatively, protease-treated cells can be extracted following Step 8 of Sections 5.6 and 5.7 as long as the volumes are scaled-up appropriately (in this case, skip to Step 5).
2. Harvest cultures at 3,000 x *g* for 30min at room temperature. Remove supernatant by pipetting.
3. Wash each cell pellet by resuspension in 45mL of PBS (pH 7.4) + 5mM $MgCl_2$ (PBS/Mg). Re-pellet at 3,000 x *g* for 20min, room temperature. Remove supernatant by pipetting.
4. Repeat Step 3 once more.

5. Pool cell pellets by resuspension in 1mL of ice-cold 2% (v/v) Triton X-114 in PBS (pH 7.4) + 0.002% (w/v) bromophenol blue. Transfer cell suspension to a fresh 1.5mL microcentrifuge tube.
6. Rotate cells overnight on a rotisserie at 4°C.
7. Centrifuge sample at 12,000 x *g* for 30min, 4°C to remove Triton X-114 insoluble material. Transfer supernatant to a fresh microcentrifuge tube. The insoluble pellet can be saved for later SDS-PAGE analysis. Optionally, supernatant can be spun again at 12,000 x *g* for 15min, 4°C to remove remaining traces of insoluble material. In this case, transfer supernatant again to a fresh tube.
8. Incubate sample at 37°C for 15min to phase-partition the Triton X-114 solution.
9. Centrifuge the sample at 16,000 x *g* for 15min at room temperature. The resulting sample should have an upper, faintly blue aqueous phase and a lower, dark blue detergent phase. Carefully transfer the upper aqueous phase (Sample AQ) to a fresh microcentrifuge tube without disturbing the lower detergent phase (Sample DE).
10. Wash the samples three times as follows. The washing process should progressively make Sample AQ less blue and Sample DE more blue.
    a. Add 100µL of ice-cold 10% (v/v) Triton X-114 in PBS (pH 7.4) + 0.002% (w/v) bromophenol blue to Sample AQ and vortex. Add 500µL of ice-cold 1% (v/v) Triton X-114 in PBS (pH 7.4) + 0.002% (w/v) bromophenol blue to Sample DE and vortex.
    b. Incubate for 15min at 37°C to phase partition the samples.

c. Centrifuge at 16,000 x *g* for 15min at room temperature. Transfer the aqueous phase of Sample AQ to a fresh tube and discard the pelleted detergent. Remove the aqueous supernatant from Sample DE and retain the detergent phase.

11. Add ice-cold 1% (v/v) Triton X-114 in PBS (pH 7.4) + 0.002% (w/v) bromophenol blue to Sample DE to a final volume of 500µL and vortex.
12. Divide Sample AQ and Sample DE evenly between four 1.5mL microcentrifuge tubes each. Add four volumes of 100% acetone (chilled to -20°C) to each sample and vortex.
13. Incubate samples overnight at -20°C.
14. Centrifuge samples at 16,000 x *g* for 30min, 4°C. Discard supernatant.
15. Wash samples by adding 1mL of 80% (v/v) acetone in Milli-Q dH_2O (chilled to -20°C) to each sample and inverting gently several times.
16. Centrifuge at 16,000 x *g* for 15min, 4°C. Carefully remove the supernatant by pipetting. Spin the samples briefly in a centrifuge to collect residual acetone at the bottom of the tubes, then remove carefully with a 20µL pipette without disturbing the protein pellets.
17. Allow the samples to air-dry on the bench with the caps open for 10min.
18. Pool like samples by resuspension in a suitable buffer and use a fraction for SDS-PAGE sample preparation (see Section 5.6). If the samples do not easily dissolve into solution, allow them to incubate with buffer in sealed tubes at 4°C for at least one hour before resuspension. Store unprocessed samples long-term at -80°C and SDS-PAGE samples at -20°C.

Chapter 6: In-depth Analysis of Selected *B. burgdorferi* Lipoproteins

Lipoproteins are essential to the survival and pathogenesis of *B. burgdorferi*; however, many of these have not been functionally characterized through experimentation. In order to better understand the biological role of the *B. burgdorferi* lipoproteome, lipoproteins were analyzed *in silico* using a variety of methods. Functional prediction using *in silico* analysis generates testable hypotheses regarding the *B. burgdorferi* lipoproteome and can guide future investigation of under-studied proteins. Of note is that lipoproteins were analyzed only from the essential genetic elements, the linear chromosome and the circular plasmid cp26, as these elements are the only ones common to all isolates of *B. burgdorferi* and, therefore, most likely to contain essential biosynthetic factors (236). Initially, the full primary sequence of each lipoprotein was analyzed with the HMMER-based HMMSCAN using all available databases and the program's default search settings (237). The top-scoring (by E-value) protein domain/family for each lipoprotein is reported in **Table 4**, with relevant domain/family accession numbers and descriptions given. Several lipoproteins were predicted to contain tetratricopeptide repeat (TPR) domains, and the identity of these lipoproteins as putative TPR proteins was confirmed by analysis using TPRpred and is recorded in **Table 5** (238). Lipoproteins with no statistically significant hits reported by HMMSCAN were then analyzed further using their predicted mature sequence with HHPred, which detects remote homology based on HMM profile-profile comparison between a multiple sequence alignment of query and deposited PDB structures (239). HHpred is able detect proteins that share tertiary structural elements despite significant differences in primary sequences. **Table 6** reports the top-scoring PDB hit for each analyzed lipoprotein. Several lipoproteins from this table were then selected for in-depth analysis, and a hypothetical *in vivo* function was formulated based on all available data.

Table 4. Analysis of Lipoprotein Genes on Essential Genetic Elements by HMMSCAN. Lipoprotein-encoding genes from the essential genetic elements of *B. burgdorferi*, the chromosome and the circular plasmid cp26, were analyzed using HMMSCAN (236, 237). Genes are reported with the top profile hit by lowest E-value. "SF#" = Superfamily number (superfam.org). Interval corresponds to amino acid region of the unprocessed protein. Genes without a significant hit are reported with "None, n/a."

	Top Hit ID	Description	Interval	E-value
BB0028	None	n/a	n/a	n/a
BB0141	TIGR01730	Efflux transporter, RND family, MFP subunit	114-306	1.8e-29
BB0144	PF04069.11	Substrate binding domain of ABC-type glycine betaine transport system	26-272	8.7e-60
BB0155	None	n/a	n/a	n/a
BB0158	None	n/a	n/a	n/a
BB0171	SF# 48452	TPR-like	28-144	3.2e-20
BB0193	None	n/a	n/a	n/a
BB0213	None	n/a	n/a	n/a
BB0215	SF# 53850	Periplasmic binding protein-like II	29-278	1.7e-56
BB0224	None	n/a	n/a	n/a
BB0227	None	n/a	n/a	n/a
BB0298	SF# 48452	TPR-like	46-211	1.1e-14
BB0323	PF01476.19	LysM domain	327-375	2.3e-08
BB0324	SF# 48452	TPR-like	25-115	2.6e-07
BB0328	PIRSF002741	Peptide binding protein, MppA type	23-528	1.2e-102
BB0329	PIRSF002741	Peptide binding protein, MppA type	14-520	1.6e-102
BB0330	PIRSF002741	Peptide binding protein, MppA type	6-533	3.4e-101
BB0352	None	n/a	n/a	n/a
BB0365	None	n/a	n/a	n/a
BB0382	PF02608.13	ABC transporter substrate-binding protein PnrA-like	27-328	9.0e-107
BB0384	PF02608.13	ABC transporter substrate-binding protein PnrA-like	29-343	3.1e-107
BB0385	PF02608.13	ABC transporter substrate-binding protein PnrA-like	46-348	6.1e-107
BB0398	SF# 48452	TPR-like	53-120, 158-190	5.9e-09
BB0456	None	n/a	n/a	n/a
BB0460	None	n/a	n/a	n/a
BB0475	None	n/a	n/a	n/a
BB0536	PF00675.19	Insulinase (Peptidase family M16)	45-183	6.3e-27
BB0542	SF# 48452	TPR-like	35-174	7.6e-16
BB0628	None	n/a	n/a	n/a
BB0652	TIGR01129	Protein-export membrane protein SecD	138-562	5.5e-141
BB0664	None	n/a	n/a	n/a
BB0689	PF00188.25	Cysteine-rich secretory protein family	34-148	3.1e-12
BB0758	None	n/a	n/a	n/a
BB0806	SF# 50998	Quinoprotein alcohol dehydrogenase-like	225-499	1.1e-10
BB0823	None	n/a	n/a	n/a
BB0832	None	n/a	n/a	n/a
BB0844	None	n/a	n/a	n/a
BBB08	None	n/a	n/a	n/a
BBB09	None	n/a	n/a	n/a
BBB25	None	n/a	n/a	n/a
BBB27	None	n/a	n/a	n/a

Table 5. Analysis of putative *B. burgdorferi* Tetratricopeptide Repeat (TPR) Lipoproteins. Lipoproteins identified in **Table 4** were further analyzed by TPRpred to determine the probability of being a true TPR protein (238).

	TPR Probability	Per-protein P-value
BB0171	100.00%	3.2e-22
BB0298	100.00%	1.7e-16
BB0324	84.53%	5.7e-09
BB0398	99.87%	1.7e-12
BB0542	100.00%	8.9e-17

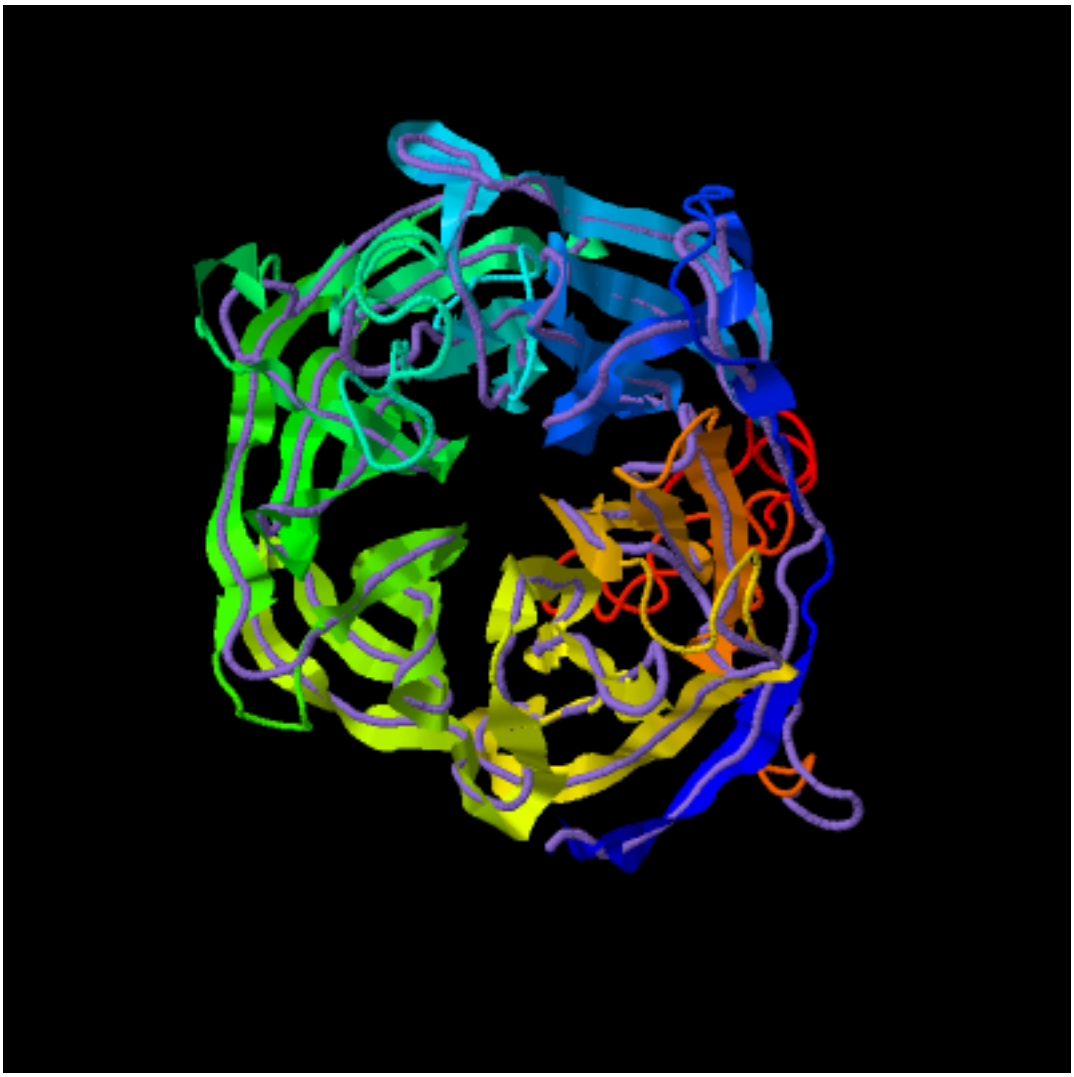
Table 6. Remote Homology Analysis of *B. burgdorferi* Lipoproteins. Lipoproteins without a detectable family/domain or with a hit resulting in an unclear function after HMMSCAN analysis (see **Table 4**) were analyzed by HHPred (239). Searches were performed against PDB profiles for conserved protein folds using the predicted mature sequence of the lipoprotein. The top-scoring hit by Probab. is reported for each lipoprotein, along with a description of the hit and the hit's score. Lipoproteins without a significant hit are denoted by "n/a."

	Top PDB#	Description	Probab.
BB0028	n/a	n/a	n/a
BB0155	3WW9_A	Pizza6 self-assembling, synthetic β-propeller protein	99.81
BB0158	n/a	n/a	n/a
BB0193	n/a	n/a	n/a
BB0213	n/a	n/a	n/a
BB0224	4HVZ_A	SIMPL domain protein, BP26, *Brucella abortus*	99.42
BB0227	3CYG_A	DUF3298/4163 protein, Fnod_1146, *Fervidobacterium nodosum* Rt17-B1	100.00
BB0352	2ZYO_A	Cyclo/maltodextrin solute-binding protein, *Thermoactinomyces vulgaris*	100.00
BB0365	n/a	n/a	n/a
BB0456	n/a	n/a	n/a
BB0460	3CYG_A	DUF3298/4163 protein, Fnod_1146, *Fervidobacterium nodosum* Rt17-B1	100.00
BB0475	n/a	n/a	n/a
BB0628	2NC8_A	LppM, Rv2171, *Mycobacterium tuberculosis*	94.70
BB0664	2PLG_A	YbjN-like protein T110839, *Synechococcus elongatus*	97.25
BB0758	n/a	n/a	n/a
BB0806	4A2L_A	Hybrid two-component system protein BT4663, *Bacteroides thetaiotaomicron*	99.97
BB0823	2KT8_A	SH3 domain protein CPE1231, *Clostridium perfringens*	96.44
BB0832	3K8G_A	Outer membrane lipoprotein TP0453, *Treponema pallidum*	96.11
BB0844	n/a	n/a	n/a
BBB08	n/a	n/a	n/a
BBB09	n/a	n/a	n/a
BBB25	n/a	n/a	n/a
BBB27	n/a	n/a	n/a

The first lipoprotein to be investigated in-depth is the product of the gene *bb0155*. This protein is well conserved only in the *Borrelia* genus based on analysis of its primary sequence using HMMER, although it appears to be somewhat conserved in certain related spirochetes such as *Treponema* (237). It may be transcribed as part of an operon with the downstream genes *bb0156* and *bb0157*, although these two gene products are likewise poorly conserved outside of *B. burgdorferi* (31, 32). A review of microarray data showed that *bb0155* appears not to be regulated by the Rrp2/RpoN/RpoS, BosR, or the Hk1/Rrp1 pathways (240). Additionally, it was found that *bb0155* was expressed in fed larvae, fed ticks, rat dialysis membrane chambers (DMCs; a facsimile for vertebrate infection), and *in vitro*; however, expression was not found to be significant different in any of the environments (19). Along with the microarray review study, this indicates that *bb0155* is likely a constitutively expressed gene. A different study that examined the expression of genes *in vitro* versus in mice showed that *bb0155* was expressed after growth in culture medium and in severe combined immunodeficiency mice, but not in immunocompetent C3H mice (241). In addition, transfer of ear tissue from infected to uninfected mice led to transient expression of *bb0155* at the 11-day and 22-day timepoints, but no expression at the 33-day timepoint. It is possible that, based on these two studies, *bb0155* is down-regulated or selected against during persistent vertebrate infection. Interestingly, passive immunization of mice resulted in down-regulation of most lipoprotein genes but not *bb0155*. Taken together, these gene regulation data indicate that the expression of *bb0155* is complex, and its expression may be an interplay of multiple factors. A genome-wide transposon mutagenesis study of *B. burgdorferi* failed to recover any *bb0155* insertion mutants (*bb0156* and *bb0157* mutants were recovered), although a separate transposon mutagenesis study found that insertional mutagenesis of *bb0155* negatively impacted the ability of the bacterium to survive on

maltose as the primary carbon source (37, 242). This may suggest that BB0155 plays a role in the metabolism of maltose or in the regulation of maltose-inducible genes in the organism. Analysis using InterPro reveals that the protein contains a predicted six-bladed, β-propeller (TolB-like) domain, IPR#011042 (243). β-propellers are a structural domain ubiquitous throughout the domains of life, with varying numbers of "blades" in the propeller and diverse functional roles (244, 245). Six-bladed β-propellers have been shown to act as structural proteins, hydrolases, and ligand-binding proteins (amongst other roles). Further analysis of BB0155 using the remote homology prediction server HHPred against a PDB profile database revealed significant homology to NHL domain, six-bladed β-propeller proteins (SCOPe# d1q7fa_, b.68.9.1; Probab=99.93, E-value=1.9e-21) (239). The best "hits" to eubacterial crystal structures were the C-terminal domain of the *Bacteroides thetaiotaomicron* protein BT3679 (PDB# 3HRP_A) and the C-terminal domain of the *Bacteroides ovatus* protein BACOVA_00264 (PDB# 4HW6_A). In-depth structural analysis of BB0155 using the I-TASSER web server found striking homology to the Brain tumor protein of *Drosophila melanogaster* (PDB# 1Q7F), a 6-bladed, NHL repeat β-propeller. As shown in **Figure 11**, the predicted structure of BB0155 and PDB#1Q7F_B align closely, matching all six blades of their β-propellers. 1Q7F belongs to the CATH superfamily 2.120.10.30 ("TolB, C-terminal domain"), which contains members with diverse functional roles. Although the diverse functions of β-propeller proteins make it impossible to ascertain the biological function of BB0155 without further experimental data, it is likely that the inner membrane localization of BB0155 means that it does not regulate translation in the same way that 1Q7F does (27, 246). Future studies will need to focus on the exact biological role of

Figure 11. Structural Analysis of BB0155 using I-TASSER. The predicted mature sequence of BB0155 was analyzed using the I-TASSER web server with the default settings (247). PDB# 1Q7F_B, belonging to the NHL domain Brain tumor protein of *Drosophila melanogaster*, was calculated to be the closest structural analog by both TM-score and RMSD. The structure of 1Q7F_B was then superimposed onto the predicted tertiary structure of BB0832, with the rainbow-colored secondary structure cartoon representing BB0832 and the purple backbone trace representing 1Q7F_B.



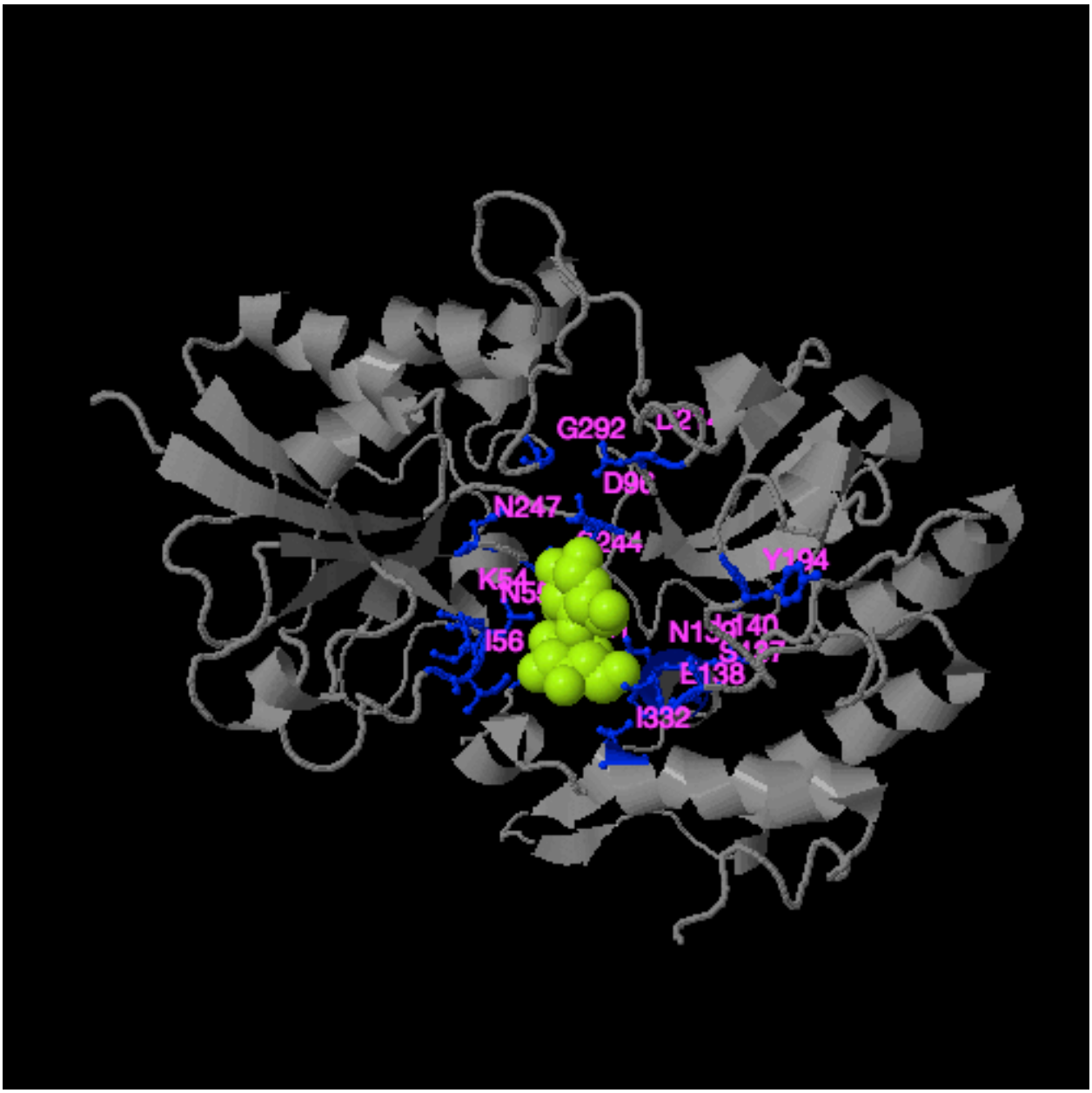
BB0155 in *B. burgdorferi* and whether its role relating to growth in maltose-containing medium is a direct or indirect effect of its disruption.

The putative lipoprotein BB0352 was similarly studied in detail. It is located in opposite orientation with the neighboring genes *bb0351* and *bb0353*, indicating that it likely is not part of an operon with surrounding genes (31, 32). No conserved domains or families were detected after a search using InterPro; likewise, a HMMER search found that only the *Borrelia* genus contains identifiable homologs at the primary sequence level (237, 243). Transposon mutagenesis experiments indicate that the gene may be essential: one study did not recover any transposon mutants, while another only recovered a single mutant 3% from the end of the gene (37, 242). Disruption of a gene within the last 10% of the reading frame is less likely to prevent the expression of a functional protein (248); this indicates that the single transposon insertion of *bb0352* may in fact result in a protein that is still biologically active. Gene regulation studies show that the gene may be part of the Rrp2/RpoN/RpoS regulon, with Rrp2 possibly the only factor responsible for its expression (240, 249). However, other studies found that *bb0352* was not differentially regulated in fed tick environments or in DMCs (19). As the Rrp2/RpoN/RpoS transcriptional cascade has often been associated with adaptation to the vertebrate host environment, and because there seems to be no differential regulation in the tick vs. vertebrate environments, these data suggest that other, unidentified factors may be additionally involved in *bb0352* gene regulation. Structural analysis of BB0352 reveals that the protein bears significant homology (E-value < 1e-30) to sugar substrate binding proteins of ABC transporters (e.g. PDB# 2ZYO_A). As shown in **Figure 12**, analysis of a MODELLER-constructed BB0352 PDB using the protein-ligand modelling server COACH demonstrates that BB0352 likely binds saccharides such as glucose, maltose, and trehalose (FINDSITE, C-score=0.70, Cluster size=30, Cluster

representative=PDB#1A7L) (250, 251). This could suggest that BB0352 similarly acts as a carbohydrate SBP in *B. burgdorferi*. However, BB0352 was found to localize to the surface of the cell's outer membrane, which means that it would not have access to ATP, and does not exist in an operon with other ABC cassettes (27, 31, 32). Rather than acting in the acquisition of nutrients, it could be that BB0352 binds sugars in order to mediate *B. burgdorferi* adhesion to host tissues. Many proteins are glycosylated with diverse carbohydrates, including galactose modification of collagen and xylose modification of proteoglycans, and such a modification could provide an attachment point for *B. burgdorferi* (252). Similar strategies have been reported for *E. coli* and *Salmonella enterica*, which bind to the extracellular matrix through oligomannoside modifications (253). Additional support of this hypothesis is found the short tether of BB0352. Secondary structure prediction of the BB0352 mature sequence using PSSPRED shows that it contains a four-residue tether, reminiscent of the similarly short tether region of decorin binding protein A (DbpA; PDB# 4ONR) (228). As the length of a surface lipoprotein's tether has been hypothesized to correlate with its biological role, it is quite possible that, like DbpA, BB0352 acts as a *B. burgdorferi* adhesin (25). Although further experimentation is needed in order to clarify the biological role of BB0352, the significant homology to known sugar-binding proteins and predicted propensity to bind multiple types of carbohydrates can help guide future study of this protein's role in *B. burgdorferi*.

Finally, the putative lipoprotein BB0832 was selected for characterization. The gene encoding this protein is located on the chromosome in a potential operon with the neighboring

Figure 12. COACH Analysis of BB0352 reveals a preference for carbohydrate binding. A tertiary structure of BB0352 was constructed by MODELLER using aligned HHPred-identified homologs (239, 251). The model was built using the automatic selection of multiple templates. The resulting model (as a PDB) was then analyzed using COACH to determine possible ligand binding based on previously characterized protein-ligand structures deposited to the PDB (250). The top-scoring hit by C-score was generated by the FINDSITE module, yielding a 30-member cluster represented by Chain A of PDB# 1A7L and a C-score of 0.70. Cluster members bound a variety of saccharides, including maltose, dextrose, trehalose, and maltotetraose. The complete list of cluster member PDBs and the input BB0352 PDB file are available upon request. The predicted binding pocket for maltose (PDB# 1A7L) and coordinating residues of BB0352 are shown above.


G292
N247
Y194
I56
E138
I332

genes encoding isoleucine-tRNA synthetase (*bb0833*) and the C subunit of the ATP-dependent Clp protease (*bb0844*) (31, 32). No transposon mutants of *bb0832* were recovered in two separate studies, suggesting that the gene is essential for *in vitro* growth (37, 242). Analysis of microarray data shows that *bb0832* is not regulated by any of the Rrp2/RpoN/RpoS, BosR, or Hk1/Rrp1 pathways (240). It also appears to be expressed in fed larvae, fed nymphs, and DMCs; yet, its expression is not significantly different between these environments (19). Analysis of BB0832 using HMMER reveals that no significant homologs exist outside of the *Borrelia* genus by primary sequence, and further analysis using HMMSCAN shows that BB0832 does not contain any identifiable conserved domains or families (237). However, BB0832 was predicted by HHPred to be homologous to PDB#3K8G_A, TP0453 from *Treponema pallidum* (Probab.=96.11, E-value=0.055) (239). TP0453 is the only known lipoprotein of the *T. pallidum* outer membrane and is not surface-exposed (254, 255). Interestingly, TP0453 contains amphipathic α-helices that permit spontaneous membrane integration of nonlipidated TP0453. Membrane insertion of nonlipidated TP0453 into membranes results in destabilization of the bilayer and increased membrane permeability. In this way, TP0453 was found to act as a pore-forming molecule in *T. pallidum*. TP0453 also contains an internal hydrophobic cavity, with a large volume exceeding 3500 $Å^3$. The authors of the studies that characterized TP0453 hypothesized that TP0453 may act as a functional homolog for LolB, in that it can spontaneously insert itself into membranes through its amphipathic α-helices. The authors postulate that this ability would explain the presence of lipoproteins in the outer membrane of *T. pallidum* despite the absence of an identified LolB homolog in the spirochete's genome. To confirm the homology between TP0453 and BB0832, the predicted mature sequence of BB0832 was analyzed using the I-TASSER web server with the default settings (**Figure 13**) (247).

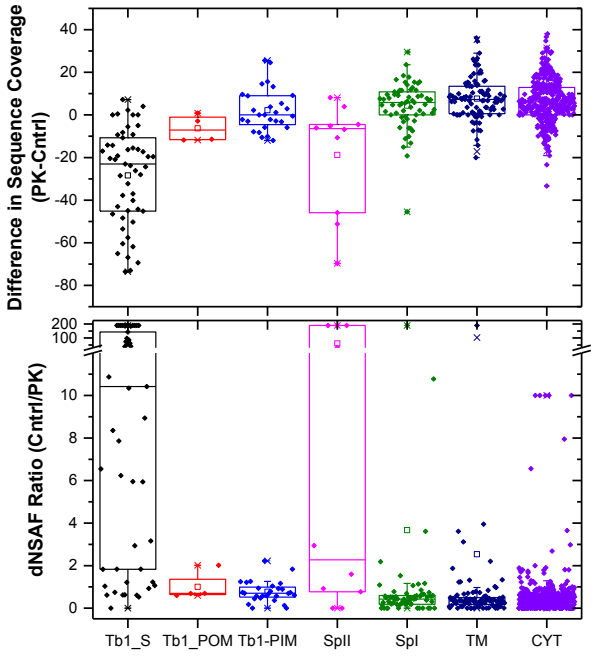
Significant homology was found between these two proteins, as a predicted structure of BB0832 was considered structurally analogous to PDB# 3K8G (TP0453) as evidenced by a TM-score of 0.829 and a RMSD of 1.38. Although I-TASSER failed to find any high-confidence ligand-binding or gene ontology assignments for BB0832, the distinct structural homology between BB0832 and TP0453 yields the hypothesis that these proteins are analogous and may function similarly *in vivo*. The propensity of TP0453 to spontaneously insert into membranes using amphipathic helices, as well as its large hydrophobic binding pocket, suggest that a *B. burgdorferi* homolog with these same properties could play a role in lipoprotein biosynthesis. Further, the lack of any recovered transposon-disrupted *bb0832* mutants suggests that the gene may be essential for *B. burgdorferi* growth. Although BB0832 was localized to the inner membrane using previous localization assays, and therefore might not act as a LolB functional homolog, it can safely be theorized that it may play an important biosynthetic role in *B. burgdorferi* and, as such, warrants further investigation in the future.

Figure 13. Analysis of BB0832 using I-TASSER. The predicted mature sequence of BB0832 was analyzed using the I-TASSER web server for structural prediction and homology detection using known PDB structures (247). Significant homology was detected with the *T. pallidum* lipoprotein TP0453, PDB# 3K8G. An overlay of the predicted structure of BB0832 (rainbow cartoon) and PDB# 3K8G (purple backbone trace) is shown above.

Appendix: Supplemental Figures and Tables

Figure S1. Statistical analyses of quantitative proteomics features for differently-located *B. burgdorferi* proteins. A. For all proteins detected by MudPIT analysis (**Table S1**), the difference in sequence coverage (% of protein sequence covered by detected peptides) observed between the proteinase K-treated and control samples were calculated (DiffSeqCov). The ratio in average dNSAF values measured in the control and proteinase K-treated samples were also calculated (dNSAF ratio). The proteins were grouped based on their known or predicted location (7 categories as defined in **Table 2** and **Table S1**) and box-plots of the distribution in DiffSeqCov and dNSAF ratios were built. **B.** The Mann−Whitney non-parametric U test was performed using OriginPro 9.1 to determine whether or not there were statistically significant differences between the distributions of the DiffSeqCov and dNSAF ratios parameters. The distributions of these parameters for proteins shown to be at the surface of *B. burgdorferi* B31-A3 cells ("S" proteins in **Table 2**) were systematically tested against the ranges of DiffSeqCov and dNSAF ratios for proteins shown (**Table 2**) or predicted to be localized elsewhere.

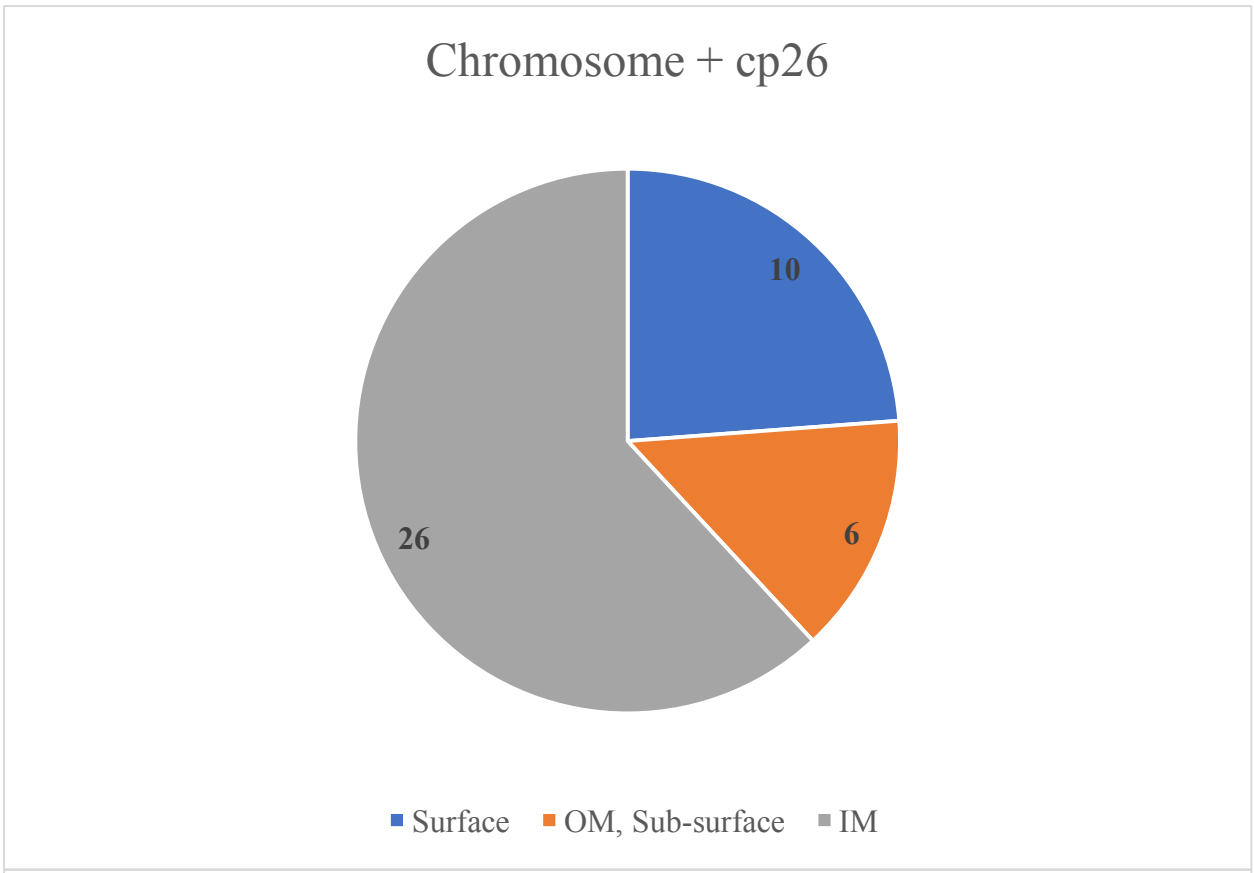
A



B

	x	y	Null Hypothesis	Alternative Hypothesis	U	Z	Exact Prob>U	Asymp. Prob>U
Difference in Sequence Coverage (PK - Control)	Table1_S	Table1_P-OM	F(x) >= G(y)	F(x) < G(y)	36	-2.12302	0.01495	0.01688
	Table1_S	Table1_P-IM	F(x) >= G(y)	F(x) < G(y)	137	-5.90927	3.88E-11	1.72E-09
	Table1_S	SpII	F(x) = G(y)	F(x) <> G(y)	177	-1.50985	0.13261	0.13108
	Table1_S	SpI	F(x) >= G(y)	F(x) < G(y)	227	-7.85903	4.82E-19	1.94E-15
	Table1_S	TM	F(x) >= G(y)	F(x) < G(y)	229	-9.13218	7.40E-27	3.36E-20
	Table1_S	CYT	F(x) >= G(y)	F(x) < G(y)	1044.5	-10.2737	5.09E-35	4.63E-25
	SpII	Table1_P-OM	F(x) = G(y)	F(x) <> G(y)	18	-0.21213	0.83916	0.832
	SpII	Table1_P-IM	F(x) >= G(y)	F(x) < G(y)	64	-2.50294	0.00523	0.00616
	SpII	SpI	F(x) >= G(y)	F(x) < G(y)	106	-3.34516	2.06E-04	4.11E-04
	SpII	TM	F(x) >= G(y)	F(x) < G(y)	122	-3.92613	8.60E-06	4.32E-05
	SpII	CYT	F(x) >= G(y)	F(x) < G(y)	550	-3.76887	2.33E-05	8.20E-05
	SpI	Table1_P-OM	F(x) <= G(y)	F(x) > G(y)	213	2.28908	0.00872	0.01104
	SpI	Table1_P-IM	F(x) = G(y)	F(x) <> G(y)	1072	1.62958	0.10298	0.10319
	SpI	TM	F(x) = G(y)	F(x) <> G(y)	2769	-1.29694	0.19494	0.19465
	SpI	CYT	F(x) = G(y)	F(x) <> G(y)	10690.5	-0.88955	0.37442	0.37371
	TM	Table1_P-OM	F(x) <= G(y)	F(x) > G(y)	359	2.68	0.00173	0.00368
	TM	Table1_P-IM	F(x) <= G(y)	F(x) > G(y)	1894	2.84539	0.00202	0.00222
	TM	CYT	F(x) = G(y)	F(x) <> G(y)	19291	0.87404	0.38255	0.3821
	CYT	Table1_P-OM	F(x) <= G(y)	F(x) > G(y)	1288	2.62773	0.00226	0.0043
	CYT	Table1_P-IM	F(x) <= G(y)	F(x) > G(y)	6603	2.57666	0.00473	0.00499
dNSAF Ratio (Control/PK)	Table1_S	Table1_P-OM	F(x) <= G(y)	F(x) > G(y)	175	2.35912	0.00712	0.00916
	Table1_S	Table1_P-IM	F(x) <= G(y)	F(x) > G(y)	1270	5.7011	2.78E-10	5.95E-09
	Table1_S	SpII	F(x) = G(y)	F(x) <> G(y)	300.5	0.88201	0.38061	0.37777
	Table1_S	SpI	F(x) <= G(y)	F(x) > G(y)	2969	7.77082	0	3.89E-15
	Table1_S	TM	F(x) <= G(y)	F(x) > G(y)	4763	8.70839	0	0
	Table1_S	CYT	F(x) <= G(y)	F(x) > G(y)	17343	10.01037	0	0
	SpII	Table1_P-OM	F(x) = G(y)	F(x) <> G(y)	29	1.20874	0.21578	0.22676
	SpII	Table1_P-IM	F(x) <= G(y)	F(x) > G(y)	204	2.10662	0.01664	0.01758
	SpII	SpI	F(x) <= G(y)	F(x) > G(y)	485.5	2.73533	2.50E-03	3.12E-03
	SpII	TM	F(x) <= G(y)	F(x) > G(y)	766.5	2.76717	2.22E-03	2.83E-03
	SpII	CYT	F(x) <= G(y)	F(x) > G(y)	2834	2.99067	9.97E-04	1.39E-03
	SpI	Table1_P-OM	F(x) >= G(y)	F(x) < G(y)	45	-2.13495	0.01464	0.01638
	SpI	Table1_P-IM	F(x) >= G(y)	F(x) < G(y)	526	-3.06138	9.60E-04	0.0011
	SpI	TM	F(x) = G(y)	F(x) <> G(y)	3433	0.96365	0.33578	0.33522
	SpI	CYT	F(x) = G(y)	F(x) <> G(y)	11447.5	-0.05475	0.95654	0.95634
	TM	Table1_P-OM	F(x) >= G(y)	F(x) < G(y)	62	-2.32492	0.00781	0.01004
	TM	Table1_P-IM	F(x) >= G(y)	F(x) < G(y)	755	-3.71601	7.23E-05	1.01E-04
	TM	CYT	F(x) = G(y)	F(x) <> G(y)	16786	-1.23175	0.21829	0.21804
	CYT	Table1_P-OM	F(x) >= G(y)	F(x) < G(y)	302	-2.02041	2.04E-02	2.17E-02
	CYT	Table1_P-IM	F(x) >= G(y)	F(x) < G(y)	3177	-3.34438	3.46E-04	4.12E-04

Figure S2. Graphical representation of putative lipoprotein genes by localization phenotype and genomic element. Putative lipoproteins were designated as belonging to either an essential genetic element or to a nonessential plasmid based on their encoding genetic element (31, 37, 217-219). Lipoproteins were then assigned a localization phenotype based on the "consensus" localization as designated in **Table 1**. Pie piece numbers indicate the number of lipoproteins belonging to each class.


Chromosome + cp26
10
6
26
Surface
OM, Sub-surface
IM

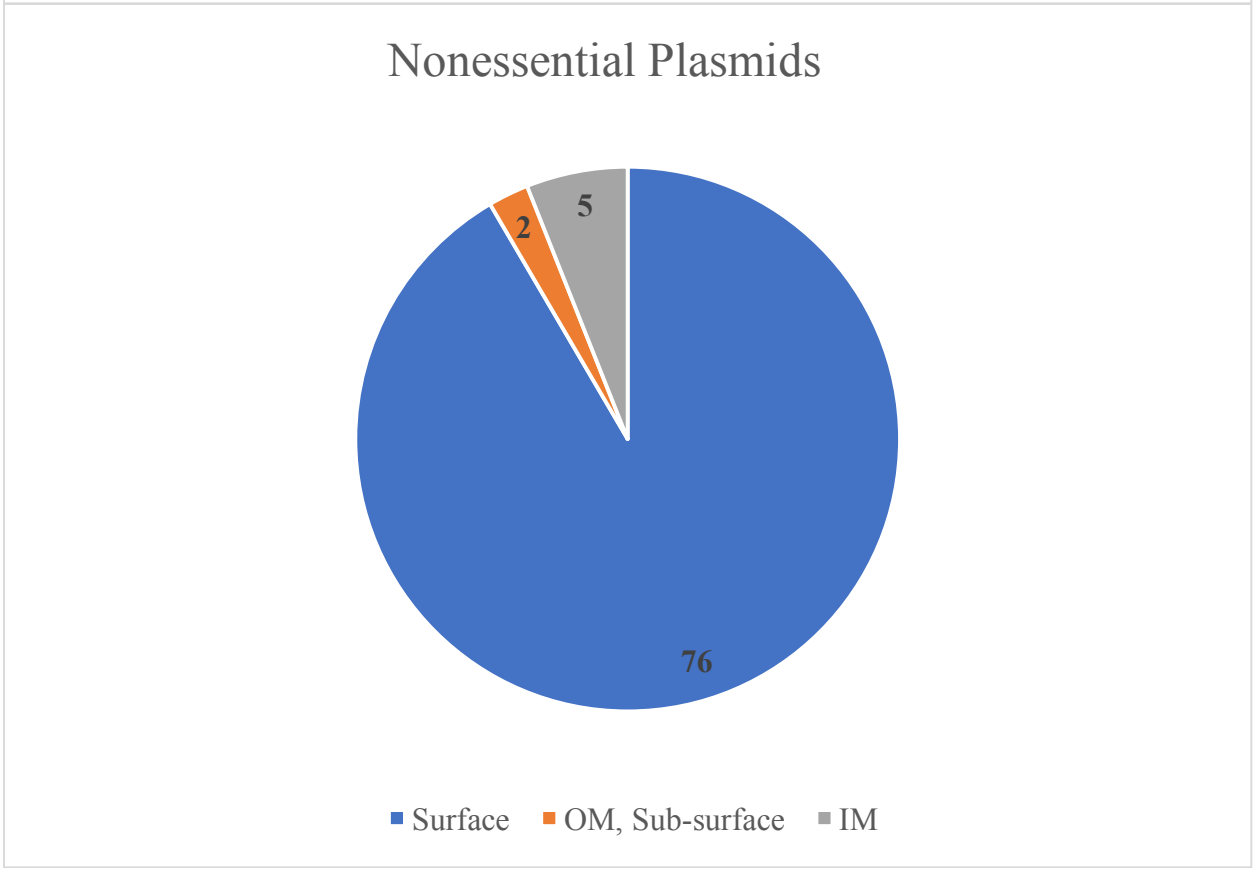
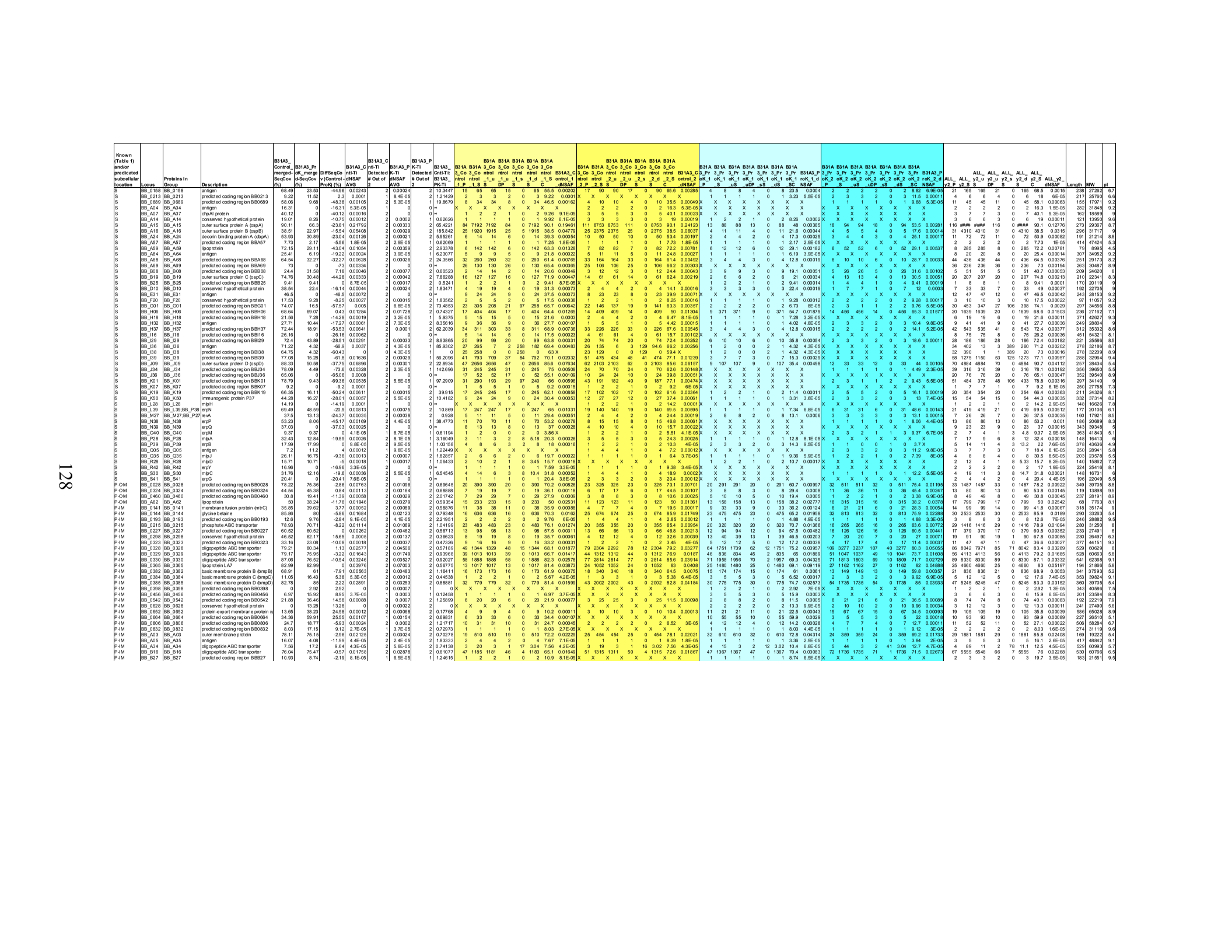

Nonessential Plasmids
5
2
76
Surface
OM, Sub-surface
IM

Table S1. Detailed peptide and spectral counts of MudPIT-detected proteins from *B. burgdorferi* B31-A3 either with or without proteolytic shaving.

Two replicate B. burgdorferi B31-A3 spirochetes samples subjected to proteinase-K as well as two untreated control samples were analyzed by MudPIT. The MS/MS datasets were searched against a protein database downloaded from the Spirochete Genome Browser, which contains accession numbers of all putative B. burgdorferi B31 ORFs (http://sgb.leibniz-fli.de/cgi/list.pl?ssi=free&c_sid=yes&sid=24&srt=ltg&.submit=Go). Exactly redundant sequences were removed from this database, leaving 1559 non-redundant Bb proteins. After adding usual contaminants and randomizing each protein entry, the total search space was 3472 amino acid sequences. The MS dataset was searched for Methionine oxidation as a differential modification. Combining all 4 runs, proteins had to be identified with at least 2 tryptic peptides of at least 7 amino acid long and/or 2 spectral counts. Proteins that were subsets of others were removed using the parsimony option in DTASelect on the proteins detected after merging all runs. Proteins that were identified by the same set of peptides (including at least one peptide unique to such protein group to distinguish between isoforms) were grouped together, and one accession number was arbitrarily considered as representative of each protein group. Half-tryptic peptides were allowed for the proteinase-K treated individual runs but did not contribute to establishing the master list of identified proteins ("ALL" columns). In each analysis, the following parameters are specified for each protein: Peptide counts (columns end with _P); Total spectral counts (_S); Spectral counts for peptides shared between protein isoforms (_sS); Peptide counts for peptides uniquely matching the protein (_uDP); Spectral counts for peptides uniquely matching the protein (_uS); Spectral counts distributed according to the spectral count contribution of peptides unique to each isoform (_dS); Sequence coverage as a percentage (_SC); Distributed Normalized Spectral Abundance Factors (_dNSAF), calculated based on distributed spectral counts and protein length. The number of times a protein is detected across replicates is listed in the "Detected # Out of" columns. dNSAF are averaged across replicate runs ("dNSAF AVG" columns). Summary statistics on False Discovery Rates (FDR, %) are calculated at the bottom of the table. A total of 621 proteins (excluding contaminants) were detected, including 83 listed in **Table 2**. Each of the detected proteins was subjected to prediction algorithms to detect potential signal peptidases I & II cleavage sites (LipoP1.0), signal peptide (SignalP4.1), and transmembrane domains (TMHMM2.0). For all detected proteins, the difference in sequence coverage (% of protein sequence covered by detected peptides, merging peptides from the 2 replicates analyzed for each condition) observed between the proteinase K-treated and control samples were calculated. The ratio in average dNSAF values measured in the control and proteinase K-treated samples were also calculated. The proteins were grouped based on their known or predicted location: 51 were tested to be surface (“S”) proteins, 4 were localized to the outer membrane (“P-OM”), and 28 were localized to the inner membrane (“P-IM”). An additional 10 proteins were predicted to contain a signal peptidase II cleavage site by LipoP1.0 (“SpII”), while another 63 were predicted to contain a signal peptide by LipoP1.0 (“SpI”) and/or SignalP4.1. Another 100 proteins were predicted to contain at least 1 transmembrane domain by TMHMM 2.0, but no signal peptide (“TM”), while the remaining 365 proteins were most likely cytoplasmic (“CYT”). The complete MudPIT mass spectrometry dataset (raw files, peak files, search files, as well as DTASelect result files) can be obtained from the MassIVE database via ftp://MSV000080434@massive.ucsd.edu (password ASD06166) and from the ProteomeXchange repository under accession number PXD005617.



Known (Table 1) and/or predicated subcellular location	Locus	Proteins In Group	Description	B31A3_Control_merged-SeqCov (%)	B31A3_ProK_merged-SeqCov (%)	DiffSeqCov (Control - ProK) (%)	B31A3_Cntl-Ti dNSAF AVG	B31A3_Cntl-Ti Detected # Out of 2	B31A3_PK-Ti dNSAF AVG	B31A3_PK-Ti Detected # Out of 2	B31A3_Cntl-Ti: B31A3_PK-Ti
P	BB_0536	BB_0536	zinc protease	67.31	65.17	-2.14	0.00669	2	0.00993	2	0.67335
SpII	BB_0383	BB_0383	basic membrane protein A (bmpA)	86.73	79.94	-6.79	0.04097	2	0.05328	2	0.76891
SpII	BB_0639	BB_0639	spermidine/putrescine ABC transp	81.9	76.72	-5.18	0.01661	2	0.01806	2	0.91971
SpII	BB_0840	BB_0840	predicted coding region BB0840	32.16	27.7	-4.46	0.00033	2	0.00021	2	1.59903
SpII	BB_A25	BB_A25	decorin binding protein B (dbpB)	20.32	9.63	-10.69	0.00016	2	5.4E-05	2	2.94444
SpII	BB_A60	BB_A60	surface lipoprotein P27	79.42	9.75	-69.67	0.00236	2	7.3E-05	2	32.3425
SpII	BB_A66	BB_A66	antigen	0	3.89	3.89	0	0	0.00004	1	0
SpII	BB_A73	BB_A73	antigen	6.08	0	-6.08	5.1E-05	1	0	0	∞
SpII	BB_J08	BB_J08	predicted coding region BBJ08	2.29	10.46	8.17	0	0	5.3E-05	1	0
SpII	BB_K47	BB_K47	predicted coding region BBK47	51.22	0	-51.22	0.0005	2	0	0	∞
SpII	BB_K49	BB_K49	predicted coding region BBK49	45.92	0	-45.92	0.00018	2	0	0	∞
SpI	BB_0016	BB_0016	glpE protein (glpE)	28.8	9.6	-19.2	8.9E-05	2	0.00036	2	0.2493
SpI	BB_0019	BB_0019	predicted coding region BB0019	27.06	15.88	-11.18	0.00026	2	0.00038	2	0.69129
SpI	BB_0027	BB_0027	predicted coding region BB0027	17.92	32.08	14.16	0.00222	2	0.00433	2	0.51236
SpI	BB_0031	BB_0031	signal peptidase I (lepB-2)	4.6	19.94	15.34	2.3E-05	1	0.00014	2	0.16912
SpI	BB_0032	BB_0032	predicted coding region BB0032	17.14	34.9	17.76	0.00018	2	0.00051	2	0.35756
SpI	BB_0034	BB_0034	predicted coding region BB0034	32.96	51.4	18.44	0.0115	2	0.03218	2	0.35736
SpI	BB_0038	BB_0038	predicted coding region BB0038	3.17	9.5	6.33	2.9E-05	1	0.00012	1	0.24576
SpI	BB_0058	BB_0058	predicted coding region BB0058	10.06	15.7	5.64	5.6E-05	1	0.00013	2	0.42748
SpI	BB_0063	BB_0063	predicted coding region BB0063	12.54	20.9	8.36	7.8E-05	2	0.00042	2	0.18483
SpI	BB_0087	BB_0087	L-lactate dehydrogenase (ldh)	42.41	40.51	-1.9	0.00045	2	0.00124	2	0.35858
SpI	BB_0126	BB_0126	predicted coding region BB0126	64.53	66.5	1.97	0.00502	2	0.01015	2	0.49468
SpI	BB_0142	BB_0142	predicted coding region BB0142	42.73	46.36	3.63	0.00137	2	0.00554	2	0.2468
SpI	BB_0159	BB_0159	predicted coding region BB0159	11.16	16.52	5.36	0.00013	2	0.00013	2	1.06349
SpI	BB_0202	BB_0202	hemolysin	3.88	10.68	6.8	1.8E-05	1	8.7E-05	1	0.2069
SpI	BB_0204	BB_0204	Lambda CII stability-governing prot	25.39	30.03	4.64	0.00028	2	0.00065	2	0.42813
SpI	BB_0209	BB_0209	predicted coding region BB0209	11.37	10.59	-0.78	0.00015	1	9.5E-05	2	1.52632
SpI	BB_0210	BB_0210	surface-located membrane protein	34.32	40.13	5.81	0.001	2	0.00181	2	0.55132
SpI	BB_0236	BB_0236	predicted coding region BB0236	3.59	12.72	9.13	1.1E-05	1	7.2E-05	2	0.15278
SpI	BB_0237	BB_0237	acid-inducible protein (act206)	31.29	31.29	0	0.00024	2	0.00053	2	0.44592
SpI	BB_0238	BB_0238	predicted coding region BB0238	0	11.83	11.83	0	0	6.1E-05	2	0
SpI	BB_0259	BB_0259	predicted coding region BB0259	35.55	36.36	0.81	0.00024	2	0.00041	2	0.5878
SpI	BB_0261	BB_0261	predicted coding region BB0261	17.83	26.52	8.69	0.00012	2	0.00028	2	0.4306
SpI	BB_0265	BB_0265	predicted coding region BB0265	0	29.48	29.48	0	0	0.0002	2	0
SpI	BB_0276	BB_0276	flagellar biosynthesis protein (fliZ)	8.17	19.71	11.54	3.6E-05	1	0.0002	1	0.18367
SpI	BB_0315	BB_0315	predicted coding region BB0315	16.23	10.09	-6.14	0.00016	1	0.00014	1	1.13287
SpI	BB_0319	BB_0319	exported protein (tpn38b)	72.57	69.43	-3.14	0.00237	2	0.00441	2	0.53661
SpI	BB_0336	BB_0336	P26	0	9.56	9.56	0	0	0.00255	2	0
SpI	BB_0359	BB_0359	carboxyl-terminal protease (ctp)	59.16	61.26	2.1	0.00151	2	0.00392	2	0.38511
SpI	BB_0368	BB_0368	glycerol-3-phosphate dehydrogena	39.39	44.08	4.69	0.00201	2	0.00359	2	0.55955
SpI	BB_0393	BB_0393	ribosomal protein L11 (rplK)	32.87	41.96	9.09	0.00026	2	0.00034	2	0.77679
SpI	BB_0395	BB_0395	preprotein translocase subunit (sec	19.64	19.64	0	0.00047	2	0.00054	2	0.85478
SpI	BB_0405	BB_0405	predicted coding region BB0405	53.2	66.01	12.81	0.00553	2	0.02236	2	0.2474
SpI	BB_0406	BB_0406	predicted coding region BB0406	38.42	38.92	0.5	0.00154	2	0.00234	2	0.65841
SpI	BB_0418	BB_0418	predicted coding region BB0418	32.46	40.35	7.89	0.00119	2	0.00229	2	0.52035
SpI	BB_0420	BB_0420	sensory transduction histidine kinas	0	4.89	4.89	0	0	1.6E-05	2	0
SpI	BB_0504	BB_0504	conserved hypothetical protein	11.18	22.75	11.57	4.4E-05	2	0.00023	2	0.19214
SpI	BB_0537	BB_0537	conserved hypothetical protein	18.22	33.64	15.42	0.00023	2	0.00034	2	0.66082
SpI	BB_0543	BB_0543	predicted coding region BB0543	46.33	56.88	10.55	0.00718	2	0.01361	2	0.5273
SpI	BB_0546	BB_0546	predicted coding region BB0546	59.09	48.95	-10.14	0.00242	2	0.00209	2	1.16211
SpI	BB_0575	BB_0575	CTP synthase (pyrG)	17.64	29.27	11.63	0.0001	2	0.0004	2	0.25743
SpI	BB_0588	BB_0588	pfs protein (pfs-2)	20.75	5.66	-15.09	0.00011	1	3.1E-05	1	3.6129
SpI	BB_0603	BB_0603	membrane-associated protein p66	48.38	57.93	9.55	0.00159	2	0.00494	2	0.32165
SpI	BB_0646	BB_0646	predicted coding region BB0646	54.74	48.32	-6.42	0.00068	2	0.00178	2	0.37892
SpI	BB_0668	BB_0668	flagellar filament outer layer protein	0	16.57	16.57	0	0	5.8E-05	2	0
SpI	BB_0714	BB_0714	predicted coding region BB0714	13.98	21.74	7.76	0.00012	2	0.00022	2	0.52727
SpI	BB_0739	BB_0739	predicted coding region BB0739	0	23.59	23.59	0	0	0.00014	2	0
SpI	BB_0744	BB_0744	antigen	0	1.57	1.57	0	0	3.4E-05	1	0
SpI	BB_0751	BB_0751	predicted coding region BB0751	71.15	70.03	-1.12	0.00397	2	0.00806	2	0.49268
SpI	BB_0776	BB_0776	predicted coding region BB0776	55.08	47.59	-7.49	0.00142	2	0.00192	2	0.73852
SpI	BB_0795	BB_0795	outer membrane protein	41.9	56.76	14.86	0.00133	2	0.00234	2	0.56899
SpI	BB_0796	BB_0796	predicted coding region BB0796	0	10.81	10.81	0	0	8.8E-05	1	0
SpI	BB_0811	BB_0811	predicted coding region BB0811	0	6.82	6.82	0	0	2.1E-05	2	0
SpI	BB_0838	BB_0838	predicted coding region BB0838	29.14	36.47	7.33	0.00025	2	0.00044	2	0.56787
SpI	BB_A52	BB_A52	outer membrane protein	17.44	24.56	7.12	0.00008	1	0.0003	2	0.26667
SpI	BB_B24	BB_B24	predicted coding region BBB24	13.1	7.14	-5.96	4.4E-05	1	0.00014	1	0.30986
SpI	BB_E09	BB_E09	protein p23	13.24	0	-13.24	0.00021	1	0	0	∞
SpI	BB_E16	BB_E16	predicted coding region BBE16	5.34	20.87	15.53	3.7E-05	1	0.00023	2	0.1588
SpI	BB_F24	BB_F24	conserved hypothetical protein	38.98	36.22	-2.76	0.00034	2	0.00056	2	0.59609
SpI	BB_J29	BB_J29;BB_J43	predicted coding region BBJ29	0	4.64	4.64	0	0	4.7E-05	1	0
SpI	BB_K45	BB_K45	immunogenic protein P37	58.71	13.23	-45.48	0.00374	2	0.00035	1	10.7752
SpI	BB_M26	BB_M26	conserved hypothetical protein	24.37	25.21	0.84	0.00017	1	7.6E-05	2	2.18421
SpI	BB_P26	BB_P26	conserved hypothetical protein	15.13	22.69	7.56	0	0	5.1E-05	2	0
SpI	BB_Q33	BB_Q33	conserved hypothetical protein	22.69	25.21	2.52	8.3E-05	1	7.6E-05	2	1.09211
TM	BB_0006	BB_0006	conserved hypothetical integral me	8.56	22.46	13.9	0.00014	2	0.00074	2	0.1886
TM	BB_0039	BB_0039	predicted coding region BB0039	43	69.6	26.6	0.00041	2	0.00182	2	0.22467
TM	BB_0044	BB_0044	predicted coding region BB0044	18.05	17.29	-0.76	5.7E-05	1	0.00025	1	0.23265
TM	BB_0054	BB_0054	predicted coding region BB0054	34.4	34.4	0	0.00146	2	0.00186	2	0.78273
TM	BB_0104	BB_0104	periplasmic serine protease DO (ht	14.91	40.58	25.67	0.00016	2	0.0007	2	0.22869
TM	BB_0106	BB_0106	predicted coding region BB0106	3.51	7.03	3.52	3.3E-05	2	0.00011	2	0.3
TM	BB_0140	BB_0140	acriflavine resistance protein (acrB)	5.79	29.25	23.46	1.8E-05	2	0.00019	2	0.09326
TM	BB_0203	BB_0203	Lambda CII stability-governing prot	32.48	28.94	-3.54	0.00038	2	0.00086	2	0.44496
TM	BB_0240	BB_0240	glycerol uptake facilitator (glpF)	7.48	7.48	0	0.00003	1	0.00053	2	0.05682
TM	BB_0257	BB_0257	cell division protein	0	14.61	14.61	0	0	0.0001	2	0
TM	BB_0263	BB_0263	signal peptidase I (lepB-3)	8.33	8.33	0	4.4E-05	1	0.00006	2	0.73333
TM	BB_0268	BB_0268	conserved hypothetical protein	35.85	37.74	1.89	0.00026	2	0.00079	2	0.32614
TM	BB_0271	BB_0271	flagellar biosynthesis protein (flhA)	17.93	32.14	14.21	0.00024	2	0.00069	2	0.34307
TM	BB_0279	BB_0279	flagellar protein (fliL)	62.92	63.48	0.56	0.01364	2	0.0184	2	0.74138
TM	BB_0280	BB_0280	flagellar motor rotation protein B (m	41.54	43.08	1.54	0.00079	2	0.00272	2	0.28876
TM	BB_0281	BB_0281	flagellar motor rotation protein A (m	15.77	50.38	34.61	0.00033	2	0.00088	2	0.37472
TM	BB_0286	BB_0286	flagellar protein (flbB)	40.1	40.58	0.48	0.00029	2	0.0011	2	0.2602
TM	BB_0332	BB_0332	oligopeptide ABC transporter	13.07	13.73	0.66	0.00017	2	0.00043	2	0.39394
TM	BB_0353	BB_0353	predicted coding region BB0353	3.34	4.67	1.33	1.2E-05	1	5.4E-05	1	0.22222
TM	BB_0354	BB_0354	predicted coding region BB0354	0	6.65	6.65	0	0	5.8E-05	2	0
TM	BB_0362	BB_0362	prolipoprotein diacylglyceryl transfe	15.85	21.04	5.19	0.0005	2	0.0011	2	0.45281
TM	BB_0401	BB_0401	glutamate transporter	0	6.75	6.75	0	0	4.1E-05	1	0
TM	BB_0469	BB_0469	signal peptidase II (lsp)	0	23.53	23.53	0	0	0.00029	2	0
TM	BB_0498	BB_0498	preprotein translocase subunit (sec	4.84	13.36	8.52	7.7E-05	2	0.00018	2	0.42778
TM	BB_0526	BB_0526	predicted coding region BB0526	12.52	5.44	-7.08	0.00016	1	0.00004	2	3.95
TM	BB_0539	BB_0539	conserved hypothetical integral me	22.41	10.78	-11.63	0.00069	2	0.00053	2	1.31238
TM	BB_0563	BB_0563	predicted coding region BB0563	16.57	25.97	9.4	0.0007	2	0.00128	2	0.55051
TM	BB_0597	BB_0597	methyl-accepting chemotaxis prote	20.54	36.46	15.92	0.00018	2	0.00083	2	0.21282
TM	BB_0600	BB_0600	predicted coding region BB0600	2.51	9.27	6.76	1.4E-05	1	5.9E-05	2	0.23729
TM	BB_0602	BB_0602	chaperonin	10.8	14.4	3.6	4.5E-05	2	0.00018	2	0.25281
TM	BB_0604	BB_0604	L-lactate permease (lctP)	10.4	10.4	0	0.00019	2	0.00052	2	0.35441
TM	BB_0645	BB_0645	PTS system	4.86	24.51	19.65	0.0001	1	0.00015	2	0.70345
TM	BB_0651	BB_0651	conserved hypothetical protein	55.24	35.24	-20	0.01146	2	0.02018	2	0.5681
TM	BB_0653	BB_0653	protein-export membrane protein (s	0	19.4	19.4	0	0	0.00016	2	0
TM	BB_0674	BB_0674	predicted coding region BB0674	0	3.45	3.45	0	0	4.7E-05	1	0
TM	BB_0680	BB_0680	methyl-accepting chemotaxis prote	13.81	24.83	11.02	9.8E-05	1	0.00022	2	0.45161
TM	BB_0681	BB_0681	methyl-accepting chemotaxis prote	14.94	18.55	3.61	8.2E-05	2	0.00017	2	0.4881
TM	BB_0709	BB_0709	conserved hypothetical protein	46.65	53.35	6.7	0.00059	2	0.0013	2	0.45119
TM	BB_0729	BB_0729	glutamate transporter (gltP)	7.56	6.7	-0.86	9.6E-05	1	0.00025	2	0.39184
TM	BB_0753	BB_0753	membrane spanning protein	6.97	15.57	8.6	6.2E-05	1	0.00021	2	0.29952

Locus	B31A3_Control_1_P	B31A3_Control_1_S	B31A3_Control_1_uS	B31A3_Control_1_uDP	B31A3_Control_1_sS	B31A3_Control_1_dS	B31A3_Control_1_SC	B31A3_Control_1_dNSAF	B31A3_Control_2_P	B31A3_Control_2_S	B31A3_Control_2_uS	B31A3_Control_2_uDP	B31A3_Control_2_sS	B31A3_Control_2_dS	B31A3_Control_2_SC	B31A3_Control_2_dNSAF
BB_0536	59	581	581	59	0	581	60.7	0.0046	71	1088	1088	71	0	1088	61.1	0.00877
BB_0383	34	1434	1432	34	2	1432	77.9	0.0312	43	2286	2286	43	0	2286	86.7	0.05074
BB_0639	34	630	630	34	0	630	70.1	0.01337	40	918	918	40	0	918	81.6	0.01985
BB_0840	13	36	36	13	0	36	32.2	0.00049	4	12	12	4	0	12	13.2	0.00017
BB_A25	2	6	6	2	0	6	10.7	0.00024	2	2	2	2	0	2	15.5	8E-05
BB_A60	14	62	62	14	0	62	48.4	0.00165	26	113	113	26	0	113	78	0.00307
BB_A66	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_A73	X	X	X	X	X	X	X	X	1	2	2	1	0	2	6.08	5.1E-05
BB_J08	1	8	0	0	8	0	2.29	X	X	X	X	X	X	X	X	X
BB_K47	12	37	12	5	25	30.8	45.4	0.00069	10	19	5	3	14	13.6	40.9	0.00031
BB_K49	10	29	4	3	25	10.3	39.6	0.00023	9	15	2	2	13	5.43	37.2	0.00012
BB_0016	2	2	2	2	0	2	16.8	0.00012	1	1	1	1	0	1	12	6E-05
BB_0019	3	8	8	3	0	8	22.9	0.00035	3	4	4	3	0	4	20.6	0.00018
BB_0027	1	3	3	1	0	3	7.08	0.0001	2	122	122	2	0	122	17.9	0.00433
BB_0031	1	1	1	1	0	1	4.6	2.3E-05	X	X	X	X	X	X	X	X
BB_0032	7	16	16	7	0	16	15.7	0.00024	4	8	8	4	0	8	14.1	0.00012
BB_0034	13	215	215	13	0	215	33	0.00887	11	336	336	11	0	336	33	0.01412
BB_0038	1	2	2	1	0	2	3.17	2.9E-05	X	X	X	X	X	X	X	X
BB_0058	4	5	5	4	0	5	10.1	5.6E-05	X	X	X	X	X	X	X	X
BB_0063	2	5	5	2	0	5	9.55	0.00011	1	2	2	1	0	2	2.99	4.5E-05
BB_0087	10	35	33	10	2	33	39.6	0.00077	3	5	5	3	0	5	17.1	0.00012
BB_0126	14	119	119	14	0	119	58.1	0.00433	13	154	154	13	0	154	57.6	0.00571
BB_0142	15	113	113	15	0	113	38.2	0.0019	12	49	49	12	0	49	29.3	0.00084
BB_0159	1	1	1	1	0	1	6.25	3.3E-05	2	7	7	2	0	7	11.2	0.00024
BB_0202	1	1	1	1	0	1	3.88	1.8E-05	X	X	X	X	X	X	X	X
BB_0204	4	10	10	4	0	10	13	0.00023	7	14	14	7	0	14	21.1	0.00033
BB_0209	2	5	5	2	0	5	11.4	0.00014	X	X	X	X	X	X	X	X
BB_0210	29	98	98	29	0	98	25.4	0.00065	29	201	201	29	0	201	29.2	0.00135
BB_0236	X	X	X	X	X	X	X	X	1	1	1	1	0	1	3.59	1.1E-05
BB_0237	6	22	22	6	0	22	20	0.00031	6	11	11	6	0	11	13.8	0.00016
BB_0238	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0259	14	40	40	14	0	40	28.2	0.0004	5	8	8	5	0	8	12.8	8.2E-05
BB_0261	5	12	12	5	0	12	13.7	0.00019	3	3	3	3	0	3	10.7	4.9E-05
BB_0265	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0276	1	1	1	1	0	1	8.17	3.6E-05	X	X	X	X	X	X	X	X
BB_0315	4	5	5	4	0	5	16.2	0.00016	X	X	X	X	X	X	X	X
BB_0319	15	100	100	15	0	100	62.6	0.00211	14	122	122	14	0	122	58.6	0.00262
BB_0336	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0359	23	127	127	23	0	127	51	0.00198	21	66	66	21	0	66	48	0.00105
BB_0368	8	133	133	8	0	133	35.8	0.00271	6	63	63	6	0	63	29.8	0.00131
BB_0393	2	4	4	2	0	4	21	0.00021	2	6	6	2	0	6	23.8	0.00032
BB_0395	2	4	4	2	0	4	19.6	0.00053	1	3	3	1	0	3	19.6	0.0004
BB_0405	19	137	137	19	0	137	50.7	0.00499	19	164	164	19	0	164	47.8	0.00608
BB_0406	6	43	43	6	0	43	35	0.00156	7	41	41	7	0	41	32	0.00152
BB_0418	8	48	48	8	0	48	26	0.00104	10	61	61	10	0	61	26.3	0.00134
BB_0420	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0504	3	4	4	3	0	4	8.63	5.8E-05	1	2	2	1	0	2	2.55	3E-05
BB_0537	4	8	8	4	0	8	18.2	0.00028	3	5	5	3	0	5	18.2	0.00018
BB_0543	8	175	175	8	0	175	46.3	0.00593	9	244	244	9	0	244	46.3	0.00842
BB_0546	15	99	99	15	0	99	48.3	0.00256	18	87	87	18	0	87	59.1	0.00229
BB_0575	6	14	14	6	0	14	15	0.00019	1	1	1	1	0	1	2.63	1.4E-05
BB_0588	3	4	4	3	0	4	20.8	0.00011	X	X	X	X	X	X	X	X
BB_0603	21	117	117	21	0	117	34.1	0.0014	25	146	146	25	0	146	44.3	0.00178
BB_0646	8	14	14	8	0	14	31.2	0.00032	13	45	45	13	0	45	43.4	0.00104
BB_0668	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0714	3	6	6	3	0	6	14	0.00014	1	4	4	1	0	4	4.04	9.3E-05
BB_0739	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0744	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0751	32	225	225	32	0	225	65.6	0.00466	28	156	156	28	0	156	64.2	0.00329
BB_0776	4	37	37	4	0	37	35.3	0.00146	6	34	34	6	0	34	38	0.00137
BB_0795	30	177	177	30	0	177	36.9	0.00159	27	117	117	27	0	117	34.5	0.00107
BB_0796	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0811	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0838	13	32	32	13	0	32	15.1	0.00021	17	45	45	17	0	45	20.7	0.0003
BB_A52	X	X	X	X	X	X	X	X	2	3	3	2	0	3	17.4	8E-05
BB_B24	1	1	1	1	0	1	13.1	4.4E-05	X	X	X	X	X	X	X	X
BB_E09	2	8	8	2	0	8	13.2	0.00021	X	X	X	X	X	X	X	X
BB_E16	X	X	X	X	X	X	X	X	1	1	1	1	0	1	5.34	3.7E-05
BB_F24	7	20	20	7	0	20	39	0.00058	2	3	3	2	0	3	7.87	8.9E-05
BB_J29	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_K45	19	158	158	19	0	158	51.9	0.00377	18	153	153	18	0	153	56.1	0.00371
BB_M26	2	3	2	1	1	2.67	24.4	0.00017	X	X	X	X	X	X	X	X
BB_P26	1	1	0	0	1	0	15.1	X	X	X	X	X	X	X	X	X
BB_Q33	2	2	1	1	1	1.33	22.7	8.3E-05	X	X	X	X	X	X	X	X
BB_0006	1	11	11	1	0	11	6.15	0.00022	2	3	3	2	0	3	8.56	6E-05
BB_0039	17	43	43	17	0	43	41.4	0.00064	3	12	12	3	0	12	5.8	0.00018
BB_0044	X	X	X	X	X	X	X	X	1	1	1	1	0	1	18.1	5.7E-05
BB_0054	7	29	29	7	0	29	34.4	0.00171	7	20	20	7	0	20	34.4	0.0012
BB_0104	5	15	15	5	0	15	14.9	0.00023	2	6	6	2	0	6	7.04	9.3E-05
BB_0106	1	4	4	1	0	4	3.51	5.2E-05	1	1	1	1	0	1	3.51	1.3E-05
BB_0140	1	2	2	1	0	2	1.83	1.4E-05	2	3	3	2	0	3	3.96	2.2E-05
BB_0203	5	15	15	5	0	15	22.2	0.00036	4	17	17	4	0	17	21.5	0.00041
BB_0240	X	X	X	X	X	X	X	X	1	1	1	1	0	1	7.48	3E-05
BB_0257	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0263	1	1	1	1	0	1	8.33	4.4E-05	X	X	X	X	X	X	X	X
BB_0268	4	8	8	4	0	8	22	0.00037	2	3	3	2	0	3	20.1	0.00014
BB_0271	6	29	29	6	0	29	17.7	0.00031	6	15	15	6	0	15	11.5	0.00016
BB_0279	7	199	199	7	0	199	51.7	0.00826	12	450	450	12	0	450	62.9	0.01902
BB_0280	6	36	36	6	0	36	31.9	0.00102	6	19	19	6	0	19	21.2	0.00055
BB_0281	3	13	13	3	0	13	12.3	0.00037	3	10	10	3	0	10	11.2	0.00029
BB_0286	4	12	12	4	0	12	35.8	0.00043	2	4	4	2	0	4	16.4	0.00015
BB_0332	4	12	12	4	0	12	13.1	0.00029	1	2	2	1	0	2	4.25	4.9E-05
BB_0353	1	1	1	1	0	1	3.34	1.2E-05	X	X	X	X	X	X	X	X
BB_0354	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0362	3	27	27	3	0	27	15.9	0.00061	2	17	17	2	0	17	12.2	0.00039
BB_0401	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0469	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0498	3	7	7	3	0	7	4.84	0.00012	1	2	2	1	0	2	1.61	3.5E-05
BB_0526	6	13	13	6	0	13	12.5	0.00016	X	X	X	X	X	X	X	X
BB_0539	7	27	27	7	0	27	10.8	0.00086	5	16	16	5	0	16	22.4	0.00052
BB_0563	2	9	9	2	0	9	9.94	0.00037	3	25	25	3	0	25	16.6	0.00104
BB_0597	11	31	31	11	0	31	18	0.00031	1	4	4	1	0	4	2.59	4.1E-05
BB_0600	1	1	1	1	0	1	2.51	1.4E-05	X	X	X	X	X	X	X	X
BB_0602	1	2	2	1	0	2	6	5.9E-05	1	1	1	1	0	1	4.8	3E-05
BB_0604	4	24	24	4	0	24	10.4	0.00035	1	1	1	1	0	1	4.4	1.5E-05
BB_0645	X	X	X	X	X	X	X	X	1	7	7	1	0	7	4.86	0.0001
BB_0651	4	115	115	4	0	115	31.4	0.00809	8	207	207	8	0	207	55.2	0.01483
BB_0653	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0674	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0680	6	10	10	6	0	10	13.8	9.8E-05	X	X	X	X	X	X	X	X
BB_0681	6	10	10	6	0	10	12.9	0.00012	3	4	4	3	0	4	8.96	4.7E-05
BB_0709	8	24	24	8	0	24	34.4	0.00052	9	30	30	9	0	30	37.9	0.00066
BB_0729	3	6	6	3	0	6	7.56	9.6E-05	X	X	X	X	X	X	X	X
BB_0753	X	X	X	X	X	X	X	X	1	2	2	1	0	2	6.97	6.2E-05

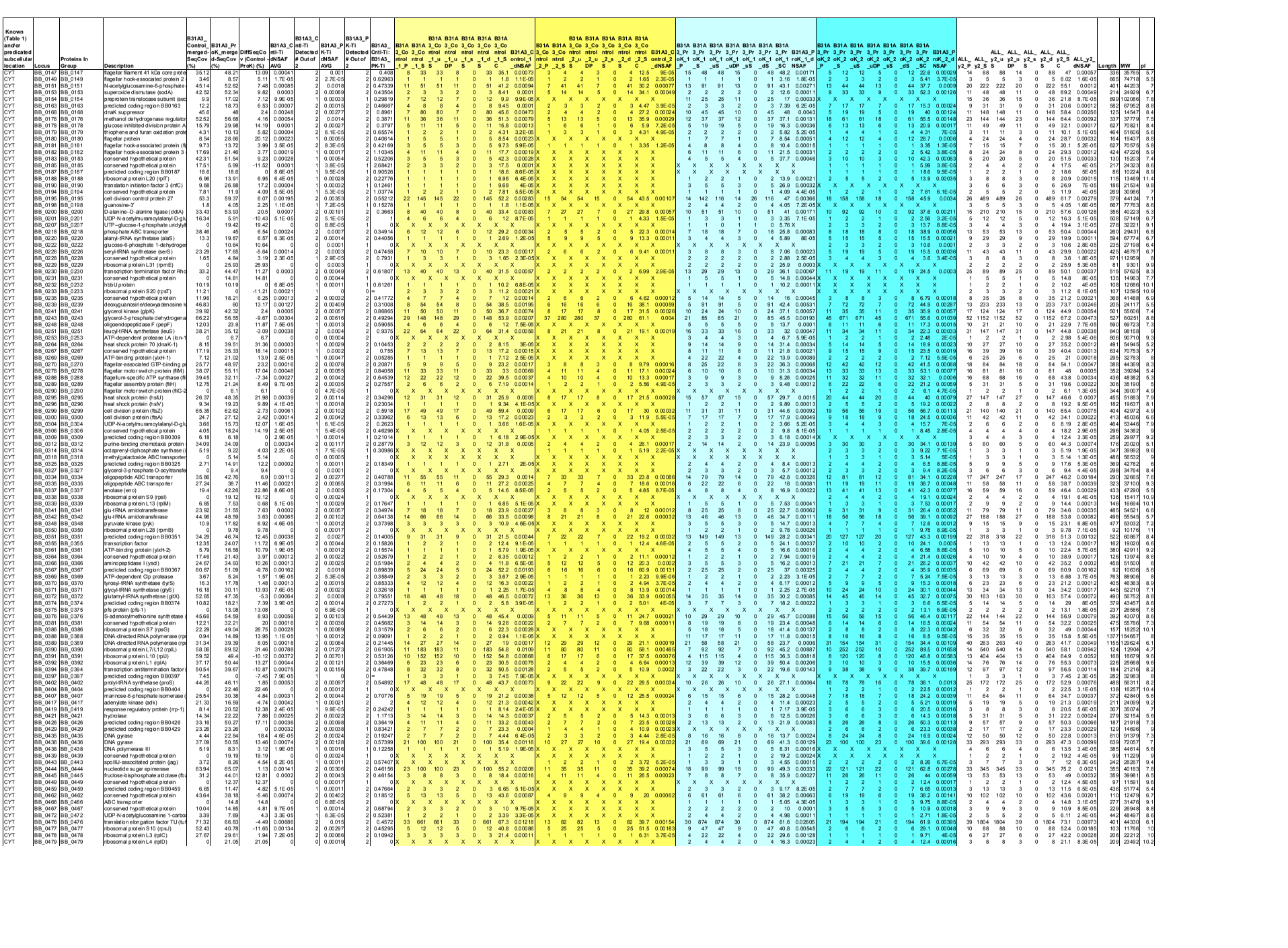
Locus	B31A3_ProK_1_P	B31A3_ProK_1_S	B31A3_ProK_1_uS	B31A3_ProK_1_uDP	B31A3_ProK_1_sS	B31A3_ProK_1_dS	B31A3_ProK_1_SC	B31A3_ProK_1_dNSAF	B31A3_ProK_2_P	B31A3_ProK_2_S	B31A3_ProK_2_uS	B31A3_ProK_2_uDP	B31A3_ProK_2_sS	B31A3_ProK_2_dS	B31A3_ProK_2_SC	B31A3_ProK_2_dNSAF
BB_0536	53	512	512	53	0	512	52.7	0.00656	97	1525	1521	97	4	1521	64.6	0.0133
BB_0383	43	1691	1691	43	0	1691	75.5	0.05963	59	1950	1950	59	0	1950	74.9	0.04694
BB_0639	43	644	644	43	0	644	70.7	0.02212	53	597	597	53	0	597	72.7	0.014
BB_0840	3	7	7	3	0	7	10.6	0.00016	9	17	17	9	0	17	27.7	0.00026
BB_A25	1	1	1	1	0	1	9.63	6.4E-05	1	1	1	1	0	1	9.63	4.4E-05
BB_A60	2	2	2	2	0	2	9.75	8.6E-05	1	2	2	1	0	2	5.42	5.9E-05
BB_A66	X	X	X	X	X	X	X	X	1	2	2	1	0	2	3.89	4E-05
BB_A73	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_J08	X	X	X	X	X	X	X	X	1	2	2	1	0	2	10.5	5.3E-05
BB_K47	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_K49	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0016	1	2	2	1	0	2	9.6	0.00019	1	8	8	1	0	8	9.6	0.00052
BB_0019	3	6	6	3	0	6	15.9	0.00042	4	7	7	4	0	7	15.9	0.00034
BB_0027	3	19	19	3	0	19	28.3	0.00107	6	197	197	6	0	197	32.1	0.00758
BB_0031	2	4	4	2	0	4	10.4	0.00015	3	5	5	3	0	5	15	0.00013
BB_0032	12	26	26	12	0	26	28.2	0.00063	12	23	23	12	0	23	24.5	0.00038
BB_0034	16	468	468	16	0	468	46.4	0.03125	23	726	726	23	0	726	48	0.0331
BB_0038	3	5	5	3	0	5	9.5	0.00012	X	X	X	X	X	X	X	X
BB_0058	6	11	11	6	0	11	11.9	0.0002	3	5	5	3	0	5	5.64	6.2E-05
BB_0063	4	10	10	4	0	10	20.9	0.00036	3	20	20	3	0	20	16.7	0.00049
BB_0087	4	11	11	4	0	11	22.2	0.00042	12	80	80	12	0	80	40.5	0.00207
BB_0126	10	120	120	10	0	120	56.7	0.00707	20	329	329	20	0	329	66.5	0.01323
BB_0142	9	178	178	9	0	178	28.4	0.00484	26	337	337	26	0	337	46.4	0.00625
BB_0159	1	2	2	1	0	2	6.7	0.00011	2	4	4	2	0	4	9.82	0.00015
BB_0202	3	3	3	3	0	3	10.7	8.7E-05	X	X	X	X	X	X	X	X
BB_0204	4	7	7	4	0	7	12.1	0.00026	11	41	41	11	0	41	26.9	0.00104
BB_0209	1	2	2	1	0	2	6.67	9.4E-05	2	3	3	2	0	3	9.02	9.6E-05
BB_0210	24	114	114	24	0	114	21.2	0.00122	54	332	330	54	2	330	38	0.00241
BB_0236	4	6	6	4	0	6	12.7	0.00011	1	3	3	1	0	3	3.59	3.7E-05
BB_0237	3	7	7	3	0	7	7.68	0.00016	11	57	57	11	0	57	28.8	0.00089
BB_0238	1	2	2	1	0	2	4.58	9.1E-05	1	1	1	1	0	1	7.25	3.1E-05
BB_0259	16	43	43	16	0	43	34.1	0.0007	5	11	11	5	0	11	13.4	0.00012
BB_0261	4	10	10	4	0	10	12	0.00026	12	17	17	12	0	17	26.5	0.0003
BB_0265	1	1	1	1	0	1	11	6.9E-05	2	7	7	2	0	7	18.5	0.00033
BB_0276	X	X	X	X	X	X	X	X	4	5	5	4	0	5	19.7	0.0002
BB_0315	X	X	X	X	X	X	X	X	1	4	4	1	0	4	10.1	0.00014
BB_0319	12	74	74	12	0	74	50.9	0.00253	28	270	270	28	0	270	69.4	0.0063
BB_0336	1	79	79	1	0	79	5.58	0.00376	2	41	41	2	0	41	9.56	0.00133
BB_0359	21	171	171	21	0	171	38.5	0.0043	32	206	206	32	0	206	59.4	0.00354
BB_0368	7	129	129	7	0	129	32	0.00425	11	130	130	11	0	130	44.1	0.00292
BB_0393	4	6	6	4	0	6	42	0.0005	1	3	3	1	0	3	11.9	0.00017
BB_0395	1	1	1	1	0	1	19.6	0.00021	3	6	6	3	0	6	19.6	0.00087
BB_0405	9	255	255	9	0	255	27.1	0.01502	31	739	739	31	0	739	66	0.02971
BB_0406	6	14	14	6	0	14	32	0.00082	9	96	96	9	0	96	35.5	0.00386
BB_0418	9	27	27	9	0	27	25.7	0.00094	20	152	152	20	0	152	40.4	0.00363
BB_0420	1	2	2	1	0	2	1.34	1.6E-05	3	3	3	3	0	3	3.55	1.6E-05
BB_0504	5	10	10	5	0	10	12.9	0.00023	7	14	14	7	0	14	16.7	0.00022
BB_0537	1	2	2	1	0	2	5.14	0.00011	7	15	15	7	0	15	33.6	0.00057
BB_0543	14	82	82	14	0	82	56.9	0.0045	23	607	607	23	0	607	56.9	0.02272
BB_0546	10	24	24	10	0	24	34.6	0.001	18	111	111	18	0	111	49	0.00317
BB_0575	8	19	19	8	0	19	19.1	0.00043	9	25	25	9	0	25	24.6	0.00038
BB_0588	X	X	X	X	X	X	X	X	1	1	1	1	0	1	5.66	3.1E-05
BB_0603	18	107	107	18	0	107	37.5	0.00207	39	591	591	39	0	591	53.2	0.0078
BB_0646	11	28	28	11	0	28	40.4	0.00102	16	102	102	16	0	102	44	0.00255
BB_0668	2	2	2	2	0	2	9.01	7E-05	2	2	2	2	0	2	7.56	4.7E-05
BB_0714	3	5	5	3	0	5	10.9	0.00019	4	10	10	4	0	10	13.4	0.00025
BB_0739	1	4	4	1	0	4	7.69	0.00025	1	1	1	1	0	1	15.9	4.2E-05
BB_0744	1	2	2	1	0	2	1.57	3.4E-05	X	X	X	X	X	X	X	X
BB_0751	22	146	145	22	1	145	60.5	0.00485	35	493	493	35	0	493	70	0.01127
BB_0776	3	19	19	3	0	19	17.7	0.00121	7	60	60	7	0	60	47.6	0.00262
BB_0795	18	69	69	18	0	69	28.6	0.001	41	377	370	41	7	370	54.6	0.00368
BB_0796	X	X	X	X	X	X	X	X	1	2	2	1	0	2	10.8	8.8E-05
BB_0811	1	1	1	1	0	1	4.69	2.5E-05	1	1	1	1	0	1	2.13	1.7E-05
BB_0838	11	22	22	11	0	22	14.6	0.00023	32	92	92	32	0	92	33.5	0.00066
BB_A52	5	10	10	5	0	10	24.6	0.00043	2	6	6	2	0	6	6.76	0.00017
BB_B24	1	2	2	1	0	2	7.14	0.00014	X	X	X	X	X	X	X	X
BB_E09	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_E16	4	6	6	4	0	6	20.9	0.00035	2	3	3	2	0	3	13.6	0.00012
BB_F24	6	15	15	6	0	15	18.9	0.00071	5	13	13	5	0	13	33.1	0.00042
BB_J29	X	X	X	X	X	X	X	X	1	2	2	1	0	2	4.64	4.7E-05
BB_K45	3	9	9	3	0	9	13.2	0.00035	X	X	X	X	X	X	X	X
BB_M26	2	2	0	0	2	0.83	25.2	8.4E-05	1	2	0	0	2	1	9.24	6.9E-05
BB_P26	1	1	0	0	1	0.33	15.1	3.3E-05	1	1	1	1	0	1	22.7	6.9E-05
BB_Q33	2	2	0	0	2	0.83	25.2	8.4E-05	1	2	0	0	2	1	9.24	6.9E-05
BB_0006	4	12	12	4	0	12	15.5	0.00038	6	50	50	6	0	50	22.5	0.00109
BB_0039	29	113	113	29	0	113	64.8	0.0027	16	57	57	16	0	57	34.4	0.00093
BB_0044	X	X	X	X	X	X	X	X	2	4	4	2	0	4	17.3	0.00025
BB_0054	4	11	11	4	0	11	32	0.00105	8	41	41	8	0	41	34.4	0.00268
BB_0104	14	48	48	14	0	48	35.4	0.00119	7	13	13	7	0	13	17.4	0.00022
BB_0106	2	5	5	2	0	5	7.03	0.00011	2	8	8	2	0	8	3.51	0.00011
BB_0140	6	11	11	6	0	11	11	0.00013	15	33	33	15	0	33	24.7	0.00026
BB_0203	2	21	21	2	0	21	9	0.00081	7	35	35	7	0	35	28.9	0.00092
BB_0240	1	4	4	1	0	4	7.48	0.00019	2	27	27	2	0	27	7.48	0.00087
BB_0257	2	3	3	2	0	3	5.97	4.6E-05	4	15	15	4	0	15	12.1	0.00016
BB_0263	1	1	1	1	0	1	8.33	7.1E-05	1	1	1	1	0	1	8.33	4.9E-05
BB_0268	4	8	8	4	0	8	23.9	0.0006	6	19	19	6	0	19	33.3	0.00098
BB_0271	10	26	26	10	0	26	18.7	0.00045	18	79	79	18	0	79	29.3	0.00092
BB_0279	14	238	238	14	0	238	56.2	0.01598	15	454	454	15	0	454	43.3	0.02081
BB_0280	9	87	87	9	0	87	31.9	0.004	10	46	46	10	0	46	36.5	0.00144
BB_0281	5	15	15	5	0	15	25.8	0.00069	8	34	34	8	0	34	43.9	0.00107
BB_0286	7	15	15	7	0	15	33.3	0.00087	6	34	34	6	0	34	40.1	0.00134
BB_0332	2	9	9	2	0	9	7.84	0.00035	4	19	19	4	0	19	13.1	0.00051
BB_0353	X	X	X	X	X	X	X	X	3	4	4	3	0	4	4.67	5.4E-05
BB_0354	1	2	2	1	0	2	3.47	6.9E-05	1	2	2	1	0	2	3.18	4.7E-05
BB_0362	3	25	25	3	0	25	15.9	0.00091	6	52	52	6	0	52	21	0.00129
BB_0401	X	X	X	X	X	X	X	X	2	2	2	2	0	2	6.75	4.1E-05
BB_0469	1	2	2	1	0	2	8.24	0.00014	2	9	9	2	0	9	23.5	0.00043
BB_0498	6	9	9	6	0	9	11.8	0.00025	3	6	6	3	0	6	7.6	0.00011
BB_0526	1	2	2	1	0	2	2.14	3.9E-05	2	3	3	2	0	3	3.29	4E-05
BB_0539	2	4	4	2	0	4	7.33	0.00021	7	24	24	7	0	24	10.8	0.00084
BB_0563	3	10	10	3	0	10	16	0.00066	5	42	42	5	0	42	26	0.00189
BB_0597	11	56	56	11	0	56	18.6	0.00091	20	67	67	20	0	67	34.6	0.00074
BB_0600	1	1	1	1	0	1	3.47	2.3E-05	2	6	6	2	0	6	5.79	9.5E-05
BB_0602	1	2	2	1	0	2	6	9.6E-05	3	8	8	3	0	8	14.4	0.00026
BB_0604	3	15	15	3	0	15	10.4	0.00036	4	42	42	4	0	42	10.4	0.00069
BB_0645	3	5	5	3	0	5	9.53	0.00012	8	11	11	8	0	11	22.8	0.00017
BB_0651	5	164	164	5	0	164	35.2	0.01867	5	279	279	5	0	279	32.4	0.02168
BB_0653	2	3	3	2	0	3	7.69	0.00012	5	7	7	5	0	7	19.4	0.00019
BB_0674	X	X	X	X	X	X	X	X	1	2	2	1	0	2	3.45	4.7E-05
BB_0680	9	15	15	9	0	15	17.9	0.00024	10	18	18	10	0	18	20.3	0.0002
BB_0681	4	7	7	4	0	7	5.82	0.00013	8	16	16	8	0	16	15.1	0.00021
BB_0709	7	31	31	7	0	31	32.1	0.00108	16	64	64	16	0	64	47.2	0.00152
BB_0729	2	6	6	2	0	6	4.1	0.00015	5	19	19	5	0	19	6.7	0.00033
BB_0753	1	3	3	1	0	3	6.97	0.00015	2	8	8	2	0	8	15.6	0.00027

Locus	ALL_y2_P	ALL_y2_S	ALL_y2_uS	ALL_y2_uDP	ALL_y2_sS	ALL_y2_dS	ALL_y2_SC	ALL_y2_dNSAF	Length	MW	pI
BB_0536	100	3634	3634	100	0	3634	71.7	0.00843	933	108458	6.3
BB_0383	49	7220	7213	49	7	7213	86.7	0.04604	339	36968	5.3
BB_0639	58	2714	2714	58	0	2714	83.9	0.01687	348	40681	5.9
BB_0840	16	70	70	16	0	70	36.6	0.00028	538	62131	8.9
BB_A25	3	10	10	3	0	10	20.3	0.00012	187	20398	9.6
BB_A60	31	178	178	31	0	178	79.4	0.00139	277	32444	9.1
BB_A66	1	2	2	1	0	2	3.89	1.1E-05	411	45874	9.2
BB_A73	1	2	2	1	0	2	6.08	1.5E-05	296	34647	8.9
BB_J08	2	10	2	1	8	2.03	12.8	1.4E-05	306	34640	8.9
BB_K47	16	56	15	5	41	41.4	51.2	0.00027	328	37091	5.9
BB_K49	15	44	6	4	38	16.6	45.9	0.00011	331	37658	6.2
BB_0016	3	13	13	3	0	13	28.8	0.00023	125	14557	10
BB_0019	5	22	22	5	0	22	27.1	0.00028	170	19274	6.1
BB_0027	5	339	339	5	0	339	32.1	0.00346	212	23865	9.1
BB_0031	5	10	10	5	0	10	24.5	6.6E-05	326	38481	8.9
BB_0032	20	72	72	20	0	72	35.7	0.00032	490	57590	9.3
BB_0034	14	1699	1699	14	0	1699	37.4	0.02054	179	19104	8.6
BB_0038	4	7	7	4	0	7	12.7	3E-05	505	59024	6.7
BB_0058	10	21	21	10	0	21	18.3	6.9E-05	656	76395	7.9
BB_0063	6	37	37	6	0	37	28.4	0.00024	335	37306	8.8
BB_0087	18	131	131	18	0	131	49.4	0.0009	316	34901	6.9
BB_0126	20	715	715	20	0	715	64.5	0.00762	203	23595	5.3
BB_0142	29	622	622	29	0	622	45.5	0.00306	440	50847	8.7
BB_0159	4	12	12	4	0	12	11.2	0.00012	224	25805	9.1
BB_0202	2	3	3	2	0	3	7.77	1.6E-05	412	46391	9.2
BB_0204	12	71	71	12	0	71	27.9	0.00048	323	37194	9.6
BB_0209	4	10	10	4	0	10	15.3	8.5E-05	255	30055	8.4
BB_0210	62	733	733	62	0	733	44.5	0.00142	1119	128086	9.2
BB_0236	4	10	10	4	0	10	12.7	3.2E-05	668	75351	8.7
BB_0237	15	83	83	15	0	83	34.6	0.00034	521	59737	9.2
BB_0238	2	3	3	2	0	3	11.8	2.5E-05	262	30379	8.3
BB_0259	23	97	97	23	0	97	44.9	0.00028	737	86337	9
BB_0261	14	41	41	14	0	41	31.1	0.00019	460	54024	9.4
BB_0265	3	8	8	3	0	8	29.5	0.0001	173	20158	8.3
BB_0276	4	5	5	4	0	5	13	5.2E-05	208	24405	5.9
BB_0315	4	9	9	4	0	9	16.2	8.5E-05	228	26880	9.4
BB_0319	25	552	552	25	0	552	72.6	0.00341	350	40130	8
BB_0336	1	2	2	1	0	2	3.98	1.7E-05	251	29765	6.8
BB_0359	37	562	562	37	0	562	61.1	0.00256	475	53542	8.3
BB_0368	13	455	455	13	0	455	49.6	0.00271	363	40697	6.2
BB_0393	4	18	18	4	0	18	39.9	0.00027	143	15168	10
BB_0395	3	14	14	3	0	14	19.6	0.00054	56	6695	9.2
BB_0405	27	1252	1252	27	0	1252	56.7	0.01334	203	22252	5.2
BB_0406	9	191	191	9	0	191	38.4	0.00204	203	22800	8.3
BB_0418	15	280	280	15	0	280	39.8	0.00177	342	38773	9
BB_0420	3	4	4	3	0	4	3.61	5.8E-06	1494	172818	8.1
BB_0504	11	28	28	11	0	28	23.9	0.00012	510	58003	7.2
BB_0537	9	30	30	9	0	30	33.6	0.0003	214	24700	9.3
BB_0543	13	1006	1006	13	0	1006	50.9	0.00998	218	24388	5.2
BB_0546	22	318	318	22	0	318	59.1	0.00241	286	32430	9.4
BB_0575	13	59	59	13	0	59	31.1	0.00024	533	59398	8
BB_0588	3	4	4	3	0	4	20.8	3.3E-05	265	29088	9.3
BB_0603	41	927	927	41	0	927	63.1	0.00325	618	68172	6.3
BB_0646	21	178	178	21	0	178	54.7	0.00118	327	37596	9.5
BB_0668	3	3	3	3	0	3	12.8	1.9E-05	344	38835	8.1
BB_0714	6	25	25	6	0	25	21.7	0.00017	322	38625	7.3
BB_0739	2	5	5	2	0	5	23.6	5.5E-05	195	23102	8.1
BB_0744	1	2	2	1	0	2	1.57	6.2E-06	700	79929	5.3
BB_0751	44	1019	1018	44	1	1018	71.2	0.00617	357	41901	6.5
BB_0776	12	144	144	12	0	144	57.8	0.00167	187	21522	9.1
BB_0795	49	735	735	49	0	735	58.3	0.00194	821	94568	6.4
BB_0796	1	2	2	1	0	2	10.8	2.3E-05	185	21446	8.3
BB_0811	2	2	2	2	0	2	6.82	9.2E-06	469	53095	8.6
BB_0838	37	183	183	37	0	183	40.8	0.00035	1146	134380	8.5
BB_A52	4	18	18	4	0	18	24.2	0.00014	281	33103	8.7
BB_B24	2	3	3	2	0	3	20.2	3.9E-05	168	20116	9.9
BB_E09	2	8	8	2	0	8	13.2	6E-05	287	34057	6.8
BB_E16	5	10	10	5	0	10	26.2	0.00011	206	24534	9.3
BB_F24	11	51	51	11	0	51	42.1	0.00043	254	29653	8.7
BB_J29	1	2	2	1	0	2	4.64	1.3E-05	345	40963	5
BB_K45	28	320	320	28	0	320	58.7	0.00223	310	35323	9.5
BB_M26	4	7	2	1	5	5	34.5	9.1E-05	119	13880	9.1
BB_P26	2	3	1	1	2	1.5	22.7	2.7E-05	119	13880	9.2
BB_Q33	4	6	1	1	5	2.5	32.8	4.5E-05	119	13877	9.1
BB_0006	7	76	76	7	0	76	24.9	0.00044	374	42911	9.4
BB_0039	37	219	219	37	0	219	71.4	0.00095	500	58503	9.1
BB_0044	3	5	5	3	0	5	24.8	8.1E-05	133	16053	4.9
BB_0054	8	99	99	8	0	99	34.4	0.00171	125	14015	4.9
BB_0104	17	82	82	17	0	82	40.6	0.00037	483	53104	9.1
BB_0106	3	18	18	3	0	18	7.03	6.8E-05	569	67849	9
BB_0140	17	48	48	17	0	48	26.9	0.0001	1036	115439	8.2
BB_0203	9	88	88	9	0	88	32.5	0.00061	311	35825	5.3
BB_0240	2	32	32	2	0	32	7.48	0.00027	254	27195	7.6
BB_0257	3	14	14	3	0	14	11.1	3.8E-05	787	90269	5.8
BB_0263	1	3	3	1	0	3	8.33	3.9E-05	168	19837	9.6
BB_0268	9	38	38	9	0	38	40.3	0.00052	159	18344	5.7
BB_0271	19	146	146	19	0	146	31	0.00045	697	76969	5.2
BB_0279	15	1310	1310	15	0	1310	73.6	0.01592	178	20060	5.4
BB_0280	12	188	188	12	0	188	52.7	0.00156	260	29206	6.1
BB_0281	8	71	71	8	0	71	40.4	0.00059	260	28354	5.3
BB_0286	10	65	65	10	0	65	47.3	0.00068	207	24579	6.3
BB_0332	6	42	42	6	0	42	13.7	0.0003	306	34061	9.8
BB_0353	4	5	5	4	0	5	8.01	1.8E-05	599	71260	9
BB_0354	2	4	4	2	0	4	6.65	2.5E-05	346	41138	9.2
BB_0362	4	108	108	4	0	108	21	0.00071	328	37595	8.9
BB_0401	2	2	2	2	0	2	6.75	1.1E-05	400	45713	9.5
BB_0469	1	10	10	1	0	10	8.24	0.00013	170	19976	9.8
BB_0498	9	24	24	9	0	24	13.4	0.00012	434	48610	9.7
BB_0526	9	18	18	9	0	18	18	6.4E-05	607	70953	8.6
BB_0539	9	71	71	9	0	71	22.4	0.00066	232	25708	9.2
BB_0563	5	86	86	5	0	86	26	0.00103	181	19824	9.9
BB_0597	24	153	153	24	0	153	34.6	0.00045	735	84570	5.6
BB_0600	4	8	8	4	0	8	11.8	3.3E-05	518	60479	6.6
BB_0602	4	13	13	4	0	13	19.2	0.00011	250	29473	9.2
BB_0604	4	82	82	4	0	82	10.4	0.00035	500	53680	9.2
BB_0645	7	21	21	7	0	21	16.7	8.8E-05	514	55843	9
BB_0651	8	761	761	8	0	761	55.2	0.01568	105	11840	9.6
BB_0653	5	10	10	5	0	10	19.4	7.2E-05	299	34057	9
BB_0674	1	2	2	1	0	2	3.45	1.2E-05	348	41679	9.5
BB_0680	15	42	42	15	0	42	25.2	0.00012	753	84579	5.1
BB_0681	13	35	35	13	0	35	23.4	0.00012	636	71460	5.9
BB_0709	16	148	148	16	0	148	53.4	0.00093	343	39462	9
BB_0729	6	31	31	6	0	31	10.4	0.00014	463	49621	9.6
BB_0753	2	13	13	2	0	13	15.6	0.00012	244	27646	9

Known (Table 1) and/or predicated subcellular location	Locus	Proteins In Group	Description	B31A3_Control_merged-SeqCov (%)	B31A3_ProK_merged-SeqCov (%)	DiffSeqCov (Control - ProK) (%)	B31A3_Cntl-Ti dNSAF AVG	B31A3_Cntl-Ti Detected # Out of 2	B31A3_PK-Ti dNSAF AVG	B31A3_PK-Ti Detected # Out of 2	B31A3_Cntl-Ti: B31A3_PK-Ti	B31A3_Control_1_P	B31A3_Control_1_S	B31A3_Control_1_uS	B31A3_Control_1_uDP	B31A3_Control_1_sS	B31A3_Control_1_dS	B31A3_Control_1_SC	B31A3_Control_1_dNSAF
TM	BB_0783	BB_0783	predicted coding region BB0783	19.29	28.17	8.88	0.0003	2	0.00036	2	0.83611	4	27	27	4	0	27	12.4	0.00051
TM	BB_0789	BB_0789	cell division protein (ftsH)	20.03	27.39	7.36	8.7E-05	2	0.00041	2	0.21481	6	11	11	6	0	11	15.7	0.00013
TM	BB_0824	BB_0824	predicted coding region BB0824	18.58	28.96	10.38	0.00018	2	0.01161	2	0.01576	2	5	5	2	0	5	10.4	0.0002
TM	BB_0843	BB_0843	conserved hypothetical integral me	3.53	3.53	0	5.4E-05	2	0.00017	2	0.32143	1	6	6	1	0	6	3.32	9.2E-05
TM	BB_B22	BB_B22	conserved hypothetical protein	5.99	14.19	8.2	0.00012	2	0.00025	2	0.45635	2	9	9	2	0	9	5.99	0.00015
TM	BB_B28	BB_B28	predicted coding region BBB28	55.56	57.97	2.41	0.00143	2	0.00214	2	0.66651	21	121	121	21	0	121	55.6	0.00216
TM	BB_B29	BB_B29	PTS system	52.95	49.08	-3.87	0.00713	2	0.02108	2	0.33805	26	677	677	26	0	677	42.3	0.00923
TM	BB_D15	BB_D15	conserved hypothetical protein	20.71	6.43	-14.28	0.00027	2	8.5E-05	1	3.11765	4	7	7	4	0	7	14.3	0.00037
TM	BB_J27	BB_J27	predicted coding region BBJ27	11.19	7.3	-3.89	0.00011	1	4.9E-05	2	2.20408	3	6	6	3	0	6	11.2	0.00011
TM	BB_K13	BB_K13	conserved hypothetical protein	14.71	43.7	28.99	9.5E-05	1	0.00103	1	0.09232	X	X	X	X	X	X	X	X
TM	BB_0252	BB_0252	conserved hypothetical integral me	7.56	8.6	1.04	5.8E-05	2	0.00016	2	0.35802	4	8	8	4	0	8	7.56	7.7E-05
TM	BB_0091	BB_0091	V-type ATPase	2.8	22.53	19.73	3.6E-05	1	0.00047	2	0.07742	1	3	3	1	0	3	2.8	3.6E-05
TM	BB_0408	BB_0408	PTS system	32.48	35.84	3.36	0.00173	2	0.00458	2	0.37656	30	186	186	30	0	186	31.7	0.0022
TM	BB_0629	BB_0629	PTS system	28.12	35.46	7.34	0.00031	2	0.0009	2	0.3456	10	20	20	10	0	20	24.3	0.00024
TM	BB_B04	BB_B04	PTS system	7	10.84	3.84	0.00013	2	0.00092	2	0.14597	2	9	9	2	0	9	5.87	0.00015
TM	BB_0071	BB_0071	predicted coding region BB0071	4.08	11.84	7.76	1.5E-05	2	0.00017	2	0.08721	1	1	1	1	0	1	2.04	1.5E-05
TM	BB_0333	BB_0333	oligopeptide ABC transporter	20.92	23.5	2.58	0.00019	2	0.00138	2	0.13881	5	13	13	5	0	13	20.6	0.00028
TM	BB_0145	BB_0145	glycine betaine	5.02	5.02	0	9.9E-05	1	0.00008	1	1.2375	1	4	4	1	0	4	5.02	9.9E-05
TM	BB_0118	BB_0118	zinc protease	26.32	32.27	5.95	0.00049	2	0.00129	2	0.37664	8	25	25	8	0	25	19.9	0.00042
TM	BB_0170	BB_0170	predicted coding region BB0170	20.23	30.21	9.98	0.00017	2	0.00051	2	0.3432	7	25	25	7	0	25	18.5	0.00027
TM	BB_0442	BB_0442	inner membrane protein	8.27	16.18	7.91	5.5E-05	2	0.00013	2	0.41353	2	5	5	2	0	5	4.78	6.8E-05
TM	BB_0157	BB_0157	predicted coding region BB0157	10.14	10.14	0	0.00011	2	0.0003	1	0.36486	1	2	2	1	0	2	10.1	0.00011
TM	BB_0217	BB_0217	phosphate ABC transporter	5.18	18.6	13.42	2.3E-05	1	0.00035	2	0.06571	X	X	X	X	X	X	X	X
TM	BB_0380	BB_0380	Mg2+ transport protein (mgtE)	4.19	10.13	5.94	4.9E-05	1	0.00004	2	1.225	2	3	3	2	0	3	4.19	4.9E-05
TM	BB_0416	BB_0416	pheromone shutdown protein (traB	39.11	31.68	-7.43	0.00067	2	0.00066	2	1.0136	16	49	49	16	0	49	36.9	0.0009
TM	BB_0766	BB_0766	colicin V production protein	8.64	8.64	0	0.00037	1	0.0001	1	3.61386	1	8	8	1	0	8	8.64	0.00036
TM	BB_0453	BB_0453	conserved hypothetical protein	0	25.36	25.36	0	0	0.00044	2	0	X	X	X	X	X	X	X	X
TM	BB_0595	BB_0595	predicted coding region BB0595	0	11.06	11.06	0	0	0.00012	1	0	X	X	X	X	X	X	X	X
TM	BB_A01	BB_A01	conserved hypothetical protein	7.45	0	-7.45	4.6E-05	2	0	0	∞	1	1	1	1	0	1	7.45	4.6E-05
TM	BB_0075	BB_0075	predicted coding region BB0075	19.4	26.87	7.47	0.00027	2	0.00028	2	0.97122	6	18	18	6	0	18	15.4	0.00028
TM	BB_0134	BB_0134	predicted coding region BB0134	4.71	3.93	-0.78	1.9E-05	1	3.1E-05	1	0.6129	1	1	1	1	0	1	4.71	1.9E-05
TM	BB_0137	BB_0137	long-chain-fatty-acid CoA ligase	32.86	50.79	17.93	0.00046	2	0.00143	2	0.32008	15	64	64	15	0	64	29.8	0.00075
TM	BB_0291	BB_0291	flagellar basal-body rod protein (fliF	30.4	40.42	10.02	0.00039	2	0.00067	2	0.57808	14	40	40	14	0	40	26.2	0.00052
TM	BB_0596	BB_0596	methyl-accepting chemotaxis prote	8.67	29.51	20.84	6.2E-05	1	0.00038	2	0.1623	4	6	6	4	0	6	8.67	6.2E-05
TM	BB_0752	BB_0752	predicted coding region BB0752	35.26	48.8	13.54	0.00035	2	0.00153	2	0.2279	11	28	28	11	0	28	30.9	0.00041
TM	BB_E19	BB_E19	conserved hypothetical protein	0	15.87	15.87	0	0	0.00013	1	0	X	X	X	X	X	X	X	X
TM	BB_U09	BB_U09	conserved hypothetical protein	11.32	26.42	15.1	0.00014	2	0.00047	2	0.29747	1	2	2	1	0	2	11.3	0.00014
TM	BB_0067	BB_0067	peptidase	5.07	13.01	7.94	3.8E-05	2	0.00025	2	0.15139	2	4	4	2	0	4	5.07	5E-05
TM	BB_0174	BB_0174	predicted coding region BB0174	5.65	5.65	0	2.5E-05	1	9.4E-05	2	0.26596	1	1	1	1	0	1	5.65	2.5E-05
TM	BB_0260	BB_0260	predicted coding region BB0260	51.63	57.86	6.23	0.00084	2	0.00265	2	0.31571	13	59	59	13	0	59	51.6	0.00129
TM	BB_0326	BB_0326	predicted coding region BB0326	1.83	11.6	9.77	1.6E-05	1	4.1E-05	2	0.39024	1	2	2	1	0	2	1.83	1.6E-05
TM	BB_0345	BB_0345	predicted coding region BB0345	50.25	50.75	0.5	0.00072	2	0.00154	2	0.46662	17	44	43	17	1	43	50.3	0.0008
TM	BB_0593	BB_0593	long-chain-fatty-acid CoA ligase	51.94	58.6	6.66	0.00161	2	0.00485	2	0.33141	28	189	189	28	0	189	50.9	0.00216
TM	BB_0665	BB_0665	predicted coding region BB0665	8.78	17.55	8.77	2.3E-05	1	0.00011	2	0.21698	1	1	1	1	0	1	8.78	2.3E-05
TM	BB_0757	BB_0757	ATP-dependent Clp protease prote	14.08	40.29	26.21	0.00014	1	0.00031	2	0.45981	2	4	4	2	0	4	14.1	0.00014
TM	BB_G33	BB_G33	conserved hypothetical protein	14.29	21.43	7.14	2.8E-05	1	0.00035	2	0.07977	7	53	0	0	53	1	14.3	2.8E-05
TM	BB_H13	BB_H13	conserved hypothetical protein	17.12	23.42	6.3	0.00186	1	0.001	2	1.87048	7	56	5	1	51	56	17.1	0.00186
TM	BB_K40	BB_K40	conserved hypothetical protein	35.87	38.59	2.72	0.00168	2	0.00696	2	0.24083	6	59	59	6	0	59	28.8	0.00237
TM	BB_L27	BB_L27	bdrP	38.66	47.94	9.28	0.00072	2	4.5E-05	2	16.0222	10	63	0	0	63	3	30.4	0.00011
TM	BB_L35	BB_L35	bdrO	16.67	32.81	16.14	0.00014	2	0.00032	1	0.44582	4	12	2	1	10	6.8	16.7	0.00026
TM	BB_M34	BB_M34	bdrK	14.86	30.18	15.32	5.6E-05	2	0.00047	2	0.11915	2	5	1	1	4	2.33	11.7	7.8E-05
TM	BB_N27	BB_N27	bdrR	27.32	36.08	8.76	3.2E-05	1	2.4E-05	2	1.33333	8	53	0	0	53	0.83	27.3	3.2E-05
TM	BB_N34	BB_N34	bdrQ	12.85	22.91	10.06	4.2E-05	1	9.5E-05	1	0.44211	2	5	0	0	5	0	8.94	X
TM	BB_O27	BB_O27	bdrN	27.8	41.46	13.66	1.2E-05	2	2.3E-05	2	0.52174	8	56	0	0	56	0.33	24.4	1.2E-05
TM	BB_O34	BB_O34	bdrM	18.95	28.95	10	4.7E-05	2	9.4E-05	2	0.5	3	6	1	1	5	2.07	15.3	8E-05
TM	BB_P34	BB_P34	bdrA	23.81	20.48	-3.33	5.3E-05	2	0.00019	2	0.28495	4	8	0	0	8	0.5	18.1	1.8E-05
TM	BB_Q34	BB_Q34	bdrW	22.27	58.4	36.13	5.2E-05	2	0.00283	1	0.01837	8	59	0	0	59	3	19.3	9.3E-05
TM	BB_R27	BB_R27	bdrH	34.09	42.61	8.52	2.5E-05	2	0.00316	1	0.00791	8	53	0	0	53	0.83	30.1	3.5E-05
TM	BB_S29	BB_S29	bdrF	23.92	38.76	14.84	8.8E-05	1	0.00104	2	0.08502	8	58	0	0	58	2.5	21.5	8.8E-05
TM	BB_S37	BB_S37	bdrE	37.76	49.49	11.73	0.00146	2	0.00592	2	0.24645	6	60	56	5	4	59.8	33.2	0.00225
CYT	BB_0002	BB_0002	beta-N-acetylhexosaminidase	0	13.45	13.45	0	0	6.4E-05	2	0	X	X	X	X	X	X	X	X
CYT	BB_0003	BB_0003	predicted coding region BB0003	2.64	14.76	12.12	2.5E-05	2	0.00026	2	0.09728	1	2	2	1	0	2	2.64	3.3E-05
CYT	BB_0005	BB_0005	tryptophanyl-tRNA synthetase (trsA	23.51	23.51	0	8.4E-05	2	0.00049	2	0.17143	4	6	6	4	0	6	23.5	0.00013
CYT	BB_0007	BB_0007	predicted coding region BB0007	0	6.32	6.32	0	0	5.7E-05	1	0	X	X	X	X	X	X	X	X
CYT	BB_0009	BB_0009	predicted coding region BB0009	20.96	41.02	20.06	0.00027	2	0.0008	2	0.33543	5	17	17	5	0	17	21	0.00038
CYT	BB_0011	BB_0011	predicted coding region BB0011	5.38	12.54	7.16	2.6E-05	1	0.00017	2	0.15029	1	1	1	1	0	1	5.38	2.6E-05
CYT	BB_0015	BB_0015	uridine kinase (udk)	19.32	27.05	7.73	0.00016	2	0.00033	2	0.49846	2	5	5	2	0	5	19.3	0.00018
CYT	BB_0020	BB_0020	pyrophosphate--fructose 6-phospha	40.72	51.17	10.45	0.0005	2	0.00104	2	0.48456	13	55	55	13	0	55	34.8	0.00073
CYT	BB_0022	BB_0022	Holliday junction DNA helicase (ruv	0	5.48	5.48	0	0	4.7E-05	1	0	X	X	X	X	X	X	X	X
CYT	BB_0023	BB_0023	Holliday junction DNA helicase (ruv	0	29.95	29.95	0	0	0.00021	2	0	X	X	X	X	X	X	X	X
CYT	BB_0025	BB_0025	conserved hypothetical protein	0	19.75	19.75	0	0	0.00013	2	0	X	X	X	X	X	X	X	X
CYT	BB_0026	BB_0026	methylenetetrahydrofolate dehydrog	36.04	32.79	-3.25	0.00038	1	0.0002	2	1.90099	8	16	16	8	0	16	36	0.00038
CYT	BB_0035	BB_0035	DNA topoisomerase IV (parC)	0	6.23	6.23	0	0	2.6E-05	2	0	X	X	X	X	X	X	X	X
CYT	BB_0042	BB_0042	phosphate transport system regula	52.02	54.71	2.69	0.00063	2	0.00087	2	0.73064	10	28	28	10	0	28	48.4	0.00093
CYT	BB_0045	BB_0045	P115 protein	0	4.52	4.52	0	0	0.00003	1	0	X	X	X	X	X	X	X	X
CYT	BB_0049	BB_0049	predicted coding region BB0049	33.33	0	-33.33	0.00171	2	0	0	∞	2	14	14	2	0	14	33.3	0.00265
CYT	BB_0055	BB_0055	triosephosphate isomerase	50.2	48.62	-1.58	0.0009	2	0.00103	2	0.87317	10	46	46	10	0	46	50.2	0.00134
CYT	BB_0056	BB_0056	phosphoglycerate kinase (pgk)	76.59	77.86	1.27	0.00131	2	0.00256	2	0.5133	23	104	104	23	0	104	67.9	0.00195
CYT	BB_0057	BB_0057	glyceraldehyde 3-phosphate dehyd	73.43	77.91	4.48	0.00255	2	0.00456	2	0.56009	22	143	143	22	0	143	70.5	0.00315
CYT	BB_0059	BB_0059	hemolysin (tlyC)	16.99	18.53	1.54	0.00016	2	0.00062	2	0.25528	3	10	10	3	0	10	17	0.00029
CYT	BB_0061	BB_0061	thioredoxin (trxA)	70.94	71.79	0.85	0.00041	2	0.00045	2	0.92601	5	9	9	5	0	9	70.9	0.00057
CYT	BB_0064	BB_0064	methionyl-tRNA formyltransferase (	17.95	30.13	12.18	0.00023	2	0.00041	2	0.55774	4	11	11	4	0	11	17.3	0.00026
CYT	BB_0065	BB_0065	polypeptide deformylase (def)	22.09	40.7	18.61	0.00039	1	0.00034	2	1.13824	2	9	9	2	0	9	22.1	0.00039
CYT	BB_0068	BB_0068	conserved hypothetical protein	33.11	44.03	10.92	0.00023	2	0.0006	2	0.3796	7	16	16	7	0	16	28.7	0.0004
CYT	BB_0070	BB_0070	conserved hypothetical protein	14.38	14.38	0	7.3E-05	2	0.0002	2	0.37056	1	2	2	1	0	2	14.4	9.7E-05
CYT	BB_0074	BB_0074	peptide chain release factor 2 (prfB	9.77	6.61	-3.16	6.4E-05	1	6.9E-05	1	0.92754	2	3	3	2	0	3	9.77	6.4E-05
CYT	BB_0084	BB_0084	aminotransferase (nifS)	41.47	55.45	13.98	0.00019	2	0.00052	2	0.37476	10	21	21	10	0	21	41.5	0.00037
CYT	BB_0086	BB_0086	conserved hypothetical protein	0	6.65	6.65	0	0	3.2E-05	1	0	X	X	X	X	X	X	X	X
CYT	BB_0088	BB_0088	GTP-binding membrane protein (le	18.78	17.89	-0.89	3.3E-05	2	0.00011	2	0.31132	1	2	2	1	0	2	5.01	2.6E-05
CYT	BB_0089	BB_0089	predicted coding region BB0089	11.42	12.96	1.54	9.1E-05	2	0.00022	2	0.40625	2	7	7	2	0	7	11.4	0.00016
CYT	BB_0092	BB_0092	V-type ATPase	7.35	0	-7.35	7.2E-05	1	0	0	∞	1	2	2	1	0	2	7.35	7.2E-05
CYT	BB_0093	BB_0093	V-type ATPase	13.13	35.02	21.89	6.8E-05	2	0.00046	2	0.1488	3	6	6	3	0	6	13.1	0.0001
CYT	BB_0094	BB_0094	V-type ATPase	17.06	37.93	20.87	0.0002	2	0.00107	2	0.18446	3	11	11	3	0	11	6.72	0.00015
CYT	BB_0095	BB_0095	predicted coding region BB0095	17.13	33.7	16.57	0.00033	1	0.00075	2	0.436	3	8	8	3	0	8	17.1	0.00033
CYT	BB_0096	BB_0096	V-type ATPase	28.5	42	13.5	0.00056	2	0.00588	2	0.09481	3	22	22	3	0	22	28.5	0.00081
CYT	BB_0101	BB_0101	asparaginyl-tRNA synthetase (asnS	11.37	8.37	-3	0.00013	1	7.7E-05	1	1.64935	3	8	8	3	0	8	11.4	0.00013
CYT	BB_0103	BB_0103	predicted coding region BB0103	55.33	62.94	7.61	0.00104	2	0.00138	2	0.75273	8	44	44	8	0	44	55.3	0.00165
CYT	BB_0105	BB_0105	methionine aminopeptidase (map)	5.98	5.98	0	5.9E-05	1	3.3E-05	1	1.78788	1	2	2	1	0	2	5.98	5.9E-05
CYT	BB_0107	BB_0107	N-utilization substance protein B (n	7.59	38.62	31.03	0.0002	1	0.00022	2	0.9148	1	4	4	1	0	4	7.59	0.0002
CYT	BB_0109	BB_0109	acetyl-CoA C-acetyltransferase (fad	47.74	50.25	2.51	0.00043	2	0.00092	2	0.46514	15	46	43	15	3	43	47.7	0.0008
CYT	BB_0110	BB_0110	predicted coding region BB0110	11.89	25.77	13.88	0.00026	2	0.0004	2	0.66582	4	13	13	4	0	13	11.9	0.00021
CYT	BB_0111	BB_0111	replicative DNA helicase (dnaB)	6.81	9.89	3.08	3.2E-05	1	0.00011	2	0.30189	2	2	2	2	0	2	6.81	3.2E-05
CYT	BB_0112	BB_0112	ribosomal protein L9 (rplI)	14.45	32.95	18.5	0.00017	2	0.00056	2	0.3059	1	7	7	1	0	7	5.2	0.0003
CYT	BB_0113	BB_0113	ribosomal protein S18 (rpsR)	0	12.5	12.5	0	0	0.00021	2	0	X	X	X	X	X	X	X	X
CYT	BB_0115	BB_0115	ribosomal protein S6 (rpsF)	0	23.74	23.74	0	0	0.00027	2	0	X	X	X	X	X	X	X	X
CYT	BB_0121	BB_0121	ribosome releasing factor (frr)	20.11	20.11	0	8.1E-05	2	0.00036	2	0.225	1	1	1	1	0	1	14.7	4E-05
CYT	BB_0122	BB_0122	translation elongation factor TS (tsf	41.22	40.5	-0.72	0.00054	2	0.00129	2	0.42203	9	36	36	9	0	36	36.6	0.00095
CYT	BB_0123	BB_0123	ribosomal protein S2 (rpsB)	80.38	75	-5.38	0.00533	2	0.00721	2	0.7387	28	260	260	28	0	260	73.5	0.00739
CYT	BB_0125	BB_0125	predicted coding region BB0125	58.33	42.92	-15.41	0.00127	2	0.00101	2	1.25893	10	57	57	10	0	57	50.4	0.00175
CYT	BB_0128	BB_0128	cytidylate kinase (cmk-1)	10.41	30.32	19.91	6.7E-05	1	0.0001	2	0.67	2	2	2	2	0	2	10.4	6.7E-05
CYT	BB_0130	BB_0130	predicted coding region BB0130	3.53	3.53	0	0.00003	1	3.2E-05	1	0.9375	X	X	X	X	X	X	X	X
CYT	BB_0131	BB_0131	recA protein (recA)	34.25	38.08	3.83	0.00022	2	0.0006	2	0.37584	7	15	15	7	0	15	34.3	0.0003
CYT	BB_0132	BB_0132	transcription elongation factor (greA	39.96	52.83	12.87	0.00061	2	0.00206	2	0.29669	31	105	105	31	0	105	39.1	0.00086
CYT	BB_0133	BB_0133	predicted coding region BB0133	23.89	49.04	25.15	0.00021	2	0.00073	2	0.29019	5	14	14	5	0	14	16.2	0.00033
CYT	BB_0135	BB_0135	histidyl-tRNA synthetase (hisS)	17.51	23.19	5.68	0.00011	2	0.00018	2	0.6	6	12	12	6	0	12	17.5	0.00019
CYT	BB_0146	BB_0146	glycine betaine	20.16	26.34	6.18	0.00015	2	0.00062	2	0.24071	5	12	12	5	0	12	20.2	0.00024

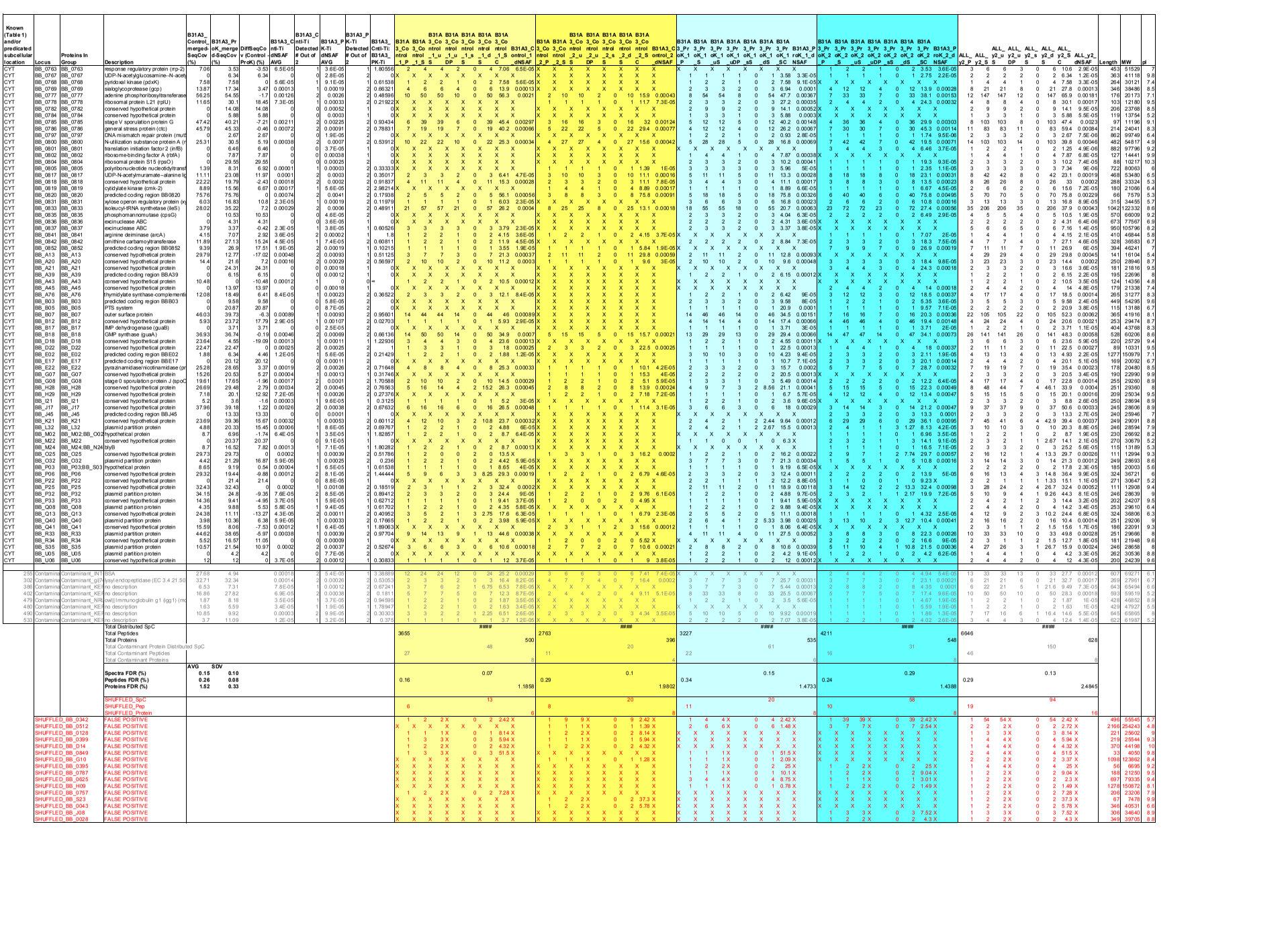
Locus	B31A3_Control_2_P	B31A3_Control_2_S	B31A3_Control_2_uS	B31A3_Control_2_uDP	B31A3_Control_2_sS	B31A3_Control_2_dS	B31A3_Control_2_SC	B31A3_Control_2_dNSAF	B31A3_ProK_1_P	B31A3_ProK_1_S	B31A3_ProK_1_uS	B31A3_ProK_1_uDP	B31A3_ProK_1_sS	B31A3_ProK_1_dS	B31A3_ProK_1_SC	B31A3_ProK_1_dNSAF
BB_0783	2	5	5	2	0	5	12.9	9.5E-05	3	8	8	3	0	8	14	0.00024
BB_0789	2	4	4	2	0	4	7.98	4.7E-05	9	16	16	9	0	16	26	0.0003
BB_0824	2	4	4	2	0	4	13.1	0.00016	4	124	124	4	0	124	23.5	0.0081
BB_0843	1	1	1	1	0	1	3.53	1.6E-05	2	6	6	2	0	6	3.53	0.00015
BB_B22	1	5	5	1	0	5	1.77	8.3E-05	3	4	4	3	0	4	8.2	0.00011
BB_B28	10	38	38	10	0	38	28.3	0.00069	17	45	45	17	0	45	56.3	0.0013
BB_B29	33	362	362	33	0	362	53	0.00503	28	861	861	28	0	861	32.5	0.01899
BB_D15	2	3	3	2	0	3	15	0.00016	1	1	1	1	0	1	6.43	8.5E-05
BB_J27	X	X	X	X	X	X	X	X	1	2	2	1	0	2	3.16	5.8E-05
BB_K13	2	3	3	2	0	3	14.7	9.5E-05	X	X	X	X	X	X	X	X
BB_0252	1	4	4	1	0	4	2.35	3.9E-05	6	18	18	6	0	18	8.6	0.00028
BB_0091	X	X	X	X	X	X	X	X	9	20	20	9	0	20	20.2	0.00039
BB_0408	22	104	104	22	0	104	30.4	0.00125	20	262	262	20	0	262	26.2	0.00501
BB_0629	9	32	32	9	0	32	21.3	0.00038	11	23	23	11	0	23	25.4	0.00044
BB_B04	3	7	7	3	0	7	7	0.00012	5	23	23	5	0	23	10.8	0.00062
BB_0071	1	1	1	1	0	1	2.04	1.5E-05	3	10	10	3	0	10	5.71	0.00024
BB_0333	2	5	5	2	0	5	10.9	0.00011	4	23	23	4	0	23	15.5	0.00079
BB_0145	X	X	X	X	X	X	X	X	1	2	2	1	0	2	5.02	8E-05
BB_0118	9	32	32	9	0	32	21.7	0.00055	9	29	29	9	0	29	25.2	0.00079
BB_0170	3	7	7	3	0	7	6.89	7.7E-05	10	34	34	10	0	34	23.3	0.0006
BB_0442	1	3	3	1	0	3	3.49	4.1E-05	4	6	6	4	0	6	10.9	0.00013
BB_0157	1	2	2	1	0	2	10.1	0.00011	X	X	X	X	X	X	X	X
BB_0217	1	1	1	1	0	1	5.18	2.3E-05	4	11	11	4	0	11	18.6	0.0004
BB_0380	X	X	X	X	X	X	X	X	1	1	1	1	0	1	1.76	2.6E-05
BB_0416	8	24	24	8	0	24	16.8	0.00045	8	14	14	8	0	14	24	0.00041
BB_0766	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0453	X	X	X	X	X	X	X	X	1	1	1	1	0	1	4.29	4.3E-05
BB_0595	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_A01	1	1	1	1	0	1	6.21	4.7E-05	X	X	X	X	X	X	X	X
BB_0075	4	16	16	4	0	16	9.17	0.00026	1	2	2	1	0	2	2.99	5.1E-05
BB_0134	X	X	X	X	X	X	X	X	1	1	1	1	0	1	3.93	3.1E-05
BB_0137	6	14	14	6	0	14	13.7	0.00017	25	74	74	25	0	74	42.5	0.0014
BB_0291	6	19	19	6	0	19	19.7	0.00025	15	32	32	15	0	32	35.2	0.00067
BB_0596	X	X	X	X	X	X	X	X	6	17	17	6	0	17	13.2	0.00028
BB_0752	4	19	19	4	0	19	15.9	0.00028	12	60	60	12	0	60	36.9	0.00143
BB_E19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_U09	1	2	2	1	0	2	11.3	0.00014	3	5	5	3	0	5	26.4	0.00056
BB_0067	1	2	2	1	0	2	2.03	2.5E-05	1	1	1	1	0	1	3.04	2E-05
BB_0174	X	X	X	X	X	X	X	X	1	2	2	1	0	2	5.65	7.9E-05
BB_0260	5	17	17	5	0	17	23.4	0.00038	13	36	36	13	0	36	41.8	0.00128
BB_0326	X	X	X	X	X	X	X	X	3	3	3	3	0	3	4.94	3.9E-05
BB_0345	4	34	34	4	0	34	12.6	0.00064	8	42	42	8	0	42	25.9	0.00126
BB_0593	14	90	90	14	0	90	29.9	0.00105	31	341	341	31	0	341	52.6	0.00632
BB_0665	X	X	X	X	X	X	X	X	3	5	5	3	0	5	17.6	0.00019
BB_0757	X	X	X	X	X	X	X	X	4	8	8	4	0	8	23.8	0.00046
BB_G33	4	28	0	0	28	0	8.65	X	7	27	2	2	25	10.3	21.4	0.00046
BB_H13	4	28	0	0	28	0	10.4	X	6	26	1	1	25	5.17	19.8	0.00028
BB_K40	3	24	24	3	0	24	27.2	0.00098	5	109	109	5	0	109	33.2	0.00708
BB_L27	8	38	1	1	37	34.2	31.4	0.00133	11	48	0	0	48	0.33	35.1	2.1E-05
BB_L35	2	6	0	0	6	0.67	8.33	2.6E-05	6	77	0	0	77	0	26	X
BB_M34	2	4	0	0	4	1	6.76	3.4E-05	5	18	2	1	16	11.5	22.1	0.00062
BB_N27	4	28	0	0	28	0	11.9	X	10	46	0	0	46	0.33	30.9	2.1E-05
BB_N34	2	4	0	0	4	1	8.38	4.2E-05	2	6	0	0	6	0	8.38	X
BB_O27	6	34	0	0	34	0.33	18.5	1.2E-05	12	50	0	0	50	0.33	36.6	1.9E-05
BB_O34	2	6	0	0	6	0.33	7.89	1.3E-05	3	5	1	1	4	1.83	12.1	0.00012
BB_P34	2	6	2	1	4	2.47	9.52	8.9E-05	3	4	1	1	3	2.17	11	0.00012
BB_Q34	5	30	0	0	30	0.33	12.6	1.1E-05	11	50	0	0	50	0	28.2	X
BB_R27	5	30	0	0	30	0.33	17.1	1.4E-05	6	27	0	0	27	0	21.6	X
BB_S29	6	33	0	0	33	0	20.1	X	10	48	3	1	45	35.5	31.6	0.00203
BB_S37	6	18	14	5	4	17.3	34.2	0.00066	10	82	10	6	72	80.5	40.8	0.00491
BB_0002	X	X	X	X	X	X	X	X	2	3	3	2	0	3	7.6	0.0001
BB_0003	1	1	1	1	0	1	2.64	1.7E-05	6	10	10	6	0	10	14.5	0.00026
BB_0005	2	2	2	2	0	2	10.2	4.3E-05	6	18	18	6	0	18	19.3	0.00061
BB_0007	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0009	3	7	7	3	0	7	12.6	0.00016	5	9	9	5	0	9	13.5	0.00032
BB_0011	X	X	X	X	X	X	X	X	2	4	4	2	0	4	12.5	0.00017
BB_0015	1	4	4	1	0	4	9.18	0.00015	1	1	1	1	0	1	7.73	5.8E-05
BB_0020	7	20	20	7	0	20	23.2	0.00027	21	58	58	21	0	58	51.2	0.00125
BB_0022	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0023	X	X	X	X	X	X	X	X	2	5	5	2	0	5	12.7	0.0003
BB_0025	X	X	X	X	X	X	X	X	1	1	1	1	0	1	8.64	4.9E-05
BB_0026	X	X	X	X	X	X	X	X	5	7	7	5	0	7	27.3	0.00027
BB_0035	X	X	X	X	X	X	X	X	1	2	2	1	0	2	2.24	3.8E-05
BB_0042	6	10	10	6	0	10	39.9	0.00034	8	20	20	8	0	20	54.7	0.00107
BB_0045	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0049	1	4	4	1	0	4	33.3	0.00077	X	X	X	X	X	X	X	X
BB_0055	5	15	15	5	0	15	26.9	0.00045	9	27	27	9	0	27	48.6	0.00128
BB_0056	15	35	35	15	0	35	50.1	0.00067	24	95	95	24	0	95	74.1	0.00289
BB_0057	19	87	87	19	0	87	66.9	0.00195	16	87	87	16	0	87	58.2	0.0031
BB_0059	1	1	1	1	0	1	8.88	2.9E-05	3	13	13	3	0	13	14.7	0.0006
BB_0061	2	4	4	2	0	4	35	0.00026	4	6	6	4	0	6	59	0.00061
BB_0064	3	8	8	3	0	8	14.1	0.00019	5	11	11	5	0	11	20.2	0.00042
BB_0065	X	X	X	X	X	X	X	X	3	5	5	3	0	5	30.8	0.00035
BB_0068	2	2	2	2	0	2	9.22	5.1E-05	5	15	15	5	0	15	28.7	0.00061
BB_0070	1	1	1	1	0	1	14.4	4.9E-05	1	3	3	1	0	3	14.4	0.00023
BB_0074	X	X	X	X	X	X	X	X	1	2	2	1	0	2	6.61	6.9E-05
BB_0084	1	1	1	1	0	1	6.16	1.8E-05	10	22	22	10	0	22	44.8	0.00062
BB_0086	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0088	3	3	3	3	0	3	13.8	4E-05	1	1	1	1	0	1	2.68	2.1E-05
BB_0089	1	1	1	1	0	1	4.94	2.3E-05	3	6	6	3	0	6	13	0.00022
BB_0092	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0093	2	2	2	2	0	2	7.83	3.5E-05	5	12	12	5	0	12	12.7	0.00033
BB_0094	6	18	18	6	0	18	16.9	0.00025	10	35	35	10	0	35	27.4	0.00076
BB_0095	X	X	X	X	X	X	X	X	4	7	7	4	0	7	19.9	0.00046
BB_0096	2	8	8	2	0	8	22.5	0.0003	7	129	129	7	0	129	40	0.00771
BB_0101	X	X	X	X	X	X	X	X	2	3	3	2	0	3	8.37	7.7E-05
BB_0103	4	11	11	4	0	11	36.6	0.00042	6	18	18	6	0	18	46.7	0.00109
BB_0105	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BB_0107	X	X	X	X	X	X	X	X	1	2	2	1	0	2	7.59	0.00016
BB_0109	2	3	3	2	0	3	12.8	5.7E-05	12	55	55	12	0	55	33.7	0.00165
BB_0110	2	19	19	2	0	19	7.27	0.00031	2	2	2	2	0	2	8.59	5.3E-05
BB_0111	X	X	X	X	X	X	X	X	2	4	4	2	0	4	6.81	0.00011
BB_0112	1	1	1	1	0	1	9.25	4.3E-05	5	8	8	5	0	8	27.8	0.00055
BB_0113	X	X	X	X	X	X	X	X	1	2	2	1	0	2	12.5	0.00025
BB_0115	X	X	X	X	X	X	X	X	2	5	5	2	0	5	23.7	0.00043
BB_0121	3	3	3	3	0	3	20.1	0.00012	4	7	7	4	0	7	14.7	0.00045
BB_0122	4	5	5	4	0	5	22.9	0.00013	11	39	39	11	0	39	36.2	0.00167
BB_0123	19	113	113	19	0	113	66.2	0.00327	23	165	165	23	0	165	60.4	0.00759
BB_0125	9	25	25	9	0	25	51.3	0.00078	5	20	20	5	0	20	30.4	0.001
BB_0128	X	X	X	X	X	X	X	X	3	3	3	3	0	3	25.3	0.00016
BB_0130	1	1	1	1	0	1	3.53	3E-05	X	X	X	X	X	X	X	X
BB_0131	3	7	7	3	0	7	18.1	0.00014	7	20	20	7	0	20	27.7	0.00065
BB_0132	13	43	43	13	0	43	20.9	0.00036	35	168	168	35	0	168	40.4	0.00223
BB_0133	2	4	4	2	0	4	7.64	9.6E-05	8	14	14	8	0	14	31.5	0.00053
BB_0135	1	1	1	1	0	1	2.84	1.6E-05	6	10	10	6	0	10	20.8	0.00026
BB_0146	1	3	3	1	0	3	6.45	6.1E-05	6	16	16	6	0	16	15.1	0.00051

Locus	B31A3_ProK_2_P	B31A3_ProK_2_S	B31A3_ProK_2_uS	B31A3_ProK_2_uDP	B31A3_ProK_2_sS	B31A3_ProK_2_dS	B31A3_ProK_2_SC	B31A3_ProK_2_dNSAF	ALL_y2_P	ALL_y2_S	ALL_y2_uS	ALL_y2_uDP	ALL_y2_sS	ALL_y2_dS	ALL_y2_SC	ALL_y2_dNSAF	Length	MW	pI
BB_0783	7	23	23	7	0	23	25.6	0.00048	10	63	63	10	0	63	32.7	0.00035	394	45766	8.4
BB_0789	10	40	40	10	0	40	16.6	0.00051	15	69	69	15	0	69	24.4	0.00023	639	70802	8.3
BB_0824	7	339	339	7	0	339	29	0.01512	5	42	42	5	0	42	18.6	0.0005	183	21175	8.8
BB_0843	3	11	11	3	0	11	3.53	0.00019	3	24	24	3	0	24	3.53	0.00011	482	52856	8.4
BB_B22	4	22	22	4	0	22	10.6	0.0004	5	39	39	5	0	39	12	0.00019	451	48977	8.5
BB_B28	20	151	151	20	0	151	52.7	0.00298	27	355	355	27	0	355	59.7	0.00186	414	49380	8.6
BB_B29	46	1539	1539	46	0	1539	48.3	0.02317	42	3112	3112	42	0	3112	53	0.01242	542	58938	8.6
BB_D15	X	X	X	X	X	X	X	X	5	11	11	5	0	11	20.7	0.00017	140	16635	9.7
BB_J27	2	2	2	2	0	2	7.3	4E-05	3	10	10	3	0	10	11.2	5.3E-05	411	46456	5.8
BB_K13	9	30	30	9	0	30	43.7	0.00103	10	33	33	10	0	33	44.5	0.0003	238	27221	9.2
BB_0252	2	4	4	2	0	4	4.56	4.3E-05	7	33	33	7	0	33	10.8	9.3E-05	767	86971	9.6
BB_0091	12	40	40	12	0	40	19.1	0.00054	13	62	62	13	0	62	22.2	0.00022	608	69125	8.3
BB_0408	33	318	318	33	0	318	32.8	0.00415	41	863	863	41	0	863	38.1	0.00299	625	67130	7.5
BB_0629	28	104	104	28	0	104	35.5	0.00136	31	178	178	31	0	178	36.4	0.00062	626	67805	8.9
BB_B04	4	66	66	4	0	66	9.71	0.00122	4	49	49	4	0	49	7	0.00024	443	48761	8.1
BB_0071	3	6	6	3	0	6	8.37	1E-04	5	9	9	5	0	9	11.6	4E-05	490	58164	9.3
BB_0333	9	84	84	9	0	84	22.9	0.00196	12	125	125	12	0	125	25.8	0.00077	349	39418	9.2
BB_0145	X	X	X	X	X	X	X	X	1	6	6	1	0	6	5.02	4.3E-05	299	33735	9
BB_0118	12	96	96	12	0	96	26.5	0.00179	18	180	180	18	0	180	35.7	0.00089	437	49741	9.2
BB_0170	9	35	35	9	0	35	22	0.00042	14	94	94	14	0	94	32.3	0.0003	682	78475	8.8
BB_0442	3	9	9	3	0	9	8.64	0.00014	6	22	22	6	0	22	13.6	8.8E-05	544	64002	8.9
BB_0157	1	5	5	1	0	5	10.1	0.0003	1	9	9	1	0	9	10.1	0.00014	138	15595	9.6
BB_0217	4	12	12	4	0	12	18.6	0.0003	4	24	24	4	0	24	18.6	0.00016	328	36113	9.3
BB_0380	2	3	3	2	0	3	8.37	5.4E-05	4	7	7	4	0	7	12.6	3.3E-05	454	50747	5.1
BB_0416	10	45	45	10	0	45	21.3	0.00091	23	128	128	23	0	128	41.6	0.00069	404	45678	9.3
BB_0766	1	2	2	1	0	2	8.64	0.0001	1	10	10	1	0	10	8.64	0.00013	162	18716	9.1
BB_0453	8	29	29	8	0	29	25.4	0.00085	8	30	30	8	0	30	25.4	0.00023	280	32302	8.6
BB_0595	3	3	3	3	0	3	11.1	0.00012	3	3	3	3	0	3	11.1	3.1E-05	208	24003	9.8
BB_A01	X	X	X	X	X	X	X	X	2	2	2	2	0	2	7.45	2.7E-05	161	17887	9.5
BB_0075	13	29	29	13	0	29	26.9	0.0005	16	63	63	16	0	63	33.5	0.00029	469	54638	9.2
BB_0134	X	X	X	X	X	X	X	X	2	2	2	2	0	2	8.64	1.1E-05	382	44325	9.3
BB_0137	23	113	113	23	0	113	44.6	0.00146	29	261	261	29	0	261	47	0.0009	630	70763	8.8
BB_0291	19	46	46	19	0	46	29.7	0.00066	27	135	135	27	0	135	41	0.00051	569	65134	5.5
BB_0596	13	42	42	13	0	42	28.4	0.00048	14	64	64	14	0	64	28.3	0.00019	715	80470	7.6
BB_0752	19	100	100	19	0	100	39.2	0.00163	28	207	207	28	0	207	54	0.00089	502	57435	8.7
BB_E19	2	4	4	2	0	4	15.9	0.00013	2	4	4	2	0	4	15.9	3.4E-05	252	29282	8.6
BB_U09	1	5	5	1	0	5	10.4	0.00038	2	11	11	2	0	11	21.7	0.00022	106	12136	8.7
BB_0067	6	35	35	6	0	35	13	0.00048	6	42	42	6	0	42	13	0.00015	592	67627	6.4
BB_0174	1	4	4	1	0	4	5.65	0.00011	1	7	7	1	0	7	5.65	5E-05	301	34488	9
BB_0260	28	166	166	28	0	166	55.5	0.00402	24	252	252	24	0	252	60.2	0.00162	337	39145	4.4
BB_0326	3	6	5	3	1	5	6.66	4.4E-05	5	10	10	5	0	10	8.27	2.3E-05	931	107524	5
BB_0345	17	89	89	17	0	89	46	0.00182	27	209	208	27	1	208	63.8	0.00113	398	46456	5.8
BB_0593	29	267	267	29	0	267	49.6	0.00338	43	881	881	43	0	881	64.2	0.00296	645	72887	9
BB_0665	1	1	1	1	0	1	8.78	2.6E-05	2	6	6	2	0	6	12.5	4.1E-05	319	37381	8.6
BB_0757	3	4	4	3	0	4	21.8	0.00016	5	15	15	5	0	15	34	0.00016	206	23206	7.9
BB_G33	11	103	2	1	101	7.77	14.7	0.00024	11	191	4	2	187	30.7	24.4	0.00025	266	30587	5.5
BB_H13	12	113	12	2	101	46.6	23.4	0.00171	10	203	18	2	185	133	23	0.00129	222	25840	5.6
BB_K40	10	154	154	10	0	154	38.6	0.00683	8	338	338	8	0	338	35.9	0.00397	184	21564	6.2
BB_L27	22	180	0	0	180	1.67	47.9	7E-05	15	226	1	1	225	12.3	55.2	0.00014	194	22421	5.2
BB_L35	7	156	1	1	155	7.6	28.7	0.00032	5	38	3	2	35	5.9	31.8	6.7E-05	192	22050	6.6
BB_M34	7	20	4	2	16	8.7	27	0.00032	6	44	20	3	24	40.5	30.2	0.00039	222	25444	5.3
BB_N27	19	164	0	0	164	0.67	36.1	2.8E-05	11	188	0	0	188	0.83	43.8	9.3E-06	194	22294	5.2
BB_N34	4	9	1	1	8	2.07	22.9	9.5E-05	3	23	0	0	23	0	12.9	X	179	20714	5.5
BB_O27	21	174	0	0	174	0.67	41.5	2.7E-05	12	211	0	0	211	0.33	43.4	3.5E-06	205	23698	5.4
BB_O34	4	13	1	1	12	1.67	24.7	7.2E-05	5	29	2	2	27	3.26	19	3.7E-05	190	21974	5.2
BB_P34	4	14	4	2	10	6.4	16.7	0.00025	6	32	7	2	25	12.7	27.1	0.00013	210	24118	5.4
BB_Q34	23	177	4	3	173	82.5	58.4	0.00283	13	212	3	1	209	29.4	28.6	0.00027	238	27307	5.2
BB_R27	16	121	17	4	104	68.1	42.6	0.00316	11	193	0	0	193	0.83	34.1	1E-05	176	20350	5.3
BB_S29	19	170	0	0	170	1	35.9	3.9E-05	12	208	3	1	205	33.3	26.8	0.00034	209	24109	5.2
BB_S37	11	175	24	6	151	166	45.9	0.00693	9	310	292	8	18	309	40.8	0.00341	196	22652	6.1
BB_0002	1	1	1	1	0	1	5.85	2.4E-05	1	2	2	1	0	2	3.51	1.3E-05	342	38465	8.7
BB_0003	5	14	14	5	0	14	13	0.00025	7	27	27	7	0	27	14.8	0.00013	454	52012	6.8
BB_0005	6	16	16	6	0	16	21.5	0.00037	8	40	40	8	0	40	32	0.00025	353	40419	9.1
BB_0007	1	2	2	1	0	2	6.32	5.7E-05	1	2	2	1	0	2	6.32	1.5E-05	285	33538	7.3
BB_0009	12	52	52	12	0	52	38	0.00127	12	83	83	12	0	83	41	0.00054	334	38543	9.3
BB_0011	2	6	6	2	0	6	5.38	0.00018	3	11	11	3	0	11	12.5	8.5E-05	279	32820	9.3
BB_0015	3	15	15	3	0	15	27.1	0.00059	3	25	25	3	0	25	27.1	0.00026	207	24006	7.6
BB_0020	11	56	56	11	0	56	28.1	0.00082	25	186	186	25	0	186	57.5	0.00073	555	62477	6.6
BB_0022	1	2	2	1	0	2	5.48	4.7E-05	1	2	2	1	0	2	5.48	1.2E-05	347	39006	5.3
BB_0023	2	3	3	2	0	3	25.9	0.00012	2	3	3	2	0	3	10.2	3.3E-05	197	22688	8.1
BB_0025	3	6	6	3	0	6	19.8	0.0002	2	5	5	2	0	5	14.8	4.5E-05	243	27034	5.5
BB_0026	2	5	5	2	0	5	9.42	0.00013	10	26	26	10	0	26	46.8	0.00018	308	34032	8.9
BB_0035	1	1	1	1	0	1	3.99	1.3E-05	1	2	2	1	0	2	2.24	6.9E-06	626	72042	8.5
BB_0042	8	18	18	8	0	18	43.5	0.00066	14	75	75	14	0	75	58.3	0.00073	223	25980	5.1
BB_0045	2	3	3	2	0	3	4.52	3E-05	1	2	2	1	0	2	2.2	5.3E-06	819	95857	5.7
BB_0049	X	X	X	X	X	X	X	X	2	18	18	2	0	18	33.3	0.001	39	4343	9
BB_0055	7	24	24	7	0	24	43.1	0.00077	13	111	111	13	0	111	60.1	0.00095	253	27759	6.2
BB_0056	21	107	107	21	0	107	59.8	0.00222	34	341	341	34	0	341	84.2	0.00188	393	42346	6.8
BB_0057	26	247	247	26	0	247	72.8	0.00602	30	562	562	30	0	562	80.3	0.00363	335	36255	8
BB_0059	3	20	20	3	0	20	17	0.00063	5	42	42	5	0	42	25.1	0.00035	259	29769	5.4
BB_0061	3	4	4	3	0	4	35	0.00028	8	22	22	8	0	22	75.2	0.00041	117	13467	4.8
BB_0064	7	15	15	7	0	15	26	0.00039	10	45	45	10	0	45	35.3	0.00031	312	35107	8.9
BB_0065	2	7	7	2	0	7	16.9	0.00033	3	18	18	3	0	18	33.7	0.00023	172	19922	7.4
BB_0068	6	21	21	6	0	21	23.6	0.00058	12	52	52	12	0	52	54.3	0.00038	293	33278	7.1
BB_0070	1	3	3	1	0	3	14.4	0.00016	1	9	9	1	0	9	14.4	0.00013	153	16765	9.7
BB_0074	X	X	X	X	X	X	X	X	2	5	5	2	0	5	9.77	3.1E-05	348	40269	8.2
BB_0084	9	21	21	9	0	21	40.8	0.00041	17	65	65	17	0	65	60	0.00033	422	48125	8.5
BB_0086	2	2	2	2	0	2	6.65	3.2E-05	2	2	2	2	0	2	6.65	8.5E-06	511	57238	9.1
BB_0088	6	13	13	6	0	13	17.9	0.00019	8	19	19	8	0	19	25	7.4E-05	559	62901	5.8
BB_0089	3	9	9	3	0	9	11.4	0.00023	3	21	21	3	0	21	11.4	0.00014	324	38100	9.2
BB_0092	X	X	X	X	X	X	X	X	1	2	2	1	0	2	7.35	2.1E-05	204	24026	9.9
BB_0093	11	31	31	11	0	31	35	0.00058	13	49	49	13	0	49	30	0.00024	434	48026	5.9
BB_0094	18	93	93	18	0	93	36.3	0.00138	16	154	154	16	0	154	37	0.0006	551	61620	5.3
BB_0095	6	23	23	6	0	23	33.7	0.00104	6	38	38	6	0	38	33.7	0.00045	181	21662	6.1
BB_0096	8	99	99	8	0	99	36.5	0.00404	8	256	256	8	0	256	42	0.00277	200	22851	7.3
BB_0101	X	X	X	X	X	X	X	X	5	11	11	5	0	11	19.7	5.1E-05	466	53600	8
BB_0103	9	40	40	9	0	40	62.9	0.00166	10	113	113	10	0	113	67.5	0.00124	197	23277	6.4
BB_0105	1	1	1	1	0	1	5.98	3.3E-05	1	3	3	1	0	3	5.98	2.6E-05	251	27711	8
BB_0107	3	5	5	3	0	5	38.6	0.00028	3	11	11	3	0	11	38.6	0.00016	145	16808	5
BB_0109	7	9	9	7	0	9	32.4	0.00018	19	110	104	19	6	104	60.3	0.00057	398	42940	7.3
BB_0110	9	41	41	9	0	41	25.8	0.00074	9	72	72	9	0	72	23.8	0.00034	454	53411	9
BB_0111	3	6	6	3	0	6	9.89	0.00011	3	12	12	3	0	12	9.89	5.7E-05	455	51662	6.7
BB_0112	5	12	12	5	0	12	26.6	0.00057	7	27	27	7	0	27	31.8	0.00034	173	19959	9.5
BB_0113	1	2	2	1	0	2	12.5	0.00017	1	4	4	1	0	4	12.5	9E-05	96	11614	10.2
BB_0115	1	2	2	1	0	2	13	0.00012	2	7	7	2	0	7	23.7	0.00011	139	16437	9.5
BB_0121	3	6	6	3	0	6	17.4	0.00027	6	17	17	6	0	17	25.5	0.0002	184	21539	8.6
BB_0122	10	33	31	10	2	31	32.3	0.00091	17	113	113	17	0	113	46.2	0.00088	279	31304	8.2
BB_0123	31	218	218	31	0	218	67.3	0.00684	40	747	747	40	0	747	80.4	0.00622	260	29676	7.4
BB_0125	8	30	30	8	0	30	42.9	0.00102	15	132	132	15	0	132	61.7	0.00119	240	27129	8.4
BB_0128	1	1	1	1	0	1	4.98	3.7E-05	4	5	5	4	0	5	20.4	4.9E-05	221	25602	9
BB_0130	1	1	1	1	0	1	3.53	3.2E-05	1	2	2	1	0	2	3.53	1.7E-05	255	29972	9.2
BB_0131	7	24	24	7	0	24	32.3	0.00054	11	66	66	11	0	66	46	0.00039	365	39949	7.4
BB_0132	46	208	208	46	0	208	47.6	0.00188	57	517	517	57	0	517	54.4	0.00124	901	105315	6.4
BB_0133	12	36	36	12	0	36	46.2	0.00094	14	68	68	14	0	68	49	0.00047	314	37176	6.1
BB_0135	3	5	5	3	0	5	5.25	8.9E-05	12	28	28	12	0	28	29.5	0.00013	457	52849	8.5
BB_0146	6	33	33	6	0	33	23.9	0.00072	10	63	63	10	0	63	31.5	0.00037	372	42481	8.7



Known (Table 1) and/or predicated subcellular location	Locus	Proteins In Group	Description	B31A3_Control_merged-SeqCov (%)	B31A3_ProK_merged-SeqCov (%)	DiffSeqCov (Control - ProK) (%)	B31A3_Cntl-Ti dNSAF AVG	B31A3_Cntl-Ti Detected # Out of 2	B31A3_PK-Ti dNSAF AVG	B31A3_PK-Ti Detected # Out of 2	B31A3_Cntl-Ti: B31A3_PK-Ti	B31A3_Control_1_P	B31A3_Control_1_S	B31A3_Control_1_uS	B31A3_Control_1_uDP	B31A3_Control_1_sS	B31A3_Control_1_dS	B31A3_Control_1_SC	B31A3_Control_1_dNSAF	B31A3_Control_2_P	B31A3_Control_2_S	B31A3_Control_2_uS	B31A3_Control_2_uDP	B31A3_Control_2_sS	B31A3_Control_2_dS	B31A3_Control_2_SC	B31A3_Control_2_dNSAF
CYT	BB_0481	BB_0481	ribosomal protein L2 (rplB)	5.05	13.72	8.67	5.3E-05	1	0.00017	1	0.30636	1	2	2	1	0	2	5.05	5.3E-05	X	X	X	X	X	X	X	X
CYT	BB_0482	BB_0482	ribosomal protein S19 (rpsS)	16.3	36.96	20.66	0.00008	1	0.00078	1	0.10256	1	1	1	1	0	1	16.3	8E-05	X	X	X	X	X	X	X	X
CYT	BB_0484	BB_0484	ribosomal protein S3 (rpsC)	3.07	19.11	16.04	0.00005	1	8.9E-05	2	0.5618	1	2	2	1	0	2	3.07	5E-05	X	X	X	X	X	X	X	X
CYT	BB_0485	BB_0485	ribosomal protein L16 (rplP)	9.42	18.84	9.42	5.5E-05	1	0.00032	2	0.17241	X	X	X	X	X	X	X	X	1	1	1	1	0	1	9.42	5.5E-05
CYT	BB_0487	BB_0487	ribosomal protein S17 (rpsQ)	8.33	11.9	3.57	0.00018	1	0.00014	1	1.26056	X	X	X	X	X	X	X	X	1	2	2	1	0	2	8.33	0.00018
CYT	BB_0488	BB_0488	ribosomal protein L14 (rplN)	0	12.9	12.9	0	0	0.00031	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0490	BB_0490	ribosomal protein L5 (rplE)	39.56	62.64	23.08	0.001	2	0.00224	2	0.44519	6	37	37	6	0	37	35.2	0.0015	3	12	12	3	0	12	13.7	0.0005
CYT	BB_0492	BB_0492	ribosomal protein S8 (rpsH)	15.91	28.03	12.12	0.00011	2	0.00048	2	0.2314	1	3	3	1	0	3	15.2	0.00017	1	1	1	1	0	1	15.9	5.7E-05
CYT	BB_0493	BB_0493	ribosomal protein L6 (rplF)	5.56	26.67	21.11	4.2E-05	1	0.00038	2	0.11082	X	X	X	X	X	X	X	X	1	1	1	1	0	1	5.56	4.2E-05
CYT	BB_0494	BB_0494	ribosomal protein L18 (rplR)	19.33	40.34	21.01	0.00012	1	0.00052	2	0.238	1	2	2	1	0	2	19.3	0.00012	X	X	X	X	X	X	X	X
CYT	BB_0495	BB_0495	ribosomal protein S5 (rpsE)	14.55	16.97	2.42	0.00013	1	0.00021	2	0.64423	2	3	3	2	0	3	14.6	0.00013	X	X	X	X	X	X	X	X
CYT	BB_0496	BB_0496	ribosomal protein L30 (rpmD)	0	36.63	36.63	0	0	0.00058	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0497	BB_0497	ribosomal protein L15 (rplO)	21.38	21.38	0	0.00013	2	0.00035	2	0.36888	1	3	3	1	0	3	15.9	0.00015	2	2	2	2	0	2	21.4	0.0001
CYT	BB_0500	BB_0500	ribosomal protein S13 (rpsM)	0	22.4	22.4	0	0	0.00039	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0501	BB_0501	ribosomal protein S11 (rpsK)	0	38.06	38.06	0	0	0.00054	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0502	BB_0502	DNA-directed RNA polymerase (rp	43.48	46.96	3.48	0.00142	2	0.0044	2	0.32213	12	109	109	12	0	109	43.5	0.00233	3	23	23	3	0	23	16.8	0.0005
CYT	BB_0503	BB_0503	ribosomal protein L17 (rplQ)	7.32	26.02	18.7	0.00006	1	0.00033	2	0.18462	1	1	1	1	0	1	7.32	6E-05	X	X	X	X	X	X	X	X
CYT	BB_0505	BB_0505	conserved hypothetical protein	0	8.02	8.02	0	0	3.8E-05	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0509	BB_0509	predicted coding region BB0509	14.56	17.18	2.62	0.00013	2	0.00021	2	0.64563	5	11	11	5	0	11	14.1	0.00019	2	4	4	2	0	4	7.4	7.2E-05
CYT	BB_0513	BB_0513	phenylalanyl-tRNA synthetase	5.3	4.17	-1.13	4.2E-05	1	4.6E-05	1	0.91304	2	3	3	2	0	3	5.3	4.2E-05	X	X	X	X	X	X	X	X
CYT	BB_0514	BB_0514	phenylalanyl-tRNA synthetase	26.68	33.57	6.89	9.9E-05	2	0.00043	2	0.23239	5	9	9	5	0	9	19.4	0.00012	3	6	6	3	0	6	12	8E-05
CYT	BB_0518	BB_0518	heat shock protein 70 (dnaK-2)	49.13	43.78	-5.35	0.00126	2	0.0012	2	1.04829	18	84	84	18	0	84	38.3	0.00098	17	130	130	17	0	130	37	0.00154
CYT	BB_0519	BB_0519	grpE protein (grpE)	57.22	50.27	-6.95	0.00094	2	0.00187	2	0.50161	9	23	23	9	0	23	45.5	0.00091	10	24	24	10	0	24	56.2	0.00097
CYT	BB_0521	BB_0521	predicted coding region BB0521	35.33	36.67	1.34	0.00022	2	0.00084	2	0.26422	3	6	6	3	0	6	24.7	0.0003	2	3	3	2	0	3	22.7	0.00015
CYT	BB_0522	BB_0522	NH(3)-dependent NAD+ synthetase	18.47	40.09	21.62	0.00047	2	0.00088	2	0.53281	4	12	12	4	0	12	18.5	0.0004	2	16	16	2	0	16	13.5	0.00054
CYT	BB_0527	BB_0527	conserved hypothetical protein	4.96	15.65	10.69	2.9E-05	1	0.00011	2	0.26852	X	X	X	X	X	X	X	X	1	1	1	1	0	1	4.96	2.9E-05
CYT	BB_0528	BB_0528	aldose reductase	30.16	37.14	6.98	0.00023	2	0.00039	2	0.57252	4	11	11	4	0	11	26	0.00026	3	8	8	3	0	8	16.5	0.00019
CYT	BB_0533	BB_0533	phnP protein (phnP)	0	12.65	12.65	0	0	6.5E-05	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0534	BB_0534	exodeoxyribonuclease III (exoA)	7.45	17.25	9.8	2.9E-05	1	0.00021	2	0.13615	1	1	1	1	0	1	7.45	2.9E-05	X	X	X	X	X	X	X	X
CYT	BB_0538	BB_0538	conserved hypothetical protein	0	14.39	14.39	0	0	0.00012	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0540	BB_0540	translation elongation factor G (fus-	35.79	24.24	-11.55	0.00031	2	0.00024	2	1.28926	14	31	31	14	0	31	27.4	0.00033	12	27	27	12	0	27	28.1	0.00029
CYT	BB_0541	BB_0541	predicted coding region BB0541	9.72	25	15.28	0.0001	1	0.0005	1	0.20683	1	1	1	1	0	1	9.72	0.0001	X	X	X	X	X	X	X	X
CYT	BB_0544	BB_0544	phosphoribosyl pyrophosphate syn	6.4	7.88	1.48	0.00016	1	2.5E-05	2	6.56	2	9	9	2	0	9	6.4	0.00016	X	X	X	X	X	X	X	X
CYT	BB_0545	BB_0545	xylulokinase (xylB)	0	6.61	6.61	0	0	5.4E-05	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0550	BB_0550	flagellar protein (flaJ)	8.97	37.93	28.96	5.2E-05	1	0.00038	2	0.13867	X	X	X	X	X	X	X	X	1	1	1	1	0	1	8.97	5.2E-05
CYT	BB_0551	BB_0551	chemotaxis response regulator (ch	15.04	18.05	3.01	8.4E-05	2	0.00012	1	0.68293	1	1	1	1	0	1	8.27	5.6E-05	1	2	2	1	0	2	6.77	0.00011
CYT	BB_0556	BB_0556	predicted coding region BB0556	0	5.54	5.54	0	0	0.00012	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0557	BB_0557	phosphocarrier protein HPr (ptsH-2	17.44	30.23	12.79	0.00018	1	0.00029	1	0.61404	X	X	X	X	X	X	X	X	1	2	2	1	0	2	17.4	0.00017
CYT	BB_0558	BB_0558	phosphoenolpyruvate-protein phos	0	7.5	7.5	0	0	7.1E-05	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0559	BB_0559	PTS system	70.9	56.08	-14.82	0.00113	2	0.00146	2	0.77266	6	26	26	6	0	26	54.5	0.00102	9	31	31	9	0	31	40.7	0.00123
CYT	BB_0560	BB_0560	heat shock protein 90 (htpG)	38	41.38	3.38	0.00052	2	0.00081	2	0.64118	20	63	63	20	0	63	35.1	0.00072	11	28	28	11	0	28	22.9	0.00032
CYT	BB_0561	BB_0561	phosphogluconate dehydrogenase	31.9	21.34	-10.56	0.00023	2	0.00024	2	0.95851	10	26	26	10	0	26	30	0.00041	1	3	3	1	0	3	1.94	4.9E-05
CYT	BB_0562	BB_0562	predicted coding region BB0562	12.22	18.33	6.11	0.00042	2	0.00036	2	1.14958	2	9	9	2	0	9	12.2	0.00037	2	11	11	2	0	11	12.2	0.00046
CYT	BB_0566	BB_0566	predicted coding region BB0566	26.21	15.53	-10.68	0.00011	2	0.00035	1	0.31034	1	2	2	1	0	2	15.5	0.00014	1	1	1	1	0	1	10.7	7.3E-05
CYT	BB_0567	BB_0567	chemotaxis histidine kinase (cheA-	28.99	22.41	-6.58	0.00012	2	0.0002	2	0.61692	11	22	22	11	0	22	26.5	0.00023	2	2	2	2	0	2	3.92	2.1E-05
CYT	BB_0568	BB_0568	protein-glutamate methylesterase (	4.42	11.43	7.01	3.9E-05	1	0.0002	2	0.19118	X	X	X	X	X	X	X	X	1	2	2	1	0	2	4.42	3.9E-05
CYT	BB_0569	BB_0569	predicted coding region BB0569	11.36	23.56	12.2	9.4E-05	2	0.00041	2	0.22871	5	11	11	5	0	11	11.4	0.00014	1	4	4	1	0	4	2.71	5.1E-05
CYT	BB_0570	BB_0570	chemotaxis response regulator (ch	54.84	45.16	-9.68	0.00049	1	0.00086	1	0.56659	X	X	X	X	X	X	X	X	3	8	8	3	0	8	54.8	0.00049
CYT	BB_0572	BB_0572	glycosyl transferase (lgtD)	24.86	22.35	-2.51	0.00018	2	0.00027	2	0.65185	7	15	15	7	0	15	21.8	0.00031	2	2	2	2	0	2	9.22	4.2E-05
CYT	BB_0573	BB_0573	ABC transporter	36.67	40.37	3.7	0.00077	2	0.00096	2	0.80249	6	34	34	6	0	34	29.6	0.00093	3	22	22	3	0	22	20.4	0.00061
CYT	BB_0578	BB_0578	methyl-accepting chemotaxis prote	21.85	20.31	-1.54	0.00013	1	0.00022	2	0.6129	6	7	7	6	0	7	21.9	0.00013	X	X	X	X	X	X	X	X
CYT	BB_0579	BB_0579	DNA polymerase III	13.7	25.06	11.36	8.6E-05	2	0.00025	2	0.34127	9	20	20	9	0	20	9.04	0.00013	4	7	7	4	0	7	6.12	4.5E-05
CYT	BB_0581	BB_0581	DNA recombinase (recG)	6.27	15.01	8.74	4.3E-05	1	9.1E-05	2	0.47253	3	5	4	3	1	4	6.27	4.3E-05	X	X	X	X	X	X	X	X
CYT	BB_0582	BB_0582	carboxypeptidase	5.34	12.98	7.64	5.6E-05	1	9.1E-05	1	0.61538	1	2	2	1	0	2	5.34	5.6E-05	X	X	X	X	X	X	X	X
CYT	BB_0585	BB_0585	UDP-N-acetylmuramoylalanine--D-g	10.86	11.09	0.23	3.3E-05	1	0.00011	2	0.29464	X	X	X	X	X	X	X	X	2	2	2	2	0	2	10.9	3.3E-05
CYT	BB_0587	BB_0587	methionyl-tRNA synthetase (metG)	24.52	28.47	3.95	0.00017	2	0.00035	2	0.47309	10	23	23	10	0	23	19.2	0.00023	4	10	10	4	0	10	8.31	0.0001
CYT	BB_0594	BB_0594	arginyl-tRNA synthetase (argS)	38.41	37.06	-1.35	0.00027	2	0.00043	2	0.6338	14	31	31	14	0	31	31.1	0.00039	5	12	12	5	0	12	11.5	0.00015
CYT	BB_0601	BB_0601	serine hydroxymethyltransferase (g	7.91	4.32	-3.59	0.00016	1	0.00002	1	7.95	2	9	9	2	0	9	7.91	0.00016	X	X	X	X	X	X	X	X
CYT	BB_0606	BB_0606	conserved hypothetical protein	37.42	29.45	-7.97	0.00023	2	0.00076	2	0.29712	5	9	9	5	0	9	37.4	0.00041	1	1	1	1	0	1	12.3	4.6E-05
CYT	BB_0608	BB_0608	aminoacyl-histidine dipeptidase (pe	21.85	24.58	2.73	0.00017	2	0.00025	2	0.65737	3	8	8	3	0	8	9.87	0.00012	4	13	13	4	0	13	17.4	0.00021
CYT	BB_0610	BB_0610	trigger factor (tig)	20.04	26.65	6.61	0.00012	2	0.00017	2	0.72781	4	7	7	4	0	7	16.7	0.00011	2	8	8	2	0	8	7.71	0.00013
CYT	BB_0612	BB_0612	ATP-dependent Clp protease	46.28	41.4	-4.88	0.00085	2	0.00105	2	0.80725	16	69	69	16	0	69	44.7	0.00119	5	29	29	5	0	29	18.8	0.00051
CYT	BB_0613	BB_0613	ATP-dependent protease LA (lon-2	17.59	23.25	5.66	0.00005	2	0.00035	2	0.14164	6	9	9	6	0	9	12.4	8.2E-05	2	2	2	2	0	2	5.17	1.9E-05
CYT	BB_0615	BB_0615	ribosomal protein S4 (rpsD)	11	28.71	17.71	3.6E-05	1	0.00058	2	0.06239	X	X	X	X	X	X	X	X	1	1	1	1	0	1	11	3.6E-05
CYT	BB_0617	BB_0617	predicted coding region BB0617	0	17.21	17.21	0	0	0.00023	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0618	BB_0618	cytidine deaminase (cdd)	33.12	9.74	-23.38	9.6E-05	2	5.3E-05	1	1.81132	2	3	3	2	0	3	20.8	0.00014	1	1	1	1	0	1	12.3	4.9E-05
CYT	BB_0619	BB_0619	conserved hypothetical protein	5.76	10.3	4.54	2.2E-05	1	5.5E-05	2	0.4	1	1	1	1	0	1	5.76	2.2E-05	X	X	X	X	X	X	X	X
CYT	BB_0621	BB_0621	4-methyl-5(b-hydroxyethyl)-thiazole	15.22	17.93	2.71	0.00016	1	0.00019	2	0.86559	2	4	4	2	0	4	15.2	0.00016	X	X	X	X	X	X	X	X
CYT	BB_0622	BB_0622	acetate kinase (ackA)	11.86	15.01	3.15	0.00018	1	0.00011	2	1.6729	3	10	10	3	0	10	11.9	0.00018	X	X	X	X	X	X	X	X
CYT	BB_0627	BB_0627	vacuolar X-prolyl dipeptidyl aminop	5.2	26.71	21.51	1.7E-05	1	0.00022	2	0.07834	1	1	1	1	0	1	5.2	1.7E-05	X	X	X	X	X	X	X	X
CYT	BB_0630	BB_0630	1-phosphofructokinase (fruK)	51.47	58.63	7.16	0.00059	2	0.00082	2	0.71255	6	16	16	6	0	16	37.1	0.00039	6	32	32	6	0	32	35.8	0.00078
CYT	BB_0636	BB_0636	glucose-6-phosphate 1-dehydroger	11.3	30.33	19.03	0.00007	2	0.00035	2	0.2	4	8	8	4	0	8	11.3	0.00012	1	1	1	1	0	1	2.72	1.6E-05
CYT	BB_0642	BB_0642	spermidine/putrescine ABC transp	17.58	17	-0.58	0.00019	2	0.00011	2	1.73874	5	13	13	5	0	13	17.6	0.00028	1	5	5	1	0	5	4.03	0.00011
CYT	BB_0644	BB_0644	conserved hypothetical protein	24.57	28.45	3.88	0.00037	2	0.00032	2	1.1358	4	18	18	4	0	18	24.6	0.00057	1	5	5	1	0	5	7.33	0.00016
CYT	BB_0647	BB_0647	ferric uptake regulation protein (fur)	10.8	5.11	-5.69	8.4E-05	1	5.7E-05	2	1.47368	1	2	2	1	0	2	10.8	8.4E-05	X	X	X	X	X	X	X	X
CYT	BB_0649	BB_0649	heat shock protein (groEL)	36.51	51.01	14.5	0.00027	2	0.0011	2	0.24292	10	29	29	10	0	29	27.7	0.00039	6	10	10	6	0	10	19.5	0.00014
CYT	BB_0650	BB_0650	predicted coding region BB0650	37.5	25	-12.5	0.00023	2	0.00038	2	0.61436	3	6	6	3	0	6	26.8	0.0004	1	1	1	1	0	1	24.1	6.7E-05
CYT	BB_0654	BB_0654	predicted coding region BB0654	12.11	25.26	13.15	7.8E-05	1	0.0003	2	0.26174	3	4	4	3	0	4	12.1	7.8E-05	X	X	X	X	X	X	X	X
CYT	BB_0655	BB_0655	heat shock protein (dnaJ-2)	0	16.67	16.67	0	0	0.00022	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0658	BB_0658	phosphoglycerate mutase (gpmA)	17.79	21.74	3.95	0.00018	1	0.00025	2	0.71429	3	6	6	3	0	6	17.8	0.00018	X	X	X	X	X	X	X	X
CYT	BB_0659	BB_0659	lysyl-tRNA synthetase	6.14	10.17	4.03	7.1E-05	1	8.4E-05	2	0.84524	2	5	5	2	0	5	6.14	7.1E-05	X	X	X	X	X	X	X	X
CYT	BB_0669	BB_0669	chemotaxis histidine kinase (cheA-	23.61	26.39	2.78	0.00012	2	0.00045	2	0.25664	10	20	20	10	0	20	16.8	0.00017	5	7	7	5	0	7	10.9	6.1E-05
CYT	BB_0670	BB_0670	purine-binding chemotaxis protein (	21.89	56.65	34.76	0.00012	2	0.00045	2	0.26622	5	11	11	5	0	11	17.2	0.00017	3	4	4	3	0	4	14	6.5E-05
CYT	BB_0671	BB_0671	chemotaxis operon protein (cheX)	68.94	72.05	3.11	0.0025	2	0.0049	2	0.51082	11	48	48	11	0	48	59.6	0.0022	9	60	60	9	0	60	66.5	0.0028
CYT	BB_0673	BB_0673	conserved hypothetical protein	0	7.6	7.6	0	0	0.00014	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0675	BB_0675	predicted coding region BB0675	0	13.94	13.94	0	0	0.00011	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0676	BB_0676	phosphoglycolate phosphatase (gp	17.73	47.27	29.54	0.00015	2	0.00079	2	0.19314	2	5	5	2	0	5	11.4	0.00017	2	4	4	2	0	4	12.7	0.00014
CYT	BB_0677	BB_0677	ribose/galactose ABC transporter	7.84	10.26	2.42	4.9E-05	2	7.9E-05	2	0.62025	2	3	3	2	0	3	7.84	4.1E-05	1	4	4	1	0	4	2.99	5.6E-05
CYT	BB_0683	BB_0683	3-hydroxy-3-methylglutaryl-CoA syr	42.01	44.96	2.95	0.00101	2	0.00172	2	0.58765	17	99	99	17	0	99	39.6	0.0018	5	12	12	5	0	12	18.9	0.00022
CYT	BB_0685	BB_0685	3-hydroxy-3-methylglutaryl-CoA red	0	3.25	3.25	0	0	5.5E-05	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0687	BB_0687	phosphomevalonate kinase	7.26	13.88	6.62	0.00012	1	0.00013	2	0.87313	1	5	5	1	0	5	7.26	0.00012	X	X	X	X	X	X	X	X
CYT	BB_0690	BB_0690	neutrophil activating protein (napA)	39.68	68.78	29.1	0.00155	2	0.00928	2	0.1667	4	15	15	4	0	15	31.2	0.00059	10	63	63	10	0	63	39.7	0.00251
CYT	BB_0691	BB_0691	translation elongation factor G (fus-	11.51	14.05	2.54	0.00005	2	0.00015	2	0.33333	3	6	6	3	0	6	6.58	6.6E-05	2	3	3	2	0	3	8.07	3.4E-05
CYT	BB_0693	BB_0693	xylose operon regulatory protein (xy	8.71	2.99	-5.72	7.3E-05	1	0.00002	1	3.65	2	4	4	2	0	4	8.71	7.4E-05	X	X	X	X	X	X	X	X
CYT	BB_0694	BB_0694	signal recognition particle protein (f	46.98	35.57	-11.41	0.00033	2	0.00046	2	0.71552	11	29	29	11	0	29	32	0.00048	7	11	11	7	0	11	26.6	0.00019
CYT	BB_0696	BB_0696	conserved hypothetical protein	50	41.46	-8.54	0.00128	2	0.001	2	1.26992	4	12	12	4	0	12	41.5	0.00108	5	16	16	5	0	16	50	0.00147
CYT	BB_0698	BB_0698	tRNA (guanine-N1)-methyltransfera	0	6.69	6.69	0	0	0.0001	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0702	BB_0702	lipopolysaccharide biosynthesis-rela	34.97	33.74	-1.23	0.00018	2	0.0003	2	0.61279	4	6	6	4	0	6	35	0.00027	1	2	2	1	0	2	9.2	9.2E-05
CYT	BB_0704	BB_0704	acyl carrier protein	48.75	35	-13.75	0.00028	2	0.00061	2	0.46053	2	3	3	2	0	3	48.8	0.00028	1	3	3	1	0	3	23.8	0.00028
CYT	BB_0705	BB_0705	ribonuclease III (rnc)	46.34	47.56	1.22	0.00291	2	0.00751	2	0.38735	8	97	97	8	0	97	41.5	0.00291	5	95	95	5	0	95	32.5	0.00291
CYT	BB_0707	BB_0707	predicted coding region BB0707	13.16	14.64	1.48	0.00013	1	0.00015	2	0.87582	5	11	11	5	0	11	13.2	0.00013	X	X	X	X	X	X	X	X
CYT	BB_0712	BB_0712	RNA polymerase sigma-70 factor (	16.8	19.65	2.85	8.8E-05	2	0.00015	2	0.60274	5	11	11	5	0	11	14.9	0.00013	2	4	4	2	0	4	4.44	4.8E-05
CYT	BB_0713	BB_0713	conserved hypothetical protein	8.7	8.3	-0.4	8.8E-05	1	0.0002	2	0.44221	2	3	3	2	0	3	8.7	8.8E-05	X	X	X	X	X	X	X	X
CYT	BB_0715	BB_0715	rod shape-determining protein (mre	53.74	57.89	4.15	0.0011	2	0.00381	2	0.28849	16	89	89	16	0	89	53.7	0.00182	4	18	18	4	0	18	18.6	0.00038
CYT	BB_0720	BB_0720	threonyl-tRNA synthetase (thrZ)	8.26	14.46	6.2	6.4E-05	1	0.0001	2	0.64	2	5	5	2	0	5	8.26	6.4E-05	X	X	X	X	X	X	X	X
CYT	BB_0725	BB_0725	conserved hypothetical protein	15.21	33.84	18.63	8.5E-05	2	0.00021	2	0.3972	2	4	4	2	0	4	15.2	0.00011	1	2	2	1	0	2	7.22	5.7E-05
CYT	BB_0726	BB_0726	ATP-binding protein (ylxH-3)	22.6	17.65	-4.95	0.00021	1	0.00041	2	0.50244	5	9	9	5	0	9	22.6	0.00021	X	X	X	X	X	X	X	X
CYT	BB_0727	BB_0727	pyrophosphate--fructose 6-phospha	70.98	68.3	-2.68	0.00192	2	0.00246	2	0.77999	30	142	142	30	0	142	68.3	0.00234	18	89	89	18	0	89	53.6	0.00149
CYT	BB_0733	BB_0733	predicted coding region BB0733	7.97	10.87	2.9	0.00008	1	0.00014	2	0.57554	2	3	3	2	0	3	7.97	8E-05	X	X	X	X	X	X	X	X
CYT	BB_0734	BB_0734	conserved hypothetical protein	3.56	5.64	2.08	2.2E-05	1	7.3E-05	1	0.30137	X	X	X	X	X	X	X	X	1	1	1	1	0	1	3.56	2.2E-05
CYT	BB_0737	BB_0737	histidine phosphokinase/phophatas	0	8.06	8.06	0	0	5.4E-05	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0738	BB_0738	valyl-tRNA synthetase (valS)	23.31	21.26	-2.05	0.00017	2	0.00019	2	0.90374	11	34	34	11	0	34	18.7	0.00029	4	6	6	4	0	6	8.23	5.2E-05
CYT	BB_0741	BB_0741	chaperonin (groES)	32.26	52.69	20.43	0.00016	2	0.00039	2	0.40816	2	3	3	2	0	3	19.4	0.00024	1	1	1	1	0	1	12.9	8.1E-05
CYT	BB_0742	BB_0742	ABC transporter	0	9.73	9.73	0	0	0.00005	2	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CYT	BB_0749	BB_0749	predicted coding region BB0749	36.28	62.79	26.51	0.0004	1	0.00044	2	0.89367	8	23	23	8	0	23	36.3	0.0004	X	X	X	X	X	X	X	X
CYT	BB_0754	BB_0754	ABC transporter	50.49	49.51	-0.98	0.00095	2	0.00126	2	0.75316	13	46	46	13	0	46	50.5	0.0011	11	33	33	11	0	33	31.1	0.0008
CYT	BB_0760	BB_0760	predicted coding region BB0760	31.45	41.13	9.68	0.00072	2	0.00205	2	0.35251	2	10	10	2	0	10	29.8	0.0006	2	14	14	2	0	14	20.2	0.00085

Locus	B31A3_ProK_1_P	B31A3_ProK_1_S	B31A3_ProK_1_uS	B31A3_ProK_1_uDP	B31A3_ProK_1_sS	B31A3_ProK_1_dS	B31A3_ProK_1_SC	B31A3_ProK_1_dNSAF	B31A3_ProK_2_P	B31A3_ProK_2_S	B31A3_ProK_2_uS	B31A3_ProK_2_uDP	B31A3_ProK_2_sS	B31A3_ProK_2_dS	B31A3_ProK_2_SC	B31A3_ProK_2_dNSAF	ALL_y2_P	ALL_y2_S	ALL_y2_uS	ALL_y2_uDP	ALL_y2_sS	ALL_y2_dS	ALL_y2_SC	ALL_y2_dNSAF	Length	MW	pI
BB_0481	3	4	4	3	0	4	13.7	0.00017	X	X	X	X	X	X	X	X	3	5	5	3	0	5	14.4	3.9E-05	277	30591	10.5
BB_0482	2	6	6	2	0	6	37	0.00078	X	X	X	X	X	X	X	X	2	7	7	2	0	7	37	0.00016	92	10410	10.2
BB_0484	2	3	3	2	0	3	10.2	0.00012	2	2	2	2	0	2	8.87	5.6E-05	4	6	6	4	0	6	17.1	4.4E-05	293	33142	10
BB_0485	2	6	6	2	0	6	18.8	0.00052	1	2	2	1	0	2	18.8	0.00012	3	9	9	3	0	9	28.3	0.00014	138	15568	10.6
BB_0487	1	1	1	1	0	1	11.9	0.00014	X	X	X	X	X	X	X	X	2	3	3	2	0	3	20.2	7.7E-05	84	9852	10
BB_0488	1	3	3	1	0	3	12.9	0.00029	1	5	5	1	0	5	12.9	0.00033	1	8	8	1	0	8	12.9	0.00014	124	13656	10.1
BB_0490	7	54	54	7	0	54	62.1	0.00355	4	21	21	4	0	21	26.4	0.00094	9	124	124	9	0	124	67	0.00147	182	20416	9.5
BB_0492	5	10	10	5	0	10	28	0.00091	1	1	1	1	0	1	15.2	6.2E-05	6	15	15	6	0	15	28	0.00025	132	14814	9.9
BB_0493	5	8	8	5	0	8	24.4	0.00053	5	5	5	5	0	5	22.8	0.00023	7	14	14	7	0	14	32.2	0.00017	180	19938	9.8
BB_0494	2	9	9	2	0	9	30.3	0.0009	2	2	2	2	0	2	21	0.00014	2	12	12	2	0	12	30.3	0.00022	119	13722	10.4
BB_0495	2	3	3	2	0	3	17	0.00022	3	4	4	3	0	4	17	0.0002	3	6	6	3	0	6	14.6	7.9E-05	165	17779	10.4
BB_0496	3	7	7	3	0	7	36.6	0.00083	1	4	4	1	0	4	9.9	0.00032	3	11	11	3	0	11	36.6	0.00024	101	11765	11.4
BB_0497	2	5	5	2	0	5	21.4	0.00041	3	5	5	3	0	5	21.4	0.00028	3	15	15	3	0	15	21.4	0.00022	145	16039	10.5
BB_0500	X	X	X	X	X	X	X	X	2	6	6	2	0	6	22.4	0.00039	2	6	6	2	0	6	22.4	0.0001	125	14078	10.8
BB_0501	3	10	10	3	0	10	38.1	0.00089	1	3	3	1	0	3	8.96	0.00018	3	13	13	3	0	13	38.1	0.00021	134	14307	10.3
BB_0502	12	192	192	12	0	192	43.2	0.00665	14	91	91	14	0	91	40.9	0.00215	18	407	407	18	0	407	51.9	0.00255	345	38685	5.4
BB_0503	4	6	6	4	0	6	26	0.00058	1	1	1	1	0	1	17.1	6.6E-05	5	8	8	5	0	8	33.3	0.00014	123	14500	11
BB_0505	1	1	1	1	0	1	2.67	4.6E-05	1	1	1	1	0	1	5.73	3.1E-05	2	2	2	2	0	2	8.02	1.7E-05	262	29138	8.3
BB_0509	5	9	9	5	0	9	14.8	0.00026	4	8	8	4	0	8	10	0.00016	9	32	32	9	0	32	22.2	0.00017	419	48742	9.2
BB_0513	X	X	X	X	X	X	X	X	2	3	3	2	0	3	4.17	4.6E-05	3	6	6	3	0	6	7.2	2.5E-05	528	59952	7
BB_0514	4	13	13	4	0	13	12.4	0.00027	10	40	40	10	0	40	31.5	0.00058	12	68	68	12	0	68	34.3	0.00026	566	65174	5.2
BB_0518	14	75	75	14	0	75	26.5	0.00141	17	77	77	17	0	77	37	0.00099	36	366	366	36	0	366	60	0.00125	635	69276	5.3
BB_0519	6	25	25	6	0	25	33.7	0.0016	13	49	49	13	0	49	50.3	0.00214	19	121	121	19	0	121	63.6	0.0014	187	21939	8.7
BB_0521	3	13	13	3	0	13	30.7	0.00104	5	12	12	5	0	12	36.7	0.00065	6	34	34	6	0	34	41.3	0.00049	150	17209	7.9
BB_0522	3	11	11	3	0	11	19.4	0.00059	9	32	32	9	0	32	34.2	0.00118	11	71	71	11	0	71	45.1	0.00069	222	25094	5.8
BB_0527	1	2	2	1	0	2	10.7	9.1E-05	2	4	4	2	0	4	15.7	0.00012	2	7	7	2	0	7	15.7	5.8E-05	262	29823	5.5
BB_0528	3	5	5	3	0	5	13	0.00019	8	23	23	8	0	23	33.3	0.0006	10	47	47	10	0	47	42.5	0.00032	315	36754	9
BB_0533	X	X	X	X	X	X	X	X	2	2	2	2	0	2	12.7	6.5E-05	2	2	2	2	0	2	12.7	1.7E-05	253	28965	7.8
BB_0534	3	5	5	3	0	5	17.3	0.00023	1	6	6	1	0	6	7.45	0.00019	3	12	12	3	0	12	17.3	0.0001	255	29756	5.3
BB_0538	X	X	X	X	X	X	X	X	1	2	2	1	0	2	14.4	0.00012	1	2	2	1	0	2	14.4	3.3E-05	132	14929	5.9
BB_0540	7	13	13	7	0	13	14	0.00022	8	22	22	8	0	22	16.9	0.00026	20	91	91	20	0	91	35.8	0.00028	693	77573	5.6
BB_0541	1	3	3	1	0	3	25	0.0005	X	X	X	X	X	X	X	X	2	4	4	2	0	4	34.7	0.00012	72	8385	6.1
BB_0544	1	1	1	1	0	1	4.19	2.9E-05	1	1	1	1	0	1	3.69	2E-05	3	11	11	3	0	11	10.6	5.9E-05	406	45749	7.7
BB_0545	X	X	X	X	X	X	X	X	2	3	3	2	0	3	6.61	5.4E-05	2	3	3	2	0	3	6.61	1.4E-05	454	51364	8.1
BB_0550	1	5	5	1	0	5	13.1	0.00041	4	6	6	4	0	6	37.9	0.00034	4	12	12	4	0	12	37.9	0.00018	145	16655	9.2
BB_0551	X	X	X	X	X	X	X	X	1	2	2	1	0	2	18.1	0.00012	3	5	5	3	0	5	33.1	8.1E-05	133	15177	8.8
BB_0556	1	3	3	1	0	3	5.54	0.00012	X	X	X	X	X	X	X	X	1	3	3	1	0	3	5.54	2.2E-05	289	34463	9
BB_0557	X	X	X	X	X	X	X	X	1	3	3	1	0	3	30.2	0.00028	2	5	5	2	0	5	47.7	0.00013	86	9386	5.5
BB_0558	X	X	X	X	X	X	X	X	3	5	5	3	0	5	7.5	7.1E-05	3	5	5	3	0	5	7.5	1.9E-05	573	64596	5.2
BB_0559	5	16	16	5	0	16	41.8	0.00101	8	44	44	8	0	44	53.4	0.0019	13	113	113	13	0	113	71.4	0.00129	189	20781	5.4
BB_0560	14	37	37	14	0	37	23.4	0.00068	24	75	75	24	0	75	34.9	0.00094	37	203	203	37	0	203	48.3	0.00068	650	75424	6.9
BB_0561	9	18	18	9	0	18	21.3	0.00046	1	1	1	1	0	1	1.51	1.8E-05	15	48	48	15	0	48	36	0.00022	464	52558	7
BB_0562	1	2	2	1	0	2	6.67	0.00013	5	13	13	5	0	13	18.3	0.00059	4	34	34	4	0	34	18.3	0.00041	180	19773	9.1
BB_0566	2	3	3	2	0	3	15.5	0.00035	X	X	X	X	X	X	X	X	3	6	6	3	0	6	26.2	0.00013	103	11837	4.8
BB_0567	4	11	11	4	0	11	10.9	0.00018	9	19	19	9	0	19	20.3	0.00022	15	53	53	15	0	53	35.3	0.00016	714	79390	5.1
BB_0568	3	7	7	3	0	7	11.4	0.00022	4	9	9	4	0	9	11.4	0.00019	3	16	16	3	0	16	7.79	9E-05	385	42691	9.3
BB_0569	8	18	18	8	0	18	14.8	0.00036	9	33	33	9	0	33	18.8	0.00046	12	66	66	12	0	66	23.6	0.00024	590	68054	5.1
BB_0570	X	X	X	X	X	X	X	X	3	13	13	3	0	13	45.2	0.00086	5	21	21	5	0	21	61.3	0.00037	124	13726	5.6
BB_0572	3	8	8	3	0	8	11.5	0.00027	5	12	12	5	0	12	18.4	0.00027	12	37	37	12	0	37	31.6	0.00022	358	42106	8.9
BB_0573	6	23	23	6	0	23	30	0.00102	6	30	30	6	0	30	32.6	0.00091	8	109	109	8	0	109	40.4	0.00087	270	30699	8.6
BB_0578	5	8	8	5	0	8	17.2	0.00025	5	9	9	5	0	9	18	0.00019	9	23	23	9	0	23	23.9	0.00013	389	44575	5.7
BB_0579	11	25	25	11	0	25	15.3	0.00026	13	35	35	13	0	35	18	0.00025	25	81	81	25	0	81	27.6	0.00015	1161	132367	6.7
BB_0581	5	7	7	5	0	7	13.4	0.00012	3	5	5	3	0	5	3.79	5.9E-05	8	15	14	8	1	14	16.2	4.4E-05	686	79044	7.3
BB_0582	2	2	2	2	0	2	13	9.1E-05	X	X	X	X	X	X	X	X	1	3	3	1	0	3	5.34	2.5E-05	262	30676	9.2
BB_0585	1	3	3	1	0	3	2.66	8E-05	2	8	8	2	0	8	8.43	0.00014	4	13	13	4	0	13	16.2	6.2E-05	451	51069	8.7
BB_0587	10	27	27	10	0	27	20.3	0.00044	9	24	24	9	0	24	16.6	0.00027	17	80	80	17	0	80	29.2	0.00024	734	85214	8.8
BB_0594	11	23	23	11	0	23	26.9	0.00047	10	28	28	10	0	28	23.4	0.00039	25	93	93	25	0	93	49.9	0.00034	591	67637	9.1
BB_0601	X	X	X	X	X	X	X	X	1	1	1	1	0	1	4.32	2E-05	2	10	10	2	0	10	7.91	5.2E-05	417	45881	7.6
BB_0606	4	14	14	4	0	14	18.4	0.00103	4	10	10	4	0	10	29.5	0.0005	8	33	33	8	0	33	48.5	0.00044	163	18141	9.3
BB_0608	3	5	5	3	0	5	11.3	0.00013	6	22	22	6	0	22	24.6	0.00038	7	48	48	7	0	48	26.5	0.00022	476	53577	5.5
BB_0610	2	4	4	2	0	4	6.83	0.00011	5	13	13	5	0	13	19.8	0.00023	10	32	32	10	0	32	32.4	0.00015	454	52906	5.1
BB_0612	19	59	59	19	0	59	41.4	0.00164	7	24	24	7	0	24	18.6	0.00046	22	181	181	22	0	181	48.8	0.00091	430	47601	7.9
BB_0613	9	31	31	9	0	31	14	0.00046	11	25	25	11	0	25	19.7	0.00025	20	67	67	20	0	67	30.1	0.00018	813	92312	6.6
BB_0615	5	12	12	5	0	12	28.7	0.00069	5	12	12	5	0	12	23	0.00047	6	23	23	6	0	23	28.7	0.00024	209	24084	10.3
BB_0617	2	2	2	2	0	2	17.2	0.0002	1	4	4	1	0	4	14.8	0.00027	2	6	6	2	0	6	17.2	0.00011	122	14613	5.5
BB_0618	X	X	X	X	X	X	X	X	1	1	1	1	0	1	9.74	5.3E-05	4	5	5	4	0	5	33.1	7E-05	154	17548	8.2
BB_0619	1	1	1	1	0	1	3.03	3.6E-05	2	3	3	2	0	3	9.7	7.4E-05	1	3	3	1	0	3	5.76	2E-05	330	37377	5.5
BB_0621	2	3	3	2	0	3	16.3	0.00019	2	4	4	2	0	4	17.9	0.00018	3	9	9	3	0	9	23.9	0.00011	184	19884	5.2
BB_0622	3	6	6	3	0	6	15	0.00017	1	2	2	1	0	2	5.33	4E-05	4	18	18	4	0	18	17.2	9.4E-05	413	46211	9.2
BB_0627	1	1	1	1	0	1	3.07	2.8E-05	5	21	21	5	0	21	23.6	0.00041	5	22	22	5	0	22	23.6	0.00011	423	47384	7.6
BB_0630	9	21	21	9	0	21	47.2	0.00082	13	31	31	13	0	31	58.3	0.00082	13	87	87	13	0	87	58	0.00061	307	33595	6.9
BB_0636	6	15	15	6	0	15	21.1	0.00038	9	19	19	9	0	19	26.8	0.00032	11	43	43	11	0	43	30.3	0.00019	478	56113	8.1
BB_0642	1	1	1	1	0	1	2.31	3.4E-05	4	8	8	4	0	8	17	0.00019	7	26	26	7	0	26	25.9	0.00016	347	39580	6.7
BB_0644	3	3	3	3	0	3	14.2	0.00015	4	14	14	4	0	14	22	0.00049	7	40	40	7	0	40	37.9	0.00037	232	25644	7
BB_0647	1	1	1	1	0	1	5.11	6.8E-05	1	1	1	1	0	1	5.11	4.6E-05	1	2	2	1	0	2	10.8	2.5E-05	176	20206	8.1
BB_0649	15	65	65	15	0	65	36	0.00143	18	51	51	18	0	51	43.3	0.00076	27	151	151	27	0	151	62.4	0.0006	545	58952	5.2
BB_0650	2	5	5	2	0	5	25	0.00053	1	3	3	1	0	3	13.4	0.00022	4	15	15	4	0	15	37.5	0.00029	112	13024	6.8
BB_0654	4	8	8	4	0	8	14.7	0.00025	7	16	16	7	0	16	22.1	0.00034	7	27	27	7	0	27	25.3	0.00015	380	45402	6.8
BB_0655	3	5	5	3	0	5	16.7	0.00022	X	X	X	X	X	X	X	X	2	4	4	2	0	4	9.78	3.1E-05	276	31404	9.5
BB_0658	4	9	9	4	0	9	21.7	0.00043	1	2	2	1	0	2	4.74	6.5E-05	5	17	17	5	0	17	26.1	0.00015	253	28943	6.8
BB_0659	3	7	6	3	1	6	10.2	0.00014	1	2	2	1	0	2	4.03	3.1E-05	3	13	13	3	0	13	8.06	5.4E-05	521	60938	8.4
BB_0669	9	34	34	9	0	34	17.5	0.00047	17	46	46	17	0	46	25.9	0.00043	22	107	107	22	0	107	30.2	0.00027	864	98353	5
BB_0670	8	13	13	8	0	13	32.8	0.00033	11	32	32	11	0	32	43.4	0.00056	16	59	59	16	0	59	50	0.00027	466	53061	4.9
BB_0671	12	61	61	12	0	61	66.5	0.00453	15	104	104	15	0	104	72.1	0.00527	16	272	272	16	0	272	74.5	0.00366	161	17614	4.5
BB_0673	1	2	2	1	0	2	7.6	0.00014	X	X	X	X	X	X	X	X	1	2	2	1	0	2	7.6	2.5E-05	171	20537	9.3
BB_0675	X	X	X	X	X	X	X	X	3	4	4	3	0	4	13.9	0.00011	1	2	2	1	0	2	4.18	1.5E-05	287	33637	9
BB_0676	6	16	16	6	0	16	36.4	0.00087	6	19	19	6	0	19	35.5	0.0007	8	43	43	8	0	43	46.8	0.00042	220	24984	5.9
BB_0677	2	3	3	2	0	3	7.84	6.7E-05	3	6	6	3	0	6	10.3	9.1E-05	3	16	16	3	0	16	10.3	6.5E-05	536	60390	9.2
BB_0683	17	93	93	17	0	93	38.8	0.00273	12	35	35	12	0	35	31.5	0.0007	25	239	239	25	0	239	45	0.00127	407	46029	6.3
BB_0685	1	2	2	1	0	2	3.25	5.5E-05	X	X	X	X	X	X	X	X	1	2	2	1	0	2	3.25	1E-05	431	48922	8.6
BB_0687	2	3	3	2	0	3	10.7	0.00011	2	6	6	2	0	6	10.4	0.00015	3	14	14	3	0	14	13.9	9.6E-05	317	35260	7.2
BB_0690	13	47	47	13	0	47	52.9	0.00297	24	361	361	24	0	361	68.8	0.01559	22	475	475	22	0	475	68.8	0.00544	189	22414	5.5
BB_0691	3	10	10	3	0	10	7.62	0.00018	5	10	10	5	0	10	11.1	0.00012	7	28	28	7	0	28	18.8	9.1E-05	669	75057	5.8
BB_0693	X	X	X	X	X	X	X	X	1	1	1	1	0	1	2.99	2E-05	3	5	5	3	0	5	11.7	2.7E-05	402	46082	7
BB_0694	9	19	19	9	0	19	20.8	0.00051	12	23	23	12	0	23	32.9	0.00042	22	81	81	22	0	81	50.3	0.00039	447	49695	9.5
BB_0696	3	9	9	3	0	9	19.5	0.00131	2	7	7	2	0	7	39	0.0007	5	44	44	5	0	44	50	0.00116	82	9253	8.1
BB_0698	1	2	2	1	0	2	6.69	0.0001	X	X	X	X	X	X	X	X	1	2	2	1	0	2	6.69	1.8E-05	239	27179	7.9
BB_0702	3	4	4	3	0	4	33.7	0.00029	3	6	6	3	0	6	33.7	0.0003	5	18	18	5	0	18	47.2	0.00024	163	19016	9.1
BB_0704	1	2	2	1	0	2	11.3	0.0003	3	9	9	3	0	9	35	0.00092	3	14	14	3	0	14	60	0.00038	80	9334	4.3
BB_0705	8	204	204	8	0	204	47.2	0.00991	8	154	154	8	0	154	44.7	0.00511	11	550	550	11	0	550	52	0.00484	246	27942	6.1
BB_0707	4	6	6	4	0	6	6.09	0.00012	6	14	14	6	0	14	11.5	0.00019	10	31	31	10	0	31	20.9	0.00011	608	71684	6.4
BB_0712	7	10	10	7	0	10	14.1	0.00019	4	8	8	4	0	8	7.61	0.0001	14	33	33	14	0	33	29.5	0.00011	631	73643	5.3
BB_0713	2	5	5	2	0	5	8.3	0.00024	2	5	5	2	0	5	5.14	0.00016	4	13	13	4	0	13	11.9	0.00011	253	29838	5.6
BB_0715	17	49	49	17	0	49	49.9	0.00162	20	265	265	20	0	265	57.9	0.00599	21	408	408	21	0	408	53.7	0.00245	361	39755	7.8
BB_0720	6	9	9	6	0	9	14.5	0.00019	1	1	1	1	0	1	1.89	1.4E-05	6	13	13	6	0	13	16.9	4.8E-05	581	68172	6.9
BB_0725	4	6	6	4	0	6	21.3	0.00027	2	5	5	2	0	5	12.6	0.00016	6	17	17	6	0	17	33.8	0.00014	263	29883	5.3
BB_0726	5	16	16	5	0	16	17.7	0.00059	2	9	9	2	0	9	11.8	0.00023	8	34	34	8	0	34	28.2	0.00023	323	36067	9.1
BB_0727	18	71	71	18	0	71	51.1	0.00189	27	166	166	27	0	166	65	0.00302	39	459	459	39	0	459	72.5	0.00222	448	49887	7.5
BB_0733	2	3	3	2	0	3	10.9	0.00013	1	5	5	1	0	5	7.25	0.00015	3	11	11	3	0	11	15.2	8.6E-05	276	31386	9.1
BB_0734	X	X	X	X	X	X	X	X	1	3	3	1	0	3	5.64	7.3E-05	2	4	4	2	0	4	9.2	2.6E-05	337	38280	9.7
BB_0737	1	1	1	1	0	1	3.88	3.6E-05	2	3	3	2	0	3	8.06	7.3E-05	1	2	2	1	0	2	3.88	1.3E-05	335	38667	8.5
BB_0738	7	13	13	7	0	13	15.7	0.00018	7	21	21	7	0	21	12.1	0.0002	17	74	74	17	0	74	28	0.00018	875	101865	7
BB_0741	1	2	2	1	0	2	8.6	0.00026	4	6	6	4	0	6	52.7	0.00053	4	12	12	4	0	12	52.7	0.00028	93	10313	7.2
BB_0742	3	4	4	3	0	4	6.55	8.5E-05	1	1	1	1	0	1	3.19	1.4E-05	3	4	4	3	0	4	6.55	1.5E-05	565	64391	6.1
BB_0749	6	12	12	6	0	12	26.1	0.00033	16	29	29	16	0	29	53.3	0.00055	18	60	60	18	0	60	65.6	0.0003	430	49352	4.7
BB_0754	10	23	23	10	0	23	36.3	0.00089	16	62	62	16	0	62	48.9	0.00164	24	164	164	24	0	164	64.1	0.00115	309	35183	9
BB_0760	4	5	5	4	0	5	28.2	0.00048	7	55	55	7	0	55	38.7	0.00362	6	79	79	6	0	79	31.5	0.00138	124	14129	6.2



LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)											Table 1																
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression				Immunogenicity	Tn mutant
CYT	-0.20091				0.12	0.11	0	o	0.174	33	0.321	25	0.882	17	0.734	0.515	N	0.57	SignalP-noTM	S2	S	S	10.3447			27	27	S2	44							+
CYT	-0.20091				0.2	0.2	0	o	0.183	20	0.331	20	0.841	3	0.654	0.483	N	0.57	SignalP-noTM		S	S	1.21429			26	26			low dNSAF ratio for S						–
CYT	-0.20091	1.77444			1.26	1.26	0	o	0.149	19	0.316	19	0.802	13	0.672	0.483	N	0.57	SignalP-noTM		S	S	19.8679	S	16368984	18	17						+			+§
SpII	2.49438	2.69529	14-15	N	0.16	0.02	0	o	0.143	22	0.229	22	0.735	1	0.377	0.298	N	0.57	SignalP-noTM	S2	S	S	∞			32	33	S2	44				+		+	+
SpII	2.91333	3.11424	19-20	K	2.79	2.79	0	o	0.13	26	0.192	11	0.466	2	0.384	0.263	N	0.51	SignalP-TM	ChpAI	S	S	∞	S	20421380	18	21						+		+	+
SpII	5.73099	3.27118	19-20	S	23.83	23.76	0	o	0.336	25	0.528	25	0.907	5	0.849	0.679	Y	0.57	SignalP-noTM		S	S†	0.62626			14	14	OrfD	143	low dNSAF ratio for S, due to pK resistance				+		+
SpII	19.6863	17.8708	16-17	K	0.1	0.1	0	o	0.164	24	0.336	19	0.807	18	0.725	0.518	N	0.57	SignalP-noTM	OspA	S	S	65.4221	S	6192088	29	31	OspAB	53		+	+			+	–
SpII	18.4788	13.5055	15-16	A	0.04	0.04	0	o	0.187	22	0.373	20	0.854	10	0.784	0.566	N	0.57	SignalP-noTM	OspB	S	S	185.842	S	6735474	32	34	OspAB	53		+	+	+		+	+
SpII	3.28639	3.4873	24-25	G	15.85	15.85	1	i12-31o	0.155	31	0.239	31	0.681	13	0.483	0.329	N	0.51	SignalP-TM	DbpA	S	S	5.95261	S	9573101	21	21						+	+		+
SpII	10.826	7.2885	22-23	N	0.65	0.65	0	o	0.516	28	0.66	28	0.947	19	0.852	0.75	Y	0.57	SignalP-noTM		S	S	0.62069	S	23303811	47	56							+	+	+
SpII	7.63463	7.83554	16-17	S	20.28	20.28	1	i7-26o	0.147	20	0.232	20	0.635	2	0.439	0.309	N	0.51	SignalP-TM		S	S	2.93378			9	18				+	+	+			+
SpII	9.13705	9.33796	27-28	S	19.44	19.4	1	o10-32i	0.151	35	0.219	33	0.411	26	0.256	0.233	N	0.51	SignalP-TM	P35	S	S	6.23077	S	16368984	34	32	P35	54						+	+
SpI	7.09235	0.72531	29-30		13.86	13.86	1	i7-29o	0.248	38	0.441	27	0.934	21	0.873	0.644	Y	0.57	SignalP-noTM	CRASP-1	S	S	24.3566	S	14607842	29	27	P35	54							+
SpII	3.20834	1.30831	24-25	A	15.61	15.61	1	i7-29o	0.135	36	0.261	27	0.629	19	0.485	0.344	N	0.51	SignalP-TM		S	S	∞	S	16368984	30	31	P35	54					+		+
SpII	3.71991	3.92082	17-18	N	22.51	22.27	1	i5-27o	0.119	56	0.217	15	0.61	6	0.483	0.315	N	0.51	SignalP-TM		S	S	0.60523			25	27			low dNSAF ratio for S, is protein pK resistant?						+
SpII	20.6353	12.9424	18-19	N	0.6	0.6	0	o	0.364	24	0.532	24	0.946	2	0.804	0.66	Y	0.57	SignalP-noTM	OspC	S	S	7.86288	S	1560779	22	22						+		+	+
SpII	1.0607	1.26161	13-14	S	4.37	1.66	0	o	0.176	23	0.348	23	0.887	3	0.734	0.529	N	0.57	SignalP-noTM		S	S†	0.5241			19	18			low dNSAF ratio for S, due to pK resistance				+		+
SpII	3.92093	4.12184	18-19	Q	15.18	15.06	1	i5-24o	0.232	26	0.31	26	0.591	5	0.449	0.361	N	0.51	SignalP-TM		S	S	1.83471			23	21			low dNSAF ratio for S						+
SpII	6.13534	6.33625	17-18	N	0.43	0.42	0	o	0.233	18	0.408	18	0.844	16	0.713	0.552	N	0.57	SignalP-noTM	P35	S	S	∞	S	21695244	28	27	P35	60				+			–
SpII	13.7282	5.76274	21-22	D	17.8	17.8	0	o	0.337	30	0.489	30	0.939	19	0.834	0.651	Y	0.57	SignalP-noTM		S	S	1.83562			11	14									+
SpII	10.917	6.37393	17-18	N	0.05	0.05	0	o	0.16	23	0.327	23	0.883	16	0.7	0.502	N	0.57	SignalP-noTM		S	S	73.4853			35	31	BB_0844	12					+		+
SpII	4.24522	4.44613	18-19	D	0.71	0.71	0	o	0.284	19	0.448	19	0.872	2	0.748	0.589	Y	0.57	SignalP-noTM	CRASP-2	S	S	0.74327	S	16925556	27	26	CRASP-2		low dNSAF ratio for S				+	+	+
SpII	7.6072	7.80811	24-25	D	18.61	18.61	1	i12-30o	0.11	29	0.144	13	0.248	9	0.217	0.171	N	0.51	SignalP-TM		S	S	5.9375			43	43									+
SpII	8.91217	9.11308	17-18	N	1.87	1.62	0	o	0.268	24	0.372	24	0.829	2	0.582	0.47	N	0.57	SignalP-noTM	P35	S	S	8.35616			29	22	P35	60							+
SpII	4.9518	3.1493	26-27	N	0.62	0.62	0	o	0.173	32	0.336	32	0.815	25	0.611	0.465	N	0.57	SignalP-noTM		S	S	62.2039			33	37	BB_0844	12					+		+
SpII	2.14854	2.34945	16-17	R	0.3	0.18	0	o	0.233	23	0.319	23	0.741	2	0.467	0.389	N	0.57	SignalP-noTM	VraA	S	S	∞	S	11179306	54	75	P35	60							na
SpII	10.0931	8.21577	17-18	T	0.25	0.25	0	o	0.48	23	0.612	23	0.929	19	0.789	0.695	Y	0.57	SignalP-noTM		S	S	8.93865			26	27	P35	60		+	+	+			na
SpI	5.99472	2.10954	29-30		18.04	18.04	1	i9-31o	0.212	27	0.435	27	0.952	25	0.869	0.639	Y	0.57	SignalP-noTM	P35	S	S	85.9302			32	37	P35	54							na
SpI	5.79867	1.77267	29-30		18.04	18.04	1	i9-31o	0.212	27	0.434	27	0.952	25	0.867	0.638	Y	0.57	SignalP-noTM		S	S	0			32	38	P35	54					+		na
SpI	7.64303	1.76749	33-34		5.63	5.63	0	o	0.196	27	0.415	27	0.925	25	0.855	0.622	Y	0.57	SignalP-noTM		S	S	56.2096			33	37	P35	54					+		na
SpII	18.2273	9.15461	19-20	V	0.57	0.57	0	o	0.445	24	0.626	24	0.963	16	0.899	0.754	Y	0.57	SignalP-noTM	OspD	S	S	22.8934	S	1398980	28	29							+		+
SpII	10.1531	7.10112	18-19	K	3.33	3.22	0	o	0.228	24	0.363	24	0.778	4	0.596	0.472	N	0.57	SignalP-noTM		S	S	142.696			40	41	CRASP-2	92					+		+
SpII	19.2032	12.8461	19-20	K	9.27	9.25	0	o	0.166	25	0.355	25	0.927	5	0.827	0.577	Y	0.57	SignalP-noTM		S	S	∞			40	35	CRASP-2	92							+
SpII	12.2834	8.03962	17-18	N	0.09	0.09	0	o	0.186	23	0.365	23	0.911	16	0.747	0.544	N	0.57	SignalP-noTM		S	S	97.2909			34	38	BB_0844	12					+		+
SpII	10.8843	11.0852	15-16	K	0.02	0.02	0	o	0.211	21	0.373	21	0.756	13	0.595	0.478	N	0.57	SignalP-noTM		S	S	∞	S	19776192	28	31	BB_K07	59						+	+
SpI	5.64382	2.39322	21-22		6.08	6	0	o	0.445	22	0.59	22	0.946	1	0.826	0.701	Y	0.57	SignalP-noTM		S	S	39.915			24	30								+	+
SpII	13.8444	14.0453	19-20	K	0.07	0.06	0	o	0.158	25	0.29	21	0.857	2	0.679	0.473	N	0.57	SignalP-noTM	P37	S	S	10.4182			37	46	P37	75							+
SpII	17.6345	17.8354	17-18	N	0.17	0.17	0	o	0.287	21	0.489	21	0.911	14	0.83	0.649	Y	0.57	SignalP-noTM	MlpH	S	S	∞			17	19	Mlp	113							–
SpII	9.64826	9.84917	23-24	K	11.39	11.39	0	o	0.116	30	0.257	27	0.763	21	0.511	0.351	N	0.51	SignalP-TM	ErpN	S	S	10.869	S	11283278	20	19	ErpA	162	identical to BB_P38/ErpA					+	+
SpII	9.09726	3.80779	19-20	K	1.69	1.67	0	o	0.371	25	0.467	25	0.893	4	0.68	0.567	N	0.57	SignalP-noTM	RevA	S	S	0.928	S	11500397	18	nd	Rev	63	low dNSAF ratio for S; native protein is resitant to pK!				+	+	+
SpII	12.6696	12.132	19-20	K	7.39	7.39	0	o	0.139	26	0.314	26	0.892	15	0.777	0.532	N	0.57	SignalP-noTM	ErpP	S	S	38.4773	S	11283278	21	20	ErpA	162						+	+
SpII	12.5726	6.71201	17-18	K	4.54	4.54	0	o	0.513	23	0.613	23	0.84	3	0.75	0.677	Y	0.57	SignalP-noTM	ErpQ	S	S	∞	S	11283278	39	55	ErpB	163						+	–
SpII	16.0992	12.6387	17-18	K	13.9	13.9	1	i5-24o	0.587	23	0.627	23	0.813	3	0.693	0.658	Y	0.57	SignalP-noTM	ErpM	S	S	0.61194	S	11283278	42	40	ErpB	163	low dNSAF ratio for S					+	+
SpII	15.779	14.4238	17-18	N	0.13	0.13	0	o	0.377	23	0.547	23	0.926	14	0.825	0.678	Y	0.57	SignalP-noTM	MlpA	S	S	3.16049			16	19	Mlp	113					+		–
SpII	9.72242	9.92333	17-18	K	2.97	2.97	0	o	0.304	23	0.446	23	0.843	20	0.664	0.548	N	0.57	SignalP-noTM	ErpB	S	S	1.03158	S	11283278	44	61	ErpB	163	low dNSAF ratio for S				+	+	–
SpII	11.9569	12.1578	15-16	N	0.03	0.03	0	o	0.219	22	0.383	22	0.852	12	0.705	0.534	N	0.57	SignalP-noTM	P35	S	S	1.22449			29	30	P35	60	low dNSAF ratio for S				+		na
SpII	13.9058	12.6473	17-18	N	0.02	0.02	0	o	0.29	23	0.461	23	0.893	14	0.761	0.602	Y	0.57	SignalP-noTM	MlpJ	S	S	1.82857			24	21	Mlp	113						+	na
SpII	16.0924	16.2933	17-18	N	0.42	0.42	0	o	0.287	21	0.492	21	0.911	14	0.847	0.659	Y	0.57	SignalP-noTM	MlpD	S	S	1.06433			16	16	Mlp	113	low dNSAF ratio for S				+		+
SpII	15.325	15.5259	19-20	K	0.11	0.11	0	o	0.166	24	0.339	23	0.832	5	0.737	0.526	N	0.57	SignalP-noTM	ErpY	S	S	∞	S	11283278	25	27	ErpG	164					+	+	+
SpII	16.4935	16.6944	17-18	N	0.17	0.17	0	o	0.287	21	0.489	21	0.912	14	0.831	0.65	Y	0.57	SignalP-noTM	MlpC	S	S	6.54545			17	16	Mlp	113					+	+	+
SpII	13.7184	9.54449	19-20	K	2.04	2.04	0	o	0.22	27	0.405	25	0.885	21	0.784	0.584	Y	0.57	SignalP-noTM	ErpG	S	S	∞	S	25864455	22	23	ErpG	164					+	+	+
SpII	5.96399	2.81488	24-25	S	21.23	21.11	1	i13-35o	0.202	33	0.269	33	0.588	11	0.383	0.311	N	0.51	SignalP-TM		P-OM	S	0.69645	P-OM	22519960	40	38			Multiple OM to	TA	TP	VT	VP		–
CYT	-0.20091				0.43	0.3	0	o	0.172	19	0.31	19	0.709	11	0.551	0.423	N	0.57	SignalP-noTM		P-OM	P-OM	0.68888	P-OM	22519960	14	16									+
SpII	3.51567	3.07065	16-17	S	0.24	0.24	0	o	0.225	28	0.364	24	0.869	15	0.706	0.525	N	0.57	SignalP-noTM		P-OM	P-OM	2.01742			28	29			dNSAF ratio close to 2.0 but P-OM			+	+		+
SpII	10.5911	7.66563	17-18	E	2.62	2.62	0	o	0.125	20	0.323	20	0.91	16	0.835	0.563	N	0.57	SignalP-noTM	Lp6.6	P-OM	P-OM	0.59354	P-OM	9009290	8	13				+	+	+			+
SpII	7.64539	7.8463	25-26	V	10.71	10.69	0	o	0.143	28	0.254	28	0.6	24	0.327	0.281	N	0.51	SignalP-TM	BesA	P-IM	P-IM	0.58876			35	39									+
SpII	5.79043	5.99134	15-16	D	0.02	0.01	0	o	0.112	41	0.195	11	0.453	1	0.393	0.268	N	0.51	SignalP-TM	ProX	P-IM	P-IM	0.79348			33	32									+§
SpII	3.75044	3.95135	19-20	K	0.38	0.25	0	o	0.138	22	0.239	22	0.591	1	0.404	0.316	N	0.57	SignalP-noTM		P-IM	P-IM	2.21951			29	28			dNSAF ratio close to 2.0 but P-IM						–
SpII	10.8726	11.0735	19-20	K	0.09	0.09	0	o	0.179	21	0.368	21	0.881	1	0.773	0.558	N	0.57	SignalP-noTM	PstS	P-IM	P-IM	1.04199			31	30								+	–
SpII	3.91814	4.11905	22-23	T	1.03	0.98	0	o	0.118	29	0.251	11	0.757	3	0.637	0.432	N	0.57	SignalP-noTM		P-IM	P-IM	0.56713	P-OM	20398211	27	26			likely mislocalized previously (Shiyong)						–
CYT	-0.20091				0.15	0.01	0	o	0.142	22	0.185	11	0.44	2	0.357	0.249	N	0.51	SignalP-TM		P-IM	P-IM	0.36623	P	16368984	26	26									–
SpII	12.336	12.5369	20-21	K	4.27	4.27	0	o	0.259	23	0.418	23	0.874	19	0.719	0.559	N	0.57	SignalP-noTM		P-IM	P-IM	0.47326	P-OM	19754308	44	14								+	–
SpII	11.5375	8.80608	23-24	I	17.25	17.23	1	i9-26o	0.281	29	0.25	29	0.383	8	0.239	0.246	N	0.51	SignalP-TM	OppA-1	P-IM	P-IM	0.57189	S	9393787	60	59	OppA	37						+	+
SpII	6.2305	6.43141	18-19	N	0.09	0.06	0	o	0.174	25	0.219	25	0.558	3	0.359	0.271	N	0.51	SignalP-TM	OppA-2	P-IM	P-IM	0.93968	S	9296108; 971	61	60	OppA	37						+	+
SpII	18.2895	13.8965	27-28	N	3.67	3.62	0	o	0.245	30	0.457	30	0.935	21	0.818	0.627	Y	0.57	SignalP-noTM	OppA-3	P-IM	P-IM	0.92027			62	57	OppA	37							+
SpII	14.3539	14.5548	21-22	T	2.83	2.76	0	o	0.166	22	0.367	22	0.912	4	0.83	0.585	Y	0.57	SignalP-noTM	IpLA7	P-IM	P-IM	0.56775	P-IM	17464050	22	21				+	+			+	+
SpII	2.54014	2.74105	14-15	F	0.62	0.49	0	o	0.151	20	0.201	20	0.384	1	0.3	0.237	N	0.51	SignalP-TM	BmpB	P-IM	P-IM	1.16411	S	18166585	38	34	Bmp	36	likely mislocalized previously; only done by IFA without proper subsurface c						–
SpII	11.0722	8.94558	16-17	F	0.9	0.18	0	o	0.172	17	0.354	17	0.927	1	0.764	0.547	N	0.57	SignalP-noTM	BmpC	P-IM	P-IM	0.44538			40	39	Bmp	36							+
CYT	-0.20091	0.75525			2.78	2.64	0	o	0.213	47	0.206	47	0.375	38	0.158	0.188	N	0.51	SignalP-TM	BmpD	P-IM	P-IM	0.88881			37	38	Bmp	36						+	–
SpII	4.42185	4.62276	19-20	N	0.07	0.07	0	o	0.199	26	0.35	26	0.872	1	0.672	0.501	N	0.57	SignalP-noTM		P-IM	P-IM	0			41	36									–
SpI	1.618	1.53405	23-24		0.01	0.01	0	o	0.485	24	0.476	24	0.752	1	0.505	0.49	N	0.57	SignalP-noTM		P-IM	P-IM	0.12458			24	23									+
CYT	-0.20091				21.06	20.72	1	i13-35o	0.135	37	0.142	37	0.209	35	0.119	0.134	N	0.51	SignalP-TM		P-IM	P-IM	1.25899			22	19						+			+§
SpII	9.14224	7.4993	16-17	S	0.96	0.96	0	o	0.33	22	0.524	22	0.936	1	0.857	0.68	Y	0.57	SignalP-noTM		P-IM	P-IM	0			27	26									–
SpII	3.72874	2.90054	17-18	L	131.52	22.3	6	i7-29o413	0.133	27	0.18	11	0.486	1	0.32	0.232	N	0.51	SignalP-TM	SecD	P-IM	nd	0.17768			65	nd			not detected by His-tag but seems to be P by MudPIT; supporte					+	–
CYT	-0.20091	2.15595			22.27	22.27	1	i7-29o	0.105	37	0.139	11	0.295	1	0.185	0.156	N	0.51	SignalP-TM		P-IM	P-IM	0.69831			26	28									–
SpII	2.28024	2.48115	21-22	L	3.86	3.32	0	o	0.149	28	0.301	28	0.88	8	0.715	0.496	N	0.57	SignalP-noTM		P-IM	P-IM	1.21717			58	53									–
SpII	3.23021	3.43112	17-18	A	3.24	3.21	0	o	0.157	30	0.286	23	0.819	2	0.711	0.486	N	0.57	SignalP-noTM		P-IM	P-IM	0.72973			31	27									–
SpII	7.67847	7.87938	16-17	K	0.03	0.03	0	o	0.218	24	0.345	24	0.766	17	0.579	0.455	N	0.57	SignalP-noTM		P-IM	P-IM	0.70278	S	22851744	19	17			likely mislocalized previously by IFA, but looks like it's resistant t					+	+
SpII	10.2964	10.4973	17-18	K	0.04	0.04	0	o	0.115	21	0.196	21	0.44	2	0.35	0.253	N	0.51	SignalP-TM	S1	P-IM	P-IM	1.83333	P	19822648	49	52									+
SpII	9.18653	0.86594	18-19	S	1.48	1.47	0	o	0.454	24	0.558	24	0.827	19	0.705	0.627	Y	0.57	SignalP-noTM	OppAV	P-IM	P-IM	0.74138	P	21628523	61	59	OppA	37					+	+	+
SpII	8.22842	8.42933	21-22	V	0.44	0.39	0	o	0.156	26	0.199	26	0.453	6	0.305	0.238	N	0.51	SignalP-TM	OppAIV	P-IM	P-IM	0.61077	P-IM	16468989	61	58	OppA	37						+	+
SpII	1.02816	1.22907	15-16	S	15.65	14.01	1	i5-22o	0.156	23	0.213	23	0.496	2	0.321	0.253	N	0.51	SignalP-TM		P-IM	P-IM	1.24615	P-OM	20398211	22	21			high dNSAF for P protein; likely mislocalized previously						+

LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)										
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used
SpII	8.10693	8.30784	22-23	V	6.29	6.08	0	o	0.14	27	0.308	27	0.93	6	0.769	0.525	N	0.57	SignalP-noTM
SpII	8.99542	9.19633	17-18	S	0.09	0.08	0	o	0.117	21	0.18	11	0.376	1	0.335	0.237	N	0.51	SignalP-TM
SpII	3.06746	3.26837	15-16	T	1.32	1.29	0	o	0.15	19	0.324	19	0.854	15	0.682	0.492	N	0.57	SignalP-noTM
SpII	4.2231	3.46124	20-21	A	15.07	14.16	1	i7-26o	0.294	26	0.374	26	0.667	16	0.471	0.41	N	0.51	SignalP-TM
SpII	9.30329	9.5042	20-21	S	14.73	14.66	1	i7-26o	0.325	32	0.316	32	0.555	16	0.354	0.33	N	0.51	SignalP-TM
SpII	4.50329	4.7042	16-17	D	0.18	0.18	0	o	0.129	23	0.269	23	0.733	13	0.515	0.384	N	0.57	SignalP-noTM
SpII	5.8237	4.71367	18-19	T	0.05	0.05	0	o	0.365	24	0.534	24	0.894	5	0.815	0.666	Y	0.57	SignalP-noTM
SpII	8.21198	4.46474	20-21	S	0.1	0.04	0	o	0.221	28	0.389	28	0.897	8	0.78	0.573	Y	0.57	SignalP-noTM
SpII	1.0979	1.29881	13-14	N	0	0	0	o	0.135	19	0.23	19	0.513	12	0.356	0.289	N	0.57	SignalP-noTM
SpII	2.89632	3.09723	24-25	S	4.49	4.49	0	o	0.111	41	0.211	18	0.535	7	0.412	0.286	N	0.51	SignalP-TM
SpII	2.89632	3.09723	24-25	S	4.49	4.49	0	o	0.111	41	0.211	18	0.535	7	0.412	0.286	N	0.51	SignalP-TM
CYT	-0.20091	0.7911			20.14	20.14	1	i5-24o	0.101	22	0.104	11	0.219	1	0.094	0.101	N	0.51	SignalP-TM
SpI	4.37766	4.57857	19-20		47.81	6.09	2	o101-123i	0.181	18	0.252	18	0.609	1	0.398	0.306	N	0.51	SignalP-TM
CYT	-0.20091	1.13792			44.36	20.47	1	i5-27o	0.168	26	0.271	26	0.518	19	0.342	0.298	N	0.51	SignalP-TM
CYT	-0.20091				22.47	20.44	1	i41-63o	0.111	66	0.182	43	0.465	35	0.18	0.181	N	0.51	SignalP-TM
CYT	-0.20091				21.58	21.53	1	i26-48o	0.107	52	0.094	66	0.098	62	0.069	0.085	N	0.51	SignalP-TM
SpI	15.9425	15.7446	21-22		80.26	30.03	4	i5-22o48-6	0.815	22	0.796	22	0.883	18	0.796	0.796	Y	0.51	SignalP-TM
CYT	-0.20091				18.29	17.81	1	i20-37o	0.158	37	0.139	11	0.26	1	0.191	0.158	N	0.51	SignalP-TM
TMH	1.64252	1.84343			4.87	4.4	0	o	0.202	20	0.404	20	0.894	1	0.834	0.606	Y	0.57	SignalP-noTM
CYT	-0.20091	0.88261			21.27	21.26	1	i29-48o	0.115	43	0.108	19	0.135	18	0.116	0.111	N	0.51	SignalP-TM
SpI	8.56896	8.76987	22-23		15.16	14.15	1	i7-25o	0.442	23	0.452	23	0.695	19	0.447	0.45	N	0.57	SignalP-noTM
CYT	-0.20091				21.8	21.78	1	i7-29o	0.122	30	0.117	11	0.219	1	0.131	0.122	N	0.51	SignalP-TM
CYT	-0.20091	2.28398			18.53	18.06	1	i25-42o	0.174	48	0.141	38	0.172	3	0.104	0.127	N	0.51	SignalP-TM
SpI	0.58978	0.7907	21-22		0.04	0	0	o	0.217	22	0.378	22	0.865	16	0.639	0.501	N	0.57	SignalP-noTM
SpI	2.35272	2.55363	23-24		72.8	25.39	3	o5-27i84-1	0.316	24	0.294	24	0.399	19	0.243	0.275	N	0.51	SignalP-TM
CYT	-0.20091				22.88	22.88	1	i7-29o	0.114	37	0.123	11	0.197	1	0.152	0.134	N	0.51	SignalP-TM
CYT	-0.20091				18.72	18.67	1	i13-30o	0.268	28	0.199	28	0.25	26	0.149	0.181	N	0.51	SignalP-TM
SpI	5.29273	4.51561	26-27		22.84	22.84	1	i7-29o	0.177	27	0.194	11	0.493	5	0.376	0.261	N	0.51	SignalP-TM
SpI	2.17122	2.37213	19-20		7.24	7.24	0	o	0.397	20	0.447	20	0.592	11	0.441	0.445	N	0.51	SignalP-TM
SpI	2.55115	2.75206	19-20		151.14	42.54	7	i5-22o27-4	0.189	20	0.329	20	0.801	1	0.618	0.436	N	0.51	SignalP-TM
SpI	0.86999	1.0709	26-27		20.01	20.01	1	i11-33o	0.222	27	0.256	27	0.395	25	0.234	0.248	N	0.51	SignalP-TM
CYT	-0.20091	2.54838			20.51	20.5	1	i21-43o	0.138	47	0.163	47	0.282	38	0.157	0.161	N	0.51	SignalP-TM
CYT	-0.20091				21.42	21.4	1	i2-24o	0.12	27	0.106	27	0.19	1	0.087	0.099	N	0.51	SignalP-TM
CYT	-0.20091				19.96	19.96	1	i2-21o	0.132	22	0.099	60	0.116	48	0.072	0.089	N	0.51	SignalP-TM
SpI	9.34817	9.54908	22-23		38.49	16.57	2	i5-22o61-8	0.497	21	0.616	21	0.867	1	0.772	0.689	Y	0.57	SignalP-noTM
CYT	-0.20091				19.51	19.06	1	o4-26i	0.11	20	0.198	16	0.494	6	0.401	0.273	N	0.51	SignalP-TM
CYT	-0.20091	0.43378			29.19	22.04	1	i5-27o	0.102	36	0.123	11	0.24	2	0.146	0.131	N	0.51	SignalP-TM
CYT	-0.20091				16.24	16.2	1	i5-27o	0.323	22	0.284	22	0.376	1	0.276	0.281	N	0.51	SignalP-TM
SpI	1.76265	1.96356	23-24		22.66	22.66	1	i7-29o	0.213	29	0.25	29	0.552	3	0.333	0.281	N	0.51	SignalP-TM
CYT	-0.20091				21.14	17.07	1	i7-26o	0.161	32	0.227	22	0.409	12	0.319	0.261	N	0.51	SignalP-TM
SpI	0.02495	0.22587	19-20		0.23	0.09	0	o	0.455	20	0.524	20	0.726	16	0.616	0.567	N	0.57	SignalP-noTM
CYT	-0.20091				22.87	22.87	1	i30-52o	0.101	22	0.092	22	0.117	20	0.076	0.086	N	0.51	SignalP-TM
SpI	5.36826	5.56917	18-19		45.88	20.17	0	o	0.254	20	0.404	20	0.792	18	0.579	0.468	N	0.51	SignalP-TM
SpI	6.89647	7.09738	23-24		37.7	11.87	0	o	0.643	24	0.761	24	0.942	9	0.884	0.819	Y	0.57	SignalP-noTM
SpI	0.83733	1.03825	20-21		0.58	0	0	o	0.244	21	0.441	21	0.893	14	0.781	0.601	Y	0.57	SignalP-noTM
SpI	3.89671	4.09762	26-27		49.65	20.44	2	o4-26i714	0.152	27	0.22	11	0.588	1	0.496	0.322	N	0.51	SignalP-TM
CYT	-0.20091				22.15	22.15	1	i2-24o	0.104	24	0.096	59	0.166	1	0.082	0.091	N	0.51	SignalP-TM
CYT	-0.20091				0.91	0.91	0	o	0.287	23	0.474	23	0.905	9	0.821	0.637	Y	0.57	SignalP-noTM
SpI	7.06226	7.26317	23-24		43.43	18.48	0	o	0.488	24	0.415	24	0.485	7	0.373	0.4	N	0.51	SignalP-TM
CYT	-0.20091				22.93	22.93	1	i13-35o	0.213	43	0.188	43	0.227	7	0.14	0.171	N	0.51	SignalP-TM
CYT	-0.20091	0.86214			18.36	18.28	1	i7-29o	0.115	29	0.16	11	0.318	8	0.27	0.201	N	0.51	SignalP-TM
CYT	-0.20091				20.29	12.29	0	o	0.49	24	0.607	24	0.937	15	0.779	0.688	Y	0.57	SignalP-noTM
SpI	1.68761	1.88852	21-22		16.83	14.61	1	i5-22o	0.418	22	0.599	22	0.941	17	0.849	0.716	Y	0.57	SignalP-noTM
CYT	-0.20091				19.1	18.35	1	i5-23o	0.22	26	0.165	26	0.26	1	0.126	0.151	N	0.51	SignalP-TM
SpI	7.11219	7.3131	22-23		0.47	0.31	0	o	0.69	23	0.78	23	0.945	21	0.894	0.833	Y	0.57	SignalP-noTM
CYT	-0.20091	0.37555			41.99	22.12	1	i7-29o	0.321	40	0.212	40	0.214	36	0.083	0.165	N	0.51	SignalP-TM
SpI	1.0601	1.26101	21-22		1.91	1.9	0	o	0.719	22	0.668	22	0.885	18	0.6	0.636	Y	0.57	SignalP-noTM
SpI	6.85167	7.05258	22-23		10.2	10.19	0	o	0.513	23	0.605	23	0.888	1	0.776	0.686	Y	0.57	SignalP-noTM
CYT	-0.20091				16.28	16.28	1	i36-53o	0.108	60	0.105	36	0.136	35	0.09	0.1	N	0.51	SignalP-TM
SpI	6.26253	6.46344	23-24		2.05	1.97	0	o	0.393	22	0.588	22	0.949	4	0.9	0.735	Y	0.57	SignalP-noTM
CYT	-0.20091	1.60177			23.01	18.77	1	i7-24o	0.236	23	0.2	23	0.281	20	0.193	0.198	N	0.51	SignalP-TM
CYT	-0.20091	0.36912			21.66	21.65	1	i7-29o	0.304	27	0.192	27	0.258	3	0.138	0.172	N	0.51	SignalP-TM
SpI	2.84501	3.04592	17-18		1.35	1.35	0	o	0.709	18	0.791	18	0.957	17	0.851	0.819	Y	0.57	SignalP-noTM
SpI	5.38267	5.58358	29-30		20.27	19.52	1	i13-32o	0.539	30	0.667	30	0.913	26	0.787	0.724	Y	0.57	SignalP-noTM
SpI	3.86015	4.06106	20-21		18.52	18.52	1	i7-26o	0.469	21	0.455	21	0.61	5	0.474	0.462	N	0.51	SignalP-TM
SpI	0.99128	1.19219	42-43		22.77	21.84	1	i20-42o	0.132	43	0.175	43	0.325	37	0.163	0.171	N	0.51	SignalP-TM
CYT	-0.20091				16.48	16.42	1	i5-22o	0.11	30	0.14	14	0.263	9	0.203	0.164	N	0.51	SignalP-TM
CYT	-0.20091				18.31	18.05	1	o4-21i	0.141	25	0.137	25	0.267	1	0.14	0.138	N	0.51	SignalP-TM
CYT	-0.20091				14.35	14.31	1	i9-31o	0.154	25	0.114	25	0.119	20	0.092	0.106	N	0.51	SignalP-TM
SpI	8.33176	8.53267	21-22		27.97	19.04	1	i7-26o	0.412	22	0.372	22	0.567	6	0.414	0.387	N	0.51	SignalP-TM
CYT	-0.20091	2.10156			20.58	20.58	1	i20-42o	0.141	49	0.15	11	0.261	1	0.236	0.182	N	0.51	SignalP-TM
SpI	6.80286	4.32924	25-26		9.88	9.88	0	o	0.381	23	0.604	23	0.975	20	0.954	0.768	Y	0.57	SignalP-noTM
SpI	5.71245	3.78786	25-26		12.77	12.77	1	i7-24o	0.423	23	0.637	23	0.98	20	0.953	0.786	Y	0.57	SignalP-noTM
SpI	6.73934	4.32574	25-26		10.29	10.29	0	o	0.381	23	0.604	23	0.976	20	0.954	0.769	Y	0.57	SignalP-noTM
TMH	0.64404	0.84495			177.43	27.39	8	i30-52o56	0.13	51	0.118	11	0.191	2	0.143	0.127	N	0.51	SignalP-TM
TMH	2.01493	2.21584			22.56	22.54	1	i25-47o	0.122	53	0.115	11	0.186	2	0.131	0.121	N	0.51	SignalP-TM
TMH	0.6806	0.88151			20.13	20.13	1	i12-29o	0.165	29	0.152	29	0.272	28	0.159	0.155	N	0.51	SignalP-TM
TMH	16.0599	16.2608			43.33	24.46	2	i9-31o57-7	0.141	31	0.107	31	0.147	1	0.068	0.093	N	0.51	SignalP-TM
TMH	0.6698	0.87072			23.02	22.33	1	i13-35o	0.245	47	0.174	47	0.261	5	0.149	0.165	N	0.51	SignalP-TM
TMH	5.63675	5.83766			20.04	20.02	1	i21-40o	0.11	41	0.118	20	0.214	17	0.135	0.124	N	0.51	SignalP-TM
TMH	0.00889	0.2098			280.52	21.75	13	i7-29o338	0.187	30	0.138	30	0.254	2	0.123	0.133	N	0.51	SignalP-TM
TMH	0.478	0.67891			23	23	1	i13-35o	0.381	31	0.129	31	0.114	5	0.056	0.102	N	0.51	SignalP-TM
TMH	13.1699	10.5466			133.02	35.42	6	i13-35o50	0.13	29	0.157	41	0.265	28	0.185	0.167	N	0.51	SignalP-TM
TMH	20.6023	20.8032			98.11	43.69	4	o10-39i46	0.151	26	0.149	26	0.238	14	0.154	0.15	N	0.51	SignalP-TM
TMH	1.54316	1.74407			22.85	22.85	1	i12-34o	0.11	37	0.165	11	0.38	2	0.27	0.204	N	0.51	SignalP-TM
TMH	13.639	13.8399			42.92	42.81	2	i13-35o40	0.118	26	0.124	11	0.183	1	0.159	0.137	N	0.51	SignalP-TM
TMH	3.78933	3.99024			160.5	37.1	8	i21-42o46	0.148	43	0.087	11	0.101	67	0.076	0.083	N	0.51	SignalP-TM
TMH	1.51692	1.71783			22.69	22.69	1	o30-52i	0.106	36	0.101	64	0.117	56	0.067	0.088	N	0.51	SignalP-TM
TMH	0.0504	0.25131			20.29	20.19	1	o25-44i	0.106	41	0.095	12	0.118	11	0.091	0.094	N	0.51	SignalP-TM
TMH	11.7254	11.9263			85.89	42.5	4	i7-29o34-5	0.143	49	0.129	49	0.187	8	0.11	0.122	N	0.51	SignalP-TM
TMH	12.5761	12.777			22.74	22.73	1	i13-35o	0.105	35	0.132	11	0.315	1	0.159	0.142	N	0.51	SignalP-TM
TMH	7.31385	5.50741			133.38	21.97	6	i9-31o100	0.123	33	0.181	33	0.516	28	0.191	0.184	N	0.51	SignalP-TM
TMH	20.5694	20.7703			139.17	34.85	6	i7-29o49-7	0.143	31	0.118	11	0.191	1	0.135	0.124	N	0.51	SignalP-TM
TMH	7.03381	7.23472			141.82	27.26	6	i40-62o84	0.103	56	0.13	23	0.219	12	0.155	0.139	N	0.51	SignalP-TM
TMH	1.95465	2.15556			132.01	26.16	5	o15-34i55	0.11	46	0.109	46	0.142	37	0.066	0.093	N	0.51	SignalP-TM
TMH	7.19592	7.39683			202.34	43.04	9	i7-26o36-5	0.407	33	0.144	33	0.224	3	0.084	0.121	N	0.51	SignalP-TM
TMH	0.72801	0.92892			81.25	19.92	4	i7-24o72-9	0.133	31	0.131	11	0.226	3	0.179	0.149	N	0.51	SignalP-TM
TMH	3.98936	4.19027			211.63	21.08	10	i20-39o71	0.11	70	0.109	12	0.152	7	0.124	0.115	N	0.51	SignalP-TM
TMH	1.12165	1.32256			21.14	21.09	1	i7-26o	0.105	28	0.113	11	0.216	2	0.12	0.116	N	0.51	SignalP-TM
TMH	7.59655	7.79746			147.63	30	7	i21-38o48	0.284	39	0.307	39	0.464	35	0.197	0.266	N	0.51	SignalP-TM
TMH	0.67129	0.8722			68.86	32.27	3	o15-32i44	0.191	33	0.187	33	0.241	28	0.169	0.18	N	0.51	SignalP-TM
TMH	3.54446	3.74537			46.44	21.77	2	o17-39i32	0.145	37	0.123	13	0.197	9	0.157	0.136	N	0.51	SignalP-TM
TMH	8.66899	7.79124			45.91	22.57	2	i9-31o436	0.138	27	0.12	11	0.208	2	0.143	0.129	N	0.51	SignalP-TM
TMH	18.8917	19.0926			53.04	37.97	3	i7-24o28-4	0.192	24	0.107	11	0.177	1	0.11	0.108	N	0.51	SignalP-TM
TMH	5.91255	6.11346			280.82	43.1	13	o4-23i28-4	0.118	30	0.096	11	0.148	3	0.092	0.095	N	0.51	SignalP-TM
TMH	11.3557	11.5566			232.38	27.44	10	o15-37i58	0.137	37	0.137	37	0.225	7	0.156	0.144	N	0.51	SignalP-TM
TMH	0.30932	0.51023			20.59	20.45	1	o20-39i	0.128	45	0.138	11	0.244	3	0.197	0.16	N	0.51	SignalP-TM
TMH	5.30133	5.50224			130.76	21.82	6	i13-35o13	0.122	34	0.15	11	0.283	5	0.242	0.184	N	0.51	SignalP-TM
TMH	1.5384	1.73931			119.22	41.55	6	i5-22o37-5	0.104	22	0.103	11	0.143	3	0.107	0.104	N	0.51	SignalP-TM
TMH	10.0546	10.2555			44.17	23.36	2	o10-32i35	0.13	30	0.106	67	0.19	1	0.087	0.099	N	0.51	SignalP-TM
TMH	10.5171	5.64673			53.4	23.62	2	o10-32i32	0.159	29	0.147	11	0.333	2	0.214	0.172	N	0.51	SignalP-TM
TMH	2.52213	2.72304			21.97	21.78	1	i9-31o	0.263	33	0.187	33	0.246	31	0.106	0.157	N	0.51	SignalP-TM
TMH	1.99117	2.19208			199.82	42.25	9	o4-26i38-5	0.111	27	0.112	38	0.163	34	0.08	0.1	N	0.51	SignalP-TM
TMH	9.6323	9.83321			135.77	27.49	6	i26-48o58	0.105	26	0.095	11	0.126	65	0.096	0.096	N	0.51	SignalP-TM

Table 1

Location	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression	Immunogenicity	Tn mutant
SpII		P	nd	0.67335			108	nd			not detected by His-tag but seems to be P by MudPIT			+

LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)											Table 1													
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/ dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression	Immunogenicity	Tn mutant
TMH	0.00747	0.20838			19.35	19.34	1	i5-23o	0.127	29	0.102	11	0.194	1	0.092	0.098	N	0.51	SignalP-TM														
TMH	10.6611	6.06579			52.77	22.23	2	i21-43o11	0.113	44	0.174	22	0.431	19	0.27	0.21	N	0.51	SignalP-TM														
TMH	5.96773	5.1117			47.48	21.94	1	i7-29o	0.153	21	0.227	21	0.582	2	0.39	0.287	N	0.51	SignalP-TM														
TMH	5.22203	5.42294			251.25	22.65	12	i13-35o83	0.119	38	0.176	11	0.381	4	0.323	0.23	N	0.51	SignalP-TM														
TMH	1.07298	1.27389			276.04	33.75	12	i23-45o50	0.108	39	0.112	70	0.157	65	0.101	0.108	N	0.51	SignalP-TM														
TMH	3.32153	3.52244			43.66	40.6	2	o5-27i40-	0.119	28	0.112	40	0.153	30	0.104	0.11	N	0.51	SignalP-TM														
TMH	6.10431	5.07437			214.47	22.88	9	o20-42i75	0.174	43	0.168	43	0.243	16	0.157	0.164	N	0.51	SignalP-TM														
TMH	2.28605	2.48696			42.85	35.45	2	o15-34i47	0.102	64	0.122	11	0.176	2	0.155	0.134	N	0.51	SignalP-TM														
TMH	6.14452	4.95182			90.87	22.02	4	i19-41o27	0.134	33	0.141	18	0.232	8	0.194	0.161	N	0.51	SignalP-TM														
TMH	2.20357	2.40448			20.11	20.1	1	o15-34i	0.134	32	0.124	32	0.186	30	0.108	0.118	N	0.51	SignalP-TM														
CYT	-0.20091				256.62	20.63	12	i7-29o206	0.107	37	0.091	11	0.1	4	0.088	0.09	N	0.51	SignalP-TM														
CYT	-0.20091				168.7	0	9	o308-325i	0.106	18	0.141	18	0.23	1	0.178	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				192.73	0	9	o297-319i	0.109	20	0.102	36	0.12	27	0.098	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				190.93	0	9	o298-320i	0.124	20	0.146	20	0.259	2	0.185	0.164	N	0.57	SignalP-noTM														
CYT	-0.20091	0.57107			200.29	22.49	9	i33-55o79	0.102	64	0.103	11	0.132	1	0.109	0.106	N	0.51	SignalP-TM														
CYT	-0.20091				157.63	1.36	7	i145-167o	0.191	23	0.31	23	0.667	17	0.502	0.4	N	0.57	SignalP-noTM														
CYT	-0.20091				138.65	22.06	7	i28-50o15	0.106	44	0.107	17	0.139	9	0.112	0.109	N	0.51	SignalP-TM														
CYT	-0.20091				131.13	19.83	6	i42-64o68	0.112	25	0.086	25	0.107	70	0.07	0.08	N	0.51	SignalP-TM														
CYT	-0.20091	0.00782			117.61	22.62	5	i7-29o102	0.122	30	0.097	61	0.108	1	0.066	0.085	N	0.51	SignalP-TM														
CYT	-0.20091				109.89	21.45	5	i16-38o40	0.113	30	0.13	11	0.289	29	0.177	0.147	N	0.51	SignalP-TM														
CYT	-0.20091	1.46371			124.08	20.91	5	i7-24o339	0.116	30	0.167	11	0.355	2	0.284	0.21	N	0.51	SignalP-TM														
CYT	-0.20091	2.00927			81.11	33.92	4	o4-26i47-	0.113	21	0.109	11	0.178	3	0.119	0.112	N	0.51	SignalP-TM														
CYT	-0.20091				101.37	0	4	i122-144o	0.102	70	0.105	15	0.139	6	0.108	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				94.05	0	4	i282-299o	0.11	56	0.104	56	0.127	52	0.083	0.094	N	0.57	SignalP-noTM														
CYT	-0.20091				87.85	0	4	o265-287i	0.121	45	0.11	45	0.128	35	0.098	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				81.76	37.33	4	i7-24o29-	0.106	26	0.096	69	0.111	6	0.061	0.083	N	0.51	SignalP-TM														
CYT	-0.20091				63.48	22.4	3	o15-36i78	0.099	45	0.092	62	0.092	56	0.056	0.078	N	0.51	SignalP-TM														
CYT	-0.20091	2.58781			93.47	23.15	3	o28-50i62	0.164	27	0.164	27	0.239	16	0.169	0.166	N	0.51	SignalP-TM														
CYT	-0.20091	0.75566			76.09	28.39	3	i45-62o67	0.157	20	0.271	20	0.578	15	0.446	0.336	N	0.51	SignalP-TM														
CYT	-0.20091				44.73	0.06	2	i381-403o	0.147	27	0.253	23	0.605	20	0.438	0.34	N	0.57	SignalP-noTM														
CYT	-0.20091				36.01	12.27	2	o43-60i15	0.133	64	0.123	64	0.181	1	0.115	0.119	N	0.57	SignalP-noTM														
CYT	-0.20091				79.82	0	2	o331-352i	0.122	55	0.111	41	0.142	31	0.094	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091	1.14429			44.59	20.81	2	i25-44o47	0.128	51	0.139	13	0.199	10	0.183	0.155	N	0.51	SignalP-TM														
CYT	-0.20091	1.26635			44.01	21.41	2	o15-33i30	0.128	21	0.138	21	0.245	2	0.166	0.148	N	0.51	SignalP-TM														
CYT	-0.20091	1.08125			43.44	21.58	2	i7-29o473	0.113	54	0.141	11	0.264	1	0.2	0.163	N	0.51	SignalP-TM														
CYT	-0.20091				40.22	0.4	2	i85-102o1	0.127	24	0.148	24	0.288	23	0.166	0.156	N	0.57	SignalP-noTM														
CYT	-0.20091				41.59	9.35	2	i52-71o76	0.106	69	0.124	51	0.187	42	0.124	0.124	N	0.51	SignalP-TM														
CYT	-0.20091				13.91	10.34	1	i44-66o	0.124	22	0.105	43	0.138	61	0.094	0.101	N	0.51	SignalP-TM														
CYT	-0.20091	2.42188			42.7	5.15	1	i138-160o	0.168	19	0.304	19	0.796	1	0.546	0.418	N	0.57	SignalP-noTM														
CYT	-0.20091				22.2	0	1	o285-307i	0.111	48	0.106	21	0.134	15	0.104	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				23.03	0	1	i329-351o	0.101	43	0.102	66	0.115	57	0.089	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				22.6	0	1	i121-143o	0.167	56	0.121	56	0.142	8	0.098	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				27.68	0	1	i330-352o	0.104	26	0.102	30	0.143	2	0.094	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				19.72	0	1	o246-268i	0.105	39	0.138	22	0.235	11	0.162	0.149	N	0.57	SignalP-noTM														
CYT	-0.20091				19.95	0	1	i92-111o	0.143	38	0.117	38	0.13	35	0.094	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				22.64	0	1	i239-261o	0.139	30	0.12	30	0.164	7	0.111	0.116	N	0.57	SignalP-noTM														
CYT	-0.20091				23.15	0	1	o193-215i	0.155	30	0.127	30	0.134	7	0.102	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				22.32	0	1	o160-182i	0.115	43	0.11	59	0.143	51	0.101	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				22.69	0	1	o171-193i	0.122	41	0.121	20	0.195	1	0.132	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				21.55	0	1	o165-184i	0.101	27	0.106	11	0.143	5	0.11	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				20.25	0	1	i197-216o	0.104	28	0.118	11	0.171	1	0.137	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				22.69	0	1	o171-193i	0.122	41	0.121	20	0.195	1	0.132	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				19.78	0	1	i154-173o	0.102	28	0.106	40	0.127	2	0.098	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				22.69	0	1	o182-204i	0.122	41	0.121	20	0.195	1	0.132	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				20.18	0	1	i165-184o	0.103	28	0.12	11	0.181	5	0.141	0.13	N	0.57	SignalP-noTM														
CYT	-0.20091				21.58	0	1	i183-205o	0.105	40	0.118	11	0.171	2	0.136	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				22.68	0	1	o215-237i	0.124	38	0.112	38	0.131	12	0.101	0.107	N	0.57	SignalP-noTM														
CYT	-0.20091				22.7	0	1	o153-175i	0.128	20	0.124	20	0.191	1	0.129	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				22.68	0	1	o186-208i	0.124	38	0.113	38	0.133	12	0.103	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				21.17	0	1	i169-188o	0.101	28	0.131	11	0.217	1	0.167	0.148	N	0.57	SignalP-noTM														
CYT	-0.20091				10.57	9.24	0	o	0.162	22	0.225	22	0.479	21	0.302	0.253	N	0.51	SignalP-TM														
CYT	-0.20091				3.46	0.59	0	o	0.109	69	0.11	11	0.159	2	0.12	0.114	N	0.57	SignalP-noTM														
CYT	-0.20091				0.39	0	0	o	0.107	30	0.136	11	0.227	2	0.185	0.159	N	0.57	SignalP-noTM														
CYT	-0.20091				0.09	0.05	0	o	0.121	29	0.111	51	0.134	39	0.1	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				13.16	13.15	0	o	0.111	37	0.107	46	0.142	36	0.085	0.099	N	0.51	SignalP-TM														
CYT	-0.20091				0.66	0.12	0	o	0.114	36	0.122	11	0.208	1	0.145	0.133	N	0.57	SignalP-noTM														
CYT	-0.20091				3.55	0	0	o	0.103	22	0.123	15	0.204	12	0.144	0.133	N	0.57	SignalP-noTM														
CYT	-0.20091				0.92	0	0	o	0.104	34	0.123	17	0.176	11	0.152	0.137	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.108	18	0.107	30	0.129	27	0.101	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				2.22	2.22	0	o	0.314	37	0.263	37	0.351	32	0.178	0.223	N	0.57	SignalP-noTM														
CYT	-0.20091				0.09	0	0	o	0.101	54	0.114	11	0.164	1	0.13	0.121	N	0.57	SignalP-noTM														
CYT	-0.20091				3.01	0.01	0	o	0.122	21	0.123	21	0.156	12	0.117	0.121	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.11	21	0.111	21	0.141	14	0.11	0.111	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.101	37	0.103	68	0.117	62	0.088	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				2.68	0	0	o	0.117	22	0.183	22	0.426	18	0.282	0.23	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.139	39	0.125	11	0.201	2	0.155	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.26	20	0.219	20	0.263	3	0.205	0.212	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0	0	o	0.102	20	0.133	11	0.223	1	0.176	0.153	N	0.57	SignalP-noTM														
CYT	-0.20091				0.07	0	0	o	0.12	40	0.111	11	0.164	3	0.129	0.12	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.13	60	0.116	60	0.115	52	0.091	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0.01	0	o	0.107	46	0.101	59	0.113	50	0.09	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.13	13	0.153	13	0.289	12	0.18	0.166	N	0.57	SignalP-noTM														
CYT	-0.20091				2.76	2.17	0	o	0.107	53	0.106	53	0.143	51	0.104	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				0.42	0.42	0	o	0.141	25	0.15	25	0.214	22	0.152	0.151	N	0.57	SignalP-noTM														
CYT	-0.20091				33.83	7.17	0	o	0.207	22	0.294	22	0.708	4	0.489	0.386	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.1	39	0.103	39	0.12	34	0.091	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0.4	0	0	o	0.1	58	0.12	11	0.178	3	0.146	0.132	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.101	38	0.108	51	0.129	44	0.094	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.102	20	0.104	20	0.135	10	0.1	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091	0.75089			3.03	1.81	0	o	0.179	20	0.347	20	0.868	1	0.705	0.515	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.102	40	0.102	58	0.117	10	0.094	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0.05	0	o	0.171	41	0.144	11	0.271	3	0.203	0.172	N	0.57	SignalP-noTM														
CYT	-0.20091				0.79	0	0	o	0.11	29	0.106	12	0.132	6	0.113	0.109	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.11	25	0.119	25	0.147	14	0.127	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	59	0.097	70	0.108	44	0.088	0.093	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.111	21	0.126	11	0.214	6	0.167	0.146	N	0.57	SignalP-noTM														
CYT	-0.20091				0.84	0.83	0	o	0.125	62	0.128	11	0.225	2	0.156	0.141	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.01	0	o	0.106	24	0.196	20	0.519	12	0.324	0.256	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.118	25	0.107	25	0.132	24	0.08	0.094	N	0.57	SignalP-noTM														
CYT	-0.20091				8.56	0.02	0	o	0.131	20	0.121	20	0.171	6	0.126	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091	1.6882			22.99	11.46	0	o	0.179	21	0.211	21	0.345	18	0.244	0.223	N	0.51	SignalP-TM														
CYT	-0.20091				0.21	0	0	o	0.128	21	0.174	13	0.405	9	0.281	0.224	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.104	45	0.102	25	0.12	42	0.091	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.101	58	0.121	18	0.172	9	0.139	0.129	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.104	43	0.104	24	0.127	13	0.093	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.101	21	0.116	53	0.174	43	0.104	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.3	0	0	o	0.1	59	0.099	21	0.127	11	0.095	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.135	18	0.127	18	0.165	12	0.112	0.12	N	0.57	SignalP-noTM														
CYT	-0.20091				1.3	0.08	0	o	0.275	43	0.18	43	0.238	42	0.108	0.146	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0.05	0	o	0.108	45	0.136	50	0.259	44	0.117	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				0.55	0.16	0	o	0.107	32	0.111	32	0.128	21	0.105	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0	0	o	0.101	37	0.099	11	0.114	1	0.097	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0	0	o	0.118	31	0.106	31	0.117	49	0.089	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.11	55	0.103	68	0.116	9	0.092	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.114	37	0.125	37	0.187	33	0.114	0.12	N	0.57	SignalP-noTM														
CYT	-0.20091				0.14	0.04	0	o	0.107	22	0.111	22	0.134	1	0.114	0.112	N	0.57	SignalP-noTM														

LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)											Table 1													
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression	Immunogenicity	Tn mutant
CYT	-0.20091				0.03	0	0	o	0.209	55	0.134	11	0.231	4	0.182	0.157	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.125	63	0.106	63	0.127	9	0.092	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.113	15	0.108	15	0.139	1	0.108	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.16	0	0	o	0.11	24	0.102	24	0.157	1	0.089	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.106	36	0.106	25	0.14	18	0.1	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.111	31	0.106	31	0.138	22	0.097	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.1	68	0.101	39	0.117	23	0.091	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.02	0	o	0.113	22	0.111	22	0.123	20	0.101	0.107	N	0.57	SignalP-noTM														
CYT	-0.20091				0.27	0.24	0	o	0.199	20	0.132	20	0.157	39	0.081	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091	2.45359			1.23	1.19	0	o	0.131	32	0.162	32	0.321	30	0.203	0.181	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.105	31	0.104	42	0.118	38	0.096	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.107	33	0.107	48	0.14	1	0.101	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0	0	o	0.114	53	0.11	20	0.145	12	0.108	0.109	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.103	58	0.122	46	0.225	40	0.098	0.111	N	0.57	SignalP-noTM														
CYT	-0.20091				1.34	0.02	0	o	0.14	29	0.184	29	0.353	23	0.216	0.199	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0.03	0	o	0.114	24	0.108	24	0.124	23	0.095	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				2.61	0.13	0	i	0.152	36	0.158	62	0.366	4	0.156	0.157	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0	0	i	0.1	57	0.114	11	0.148	1	0.132	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.106	25	0.099	69	0.12	49	0.088	0.094	N	0.57	SignalP-noTM														
CYT	-0.20091				0.56	0	0	o	0.102	20	0.104	59	0.121	48	0.087	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.104	20	0.101	29	0.116	24	0.089	0.095	N	0.57	SignalP-noTM														
CYT	-0.20091				0.14	0.01	0	o	0.116	39	0.124	39	0.173	32	0.115	0.12	N	0.57	SignalP-noTM														
CYT	-0.20091				0.29	0.04	0	o	0.121	35	0.123	53	0.223	45	0.103	0.114	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.105	20	0.119	20	0.167	10	0.135	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.16	0	o	0.106	31	0.122	46	0.174	50	0.131	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				0.15	0.02	0	o	0.104	25	0.114	12	0.149	2	0.133	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				3.33	3.26	0	o	0.103	49	0.115	54	0.162	48	0.111	0.113	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0	0	o	0.103	21	0.098	67	0.113	8	0.092	0.095	N	0.57	SignalP-noTM														
CYT	-0.20091				3.25	0	0	o	0.123	46	0.107	20	0.166	1	0.11	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.111	32	0.131	11	0.21	2	0.171	0.15	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.105	50	0.108	28	0.133	23	0.101	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0.01	0	o	0.103	59	0.11	11	0.159	5	0.128	0.119	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	52	0.117	12	0.15	9	0.136	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.116	21	0.117	21	0.143	27	0.108	0.113	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.208	31	0.196	31	0.277	7	0.205	0.2	N	0.57	SignalP-noTM														
CYT	-0.20091				0.35	0.35	0	o	0.112	57	0.106	22	0.122	25	0.1	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.59	0	0	o	0.158	19	0.141	19	0.181	2	0.137	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				3.37	2.45	0	o	0.098	45	0.101	13	0.124	3	0.107	0.103	N	0.51	SignalP-TM														
CYT	-0.20091				5.46	0	0	o	0.104	21	0.103	32	0.141	3	0.095	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0.35	0	0	o	0.113	49	0.106	28	0.125	21	0.103	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				4.21	2.59	0	o	0.133	44	0.131	44	0.167	50	0.111	0.122	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.01	0	o	0.129	21	0.149	21	0.235	18	0.185	0.166	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	25	0.101	34	0.112	27	0.083	0.092	N	0.57	SignalP-noTM														
CYT	-0.20091				1.46	0.09	0	o	0.107	49	0.104	11	0.145	1	0.108	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0.26	0.01	0	o	0.107	39	0.12	39	0.183	4	0.134	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	10	0.108	43	0.124	37	0.085	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0.08	0.01	0	o	0.11	43	0.112	22	0.181	39	0.118	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.127	24	0.105	24	0.12	57	0.087	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.128	39	0.126	39	0.158	29	0.103	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.107	36	0.102	57	0.129	53	0.09	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.2	0.18	0	o	0.261	21	0.203	21	0.342	48	0.173	0.189	N	0.57	SignalP-noTM														
CYT	-0.20091	1.59468			2.42	0.04	0	o	0.133	41	0.129	26	0.2	21	0.153	0.14	N	0.57	SignalP-noTM														
CYT	-0.20091				0.8	0	0	o	0.199	64	0.137	64	0.187	19	0.115	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				2.91	0	0	o	0.101	41	0.106	41	0.139	1	0.105	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				0.51	0	0	o	0.119	27	0.115	11	0.229	1	0.119	0.117	N	0.57	SignalP-noTM														
CYT	-0.20091				1.94	0.49	0	o	0.156	35	0.146	35	0.186	34	0.131	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				0.16	0.07	0	o	0.137	47	0.121	47	0.148	2	0.099	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				1.24	0.01	0	o	0.103	29	0.134	12	0.247	8	0.186	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				0.69	0	0	o	0.103	47	0.1	69	0.107	61	0.092	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				0.11	0.11	0	o	0.119	17	0.197	17	0.409	3	0.341	0.25	N	0.51	SignalP-TM														
CYT	-0.20091				1.63	0	0	o	0.131	40	0.122	40	0.133	39	0.097	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.137	56	0.121	11	0.163	1	0.148	0.134	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.115	36	0.126	22	0.186	7	0.148	0.136	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.12	50	0.107	25	0.114	22	0.097	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.15	0.02	0	i	0.113	24	0.155	11	0.334	2	0.239	0.195	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.117	49	0.118	11	0.192	3	0.136	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.139	24	0.132	53	0.199	37	0.139	0.135	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0	0	o	0.102	25	0.101	70	0.139	24	0.097	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.129	23	0.113	23	0.156	1	0.101	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	i	0.106	67	0.128	14	0.189	52	0.165	0.145	N	0.57	SignalP-noTM														
CYT	-0.20091				1.33	0	0	o	0.128	18	0.165	18	0.367	17	0.21	0.186	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.105	16	0.12	16	0.162	10	0.135	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				12.28	0.02	0	o	0.136	28	0.14	28	0.264	7	0.162	0.151	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.122	47	0.123	47	0.159	34	0.104	0.114	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.122	43	0.111	43	0.144	12	0.105	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.102	47	0.105	38	0.142	27	0.092	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				5.7	5.59	0	o	0.129	25	0.204	11	0.595	1	0.388	0.29	N	0.57	SignalP-noTM														
CYT	-0.20091				0.75	0.74	0	o	0.112	36	0.127	11	0.222	5	0.17	0.147	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.135	26	0.111	26	0.146	32	0.101	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0.5	0.49	0	o	0.125	31	0.163	11	0.312	6	0.277	0.216	N	0.57	SignalP-noTM														
CYT	-0.20091				0.82	0	0	o	0.161	32	0.126	32	0.137	2	0.101	0.114	N	0.57	SignalP-noTM														
CYT	-0.20091				0.14	0.01	0	o	0.143	59	0.117	59	0.161	7	0.102	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.01	0	o	0.128	60	0.112	60	0.113	53	0.085	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				17.32	0	0	o	0.108	28	0.105	14	0.122	11	0.111	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.115	52	0.165	16	0.342	8	0.272	0.215	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0	0	o	0.103	27	0.11	35	0.136	26	0.102	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	29	0.1	34	0.112	32	0.084	0.092	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.17	0	o	0.161	39	0.159	51	0.276	46	0.109	0.136	N	0.57	SignalP-noTM														
CYT	-0.20091				0.11	0	0	o	0.11	44	0.105	11	0.125	6	0.113	0.109	N	0.57	SignalP-noTM														
CYT	-0.20091				0.21	0	0	o	0.138	37	0.113	37	0.121	36	0.093	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0.01	0	o	0.116	33	0.12	18	0.159	11	0.129	0.124	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0.04	0	o	0.271	22	0.403	22	0.722	11	0.582	0.487	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.06	0	o	0.11	34	0.112	51	0.142	41	0.093	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				13.97	0.56	0	o	0.117	48	0.194	13	0.456	9	0.375	0.279	N	0.57	SignalP-noTM														
CYT	-0.20091				3.35	0	0	o	0.103	21	0.095	31	0.103	70	0.086	0.091	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.113	47	0.109	47	0.113	40	0.093	0.101	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.106	37	0.096	68	0.105	44	0.081	0.089	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0.04	0	o	0.11	39	0.155	11	0.365	1	0.226	0.189	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.137	17	0.13	17	0.205	10	0.116	0.124	N	0.57	SignalP-noTM														
CYT	-0.20091				0.08	0.03	0	o	0.107	23	0.102	23	0.132	1	0.096	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0.02	0	o	0.105	36	0.11	41	0.132	35	0.105	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				2.38	0.02	0	o	0.111	56	0.136	11	0.259	2	0.185	0.159	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.105	39	0.105	26	0.127	20	0.101	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0.02	0	o	0.111	46	0.099	46	0.104	15	0.09	0.095	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.107	56	0.1	32	0.13	6	0.095	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.22	0.01	0	o	0.139	18	0.144	18	0.244	1	0.155	0.149	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.137	45	0.131	45	0.189	43	0.102	0.117	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.101	55	0.111	14	0.156	6	0.123	0.117	N	0.57	SignalP-noTM														
CYT	-0.20091				13.02	2.05	0	o	0.109	63	0.1	63	0.117	49	0.087	0.094	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.105	29	0.122	11	0.187	4	0.152	0.136	N	0.57	SignalP-noTM														
CYT	-0.20091				0.18	0.01	0	o	0.102	42	0.121	11	0.219	1	0.135	0.128	N	0.57	SignalP-noTM														
CYT	-0.20091				9.03	0	0	o	0.1	34	0.106	26	0.131	12	0.103	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				0.59	0.33	0	o	0.185	24	0.193	24	0.417	45	0.214	0.203	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.01	0	o	0.113	10	0.135	13	0.227	9	0.183	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.113	30	0.115	11	0.201	3	0.131	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0.03	0	o	0.105	20	0.126	17	0.177	16	0.151	0.138	N	0.57	SignalP-noTM														
CYT	-0.20091				0.07	0	0	o	0.104	31	0.11	57	0.139	48	0.108	0.109	N	0.57	SignalP-noTM														

LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)											Table 1													
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/ dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression	Immunogenicity	Tn mutant
CYT	-0.20091				0.05	0	0	o	0.103	21	0.163	11	0.346	1	0.258	0.208	N	0.57	SignalP-noTM														
CYT	-0.20091				0.37	0.34	0	o	0.109	55	0.12	55	0.164	47	0.12	0.12	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.103	46	0.101	23	0.127	19	0.104	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.119	50	0.151	11	0.334	1	0.218	0.183	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.124	48	0.107	48	0.117	29	0.097	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.254	36	0.167	36	0.198	9	0.126	0.148	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.103	46	0.111	11	0.172	3	0.127	0.119	N	0.57	SignalP-noTM														
CYT	-0.20091				0.2	0	0	o	0.106	18	0.114	11	0.16	2	0.132	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.106	18	0.104	50	0.122	40	0.095	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				0.09	0	0	i	0.121	44	0.139	12	0.222	2	0.196	0.166	N	0.57	SignalP-noTM														
CYT	-0.20091				0.28	0.21	0	o	0.123	34	0.149	34	0.247	22	0.162	0.155	N	0.57	SignalP-noTM														
CYT	-0.20091				0.08	0.08	0	o	0.104	60	0.159	11	0.331	1	0.248	0.201	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	35	0.135	12	0.205	6	0.182	0.157	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.217	46	0.157	46	0.201	29	0.12	0.14	N	0.57	SignalP-noTM														
CYT	-0.20091				0.11	0.1	0	o	0.117	49	0.13	49	0.206	48	0.126	0.128	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	61	0.109	61	0.132	43	0.096	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.116	33	0.192	16	0.544	12	0.361	0.271	N	0.57	SignalP-noTM														
CYT	-0.20091				0.63	0.02	0	o	0.109	52	0.111	52	0.146	1	0.106	0.109	N	0.57	SignalP-noTM														
CYT	-0.20091				2.45	2.28	0	o	0.331	18	0.349	18	0.429	14	0.361	0.354	N	0.51	SignalP-TM														
CYT	-0.20091				0.05	0	0	o	0.108	28	0.12	19	0.186	18	0.142	0.13	N	0.57	SignalP-noTM														
CYT	-0.20091				2.43	0	0	o	0.102	25	0.104	14	0.121	9	0.109	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.118	18	0.116	18	0.146	2	0.12	0.118	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.099	31	0.122	18	0.191	13	0.133	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.15	0	o	0.109	9	0.112	51	0.144	43	0.103	0.107	N	0.57	SignalP-noTM														
CYT	-0.20091				0.2	0	0	o	0.102	53	0.103	53	0.137	38	0.089	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				5.88	1.75	0	o	0.446	22	0.285	22	0.392	19	0.169	0.231	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.02	0	o	0.108	29	0.153	11	0.298	1	0.227	0.188	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0.02	0	o	0.124	30	0.166	30	0.386	25	0.202	0.183	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.101	62	0.134	11	0.248	3	0.178	0.155	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.104	24	0.121	22	0.188	14	0.133	0.127	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.15	36	0.115	36	0.144	1	0.099	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.108	42	0.104	42	0.107	35	0.089	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0	0	o	0.127	22	0.136	11	0.273	20	0.181	0.157	N	0.57	SignalP-noTM														
CYT	-0.20091				13.78	0.03	0	o	0.113	27	0.138	16	0.239	14	0.182	0.159	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0.03	0	o	0.131	30	0.145	15	0.274	9	0.203	0.172	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.126	43	0.12	43	0.16	40	0.085	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.19	0.01	0	o	0.109	26	0.145	11	0.324	2	0.202	0.172	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.127	22	0.121	12	0.165	3	0.146	0.133	N	0.57	SignalP-noTM														
CYT	-0.20091				0.34	0	0	o	0.103	26	0.105	11	0.14	1	0.11	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.169	26	0.148	26	0.163	1	0.128	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				6.5	5.42	0	o	0.113	59	0.104	40	0.139	1	0.082	0.096	N	0.51	SignalP-TM														
CYT	-0.20091				0.77	0.12	0	o	0.141	20	0.176	20	0.332	15	0.196	0.185	N	0.57	SignalP-noTM														
CYT	-0.20091	1.51431			41.49	20.78	0	o	0.222	21	0.327	21	0.736	11	0.52	0.418	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0.03	0	o	0.222	60	0.15	60	0.128	5	0.09	0.122	N	0.57	SignalP-noTM														
CYT	-0.20091				0.07	0	0	o	0.107	70	0.101	37	0.111	33	0.087	0.095	N	0.57	SignalP-noTM														
CYT	-0.20091				0.94	0.01	0	o	0.139	22	0.134	22	0.189	17	0.131	0.132	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0	0	o	0.122	26	0.098	32	0.128	1	0.075	0.087	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.156	33	0.128	20	0.16	2	0.123	0.126	N	0.57	SignalP-noTM														
CYT	-0.20091				12.98	0.02	0	o	0.237	26	0.23	26	0.392	19	0.192	0.212	N	0.57	SignalP-noTM														
CYT	-0.20091				0.46	0	0	o	0.118	33	0.117	33	0.14	20	0.113	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				0.12	0	0	o	0.108	36	0.18	11	0.444	4	0.346	0.258	N	0.57	SignalP-noTM														
CYT	-0.20091				4.96	0	0	o	0.127	29	0.163	21	0.321	18	0.267	0.212	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.107	44	0.104	23	0.122	14	0.095	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				2.83	2.83	0	o	0.361	19	0.365	19	0.522	2	0.393	0.375	N	0.51	SignalP-TM														
CYT	-0.20091				14.06	11.82	0	o	0.116	25	0.111	11	0.175	1	0.122	0.115	N	0.51	SignalP-TM														
CYT	-0.20091				0.48	0.47	0	o	0.207	34	0.202	21	0.471	9	0.373	0.282	N	0.57	SignalP-noTM														
CYT	-0.20091				0.11	0.02	0	o	0.103	30	0.161	20	0.351	12	0.228	0.193	N	0.57	SignalP-noTM														
CYT	-0.20091				0.49	0	0	o	0.117	48	0.109	48	0.116	46	0.095	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.09	0	o	0.119	24	0.149	43	0.349	37	0.155	0.152	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.101	33	0.105	20	0.122	11	0.107	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.104	28	0.104	35	0.145	27	0.101	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.92	0.81	0	o	0.113	34	0.161	21	0.361	2	0.244	0.2	N	0.57	SignalP-noTM														
CYT	-0.20091				0.08	0.05	0	o	0.106	57	0.12	11	0.196	3	0.141	0.13	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.104	28	0.158	11	0.37	1	0.235	0.194	N	0.57	SignalP-noTM														
CYT	-0.20091				0.22	0.02	0	o	0.109	29	0.121	11	0.207	2	0.145	0.132	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0	0	o	0.103	31	0.113	42	0.166	40	0.107	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.109	52	0.124	13	0.174	1	0.155	0.138	N	0.57	SignalP-noTM														
CYT	-0.20091				6.48	0.01	0	o	0.119	31	0.117	31	0.167	8	0.101	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				8.04	0.06	0	o	0.14	25	0.203	25	0.457	1	0.332	0.263	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.12	40	0.114	40	0.156	1	0.103	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				1.31	0.02	0	o	0.133	58	0.114	11	0.149	2	0.13	0.121	N	0.57	SignalP-noTM														
CYT	-0.20091				0.27	0.06	0	o	0.109	40	0.125	22	0.202	3	0.155	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				1.31	1.28	0	o	0.102	46	0.111	28	0.134	18	0.108	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0.03	0	o	0.168	36	0.177	18	0.36	15	0.241	0.207	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.109	39	0.106	57	0.115	53	0.089	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.108	3	0.107	29	0.174	2	0.11	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.117	17	0.131	17	0.234	1	0.155	0.143	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0.02	0	o	0.107	35	0.125	40	0.172	33	0.124	0.125	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.105	43	0.105	68	0.117	56	0.097	0.101	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.124	67	0.106	22	0.134	13	0.1	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.21	0.21	0	o	0.188	39	0.137	39	0.113	36	0.09	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				0.8	0	0	o	0.115	20	0.106	41	0.121	35	0.087	0.097	N	0.57	SignalP-noTM														
CYT	-0.20091				0.07	0	0	o	0.111	21	0.114	21	0.13	15	0.107	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.43	0.27	0	o	0.117	30	0.115	30	0.231	49	0.1	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.2	0.2	0	o	0.265	20	0.259	20	0.382	19	0.261	0.259	N	0.51	SignalP-TM														
CYT	-0.20091				0.56	0	0	o	0.123	25	0.125	11	0.195	1	0.155	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				0.75	0.64	0	o	0.124	31	0.157	18	0.322	15	0.217	0.186	N	0.57	SignalP-noTM														
CYT	-0.20091				0.23	0	0	o	0.157	36	0.115	36	0.112	11	0.089	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				4.16	0.08	0	o	0.121	30	0.151	17	0.34	16	0.224	0.185	N	0.57	SignalP-noTM														
CYT	-0.20091				11.68	0.52	0	o	0.149	45	0.117	45	0.113	18	0.093	0.105	N	0.57	SignalP-noTM														
CYT	-0.20091				18.5	1.03	0	o	0.113	22	0.11	22	0.146	1	0.108	0.109	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.02	0	o	0.105	46	0.112	11	0.194	1	0.117	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				2.61	0	0	o	0.109	33	0.108	12	0.141	12	0.117	0.112	N	0.57	SignalP-noTM														
CYT	-0.20091				0.83	0.03	0	o	0.137	33	0.189	33	0.384	29	0.209	0.199	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.136	45	0.118	13	0.16	8	0.132	0.124	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	22	0.107	32	0.145	69	0.095	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.01	0	o	0.119	26	0.179	11	0.396	1	0.316	0.243	N	0.57	SignalP-noTM														
CYT	-0.20091				0.23	0.04	0	o	0.112	39	0.106	39	0.127	1	0.099	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	53	0.107	69	0.122	65	0.095	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.36	0	0	o	0.109	49	0.127	22	0.197	18	0.152	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				0.57	0.25	0	o	0.115	22	0.188	18	0.432	10	0.32	0.237	N	0.51	SignalP-TM														
CYT	-0.20091				0	0	0	o	0.101	27	0.102	11	0.121	2	0.105	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.099	39	0.104	37	0.127	30	0.095	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				1.71	0.39	0	o	0.135	51	0.131	12	0.212	4	0.171	0.15	N	0.57	SignalP-noTM														
CYT	-0.20091				1.35	0.03	0	o	0.155	37	0.13	37	0.144	33	0.103	0.117	N	0.57	SignalP-noTM														
CYT	-0.20091				0.16	0.05	0	o	0.113	21	0.174	11	0.409	1	0.292	0.229	N	0.57	SignalP-noTM														
CYT	-0.20091				0.48	0.03	0	o	0.123	24	0.133	11	0.212	1	0.175	0.153	N	0.57	SignalP-noTM														
CYT	-0.20091				0.08	0	0	o	0.106	42	0.107	69	0.138	2	0.101	0.104	N	0.57	SignalP-noTM														
CYT	-0.20091				0.51	0.18	0	o	0.103	62	0.158	11	0.311	2	0.249	0.201	N	0.57	SignalP-noTM														
CYT	-0.20091				0.21	0.19	0	o	0.13	40	0.138	40	0.171	28	0.131	0.135	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0.05	0	o	0.119	44	0.252	20	0.669	18	0.569	0.401	N	0.57	SignalP-noTM														
CYT	-0.20091				1.74	0.1	0	o	0.107	49	0.111	42	0.144	15	0.105	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.135	36	0.121	36	0.165	2	0.104	0.113	N	0.57	SignalP-noTM														
CYT	-0.20091				0.05	0.02	0	o	0.172	21	0.244	21	0.468	17	0.317	0.278	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.106	24	0.099	43	0.107	41	0.09	0.095	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.122	27	0.158	11	0.355	2	0.248	0.2	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0	0	o	0.104	47	0.113	31	0.177	45	0.107	0.11	N	0.57	SignalP-noTM														

LipoP1.0 (http://www.cbs.dtu.dk/services/LipoP/)					TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/)				SignalP4.1 gram- predictions (http://www.cbs.dtu.dk/services/SignalP/)											Table 1													
Location	Score	Margin	Cleavage	Pos+2	ExpAA	First60	PredHel	Topology	Cmax	pos	Ymax	pos	Smax	pos	Smean	D	SignalP4.1 Consensus	Dmaxcut	Networks-used	Protein Name	Consens. Loc.	His-tag Loc.	dNSAF-pK/dNSAF+pK	Previous Location	References for Prev. Loc. (PMID)	Predicted MW (kDa)	Observed M.W. (kDa)	Paralogous Family (Casjens 2000)	Number of Paralogs	Comments	in vivo Differential Expression	Immunogenicity	Tn mutant
CYT	-0.20091				0.08	0	0	o	0.107	10	0.107	40	0.125	35	0.09	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				20.09	4.89	0	o	0.121	21	0.111	11	0.171	1	0.119	0.115	N	0.57	SignalP-noTM														
CYT	-0.20091				7.65	7.65	0	o	0.202	39	0.275	39	0.577	29	0.35	0.31	N	0.57	SignalP-noTM														
CYT	-0.20091				0.39	0	0	o	0.111	32	0.116	32	0.158	29	0.099	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				0.25	0.08	0	i	0.117	40	0.135	11	0.207	8	0.183	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	31	0.101	65	0.11	5	0.094	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				3.03	2.72	0	o	0.122	58	0.135	11	0.236	1	0.183	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				0.77	0.61	0	o	0.111	53	0.103	59	0.116	52	0.088	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				4.13	4.13	0	i	0.113	36	0.138	20	0.282	15	0.17	0.153	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.02	0	o	0.111	37	0.144	11	0.301	1	0.198	0.17	N	0.57	SignalP-noTM														
CYT	-0.20091				1.3	0.25	0	o	0.125	36	0.136	36	0.263	32	0.159	0.146	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.107	45	0.104	31	0.121	56	0.101	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.94	0	0	o	0.109	54	0.112	16	0.156	31	0.124	0.118	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.105	33	0.157	11	0.303	3	0.25	0.201	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	i	0.105	43	0.108	43	0.139	39	0.095	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				0.34	0.06	0	o	0.143	32	0.148	41	0.342	39	0.146	0.147	N	0.57	SignalP-noTM														
CYT	-0.20091				6.73	0.34	0	o	0.12	26	0.137	35	0.251	10	0.153	0.145	N	0.57	SignalP-noTM														
CYT	-0.20091				0.07	0.06	0	o	0.107	26	0.129	11	0.194	8	0.173	0.15	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0.01	0	o	0.122	38	0.117	12	0.162	1	0.134	0.125	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	i	0.119	38	0.106	38	0.106	64	0.093	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				13.23	0	0	o	0.112	42	0.126	11	0.249	1	0.154	0.139	N	0.57	SignalP-noTM														
CYT	-0.20091				2.89	0	0	o	0.104	51	0.111	11	0.159	3	0.124	0.117	N	0.57	SignalP-noTM														
CYT	-0.20091				0.3	0.15	0	o	0.107	66	0.103	35	0.141	2	0.102	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.11	0	0	o	0.162	19	0.158	19	0.198	11	0.134	0.146	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.128	47	0.107	11	0.145	2	0.112	0.11	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.101	30	0.125	11	0.2	2	0.153	0.138	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.137	38	0.144	11	0.349	1	0.185	0.163	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.104	52	0.104	11	0.125	1	0.11	0.107	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0.03	0	o	0.105	29	0.108	60	0.149	1	0.098	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				16.35	1.55	0	o	0.153	47	0.205	47	0.43	35	0.223	0.214	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	66	0.107	24	0.129	17	0.105	0.106	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.105	55	0.107	44	0.121	15	0.099	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0.17	0.17	0	o	0.114	32	0.132	20	0.185	1	0.162	0.146	N	0.57	SignalP-noTM														
CYT	-0.20091				0.25	0	0	o	0.106	46	0.114	46	0.146	39	0.11	0.112	N	0.57	SignalP-noTM														
CYT	-0.20091				0.06	0	0	o	0.122	29	0.108	29	0.155	1	0.09	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0	0	o	0.101	32	0.101	32	0.121	3	0.097	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	i	0.387	22	0.206	22	0.136	20	0.104	0.158	N	0.57	SignalP-noTM														
CYT	-0.20091				0.19	0.19	0	o	0.108	36	0.121	28	0.193	23	0.14	0.13	N	0.57	SignalP-noTM														
CYT	-0.20091				0.5	0.33	0	o	0.104	21	0.151	21	0.257	9	0.215	0.181	N	0.57	SignalP-noTM														
CYT	-0.20091				0.1	0.01	0	o	0.122	50	0.131	50	0.27	49	0.118	0.124	N	0.57	SignalP-noTM														
CYT	-0.20091				6.73	2.55	0	o	0.185	23	0.33	23	0.668	15	0.533	0.425	N	0.57	SignalP-noTM														
CYT	-0.20091				0.04	0.04	0	o	0.105	29	0.137	11	0.247	3	0.194	0.164	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.112	24	0.104	24	0.131	10	0.1	0.102	N	0.57	SignalP-noTM														
CYT	-0.20091				3.35	0	0	o	0.103	55	0.126	11	0.21	4	0.155	0.14	N	0.57	SignalP-noTM														
CYT	-0.20091				0.34	0.34	0	o	0.133	21	0.186	19	0.325	17	0.253	0.211	N	0.51	SignalP-TM														
CYT	-0.20091				0.1	0	0	o	0.102	21	0.11	21	0.149	24	0.117	0.113	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.102	18	0.123	11	0.228	2	0.156	0.138	N	0.57	SignalP-noTM														
CYT	-0.20091				2.82	2.81	0	o	0.116	44	0.182	11	0.397	3	0.325	0.249	N	0.57	SignalP-noTM														
CYT	-0.20091				5.84	4.86	0	o	0.113	33	0.132	21	0.199	28	0.151	0.141	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	i	0.101	44	0.134	11	0.215	3	0.187	0.159	N	0.57	SignalP-noTM														
CYT	-0.20091				0.47	0.38	0	o	0.127	47	0.176	21	0.392	12	0.279	0.225	N	0.57	SignalP-noTM														
CYT	-0.20091				3.44	3.39	0	o	0.118	33	0.159	17	0.309	13	0.248	0.201	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.108	30	0.104	11	0.147	3	0.111	0.108	N	0.57	SignalP-noTM														
CYT	-0.20091				3.89	3.89	0	o	0.111	33	0.156	21	0.274	13	0.221	0.186	N	0.57	SignalP-noTM														
CYT	-0.20091				1.22	1.2	0	o	0.146	29	0.178	29	0.311	13	0.22	0.198	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.1	43	0.109	45	0.135	13	0.096	0.103	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.121	45	0.107	22	0.134	14	0.094	0.101	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0.02	0	o	0.106	26	0.118	26	0.23	18	0.129	0.123	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0.01	0	o	0.252	21	0.26	21	0.483	19	0.258	0.259	N	0.57	SignalP-noTM														
CYT	-0.20091				0.96	0.78	0	o	0.134	47	0.167	24	0.343	23	0.219	0.191	N	0.57	SignalP-noTM														
CYT	-0.20091				0.03	0	0	o	0.101	54	0.104	69	0.112	61	0.092	0.098	N	0.57	SignalP-noTM														
CYT	-0.20091				0.01	0	0	o	0.111	66	0.105	66	0.127	40	0.093	0.099	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.121	45	0.107	22	0.134	14	0.094	0.101	N	0.57	SignalP-noTM														
CYT	-0.20091	1.474			0.02	0.02	0	o	0.277	21	0.3	21	0.575	19	0.324	0.311	N	0.57	SignalP-noTM														
CYT	-0.20091				1.14	1.14	0	o	0.125	40	0.18	24	0.331	12	0.241	0.208	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	55	0.114	11	0.212	1	0.123	0.118	N	0.57	SignalP-noTM														
CYT	-0.20091				1.48	1.47	0	o	0.106	51	0.12	14	0.169	8	0.144	0.131	N	0.57	SignalP-noTM														
CYT	-0.20091				0.02	0	0	o	0.109	66	0.105	66	0.13	40	0.094	0.1	N	0.57	SignalP-noTM														
CYT	-0.20091				1.52	1.5	0	o	0.132	33	0.178	24	0.346	23	0.242	0.208	N	0.57	SignalP-noTM														
CYT	-0.20091				0	0	0	o	0.1	32	0.101	32	0.111	22	0.09	0.096	N	0.57	SignalP-noTM														
CYT	-0.20091				6.8	6.69	0	o	0.101	33	0.107	11	0.142	1	0.115	0.11	N	0.51	SignalP-TM														
CYT	-0.20091				0	0	0	o	0.1	21	0.115	35	0.146	29	0.113	0.114	N	0.57	SignalP-noTM														
CYT	-0.20091				4.43	1.66	0	o	0.134	40	0.189	24	0.384	23	0.263	0.224	N	0.57	SignalP-noTM														
CYT	-0.20091				8.38	8.36	0	o	0.106	32	0.182	13	0.415	9	0.332	0.252	N	0.57	SignalP-noTM														
CYT	-0.20091				0.15	0	0	o	0.102	70	0.114	11	0.21	1	0.121	0.117	N	0.57	SignalP-noTM														

References

1. **Benach JL, Garcia-Monco JC.** 2010. The Worldwide Saga of Lyme Borreliosis. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.
2. **Wormser GP, Wormser V.** 2016. Did Garin and Bujadoux Actually Report a Case of Lyme Radiculoneuritis? Open Forum Infect Dis **3:**ofw085.
3. **Garin C, Bujadoux A.** 1993. Paralysis by ticks. 1922. Clin Infect Dis **16:**168-169.
4. **Askani H.** 1936. Zur Ätiologie des Erythema chronicum migrans Dermatol Wochenschr **102:**125-131.
5. **Keller A, Graefen A, Ball M, Matzas M, Boisguerin V, Maixner F, Leidinger P, Backes C, Khairat R, Forster M, Stade B, Franke A, Mayer J, Spangler J, McLaughlin S, Shah M, Lee C, Harkins TT, Sartori A, Moreno-Estrada A, Henn B, Sikora M, Semino O, Chiaroni J, Rootsi S, Myres NM, Cabrera VM, Underhill PA, Bustamante CD, Vigl EE, Samadelli M, Cipollini G, Haas J, Katus H, O'Connor BD, Carlson MR, Meder B, Blin N, Meese E, Pusch CM, Zink A.** 2012. New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. Nat Commun **3:**698.
6. **Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, Steele FM.** 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum **20:**7-17.
7. **Steere AC, Batsford WP, Weinberg M, Alexander J, Berger HJ, Wolfson S, Malawista SE.** 1980. Lyme carditis: cardiac abnormalities of Lyme disease. Ann Intern Med **93:**8-16.
8. **Steere AC, Malawista SE, Newman JH, Spieler PN, Bartenhagen NH.** 1980. Antibiotic therapy in Lyme disease. Ann Intern Med **93:**1-8.
9. **Steere AC, Broderick TF, Malawista SE.** 1978. Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am J Epidemiol **108:**312-321.
10. **Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP.** 1982. Lyme disease-a tick-borne spirochetosis? Science **216:**1317-1319.
11. **Benach JL, Bosler EM, Hanrahan JP, Coleman JL, Habicht GS, Bast TF, Cameron DJ, Ziegler JL, Barbour AG, Burgdorfer W, Edelman R, Kaslow RA.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med **308:**740-742.
12. **Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE.** 1983. The spirochetal etiology of Lyme disease. N Engl J Med **308:**733-740.
13. **Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ.** 1984. Borrelia burgdorferi sp. nov.: Etiologic Agent of Lyme Disease. International Journal of Systematic and Evolutionary Microbiology **34:**496-497.
14. **CDC.** December 14, 2016. Lyme Disease Frequently Asked Questions (FAQ). https://www.cdc.gov/lyme/faq/index.html. Accessed February 7, 2017.
15. **NIAID.** October 26, 2016. NIAID Emerging Infectious Diseases/Pathogens. https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens. Accessed February 7, 2017.
16. **Pal U, Fikrig E.** 2010. Tick Interactions. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.

17. **Radolf JD, Caimano MJ, Stevenson B, Hu LT.** 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat Rev Microbiol **10:**87-99.
18. **Barthold SW, Cadavid D, Philipp MT.** 2010. Animal Models of Borreliosis. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.
19. **Iyer R, Caimano MJ, Luthra A, Axline D, Jr., Corona A, Iacobas DA, Radolf JD, Schwartz I.** 2015. Stage-specific global alterations in the transcriptomes of Lyme disease spirochetes during tick feeding and following mammalian host adaptation. Mol Microbiol **95:**509-538.
20. **Francis E.** 1938. Longevity of the Tick Ornithodoros turicata and of Spirochaeta recurrentis within This Tick. Public Health Reports (1896-1970) **53:**2220-2241.
21. **Ribeiro JM, Alarcon-Chaidez F, Francischetti IM, Mans BJ, Mather TN, Valenzuela JG, Wikel SK.** 2006. An annotated catalog of salivary gland transcripts from Ixodes scapularis ticks. Insect Biochem Mol Biol **36:**111-129.
22. **Pal U, Yang X, Chen M, Bockenstedt LK, Anderson JF, Flavell RA, Norgard MV, Fikrig E.** 2004. OspC facilitates Borrelia burgdorferi invasion of Ixodes scapularis salivary glands. J Clin Invest **113:**220-230.
23. **Setubal JC, Reis M, Matsunaga J, Haake DA.** 2006. Lipoprotein computational prediction in spirochaetal genomes. Microbiology **152:**113-121.
24. **Schutzer SE, Fraser-Liggett CM, Casjens SR, Qiu WG, Dunn JJ, Mongodin EF, Luft BJ.** 2011. Whole-genome sequences of thirteen isolates of Borrelia burgdorferi. J Bacteriol **193:**1018-1020.
25. **Zuckert WR.** 2014. Secretion of bacterial lipoproteins: through the cytoplasmic membrane, the periplasm and beyond. Biochim Biophys Acta **1843:**1509-1516.
26. **Narita SI, Tokuda H.** 2016. Bacterial lipoproteins; biogenesis, sorting and quality control. Biochim Biophys Acta doi:10.1016/j.bbalip.2016.11.009.
27. **Dowdell AS, Murphy MD, Azodi C, Swanson SK, Florens L, Chen S, Zuckert WR.** 2017. Comprehensive Spatial Analysis of the Borrelia burgdorferi Lipoproteome Reveals a Compartmentalization Bias toward the Bacterial Surface. J Bacteriol **199**.
28. **Kumru OS, Schulze RJ, Rodnin MV, Ladokhin AS, Zuckert WR.** 2011. Surface localization determinants of Borrelia OspC/Vsp family lipoproteins. J Bacteriol **193:**2814-2825.
29. **Schulze RJ, Chen S, Kumru OS, Zuckert WR.** 2010. Translocation of Borrelia burgdorferi surface lipoprotein OspA through the outer membrane requires an unfolded conformation and can initiate at the C-terminus. Mol Microbiol **76:**1266-1278.
30. **Schulze RJ, Zuckert WR.** 2006. Borrelia burgdorferi lipoproteins are secreted to the outer surface by default. Mol Microbiol **59:**1473-1484.
31. **Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson RJ, Haft D, Hickey E, Gwinn M, White O, Fraser CM.** 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete Borrelia burgdorferi. Mol Microbiol **35:**490-516.
32. **Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J,**

Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC. 1997. Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. Nature **390:**580-586.

33. **Tam R, Saier MH, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol Rev **57:**320-346.
34. **Bono JL, Tilly K, Stevenson B, Hogan D, Rosa P.** 1998. Oligopeptide permease in Borrelia burgdorferi: putative peptide-binding components encoded by both chromosomal and plasmid loci. Microbiology **144 (Pt 4):**1033-1044.
35. **Kornacki JA, Oliver DB.** 1998. Lyme disease-causing Borrelia species encode multiple lipoproteins homologous to peptide-binding proteins of ABC-type transporters. Infect Immun **66:**4115-4122.
36. **Wang XG, Kidder JM, Scagliotti JP, Klempner MS, Noring R, Hu LT.** 2004. Analysis of differences in the functional properties of the substrate binding proteins of the Borrelia burgdorferi oligopeptide permease (Opp) operon. J Bacteriol **186:**51-60.
37. **Lin T, Gao L, Zhang C, Odeh E, Jacobs MB, Coutte L, Chaconas G, Philipp MT, Norris SJ.** 2012. Analysis of an ordered, comprehensive STM mutant library in infectious Borrelia burgdorferi: insights into the genes required for mouse infectivity. PLoS One **7:**e47532.
38. **Lin B, Short SA, Eskildsen M, Klempner MS, Hu LT.** 2001. Functional testing of putative oligopeptide permease (Opp) proteins of Borrelia burgdorferi: a complementation model in opp(-) Escherichia coli. Biochim Biophys Acta **1499:**222-231.
39. **Medrano MS, Ding Y, Wang XG, Lu P, Coburn J, Hu LT.** 2007. Regulators of expression of the oligopeptide permease A proteins of Borrelia burgdorferi. J Bacteriol **189:**2653-2659.
40. **Wang XG, Lin B, Kidder JM, Telford S, Hu LT.** 2002. Effects of environmental changes on expression of the oligopeptide permease (opp) genes of Borrelia burgdorferi. J Bacteriol **184:**6198-6206.
41. **Ouyang Z, Kumar M, Kariu T, Haq S, Goldberg M, Pal U, Norgard MV.** 2009. BosR (BB0647) governs virulence expression in Borrelia burgdorferi. Mol Microbiol **74:**1331-1343.
42. **Raju BV, Esteve-Gassent MD, Karna SL, Miller CL, Van Laar TA, Seshu J.** 2011. Oligopeptide permease A5 modulates vertebrate host-specific adaptation of Borrelia burgdorferi. Infect Immun **79:**3407-3420.
43. **Brautigam CA, Ouyang Z, Deka RK, Norgard MV.** 2014. Sequence, biophysical, and structural analyses of the PstS lipoprotein (BB0215) from Borrelia burgdorferi reveal a likely binding component of an ABC-type phosphate transporter. Protein Sci **23:**200-212.
44. **Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer KH, Ludwig W, Glockner FO, Rossello-Mora R.** 2008. The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst Appl Microbiol **31:**241-250.
45. **Barbour AG, Tessier SL, Todd WJ.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect Immun **41:**795-804.
46. **Brandt ME, Riley BS, Radolf JD, Norgard MV.** 1990. Immunogenic integral membrane proteins of Borrelia burgdorferi are lipoproteins. Infect Immun **58:**983-991.

47. **Erdile LF, Brandt MA, Warakomski DJ, Westrack GJ, Sadziene A, Barbour AG, Mays JP.** 1993. Role of attached lipid in immunogenicity of Borrelia burgdorferi OspA. Infect Immun **61:**81-90.
48. **Centers for Disease C, Prevention.** 1999. Availability of Lyme disease vaccine. MMWR Morb Mortal Wkly Rep **48:**35-36, 43.
49. **Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA.** 1995. Induction of an outer surface protein on Borrelia burgdorferi during tick feeding. Proc Natl Acad Sci U S A **92:**2909-2913.
50. **Pal U, de Silva AM, Montgomery RR, Fish D, Anguita J, Anderson JF, Lobet Y, Fikrig E.** 2000. Attachment of Borrelia burgdorferi within Ixodes scapularis mediated by outer surface protein A. J Clin Invest **106:**561-569.
51. **Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, Desilva AM, Bao F, Yang X, Pypaert M, Pradhan D, Kantor FS, Telford S, Anderson JF, Fikrig E.** 2004. TROSPA, an Ixodes scapularis receptor for Borrelia burgdorferi. Cell **119:**457-468.
52. **Yang XF, Pal U, Alani SM, Fikrig E, Norgard MV.** 2004. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. J Exp Med **199:**641-648.
53. **Becker M, Bunikis J, Lade BD, Dunn JJ, Barbour AG, Lawson CL.** 2005. Structural investigation of Borrelia burgdorferi OspB, a bactericidal Fab target. J Biol Chem **280:**17363-17370.
54. **Bergstrom S, Bundoc VG, Barbour AG.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete Borrelia burgdorferi. Mol Microbiol **3:**479-486.
55. **Li H, Dunn JJ, Luft BJ, Lawson CL.** 1997. Crystal structure of Lyme disease antigen outer surface protein A complexed with an Fab. Proc Natl Acad Sci U S A **94:**3584-3589.
56. **Tilly K, Bestor A, Rosa PA.** 2016. Functional Equivalence of OspA and OspB, but Not OspC, in Tick Colonization by Borrelia burgdorferi. Infect Immun **84:**1565-1573.
57. **Probert WS, Johnson BJ.** 1998. Identification of a 47 kDa fibronectin-binding protein expressed by Borrelia burgdorferi isolate B31. Mol Microbiol **30:**1003-1015.
58. **Li X, Liu X, Beck DS, Kantor FS, Fikrig E.** 2006. Borrelia burgdorferi lacking BBK32, a fibronectin-binding protein, retains full pathogenicity. Infect Immun **74:**3305-3313.
59. **Guo BP, Norris SJ, Rosenberg LC, Hook M.** 1995. Adherence of Borrelia burgdorferi to the proteoglycan decorin. Infect Immun **63:**3467-3472.
60. **Blevins JS, Hagman KE, Norgard MV.** 2008. Assessment of decorin-binding protein A to the infectivity of Borrelia burgdorferi in the murine models of needle and tick infection. BMC Microbiol **8:**82.
61. **Brissette CA, Verma A, Bowman A, Cooley AE, Stevenson B.** 2009. The Borrelia burgdorferi outer-surface protein ErpX binds mammalian laminin. Microbiology **155:**863-872.
62. **Stevenson B, Bono JL, Schwan TG, Rosa P.** 1998. Borrelia burgdorferi erp proteins are immunogenic in mammals infected by tick bite, and their synthesis is inducible in cultured bacteria. Infect Immun **66:**2648-2654.
63. **Carroll JA, Garon CF, Schwan TG.** 1999. Effects of environmental pH on membrane proteins in Borrelia burgdorferi. Infect Immun **67:**3181-3187.
64. **Schwan TG, Piesman J.** 2000. Temporal changes in outer surface proteins A and C of the lyme disease-associated spirochete, Borrelia burgdorferi, during the chain of infection in ticks and mice. J Clin Microbiol **38:**382-388.

65. **Hovius JW, de Jong MA, den Dunnen J, Litjens M, Fikrig E, van der Poll T, Gringhuis SI, Geijtenbeek TB.** 2008. Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. PLoS Pathog **4:**e31.
66. **Garg R, Juncadella IJ, Ramamoorthi N, Ashish, Ananthanarayanan SK, Thomas V, Rincon M, Krueger JK, Fikrig E, Yengo CM, Anguita J.** 2006. Cutting edge: CD4 is the receptor for the tick saliva immunosuppressor, Salp15. J Immunol **177:**6579-6583.
67. **Anguita J, Ramamoorthi N, Hovius JW, Das S, Thomas V, Persinski R, Conze D, Askenase PW, Rincon M, Kantor FS, Fikrig E.** 2002. Salp15, an ixodes scapularis salivary protein, inhibits CD4(+) T cell activation. Immunity **16:**849-859.
68. **Schuijt TJ, Hovius JW, van Burgel ND, Ramamoorthi N, Fikrig E, van Dam AP.** 2008. The tick salivary protein Salp15 inhibits the killing of serum-sensitive Borrelia burgdorferi sensu lato isolates. Infect Immun **76:**2888-2894.
69. **Onder O, Humphrey PT, McOmber B, Korobova F, Francella N, Greenbaum DC, Brisson D.** 2012. OspC is potent plasminogen receptor on surface of Borrelia burgdorferi. J Biol Chem **287:**16860-16868.
70. **Coleman JL, Sellati TJ, Testa JE, Kew RR, Furie MB, Benach JL.** 1995. Borrelia burgdorferi binds plasminogen, resulting in enhanced penetration of endothelial monolayers. Infect Immun **63:**2478-2484.
71. **Grimm D, Tilly K, Byram R, Stewart PE, Krum JG, Bueschel DM, Schwan TG, Policastro PF, Elias AF, Rosa PA.** 2004. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc Natl Acad Sci U S A **101:**3142-3147.
72. **Hellwage J, Meri T, Heikkila T, Alitalo A, Panelius J, Lahdenne P, Seppala IJ, Meri S.** 2001. The complement regulator factor H binds to the surface protein OspE of Borrelia burgdorferi. J Biol Chem **276:**8427-8435.
73. **Kraiczy P, Skerka C, Kirschfink M, Brade V, Zipfel PF.** 2001. Immune evasion of Borrelia burgdorferi by acquisition of human complement regulators FHL-1/reconectin and Factor H. Eur J Immunol **31:**1674-1684.
74. **Brooks CS, Vuppala SR, Jett AM, Alitalo A, Meri S, Akins DR.** 2005. Complement regulator-acquiring surface protein 1 imparts resistance to human serum in Borrelia burgdorferi. J Immunol **175:**3299-3308.
75. **von Lackum K, Miller JC, Bykowski T, Riley SP, Woodman ME, Brade V, Kraiczy P, Stevenson B, Wallich R.** 2005. Borrelia burgdorferi regulates expression of complement regulator-acquiring surface protein 1 during the mammal-tick infection cycle. Infect Immun **73:**7398-7405.
76. **Hartmann K, Corvey C, Skerka C, Kirschfink M, Karas M, Brade V, Miller JC, Stevenson B, Wallich R, Zipfel PF, Kraiczy P.** 2006. Functional characterization of BbCRASP-2, a distinct outer membrane protein of Borrelia burgdorferi that binds host complement regulators factor H and FHL-1. Mol Microbiol **61:**1220-1236.
77. **Siegel C, Hallstrom T, Skerka C, Eberhardt H, Uzonyi B, Beckhaus T, Karas M, Wallich R, Stevenson B, Zipfel PF, Kraiczy P.** 2010. Complement factor H-related proteins CFHR2 and CFHR5 represent novel ligands for the infection-associated CRASP proteins of Borrelia burgdorferi. PLoS One **5:**e13519.
78. **Hallstrom T, Siegel C, Morgelin M, Kraiczy P, Skerka C, Zipfel PF.** 2013. CspA from Borrelia burgdorferi inhibits the terminal complement pathway. MBio **4**.

79. **Coleman AS, Yang X, Kumar M, Zhang X, Promnares K, Shroder D, Kenedy MR, Anderson JF, Akins DR, Pal U.** 2008. Borrelia burgdorferi complement regulator-acquiring surface protein 2 does not contribute to complement resistance or host infectivity. PLoS One **3:**3010e.
80. **Woodman ME, Cooley AE, Miller JC, Lazarus JJ, Tucker K, Bykowski T, Botto M, Hellwage J, Wooten RM, Stevenson B.** 2007. Borrelia burgdorferi binding of host complement regulator factor H is not required for efficient mammalian infection. Infect Immun **75:**3131-3139.
81. **Zhang JR, Hardham JM, Barbour AG, Norris SJ.** 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. Cell **89:**275-285.
82. **Bankhead T, Chaconas G.** 2007. The role of VlsE antigenic variation in the Lyme disease spirochete: persistence through a mechanism that differs from other pathogens. Mol Microbiol **65:**1547-1558.
83. **Zhang JR, Norris SJ.** 1998. Kinetics and in vivo induction of genetic variation of vlsE in Borrelia burgdorferi. Infect Immun **66:**3689-3697.
84. **Indest KJ, Howell JK, Jacobs MB, Scholl-Meeker D, Norris SJ, Philipp MT.** 2001. Analysis of Borrelia burgdorferi vlsE gene expression and recombination in the tick vector. Infect Immun **69:**7083-7090.
85. **Coutte L, Botkin DJ, Gao L, Norris SJ.** 2009. Detailed analysis of sequence changes occurring during vlsE antigenic variation in the mouse model of Borrelia burgdorferi infection. PLoS Pathog **5:**e1000293.
86. **Katona LI, Beck G, Habicht GS.** 1992. Purification and immunological characterization of a major low-molecular-weight lipoprotein from Borrelia burgdorferi. Infect Immun **60:**4995-5003.
87. **Lahdenne P, Porcella SF, Hagman KE, Akins DR, Popova TG, Cox DL, Katona LI, Radolf JD, Norgard MV.** 1997. Molecular characterization of a 6.6-kilodalton Borrelia burgdorferi outer membrane-associated lipoprotein (lp6.6) which appears to be downregulated during mammalian infection. Infect Immun **65:**412-421.
88. **Promnares K, Kumar M, Shroder DY, Zhang X, Anderson JF, Pal U.** 2009. Borrelia burgdorferi small lipoprotein Lp6.6 is a member of multiple protein complexes in the outer membrane and facilitates pathogen transmission from ticks to mice. Mol Microbiol **74:**112-125.
89. **Yang X, Promnares K, Qin J, He M, Shroder DY, Kariu T, Wang Y, Pal U.** 2011. Characterization of multiprotein complexes of the Borrelia burgdorferi outer membrane vesicles. J Proteome Res **10:**4556-4566.
90. **Freeman D, Kumru O, Middaugh C, Joshi S, Zückert W.** 2014. Low resolution structural determination of lipoprotein6.6: a membrane component of Borrelia burgdorferi (753.2). The FASEB Journal **28**.
91. **Lam TT, Nguyen TP, Fikrig E, Flavell RA.** 1994. A chromosomal Borrelia burgdorferi gene encodes a 22-kilodalton lipoprotein, P22, that is serologically recognized in Lyme disease. J Clin Microbiol **32:**876-883.
92. **Wallich R, Simon MM, Hofmann H, Moter SE, Schaible UE, Kramer MD.** 1993. Molecular and immunological characterization of a novel polymorphic lipoprotein of Borrelia burgdorferi. Infect Immun **61:**4158-4166.

93. **von Lackum K, Ollison KM, Bykowski T, Nowalk AJ, Hughes JL, Carroll JA, Zuckert WR, Stevenson B.** 2007. Regulated synthesis of the Borrelia burgdorferi inner-membrane lipoprotein IpLA7 (P22, P22-A) during the Lyme disease spirochaete's mammal-tick infectious cycle. Microbiology **153:**1361-1371.
94. **Babb K, Bykowski T, Riley SP, Miller MC, Demoll E, Stevenson B.** 2006. Borrelia burgdorferi EbfC, a novel, chromosomally encoded protein, binds specific DNA sequences adjacent to erp loci on the spirochete's resident cp32 prophages. J Bacteriol **188:**4331-4339.
95. **Babb K, McAlister JD, Miller JC, Stevenson B.** 2004. Molecular characterization of Borrelia burgdorferi erp promoter/operator elements. J Bacteriol **186:**2745-2756.
96. **Pal U, Dai J, Li X, Neelakanta G, Luo P, Kumar M, Wang P, Yang X, Anderson JF, Fikrig E.** 2008. A differential role for BB0365 in the persistence of Borrelia burgdorferi in mice and ticks. J Infect Dis **197:**148-155.
97. **Yang X, Hegde S, Shroder DY, Smith AA, Promnares K, Neelakanta G, Anderson JF, Fikrig E, Pal U.** 2013. The lipoprotein La7 contributes to Borrelia burgdorferi persistence in ticks and their transmission to naive hosts. Microbes Infect **15:**729-737.
98. **Hubner A, Revel AT, Nolen DM, Hagman KE, Norgard MV.** 2003. Expression of a luxS gene is not required for Borrelia burgdorferi infection of mice via needle inoculation. Infect Immun **71:**2892-2896.
99. **Barbour AG, Jasinskas A, Kayala MA, Davies DH, Steere AC, Baldi P, Felgner PL.** 2008. A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with Borrelia burgdorferi. Infect Immun **76:**3374-3389.
100. **Eggers CH, Kimmel BJ, Bono JL, Elias AF, Rosa P, Samuels DS.** 2001. Transduction by phiBB-1, a bacteriophage of Borrelia burgdorferi. J Bacteriol **183:**4771-4778.
101. **Marconi RT, Earnhart CG.** 2010. Lyme Disease Vaccines. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.
102. **Terekhova D, Iyer R, Wormser GP, Schwartz I.** 2006. Comparative genome hybridization reveals substantial variation among clinical isolates of Borrelia burgdorferi sensu stricto with different pathogenic properties. J Bacteriol **188:**6124-6134.
103. **de Silva AM, Telford SR, 3rd, Brunet LR, Barthold SW, Fikrig E.** 1996. Borrelia burgdorferi OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J Exp Med **183:**271-275.
104. **de Silva AM, Zeidner NS, Zhang Y, Dolan MC, Piesman J, Fikrig E.** 1999. Influence of outer surface protein A antibody on Borrelia burgdorferi within feeding ticks. Infect Immun **67:**30-35.
105. **Sigal LH, Zahradnik JM, Lavin P, Patella SJ, Bryant G, Haselby R, Hilton E, Kunkel M, Adler-Klein D, Doherty T, Evans J, Molloy PJ, Seidner AL, Sabetta JR, Simon HJ, Klempner MS, Mays J, Marks D, Malawista SE.** 1998. A vaccine consisting of recombinant Borrelia burgdorferi outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. N Engl J Med **339:**216-222.
106. **Steere AC, Sikand VK, Meurice F, Parenti DL, Fikrig E, Schoen RT, Nowakowski J, Schmid CH, Laukamp S, Buscarino C, Krause DS.** 1998. Vaccination against Lyme

disease with recombinant Borrelia burgdorferi outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. N Engl J Med **339:**209-215.
107. **Wilske B, Busch U, Fingerle V, Jauris-Heipke S, Preac Mursic V, Rossler D, Will G.** 1996. Immunological and molecular variability of OspA and OspC. Implications for Borrelia vaccine development. Infection **24:**208-212.
108. **Lathrop SL, Ball R, Haber P, Mootrey GT, Braun MM, Shadomy SV, Ellenberg SS, Chen RT, Hayes EB.** 2002. Adverse event reports following vaccination for Lyme disease: December 1998-July 2000. Vaccine **20:**1603-1608.
109. **Nigrovic LE, Thompson KM.** 2007. The Lyme vaccine: a cautionary tale. Epidemiol Infect **135:**1-8.
110. **Trollmo C, Meyer AL, Steere AC, Hafler DA, Huber BT.** 2001. Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 alpha L is a partial agonist for outer surface protein A-reactive T cells. J Immunol **166:**5286-5291.
111. **Kalish RA, Leong JM, Steere AC.** 1993. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of Borrelia burgdorferi. Infect Immun **61:**2774-2779.
112. **Willett TA, Meyer AL, Brown EL, Huber BT.** 2004. An effective second-generation outer surface protein A-derived Lyme vaccine that eliminates a potentially autoreactive T cell epitope. Proc Natl Acad Sci U S A **101:**1303-1308.
113. **Livey I, O'Rourke M, Traweger A, Savidis-Dacho H, Crowe BA, Barrett PN, Yang X, Dunn JJ, Luft BJ.** 2011. A new approach to a Lyme disease vaccine. Clin Infect Dis **52 Suppl 3:**s266-270.
114. **Earnhart CG, Buckles EL, Dumler JS, Marconi RT.** 2005. Demonstration of OspC type diversity in invasive human lyme disease isolates and identification of previously uncharacterized epitopes that define the specificity of the OspC murine antibody response. Infect Immun **73:**7869-7877.
115. **Wang G, van Dam AP, Dankert J.** 1999. Evidence for frequent OspC gene transfer between Borrelia valaisiana sp. nov. and other Lyme disease spirochetes. FEMS Microbiol Lett **177:**289-296.
116. **Theisen M, Frederiksen B, Lebech AM, Vuust J, Hansen K.** 1993. Polymorphism in ospC gene of Borrelia burgdorferi and immunoreactivity of OspC protein: implications for taxonomy and for use of OspC protein as a diagnostic antigen. J Clin Microbiol **31:**2570-2576.
117. **Earnhart CG, Buckles EL, Marconi RT.** 2007. Development of an OspC-based tetravalent, recombinant, chimeric vaccinogen that elicits bactericidal antibody against diverse Lyme disease spirochete strains. Vaccine **25:**466-480.
118. **Earnhart CG, Marconi RT.** 2007. An octavalent lyme disease vaccine induces antibodies that recognize all incorporated OspC type-specific sequences. Hum Vaccin **3:**281-289.
119. **LaFleur RL, Callister SM, Dant JC, Wasmoen TL, Jobe DA, Lovrich SD.** 2015. Vaccination with the ospA- and ospB-Negative Borrelia burgdorferi Strain 50772 Provides Significant Protection against Canine Lyme Disease. Clin Vaccine Immunol **22:**836-839.
120. **Kolb P, Wallich R, Nassal M.** 2015. Whole-Chain Tick Saliva Proteins Presented on Hepatitis B Virus Capsid-Like Particles Induce High-Titered Antibodies with Neutralizing Potential. PLoS One **10:**e0136180.

121. **Silhavy TJ, Kahne D, Walker S.** 2010. The bacterial cell envelope. Cold Spring Harb Perspect Biol **2:**a000414.
122. **Cowles CE, Li Y, Semmelhack MF, Cristea IM, Silhavy TJ.** 2011. The free and bound forms of Lpp occupy distinct subcellular locations in Escherichia coli. Mol Microbiol **79:**1168-1181.
123. **Konovalova A, Perlman DH, Cowles CE, Silhavy TJ.** 2014. Transmembrane domain of surface-exposed outer membrane lipoprotein RcsF is threaded through the lumen of beta-barrel proteins. Proc Natl Acad Sci U S A **111:**E4350-4358.
124. **Michel LV, Shaw J, MacPherson V, Barnard D, Bettinger J, D'Arcy B, Surendran N, Hellman J, Pichichero ME.** 2015. Dual orientation of the outer membrane lipoprotein Pal in Escherichia coli. Microbiology **161:**1251-1259.
125. **Webb CT, Selkrig J, Perry AJ, Noinaj N, Buchanan SK, Lithgow T.** 2012. Dynamic association of BAM complex modules includes surface exposure of the lipoprotein BamC. J Mol Biol **422:**545-555.
126. **Konovalova A, Silhavy TJ.** 2015. Outer membrane lipoprotein biogenesis: Lol is not the end. Philos Trans R Soc Lond B Biol Sci **370**.
127. **Casjens SR, Eggers CH, Schwartz I.** 2010. *Borrelia* Genomics: Chromosome, Plasmids, Bacteriophages and Genetic Variation. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.
128. **Shruthi H, Babu MM, Sankaran K.** 2010. TAT-pathway-dependent lipoproteins as a niche-based adaptation in prokaryotes. J Mol Evol **70:**359-370.
129. **Froderberg L, Houben EN, Baars L, Luirink J, de Gier JW.** 2004. Targeting and translocation of two lipoproteins in Escherichia coli via the SRP/Sec/YidC pathway. J Biol Chem **279:**31026-31032.
130. **Dalbey RE, Kuhn A, Zhu L, Kiefer D.** 2014. The membrane insertase YidC. Biochim Biophys Acta **1843:**1489-1496.
131. **van der Sluis EO, Driessen AJ.** 2006. Stepwise evolution of the Sec machinery in Proteobacteria. Trends Microbiol **14:**105-108.
132. **Sankaran K, Wu HC.** 1994. Lipid modification of bacterial prolipoprotein. Transfer of diacylglyceryl moiety from phosphatidylglycerol. J Biol Chem **269:**19701-19706.
133. **Vogeley L, El Arnaout T, Bailey J, Stansfeld PJ, Boland C, Caffrey M.** 2016. Structural basis of lipoprotein signal peptidase II action and inhibition by the antibiotic globomycin. Science **351:**876-880.
134. **Denham EL, Ward PN, Leigh JA.** 2008. Lipoprotein signal peptides are processed by Lsp and Eep of Streptococcus uberis. J Bacteriol **190:**4641-4647.
135. **Inukai M, Takeuchi M, Shimizu K, Arai M.** 1978. Mechanism of action of globomycin. J Antibiot (Tokyo) **31:**1203-1205.
136. **Carter CJ, Bergstrom S, Norris SJ, Barbour AG.** 1994. A family of surface-exposed proteins of 20 kilodaltons in the genus Borrelia. Infect Immun **62:**2792-2799.
137. **Ichihara S, Beppu N, Mizushima S.** 1984. Protease IV, a cytoplasmic membrane protein of Escherichia coli, has signal peptide peptidase activity. J Biol Chem **259:**9853-9857.
138. **Saito A, Hizukuri Y, Matsuo E, Chiba S, Mori H, Nishimura O, Ito K, Akiyama Y.** 2011. Post-liberation cleavage of signal peptides is catalyzed by the site-2 protease (S2P) in bacteria. Proc Natl Acad Sci U S A **108:**13740-13745.

139. **Akiyama Y, Kanehara K, Ito K.** 2004. RseP (YaeL), an Escherichia coli RIP protease, cleaves transmembrane sequences. EMBO J **23:**4434-4442.
140. **Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H.** 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol **2:**2006 0008.
141. **Kanehara K, Ito K, Akiyama Y.** 2002. YaeL (EcfE) activates the sigma(E) pathway of stress response through a site-2 cleavage of anti-sigma(E), RseA. Genes Dev **16:**2147-2155.
142. **Caimano MJ, Eggers CH, Gonzalez CA, Radolf JD.** 2005. Alternate sigma factor RpoS is required for the in vivo-specific repression of Borrelia burgdorferi plasmid lp54-borne ospA and lp6.6 genes. J Bacteriol **187:**7845-7852.
143. **Hantke K, Braun V.** 1973. Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the Escherichia coli outer membrane. Eur J Biochem **34:**284-296.
144. **Vidal-Ingigliardi D, Lewenza S, Buddelmeijer N.** 2007. Identification of essential residues in apolipoprotein N-acyl transferase, a member of the CN hydrolase family. J Bacteriol **189:**4456-4464.
145. **Tschumi A, Nai C, Auchli Y, Hunziker P, Gehrig P, Keller P, Grau T, Sander P.** 2009. Identification of apolipoprotein N-acyltransferase (Lnt) in mycobacteria. J Biol Chem **284:**27146-27156.
146. **Robichon C, Vidal-Ingigliardi D, Pugsley AP.** 2005. Depletion of apolipoprotein N-acyltransferase causes mislocalization of outer membrane lipoproteins in Escherichia coli. J Biol Chem **280:**974-983.
147. **Narita S, Tokuda H.** 2011. Overexpression of LolCDE allows deletion of the Escherichia coli gene encoding apolipoprotein N-acyltransferase. J Bacteriol **193:**4832-4840.
148. **LoVullo ED, Wright LF, Isabella V, Huntley JF, Pavelka MS, Jr.** 2015. Revisiting the Gram-negative lipoprotein paradigm. J Bacteriol **197:**1705-1715.
149. **Chahales P, Thanassi DG.** 2015. A more flexible lipoprotein sorting pathway. J Bacteriol **197:**1702-1704.
150. **Yamaguchi K, Yu F, Inouye M.** 1988. A single amino acid determinant of the membrane localization of lipoproteins in E. coli. Cell **53:**423-432.
151. **Seydel A, Gounon P, Pugsley AP.** 1999. Testing the '+2 rule' for lipoprotein sorting in the Escherichia coli cell envelope with a new genetic selection. Mol Microbiol **34:**810-821.
152. **Terada M, Kuroda T, Matsuyama SI, Tokuda H.** 2001. Lipoprotein sorting signals evaluated as the LolA-dependent release of lipoproteins from the cytoplasmic membrane of Escherichia coli. J Biol Chem **276:**47690-47694.
153. **Hara T, Matsuyama S, Tokuda H.** 2003. Mechanism underlying the inner membrane retention of Escherichia coli lipoproteins caused by Lol avoidance signals. J Biol Chem **278:**40408-40414.
154. **Narita S, Tokuda H.** 2007. Amino acids at positions 3 and 4 determine the membrane specificity of Pseudomonas aeruginosa lipoproteins. J Biol Chem **282:**13372-13378.
155. **Tanaka SY, Narita S, Tokuda H.** 2007. Characterization of the Pseudomonas aeruginosa Lol system as a lipoprotein sorting mechanism. J Biol Chem **282:**13379-13384.

156. **Kumru OS, Schulze RJ, Slusser JG, Zuckert WR.** 2010. Development and validation of a FACS-based lipoprotein localization screen in the Lyme disease spirochete Borrelia burgdorferi. BMC Microbiol **10:**277.
157. **Ito Y, Matsuzawa H, Matsuyama S, Narita S, Tokuda H.** 2006. Genetic analysis of the mode of interplay between an ATPase subunit and membrane subunits of the lipoprotein-releasing ATP-binding cassette transporter LolCDE. J Bacteriol **188:**2856-2864.
158. **Yasuda M, Iguchi-Yokoyama A, Matsuyama S, Tokuda H, Narita S.** 2009. Membrane topology and functional importance of the periplasmic region of ABC transporter LolCDE. Biosci Biotechnol Biochem **73:**2310-2316.
159. **Mizutani M, Mukaiyama K, Xiao J, Mori M, Satou R, Narita S, Okuda S, Tokuda H.** 2013. Functional differentiation of structurally similar membrane subunits of the ABC transporter LolCDE complex. FEBS Lett **587:**23-29.
160. **Okuda S, Tokuda H.** 2009. Model of mouth-to-mouth transfer of bacterial lipoproteins through inner membrane LolC, periplasmic LolA, and outer membrane LolB. Proc Natl Acad Sci U S A **106:**5877-5882.
161. **Narita S, Tanaka K, Matsuyama S, Tokuda H.** 2002. Disruption of lolCDE, encoding an ATP-binding cassette transporter, is lethal for Escherichia coli and prevents release of lipoproteins from the inner membrane. J Bacteriol **184:**1417-1422.
162. **Matsuyama S, Tajima T, Tokuda H.** 1995. A novel periplasmic carrier protein involved in the sorting and transport of Escherichia coli lipoproteins destined for the outer membrane. EMBO J **14:**3365-3372.
163. **Takeda K, Miyatake H, Yokota N, Matsuyama S, Tokuda H, Miki K.** 2003. Crystal structures of bacterial lipoprotein localization factors, LolA and LolB. EMBO J **22:**3199-3209.
164. **Remans K, Pauwels K, van Ulsen P, Buts L, Cornelis P, Tommassen J, Savvides SN, Decanniere K, Van Gelder P.** 2010. Hydrophobic surface patches on LolA of Pseudomonas aeruginosa are essential for lipoprotein binding. J Mol Biol **401:**921-930.
165. **Matsuyama S, Yokota N, Tokuda H.** 1997. A novel outer membrane lipoprotein, LolB (HemM), involved in the LolA (p20)-dependent localization of lipoproteins to the outer membrane of Escherichia coli. EMBO J **16:**6947-6955.
166. **Tajima T, Yokota N, Matsuyama S, Tokuda H.** 1998. Genetic analyses of the in vivo function of LolA, a periplasmic chaperone involved in the outer membrane localization of Escherichia coli lipoproteins. FEBS Lett **439:**51-54.
167. **Tsukahara J, Mukaiyama K, Okuda S, Narita S, Tokuda H.** 2009. Dissection of LolB function--lipoprotein binding, membrane targeting and incorporation of lipoproteins into lipid bilayers. FEBS J **276:**4496-4504.
168. **Taniguchi N, Matsuyama S, Tokuda H.** 2005. Mechanisms underlying energy-independent transfer of lipoproteins from LolA to LolB, which have similar unclosed {beta}-barrel structures. J Biol Chem **280:**34481-34488.
169. **Hayashi Y, Tsurumizu R, Tsukahara J, Takeda K, Narita S, Mori M, Miki K, Tokuda H.** 2014. Roles of the protruding loop of factor B essential for the localization of lipoproteins (LolB) in the anchoring of bacterial triacylated proteins to the outer membrane. J Biol Chem **289:**10530-10539.
170. **Okuda S, Tokuda H.** 2011. Lipoprotein sorting in bacteria. Annu Rev Microbiol **65:**239-259.

171. **Cho SH, Szewczyk J, Pesavento C, Zietek M, Banzhaf M, Roszczenko P, Asmar A, Laloux G, Hov AK, Leverrier P, Van der Henst C, Vertommen D, Typas A, Collet JF.** 2014. Detecting envelope stress by monitoring beta-barrel assembly. Cell **159:**1652-1664.
172. **Hooda Y, Lai CC, Judd A, Buckwalter CM, Shin HE, Gray-Owen SD, Moraes TF.** 2016. Slam is an outer membrane protein that is required for the surface display of lipidated virulence factors in Neisseria. Nat Microbiol **1:**16009.
173. **Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG.** 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol **7:**539.
174. **Chen S, Zuckert WR.** 2011. Probing the Borrelia burgdorferi surface lipoprotein secretion pathway using a conditionally folding protein domain. J Bacteriol **193:**6724-6732.
175. **Barbour AG.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med **57:**521-525.
176. **Lukehart SA, Marra CM.** 2007. Isolation and laboratory maintenance of Treponema pallidum. Curr Protoc Microbiol **Chapter 12:**Unit 12A 11.
177. **Zuckert WR.** 2007. Laboratory maintenance of Borrelia burgdorferi. Curr Protoc Microbiol **Chapter 12:**Unit 12C 11.
178. **Samuels DS.** 1995. Electrotransformation of the spirochete Borrelia burgdorferi. Methods Mol Biol **47:**253-259.
179. **Stewart PE, Thalken R, Bono JL, Rosa P.** 2001. Isolation of a circular plasmid region sufficient for autonomous replication and transformation of infectious Borrelia burgdorferi. Mol Microbiol **39:**714-721.
180. **Bunikis J, Barbour AG.** 1999. Access of antibody or trypsin to an integral outer membrane protein (P66) of Borrelia burgdorferi is hindered by Osp lipoproteins. Infect Immun **67:**2874-2883.
181. **Skare JT, Shang ES, Foley DM, Blanco DR, Champion CI, Mirzabekov T, Sokolov Y, Kagan BL, Miller JN, Lovett MA.** 1995. Virulent strain associated outer membrane proteins of Borrelia burgdorferi. J Clin Invest **96:**2380-2392.
182. **Norris SJ, Coburn J, Leong JM, Hu LT, Höök M.** 2010. Pathobiology of Lyme Disease Borrelia. *In* Radolf JD, Samuels DS (ed), Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press.
183. **Stevenson B, Zuckert WR, Akins DR.** 2000. Repetition, conservation, and variation: the multiple cp32 plasmids of Borrelia species. J Mol Microbiol Biotechnol **2:**411-422.
184. **Elias AF, Stewart PE, Grimm D, Caimano MJ, Eggers CH, Tilly K, Bono JL, Akins DR, Radolf JD, Schwan TG, Rosa P.** 2002. Clonal polymorphism of Borrelia burgdorferi strain B31 MI: implications for mutagenesis in an infectious strain background. Infect Immun **70:**2139-2150.
185. **Bunikis I, Kutschan-Bunikis S, Bonde M, Bergstrom S.** 2011. Multiplex PCR as a tool for validating plasmid content of Borrelia burgdorferi. J Microbiol Methods **86:**243-247.
186. **Carroll JA.** 2010. Methods of identifying membrane proteins in spirochetes. Curr Protoc Microbiol **Chapter 12:**Unit12C 12.

187. **El-Hage N, Babb K, Carroll JA, Lindstrom N, Fischer ER, Miller JC, Gilmore RD, Jr., Mbow ML, Stevenson B.** 2001. Surface exposure and protease insensitivity of Borrelia burgdorferi Erp (OspEF-related) lipoproteins. Microbiology **147:**821-830.
188. **Ebeling W, Hennrich N, Klockow M, Metz H, Orth HD, Lang H.** 1974. Proteinase K from Tritirachium album Limber. Eur J Biochem **47:**91-97.
189. **Sweeney PJ, Walker JM.** 1993. Proteinase K (EC 3.4.21.14). Methods Mol Biol **16:**305-311.
190. **Laemmli UK.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680-685.
191. **Barbour AG, Hayes SF, Heiland RA, Schrumpf ME, Tessier SL.** 1986. A Borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect Immun **52:**549-554.
192. **Washburn MP, Wolters D, Yates JR, 3rd.** 2001. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol **19:**242-247.
193. **Florens L, Washburn MP.** 2006. Proteomic analysis by multidimensional protein identification technology. Methods Mol Biol **328:**159-175.
194. **Zhang Y, Wen Z, Washburn MP, Florens L.** 2010. Refinements to label free proteome quantitation: how to deal with peptides shared by multiple proteins. Anal Chem **82:**2272-2281.
195. **Chen S, Kumru OS, Zuckert WR.** 2011. Determination of Borrelia surface lipoprotein anchor topology by surface proteolysis. J Bacteriol **193:**6379-6383.
196. **Zuckert WR, Lloyd JE, Stewart PE, Rosa PA, Barbour AG.** 2004. Cross-species surface display of functional spirochetal lipoproteins by recombinant Borrelia burgdorferi. Infect Immun **72:**1463-1469.
197. **Notredame C, Higgins DG, Heringa J.** 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol **302:**205-217.
198. **Kumru OS, Bunikis I, Sorokina I, Bergstrom S, Zuckert WR.** 2011. Specificity and role of the Borrelia burgdorferi CtpA protease in outer membrane protein processing. J Bacteriol **193:**5759-5765.
199. **Ostberg Y, Carroll JA, Pinne M, Krum JG, Rosa P, Bergstrom S.** 2004. Pleiotropic effects of inactivating a carboxyl-terminal protease, CtpA, in Borrelia burgdorferi. J Bacteriol **186:**2074-2084.
200. **Lin YP, Chen Q, Ritchie JA, Dufour NP, Fischer JR, Coburn J, Leong JM.** 2015. Glycosaminoglycan binding by Borrelia burgdorferi adhesin BBK32 specifically and uniquely promotes joint colonization. Cell Microbiol **17:**860-875.
201. **Bunikis J, Noppa L, Ostberg Y, Barbour AG, Bergstrom S.** 1996. Surface exposure and species specificity of an immunoreactive domain of a 66-kilodalton outer membrane protein (P66) of the Borrelia spp. that cause Lyme disease. Infect Immun **64:**5111-5116.
202. **Hughes JL, Nolder CL, Nowalk AJ, Clifton DR, Howison RR, Schmit VL, Gilmore RD, Jr., Carroll JA.** 2008. Borrelia burgdorferi surface-localized proteins expressed during persistent murine infection are conserved among diverse Borrelia spp. Infect Immun **76:**2498-2511.
203. **Lenhart TR, Kenedy MR, Yang X, Pal U, Akins DR.** 2012. BB0324 and BB0028 are constituents of the Borrelia burgdorferi beta-barrel assembly machine (BAM) complex. BMC Microbiol **12:**60.

204. **Pal U, Wang P, Bao F, Yang X, Samanta S, Schoen R, Wormser GP, Schwartz I, Fikrig E.** 2008. Borrelia burgdorferi basic membrane proteins A and B participate in the genesis of Lyme arthritis. J Exp Med **205:**133-141.
205. **Das S, Shraga D, Gannon C, Lam TT, Feng S, Brunet LR, Telford SR, Barthold SW, Flavell RA, Fikrig E.** 1996. Characterization of a 30-kDa Borrelia burgdorferi substrate-binding protein homologue. Res Microbiol **147:**739-751.
206. **Hu LT, Pratt SD, Perides G, Katz L, Rogers RA, Klempner MS.** 1997. Isolation, cloning, and expression of a 70-kilodalton plasminogen binding protein of Borrelia burgdorferi. Infect Immun **65:**4989-4995.
207. **Bestor A, Rego RO, Tilly K, Rosa PA.** 2012. Competitive advantage of Borrelia burgdorferi with outer surface protein BBA03 during tick-mediated infection of the mammalian host. Infect Immun **80:**3501-3511.
208. **Zhang X, Yang X, Kumar M, Pal U.** 2009. BB0323 function is essential for Borrelia burgdorferi virulence and persistence through tick-rodent transmission cycle. J Infect Dis **200:**1318-1330.
209. **Kariu T, Yang X, Marks CB, Zhang X, Pal U.** 2013. Proteolysis of BB0323 results in two polypeptides that impact physiologic and infectious phenotypes in Borrelia burgdorferi. Mol Microbiol **88:**510-522.
210. **Dunn JP, Kenedy MR, Iqbal H, Akins DR.** 2015. Characterization of the beta-barrel assembly machine accessory lipoproteins from Borrelia burgdorferi. BMC Microbiol **15:**70.
211. **Perez-Bercoff A, Koch J, Burglin TR.** 2006. LogoBar: bar graph visualization of protein logos with gaps. Bioinformatics **22:**112-114.
212. **Lauber F, Cornelis GR, Renzi F.** 2016. Identification of a New Lipoprotein Export Signal in Gram-Negative Bacteria. MBio **7**.
213. **Lauber F, Cornelis GR, Renzi F.** 2016. Erratum for Lauber et al., Identification of a New Lipoprotein Export Signal in Gram-Negative Bacteria. MBio **7**.
214. **Brokx SJ, Ellison M, Locke T, Bottorff D, Frost L, Weiner JH.** 2004. Genome-wide analysis of lipoprotein expression in Escherichia coli MG1655. J Bacteriol **186:**3254-3258.
215. **Tokuda H, Matsuyama S, K. T-M.** 2007. Structure, function, and transport of lipoproteins in Escherichia coli, p 67-69. *In* M. E (ed), The Periplasm. ASM Press.
216. **Howe TR, Mayer LW, Barbour AG.** 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science **227:**645-646.
217. **Labandeira-Rey M, Skare JT.** 2001. Decreased infectivity in Borrelia burgdorferi strain B31 is associated with loss of linear plasmid 25 or 28-1. Infect Immun **69:**446-455.
218. **Purser JE, Norris SJ.** 2000. Correlation between plasmid content and infectivity in Borrelia burgdorferi. Proc Natl Acad Sci U S A **97:**13865-13870.
219. **Schwan TG, Burgdorfer W, Garon CF.** 1988. 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.
220. **Zuckert WR, Meyer J.** 1996. Circular and linear plasmids of Lyme disease spirochetes have extensive homology: characterization of a repeated DNA element. J Bacteriol **178:**2287-2298.

221. **Alva V, Nam SZ, Soding J, Lupas AN.** 2016. The MPI bioinformatics Toolkit as an integrative platform for advanced protein sequence and structure analysis. Nucleic Acids Res **44:**W410-415.
222. **Revel AT, Blevins JS, Almazan C, Neil L, Kocan KM, de la Fuente J, Hagman KE, Norgard MV.** 2005. bptA (bbe16) is essential for the persistence of the Lyme disease spirochete, Borrelia burgdorferi, in its natural tick vector. Proc Natl Acad Sci U S A **102:**6972-6977.
223. **Fikrig E, Barthold SW, Marcantonio N, Deponte K, Kantor FS, Flavell RA.** 1992. Roles of OspA, OspB, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. Infect Immun **60:**657-661.
224. **Gilmore RD, Jr., Kappel KJ, Dolan MC, Burkot TR, Johnson BJ.** 1996. Outer surface protein C (OspC), but not P39, is a protective immunogen against a tick-transmitted Borrelia burgdorferi challenge: evidence for a conformational protective epitope in OspC. Infect Immun **64:**2234-2239.
225. **Xu H, He M, He JJ, Yang XF.** 2010. Role of the surface lipoprotein BBA07 in the enzootic cycle of Borrelia burgdorferi. Infect Immun **78:**2910-2918.
226. **Wada R, Matsuyama S, Tokuda H.** 2004. Targeted mutagenesis of five conserved tryptophan residues of LolB involved in membrane localization of Escherichia coli lipoproteins. Biochem Biophys Res Commun **323:**1069-1074.
227. **Wilson MM, Anderson DE, Bernstein HD.** 2015. Analysis of the outer membrane proteome and secretome of Bacteroides fragilis reveals a multiplicity of secretion mechanisms. PLoS One **10:**e0117732.
228. **Yan R, Xu D, Yang J, Walker S, Zhang Y.** 2013. A comparative assessment and analysis of 20 representative sequence alignment methods for protein structure prediction. Sci Rep **3:**2619.
229. **Lenhart TR, Akins DR.** 2010. Borrelia burgdorferi locus BB0795 encodes a BamA orthologue required for growth and efficient localization of outer membrane proteins. Mol Microbiol **75:**692-709.
230. **Iqbal H, Kenedy MR, Lybecker M, Akins DR.** 2016. The TamB ortholog of Borrelia burgdorferi interacts with the beta-barrel assembly machine (BAM) complex protein BamA. Mol Microbiol **102:**757-774.
231. **Fukunaga M, Takahashi Y, Tsuruta Y, Matsushita O, Ralph D, McClelland M, Nakao M.** 1995. Genetic and phenotypic analysis of Borrelia miyamotoi sp. nov., isolated from the ixodid tick Ixodes persulcatus, the vector for Lyme disease in Japan. Int J Syst Bacteriol **45:**804-810.
232. **Scoles GA, Papero M, Beati L, Fish D.** 2001. A relapsing fever group spirochete transmitted by Ixodes scapularis ticks. Vector Borne Zoonotic Dis **1:**21-34.
233. **Kingry LC, Batra D, Replogle A, Rowe LA, Pritt BS, Petersen JM.** 2016. Whole Genome Sequence and Comparative Genomics of the Novel Lyme Borreliosis Causing Pathogen, Borrelia mayonii. PLoS One **11:**e0168994.
234. **Pritt BS, Respicio-Kingry LB, Sloan LM, Schriefer ME, Replogle AJ, Bjork J, Liu G, Kingry LC, Mead PS, Neitzel DF, Schiffman E, Hoang Johnson DK, Davis JP, Paskewitz SM, Boxrud D, Deedon A, Lee X, Miller TK, Feist MA, Steward CR, Theel ES, Patel R, Irish CL, Petersen JM.** 2016. Borrelia mayonii sp. nov., a member of the Borrelia burgdorferi sensu lato complex, detected in patients and ticks in the upper midwestern United States. Int J Syst Evol Microbiol **66:**4878-4880.

235. **Roche.** January 2011. Pronase, Nuclease-free, isolated form Streptomyces griseus, Lyophilized powder. Accessed 11 March 2017.
236. **Byram R, Stewart PE, Rosa P.** 2004. The essential nature of the ubiquitous 26-kilobase circular replicon of Borrelia burgdorferi. J Bacteriol **186:**3561-3569.
237. **Finn RD, Clements J, Arndt W, Miller BL, Wheeler TJ, Schreiber F, Bateman A, Eddy SR.** 2015. HMMER web server: 2015 update. Nucleic Acids Res **43:**W30-38.
238. **Karpenahalli MR, Lupas AN, Soding J.** 2007. TPRpred: a tool for prediction of TPR-, PPR- and SEL1-like repeats from protein sequences. BMC Bioinformatics **8:**2.
239. **Soding J, Biegert A, Lupas AN.** 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res **33:**W244-248.
240. **Iyer R, Schwartz I.** 2016. Microarray-Based Comparative Genomic and Transcriptome Analysis of Borrelia burgdorferi. Microarrays (Basel) **5**.
241. **Liang FT, Nelson FK, Fikrig E.** 2002. Molecular adaptation of Borrelia burgdorferi in the murine host. J Exp Med **196:**275-280.
242. **Troy EB, Lin T, Gao L, Lazinski DW, Lundt M, Camilli A, Norris SJ, Hu LT.** 2016. Global Tn-seq analysis of carbohydrate utilization and vertebrate infectivity of Borrelia burgdorferi. Mol Microbiol **101:**1003-1023.
243. **Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang HY, Dosztanyi Z, El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R, Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y, Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C, Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N, Thomas PD, Tosatto SC, Wu CH, Xenarios I, Yeh LS, Young SY, Mitchell AL.** 2017. InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res **45:**D190-D199.
244. **Chaudhuri I, Soding J, Lupas AN.** 2008. Evolution of the beta-propeller fold. Proteins **71:**795-803.
245. **Chen CK, Chan NL, Wang AH.** 2011. The many blades of the beta-propeller proteins: conserved but versatile. Trends Biochem Sci **36:**553-561.
246. **Edwards TA, Wilkinson BD, Wharton RP, Aggarwal AK.** 2003. Model of the brain tumor-Pumilio translation repressor complex. Genes Dev **17:**2508-2513.
247. **Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y.** 2015. The I-TASSER Suite: protein structure and function prediction. Nat Methods **12:**7-8.
248. **Gallagher LA, Ramage E, Jacobs MA, Kaul R, Brittnacher M, Manoil C.** 2007. A comprehensive transposon mutant library of Francisella novicida, a bioweapon surrogate. Proc Natl Acad Sci U S A **104:**1009-1014.
249. **Ouyang Z, Blevins JS, Norgard MV.** 2008. Transcriptional interplay among the regulators Rrp2, RpoN and RpoS in Borrelia burgdorferi. Microbiology **154:**2641-2658.
250. **Yang J, Roy A, Zhang Y.** 2013. Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics **29:**2588-2595.
251. **Sali A, Potterton L, Yuan F, van Vlijmen H, Karplus M.** 1995. Evaluation of comparative protein modeling by MODELLER. Proteins **23:**318-326.
252. **Moremen KW, Tiemeyer M, Nairn AV.** 2012. Vertebrate protein glycosylation: diversity, synthesis and function. Nat Rev Mol Cell Biol **13:**448-462.

253. **Kukkonen M, Raunio T, Virkola R, Lahteenmaki K, Makela PH, Klemm P, Clegg S, Korhonen TK.** 1993. Basement membrane carbohydrate as a target for bacterial adhesion: binding of type I fimbriae of Salmonella enterica and Escherichia coli to laminin. Mol Microbiol **7:**229-237.
254. **Luthra A, Zhu G, Desrosiers DC, Eggers CH, Mulay V, Anand A, McArthur FA, Romano FB, Caimano MJ, Heuck AP, Malkowski MG, Radolf JD.** 2011. The transition from closed to open conformation of Treponema pallidum outer membrane-associated lipoprotein TP0453 involves membrane sensing and integration by two amphipathic helices. J Biol Chem **286:**41656-41668.
255. **Hazlett KR, Cox DL, Decaffmeyer M, Bennett MP, Desrosiers DC, La Vake CJ, La Vake ME, Bourell KW, Robinson EJ, Brasseur R, Radolf JD.** 2005. TP0453, a concealed outer membrane protein of Treponema pallidum, enhances membrane permeability. J Bacteriol **187:**6499-6508.