

1 **Table S1.** Primers used in this study.

| Primer | Description | Sequence (5'-3') | Reference |
|--------|---|--|---------------------|
| P83 | Forward primer for amplification of <i>gent</i> for IVET reporter cassette | CCT CGA GCT CGT TTA AAC TAA CTG ACT AGG AGG GAG GTT TCC ATA TGT TAC GC | This study |
| P84 | Reverse primer for amplification of <i>gent</i> for IVET reporter cassette | CCT CGG TAC CTT AGG TGG CGG TAC TTG GGT | This study |
| P86 | Forward primer for amplification of <i>pncA</i> for IVET reporter cassette | CCT CGG TAC CGA GGG AGG TTT CCA TGT GGC ACT TAT TTT AAT AGA TAT ACA AAA TGA TTT TTT AG | This study |
| P87 | Reverse primer for amplification of <i>pncA</i> for IVET reporter cassette | CCT CAC CGG TTT ATA TAT TAA GCT TAC TTT GGC TG | This study |
| P129 | Forward primer for amplification of <i>flaB</i> promoter fragment | ATA TCA TTC CTC CAT GAT AAA ATT TAA ATT TCT GAC | This study |
| P130 | Reverse primer for amplification of <i>flaB</i> promoter fragment | CAT CAC TCA ACA GTC CTT TTA ATC TAA TGT CAG G | This study |
| P93 | Forward primer for amplification of cloned DNA fragment from IVET reporter plasmids | GTCAGGGCCGAGCCTACATGTGC | This study |
| P155 | Reverse primer for amplification of cloned DNA fragment from IVET reporter plasmids | CACACAGGAAACAGCTATGACCATG | This study |
| P230 | Sequencing primer for identification of cloned DNA fragment in IVET reporter plasmids | GCCCTGCTGCGT AACATCGTTGC | This study |
| P54 | Forward primer for amplification of kan | CATATGAGCCATATTCAACGGGAAACG | [Bono et al., 2000] |
| P55 | Reverse primer for amplification of kan | AAAGCCGTTTCTGTAATGAAGGAG | [Bono et al., 2000] |

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4 **SUPPLEMENTAL FIGURE LEGEND**

5 **Figure S1. Multiplex PCR profile of endogenous *B. burgdorferi* plasmids.**

6 Agarose gel image of multiplex PCR products after screening wildtype 5A10 (WT) and 17
7 representative pre-infection IVET clones for the presence of endogenous *B. burgdorferi* linear
8 (A), and circular (B) plasmids. Left panels show schematic gel identifying the expected bands
9 corresponding to the plasmids in 5A10. Positions of the 500bp and 100bp molecular weight
10 markers are shown for both schematic and gel images. All clones possessed an identical
11 endogenous plasmid profile compared to the 5A10 parent strain with the exception of one clone
12 that had lost the linear plasmid lp28-2, one clone that lacked the circular plasmid cp32-7, and
13 several clones that had lost lp5 and cp9.

A

Size (bp)

500

100

← lp21
 ← lp28-3
 ← lp38
 ← lp28-1
 ← lp36
 ← lp17
 ← lp54
 ← lp28-2
 ← lp28-4
 ← lp5

IVET Clone

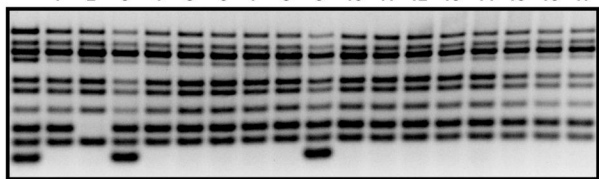
WT

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Size (bp)

500

100

**B**

Size (bp)

500

100

← cp32-8
 ← cp32-1
 ← cp26
 ← cp32-4
 ← cp32-6
 ← cp32-9
 ← cp9
 ← cp32-7
 ← cp32-3

IVET Clone

WT

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Size (bp)

500

100

