Polyploidy, regular patterning of genome copies, and unusual control of DNA partitioning in the Lyme disease spirochete

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SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY NOTES

Specific labeling of *B. burgdorferi* DNA loci using endogenous and heterologous ParB/parS systems

ParB proteins specifically recognize their cognate parS sequence and spread onto adjacent DNA sequences\(^1\)\(^-\)\(^3\). Due to this property, expression of a fluorescent protein-tagged ParB protein leads to accumulation of its fluorescence into a diffraction-limited signal that pinpoints the subcellular location of the DNA locus that contains the parS sequence\(^4\).

We have adapted this method for use in *B. burgdorferi*, whose chromosome contains a single predicted parS sequence\(^3\) located within the par locus, 6 kilobases to the left of oriC (Supplementary Fig. 1a). We labeled this parS sequence either by replacing the native parB gene (bb0434) with the mcherry-parB translational fusion, yielding knock-in strains (Fig. 1a, Supplementary Fig. 1c, Supplementary Data 1), or by driving expression of mcherry-parB or msfgfp-parB from a multi-copy shuttle vector (SV) using the weak promoter P\(_{0826}\)\(^5\) (Supplementary Fig. 1c, Supplementary Data 1). mCherry-ParB fluorescent foci formed only when parS was present on the *B. burgdorferi* chromosome (Supplementary Fig. 1b).

To label an additional *B. burgdorferi* locus, we first inserted the parS sequence of *E. coli* plasmid P1\(^2\), hereafter referred to as parS\(^{P1}\), into the *B. burgdorferi* genome. We then expressed an msfgfp fusion to the parB gene of plasmid P1 (msfgfp-parB\(^{P1}\)) from the same multi-copy shuttle vector that contained the mcherry-parB expression cassette (Supplementary Data 1, Supplementary Fig. 1b). We drove expression of msfgfp-parB\(^{P1}\) using the intermediate strength promoter P\(_{0031}\)\(^5\). The expressed msfGFP-ParB\(^{P1}\) formed fluorescent puncta only when parS\(^{P1}\) was also present in a
given *B. burgdorferi* strain (Supplementary Fig. 1b), regardless of whether the chromosomal
*parS* site was present or not (Supplementary Fig. 1b,d), confirming that labeling of the two *parS*
sequences by their tagged cognate ParB proteins was independent and specific.

This conclusion was further strengthened by quantitative analyses of images of strain
CJW_Bb205 (Supplementary Fig. 1d-f). In this strain, mCherry-ParB foci, which pinpoint the
subcellular location of *par* loci, and msfGFP-ParB$^{P1}$ foci, which pinpoint the subcellular location
of *uvrC* loci (Supplementary Fig. 1a), colocalized almost perfectly (Supplementary Fig. 1d-e).
The *par* and *uvrC* loci are 24 kbp away from each other and 6 and 18 kbp away from *oriC*,
respectively (Supplementary Fig. 1a). Thus, both labels approximate the subcellular location of
*oriC*. Importantly, copy numbers of the *par* and *uvrC* loci were similar (Supplementary Fig. 1f).

**Detection of oriC loci in multiple *B. burgdorferi* strains**

We localized the *oriC* locus in several *B. burgdorferi* strains that were derived from the B31
isolate, which is the type strain, as well as from other isolates, namely N40, 297, Sh-2-82, and
JD1. For the B31-derived strains, we used the B31-A3-68-Δbbe02 genetic background (strains
S9 and K2, see Supplementary Data 1), which is easily transformable and fully capable of
completing the tick-mouse transmission cycle$^{6,7}$. The S9 derivatives CJW_Bb379 and
CJW_Bb474 both carry a replacement of the *parB* gene with an *mcherry-parB* fusion driven by
the native *parB* promoter, and are therefore labeled as knock-in (KI) strains (Fig. 1b,
Supplementary Fig. 1c). CJW_Bb474 additionally expresses free GFP, driven by the P$_{flaB}$
promoter, and inserted into endogenous plasmid cp26 (Fig. 1a, Supplementary Data 1). Strains
CJW_Bb339 and CJW_Bb340 are also derived from the infectious K2 and S9 strains,
respectively, but express mcherry-parB as a second parB copy, in trans, from a shuttle vector (Supplementary Data 1). Strains CJW_Bb339, CJW_Bb340, CJW_Bb379, and CJW_Bb474 each has an almost complete complement of endogenous plasmids. They only lack plasmids cp9, lp5, and lp56 (Supplementary Data 1), which are also absent from the parental strains S9 and K2 and are not required for completion of the tick-mouse transmission cycle. We therefore refer to these strains as having an infectious background, which we experimentally demonstrated for strain CJW_Bb474 (see below).

We determined that the other B31-derived strains have lost multiple endogenous plasmids (Supplementary Data 1) during their generation and/or the generation of their parental strains. At most, strain CJW_Bb075 carries 11 endogenous plasmids, while strain CJW_Bb344 only carries two endogenous plasmids, cp26 and cp32-3 (Supplementary Data 1). They all expressed tagged ParB proteins (mCherry-ParB or msfGFP-ParB) from a shuttle vector (Supplementary Data 1). Lastly, we localized oriC loci in several other B. burgdorferi strains, including the widely studied N40, 297, and JD1 isolate backgrounds (Fig. 1b, Supplementary Fig. 1c, Supplementary Data 1). We did not determine the endogenous plasmid content of the clones derived from the non-B31 isolates as there are no available characterized sets of primers for multiplex PCR detection of the native plasmids of these strains.

Recapitulation of the mouse-tick transmission cycle using strain CJW_Bb474

Strain CJW_Bb474 was used to image the chromosomal copy number in the tick (Fig. 3). Since this strain carries genetic modifications, it was important to assess whether it can reproduce the mouse-tick transmission cycle. Two modifications, inactivation of gene bbe02 and constitutive
expression of GFP from cp26, did not affect \textit{B. burgdorferi}'s ability to complete its transmission cycle when previously tested in several strain backgrounds\textsuperscript{6,7,9,10,14,15}. The third modification, replacement of \textit{parB} with \textit{mcherry-parB}, has not been previously tested. Supplementary Fig. 1i depicts our experimental setup. Mice were infected with \textit{B. burgdorferi} by needle inoculation (step a). Naïve tick larvae were allowed to feed on these infected mice and thus to acquire \textit{B. burgdorferi} (step b). These colonized larvae molted into unfed nymphs (step c), which were then allowed to feed on and transmit \textit{B. burgdorferi} to naïve mice (step d). Infection of mice was confirmed by tissue biopsy culture in BSK-II medium (stages I and V). \textit{B. burgdorferi} acquisition by, and stable colonization of, ticks were assessed in fed larvae, unfed nymphs, and fed nymphs (stages II through IV) by crushing ticks in BSK-II then using the resulting tick extracts to inoculate liquid BSK-II cultures or embedding them in semisolid BSK-agarose plates. Spirochete outgrowth in the BSK-II medium or colony formation in the BSK-agarose plates were deemed evidence that the ticks were colonized by \textit{B. burgdorferi}. All the mice exposed to strain CJW_Bb474, as well as those exposed to the CJW_Bb473 control strain, which only expresses GFP from cp26, were successfully infected (Supplementary Fig. 1j). All the ticks exposed to CJW_Bb474 were also infected, as were most of the ticks exposed to the CJW_Bb473 control (Supplementary Fig. 1j). Additionally, spirochete loads in unfed nymphs were close to $10^2$ cfu/tick for both strains (Supplementary Fig. 1k). These loads increased to above $10^5$ cfu/tick in fed nymphs assayed 10 days after completion of nymphal feeding (Supplementary Fig. 1k). The spirochete burdens that we measured in unfed and fed nymphs are similar to those previously measured in ticks colonized with the parental strain S9\textsuperscript{16-18}. These results indicate that strain CJW_Bb474 is fully capable of completing the mouse-tick transmission cycle.
Supplementary Figure 1. *B. burgdorferi* cells carry multiple chromosome copies

**a.** Schematic of chromosomal loci localized in this study (not drawn to scale). oriC was localized either by labeling the par locus through the expression of fluorescently tagged ParB (red) or by insertion of parS<sup>P1</sup> downstream of uvrC, which is located 52% along the length of the chromosome, followed by expression of msfGFP-ParB<sup>P1</sup>. The telomeres were labeled by insertion of parS<sup>P1</sup> at the phoU or lptD loci, which are located at 5% or 98% along the length of the chromosome, respectively, followed by expression of msfGFP-ParB<sup>P1</sup>. Distances between the labeled DNA loci and the oriC or terC loci, are shown in kilobase pairs (kbp).

**b.** Images showing that mCherry-ParB and msfGFP-ParB<sup>P1</sup> specifically recognize their cognate parS sites. mCherry-ParB and msfGFP-ParB<sup>P1</sup> were expressed from the same shuttle vector (see methods). The strains are, from top to bottom: CJW_Bb211, CJW_Bb534, CJW_Bb532, and CJW_Bb533. Presence of endogenous parB or parS, and chromosomal insertion of parS<sup>P1</sup> are indicated at the left.

**c.** Localization of mCherry-ParB or msfGFP-ParB at oriC regions in various strain backgrounds. Tagged ParB was expressed either by knock-in of mcherry-parB at the gene locus or in trans, from a shuttle vector. Strain CJW_Bb142 expressed msfgfp-parB from a shuttle vector. Strain backgrounds are shown at the left. The CJW_Bb number of each strain is listed on the phase-contrast image.

**d.** Images of a cell of strain CJW_Bb205 showing the oriC region co-labeled by expression of mCherry-ParB, which binds to the endogenous parS site located within the par locus, and msfGFP-ParB<sup>P1</sup>, which binds to the par<sub>S</sub><sup>P1</sup> sequence introduced at the oriC-proximal uvrC locus, as shown in (a).
e. Fluorescence intensity profiles along the cell length for the cell shown in (d).

f. Boxplot showing the number of oriC copies per cell based on the labeling of the par locus (red) or of the uvrC locus (blue) in strain CJW_Bb205. Shown are the mean of the data (middle line), the 25 to 75 percentiles of the data (box), and the 2.5 to 97.5 percentiles of the data (whiskers).

g. Images showing DNA fluorescence in situ hybridization (FISH) staining of the repetitive sequence found within the right telomere of the chromosome of strain 297. Strain B31e2, which does not contain this repetitive sequence, serves as a negative control. Cell outlines are in green.

h. qPCR-based quantification of chromosomal copy numbers per cell in strain CJW_Bb339 at different culture densities. flaB and recA gene copy numbers per cell (mean ± standard deviations of measurements done in three replicate cultures) are shown in blue and red, respectively. Culture densities (mean ± standard deviation) are in black. Values do not account for losses that may have occurred during sample prep.

i. Schematic of the experimental workflow used to test the transmission of B. burgdorferi strains CJW_Bb473 and CJW_Bb474 between ticks and mice. Roman numerals depict the stages at which infection of mice or colonization of ticks by B. burgdorferi was assessed.

j. Summary of infection or colonization readouts as assayed at the stages depicted in (i). Assay methods are given for each stage. Shown are numbers of positive animals (mice or ticks) over numbers of assayed animals.

k. Plot showing B. burgdorferi loads in nymphs prior to nymphal stage feeding (unfed) or 10 days after nymphal feeding drop-off (fed). Individual data points, as well as the mean values ± standard deviation, are plotted. For unfed nymphs colonized with strain CJW_Bb473, one of the
nymphs contained no spirochetes (see Source Data). This data point could not be plotted on a log scale but is included in the calculation of the mean.

Source data for panels f, h, and k are provided as a Source Data file. The numbers (n) of cells analyzed and the number of replicates are provided in Supplementary Data 2.

**Supplementary Figure 2. B. burgdorferi contains multiple copies of its endogenous plasmids**

a. Boxplots showing the quantification of various characteristics (plasmid copies per cell; plasmid copies per 10 μm of cell length; plasmid to oriC ratios; oriC copies per cell; oriC copies per 10 μm of cell length, and cell length) for strains in which an endogenous plasmid is labeled by insertion of parS\(^{P1}\) and expression of msfGFP-ParB\(^{P1}\), while oriC is labeled by expression of mCherry-ParB. Strains are, from left to right: CJW_Bb207, CJW_Bb526, CJW_Bb274, CJW_Bb489, CJW_Bb271, CJW_Bb241, CJW_Bb325, CJW_Bb272, CJW_Bb261, CJW_Bb326, CJW_Bb203, CJW_Bb501, CJW_Bb515, CJW_Bb517, CJW_Bb516, and CJW_Bb518. Selected images for each of these strains are provided in Fig. 2a. Shown are the mean of the data (middle line), the 25 to 75 percentiles of the data (box), and the 2.5 to 97.5 percentiles of the data (whiskers).

b. An exponentially growing culture of strain CJW_Bb203, in which oriC is labeled by expression of mCherry-ParB and cp26 is labeled using the msfGFP-ParB\(^{P1}/\text{parS}\(^{P1}\) system, was diluted to \(10^3\) cells/mL, then imaged daily from day 4 through day 8 of growth in culture. Shown is the oriC copy number per cell (red, mean ± standard deviation), the cp26 copy number per cell (blue, mean ± standard deviation) and the culture density (black, in cells/mL) at the indicated times.
Source data are provided as a Source Data file. The numbers \((n)\) of cells analyzed and the number of replicates are provided in Supplementary Data 2.

**Supplementary Figure 3. Chromosome and plasmid copy numbers correlate with cell length**

**a.** Correlations between \(oriC\) copy number per cell and cell length in the indicated strains, which are also shown and analyzed in Fig. 1b and Supplementary Fig. 1c. \(r\), Spearman’s correlation coefficient.

**b.** Same as in (a), except for strains CJW_Bb074 and CJW_Bb142. These strains have longer characteristic cell lengths, which is reflected in the range used for the x-axis.

**c.** Correlations between plasmid copy number per cell and cell length in a subset of the strains described in Supplementary Fig. 2a and Fig. 2a. The analyzed plasmid is listed in blue, while the Spearman’s correlation coefficient \(r\) is in burgundy.

**d.** Correlations between plasmid copy number per cell and cell length in the remaining strains described in Supplementary Fig. 2a and Fig. 2a and not included in (c). These plasmids have fewer copies per cell. The analyzed plasmid is listed in blue, while the Spearman’s correlation coefficient \(r\) is in burgundy.

Source data are provided as a Source Data file. The numbers \((n)\) of cells analyzed and the number of replicates are provided in Supplementary Data 2.
Supplementary Figure 4. ParZ is a novel centromere-binding protein that controls oriC segregation

a. Whole genome ChIP-seq profiles for strains expressing free GFP (CJW_Bb473), ParZ-msfGFP (CJW_Bb378), mCherry-ParB (CJW_Bb379), or ParA-msfGFP (CJW_Bb488). The x-axis shows the chromosome coordinates followed by the concatenated endogenous plasmids of strain S9 in the order: lp28_3, lp25, lp28_2, lp38, lp36, lp28_4, lp54, cp26, lp17, lp28_1, cp32_1, cp32_3, cp32_4, cp32_6, cp32_7, cp32_8, cp32_9, and lp21. The vertical dotted lines indicate the boundary between chromosomal and plasmid sequences in the concatenated genome. Two replicates are shown for each strain. No ChIP-seq peaks are seen in the free GFP control. The peaks visible in the other traces correspond to the par locus (also see Fig. 6). The endogenous P_{flaB} and flaBt sequences were computationally removed from the flaB locus on the chromosome sequence before read mapping to prevent erroneous mapping of ChIP-seq reads to the flaB locus (see the Online Methods for a detailed explanation).

b. ChIP-seq profiles of ParZ-msfGFP and mCherry-ParB binding to the par locus in strain CJW_Bb403, which expresses both protein fusions.

c. ChIP-seq profile of ParZ-msfGFP binding to the genome of strain CJW_Bb101, which carries parZ-msfGfp on a shuttle vector. The two peaks of binding are at the chromosomal par locus and on the shuttle vector, as indicated. See panels (d) and (e) for detailed views of binding to these genome regions. Strain CJW_Bb101 lacks all endogenous plasmids except lp54, cp26, lp17, cp32-1, cp32-3, and cp32-4. The sequences corresponding to these plasmids were concatenated in this order, followed by the sequence of the pBSV2G_P_{0826}-RBS-ParZ-msfGFP^{Bb} shuttle vector to generate the plasmid portion of the genome of strain CJW_Bb101. The vertical dotted line indicates the boundary between chromosomal and plasmid sequences in the concatenated...
genome. The endogenous P<sub>0826</sub> and P<sub>flgB</sub> sequences were removed from the chromosome before the mapping of the reads to prevent erroneous mapping of ChIP-seq reads to these chromosomal loci (see the Online Methods for a detailed explanation).

d. Detailed view of panel (c) showing the binding of ParZ-msfGFP to the chromosomal par locus.

e. Detailed view of panel (c) showing the binding of ParZ-msfGFP to sequences within the shuttle vector.

f. Images of a cell of strain CJW_Bb101. Arrowheads pinpoint four of the many densely packed ParZ-msfGFP puncta that can be detected in cells of this strain.

g. Images of a cell of a strain CJW_Bb571 which expresses ParZ-msfGFP from the parZ gene locus and carries an empty shuttle vector.

h. ChIP-seq profiles showing an overlay of the landscape binding pattern of free GFP, ParZ-msfGFP, mCherry-ParB, and ParA-msfGFP to the concatenated endogenous plasmid sequences. Traces are those of Replicate 2 shown in (a).

**Supplementary Figure 5. Phylogenetic analyses of Par proteins**

a. Alignment of the indicated chromosomally expressed ParB sequences. ParB domains are highlighted at the bottom.

b. Alignment of indicated chromosomally expressed ParA sequences. The numbers at the right indicate the same species as those listed in (a), at the right.

c. Table showing the distance in base pairs (bp) between parA and parB homologs in the par loci of representative spirochete bacteria. parA and parB are found in the same orientation with a
short genomic distance separating the two genes, which is suggestive of an operon structure. A negative value indicates overlap of the coding regions of the two genes.

d. Organization of the par loci of the indicated Lyme disease spirochete strains as visualized using the BorreliaBase genome browser19.

e. Alignment of 65 Borrelliaceae ParZ sequences. Putative ParZ domains are highlighted at the bottom. Sequences belonging to Lyme disease and relapsing fever spirochetes are marked at the right.

Supplementary Figure 6. Characterization of B. burgdorferi strains expressing tagged Par proteins and/or carrying par gene mutations.

a. ParA-msfGFP signal concentrations in individual cells of strains carrying parA-msfgfp as the single parA copy at its native locus (knock-in strains). par locus mutations present in these strains are indicated at the bottom. From left to right, the strains are: CJW_Bb488, CJW_Bb520, CJW_Bb519, CJW_Bb610. Shown are individual data points as well as means ± standard deviations. A.U., arbitrary units.

b. Same as in (a), but also including strains carrying parA-msfgfp on a shuttle vector. From left to right, the strains are: CJW_Bb488, CJW_Bb520, CJW_Bb519, CJW_Bb610, CJW_Bb219, CJW_Bb218, CJW_Bb256, and CJW_Bb255. Please note the y-axis range is different than in (a).

c. Phase contrast and fluorescence micrographs of strains expressing ParA-msfGFP from a shuttle vector and carrying the indicated par locus mutations. From top to bottom, the strains are: CJW_Bb219, CJW_Bb218, CJW_Bb256, and CJW_Bb255.
d. Plot showing relative mRNA levels for parZ and parZΔN20 determined by qRT-PCR in the indicated strains. Shown are the individual values and the means from two replicates. Mann-Whitney test comparing parZΔN20 expression (strain CJW_Bb610) with parZ expression (strain CJW_Bb488) yielded \( p = 0.33 \).

e. Plots comparing cell length, oriC copy numbers per cell, oriC copies per 10 μm of cell length, and abundance of cells without oriC foci for control and mutant strains analyzed in Figs. 6, 8, and 9. The boxplots depict the mean of the data (middle line), the 25 to 75 percentiles of the data (box), and the 2.5 to 97.5 percentiles of the data (whiskers). The numbers on the bottom graph represent the number of cells without oriC foci and the total number of cells analyzed for each strain. The nature of the oriC label is listed at the top. Mutations introduced into the strains are listed at the bottom. From the left, the following strains were used: CJW_Bb378, CJW_Bb490, CJW_Bb524, CJW_Bb616, CJW_Bb603, CJW_Bb602, CJW_Bb379, CJW_Bb525, CJW_Bb626, and CJW_Bb604.

f. Phase contrast and fluorescent images of a cell of strain CJW_Bb626 that expresses mCherry-ParB and carries the ΔparZ mutation.

g. Plot showing the growth kinetics of the indicated strains in semisolid BSK-agarose media. Approximately 50 cells of each strain were plated in triplicate. The plates were then inspected daily from day 5 after plating onwards and visible colonies were counted on each day. The plating efficiency measured on a given day was calculated by dividing the number of colonies counted on that day by the maximum number of colonies counted on the same plate during the 9-day course of the experiment. Shown are means ± standard deviations.
h. Growth curves of the indicated strains in BSK-II medium. Cultures were inoculated in triplicate from exponentially growing parental cultures and then cell densities were determined daily by direct counting under darkfield illumination. Shown are means ± standard deviations. Source data for panels a,b,d,e,g, and h are provided as a Source Data file. The numbers (n) of cells analyzed and the number of replicates are provided in Supplementary Data 2.

**Supplementary Figure 7. ParZ-like sequences can be found in Firmicutes, Fusobacteria, and their phages**

Blast searches were performed using *B. burgdorferi* ParZ as bait. No hits were obtained among archaeal and eukaryotic proteins. Hits obtained among bacterial chromosome-encoded proteins are in red, while those obtained among bacteriophage-encoded proteins are in cyan. Letters highlight the Firmicutes and Fusobacteria phyla, or the Borreliaceae family, while the numbers highlight the indicated genera.

**Supplementary Figure 8. Schematic pedigree of genetic changes engineered at the par and smc loci**

a. Depiction of genetic changes at the *par* locus. Genes affected by genetic modifications are in orange. Genes flanking the modified region and not affected by the changes are in gray. WT, wild type. *aacC1*, gentamicin resistance cassette. *aphI*, kanamycin resistance cassette. The promoters and transcriptional terminators present in the antibiotic resistance cassettes are not shown. Features are not drawn to scale. The lines starting from the WT locus at the left depict the order in which successive genetic modifications were introduced. *, *parZΔN20*. 
b. Same as in (a) but for the *smc* locus. *hyp*, gene *bb0044* of hypothetical function. *, a short sequence encoding the C-terminus of SMC was not deleted to avoid inactivating the promoter upstream of gene *bb0044*.

a. and b. Please note that individual strains (see Supplementary Data 1) may carry genetic modification at a single locus or multiple loci, including the chromosomal *phoU*, *uvrC*, or *lptD* loci, or plasmid-specific loci.
**Supplementary Figure 1**

### a. Genetic Features

| Strain       | Genetic Features       | I (Culture) | II (Culture/Plating) | III (Culture/Plating) | IV (Culture/Plating) | V (Culture) |
|--------------|------------------------|-------------|----------------------|-----------------------|----------------------|-------------|
| CJW_Bb473    | gfp                    | 5/5         | 5/9                  | 5/6                   | 6/6                  | 2/2         |
| CJW_Bb474    | gfp; mcherry-parB      | 5/5         | 9/9                  | 6/6                   | 6/6                  | 2/2         |

### b. Knock-in

| Strain       | Genetic Features       | Phase | oriC | mCherry-ParB | msfGFP-ParBP<sup>+</sup> |
|--------------|------------------------|------|------|--------------|--------------------------|
| CJW_Bb473    | gfp                    | -    | -    | +            | -                        |
| CJW_Bb474    | gfp; mcherry-parB      | -    | -    | +            | -                        |

### c. Infectious background

- Reduced genome
- B31 strains
- Non-B31 strains

### d. DNA

| Stage | Method | Infectious load (cfu/tick) |
|-------|--------|---------------------------|
| I     | a. needle inoculation | Unfed larvae | Fed larvae | Nymphs |
| II    | b. acquisition        | Infected mice | Infected mice | Naive mice |
| III   | c. moult               | Fed nymphs | Fed nymphs | Unfed nymphs |
| IV    | d. transmission        | Fed nymphs | Fed nymphs | Unfed nymphs |

### e. Locus copies/cell

| Day | oriC (par) | oriC (uvrC) |
|-----|------------|-------------|
| 0   | 10<sup>7</sup> | 10<sup>6</sup> |
| 1   | 10<sup>8</sup> | 10<sup>7</sup> |
| 2   | 10<sup>9</sup> | 10<sup>8</sup> |
| 3   | 10<sup>10</sup> | 10<sup>9</sup> |
| 4   | 10<sup>11</sup> | 10<sup>10</sup> |
| 5   | 10<sup>12</sup> | 10<sup>11</sup> |

### f. Signal intensity (A.U.)

| Cell length (µm) | mCherry-ParB | msfGFP-ParBP<sup>+</sup> |
|------------------|--------------|--------------------------|
| 0                | 0            | 0                        |
| 1                | 0            | 0                        |
| 2                | 0            | 0                        |
| 3                | 0            | 0                        |
| 4                | 0            | 0                        |
| 5                | 0            | 0                        |
| 6                | 0            | 0                        |
| 7                | 0            | 0                        |
| 8                | 0            | 0                        |
| 9                | 0            | 0                        |
| 10               | 0            | 0                        |
| 11               | 0            | 0                        |
| 12               | 0            | 0                        |
| 13               | 0            | 0                        |
| 14               | 0            | 0                        |
| 15               | 0            | 0                        |

### g. Flavobacterial loads (cfu/tick)

- CJW_Bb473 (control)
- CJW_Bb474 (mCherry-ParB)
Supplementary Figure 2

(a) Plasmid copies/cell vs. cell length (µm)

(b) Culture density (cells/mL) and orC copies/cell vs. Day

- Exponential growth
- Stationary phase

Legend:
- Culture density (cells/mL)
- orC copies/cell
- cp26 copies/cell

Plasmid labeled:
- lp17, lp21, lp25, lp28-1, lp28-2, lp28-3, lp28-4, lp36, lp38, lp54, cp26, cp32-1, cp32-3, cp32-4, cp32-7, cp32-8

oriC ratio, oriC density (copies / 10 µm)
Supplementary Figure 5

### Table: parA-parB distance (bp)

| Species (strain) | parA-parB distance (bp) |
|------------------|-------------------------|
| *Brachyspira hyodysenteriae* (WA1) | 100 |
| *Leptospira interrogans* ser. *Copenhageni* (Piyasena) | 3 |
| *Leptospira biflexa* ser. *Patoc* (Patoc 1) | 4 |
| *Treponema denticola* (ATCC 35405) | -7 |
| *Treponema pallidum* subsp. *pallidum* (Nichols) | -13 |
| *Spirochaeta africana* (DSM 8902) | -22 |
| *Sphaerochaeta globosa* (Buddy) | -19 |
| *Salinispira pacifica* (L21-Rpul-D2) | 32 |

### Diagrams:

- Graphical representation of parA-parB distance for various species.
- Analysis of residue similarity between parA and parB amino acid positions.
- Comparative analysis of *B. burgdorferi* strains and other Lyme disease Borrelia species.
A. **Firmicutes**
1. *Streptococcus*
2. *Enterococcus*
3. *Staphylococcus*
4. *Limosilactobacillus*
5. miscellaneous *Eubacteriales*
6. *Bacillus*
7. miscellaneous *Bacillales*
8. *Bacillus*
9. miscellaneous *Firmicutes*

B. **Borreliaceae**
10. Lyme disease *Borrelia*
11. relapsing fever *Borrelia*

C. **Fusobacteria**

**Chromosomal sequences**
**Phage sequences**
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