Supplementary Material

Displacement of pBSV2Bh*resT* by pNB12

Clones resulting from transformation of GCB51 with pNB12 were found to contain both pBSV2Bh*resT* (Km^R) and pNB12 (Sm^R). As pNB12 contains the same replication origin as pBSV2*BhresT* it was thought that these two plasmids should be incompatible and under selection for streptomycin, the selectable marker on pNB12, that pBSV2*BhresT* should be displaced from the cell (1-3). However, it has also been reported that the plasmid pKFSS1, from which pJSB104 is derived, is co-maintained with pBSV2 despite the fact that the two plasmids share the same replication origin and should be incompatible (4). Indeed, this is what was observed when GCB51 was transformed with pNB12. By increasing the amount of streptomycin our goal was to increase the number of copies of pNB12 relative to pBSV2*BhresT* until the latter was ultimately displaced from the cell. The pBSV2Bh*resT* plasmid was displaced by serial passage in increasing concentrations of streptomycin : 2.5 mg/ml, 4 mg/ml, and 6 mg/ml. After each passage the cultures were plated in 96 well liquid plates at the same concentration of streptomycin and allowed to grow to late log phase. The bacteria were plated at a concentration of approximately 2.8 spirochetes/ml in 250 µl aliquots and allowed to grow until the wells turned yellow. These were then replica plated in 96 well liquid plates in fresh BSK-II containing 200 µg/ml kanamycin to screen for clones that were resistant to streptomycin but sensitive to kanamycin. Clones which grew in the presence of streptomycin but not in the presence of kanamycin were identified as potential displacement clones and screened by PCR for the loss of the *B. hermsii resT* gene using the primers B322 and B323, which are unique to the *B. hermsii resT* gene. Multiple clones were eventually recovered at 6 mg/ml streptomycin. Twelve were screened for the absence of *B. hermsii resT;* all clones screened were negative for the presence of *B. hermsii resT.* This represents a new approach for plasmid displacement in *B. burgdorferi.*

Table S1. Primers

Primers used in construction of an inducible *resT* expression vector

Product	Primer	Target	Sequence[1]
PpQE30-resT	B2145	*PpQE30*	BamHI - **ggatcc**tctagaaaatcataaaaaatttatttgc
	B2149	*PpQE30* + *resT*	CTTCACTTTTGGAGGCATatgtaatttctcctctttaatgaattc
	B2150	*PpQE30* + *resT*	gaattcattaaagaggagaaattacatATGCCTCCAAAAGTGAAG
	B2169	*resT*	XhoI - **ctcgag**CTATAGCTTATAATTAAAAATTATTGATAAGTATTCTG
pJSB104MCS	B2155	pJLB12g MCS	HindIII - **aagctt**CTCGAGATCTATCG
	B2156		CGAATTCGAGCTCCC

[1] Lower case text indicates *PpQE30,* bold text represents added restriction site. All sequences are 5' to 3'.

Primers used for deletion of wild-type *resT*

Product	Primer	Target	Sequence[1]
ΔresT::aadA	B2209	*bbb04*	GTTTAACAAGTACCTTAACCTTATTTTTTG
	B2210	*bbb04* + *aadA*	gatgctcgatgagtttttctaaTTTTTGTTTTTTTAAATTCAGGC
	B2211	*bbb04* + *aadA*	GCCTGAATTTAAAAAAACAAAAAttagaaaaactcatcgagcatc
	B2212	*aadA* + *bbb02*	CTCTACTTTTGCAGGAAAAAGAATAGtacccgagcttcaagga
	B2213	*aadA* + *bbb02*	tccttgaagctcgggtaCTATTCTTTTTCCTGCAAAAGTAGAG
	B2214	*bbb02*	CAAATCTTGATTCTAATATTAGGGC

[1] Lower case text represents *aadA* sequence+A48

Primers used for Southern blotting

Target	Probe Generated	Primer	Sequence
lp17	12,980-13,727	B2275	GCTAAGACAGGAATTTTCCG
		B2276	GCAAATTCAAATCCCAATTTC
	4,854-5,431	B2317	CAATTATTGTATAGCTATCCAGAATAAAGG
		B2318	GTAAAATATATTTTATTGATGAAAATTTGG
lp28-2	18,725-19,374	B2295	GGTGCATTTTTAGTAATTTCATACTC
		B2296	GATACTAAAAAATTTTGTATTCCAGATGG
	26,461-26,960	B2331	GGAGGTAAGGGGAGCAAG
		B2332	AGTGTGGAGATACAAAAACACAC

Primers used for screening

Primer	Sequence	Target
B2177	ATACGAATATGATTTTTATCTGCG	Reverse primer internal to *resT* used for sequencing
B2178	ATACCTATATTGAAATAATTAAGCTTCTACTG	Forward primer internal to *resT* used for sequencing
B2180	CCAAATAATGCCCGTG	Backbone of pJSB104 flanking the expression site
B2181	CTTTCATGCGCTTAACG	
B2251	GAAATGTAAATCGTTCTATTGTCATTAG	cp26 flanking the *resT* locus
B2252	GGTTGTCCATGGAACCAG	
B322	CGACGTCGACGATAAGGATATACAAGTTTCTAC	Specific for *Borrelia hermsii resT*
B323	CTCCGTCGACGGATATCGAACATAAAAGCA	

Table S2. Strains and plasmids

B. burgdorferi

Strain (GCB)	Description	Reference
908	B31-A, wild-type *resT* parent strain	(5)
2127	GCB2093 with complete *resT* deletion	This study
51	Partial *resT* deletion complemented by *Borrelia hermsii resT*	(6)
2103	GCB51 with pBSV2*BhresT* displaced by pNB12	This study

E. coli

Strain (GCE)	Plasmid	Description	Strain background	Reference
2753	pJSB104	Carries IPTG inducible expression vector	DH5α	(7)
2755	pJSB104MCS	Carries pJSB104MCS	DH5α	This study
2773	pNB12	Inducible *resT* expression plasmid	DH5α	
2769	pNB13	Plasmid for targeted knock-out of the *bbb03* locus through homologous recombination	DH5α	

Plasmid	Selectable Marker	Description	Reference
pJSB104	Strep	IPTG inducible expression vector	(7)
pBSV2*BhresT*	Kan	Plasmid constitutively expressing *resT* from *Borrelia hermsii*	(6)
pJLB12g	Kan	Plasmid for targeted mutagenesis of *B. burgdorferi*	(5)
pJSB104MCS	Strep	pJSB104 with the MCS from pJLB12g inserted at the HindIII site	This study
pNB12	Strep	pJSB104MCS containing *resT* in the expression site	
pNB13	Strep	*resT* KO plasmid with 500 bp on either side of *resT* locus flanking *kan* cassette	

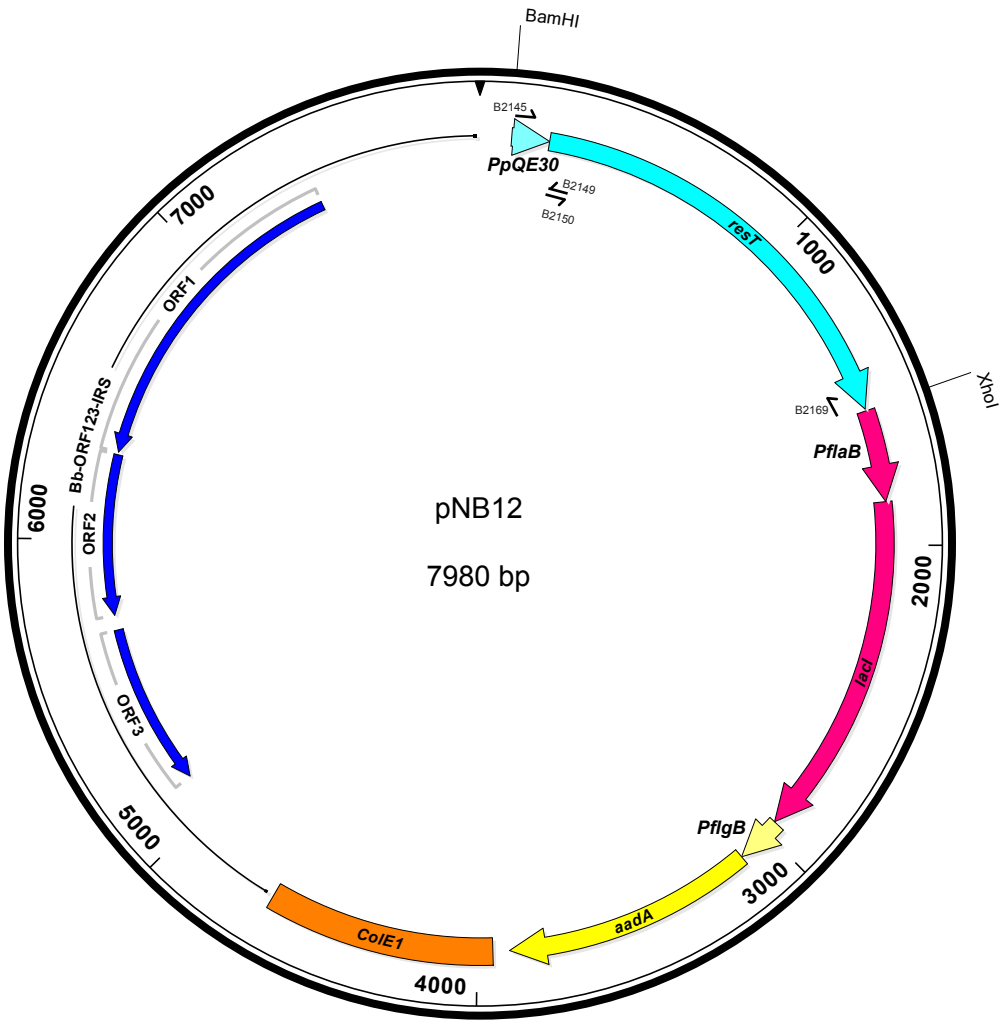

Fig. S1. Map of pNB12. Wild-type *resT* was amplified from B31-A (GCB908) genomic DNA and inserted into the inducible expression plasmid pJSB104 (7) to generate plasmid pNB12 (see Materials and Methods for plasmid construction). Briefly, the *resT* gene is under control of the *PpQE30* promoter, which is controlled by the *lac* repressor and two *lac* operators upstream of the *resT* gene. The plasmid is selectable with streptomycin due to the *aadA* gene. *E. coli* and *B. burgdorferi* replication regions are also shown.

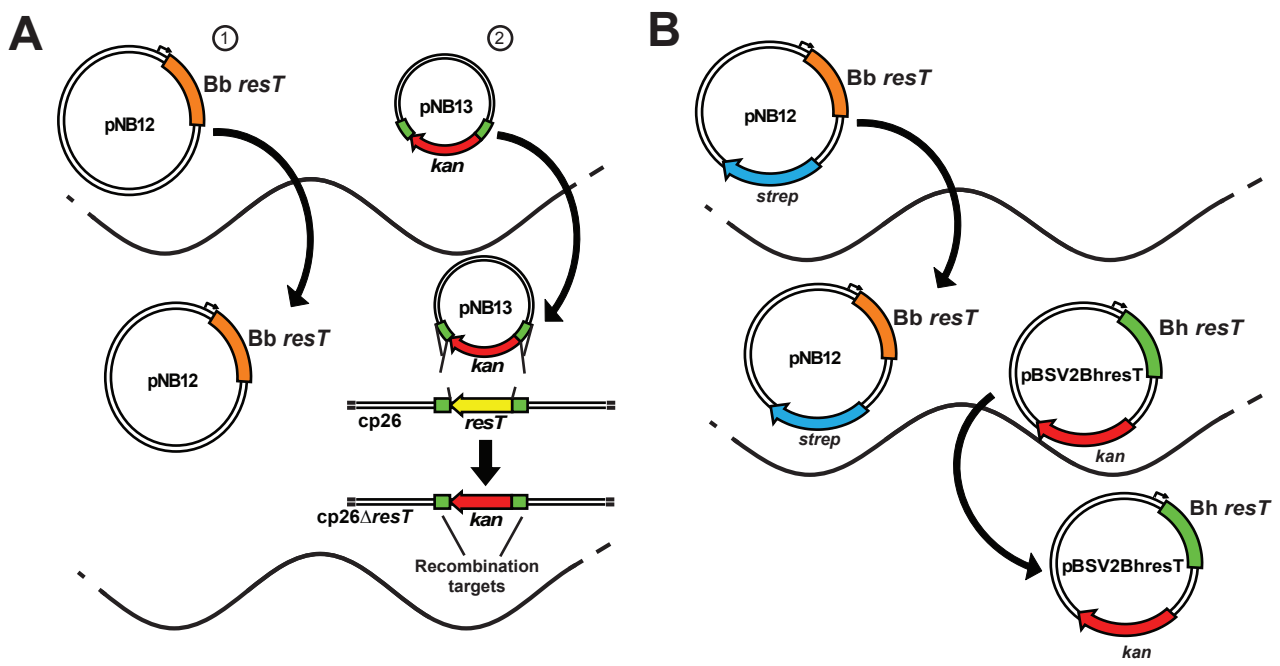

Fig. S2. Strategies for generating a conditional *resT* mutant *B. burgdorferi*. **A)** In strain B31-A *resT* was knocked-out in a two step process. First *B. burgdorferi* was transformed with the plasmid pNB12 which contains a *resT* gene under the IPTG inducible *PpQE30* promoter. Next, the strain was transformed with the recombination plasmid pNB13 which targets the native *resT* on cp26, completely deleting the gene through allelic exchange and replacing it with a kanamycin resistance cassette (GCB2127). **B)** pNB12 was also used to transform the strain GCB51, a B31-A derivative which contains a partial deletion of the native *resT* on cp26, but which is complemented by the plasmid pBSV2Bh*resT*, which expresses the related *Borrelia hermsii resT* (6). As the two plasmids have the same backbone, the streptomycin resistant plasmid carrying an inducible *resT gene* was expected to displace the kanamycin resistant plasmid carrying the *B. hermsii resT* gene. However this was not the case and displacement of pBSV2Bh*resT* was accomplished (GCB2103) through growth of the strain in increasing concentration of streptomycin (see text).

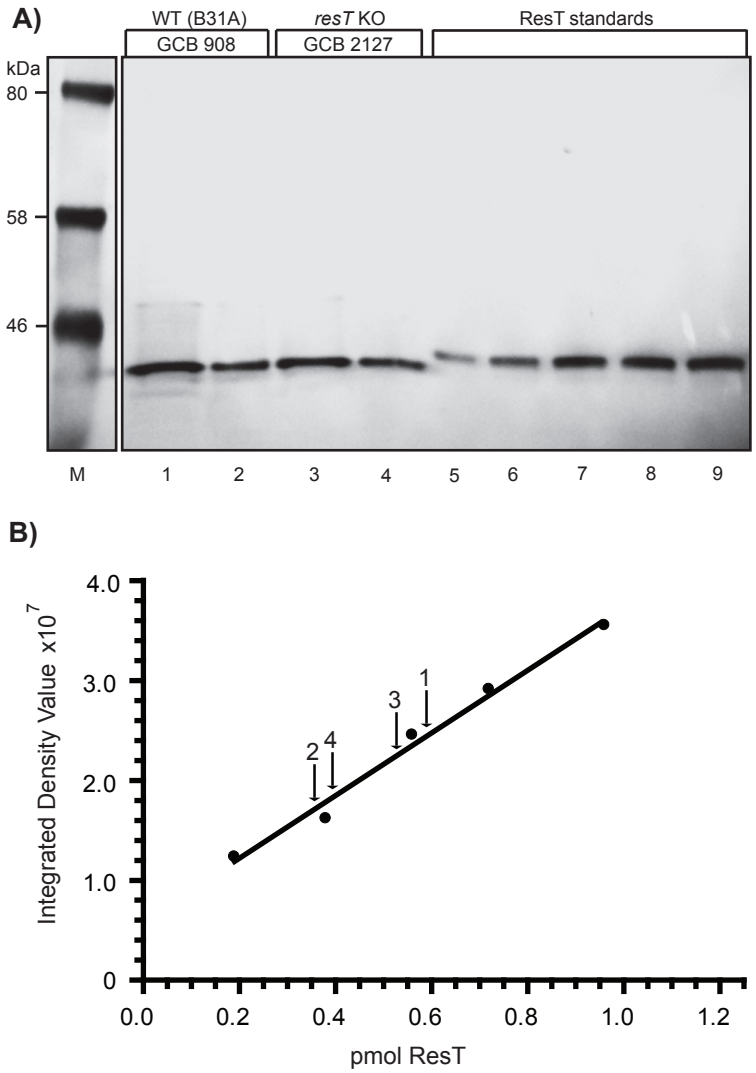

Fig. S3. Western blot for determination of ResT abundance. **A)** To determine the cellular levels of ResT, a Western blot was performed on whole *B. burgdorferi* cell lysates using the high-passage wild-type parent strain B31-A (GCB908) and the ResT-inducible strain (GCB2127) along with known amounts of purified ResT. Band intensities were quantified AlphaEaseFC software and a FluorChem8900 Imager (Alpha Innotech). M Indicates the prestained protein marker used. Lanes 1 and 2 show wild type lysates (B31A) loaded at $2.0x10^7$ and $1.0x10^7$ spirochetes per well, respectively. Lanes 3 and 4 contained the inducible strain (GCB2127) loaded at $2.0x10^7$ and $1.0x10^7$ spirochetes per well, respectively. Lanes 5 - 9 contained 0.19, 0.38, .56, .72 and .96 pmoles of purified recombinant his-tagged ResT, respectively (kindly provided by Kerri Kobryn). **B)** Densitometric analysis of bands 5-9 shown in part A plotted as a standard curve used for calculation of ResT levels in wild-type strain (GCB908) and inducible strain (GCB2127). Numbers 1, 2, 3, and 4 correspond to the lanes in part A with arrows showing the amount calculated using the standard curve. The mean and standard deviation for ResT abundance was calculated as 14,000 ± 2,000 monomers per spirochete in the conditional expression strain and 15,000 ± 1,000 monomers per spirochete in the parental strain.

References

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