

Supporting Information File S1

Analysis of an ordered, comprehensive STM mutant library in infectious *Borrelia burgdorferi*: insights into the genes required for mouse infectivity

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DIRECTORY	Pages
Table 1. Transposon insertions obtained in <i>B. burgdorferi</i> genes with predicted functions.	2-24
Table 2. Genes with a single insertion in the last 10% of the reading frame.	25-26
Table 3. Summary of mouse infectivity results obtained for STM clones with insertions in cp26.	27-28
Table 4. Oligonucleotides used in this study.	29
Figure 1. Construction of signature-tagged suicide himar1 transposon vectors pGKTSTM1-pGKTSTM11 for transposon mutagenesis of infectious <i>Borrelia burgdorferi</i> .	30
Figure 2. Distribution of transposon insertions in <i>B. burgdorferi</i> genes.	31
Figure 3. Transposon insertion sites for each of the plasmids present in <i>B. burgdorferi</i> B31 5A18NP1.	32-52
Figure 4. Distribution of individual MFI values obtained with high, intermediate, and low infectivity STM transposon mutant clones.	53
Figure 5. MFI values obtained by Luminex-based STM analysis are reproducible.	54
Figure 6. Sampling error may contribute to the variability of Luminex-based MFI values obtained in mouse infectivity analyses.	54
Figure 7. Heat map representations of the mouse infectivity of transposon mutants in the genes of <i>B. burgdorferi</i> plasmids.	55-60
Figure 8. Heat map representations of the mouse infectivity of transposon mutants in genes by functional group.	61-66
Spreadsheet S1. Transposon insertion sites in 4,479 clones derived by electroporation of <i>B. burgdorferi</i> 5A18NP1 with the <i>Himar1</i> suicide vector pGKT. (Excel format)	Separate Excel File
Spreadsheet S2. Mouse infectivity results obtained for 434 <i>B. burgdorferi</i> STM mutants, using Luminex STM analysis. (Excel format)	Separate Excel File

Table 1. Transposon insertions obtained in *B. burgdorferi* genes with predicted functions.^a Genes are arranged into broad (green) and narrow (blue) functional groups.

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
Amino acid biosynthesis				1	0	0	0.00
Serine family	Chr	BB601	serine OH methyltransferase (glyA)				
Biosynthesis of cofactors, prosthetic groups, and carriers				8	2	3	0.25
Folic acid				1	0	0	0.00
	Chr	BB026	methylenetetrahydrofolate dehydrogenase (fold)				
Heme and porphyrin				2	2	0	1.00
	Chr	BB197	protoporphyrinogen oxidase		Yes		
	Chr	BB656	oxygen-independent coproporphyrinogen III oxidase		Yes		
Menaquinone and ubiquinone				1	0	0	0.00
	Chr	BB314	octaprenyl-diP synthase (ispB)				
Pantothenate				1	0	1	0.00
	Chr	BB812	pantothenate metabolism flavoprotein (dfp)			Yes (1.00)	
Pyridoxine				1	0	0	0.00
	Chr	BB768	pyridoxal kinase (pdxK)				
Thiamine				1	0	1	0.00
	Chr	BB621	4-methyl-5 (β-hydroxyethyl)-thiozole monophosphate biosynthesis protein (thiJ)			Yes (0.92)	
Pyridine nucleotides				1	0	1	0.00
	Chr	BB522	NH ₃ -dep NAD ⁺ synthase			Yes (0.99)	
Cell envelope				116	51	2	0.44
Membrane proteins, lipoproteins, and porins				44	28	0	0.64
	Chr	BB10	surface-located membrane protein 1 (Imp1)				
	Chr	BB108	basic membrane protein				
	Chr	BB158	S2 protein		Yes		
	Chr	BB167	outer membrane protein (tpn50)		Yes		
	Chr	BB319	exported protein (tpn38b)		Yes		
	Chr	BB347	fibronectin/fibrinogen-binding protein		Yes		
	Chr	BB365	lipoprotein LA7		Yes		
	Chr	BB382	basic membrane protein B (bmpB)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB383	basic membrane protein A (bmpA)				
	Chr	BB384	basic membrane protein C (bmpC)		Yes		
	Chr	BB385	basic membrane protein D (bmpD)				
	Chr	BB442	inner membrane protein				
	Chr	BB603	membrane-associated protein p66				
	Chr	BB735	rare lipoprotein A (rlpA)		Yes		
	Chr	BB753	membrane spanning protein				
	Chr	BB795	outer membrane protein				
	cp09	BBC06	exported protein A (eppA)		Yes		
	cp26	BBB07	outer surface protein		Yes		
	cp26	BBB19	outer surface protein C (ospC)		Yes		
	lp25	BBE09	protein p23		Yes		
	lp28-1	BBF01	arthritis-related protein (arp)		Yes		
	lp28-1	BBF22	protein p23		Yes		
	lp28-1	BBF32	vis recombination cassette Vls3-16		Yes		
	lp28-3	BBH20	outer membrane porin (oms28)		Yes		
	lp28-3	BBH21	outer membrane porin (oms28)				
	lp36	BBK52	protein p23				
	lp36	BBK53	outer membrane protein				
	lp38	BBJ09	outer surface protein D (ospD)		Yes		
	lp38	BBJ50	outer membrane protein				
	lp38	BBJ51	visE paralog, authentic frameshift		Yes		
	lp38	BBJ52	visE paralog, authentic frameshift				
	lp54	BBA03	outer membrane protein		Yes		
	lp54	BBA04	S2 protein		Yes		
	lp54	BBA05	S1 protein		Yes		
	lp54	BBA15	outer surface protein A (ospA)				
	lp54	BBA16	outer surface protein B (ospB)		Yes		
	lp54	BBA24	decorin binding protein A (dbpA)		Yes		
	lp54	BBA25	decorin binding proteinB (dbpB)		Yes		
	lp54	BBA36	lipoprotein		Yes		

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	lp54	BBA52	outer membrane protein				
	lp54	BBA59	lipoprotein		Yes		
	lp54	BBA60	surface lipoprotein P27		Yes		
	lp54	BBA62	lipoprotein		Yes		
	lp54	BBA74	outer membrane porin (oms28)		Yes		
Murein sacculus and peptidoglycan				20	2	2	0.10
	Chr	BB100	glutamate racemase (murl)				
	Chr	BB136	penicillin-binding protein (pbp-1)				
	Chr	BB160	alanine racemase (alr)				
	Chr	BB200	D-alanine-D-alanine ligase (ddlA)				
	Chr	BB201	UDP-N-Acmuramoylalanyl-D-glutamate—2,6-diaminopimelate ligase (murE)				
	Chr	BB303	phospho-N-Acmuramoyl-pentapeptidetransferase (mraY)				
	Chr	BB304	UDP-N-Acmuramoylalanyl-D-glutamyl-2,6-diamino-pimelate-D-alanyl-D-alanine ligase (murF)				
	Chr	BB472	UDP-NAG 1-carboxy-vinyl transferase (murA)				
	Chr	BB582	CPDase		Yes		
	Chr	BB585	UDP-N-Acmuramoylalanine-D-glutamate ligase (murD)			Yes (0.99)	
	Chr	BB598	UDP-N-Acmuramate Dase (murB)				
	Chr	BB605	serine-type D-Ala-D-Ala CPDase (dacA)		Yes		
	Chr	BB625	N-Acmuramoyl-L-alanine amidase				
	Chr	BB715	rod shape-determining protein (mreB-1)				
	Chr	BB716	rod shape-determining protein (mreC)				
	Chr	BB718	penicillin-binding protein (pbp-2)				
	Chr	BB719	rod shape-determining protein (mreB-2)				
	Chr	BB732	penicillin-binding protein (pbp-3)				
	Chr	BB767	UDP-N-Acglucosamine-N-Acmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol NAG transferase (murG)			Yes (0.97)	
	Chr	BB817	UDP-N-Acmuramate-alanine ligase (murC)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
Surface polysaccharides, lipopolysaccharides and antigens				16	13	0	0.81
	Chr	BB572	glycosyl transferase (lgtD)				
	Chr	BB744	antigen, p83/100		Yes		
	lp25	BBE31	antigen, P35				
	lp28-3	BBH32	antigen, P35		Yes		
	lp28-4	BBI36	antigen, P35				
	lp36	BBK15	antigen, P35, putative		Yes		
	lp36	BBK32	immunogenic protein P35		Yes		
	lp36	BBK37	immunogenic protein P37		Yes		
	lp36	BBK45	immunogenic protein P37		Yes		
	lp36	BBK46	immunogenic protein P37		Yes		
	lp36	BBK48	immunogenic protein P37		Yes		
	lp36	BBK50	immunogenic protein P37		Yes		
	lp38	BBJ41	antigen P35, putative		Yes		
	lp54	BBA64	antigen P35		Yes		
	lp54	BBA66	antigen P35		Yes		
	lp54	BBA73	antigen P35, putative		Yes		
Flagella				36	8	0	0.22
	Chr	BB147	flagellar filament 41 kDa core protein (flaB)				
	Chr	BB149	flgr hook-associated protein 2 (fliD2)				
	Chr	BB180	flgr protein				
	Chr	BB181	flgr hook-associated protein (flgK)				
	Chr	BB182	flgr hook-associated protein 3 (flgL)				
	Chr	BB221	flgr motor switch protein (fliG-1)		Yes		
	Chr	BB270	flgr-associated GTP-binding protein (flhF)				
	Chr	BB271	flgr biosynthesis protein (flhA)				
	Chr	BB272	flgr biosynthesis protein (flhB)				
	Chr	BB273	flgr biosynthesis protein (fliR)				
	Chr	BB274	flgr biosynthesis protein (fliQ)				
	Chr	BB275	flgr biosynthesis protein (fliP)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB276	flgr biosynthesis protein (fliZ)		Yes		
	Chr	BB277	flgr motor switch protein (fliN)				
	Chr	BB278	flgr motor switch protein (fliM)				
	Chr	BB279	flgr protein (fliL)				
	Chr	BB280	flgr motor rotation protein B (motB)				
	Chr	BB281	flgr motor rotation protein A (motA)				
	Chr	BB282	flgr protein (flbD)				
	Chr	BB283	flgr hook protein (flgE)				
	Chr	BB284	flgr hook assembly protein (flgD)				
	Chr	BB285	flgr protein (flbC)				
	Chr	BB286	flgr protein (flbB)				
	Chr	BB287	flgr protein (flbA)		Yes		
	Chr	BB288	flgr-specific ATPase (fliI)		Yes		
	Chr	BB289	flgr assembly protein (fliH)		Yes		
	Chr	BB290	flgr motor switch protein (fliG-2)				
	Chr	BB291	flgr basal-body rod protein (fliF)				
	Chr	BB292	flgr hook-basal body complex protein (fliE)				
	Chr	BB293	flgr basal-body rod protein (flgC)				
	Chr	BB294	flgr basal-body rod protein (flgB)		Yes		
	Chr	BB550	flgr protein (flaJ)				
	Chr	BB668	flgr filament outer layer protein (flaA)		Yes		
	Chr	BB772	flgr P-ring protein (flgI)		Yes		
	Chr	BB774	flgr basal-body rod protein (flgG)				
	Chr	BB775	flgr hook-basal body complex protein (flhO)				
Cellular processes				66	27	2	0.41
General				2	0	0	0.00
	Chr	BB660	GTP-binding protein (era)				
	Chr	BB781	GTP-binding protein (obg)				
Chemotaxis				19	14	0	0.74
	Chr	BB040	chemotaxis protein methyltransferase (cheR-1)				
	Chr	BB312	purine-B chemotaxis protein (cheW-1)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB414	chemotaxis protein methyltransferase (cheR-2)		Yes		
	Chr	BB415	protein-glutamate methylesterase (cheB-1)		Yes		
	Chr	BB551	chemotaxis response regulator (cheY-1)				
	Chr	BB565	purine-B chemotaxis protein (cheW-2)		Yes		
	Chr	BB567	chemotaxis histidine kinase (cheA-1)		Yes		
	Chr	BB568	protein-glutamate methylesterase (cheB-2)		Yes		
	Chr	BB570	chemotaxis response regulator (cheY-2)		Yes		
	Chr	BB578	MC protein (mcp-1)		Yes		
	Chr	BB596	MC protein (mcp-2)				
	Chr	BB597	MC protein (mcp-3)		Yes		
	Chr	BB606	chemoreceptor glutamine deamidase (cheD)		Yes		
	Chr	BB669	chemotaxis histidine kinase (cheA-2)		Yes		
	Chr	BB670	purine-B chemotaxis protein (cheW-3)		Yes		
	Chr	BB671	chemotaxis operon protein (cheX)		Yes		
	Chr	BB672	chemotaxis response regulator (cheY-3)				
	Chr	BB680	MC protein (mcp-4)		Yes		
	Chr	BB681	MC protein (mcp-5)		Yes		
Cell division				13	2	1	0.15
	Chr	BB058	cell division control protein 27				
	Chr	BB195	cell division control protein				
	Chr	BB361	cell division inhibitor		Yes		
	Chr	BB299	cell division protein (ftsZ)				
	Chr	BB789	cell division protein (ftsH)			Yes (0.96)	
	Chr	BB076	cell division protein				
	Chr	BB257	cell division protein				
	Chr	BB300	cell division protein (ftsA)				
	Chr	BB302	cell division protein (ftsW)				
	Chr	BB301	cell division protein (divIB)				
	Chr	BB313	cell division protein (ftsJ)				
	Chr	BB434	stage 0 sporulation protein J (spoJ)		Yes		
	lp28-2	BBG08	stage 0 sporulation protein J (spoJ)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
Cell killing				5	3	0	0.60
	Chr	BB059	hemolysin (tlyC)				
	Chr	BB117	hemolysin III (yplQ)		Yes		
	Chr	BB143	alpha-hemolysin (hlyA)				
	Chr	BB202	hemolysin		Yes		
	Chr	BB506	hemolysin (tlyA)		Yes		
Chaperones				11	5	1	0.45
	Chr	BB264	heat shock protein 70 (dnaK-1)		Yes		
	Chr	BB295	heat shock protein (hslU)		Yes		
	Chr	BB296	heat shock protein (hslV)		Yes		
	Chr	BB517	heat shock protein (dnaJ-1)				
	Chr	BB518	heat shock protein 70 (dnaK-2)				
	Chr	BB519	grpE protein (grpE)				
	Chr	BB560	heat shock protein 90 (htpG)		Yes		
	Chr	BB602	chaperonin			Yes (1.00)	
	Chr	BB649	heat shock protein (groEL)				
	Chr	BB655	heat shock protein (dnaJ-2)		Yes		
	Chr	BB741	chaperonin (groES)				
Detoxification				3	1	0	0.33
	Chr	BB153	superoxide dismutase (sodA)		Yes		
	Chr	BB179	thiophene and furan oxidation protein (thdF)				
	Chr	BB690	neutrophil activating protein (napA)				
Protein and peptide secretion				11	1	0	0.09
	Chr	BB030	signal peptidase I (lepB-1)				
	Chr	BB031	signal peptidase I (lepB-2)				
	Chr	BB154	preprotein translocase sub (secA)				
	Chr	BB263	signal peptidase I (lepB-3)		Yes		
	Chr	BB362	prolipoprotein diacylglycerol transferase (lgt)				
	Chr	BB395	preprotein translocase sub (secE)				
	Chr	BB498	preprotein translocase sub (secY)				
	Chr	BB610	trigger factor (tig)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB652	protein-export membrane protein (secD)				
	Chr	BB653	protein-export membrane protein (secF)				
	Chr	BB694	signal recognition particleprotein (ffh)				
Transformation				2	1	0	0.50
	Chr	BB591	competence locus E		Yes		
	Chr	BB798	competence protein F				
Central intermediary metabolism				11	5	0	0.45
General				3	2	0	0.67
	Chr	BB241	glycerol kinase (glpK)		Yes		
	Chr	BB243	glycerol-3-P dehydrogenase, anaerobic (glpA)		Yes		
	Chr	BB376	SAM synthase (metK)				
Amino sugars				2	0	0	0.00
	Chr	BB151	N-acetylglucosamine-6-P-deAcase (nagA)				
	Chr	BB152	glucosamine-6-P-isomerase (nagB)				
Degradation of polysaccharides				2	2	0	1.00
	Chr	BB002	b-N-acetylhexosaminidase		Yes		
	Chr	BB620	b-glucosidase		Yes		
Phosphorus compounds				1	0	0	0.00
	Chr	BB533	phnP protein (phnP)				
Polysaccharides (cytoplasmic)				3	1	0	0.33
	Chr	BB004	phosphoglucomutase (femD)				
	Chr	BB166	4-a—glucanotransferase (malQ)		Yes		
	Chr	BB835	phosphomannomutase (cpsG)				
Energy metabolism				26	2	1	0.08
Anaerobic				1	0	0	0.00
	Chr	BB728	NADH oxidase, water-forming (nox)				
Amino acid and amines				2	1	0	0.50
	Chr	BB841	arginine deiminase (arcA)		Yes		
	Chr	BB842	ornithine carbamoyl transferase (arcB)				
Anaerobic				2	0	0	0.00
	Chr	BB016	glpE protein (glpE)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB087	L-lactate dehydrogenase (ldh)				
ATP-proton motive force interconversion				6	0	0	0.00
	Chr	BB090	V-type ATPase, sub K (atpK)				
	Chr	BB091	V-type ATPase, sub I (atpI)				
	Chr	BB092	V-type ATPase, sub D (atpD)				
	Chr	BB093	V-type ATPase, sub B (atpB)				
	Chr	BB094	V-type ATPase, sub A (atpA)				
	Chr	BB096	V-type ATPase, sub E (atpE)				
Electron transport				2	0	0	0.00
	Chr	BB061	thioredoxin (trxA)				
	Chr	BB515	thioredoxin reductase (trxB)				
Fermentation				2	0	0	0.00
	Chr	BB589	phosphate acetyltransferase (pta)				
	Chr	BB622	acetate kinase (ackA)				
Glycolysis				11	1	1	0.09
	Chr	BB020	pyroP-fructose 6-P1-PP transferase sub (pfpB)				
	Chr	BB055	triosePisomerase				
	Chr	BB056	phosphoglycerate kinase (pgk) {Bb}				
	Chr	BB057	glyceraldehyde 3-P dehydrogenase (gap)				
	Chr	BB337	enolase (eno)				
	Chr	BB348	pyruvate kinase (pyk)				
	Chr	BB445	fructose-bisPaldolase (fba)				
	Chr	BB630	1-phosphofructo kinase (fruK)		Yes		
	Chr	BB658	phosphoglycerate mutase (gpmA)				
	Chr	BB727	pyroP-fructose 6-P1-PP transferase (pfk)				
	Chr	BB730	glucose-6-P-isomerase (pgi)			Yes (0.97)	
Pentose phosphate pathway				4	0	0	0.00
	Chr	BB222	glucose-6-P1-dehydrogenase				
	Chr	BB561	phosphogluconate dehydrogenase (gnd)				
	Chr	BB636	glucose-6-P1-dehydrogenase (zwf)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB657	ribose 5-Pisomerase (rpi)				
Sugars				5	2	0	0.40
	Chr	BB207	UTP-glucose-1-Puridylyl transferase				
	Chr	BB407	mannose-6-isomerase (manA)				
	Chr	BB444	nucleotide sugar epimerase				
	Chr	BB545	xylulokinase (xylB)		Yes		
	Chr	BB676	phosphoglycolate P phosphatase (gph)		Yes		
Fatty acid and phospholipid metabolism (General)				15	1	0	0.07
	Chr	BB037	1-acyl-sn-glycerol-3-PAc transferase (plsC)				
	Chr	BB109	Ac-CoAC-Ac transferase (fadA)				
	Chr	BB119	phosphatidate cytidylase (cdsA), AFS				
	Chr	BB137	long-chain-fatty-acid CoAligase				
	Chr	BB249	phosphatidyl transferase				
	Chr	BB327	glycerol-3-PO-acyl transferase				
	Chr	BB368	glycerol-3-P dehydrogenase, NAD (P)+ (gpsA)		Yes		
	Chr	BB593	long-chain-fatty-acid CoAligase				
	Chr	BB683	3-OH-3-methylglutaryl-CoA synthase				
	Chr	BB685	3-OH-3-methylglutaryl-CoA reductase (mvaA)				
	Chr	BB686	mevalonate pyroPDCase				
	Chr	BB687	phosphomevalonate kinase				
	Chr	BB688	melvalonate kinase				
	Chr	BB704	acyl carrier protein				
	Chr	BB721	CDP-diacylglycerol-glycerol-3-P 3-phosphatidyl transferase				
Purines, pyrimidines, nucleosides, nucleotides				19	7	0	0.37
Nucleotide and nucleoside interconversion				6	1	0	0.17
	Chr	BB128	cytidylate kinase (cmk-1)				
	Chr	BB417	adenylate kinase (adk)				
	Chr	BB463	nucleoside-dikinase (ndk)		Yes		
	Chr	BB571	uridylate kinase (smbA)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB793	thymidylate kinase (tmk)				
	Chr	BB819	cytidylate kinase (cmk-2)				
Purine ribonucleotide biosynthesis				3	2	0	0.67
	Chr	BB544	phosphoribosyl pyrophosphatase (prs)				
	cp26	BBB17	IMP dehydrogenase (guaB)		Yes		
	cp26	BBB18	GMP synthase (guaA)		Yes		
Pyrimidine ribonucleotide biosynthesis				1	0	0	0.00
	Chr	BB575	CTP synthase (pyrG)				
Salvage of nucleosides and nucleotides				9	4	0	0.44
	Chr	BB015	uridine kinase (udk)				
	Chr	BB239	deoxyguanosine/deoxyadenosine kinase (I) sub 2 (dck)				
	Chr	BB375	pfs protein (pfs-1)				
	Chr	BB588	pfs protein (pfs-2)		Yes		
	Chr	BB618	cytidine deaminase (cdd)		Yes		
	Chr	BB777	adenine phosphoribosyl transferase (apt)				
	Chr	BB791	thymidine kinase (tdk)				
	lp28-3	BBH33	adenine deaminase (adeC), paralog of BBK17		Yes		
	lp36	BBK17	adenine deaminase (adeC), paralog of BBH33		Yes		
Regulatory functions (General)				15	6	0	0.40
	Chr	BB042	P transport system regulatory protein (phoU)		Yes		
	Chr	BB176	methanol dehydrogenase regulator (moxR)				
	Chr	BB184	carbon storage regulator (csrA)				
	Chr	BB198	guanosine-3',5'-bis (diP) 3'-pyrophosphohydrolase (spoT)		Yes		
	Chr	BB379	protein kinase C1 inhibitor (pkci)				
	Chr	BB416	pheromone shutdown protein (traB)				
	Chr	BB419	response regulatory protein (rrp-1)		Yes		
	Chr	BB420	sensory transduction histidine kinase (hk-1)		Yes		
	Chr	BB647	ferric uptake regulation protein (fur)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB693	xylose operon regulatory protein (xylR-1)				
	Chr	BB737	histidine phospho kinase/phosphatase putative				
	Chr	BB763	response regulatory protein (rrp-2)				
	Chr	BB764	sensory transduction histidine kinase (hk-2)				
	Chr	BB831	xylose operon regulatory protein (xylR-2)		Yes		
	lp54	BBA07	chpAI protein		Yes		
DNA metabolism				46	28	1	0.61
Degradation of DNA				2	2	0	1.00
	Chr	BB411	endonuclease precursor (nucA)		Yes		
	Chr	BB745	endonuclease III (nth)		Yes		
DNA replication				18	5	1	0.28
	Chr	BB014	primosomal protein N (priA)		Yes		
	Chr	BB035	DNA topoisomerase IV (parC)				
	Chr	BB036	DNA topoisomerase IV (parE)				
	Chr	BB111	replicative DNA helicase (dnaB)			Yes (1.00)	
	Chr	BB177	glucose-inhibited div protein B (gidB)		Yes		
	Chr	BB178	glucose-inhibited div protein A (gidA)				
	Chr	BB435	DNA gyrase, sub A (gyrA)				
	Chr	BB436	DNA gyrase, sub B (gyrB)				
	Chr	BB437	chromosomal replication initiation protein (dnaA)				
	Chr	BB438	DNA polymerase III, subunit beta (dnaN)				
	Chr	BB461	DNA polymerase III, sub gamma/tau (dnaX)				
	Chr	BB534	exodeoxyribonuclease III (exoA)				
	Chr	BB552	DNA ligase (lig)				
	Chr	BB579	DNA polymerase III, sub alpha (dnaE)				
	Chr	BB710	DNA primase (DNA G)				
	Chr	BB827	ATP-dep helicase (hrpA)		Yes		
	Chr	BB828	DNA topoisomerase I (topA)		Yes		
	lp28-2	BBG32	replicative DNA helicase		Yes		
DNA recombination and repair				21	16	0	0.76
	Chr	BB022	Holliday junction DNA helicase (ruvB)		Yes		

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB023	Holliday junction DNA helicase (ruvA)		Yes		
	Chr	BB053	uracil DNA glycosylase (ung)		Yes		
	Chr	BB098	DNA mismatch repair protein		Yes		
	Chr	BB114	ss DNA-binding protein (ssb)				
	Chr	BB131	recA protein (recA)				
	Chr	BB211	DNA mismatch repair protein (mutL)		Yes		
	Chr	BB254	ss-DNA-specific exonuclease (recJ)		Yes		
	Chr	BB344	DNA helicase (uvrD)		Yes		
	Chr	BB457	excinuclease ABC, sub C (uvrC)		Yes		
	Chr	BB581	DNA recombinase (recG)		Yes		
	Chr	BB607	rep helicase, ss DNA-dep ATPase (rep)		Yes		
	Chr	BB623	transcription-repair coupling factor (mfd)		Yes		
	Chr	BB632	exodeoxyribonuclease V, a chain (recD)		Yes		
	Chr	BB633	exodeoxyribonuclease V, b chain (recB)				
	Chr	BB634	exodeoxyribonuclease V g chain (recC)				
	Chr	BB797	DNA mismatch repair protein (mutS)		Yes		
	Chr	BB829	exonuclease SbcD (sbcD)		Yes		
	Chr	BB830	exonuclease SbcC (sbcC)		Yes		
	Chr	BB836	excinuclease ABC, sub B (uvrB)		Yes		
	Chr	BB837	excinuclease ABC, sub A (uvrA)				
DNA restriction and modification				5	5	0	1.00
	Chr	BB421	DNA 3-methyladenine glycosylase		Yes		
	Chr	BB422	DNA 3-methyladenine glycosylase (mag)		Yes		
	lp25	BBE02	putative restriction-modification enzyme				
	lp25	BBE29	adenine specific DNA methyltransferase		Yes		
	lp28-3	BBH09	putative restriction-modification enzyme		Yes		
Transcription				19	4	0	0.21
General				1	0	0	0.00
	Chr	BB052	spoU protein (spoU)				
Degradation of RNA				4	1	0	0.25
	Chr	BB046	ribonuclease H (rnhB)		Yes		

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB441	ribonuclease P protein component (rnpA)				
	Chr	BB705	ribonuclease III (rnc)				
	Chr	BB805	polyribonucleotide nucleotidyl transferase (pnpA)				
DNA-dependent RNA polymerase				6	2	0	0.33
	Chr	BB388	DNA-directed RNA polymerase (rpoC)				
	Chr	BB389	DNA-directed RNA polymerase (rpoB)				
	Chr	BB450	RNA polymerase sigma-54 factor (ntrA)		Yes		
	Chr	BB502	DNA-directed RNA polymerase (rpoA)				
	Chr	BB712	RNA polymerase sigma-70 factor (rpoD)				
	Chr	BB771	RNA polymerase sigma factor (rpoS)		Yes		
Transcription factors				6	0	0	0.00
	Chr	BB107	N utilization substance protein B (nusB)				
	Chr	BB132	transcription elongation factor (greA)				
	Chr	BB230	transcription termination factor Rho (rho)				
	Chr	BB355	transcription factor				
	Chr	BB394	transcription antitermination factor (nusG)				
	Chr	BB800	N-utilization substance protein A (nusA)				
RNA processing				2	1	0	0.50
	Chr	BB516	RNA methyltransferase, TrmH family, group 3		Yes		
	Chr	BB706	polynucleotide adenyl transferase (papS)				
Translation				124	12	1	0.10
General				2	1	0	0.50
	Chr	BB590	dimethyladenosine transferase (ksgA)		Yes		
	Chr	BB802	ribosome-B factor A (rbfA)				
Amino acyl tRNA synthetases				20	0	0	0.00
	Chr	BB005	tryptophanyl-tRNA synthase (trsA)				
	Chr	BB101	asparaginyl-tRNA synthase (asnS)				
	Chr	BB135	histidyl-tRNA synthase (hisS)				
	Chr	BB220	alanyl-tRNA synthase (alaS)				
	Chr	BB226	seryl-tRNA synthase (serS)				
	Chr	BB251	leucyl-tRNA synthase (leuS)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB370	tyrosyl-tRNA synthase (tyrS)				
	Chr	BB371	glycyl-tRNA synthase (glyS)				
	Chr	BB372	glutamyl-tRNA synthase (gltX)				
	Chr	BB402	prolyl-tRNA synthase (proS)				
	Chr	BB446	aspartyl-tRNA synthase (aspS)				
	Chr	BB513	phenylalanyl-tRNA synthase, a sub (pheS)				
	Chr	BB514	phenylalanyl-tRNA synthase, b sub (pheT)				
	Chr	BB587	methionyl-tRNA synthase (metG)				
	Chr	BB594	arginyl-tRNA synthase (argS)				
	Chr	BB599	cysteinyl-tRNA synthase (cysS)				
	Chr	BB659	lysyl-tRNA synthase				
	Chr	BB720	threonyl-tRNA synthase (thrZ)				
	Chr	BB738	valyl-tRNA synthase (valS)				
	Chr	BB833	isoleucyl-tRNA synthase (ileS)				
Degradation of proteins, peptides, and glycopeptides				21	8	1	0.38
	Chr	BB067	peptidase				
	Chr	BB069	aminopeptidase II				
	Chr	BB104	periplasmic serine protease DO (htrA)				
	Chr	BB118	RIP metalloprotease (rseP)		Yes		
	Chr	BB203	Lambda CII stability-governing protein (hflK)		Yes		
	Chr	BB204	Lambda CII stability-governing protein (hflC)		Yes		
	Chr	BB248	oligoendopeptidase F (pepF)				
	Chr	BB253	ATP-dep protease LA (lon-1)				
	Chr	BB359	carboxyl-terminal protease (ctp)			Yes (1.00)	
	Chr	BB366	aminopeptidase I (yscI)				
	Chr	BB369	ATP-dep Clp protease, sub A (clpA)				
	Chr	BB430	proline dipeptidase (pepQ)		Yes		
	Chr	BB536	zinc protease		Yes		
	Chr	BB608	aminoacyl-histidine dipeptidase (pepD)		Yes		
	Chr	BB611	ATP-dep Clp protease proteolytic component (clpP-1)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB612	ATP-dep Clp protease, sub X (clpX)				
	Chr	BB613	ATP-dep protease LA (lon-2)		Yes		
	Chr	BB627	vacuolar X-prolyl dipeptidyl aminopeptidase I (pepX)		Yes		
	Chr	BB757	ATP-dep Clp protease proteolytic component (clpP-2)				
	Chr	BB769	sialoglycoprotease (gcp)				
	Chr	BB834	ATP-dep Clp protease, sub C (clpC)				
Nucleoproteins				1	0	0	0.00
	Chr	BB232	hbbU protein				
Protein modification				3	0	0	0.00
	Chr	BB065	polypeptide deformylase (def)				
	Chr	BB105	methionine aminopeptidase (map)				
	Chr	BB648	serine/threonine kinase				
Ribosomal proteins: synthesis and modification				53	0	0	0.00
	Chr	BB112	ribosomal protein L9 (rplI)				
	Chr	BB113	ribosomal protein S18 (rpsR)				
	Chr	BB115	ribosomal protein S6 (rpsF)				
	Chr	BB123	ribosomal protein S2 (rpsB)				
	Chr	BB127	ribosomal protein S1 (rpsA)				
	Chr	BB188	ribosomal protein L20 (rplT)				
	Chr	BB189	ribosomal protein L35 (rpmI)				
	Chr	BB229	ribosomal protein L31 (rpmE)				
	Chr	BB233	ribosomal protein S20 (rpsT)				
	Chr	BB256	ribosomal protein S21 (rpsU)				
	Chr	BB338	ribosomal protein S9 (rpsI)				
	Chr	BB339	ribosomal protein L13 (rplM)				
	Chr	BB350	ribosomal protein L28 (rpmB)				
	Chr	BB386	ribosomal protein S7 (rpsG)				
	Chr	BB387	ribosomal protein S12 (rpsL)				
	Chr	BB390	ribosomal protein L7/L12 (rplL)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB391	ribosomal protein L10 (rplJ)				
	Chr	BB392	ribosomal protein L1 (rplA)				
	Chr	BB393	ribosomal protein L11 (rplK)				
	Chr	BB396	ribosomal protein L33 (rpmG)				
	Chr	BB440	ribosomal protein L34 (rpmH)				
	Chr	BB477	ribosomal protein S10 (rpsJ)				
	Chr	BB478	ribosomal protein L3				
	Chr	BB479	ribosomal protein L4 (rplD)				
	Chr	BB480	ribosomal protein L23 (rplW)				
	Chr	BB481	ribosomal protein L2 (rplB)				
	Chr	BB482	ribosomal protein S19 (rpsS)				
	Chr	BB483	ribosomal protein L22 (rplV)				
	Chr	BB484	ribosomal protein S3 (rpsC)				
	Chr	BB485	ribosomal protein L16 (rplP)				
	Chr	BB486	ribosomal protein L29 (rpmC)				
	Chr	BB487	ribosomal protein S17 (rpsQ)				
	Chr	BB488	ribosomal protein L14 (rplN)				
	Chr	BB489	ribosomal protein L24 (rplX)				
	Chr	BB490	ribosomal protein L5 (rplE)				
	Chr	BB491	ribosomal protein S14 (rpsN)				
	Chr	BB492	ribosomal protein S8 (rpsH)				
	Chr	BB493	ribosomal protein L6 (rplF)				
	Chr	BB494	ribosomal protein L18 (rplR)				
	Chr	BB495	ribosomal protein S5 (rpsE)				
	Chr	BB496	ribosomal protein L30 (rpmD)				
	Chr	BB497	ribosomal protein L15 (rplO)				
	Chr	BB499	ribosomal protein L36 (rpmJ)				
	Chr	BB500	ribosomal protein S13 (rpsM)				
	Chr	BB501	ribosomal protein S11 (rpsK)				
	Chr	BB503	ribosomal protein L17 (rplQ)				
	Chr	BB615	ribosomal protein S4 (rpsD)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB695	ribosomal protein S16 (rpsP)				
	Chr	BB699	ribosomal protein L19 (rplS)				
	Chr	BB703	ribosomal protein L32 (rpmF)				
	Chr	BB778	ribosomal protein L21 (rplU)				
	Chr	BB780	ribosomal protein L27 (rpmA)				
	Chr	BB804	ribosomal protein S15 (rpsO)				
tRNA modification				12	2	0	0.17
	Chr	BB012	pseudouridylate synthase I (hisT)				
	Chr	BB021	SAM: tRNAribosyl transferase-isomerase		Yes		
	Chr	BB064	methionyl-tRNAformyl transferase (fmt)				
	Chr	BB084	aminotransferase (nifS)				
	Chr	BB341	glu-tRNAamido transferase, sub B (gatB)				
	Chr	BB342	glu-tRNAamido transferase, sub A (gatA)				
	Chr	BB343	glu-tRNAamido transferase, sub C (gatC)				
	Chr	BB698	tRNA (guanine-N1)-M transferase (trmD)				
	Chr	BB787	peptidyl-tRNAhydrolase (pth)				
	Chr	BB803	tRNApseudouridine 55 synthase (truB)		Yes		
	Chr	BB809	tRNA-guanine transglycosylase (tgt)				
	Chr	BB821	2-methylthio-N6-isopentyladenosine tRNA modification enzyme (miaA)				
Translation factors				12	1	0	0.08
	Chr	BB074	peptide chain release factor 2 (prfB)				
	Chr	BB088	GTP-B membrane protein (lepA)		Yes		
	Chr	BB121	ribosome releasing factor (frr)				
	Chr	BB122	translation elongation factor TS (tsf)				
	Chr	BB169	translation initiation factor 1 (infA)				
	Chr	BB190	translation initiation factor 3 (infC)				
	Chr	BB196	peptide chain release factor 1 (prfA)				
	Chr	BB214	translation elongation factor P (efp)				
	Chr	BB476	translation elongation factor TU (tuf)				
	Chr	BB540	translation elongation factor G (fus-1)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB691	translation elongation factor G (fus-2)				
	Chr	BB801	translation initiation factor 2 (infB)				
Transport and binding proteins				61	28	4	0.59
General				8	3	1	0.38
	Chr	BB080	ABC transporter, ATP-binding protein				
	Chr	BB269	ATP-binding protein (ylxH-1)				
	Chr	BB466	ABC transporter, ATP-binding protein				
	Chr	BB573	ABC transporter, ATP-binding protein		Yes		
	Chr	BB726	ATP-binding protein (ylxH-2)		Yes		
	Chr	BB742	ABC transporter, ATP-binding protein			Yes (0.95)	
	Chr	BB754	ABC transporter, ATP-binding protein				
	lp38	BBJ26	ABC transporter, ATP-binding protein		Yes		
Amino acids, peptide, and amines				20	7	1	0.35
	Chr	BB144	Glycine/betaine/L-proline binding proteinABC transporter, binding protein (proX)			Yes (0.99)	
	Chr	BB145	Glycine/betaine/L-proline binding proteinABC transporter, permease protein (proW)		Yes		
	Chr	BB146	Glycine/betaine/L-proline binding proteinABC transporter,ATP-binding protein (proV)				
	Chr	BB328	oligopeptide ABC transporter, periplasmic binding protein (oppA-1)		Yes		
	Chr	BB329	oligopeptide ABC transporter, periplasmic binding protein (oppA-2)		Yes		
	Chr	BB330	oligopeptide ABC transporter, periplasmic binding protein (oppA-3)		Yes		
	Chr	BB332	oligopeptide ABC transporter, permease protein (oppB-1)				
	Chr	BB333	oligopeptide ABC transporter, permease protein (oppC-1)				
	Chr	BB334	oligopeptide ABC transporter, ATP-binding protein (oppD)				
	Chr	BB335	oligopeptide ABC transporter,ATP-binding protein (oppF)				
	Chr	BB401	glutamate transporter, putative				
	Chr	BB639	spermidine/putrescine ABC transporter, periplasmic binding protein (potD)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB640	spermidine/putrescine ABC transporter, permease protein (potC)				
	Chr	BB641	spermidine/putrescine ABC transporter, permease protein (potB)				
	Chr	BB642	spermidine/putrescine ABC transporter, ATP-binding protein (potA)				
	Chr	BB729	glutamate transporter (gltP)		Yes		
	Chr	BB746	oligopeptide ABC transporter, permease protein (oppC-2)				
	Chr	BB747	oligopeptide ABC transporter, permease protein (oppB-2)				
	cp26	BBB16	oligopeptide ABC transporter, periplasmic binding protein (oppA-4)		Yes		
	lp54	BBA34	oligopeptide ABC transporter, periplasmic binding protein (oppA-5)		Yes		
Anions				4	0	0	0.00
	Chr	BB215	phosphate ABC transporter, periplasmic P-binding protein (pstS)				
	Chr	BB216	phosphate ABC transporter, permease protein (pstC)				
	Chr	BB217	phosphate ABC transporter, permease protein (pstA)				
	Chr	BB218	phosphate ABC transporter, ATP-binding protein (pstB)				
Carbohydrates, organic alcohols, and acids				19	12	1	0.63
	Chr	BB116	PTS system, mal/glu-specific IIABC (malX)		Yes		
	Chr	BB240	glycerol uptake facilitator (glpF)		Yes		
	Chr	BB318	methylgalactoside ABC transporter, ATP-binding protein (mglA)		Yes		
	Chr	BB408	PTS system, fru-specific IIABC (fruA-1)		Yes		
	Chr	BB448	phosphocarrier protein HPr (ptsH-1)		Yes		
	Chr	BB557	phosphocarrier protein HPr (ptsH-2)				
	Chr	BB558	phosphoenolpyruvate-protein Ppase (ptsI)				
	Chr	BB559	PTS system, glu-specific IIA (crr)				
	Chr	BB604	L-lactate D605 permease (lctP)		Yes		
	Chr	BB629	PTS system, fru-specific IIABC (fruA-2)		Yes		

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB645	PTS system, glu-specific IIBC (ptsG)		Yes		
	Chr	BB677	ribose-galactose ABC transporter, ATP-binding protein (mglA)				
	Chr	BB678	ribose-galactose ABC transporter, permease protein (rbsC-1)				
	Chr	BB679	ribose-galactose ABC transporter, permease protein (rbsC-2)				
	Chr	BB814	pantothenate permease (panF)			Yes (0.99)	
	cp26	BBB04	PTS system IIC chitibiose transporter protein chbC		Yes		
	cp26	BBB05	PTS system IIA chitibiose transporter protein chbA		Yes		
	cp26	BBB06	PTS system IIB chitibiose transporter protein chbB		Yes		
	cp26	BBB29	PTS system, glu-specific IIBC		Yes		
Cations				6	3	1	0.50
	Chr	BB164	Na ⁺ /Ca ⁺ exchange protein			Yes (0.90)	
	Chr	BB380	Mg ²⁺ transport protein (mgtE)				
	Chr	BB447	Na ⁺ /H ⁺ antiporter (napA)		Yes		
	Chr	BB637	Na ⁺ /H ⁺ antiporter (nhaC-1)		Yes		
	Chr	BB638	Na ⁺ /H ⁺ antiporter (nhaC-2)		Yes		
	Chr	BB724	K ⁺ transport protein (ntpJ)				
Other				4	3	0	0.75
	Chr	BB140	Borrelia efflux system protein B (besB)		Yes		
	Chr	BB141	Borrelia efflux system protein A (besA)		Yes		
	Chr	BB142	Borrelia efflux system protein C (besC)		Yes		
	Chr	BB451	chromate transport protein				
Other categories				18	9	0	0.50
Adaptations and atypical conditions				4	0	0	0.00
	Chr	BB237	acid-inducible protein (act206)				
	Chr	BB785	stage V sporulation protein G				
	Chr	BB786	general stress protein (ctc)				
	Chr	BB810	virulence factor mviN protein (mviN)				
Colicin-related functions				2	0	0	0.00
	Chr	BB546	outer membrane integrity (tolA)				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	Chr	BB766	colicin V production protein				
Drug and analog sensitivity				4	1	0	0.25
	Chr	BB258	bacitracin resistance protein (bacA)				
	Chr	BB586	femA protein (femA)				
	lp25	BBE22	pyrazinamidase/nicotinamidase (pncA)		Yes		
	lp28-4	BBI26	multidrug-efflux transporter				
Transposon-related functions				8	8	0	1.00
	lp17	BBD20	transposon-like protein, authentic frameshift		Yes		
	lp17	BBD23	transposon-like protein, authentic frameshift		Yes		
	lp28-1	BBF18	transposon-like protein		Yes		
	lp28-1	BBF19	transposon-like protein, authentic frameshift		Yes		
	lp28-2	BBG05	transposon-like protein		Yes		
	lp28-3	BBH40	transposon-like protein,		Yes		
	lp36	BBK25	transposon-like protein, authentic frameshift		Yes		
	lp38	BBJ05	transposon-like protein, pseudogene		Yes		
Annotated, but unknown function				19	8	0	0.42
	Chr	BB033	small protein (smpB)				
	Chr	BB045	P115 protein		Yes		
	Chr	BB168	dnaK suppressor, putative				
	Chr	BB219	gufA protein				
	Chr	BB250	dedA protein (dedA)				
	Chr	BB297	smg protein		Yes		
	Chr	BB336	P26		Yes		
	Chr	BB363	periplasmic protein		Yes		
	Chr	BB443	spoIIJ-associated protein (jag)				
	Chr	BB454	lipopolysaccharide biosynthesis-related protein				
	Chr	BB508	GTP-binding protein				
	Chr	BB524	inositol monophosphate phosphatase		Yes		
	Chr	BB528	aldose reductase, putative		Yes		
	Chr	BB684	carotenoid biosynthesis protein, putative				
	Chr	BB702	lipopolysaccharide biosynthesis-related protein				

Predicted function	Replicon	Gene	Description	No. of genes	Gene(s) disrupted ^b	Disrupted only in last 10% of gene (Insertion ratio)	Proportion of genes disrupted ^b
	cp09	BBC09	rev protein (rev)		Yes		
	cp09	BBC10	rev protein (rev)		Yes		
	lp28-4	BBI06	pfs protein (pfs)				
	lp54	BBA76	thy1 protein (thy1)				
Total				564	190	14	0.34

a Functional groups and assigned genes adapted from Fraser et al. (4).

b Excluding genes disrupted only in the last 10% of the open reading frame

Table 2. Genes with a single insertion in the last 10% of the reading frame.

Replicon	Gene	Name of clone	Insertion Site	Tn Or.	Inserts per kb DNA	Locus size (bp)	Gene Or.	Insertion Ratio	Description	Common Name
Chrom	BB0085	T11TC194	81399	Reverse	2.35	426	Forward	0.98	hypothetical protein	
Chrom	BB0089	T09TC444	85935	Reverse	1.03	975	Reverse	0.94	hypothetical protein	
Chrom	BB0111	T06TC366	109676	Reverse	0.73	1368	Reverse	1.00	replicative DNA helicase	dnaB
Chrom	BB0144	T06TC417	144595	Reverse	1.15	873	Reverse	0.99	glycine betaine, L-proline ABC transporter, glycine/betaine/L-proline-binding protein	proX
Chrom	BB0164	T11P02D04	164997	Reverse	0.99	1014	Reverse	0.91	Na ⁺ /Ca ²⁺ exchange protein, putative	
Chrom	BB0245	T08TC689	250672	Reverse	1.80	555	Reverse	0.97	hypothetical protein	
Chrom	BB0256	T11TC311	267273	Forward	4.76	210	Forward	0.99	ribosomal protein S21	rpsU
Chrom	BB0352	T03TC295	362288	Reverse	0.88	1134	Forward	0.97	hypothetical protein	
Chrom	BB0359	T06TC006	366965	Forward	0.70	1428	Reverse	1.00	carboxyl-terminal protease	ctp
Chrom	BB0374	T08P01E10	385422	Forward	0.88	1140	Forward	1.00	hypothetical protein	
Chrom	BB0433	T04TC385	450979	Reverse	10.10	99	Reverse	0.93	hypothetical protein	
Chrom	BB0522	T04TC403	533599	Forward	1.49	669	Forward	0.99	NH(3)-dependent NAD ⁺ synthetase	
Chrom	BB0527	T07TC091	538284	Forward	1.27	789	Forward	0.97	conserved hypothetical protein	
Chrom	BB0542	T05TC330	553009	Forward	1.73	579	Forward	0.98	hypothetical protein	
Chrom	BB0545	T03TC312	556559	Forward	0.73	1365	Forward	0.98	xylulokinase	xylB
Chrom	BB0585	T08TC446	603714	Reverse	0.74	1356	Forward	0.99	UDP-N-acetylmuramoylalanine--D-glutamate ligase	murD
Chrom	BB0602	T05TC458	627036	Reverse	1.33	753	Forward	1.00	DnaJ domain containing protein	
Chrom	BB0621	T05TC615	649064	Reverse	1.80	555	Forward	0.92	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein	thiJ
Chrom	BB0631	T08P01B02	662623	Reverse	3.17	315	Reverse	0.94	hypothetical protein	
Chrom	BB0663	T08TC335	702261	Reverse	2.22	450	Forward	0.97	hypothetical protein	
Chrom	BB0689	T06TC547	730665	Reverse	2.14	468	Reverse	0.93	hypothetical protein	
Chrom	BB0730	T09TC447	769586	Reverse	0.63	1599	Reverse	0.98	glucose-6-phosphate isomerase	pgi
Chrom	BB0733	T11P01A09	775396		1.20	831	Forward	0.92	hypothetical protein	
Chrom	BB0742	T05TC553	784934	Reverse	0.59	1698	Forward	0.95	ABC transporter, ATP-binding protein	
Chrom	BB0767	T04TC203	808494	Reverse	0.92	1092	Reverse	0.97	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	murG
Chrom	BB0789	T11TC038	827548	Forward	0.52	1920	Forward	0.96	cell division protein	ftsH
Chrom	BB0812	T09TC008	858940	Reverse	0.85	1173	Reverse	1.00	pantothenate metabolism flavoprotein	dfp
Chrom	BB0814	T06TC081	861759	Reverse	0.75	1335	Forward	0.99	pantothenate permease	panF
Chrom	BB0823	T04TC350	867778	Reverse	2.69	372	Reverse	0.91	hypothetical protein	
cp26	BBB03	T08TC298	922	Reverse	0.74	1350	Reverse	0.94	hypothetical protein	
cp32-1, cp32-3 or cp32-8	BBP11	T10P01C12	7729	Reverse	0.90	1113	Forward	0.92	hypothetical protein; identical sequence, position in all three plasmids	
cp32-3	BBS21	T09TC133	14312	Forward	1.88	531	Forward	1.00	conserved hypothetical protein	
cp32-3	BBS27	T08TC557	16878	Forward	3.14	318	Forward	0.92	hypothetical protein	
cp32-4	BBR09	T11TC449	6147	Forward	2.56	390	Forward	0.95	conserved hypothetical protein	
cp32-4	BBR40	T09TC232	25967	Reverse	9.52	105	Forward	0.98	erpH protein	erpH
cp32-6	BBM09	T11TC021	6144	Reverse	2.56	390	Forward	0.95	conserved hypothetical protein	
cp32-7	BBO13	T05TC227	8696	Forward	2.19	456	Forward	0.93	hypothetical protein	
cp32-7	BBO41	T11TC493	28012	Reverse	8.77	114	Reverse	0.93	hypothetical protein	
cp32-9	BBN05	T11P02B02	3940	Reverse	1.64	611	Forward	0.98	hypothetical protein, paralogous family 148, authentic frameshift	
lp17	BBD15	T08TC225	9614	Forward	2.36	423	Reverse	0.95	hypothetical protein, paralogous family 85	

Table 2, ctd. Genes with a single insertion in last 10% of reading frame.										
Replicon	Gene	Name of clone	Insertion Site	Tn Or.	Inserts per kb DNA	Locu s size (bp)	Gene Or.	Insertion Ratio	Description	Common Name
lp21	BBU03	T09TC289	1239	Reverse	8.33	120	Reverse	0.99	hypothetical protein	
lp25	BBE04.1	T04TC095	5726	Forward	2.77	361	Forward	0.97	protein p23, pseudogene	
lp25	BBE18	T04TC328	11521	Reverse	1.72	582	Reverse	0.96	conserved hypothetical protein	
lp28-1	BBF07	T11TC414	3411	Reverse	8.33	120	Reverse	0.99	hypothetical protein	
lp28-1	BBF27	T10TC112	15769	Forward	5.85	171	Reverse	0.92	hypothetical protein	
lp28-2	BBG33	T10TC240	28777	Reverse	1.25	801	Forward	0.93	conserved hypothetical protein	
lp28-3	BBH09.1	T11TC342	7816	Reverse	3.55	282	Reverse	0.98	conserved hypothetical protein, pseudogene	
lp36	BBK42	T04TC031	26586	Reverse	4.57	219	Reverse	1.00	hypothetical protein	
lp36	BBK42.1	T10TC040	27077	Forward	6.02	166	Forward	0.98	conserved hypothetical protein	
lp38	BBJ51	T03TC222	37384	Forward	0.87	1144	Reverse	1.00	vlsE paralog, authentic frameshift	
lp54	BBA35	T10TC201	23282	Reverse	9.01	111	Reverse	0.99	hypothetical protein	

Table 3. Summary of mouse infectivity results obtained for <i>B. burgdorferi</i> STM clones with insertions in cp26 genes.																			
								No. Tissues Positive/Total Examined ^a											
								2 Weeks Post Inoculation						4 Weeks Post Inoculation					
Clone Name	Plasmid Missing	Replicon	Gene	Insertion Ratio	Description	Common Name	STM Set	Bladder	Ear	Heart	Joint	Inoc. Site	All Sites	Bladder	Ear	Heart	Joint	Inoc. Site	All Sites
T09TC018	None	cp26	BBB01	0.11	Acylphosphatase		36	3/3	3/3	3/3	3/3	3/3	15/15	2/3	3/3	3/3	2/3	3/3	13/15
3TC076	lp5, lp21	cp26	BBB02	0.44	Conserved hypothetical protein		36	0/3	0/3	0/3	1/3	2/3	3/15	0/3	0/3	0/3	0/3	0/3	0/15
T08TC298	cp9, lp5	cp26	BBB03	0.94	Telomere resolvase	resT	37	3/3	1/3	1/3	3/3	3/3	11/15	2/3	1/3	0/3	3/3	3/3	9/15
T09TC043	lp5	cp26	BBB04	0.20	PTS system IIC chitibiose transporter protein	chbC	42	2/3	2/3	3/3	3/3	3/3	13/15	3/3	3/3	1/3	3/3	3/3	13/15
T08P01C04	lp21, lp5	cp26	BBB04	0.23	PTS system IIC chitibiose transporter protein	chbC	9	3/3	3/3	3/3	3/3	ND	12/12	3/3	2/3	0/3	3/3	ND	8/12
T05TC295	cp9, lp5	cp26	BBB05	0.42	PTS system IIA chitibiose transporter protein	chbA	37	3/3	3/3	1/3	3/3	3/3	13/15	0/3	3/3	0/3	3/3	1/3	7/15
T08TC580	lp5	cp26	BBB06	0.13	PTS system IIB chitibiose transporter protein	chbB	39	0/3	0/3	1/3	0/3	1/3	2/15	0/3	1/3	1/3	0/3	0/3	2/15
T02P01C06	lp5	cp26	BBB06	0.86	PTS system IIB chitibiose transporter protein	chbB	2	0/3	0/3	0/3	0/3	ND	0/12	ND	ND	ND	ND	ND	ND
T07TC077	lp5	cp26	BBB07	0.11	Putative alpha3-beta1 integrin-binding outer surface protein		37	0/3	0/3	1/3	1/3	3/3	5/15	0/3	1/3	1/3	0/3	0/3	2/15
T11TC010	lp5	cp26	BBB08	0.10	Lipoprotein, putative		24	3/3	3/3	2/3	3/3	3/3	14/15	1/3	2/3	1/3	3/3	3/3	10/15
T11TC010	lp5	cp26	BBB08	0.10	Lipoprotein, putative		36	3/3	2/3	1/3	3/3	3/3	12/15	0/3	1/3	1/3	2/3	2/3	6/15
T08TC506	None	cp26	BBB09	0.09	Lipoprotein, putative		26	0/3	0/3	0/3	0/3	0/3	0/15	0/3	0/3	0/3	0/3	0/3	0/15
T03TC124	None	cp26	BBB09	0.28	Lipoprotein, putative		37	0/3	0/3	0/3	0/3	1/3	1/15	0/3	0/3	0/3	1/3	0/3	1/15
T03TC311	lp5	cp26	BBB12	0.27	Conserved hypothetical protein, protein family 32	PF32	35	0/3	0/3	0/3	0/3	0/3	0/15	0/3	0/3	0/3	0/3	0/3	0/15
T10TC205	lp5	cp26	BBB13	0.46	Putative plasmid partition protein, protein family 49	PF49	37	0/3	0/3	0/3	0/3	0/3	0/15	0/3	0/3	0/3	0/3	0/3	0/15
T06TC154	lp5	cp26	BBB14	0.43	Conserved hypothetical protein		33	0/3	0/3	0/3	0/3	0/3	0/15	0/3	0/3	0/3	0/3	1/3	1/15
T07TC044	None	cp26	BBB14	0.11	Conserved hypothetical protein		25	0/3	1/3	1/3	0/3	0/3	2/15	0/3	0/3	0/3	0/3	0/3	0/15
T07TC044	None	cp26	BBB14	0.11	Conserved hypothetical protein		36	1/3	0/3	0/3	1/3	1/3	3/15	0/3	0/3	0/3	0/3	0/3	0/15
T11TC530	None	cp26	BBB15	0.06	hypothetical protein		37	3/3	2/3	3/3	3/3	3/3	14/15	2/3	3/3	3/3	3/3	2/3	13/15
T05P1D02	None	cp26	BBB16	0.06	Oligopeptide ABC transporter, periplasmic binding protein	oppAIV	7	1/3	1/3	2/3	2/3	0/3	6/15	2/3	3/3	2/3	3/3	NA	10/12
T10P01A05	None	cp26	BBB16	0.17	Oligopeptide ABC transporter, periplasmic binding protein	oppAIV	2	0/3	0/3	0/3	0/3	0/3	0/15	ND	ND	ND	ND	ND	ND
T06TC037	None	cp26	BBB16	0.45	Oligopeptide ABC transporter, periplasmic binding protein	oppAIV	27	3/3	0/3	2/3	3/3	2/3	10/15	0/3	0/3	0/3	3/3	3/3	6/15
T06TC037	None	cp26	BBB16	0.17	Oligopeptide ABC transporter, periplasmic binding protein	oppAIV	36	3/3	0/3	0/3	3/3	3/3	9/15	3/3	2/3	2/3	3/3	3/3	13/15
T06TC118	cp32-3, lp5	cp26	BBB17	0.41	Inosine-5-monophosphate dehydrogenase	guaB	37	0/3	0/3	1/3	1/3	2/3	4/15	0/3	1/3	1/3	1/3	1/3	4/15
T04TC173	None	cp26	BBB18	0.18	GMP synthase	guaA	37	0/3	0/3	1/3	0/3	0/3	1/15	0/3	0/3	0/3	0/3	0/3	0/15
T08TC072	lp5	cp26	BBB19	0.07	Outer surface protein C	ospC	19	0/3	0/3	1/3	0/3	0/3	1/15	0/3	1/3	0/3	0/3	0/3	1/15
T08TC175	None	cp26	BBB19	0.51	Outer surface protein C	ospC	36	0/3	0/3	0/3	0/3	0/3	0/15	0/3	0/3	0/3	0/3	0/3	0/15
T07TC104	lp5	cp26	BBB20	0.41	Conserved hypothetical protein	CHP	35	2/3	2/3	3/3	3/3	3/3	13/15	2/3	3/3	1/3	2/3	3/3	11/15

Table 3, ctd. Summary of mouse infectivity results obtained for <i>B. burgdorferi</i> STM clones with insertions in cp26 genes.																			
								No. Tissues Positive/Total Examined ^a											
								2 Weeks Post Inoculation						4 Weeks Post Inoculation					
Clone Name	Plasmid Missing	Replicon	Gene	Insertion Ratio	Description	Common Name	STM Set	Bladder	Ear	Heart	Joint	Inoc. Site	All Sites	Bladder	Ear	Heart	Joint	Inoc. Site	All Sites
T08TC648	lp5	cp26	BBB21	0.76	Conserved hypothetical protein	CHP	50	3/3	1/3	3/3	3/3	3/3	13/15	3/3	3/3	2/3	3/3	3/3	14/15
T07TC086	cp9, lp5	cp26	BBB22	0.15	Purine permease G1	pbuG1	34	0/3	0/3	0/3	0/3	0/3	0/15	1/3	3/3	1/3	1/3	3/3	9/15
T06TC015	lp5	cp26	BBB22	0.44	Purine permease G1	pbuG1	32	1/3	0/3	0/3	1/3	1/3	3/15	0/3	0/3	0/3	0/3	1/3	1/15
T03TC156	lp5, cp9	cp26	BBB23	0.19	Purine permease G2	pbuG2	34	0/3	0/3	0/3	0/3	0/3	0/15	0/3	1/3	1/3	1/3	0/3	3/15
T05TC179	lp21, cp32-3	cp26	BBB24	0.15	Conserved hypothetical protein	CHP	36	2/3	3/3	2/3	3/3	3/3	13/15	2/3	3/3	3/3	3/3	3/3	14/15
T03P01A07	None	cp26	BBB25	0.5	Conserved hypothetical protein	CHP	3	2/2	0/2	0/2	1/2	ND	3/12	ND	ND	ND	ND	ND	ND
T10TC048	cp9, lp5, lp28-2	cp26	BBB25	0.67	Conserved hypothetical protein	CHP	36	1/3	1/3	1/3	3/3	3/3	9/15	1/3	0/3	0/3	3/3	1/3	5/15
T09TC218	lp5	cp26	BBB27	0.1	Lipoprotein, putative	LP	37	1/3	1/3	2/3	3/3	3/3	10/15	0/3	0/3	0/3	1/3	2/3	3/15
T09TC218	lp5	cp26	BBB27	0.1	Lipoprotein, putative	LP	19	3/3	0/3	2/3	2/3	3/3	10/15	1/3	1/3	2/3	3/3	3/3	10/15
T06TC312	cp9, lp5	cp26	BBB28	0.27	Conserved hypothetical protein (Borrelia only); putative ankyrin repeat protein	CHP	34	2/3	0/3	0/3	2/3	3/3	7/15	1/3	2/3	2/3	2/3	3/3	10/15
T04TC008	None	cp26	BBB29	0.17	PTS system, IIBC component	malX	7	0/3	0/3	0/3	1/3	ND	1/12	0/3	1/3	1/3	1/3	NA	3/12
T04TC008	None	cp26	BBB29	0.17	PTS system, IIBC component	malX	36	0/3	2/3	3/3	3/3	3/3	11/15	0/3	1/3	3/3	2/3	2/3	8/15

a Results obtained by direct extraction of DNA from tissue are shown. Tissue samples were considered positive if MFI > 100.

Table 4. Oligonucleotides used in this study. Underlined regions indicate 7-bp signature tags. See Materials and Methods for details regarding STM plasmid construction and Luminex detection methods.	
Name	Nucleotide Sequences
Tag 1 Construct Oligos	5' - TCGGATCCCTGAACGTTTAAAGCGCGGTACGGTACC - 3' 3' - CGAGCCTAGGGACTTGCAAATTCGCGCCATGCCATGGAAT - 5'
Tag 2 Construct Oligos	5' - TCGATCCCAGGCTATTTAAGCGCGGTACCTCGAGGCATGCGGTAC - 3' 3' - CGAGCCTAGGTCCGATAAAATTCGCGCCATGGAGCTCCGTACGC - 5'
Tag 3 Construct Oligos	5' - GATCCCAGACTTTTAAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGCTCTGAAAATTCGCGCCATGGAGCTCC - 5'
Tag 4 Construct Oligos	5' - GATCCCCACGTTATTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGGTGCAATAATTCGCGCCATGGAGCTCC - 5'
Tag 5 Construct Oligos	5' - GATCCCTACCAGTTTAAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGATGGTCAAATTCGCGCCATGGAGCTCC - 5'
Tag 6 Construct Oligos	5' - GATCCCAACATGCTTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGGTGTACGAATTCGCGCCATGGAGCTCC - 5'
Tag 7 Construct Oligos	5' - GATCCCGGTTACATTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGCCAATGTAATTCGCGCCATGGAGCTCC - 5'
Tag 8 Construct Oligos	5' - GATCCCCGAGATTTTAAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGGCTCTAAAATTCGCGCCATGGAGCTCC - 5'
Tag 9 Construct Oligos	5' - GATCCCTTGCCTATTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGAACGGATAATTCGCGCCATGGAGCTCC - 5'
Tag 10 Construct Oligos	5' - GATCCCACGATTGTTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGTGCTAACAATTCGCGCCATGGAGCTCC - 5'
Tag 11 Construct Oligos	5' - GATCCCGTCTTGATTAAGCGCGGTACCTCGAGGCATG - 3' 3' - GGCAGAACTAATTCGCGCCATGGAGCTCC - 5'
Clone sequencing primer	5' - CAGCAACGCGGCCTTTTACG - 3'
Forward Primer – STM PCR amplification step	5' - TACAGCGTGAGCTATGAGAAAGCG - 3'
Reverse Primer – STM PCR amplification step	5' - CCTAAGGTACCGCATGCCT - 3'
Primers for tag-specific labeling step^a	
Tag1 (tLUA_64)	5' - CTACATATTCAAATTACTACTTACA/CCGCGCTTAAACGTTCA - 3'
Tag2 (tLUA_24)	5' - TCAATTACCTTTTCAATACAATACA/CCGCGCTTAAATAGCCT - 3'
Tag3 (tLUA_36)	5' - CAATTCAATTCATTACAAATCAATA/CCGCGCTTAAAAGTCTC - 3'
Tag4 (tLUA_82)	5' - TACATACACTAATAACATACTCATA/CCGCGCTTAATAACGTG - 3'
Tag5 (tLUA_12)	5' - TACACTTTCTTTCTTTCTTTCTTTA/CCGCGCTTAAACTGGTA - 3'
Tag6 (tLUA_30)	5' - TTACCTTTATACCTTTCTTTTACA/CCGCGCTTAAGCATGTT - 3'
Tag7 (tLUA_68)	5' - TCATAATCTCAACAATCTTTCTTTA/CCGCGCTTAATGTAACC - 3'
Tag8 (tLUA_55)	5' - TATATACACTTCTCAATAACTAACA/CCGCGCTTAAATCTCG - 3'
Tag9 (tLUA_52)	5' - TCAATCATCTTTATACTTACAATA/CCGCGCTTAATAGGCAA - 3'
Tag10 (tLUA_2)	5' - CTTTATCAATACATACTACAATCAA/CCGCGCTTAAACAATCGT - 3'
Tag11 (tLUA_80)	5' - CTAATAACAATAATCTAATAACA/CCGCGCTTAATCAAGAC - 3'
Universal (tLUA_14)	5' - CTACTATACATCTTACTATACTTTC/GGTCTCCTAAGGTACCG - 3'

a The Luminex FlexMAP™ microsphere specificity is indicated in parentheses. The forward slash represents the demarcation point between the Luminex FlexMap™ oligonucleotide-specific sequence and the STM tag-specific primer sequence.

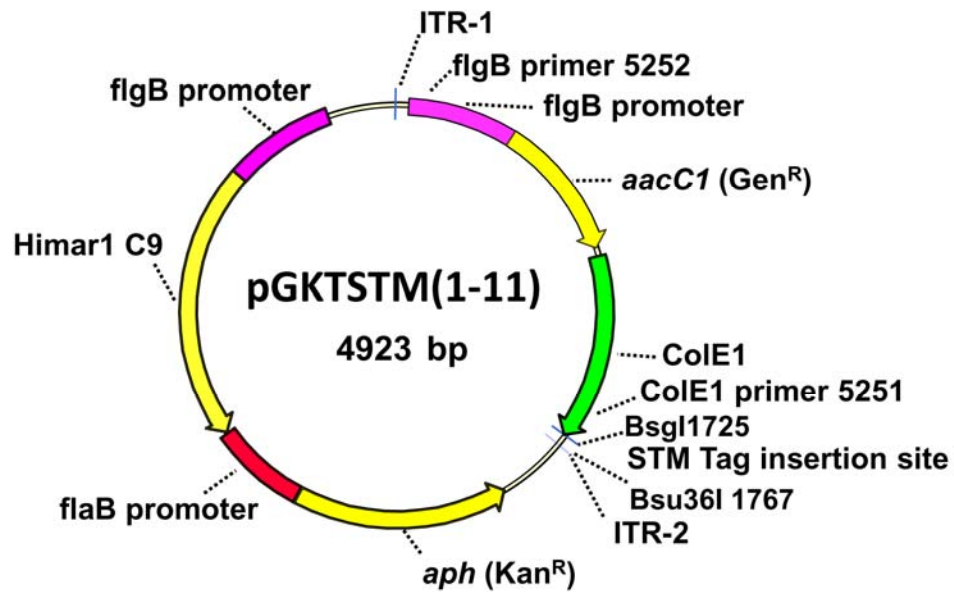


Fig. 1. Construction of signature-tagged suicide himar1 transposon vectors pGKTSTM1-pGKTSTM11 for transposon mutagenesis of infectious *Borrelia burgdorferi*. The signature-tagged suicide vectors pGKTSTM1-pGKTSTM11 vectors were constructed by inserting STM tags into a region between the ColE1 origin and Inverted Terminal Repeat 2 (ITR2) in Himar1 transposon vector pGKT (2).

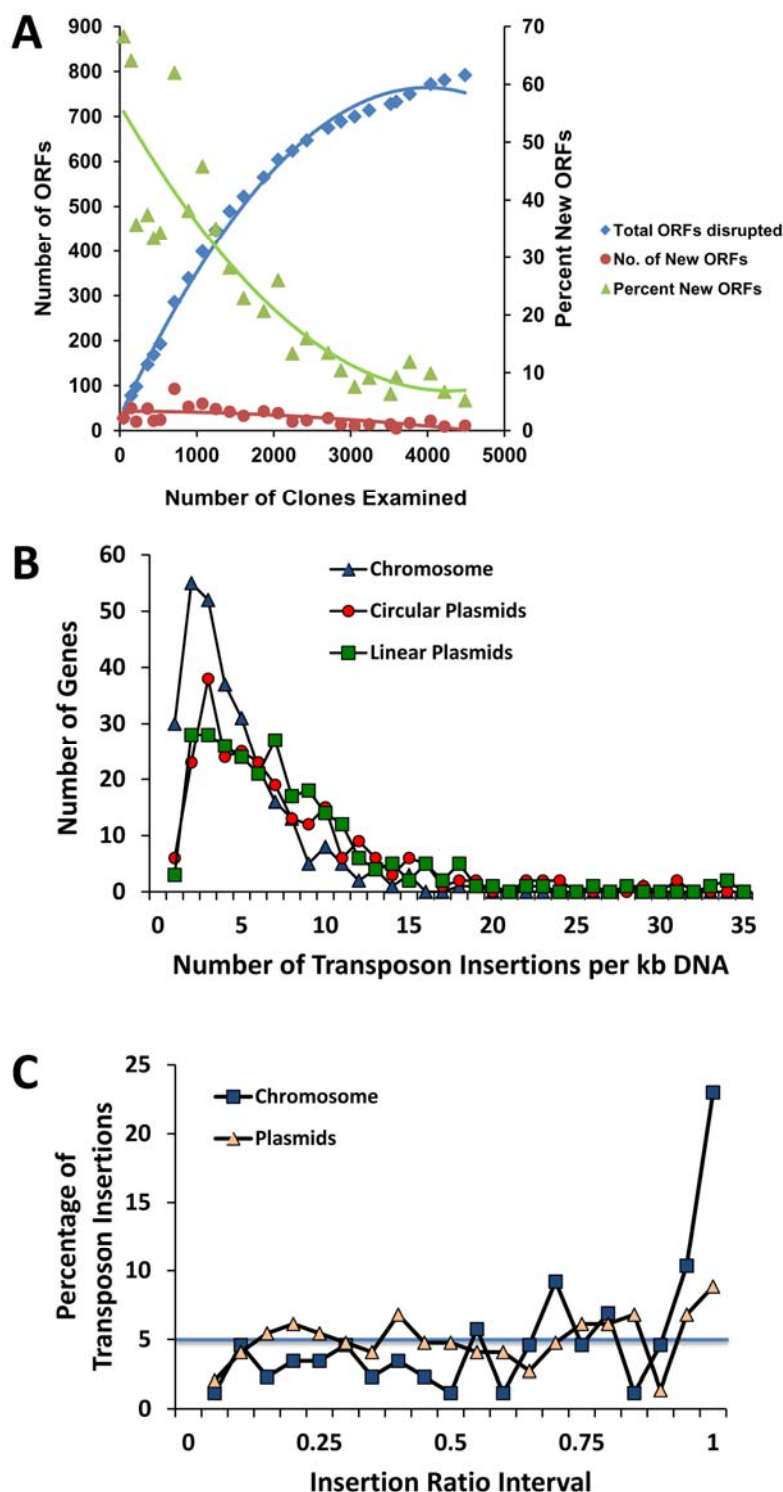
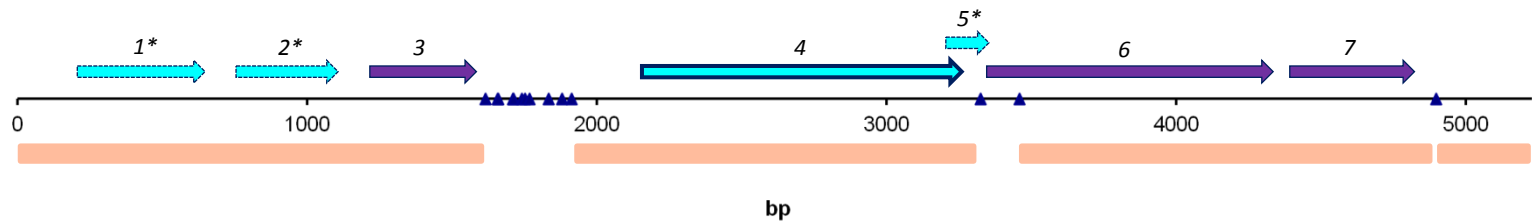


Fig. 2. Characteristics of transposon insertions in *B. burgdorferi* genes. (A) The number of genes with transposon insertions approaches saturation. Each data point represents a separate sequencing run for insertion site determination, which typically included 1-3 transformations. Total ORFs disrupted is the cumulative number of ORFs with insertions (final N = 790). Number of new ORFs is the number of ORFs in each sequencing run in which the ORF was not disrupted previously. Percent new ORFs represents the percentage of clones in a sequencing run that were in a newly disrupted ORF. Trendlines were generated in Microsoft Excel using the polynomial function. (B) Number of transposon insertions per kb DNA for genes in the chromosome, circular plasmids, and linear plasmids. Results for the 790 predicted genes with insertions are shown. (C) Preferential localization of transposon insertions in the last 10% of the open reading frame for the 233 genes that had only one insertion. Results are shown as the percentage of insertions in the insertion ratio intervals 0-0.05, 0.05-0.10, etc.

Fig. 3. Maps indicating the locations of STM transposon insertion sites in the plasmids of *B. burgdorferi* 5A18NP1. The genes are indicated as arrows; ORFs with dashed borders and an asterisk after the gene designation are pseudogenes. The key at the bottom of the figure indicates the color codes for four paralogous gene families (PFs) that have been postulated to be involved in plasmid replication or partitioning. The triangles on the line underlying the genes represent transposon insertion sites. Regions lacking transposon insertions (and thus containing genes potentially required for *B. burgdorferi* survival or plasmid retention) are marked as orange rectangles below the transposon insertion sites. The final panel is a histogram of the distribution of insertion sites at 500 bp intervals in the chromosome.

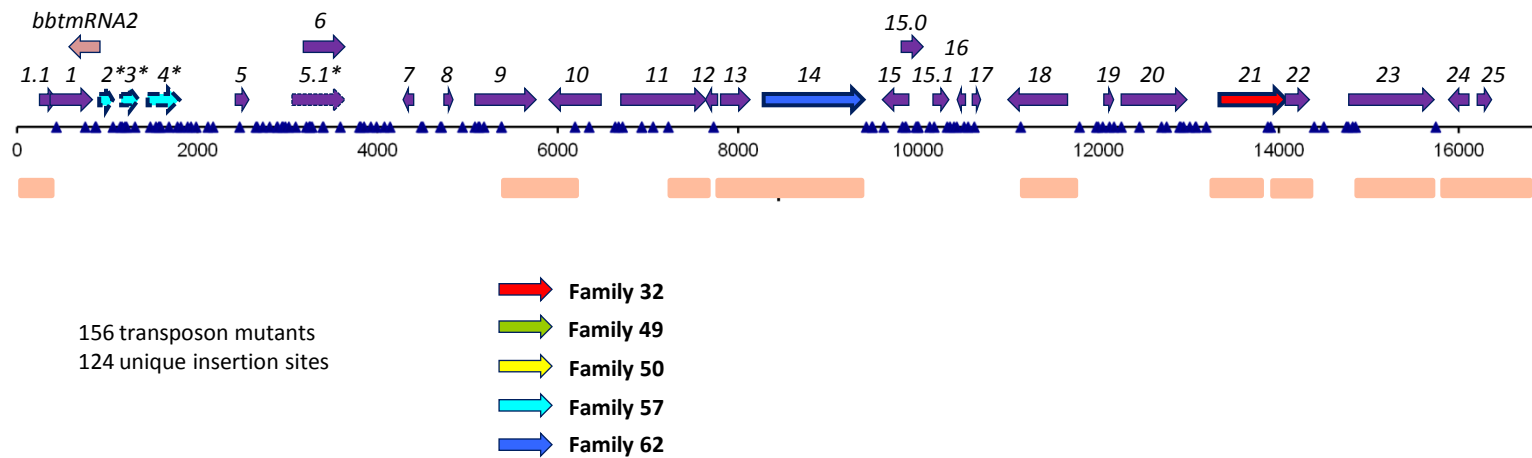
Ip5 (Plasmid T) (5,228 bp)



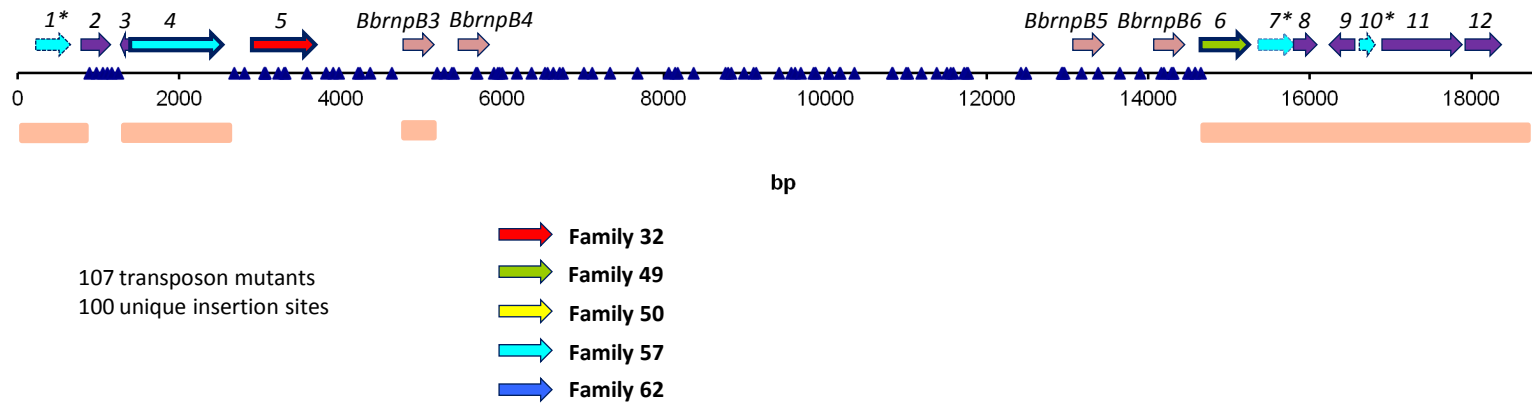
14 transposon mutants
14 unique insertion sites

- ➡ Family 32
- ➡ Family 49
- ➡ Family 50
- ➡ Family 57
- ➡ Family 62

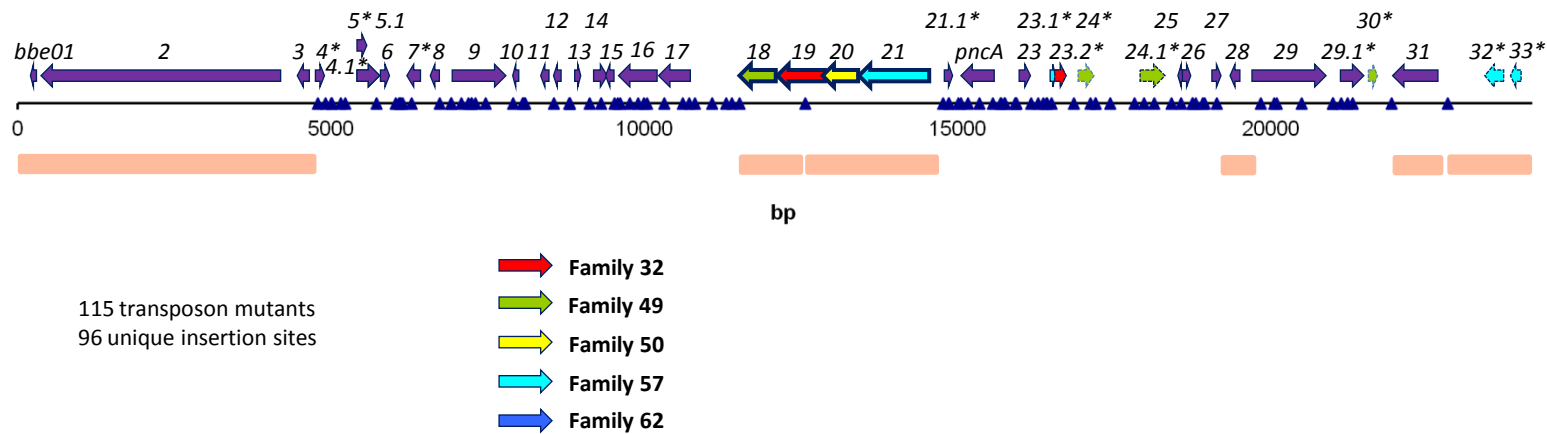
Ip17 (Plasmid D) (16,823 bp)



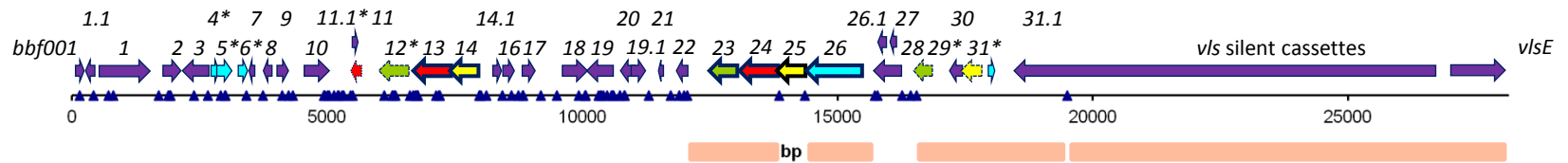
Ip21 (Plasmid U) (18,753 bp)



Ip25 (Plasmid E) (24,177 bp)



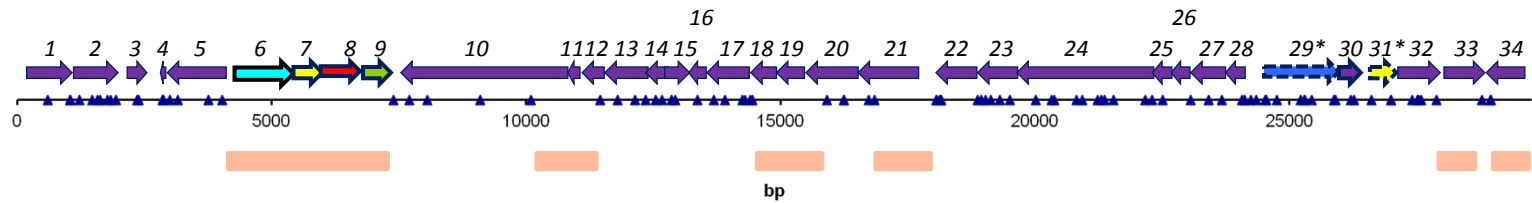
Ip28-1 (Plasmid F) (28,155 bp)



97 transposon mutants
82 unique insertion sites

- ➡ Family 32
- ➡ Family 49
- ➡ Family 50
- ➡ Family 57
- ➡ Family 62

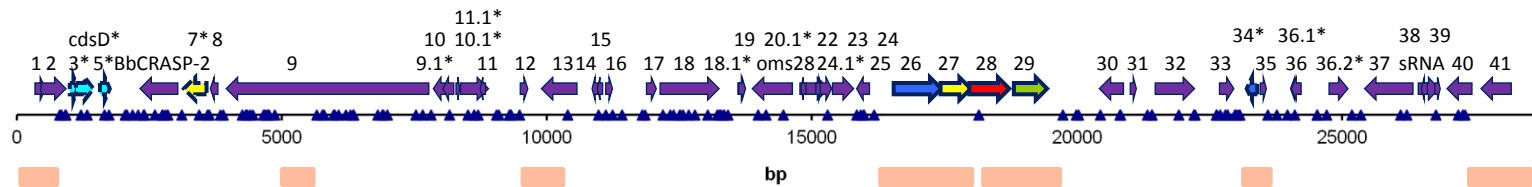
Ip28-2 (Plasmid G) (29,766 bp)



104 transposon mutants
96 unique insertion sites

- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

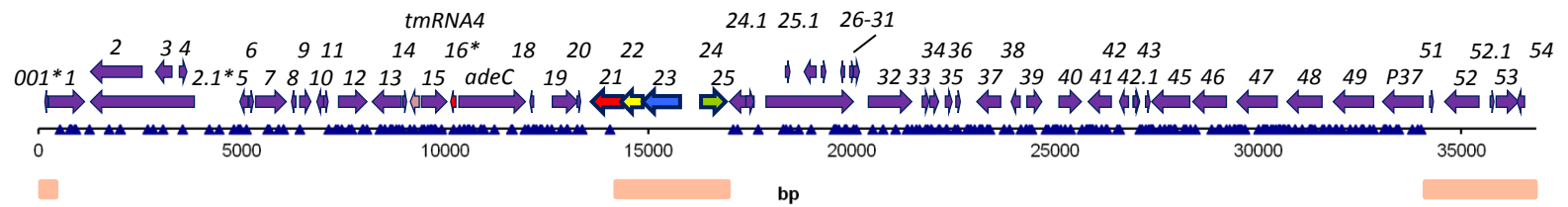
Ip28-3 (Plasmid H) (28,601 bp)



160 transposon mutants
134 unique insertion sites

- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

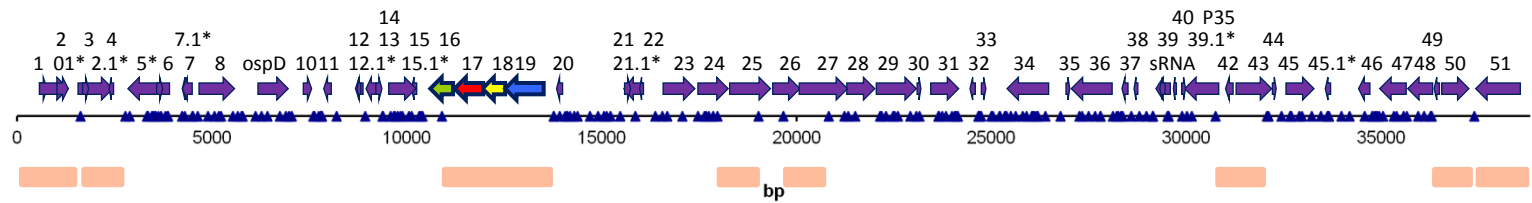
Ip36 (Plasmid K) (29,766 bp)



285 transposon mutants
266 unique insertion sites

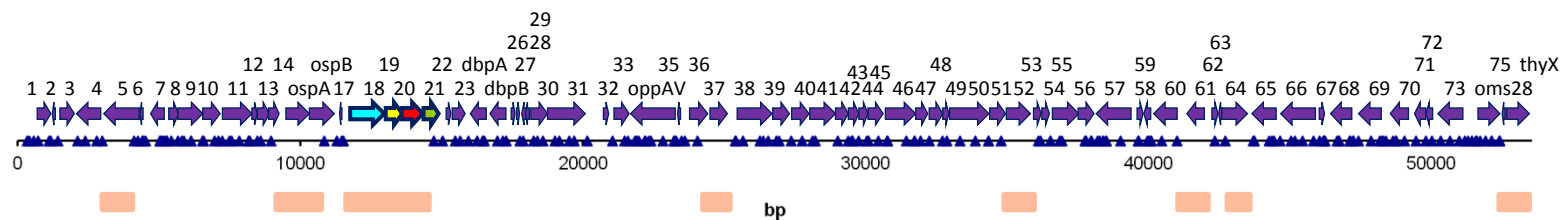
-  Family 32
-  Family 49
-  Family 50
-  Family 57
-  Family 62

Ip38 (Plasmid J) (38,829 bp)



- 214 transposon mutants
185 unique insertion sites
-  Family 32
 -  Family 49
 -  Family 50
 -  Family 57
 -  Family 62

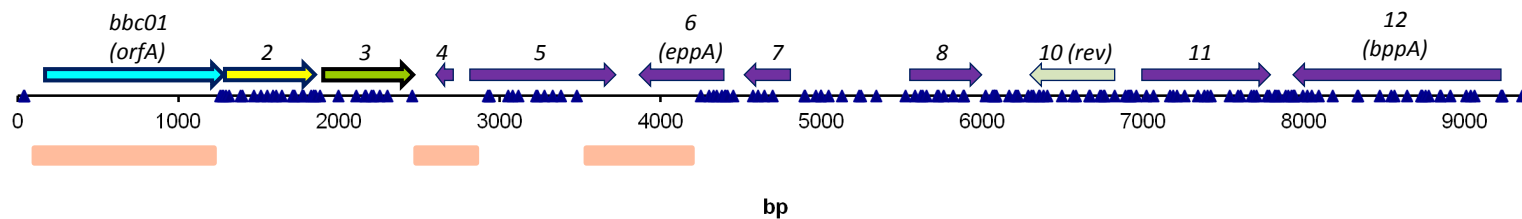
Ip54 (Plasmid A) (53,561 bp)



223 transposon mutants
195 unique insertion sites

- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

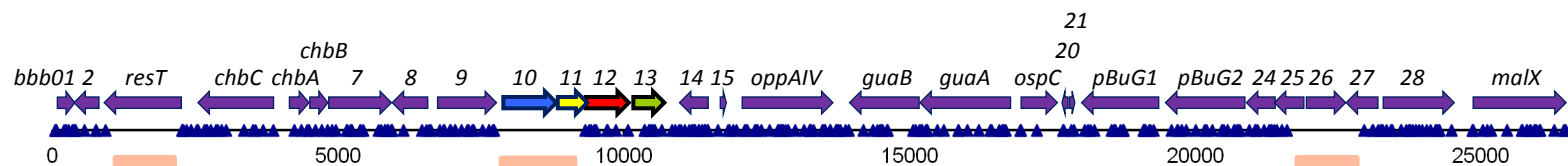
cp9 (Plasmid C) (9,386 bp)



- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

225 transposon mutants
169 unique sites

cp26 (Plasmid B) (26,498 bp)

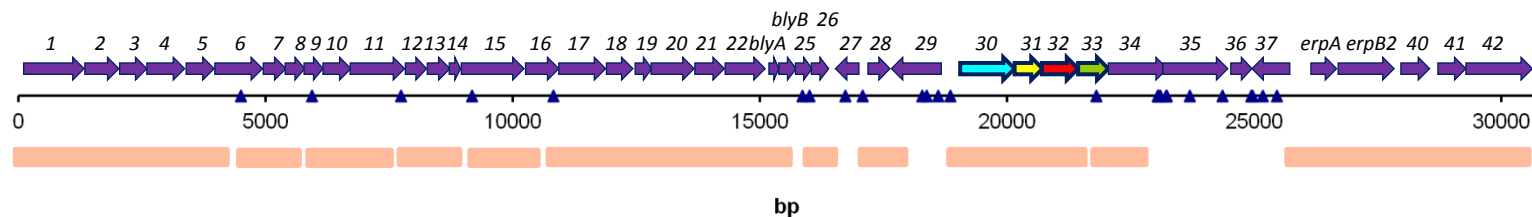


bp

372 transposon mutants
302 unique insertion sites

- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

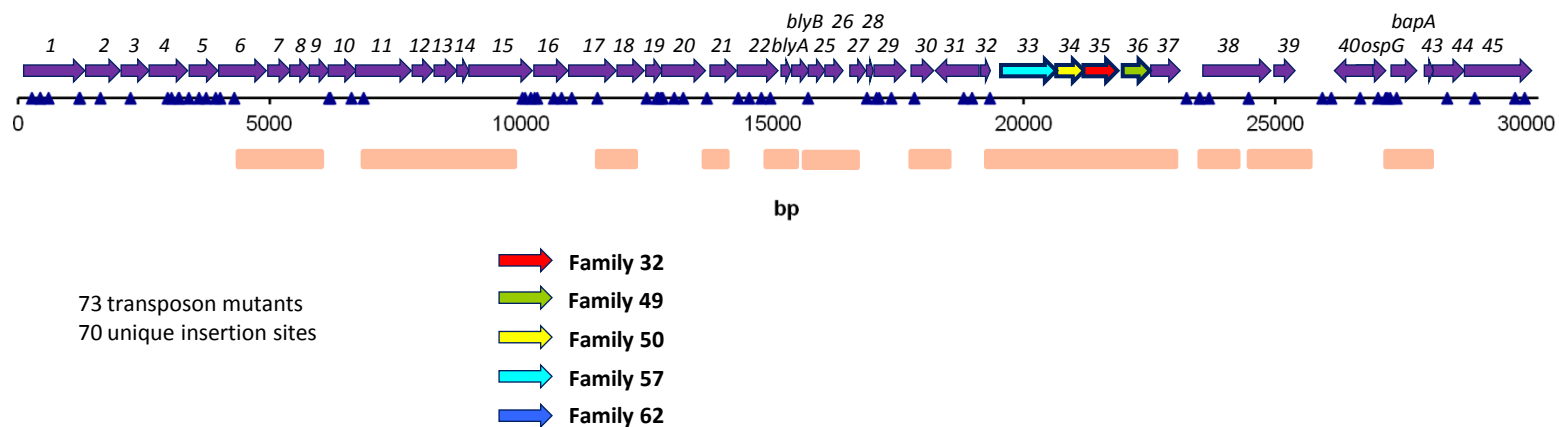
cp32-1 (Plasmid P) (30,750 bp)



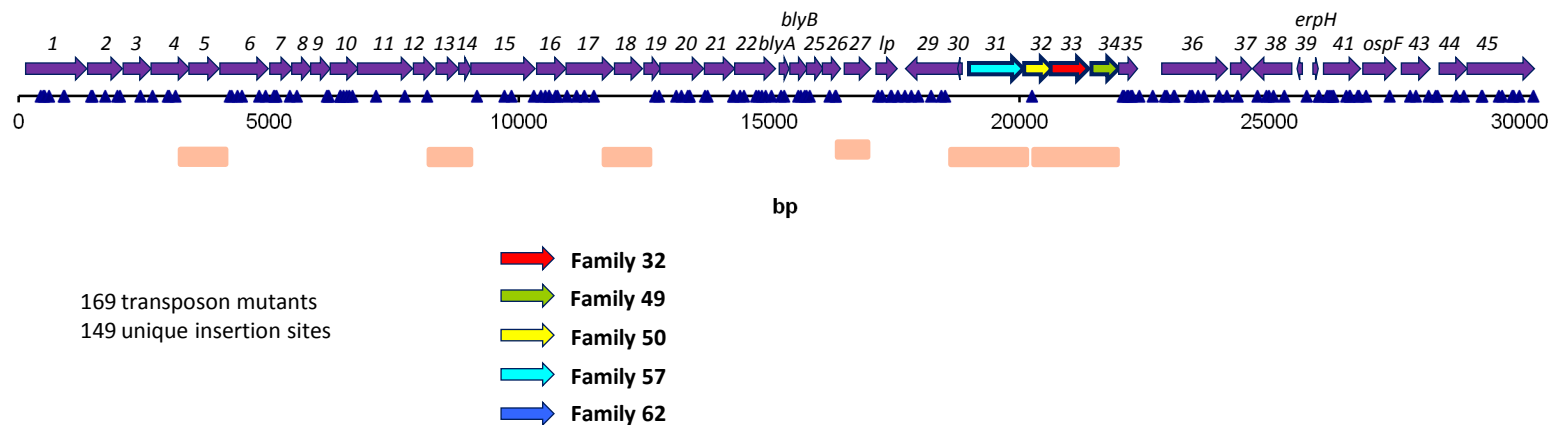
28 transposon mutants
26 unique insertion sites

- ➔ Family 32
- ➔ Family 49
- ➔ Family 50
- ➔ Family 57
- ➔ Family 62

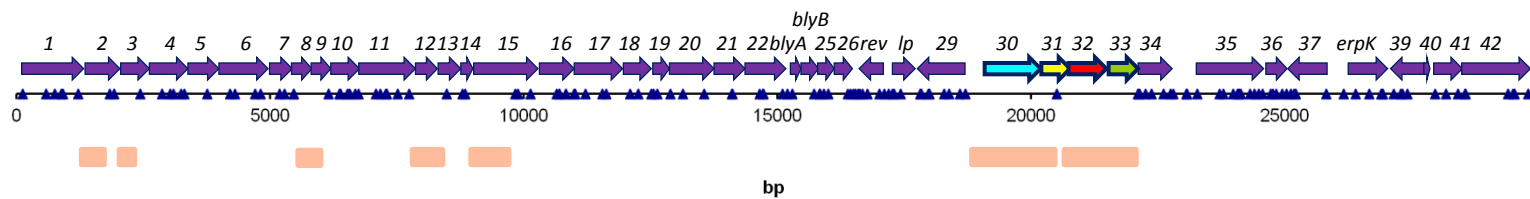
cp32-3 (Plasmid S) (30,233 bp)



cp32-4 (Plasmid R) (30,299 bp)



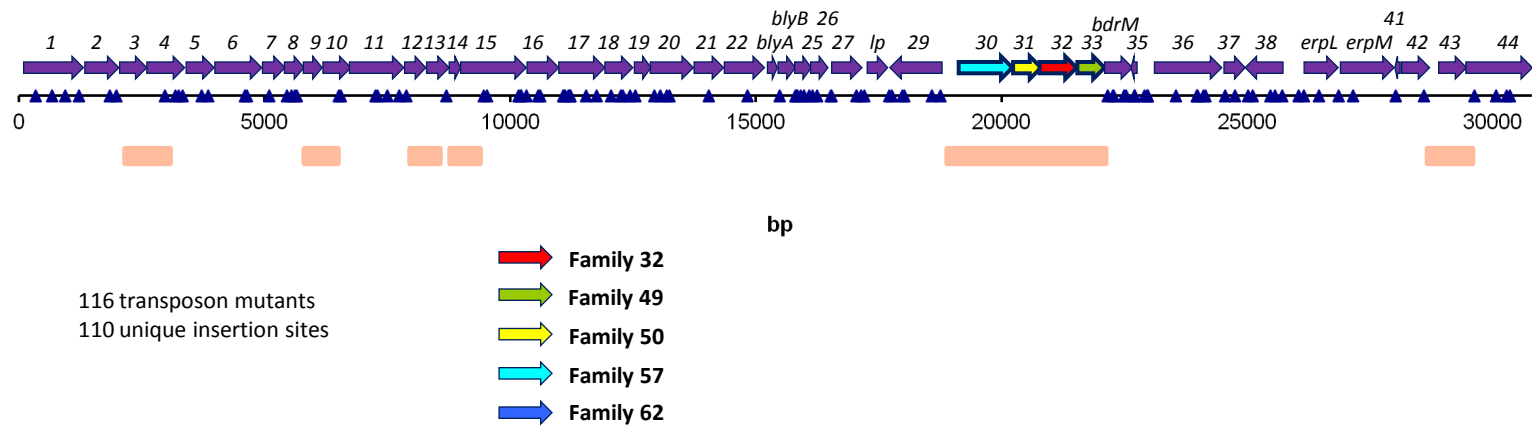
cp32-6 (Plasmid M) (29,838 bp)



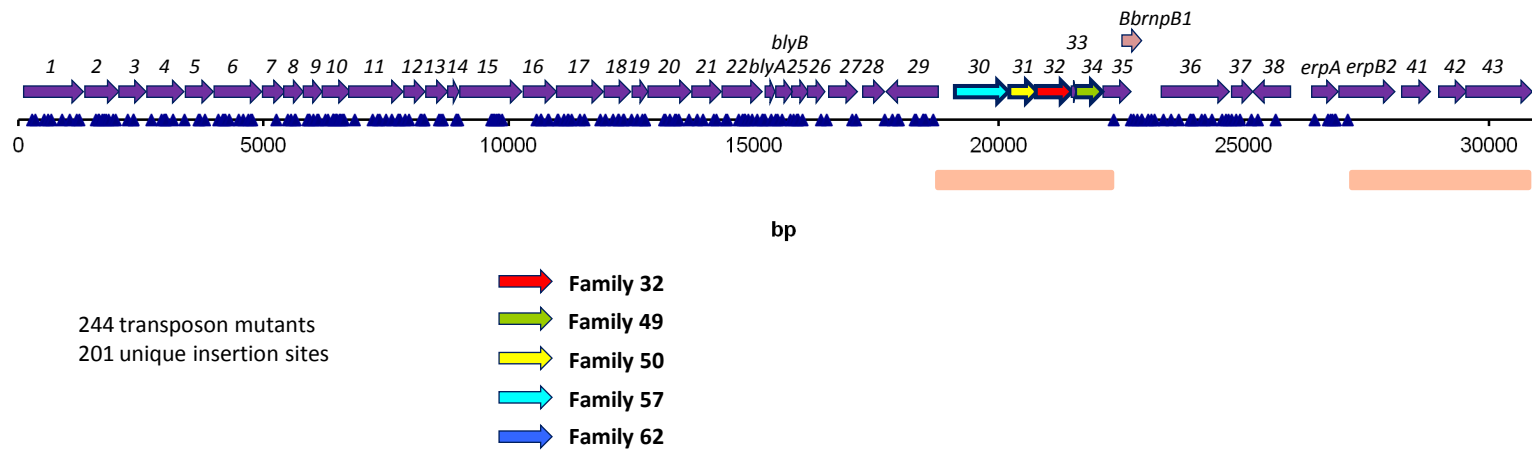
163 transposon mutants
148 unique insertion sites

- ➡ Family 32
- ➡ Family 49
- ➡ Family 50
- ➡ Family 57
- ➡ Family 62

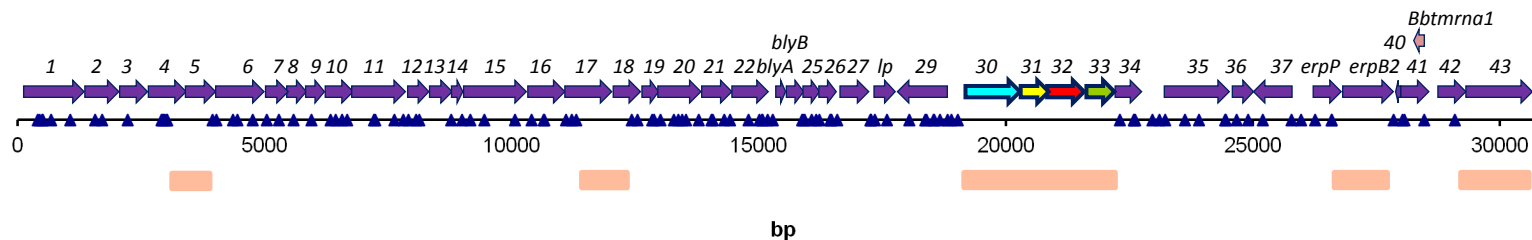
cp32-7 (Plasmid O) (30,800 bp)



cp32-8 (Plasmid L) (30,885 bp)



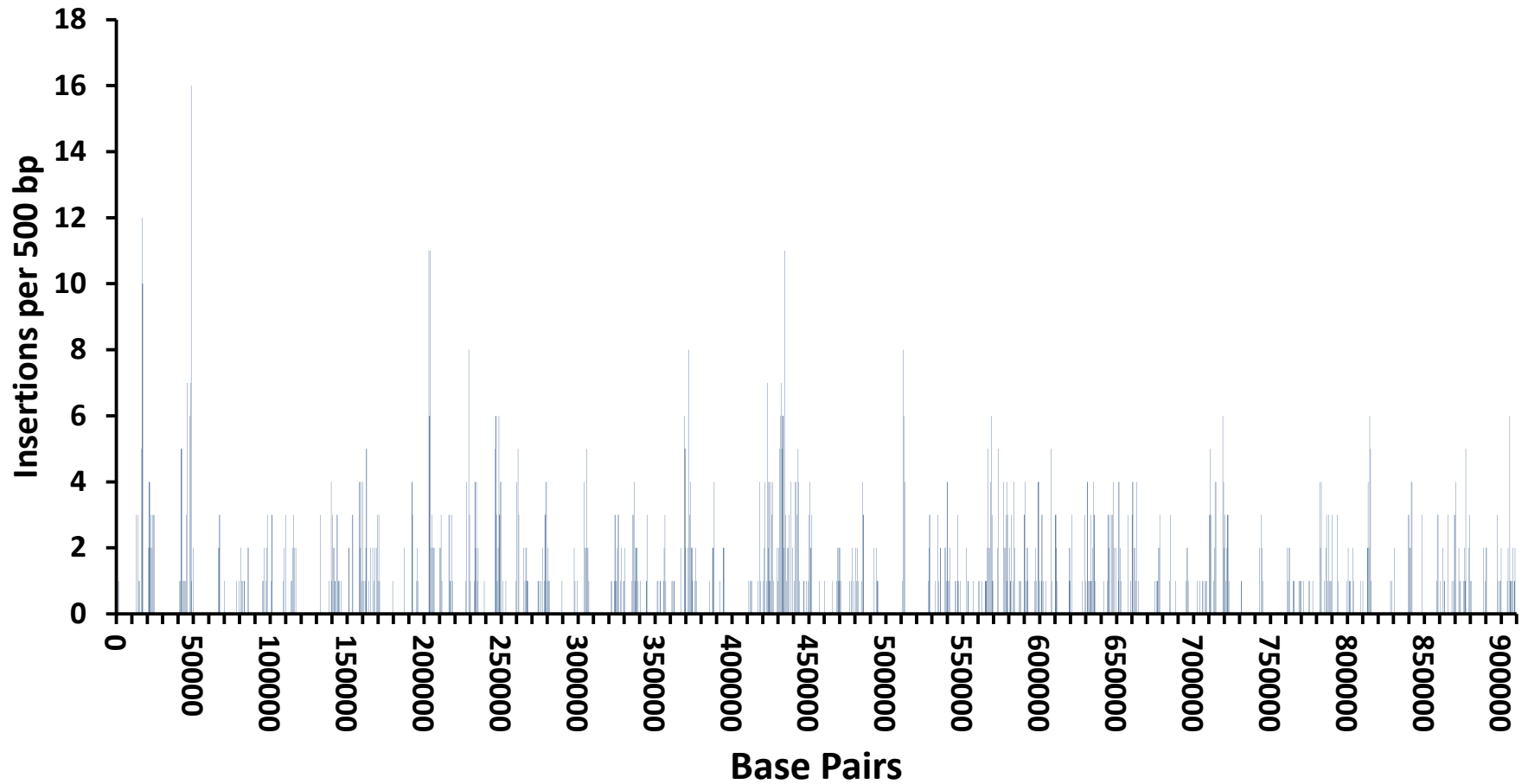
cp32-9 (Plasmid N) (30,651 bp)



128 transposon mutants
114 unique insertion sites

- ➡ Family 32
- ➡ Family 49
- ➡ Family 50
- ➡ Family 57
- ➡ Family 62

Distribution of Transposon Insertion Sites - Chromosome



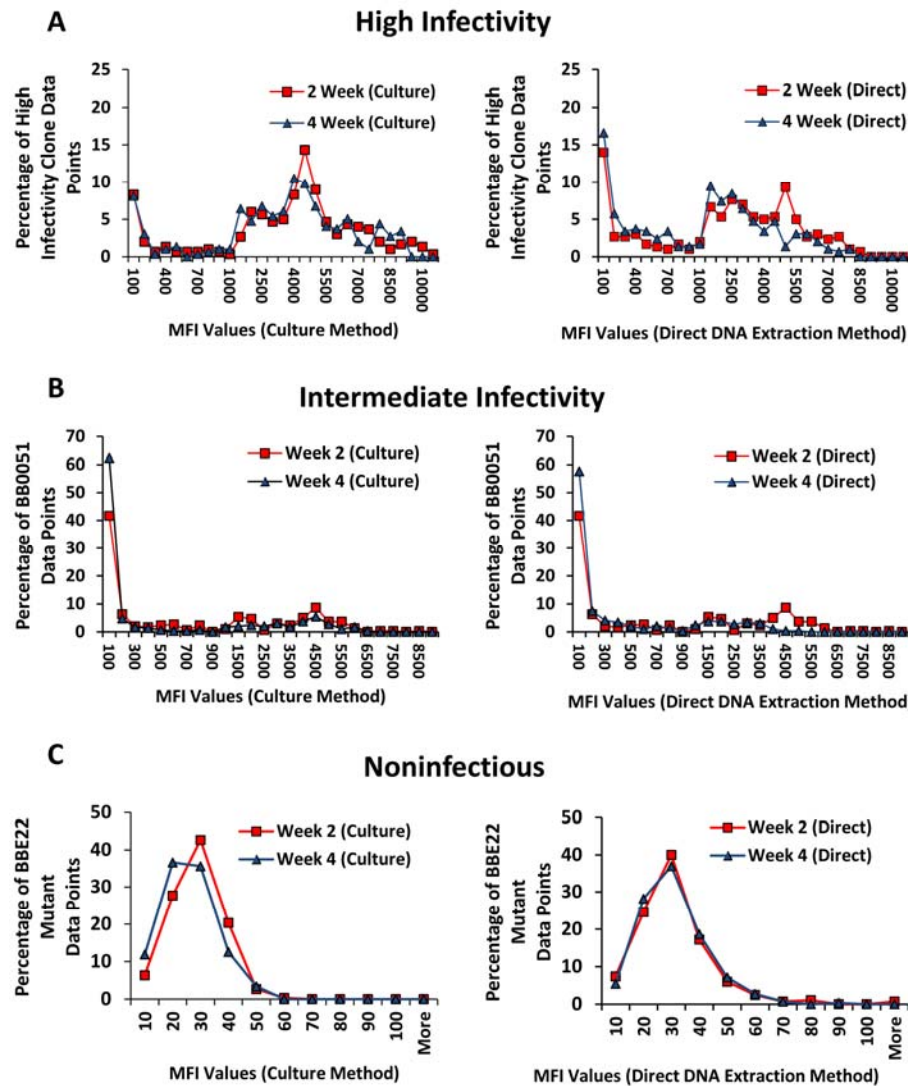


Fig. 4. Distribution of individual MFI values obtained with high, intermediate, and low infectivity STM transposon mutant clones. Results are shown for (a) the 20 clones that had the highest mean MFI values, (b) the intermediate infectivity BB0051 mutant T02P01A01, and (c) the low infectivity bbe02 (pncA) mutant T01P01A11. T01P01A11 and T02P01A01 are the negative and positive controls used in STM experiments. The data are subdivided into culture and direct DNA extraction methods, and into 2 week and 4 week samples. The minimum number of individual data points for each histogram is 286.

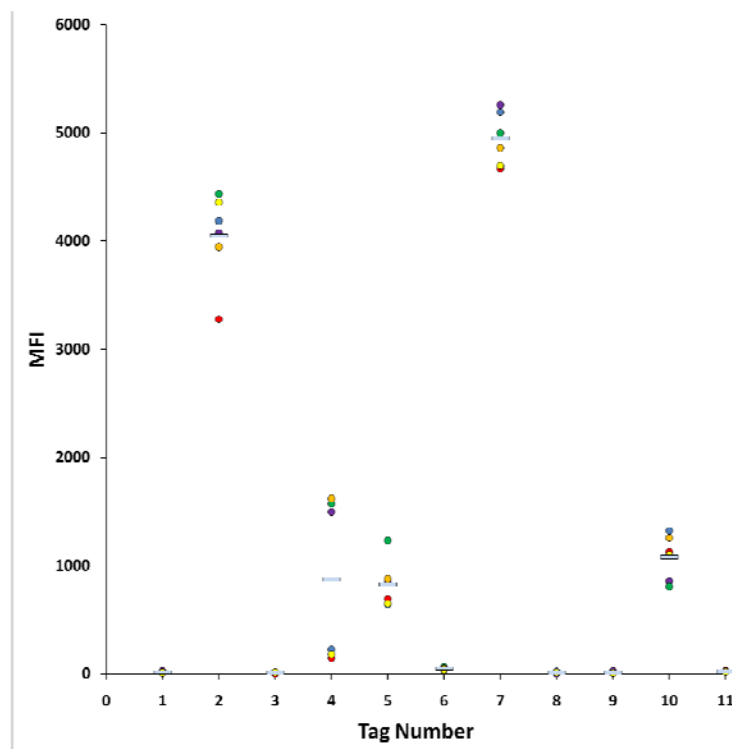


Fig. 5. MFI values obtained by Luminex-based STM analysis are reproducible. The results of six replicate determinations using Mouse 1 2 week direct tissue DNA extraction sample from Set 51 are shown. The X axis represents the 11 clones used to inoculate the animals. Consistent results were obtained in the 6 replicates.

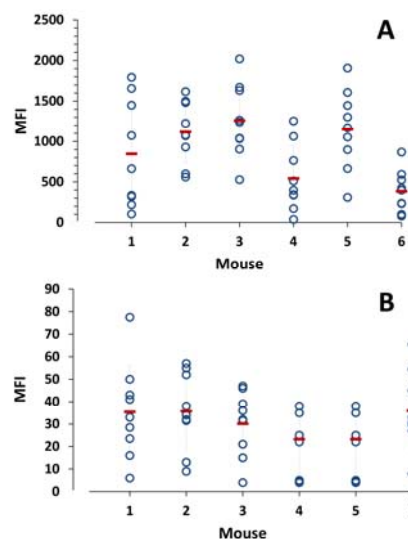


Fig. 6. Sampling error may contribute to the variability of Luminex-based MFI values obtained in mouse infectivity analyses. The STM Set 51 skin inoculation site tissue from 6 mice were used; mice 1-3 were infected for 2 weeks, whereas mice 4-6 were infected for 4 weeks prior to analysis. For each mouse, the skin inoculation site was subdivided into 9 equally-sized pieces, and each piece was processed separately by the direct DNA extraction method and evaluated for clone content by Luminex STM analysis. The results presented are for (a) the relatively high infectivity clone T07TC483 and (b) the low infectivity pncA mutant T01P01A01 (note difference in Y-axis scale). The MFI values obtained for each tissue specimen are shown; the red line indicates the mean value.

Figure 7. Heat map representations of the mouse infectivity of transposon mutants in the genes of *B. burgdorferi* plasmids. The plasmids shown are (A) cp26, (B) lp25, (C) lp28-1, (D) lp36, and (E) lp54. For each clone, results from Luminex STM analysis (see Fig. 4, Table S3) were grouped by week post inoculation (2 weeks or 4 weeks) and by DNA preparation method (Culture = use of organisms cultured from tissue; Tissue = use of DNA extracted directly from tissue). The data in each group were scored according to the percentage of samples with MFI values above the negative threshold (100) or to the mean MFI value; each result was then color coded as indicated in the key. Each colored box in the figure corresponds to 12 to 15 data points (typically 5 tissues from 3 mice). The composite results obtained in all experiments for the positive (*bb0051*) and negative (*pncA*) control mutants are provided at the right side of each figure. HP, hypothetical protein; CHP, conserved hypothetical protein; LP, lipoprotein; OMP, outer membrane protein; OSP, outer surface protein. Keys that indicate the clones and STM experiments utilized in each figure panel are provided at <http://www.uth.tmc.edu/pathology/borrelia/>.

A

cp26

Sample Type		Gene (Insertion ratio) Gene Name																																													
Week PI		BBB01 (0.11) <i>acyp</i>	BBB02 (0.44) <i>CHP</i>	BBB03 (0.94) <i>resT</i>	BBB04 (0.20) <i>chbC</i>	BBB04 (0.23) <i>chbC</i>	BBB05 (0.42) <i>chbA</i>	BBB06 (0.13) <i>chbB</i>	BBB06 (0.86) <i>chbB</i>	BBB07 (0.11) <i>osp</i>	BBB08 (0.10) <i>LP</i>	BBB08 (0.10) <i>LP</i>	BBB09 (0.09) <i>LP</i>	BBB09 (0.28) <i>LP</i>	BBB12 (0.27) <i>PF32</i>	BBB13 (0.46) <i>PF49</i>	BBB14 (0.11) <i>CHP</i>	BBB14 (0.11) <i>CHP</i>	BBB14 (0.43) <i>CHP</i>	BBB15 (0.06) <i>CHP</i>	BBB16 (0.06) <i>oppA-4</i>	BBB16 (0.17) <i>oppA-4</i>	BBB16 (0.17) <i>oppA-4</i>	BBB17 (0.41) <i>guaB</i>	BBB18 (0.18) <i>guaA</i>	BBB19 (0.07) <i>ospC</i>	BBB19 (0.51) <i>ospC</i>	BBB20 (0.41) <i>CHP</i>	BBB21 (0.76) <i>CHP</i>	BBB22 (0.15) <i>pbuG1</i>	BBB22 (0.44) <i>pbuG1</i>	BBB23 (0.19) <i>pbuG2</i>	BBB24 (0.15) <i>CHP</i>	BBB25 (0.50) <i>CHP</i>	BBB25 (0.67) <i>CHP</i>	BBB27 (0.10) <i>LP</i>	BBB27 (0.10) <i>LP</i>	BBB28 (0.27) <i>CHP</i>	BBB29 (0.17) <i>malX</i>	BBB29 (0.17) <i>malX</i>	BB0051 <i>CHP</i> (0.79) (+)	BBE22 (0.44) <i>pncA</i> (-)					
		Percent Sites Positive																																													
T	2																																														
T	4								ND												ND															ND											
C	2				ND																										ND																
C	4				ND				ND												ND										ND					ND											
Overall																																															
		Mean MFI Value																																													
T	2																																														
T	4								ND												ND															ND											
C	2				ND																										ND																
C	4				ND				ND												ND										ND					ND											
Overall																																															

Percent Positive	81 - 100	61 - 80	41 - 60	21 - 40	0 - 20

Mean MFI	>1000	501-1000	100-500	<100

B

Ip25

		Gene (Insertion ratio) Gene Name																					
Sample Type	Week PI	BBE04* (0.19) CHP	BBE04.1 (0.97)	BBE07* (0.56) pfs	BBE09 (0.34) P23	BBE10 (0.68) CHP	BBE12 (0.74) CHP	BBE14 (0.67) HP	BBE16 (0.67) bptA	BBE17 (0.78) CHP	BBE18 (0.96) PF49	BBE19 (0.37) PF32	BBE21.1* (0.67) TPR	BBE22 (0.44) pncA	BBE24* (0.81) CHP	BBE24.1* (0.57) CHP	BBE25 (0.38) HP	BBE27 (0.55) CHP	BBE29 (0.11) CHP	BBE29.1* (0.30) CHP	BB0051 (0.79) CHP (+)	BBE22 (0.44) pncA (-)	
		Percent Sites Positive																					
T	2																						
T	4																						
C	2									ND													
C	4									ND													
Overall																							
		Mean MFI Value																					
T	2																						
T	4																						
C	2									ND													
C	4									ND													
Overall																							

Percent Positive	81 - 100	61 - 80	41 - 60	21 - 40	0 - 20

Mean MFI	>1000	501-1000	100-500	<100

C

Ip28-1

		Gene (Insertion ratio) Gene Name																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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Mean MFI	>1000	501-1000	100-500	<100

D

Ip36

		Gene (Insertion ratio) Gene Name																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
Sample Type	Week PI	BBK01 (0.66) LP	BBK02.1* (0.55) CHP	BBK03 (0.41) CHP	BBK04 (0.32)CHP	BBK05 (0.73) HP	BBK07 (0.19) HP	BBK09 (0.21) CHP	BBK12 (0.41) LP	BBK13 (0.12) CHP	BBK13 (0.25) CHP	BBK14 (0.15) CHP	BBK15 (0.57) P35	BBK16* (0.21) CHP	BBK17 (0.18) <i>adeC</i>	BBK17 (0.23) <i>adeC</i>	BBK19 (0.02) LP	BBK25 (0.63) TLP	BBK25.1 (0.20) CHP	BBK26 (0.30) HP	BBK27 (0.35) CHP	IR BBK27-28 (0.58)	BBK29 (0.25) CHP	BBK31 (0.13) HP	BBK32 (0.35) <i>bbk32</i>	BBK33 (0.57) CHP	BBK34 (0.21) CHP	BBK35 (0.19) CHP	BBK36 (0.53) HP	BBK37 (0.53) P37	BBK39 (0.22) CHP	BBK40 (0.27) CHP	BBK41 (0.41) CHP	BBK42 (0.99) CHP	BBK42.1 (0.98) CHP	BBK44 (0.50) HP	BBK45 (0.10) P37	BBK45 (0.38) P37	BBK46 (0.17) P37	BBK47 (0.36) CHP	BBK48 (0.31) P37	BBK49 (0.24) CHP	BBK50 (0.29) P37	tmRNA4 (0.36) sRNA	IR BBK41-42	BB0051 CHP (0.79) (+)	BBE22 (0.44) <i>pncA</i> (-)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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Percent Positive	81 - 100	61 - 80	41 - 60	21 - 40	0 - 20

Mean MFI	>1000	501-1000	100-500	<100

Ip54

Percent Positive	81 - 100	61 - 80	41 - 60	21 - 40	0 - 20

Mean MFI	>1000	501-1000	100-500	<100

Figure 8. Heat map representations of the mouse infectivity of transposon mutants in genes by functional group. Gene groups shown include those involved in (A) chemotaxis, (B) flagellar structure and assembly, (C) phosphoenol pyruvate phosphotransferase system (PEP-PTS), (D) other transport systems, and (E) plasmid maintenance. For each clone, results from Luminex STM analysis (see Fig. 4, Table S3) were grouped by week post inoculation (2 weeks or 4 weeks) and by DNA preparation method (Culture = use of organisms cultured from tissue; Tissue = use of DNA extracted directly from tissue). The data in each group were scored according to the percentage of samples with MFI values above the negative threshold (100) or to the mean MFI value; each result was then color coded as indicated in the key. Each colored box in the figure corresponds to 12 to 15 data points (typically 5 tissues from 3 mice). The composite results obtained in all experiments for the positive (*bb0051*) and negative (*pncA*) control mutants are provided at the right side of each figure. HP, hypothetical protein; CHP, conserved hypothetical protein; LP, lipoprotein; OMP, outer membrane protein; OSP, outer surface protein. Keys that indicate the clones and STM experiments utilized in each figure panel are provided at <http://www.uth.tmc.edu/pathology/borrelia/>.

A

Chemotaxis

Sample Type	Week PI	Gene (Insertion Ratio) Gene Name																						BB0051 (0.79) CHP (+)	BBE22 (0.44) pncA (-)
		BB0414 (0.37) cheR-2	BB0415 (0.60) cheB-1	BB0565 (0.15) cheW-2	BB0566 (0.54) sulP	BB0567 (0.07) cheA-1	BB0567 (0.08) cheA-1	BB0567 (0.46) cheA-1	BB0568 (0.26) cheB-2	BB0570 (0.59) cheY-2	BB0669 (0.26) cheA-2	BB0670 (0.54) cheW-3	BB0671 (0.35) cheX	BB0671 (0.48) cheX	BB0578 (0.50) mcp1	BB0597 (0.37) mcp3	BB0680 (0.00) mcp4	BB0680 (0.00) mcp4	BB0680 (0.05) mcp4	BB0680 (0.05) mcp4	BB0680 (0.41) mcp4	BB0681 (0.19) mcp5	BB0681 (0.38) mcp5		
		Percent Sites Positive																							
T	2																								
T	4																					ND			
C	2				ND																ND				
C	4				ND																ND	ND			
Overall																									
		Mean MFI Value																							
T	2																								
T	4																					ND			
C	2				ND																ND				
C	4				ND																ND	ND			
Overall																									

Percent Positive	81-100	61-80	41-60	21-40	0-20

Mean MFI	>1000	501-1000	100-500	<100

B

Flagellar Structure and Assembly

Sample Type	Week PI	Gene (Insertion Ratio) Gene Name										BB0051 (0.79) <i>CHP</i> (+)	BBE22 (0.44) <i>pncA</i> (-)
		BB0149 (0.28) <i>fliD</i>	BB0221 (0.33) <i>fliG-1</i>	BB0276 (0.75) <i>fliZ</i>	BB0287 (0.65) <i>flbA</i>	BB0288 (0.79) <i>fliI</i>	BB0289 (0.29) <i>fliH</i>	BB0294 (0.00) <i>flgB</i>	BB0668 (0.23) <i>flaA</i>	BB0772 (0.51) <i>flgI</i>	BB0772 (0.67) <i>flgI</i>		
Percent Sites Positive													
T	2												
T	4												
C	2							ND					
C	4							ND					
Overall													
Mean MFI Value													
T	2												
T	4												
C	2							ND					
C	4							ND					
Overall													

Percent Positive	81-100	61-80	41-60	21-40	0-20

Mean MFI	>1000	501-1000	100-500	<100

C

PEP-PTS

Sample Type		Week PI	Replicon, Gene (Insertion Ratio) Gene Name																
			Chrom, BB0645 (0.45) <i>ptsG</i>	Chrom, BB0448 (0.64) <i>ptsH-1</i>	Chrom, BB0116 (0.05) <i>malX-1</i>	Chrom, BB0116 (0.52) <i>malX-1</i>	Chrom, BB0116 (0.75) <i>malX-1</i>	cp26, BBB29 (0.17) <i>malX-2</i>	cp26, BBB29 (0.17) <i>malX-2</i>	cp26, BBB04 (0.20) <i>chbC</i>	cp26, BBB04 (0.23) <i>chbC</i>	cp26, BBB05 (0.42) <i>chbA</i>	cp26, BBB06 (0.13) <i>chbB</i>	cp26, BBB06 (0.86) <i>chbB</i>	Chrom, BB0408 (0.12) <i>fruA-1</i>	Chrom, BB0629 (0.20) <i>fruA-2</i>	Chrom, BB0629 (0.20) <i>fruA-2</i>	BB0051 (0.79) CHP (+)	BBE22 (0.44) <i>pncA</i> (-)
Percent Sites Positive																			
T	2																		
T	4												ND						
C	2		ND						ND										
C	4		ND						ND				ND						
Overall																			
Mean MFI Value																			
T	2												ND						
T	4												ND						
C	2		ND						ND										
C	4		ND						ND										
Overall																			
Percent Positive		81-100		61-80		41-60		21-40		0-20									
Mean MFI		>1000		501-1000		100-500		<100											

D

Other Transport Systems

Sample Type	Week PI	Replicon, Gene (Insertion Ratio)	Gene Name
		Chrom, BB0042 (0.24) <i>phoU</i>	
		Chrom, BB0140 (0.15) <i>BesB</i>	
		Chrom, BB0141 (0.06) <i>BesA</i>	
		Chrom, BB0142 (0.73) <i>BesC</i>	
		Chrom, BB0164 (0.90)	
		Chrom, BB0240 (0.17) <i>gfpF</i>	
		Chrom, BB0604 (0.34) <i>lcpP</i>	
		Chrom, BB0604 (0.34) <i>lcpP</i>	
		Chrom, BB0604 (0.03) <i>lcpP</i>	
		Chrom, BB0637 (0.60) <i>rhaC-1</i>	
		Chrom, BB0638 (0.27) <i>rhaC-2</i>	
		Chrom, BB0729 (0.69) <i>gltP</i>	
		Chrom, BB0814 (0.99) <i>panF</i>	
		cp26, BB022 (0.15) <i>pbuG1</i>	
		Chrom, BB0144 (0.99) <i>proX</i>	
		Chrom, BB0145 (0.73) <i>proW</i>	
		Chrom, BB0318 (0.15) <i>mgIA</i>	
		Chrom, BB0573 (0.65)	
		Chrom, BB0573 (0.65)	
		lp38, BBJ26 (0.48)	
		Chrom, BB0328 (0.54) <i>oppA-1</i>	
		Chrom, BB0328 (0.83) <i>oppA-1</i>	
		Chrom, BB0329 (0.77) <i>oppA-2</i>	
		Chrom, BB0330 (0.24) <i>oppA-3</i>	
		cp26, BB016 (0.06) <i>oppA-4</i>	
		cp26, BB016 (0.17) <i>oppA-4</i>	
		cp26, BB016 (0.17) <i>oppA-4</i>	
		lp54, BBA34 (0.24) <i>oppA-5</i>	
		lp54, BBA34 (0.51) <i>oppA-5</i>	
		BB0051 (0.79) <i>CHP (+)</i>	
		BB022 (0.44) <i>pncA (-)</i>	
Percent Sites Positive			
T	2		
T	4		
C	2		
C	4		
Overall			
Mean MFI Value			
T	2		
T	4		
C	2		
C	4		
Overall			

Percent Positive	81-100	61-80	41-60	21-40	0-20

Mean MFI	>1000	501-1000	100-500	<100

Plasmid Maintenance

Sample Type		Week PI	Replicon, Gene (Insertion Ratio) Gene Name								BB0051 (0.79) CHP (+)	BBE22 (0.44) <i>pncA</i> (-)
			cp26, BBB12 (0.27) PF32	cp26, BBB13 (0.46) PF49	lp17, BBD21 (0.73) PF32	lp21, BBU05 (0.23) PF32	lp25, BBE18 (0.96) PF49	lp25, BBE19 (0.37) PF32	lp28-1, BBF13 (0.25) PF32	lp28-1, BBF25 (0.87) PF50		
Percent Sites Positive												
T	2											
T	4											
C	2			ND	ND				ND			
C	4			ND	ND				ND			
Overall												
Mean MFI Value												
T	2											
T	4											
C	2			ND	ND				ND			
C	4			ND	ND				ND			
Overall												

Percent Positive	81-100	61-80	41-60	21-40	0-20

Mean MFI	>1000	501-1000	100-500	<100