

Bgp	1	MNNCLIKFFIFLLVFSNSYVAFSKN-----VNVLIVTAMDS <u>EF</u> FDQINKLMSNKEE	50
MtnN	1	MKIKNIANILIFFLSIVLNSWGNESKINTPNENSQSNILIIISATKA <u>E</u> TTEEINKIIQNKKY	60
Pfs	1	MILIIISAMQ <u>EF</u> SEEINKILDNKEE	24
		+***** * +***** **	
Bgp	51	IVLKEYGLN <u>KKILKGL</u> SNRNVNVMVICG <u>V</u> GKVNAGVWTSYILSKYNI SHVINS <u>G</u> VAGGVV	110
MtnN	61	ISIEEH <u>RRKKK</u> IAIGKLMDDHNIITTIATG <u>V</u> GKINTALWTSYIISKYKISHIINAG <u>V</u> ASGIY	120
Pfs	25	IVLNDYLEN <u>KKIYKGL</u> IKGQVISLTTG <u>I</u> GKVNAAATWSSQIISKYKITHIINS <u>G</u> SSGGIK	84
		* + + *** ** + + + + + + + + + + * + * + * + * + * + + + + + + + + +	
Bgp	111	SAKYKDIKVGDVVVSSEVAYHDVDLTK <u>E</u> GYKVGQLTGGLPQKFNANKNLIKNAIEAIKSK	170
MtnN	121	SDKNFKIKIGDVVISTETTSYDFDLH <u>R</u> EGYEIGHVP-EHPKKFKANTALIRKTSKIKIN-	178
Pfs	85	ENSN--LKILDIIVSSETAYYDFDLTK <u>E</u> GHKIGQVP-NLPQKFKADEELLKKVANIVDNK	141
		+*+ *+***** + * ** +** +*** *+*** *+*** * +**	
Bgp	171	VGGSNAYSGLIVSGDQ <u>E</u> IDPTY-INKIIGNFKDVI AVE <u>M</u> EGAAGHVSHMFNIPFIV <u>I</u> RS	229
MtnN	179	--NITSYMGLIITGDQ <u>E</u> IDHQT-FQEIPPEEFENAI AID <u>M</u> ESAAMAQVAYGFKIPFII <u>I</u> RS	235
Pfs	142	LLNIDIHIGLILTGDQ <u>E</u> VDNKNELETIKKNFKDALAVD <u>M</u> EGAAGIAQVAHIFKIPFII <u>I</u> RS	201
		+ ****+*****+ * * +***** ** +** * ****+**	
Bgp	230	IS <u>D</u> IVNKEGNEVEY <u>S</u> KFSKIAAFNSAKVVQ <u>E</u> ILRKL* 266	
MtnN	236	IS <u>D</u> IVNNENNYDDY <u>K</u> FLKKASSSSAKIVENLIKLM* 272	
Pfs	202	IS <u>D</u> LPNNKDNHIDEN <u>K</u> FLKTSSINSKMTKELIRLI* 238	
		***** * * +* ** * +* ** * +* +* +* +*	

Supplementary Fig. S1. Clustal W sequence alignment of homologous *B. burgdorferi* MTA/SAH nucleosidases. The three proteins of *B. burgdorferi*, exported Bgp and MtnN and cytoplasmic Pfs, show homology to the MTA/SAH nucleosidase enzyme present in a variety of bacteria. The amino acids identified on the basis of the crystal structure and enzymatic activity determination of *E. coli* enzyme are indicated. Conserved amino acids involved in substrate binding are underlined, while bold underlined letters indicate catalytic residues present in the enzyme active site. Identical residues are marked by asterisk, and conserved but non-identical residues are indicated by “+”. Boxed residues depict the putative heparin-binding region of Bgp and differences with other two proteins in the region.

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N40 dbpA      10      20      30      40      50      60
N40D10 dbpA  ATGAATAAATATCAAAAAAATTTCAAATCTTTAAATTTTAAAAATTTACTTAAACTAAGT
N40B dbpA    ATGAATAAATATCAAAAAAATTTCAAATCTTTAAATTTTAAAAATTTACTTAAACTAAGT
N40C dbpA    ATGAATAAATATCAAAAAAATTTCAAATCTTTAAATTTTAAAAATTTACTTAAACTAAGT
ATGAATAAATATCAAAAAAATTTCAAATCTTTAAATTTTAAAAATTTACTTAAACTAAGT

N40 dbpA      70      80      90     100     110     120
N40D10 dbpA  TTACTTTGTTGCCCTCATATCATGCGGATTA AAAAGGAGAAACAAAAATCATATTAGAACGA
N40B dbpA    TTACTTTGTTGCCCTCATATCATGCGGATTA AAAAGGAGAAACAAAAATCATATTAGAACGA
N40C dbpA    TTACTTTGTTGCCCTCATATCATGCGGATTA AAAAGGAGAAACAAAAATCATATTAGAACGA
TTACTTTGTTGCCCTCATATCATGCGGATTA AAAAGGAGAAACAAAAATCATATTAGAACGA

N40 dbpA      130     140     150     160     170     180
N40D10 dbpA  AGCGCTAAAGACATTTACAGATGAAATAAATAAAATTA AAAAAGACGCTGCTGATAACAAT
N40B dbpA    AGCGCTAAAGACATTTACAGATGAAATAAATAAATAAAATTA AAAAAGACGCTGCTGATAACAAT
N40C dbpA    AGCGCTAAAGACATTTACAGATGAAATAAATAAATAAATAAAATTA AAAAAGACGCTGCTGATAACAAT
AGCGCTAAAGACATTTACAGATGAAATAAATAAATAAATAAATAAATAA AAAAAGACGCTGCTGATAACAAT

N40 dbpA      190     200     210     220     230     240
N40D10 dbpA  GTAAATTTTGCTGCTTTTACAGATAGTGAAACAGGTAGCAAGGTATCAGAAAAATTCATTG
N40B dbpA    GTAAATTTTGCTGCTTTTACAGATAGTGAAACAGGTAGCAAGGTATCAGAAAAATTCATTG
N40C dbpA    GTAAATTTTGCTGCTTTTACAGATAGTGAAACAGGTAGCAAGGTATCAGAAAAATTCATTG
GTAAATTTTGCTGCTTTTACAGATAGTGAAACAGGTAGCAAGGTATCAGAAAAATTCATTG

N40 dbpA      250     260     270     280     290     300
N40D10 dbpA  ATACTTTGAAGCAAAAAGTGCAGCTACTACAGTAGCAGAAAAATTTGTAACAGCGGATCGAA
N40B dbpA    ATACTTTGAAGCAAAAAGTGCAGCTACTACAGTAGCAGAAAAATTTGTAACAGCGGATCGAA
N40C dbpA    ATACTTTGAAGCAAAAAGTGCAGCTACTACAGTAGCAGAAAAATTTGTAACAGCGGATCGAA
ATACTTTGAAGCAAAAAGTGCAGCTACTACAGTAGCAGAAAAATTTGTAACAGCGGATCGAA

N40 dbpA      310     320     330     340     350     360
N40D10 dbpA  GGGGAAGCTACAAAACTTAAAAAGACTGGAAGTAGTGGTGAATTTCTCAGCAATGTACAAC
N40B dbpA    GGGGAAGCTACAAAACTTAAAAAGACTGGAAGTAGTGGTGAATTTCTCAGCAATGTACAAC
N40C dbpA    GGGGAAGCTACAAAACTTAAAAAGACTGGAAGTAGTGGTGAATTTCTCAGCAATGTACAAC
GGGGAAGCTACAAAACTTAAAAAGACTGGAAGTAGTGGTGAATTTCTCAGCAATGTACAAC

N40 dbpA      370     380     390     400     410     420
N40D10 dbpA  ATGATGCTTGAGGTCTCAGGCCCATTTAGAAGAATTAGGAGTACTAAGAATGACAAAGCA
N40B dbpA    ATGATGCTTGAGGTCTCAGGCCCATTTAGAAGAATTAGGAGTACTAAGAATGACAAAGCA
N40C dbpA    ATGATGCTTGAGGTCTCAGGCCCATTTAGAAGAATTAGGAGTACTAAGAATGACAAAGCA
ATGATGCTTGAGGTCTCAGGCCCATTTAGAAGAATTAGGAGTACTAAGAATGACAAAGCA

N40 dbpA      430     440     450     460     470     480
N40D10 dbpA  GTTACAGATGCGGCTGAACAACACCCCTACAACCTACAGCTGAAGGAATACTTGAAATTGCT
N40B dbpA    GTTACAGATGCGGCTGAACAACACCCCTACAACCTACAGCTGAAGGAATACTTGAAATTGCT
N40C dbpA    GTTACAGATGCGGCTGAACAACACCCCTACAACCTACAGCTGAAGGAATACTTGAAATTGCT
GTTACAGATGCGGCTGAACAACACCCCTACAACCTACAGCTGAAGGAATACTTGAAATTGCT

N40 dbpA      490     500     510     520     530     540
N40D10 dbpA  AAAAAAATGAAAAAATAAATAAAGGGTTTCATACAAAAAACTACTGCGCCCTTGAAAAAG
N40B dbpA    AAAAAAATGAAAAAATAAATAAAGGGTTTCATACAAAAAACTACTGCGCCCTTGAAAAAG
N40C dbpA    AAAAAAATGAAAAAATAAATAAAGGGTTTCATACAAAAAACTACTGCGCCCTTGAAAAAG
AAAAAATGAAAAAATAAATAAAGGGTTTCATACAAAAAACTACTGCGCCCTTGAAAAAG

N40 dbpA      550     560     570     580     590     600
N40D10 dbpA  AAGAAAAATCCTAATTTTACTGATGAAAAATGCAAAAAATAACTAA
N40B dbpA    AAGAAAAATCCTAATTTTACTGATGAAAAATGCAAAAAATAACTAA
N40C dbpA    AAGAAAAATCCTAATTTTACTGATGAAAAATGCAAAAAATAACTAA
AAGAAAAATCCTAATTTTACTGATGAAAAATGCAAAAAATAACTAA

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Supplementary Fig. S2. Clustal W sequence alignment of primary *dbpA* gene sequence of N40 *B. burgdorferi* strains. Sequence of the PCR amplified DNA using the primers flanking the *dbpA* gene of B31 strain using hifidelity herculase enzyme (Agilent Technologies Inc., CA) was used to derive the *dbpA* gene sequence and was compared using Clustal W program.

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10          20          30          40          50          60
CN40 ospC ATGAAAAAGAATACATTTAAGTGCAATATTAATGACTTTATTTTTATTTATATCTTGTAAAT
N40D10 ospC ATGAAAAAGAATACATTTAAGTGCAATATTAATGACTTTATTTTTATTTATATCTTGTAAAT
N40B ospC ATGAAAAAGAATACATTTAAGTGCAATATTAATGACTTTATTTTTATTTATATCTTGTAAAT
N40C ospC ATGAAAAAGAATACATTTAAGTGCAATATTAATGACTTTATTTTTATTTATATCTTGTAAAT

70          80          90          100         110         120
CN40 ospC AATTCAGGAAAGATGGGAATGCACTCTGCAAATTCCTGCTGATGAGTCTGTTAAAGGGCCT
N40D10 ospC AATTCAGGAAAGATGGGAATGCACTCTGCAAATTCCTGCTGATGAGTCTGTTAAAGGGCCT
N40B ospC AATTCAGGAAAGATGGGAATGCACTCTGCAAATTCCTGCTGATGAGTCTGTTAAAGGGCCT
N40C ospC AATTCAGGAAAGATGGGAATGCACTCTGCAAATTCCTGCTGATGAGTCTGTTAAAGGGCCT

130         140         150         160         170         180
CN40 ospC AATCTTTACAGAAATAAGTAAAAAAATTACAGAATCTAACCGCAGTTGTTCTG GCCGTGAAA
N40D10 ospC AATCTTTACAGAAATAAGTAAAAAAATTACAGAATCTAACCGCAGTTGTTCTG GCCGTGAAA
N40B ospC AATCTTTACAGAAATAAGTAAAAAAATTACAGAATCTAACCGCAGTTGTTCTG GCCGTGAAA
N40C ospC AATCTTTACAGAAATAAGTAAAAAAATTACAGAATCTAACCGCAGTTGTTCTG GCCGTGAAA

190         200         210         220         230         240
CN40 ospC GAAAGTTGAGACTTACTTTCATCTATAGATGAAC TTGCTACCAAAGCTATTGGTAA AAAAA
N40D10 ospC GAAAGTTGAGACTTACTTTCATCTATAGATGAAC TTGCTACCAAAGCTATTGGTAA AAAAA
N40B ospC GAAAGTTGAGACTTACTTTCATCTATAGATGAAC TTGCTACCAAAGCTATTGGTAA AAAAA
N40C ospC GAAAGTTGAGACTTACTTTCATCTATAGATGAAC TTGCTACCAAAGCTATTGGTAA AAAAA

250         260         270         280         290         300
CN40 ospC ATAGGCCAAATATGGTTTAAAGGCCAATCAGAGTAAAAACAATCATTGTTATCAGGAGCT
N40D10 ospC ATAGGCCAAATATGGTTTAAAGGCCAATCAGAGTAAAAACAATCATTGTTATCAGGAGCT
N40B ospC ATAGGCCAAATATGGTTTAAAGGCCAATCAGAGTAAAAACAATCATTGTTATCAGGAGCT
N40C ospC ATAGGCCAAATATGGTTTAAAGGCCAATCAGAGTAAAAACAATCATTGTTATCAGGAGCT

310         320         330         340         350         360
CN40 ospC TATGCAATATCTGACCTAATAGCAGAAAAATTAATGTATTGAAAAATTCAGAGAATTA
N40D10 ospC TATGCAATATCTGACCTAATAGCAGAAAAATTAATGTATTGAAAAATTCAGAGAATTA
N40B ospC TATGCAATATCTGACCTAATAGCAGAAAAATTAATGTATTGAAAAATTCAGAGAATTA
N40C ospC TATGCAATATCTGACCTAATAGCAGAAAAATTAATGTATTGAAAAATTCAGAGAATTA

370         380         390         400         410         420
CN40 ospC AAGGAAAAATTGATACGCTAAAGCAATGTTCTACAGAAATTTACTGATAAACTAAAAAGT
N40D10 ospC AAGGAAAAATTGATACGCTAAAGCAATGTTCTACAGAAATTTACTGATAAACTAAAAAGT
N40B ospC AAGGAAAAATTGATACGCTAAAGCAATGTTCTACAGAAATTTACTGATAAACTAAAAAGT
N40C ospC AAGGAAAAATTGATACGCTAAAGCAATGTTCTACAGAAATTTACTGATAAACTAAAAAGT

430         440         450         460         470         480
CN40 ospC GAAACATGCAGTGTGGTCTGGTACAAATC-----TACTGATGATAAATGCAAAAGAGCTT
N40D10 ospC GAAACATGCAGTGTGGTCTGGTACAAATC-----TACTGATGATAAATGCAAAAGAGCTT
N40B ospC GAAACATGCAGTGTGGTCTGGTACAAATC-----TACTGATGATAAATGCAAAAGAGCTT
N40C ospC GAAACATGCAGTGTGGTCTGGTACAAATC-----TACTGATGATAAATGCAAAAGAGCTT

490         500         510         520         530         540
CN40 ospC ATTTTAAAAAACAATGCACTTAAAGATAAAGGGTGTGCTCAGAACTTTGAAAAAGTTATTTAAA
N40D10 ospC ATTTTAAAAAACAATGCACTTAAAGATAAAGGGTGTGCTCAGAACTTTGAAAAAGTTATTTAAA
N40B ospC ATTTTAAAAAACAATGCACTTAAAGATAAAGGGTGTGCTCAGAACTTTGAAAAAGTTATTTAAA
N40C ospC ATTTTAAAAAACAATGCACTTAAAGATAAAGGGTGTGCTCAGAACTTTGAAAAAGTTATTTAAA

550         560         570         580         590         600
CN40 ospC GCGGTAAGAAACTTATCAAAAAGCAGCTCAAGAACACATTAATAAATGCTTTAAAGAGCTT
N40D10 ospC GCGGTAAGAAACTTATCAAAAAGCAGCTCAAGAACACATTAATAAATGCTTTAAAGAGCTT
N40B ospC GCGGTAAGAAACTTATCAAAAAGCAGCTCAAGAACACATTAATAAATGCTTTAAAGAGCTT
N40C ospC GCGGTAAGAAACTTATCAAAAAGCAGCTCAAGAACACATTAATAAATGCTTTAAAGAGCTT

610         620         630         640         650         660
CN40 ospC ACAAGTCCTATTTGTGGCAGAAAGTCCAAAAAAACCTTAA
N40D10 ospC ACAAGTCCTATTTGTGGCAGAAAGTCCAAAAAAACCTTAA
N40B ospC ACAAGTCCTATTTGTGGCAGAAAGTCCAAAAAAACCTTAA
N40C ospC ACAAGTCCTATTTGTGGCAGAAAGTCCAAAAAAACCTTAA

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Supplementary Fig. S3. Clustal W sequence alignment of primary *ospC* gene sequence of N40 *B. burgdorferi* strains. Sequence of the PCR amplified DNA using the primers flanking the *ospC* gene of B31 strain using hifidelity herculase enzyme (Agilent Technologies Inc., CA) was used to derive the *ospC* gene sequence and was compared using Clustal W program.

Supplementary Table S1: Primers used for PCR detection of putative endogenous plasmids of *B. burgdorferi* strain N40B.

Plasmid (size in N40B)	Sequence	Amplicon coordinates (size in bp)	Gene	T _m (°C)
cp9 (8722)	CGTACGGTGTAGTTCTTGCAATAG	5634-6104 (471)	C08-C11 gap	66.5
	TTAAAAAACGGTGCAATATATGAC			64.3
cp26 (26498)	ATAGCCCATTCAGACATTAAACCGCCT	13982-14375 (394)	BBB17	76.8
	AGTTCCCCAAATAACAGCAATCTGCGA			77.4
cp32-2/7 (17483)	GGAATGTATTAATTGATAATTCAG	8753-9127 (375)	BBO32	47.7
	GCGAACTAAATAGTGCCTTATGGG			58.0
cp32-4 (15915)	ATGAACAATAAAGTGTTGATTGTAG	6680-7052 (373)	BBR33-like	61.0
	TTTCTGCTTGAATAGGTATTATAAC			60.7
cp32-5 (29615)	GCCCATTTTTTCAGCAGTTATGGGTA	20692-21205 (514)	BBR33-like	64.1
	GGGAAAAAATACCCGGAACAAGGA			65.2
cp32-9 (30658)	TATCAAAAAAGTGCTGTTTTATAG	21454-21721 (268)	BBN32- BBN33	50.2
	TAATCTCAAATATTCTTCTTTATG			46.2
cp32-10 (30154)	CTTCGAGGAACTTTATTACTTTATC	16607-17054 (448)	Rev (<i>orf3</i>)	61.7
	AAATTATTTTTTGCATCAATGTTAT			62.9
cp32-12 (27767)	AGTGAAGGCTCAAATTACAAGAAG	25112-25500 (389)	Erp27	66.4
	ATACCAAGCCCTAATGAATTAACAC			66.1
lp17 (20672)	ACTGCAATCTGCCAAGCTACATAATC	11193-11473 (281)	BBD12	73.8
	GATAAGGACGGTTGTCTACATGGATTG			72.0
lp25 (23394)	TTGCTGCCATTTCTCACTTGGTAA	9588-9764 (177)	BBE16	65.0
	ATAAAAGCGACAGGTTATCGTGCAG			64.6
lp28-2 (29521)	CCCTCATCAAGTTTTCCATGTGTTTT	13961-14457 (497)	BBG17	69.7
	GAAGCAAGCGGTAGTTACAGTGCAG			74.7
lp28-3*	GTAGCAATATACTTGTGCTAGAGG	(Not present in N40B) (816)	BBH06	62.0
	TCTCTTTTGATAAATTGGCTTAAG			56.6
lp28-4 (29424)	TTACCTTAGCTAATCTATTTATCGACAC	10779-10992 (214)	Non-coding region	65.3
	TAAAGCGCGGAGTTTTCGGCTG			75.9
lp28-5 (26624)	CGGCTTATGGCGGTGGAGTTGGA	11029-11599 (571)	Y06-Y07	68.3
	GCGGATTTGCTGTATCTTTTACTTT			62.9
lp36 (29194)	CCCTACCAAATTCAAGTCCAGCAAT	21063-21596 (534)	Non-coding region	64.1
	CACCGAACCAGTATGAATTGCCAT			65.2
lp38 (37903)	AGCGGCAGAACAAAACATGCAAAAACCTG	6476-6603 (128)	BBJ09 (<i>ospD</i>)	71.8
	TCCAAGCTATTTCTACGGCCTCTTTAGC			70.0
lp54 (53799)	GCAAAAATGTTAGCAGCCTTGACGAGAAA	9504-9612 (109)	BBA15 (<i>ospA</i>)	72.5
	TAGATCGTACTTGCCGCTTTGTTTTT			67.8

Supplementary Table S2: Primers used for PCR amplification of the specific genes

Plasmid	Sequence	Gene	T _m (°C)
lp17	ATGATAATAAAAATAAAAATAATGTC	BBD14	45.1
	AATTTTGATTAATTTTAATTTTGCTGATTCTTTTAAAACCT		59.0
lp25	TATAAATAGTAGTTTCTAATTAAATACTATTGAA	BBE22	52.8
	AATAATTTCTTTGATTAACCAACTTCAAATTA		54.2
lp28-2	GTGGAAATAAATTTACAAAGTAAAT	BBG02	46.4
	CTATTTTTTTTTGTATGCCAATT		45.3
lp28-3*	ATCTCTAAAGATTTTAGCAGGGGAGAA	BBH06	56.9
	CCTTGTGATCTATAATAAAGTTTGCTTAATAG		56.5
lp28-4	ATGAAAATCAAAAATATAGCAAACATA	BBI06 signal peptide seq.	50.1
	TTCTATTGAAATATATTTTTTGTTTTGAAT		50.3
lp36	ATGATTTATTCATAAGATATGAA	BBK32	43.5
	TTAGTACCAAACGCCATTCTTGTCATGA		58.9
lp38	ATGAAAAAATTAATAAAAATACT	BBJ09 (<i>ospD</i>)	40.7
	TTAAGTATTTAACAAGGCCACA		48.5
lp54	TGTAGTATTGGATTAGT	BBA25 (<i>dbpB</i>)	36.4
	ACTAATCCAATACTACA		36.4
lp21	AATTAAGTATGTGGAGGATATATATATGAGCA	BBU06	56.5
	TCCTTTACTTTTAATTTAATTTCTTTCATTATCATATTTAG		57.6
lp28-1	TTTCAAGTGCAATTTTATTAACAACCTTCT	VlsE1	54.9
	GCAGCTTCAACAATCTCCTTTAT		53.4
lp28-4	CGGGATCCAATATTTTAATAATCTCAGCTACA	Soluble BBI06 (for recombinant protein prep)	59.4
	AACTGCAGTTACATTAACTTAATTAAGTTTTCTA		56.9
lp54	TTAGCTCGTCCTAATATTTACAATT	For <i>dbpA</i> sequencing	51.4
	TATGTCTTGATTATCGGGCGAAGAGT		58.4
cp26	TTAATTTTAGCATATTTGGCTTTGCTTAT	For <i>ospC</i> sequencing	55.3
	TCCAGTACTTTTTTAAAACAAATTAATCTT		53.2

Supplementary Table S3: MALDI-MS analysis of eight protein spots recovered from Two-dimensional electrophoresis gels of cN40 and N40D10/E9 total proteins followed by digestion with trypsin and identification of the proteins by peptide fingerprinting.

Spot #	Protein Identified	NCBI Accession #
1	Flagellin (BB_0147) Basic membrane protein A (X97240.1, homolog of BB_0383)	P11089 GI:2598245
2	Basic membrane protein D (BbuJD1_0385 or BB_0385) 60kDa chaperonin (GroEL) (BB_0649)	E4S1L1 P0C923
3	Tentative: Integral membrane protein (BB_0603)	GI:239835986
4	Outer surface protein D (BB_J09)	GI:506387
5	30S ribosomal protein S4 (BbuZS7_0632 or BB_0615) Phosphoglycolate phosphatase (BBU29805_0719 or BB_0676) Hypothetical protein (BBU29805_K10 or BB_K13)	B7J2H5 GI:226320487 GI:226315606
6	Outer surface protein C (BBU29805_B19 homolog of BB_B19) Neutrophil activating protein (BB_0690)	GI:226246807 GI:15595035
7	Neutrophil activating protein (BB_0690)	GI:15595035
8	Outer surface 22 kD lipoprotein (BB_0365) Outer membrane protein (BBUWI9123_A0003 or BB_A03)	P0CL67 GI:224796629