

Supplementary information, Minnen *et al.* SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*.

## Supporting Experimental Procedures:

### Strains and growth conditions

Bacterial strains and plasmids used in this study are listed in Table 1. *S. pneumoniae* strains were grown as standing cultures in M17 broth (Terzaghi and Sandine, 1975) containing 0.5% (w/v) glucose (GM17) (for ChIP analysis) or in complex C+Y medium (Martin *et al.*, 1995) at 30°C or 37°C (for microscopy). For growth on plates 1% (v/v) of defibrinated sheep blood was added to GM17 agar. When applicable, ZnSO<sub>4</sub> was added to liquid medium at a final concentration ranging between 0.05 mM and 0.20 mM. *E. coli* EC1000 was grown at 37°C in a shaking incubator in TY broth (Bacto-Tryptone (1%), Bacto-yeast extract (0.5%) and 1% NaCl). When appropriate, antibiotics were added in the following concentrations for *E. coli*: ampicillin (amp) at 100 µg/ml and spectinomycin (spec) at 50 µg/ml. For *S. pneumoniae* the following concentrations were used: tetracycline (tet) 1 µg/ml, trimethoprim (trmp) 18 µg/ml, spectinomycin 100 µg/ml. For *S. pneumoniae*, storage of mid-exponential phase cultures was done by growing cells in GM17 or C+Y at 37°C to an OD<sub>600nm</sub> of approximately 0.2 and 0.4, respectively. The cells were centrifuged for 2 min at 14000 rpm and the cell pellet was resuspended in a volume of fresh medium containing 14.5% glycerol (v/v) that would result in an OD<sub>600nm</sub> of exactly 0.2 and 0.4, respectively. The cells were then aliquoted and stored at -80°C.

### Construction of plasmids and strains

To construct plasmid pAM6, carrying *gfp* fused to *smc* under the control of the zinc-inducible *czcD* promoter (noted P<sub>Zn</sub>), a PCR with the primers 63 smc-F+XbaI and 64 smc-R+NotI (Table S1) was performed, using chromosomal DNA of strain D39 as a template. The amplified fragment was subsequently cleaved with XbaI and NotI and ligated into the SpeI and NotI sites of plasmid pJWV25 (Eberhardt *et al.*, 2009) resulting in plasmid pAM6. The correct sequence of the plasmid was verified by sequencing using primers 103 pPP2-R, 71 *gfp*-F seq pJWV25, 20 smc-up-R+BamHI, 21 smc up F *gfp*, 22 smc *gfp* check F, 88 smc in seq F, 89 smc in seq2 F, 90 smc in seq R and 91 smc in seq2 R.

To construct plasmid pAM9, carrying *gfp* fused to *scpB* under the control of the P<sub>Zn</sub>, a PCR with the primers 69 scpB-F+SpeI and 70 scpB-R+NotI (Table S1) was performed, using chromosomal DNA of strain D39 as a template. The amplified fragment was subsequently cleaved with SpeI and NotI and ligated into the corresponding sites of plasmid pJWV25 (Eberhardt *et al.*, 2009) resulting in plasmid pAM9. The correct sequence of the plasmid was verified by sequencing using primers 103 pPP2-R and 71 *gfp*-F seq pJWV25.

Strains AM10 (P<sub>Zn</sub>-*gfp*), AM13 (P<sub>Zn</sub>-*gfp*-*scpB*) and AM14 (P<sub>Zn</sub>-*gfp*-*smc*)

*S. pneumoniae* strains AM10, AM13 and AM14 were obtained by a double crossover recombination event between the *bgaA* regions located on plasmids pJWV25, pAM9 and pAM6, respectively, and the chromosomal *bgaA* locus. Transformants were selected on GM17 blood agar plates containing tetracycline (1 µg/ml) and correct integration was verified by PCR using primers 112 BgaA\_down check R and 113 TetM check-F.

#### Strain AM39 ( $\Delta smc::trmp$ )

The gene that encodes SMC was deleted from strain D39 by replacement with a trimethoprim resistance cassette. To prevent any polar effects of the gene replacement, the trimethoprim resistance gene was inserted without its own promoter and terminator using three-way long flanking PCR. This was done by amplifying the region downstream of *smc* with primers 18 *smc*-down-F+KpnI and 10 *smc*-up-F and the region upstream of *smc* with primers 17 *smc*-R+KpnI KO and 16 *smc*-up-F KO using chromosomal DNA of strain D39 as a template. The trimethoprim resistance cassette, without its promoter and terminator, was amplified from plasmid pKOT (Hendriksen *et al.*, 2008) with primers 83 *trmp*-F + KpnI and 84 *trmp*-R + KpnI. The 3 PCR products were digested with KpnI and ligated together. The ligation mixture was subsequently transformed to strain D39. Transformants were selected on GM17 blood agar plates containing trimethoprim (18  $\mu$ g/ml), after overnight incubation at 37 °C. Correct integration was verified by PCR using primer pairs 60 *trmp* F seq and 14 *smc* check F, 19 *smc*-check-F KO and 45 *trmp* R + NotI, 89 *smc* in seq2 F and 90 *smc* in seq R and with 60 *trmp* F seq and 14 *smc* check F. The construct was sequence verified. Chromosomal DNA of PCR verified mutants was backcrossed to D39 to check for normal transformation efficiencies and lack of second site suppressor mutants, basically as described (Caymaris *et al.*, 2010). Southern blots were performed on chromosomal DNA of three mutants that appeared correct by PCR to independently verify the replacement and deletion of *smc* on the *S. pneumoniae* genome, using a probe upstream of *smc* and a probe inside the coding region of *smc* and using EcoRV as restriction enzyme, as shown in Figure S6.

#### Strain MT1 (*parB-spec*)

A PCR using primers 1 *parB*-up-F and 2 *parB*-up-R+Ascl and a PCR using primers 3 *parB*-down-F+NotI and 4 *parB*-down-R was performed on D39 chromosomal DNA. Another PCR using primers 38 *spec*-F-Ascl and 39 *spec*-R-NotI was performed using plasmid pORI38 (Leenhouts *et al.*, 1996) as a template. PCR product *parB*-up was digested with Ascl, PCR product *parB*-down was digested with NotI and PCR product *spec* was digested with Ascl/NotI. The 3 PCR products were ligated together. The ligation was then amplified with PCR using the outside primers (1 and 4). The obtained PCR product was transformed to D39 competent cells. Transformants were selected on GM17 blood agar plates containing spectinomycin (100  $\mu$ g/ml), after overnight incubation at 37 °C. Correct integration of the spectinomycin resistance cassette just after the *parB* locus was verified by PCR using primers 8 *parB*-check-F and 9 *parB*-check-R. The construct was sequence verified.

#### Strain MT2 (*parB-gfp-spec*)

A PCR using primers 1 *parB*-up-F and 6 *parB*-R+linker+BamHI and a PCR using primers 5 *parB*-F-down+EcoRI and 4 *parB*-down-R was performed using chromosomal DNA of strain MT1 as a template. The *parB* reverse primer contained a sequence which encodes for the following flexible domain breaking linker: GSGGEAAKGS (Arai *et al.*, 2001). Another PCR using

Supplementary information, Minnen *et al.* SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*.

primers 46 *gfp\_sf-F+BamHI* and 47 *gfp\_sf-R+EcoRI* was performed using plasmid pUC57-*gfp* as a template (Veening, unpublished). PCR product *parB-up* was digested with BamHI, PCR product *parB-down* was digested with EcoRI and PCR product *gfp* was digested with BamHI/EcoRI. The 3 PCR products were ligated together. The ligation product was transformed to D39 competent cells and transformants were selected on GM17 blood agar plates containing spectinomycin (100 µg/ml), after overnight incubation at 37 °C. Correct integration of the *parB-gfp* fusion was verified by PCR using primers 8 *parB-check-F* and 9 *parB-check-R*. The construct was sequence verified.

#### Strain MT3 ( $\Delta(\textit{parB})\text{-spec}$ )

A PCR using primers 1 *parB-up-F* and 7 *parB-R+EcoRI-KO* and a PCR using primers 5 *parB-F-down+EcoRI* and 4 *parB-down-R* was performed using chromosomal DNA of strain MT1 as a template. PCR products *parB-up* and *parB-down* were digested with EcoRI. The 2 PCR products were ligated to each other. The ligation product was transformed to D39 competent cells. Transformants were selected on GM17 blood agar plates containing spectinomycin (100 µg/ml), after overnight incubation at 37 °C. Correct deletion of *parB* was verified by PCR using primers 8 *parB-check-F* and 9 *parB-check-R* and the locus was verified by sequencing.

#### JWV415 ( $\Delta(\textit{parB})\text{-spec}$ , $\Delta\textit{smc}::\textit{trmp}$ )

Chromosomal DNA of strain MT3 was transformed to strain AM39 and transformants were selected on GM17 blood agar plates containing spectinomycin (100 µg/ml), after overnight incubation at 37 °C. Transformants were checked for the presence of trimethoprim resistance and both the *smc* and the *parB* deletions were verified by PCR.

#### AM15 ( $P_{Zn}\text{-gfp}\text{-}\textit{scpB}$ , $\Delta(\textit{parB})\text{-spec}$ ) and AM16 ( $P_{Zn}\text{-gfp}\text{-}\textit{smc}$ , $\Delta(\textit{parB})\text{-spec}$ )

Chromosomal DNA of strain MT3 was transformed to strains AM13 and AM14, respectively, and transformants were selected on GM17 blood agar plates containing spectinomycin (100 µg/ml), after overnight incubation at 37 °C. Transformants were checked for the presence of tetracylin resistance and both the presence of the *gfp* fusion and the *parB* deletion were verified by PCR.

### Chromatin Immuno-Precipitation (ChIP)

Cells were grown to mid-exponential phase (OD<sub>600nm</sub> ~0.2) in GM17 medium at 37°C (with 0.15 mM ZnSO<sub>4</sub> where relevant) and 84 ml of culture was mixed by inverting with 8.4 ml of fixing solution (50 mM Tris pH 8.0, 100 mM NaCl, 0.5 mM EGTA, 1 mM EDTA, 30% (v/v) formaldehyde) and incubated at room temperature for 30 min. Cells were centrifuged for 3 min at 4°C at 6000 rpm and resuspended in 30 ml ice cold PBS. Cells were washed again with PBS and the pellet was resuspended in 1.5ml of TSEMS (50 mM Tris/HCl pH 7.4, 50 mM NaCl, 10 mM EDTA, 0.5 M Sucrose and 1 mM PMSF). Cells were washed twice more with TSEMS and the pellets aliquoted in three portions and were snap frozen in liquid nitrogen and subsequently stored at -80°C. One aliquot of each strain was resuspended in 2 ml ice cold lysis buffer (50 mM HEPES-KOH, pH 7.55, 140 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium deoxycholate and RNase A (100 µg/ml))

and transferred to a 5 ml plastic tube. Cells were sonicated on ice using a Sonics vibra cell vcx 130. Pulse was on for 30 sec, then off for 30 sec and this was repeated for a total time of 3 min with the amplitude set to 75%. The lysates were transferred to 2 ml tubes and centrifuged for 10 min at 4°C at 13200 rpm. The supernatant was transferred to a clean 2 ml tube and centrifuged for 10 min 4°C 13200 rpm. The supernatant was pre-cleared with 100 µl proteinG coupled Dyna Beads (Invitrogen) and incubated on a turning wheel with powersetting 18 for 1 hour at 4°C (cold room). Beads were pelleted using a magnet and the precleared supernatant was transferred to a new 2 ml eppendorf tube. 200 µl of the supernatant was kept at -20°C as the Whole Cell Extract (WCE) control. To 1500 µl of the precleared supernatant, 10 µl of anti-GFP antibodies (rabbit serum, polyclonal, Invitrogen A-6455) was added and incubated on a turning wheel for 2 hrs at 4°C. 100 µl of proteinG coupled Dyna Beads (invitrogen) were added to the samples and incubated for 12 hours or more (overnight) on the turning wheel at 4°C. The beads were collected using a magnet and the supernatant was discarded. The beads were washed once for 5 min (vortex approximately 800 rpm) at room temperature with 1 ml lysis buffer containing 500 mM NaCl. The beads were pelleted with a magnet and subsequently washed with 1 ml wash buffer. Beads were pelleted and resuspended in 520 µl TES buffer. The WCE samples were thawed and combined with 300 µl TES and 20 µl 10% SDS. The samples were kept shaking at 65°C (little oven) for 6 hours (to overnight). This eluted the DNA from the beads and reversed cross links. Beads were collected using a magnet and 500 µl of the supernatants was transferred to a fresh 2 ml eppendorf cup. 12.5 µl Proteinase K (20 mg/ml in TE) was added to the samples and shaken for 2 hours at 37°C. 520 µl phenol was added and the solution was mixed by inverting and centrifuged for 2 min at max. speed. 450 µl of the top layer was pipetted off and transferred to a clean 1.5 ml eppendorf cup. 450 µl chloroform was added and the solution was mixed by inverting, spinned for 2 min at max. speed, 400 µl of the top layer was collected and transferred to a clean 1.5 ml eppendorfcup. 1 µl glycogen (20 mg/ml from Roche) was added, mixed by inverting and 40 µl sodium acetate, pH 5.3, was added and mixed by inverting. 1 ml of pure ethanol was added, mixed by inverting and incubated for 20 min at 20°C. The mixture was centrifuged for 15 min at 4°C max speed, the supernatant was carefully removed. 200 µl of 70% ethanol was added and the cup was inverted several times, subsequently centrifuged for 15 min at 4°C and the supernatant was removed. The pellet was air dried at 45°C and resuspended in 50 µl TE, pH 8 for 15 min at 45°C. The ChIP samples were stored in -20°C, 5 µl of the WCE was diluted tenfold and also stored in -20°C. To the remaining 45 µl, RNaseA (1 µl of 10 mg/ml stock) was added and incubated for 30 min at 37°C. The sample was run on a 2% agarose gel to check the sheering of DNA. 1 µl of the ChIP samples (1:25) and the diluted WCE (1:250) were used for qPCR in a 25 µl reaction.

### **Fluorescence Microscopy**

Cells were grown at 30°C (or at 37°C for strain AM13) in plastic 5 ml capped tubes, filled to a maximum of 3 ml to allow for enough air for proper GFP folding, basically as described previously (Eberhardt *et al.*, 2009). When appropriate, ZnSO<sub>4</sub> was added prior to imaging (0.15 mM for GFP-SMC and

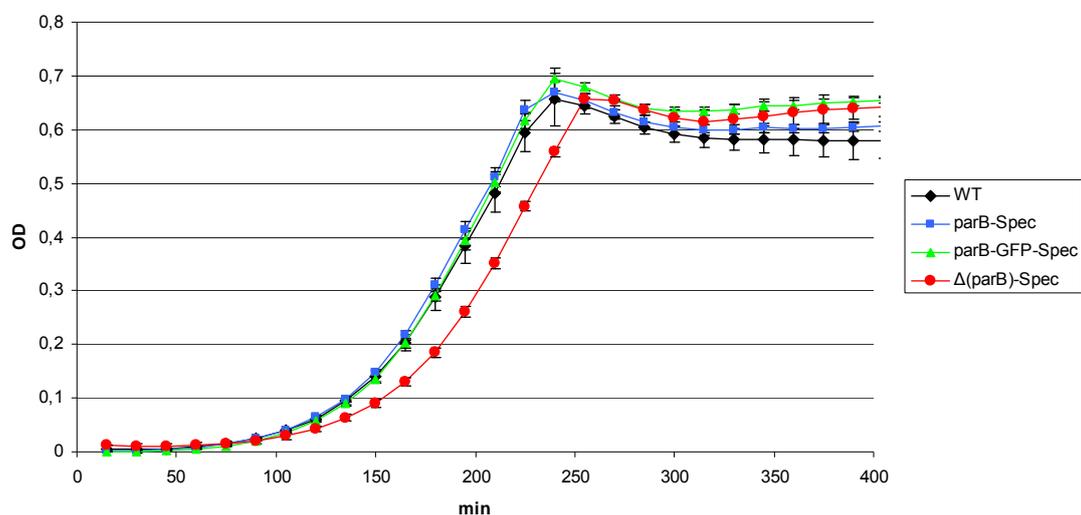
Supplementary information, Minnen *et al.* SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*.

0.05 mM for GFP-ScpB). Strain AM13 (GFP-ScpB) and AM15 (GFP-ScpB,  $\Delta parB$ ) were induced with 0.05 mM ZnSO<sub>4</sub> and grown at 37°C to prevent the formation of inclusion bodies (not shown). 1 ml of mid-exponential growing cells (OD<sub>600nm</sub> between 0.30 and 0.45) were washed and resuspended in 100  $\mu$ l of PBS (when applicable, 2  $\mu$ g/ml of DAPI (Invitrogen) was added) of which 0.4  $\mu$ l was subsequently spotted onto an agarose containing microscope slide.

Microscopy pictures were taken with a Deltavision (Applied Precision) IX71 Microscope (Olympus), using a CoolSNAP HQ2 camera (Princeton Instruments) and a 300W Xenon light source through a 100x oil immersion objective (phase contrast). For GFP signals, a Chroma filterset was used with excitation wavelengths between 450 and 490 nm and emission at 500 and 550 nm. For DAPI, excitation wavelengths were between 340 and 380 nm and emission between 432 and 482 nm. For phase contrast images, the exposure time was 0.1 sec with 32% APLLC White LED light, for GFP and DAPI the exposure time was between 1 and 2 sec with 100% Xenon light. Images were deconvolved using softWoRx 3.6.0 (Applied Precision) and modified for publication using ImageJ (<http://rsb.info.nih.gov/ij/>) and CorelDRAW X3 (Corel Corporation).

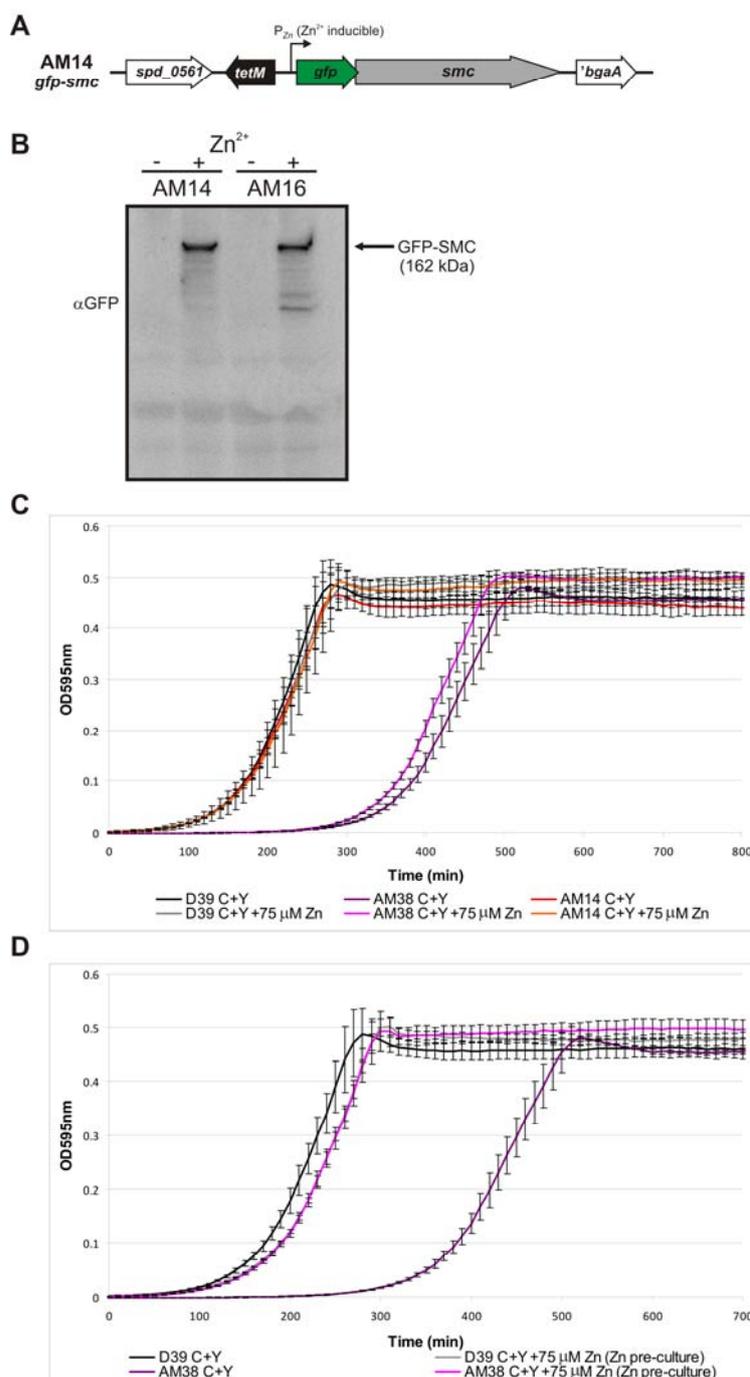
## Supporting Figures:

Minnen *et al*\_Figure\_S1



**Fig S1: The ParB-GFP fusion is functional.** The strains used in this study were D39 (WT, black diamond) and its derivatives: MT1 (*parB-spec*, blue square), MT2 (*parB-gfp-spec*, green triangle) and MT3 ( $\Delta$ (*parB*)-*spec*, red circle). For inoculation, stocks of bacteria grown in C+Y medium to an OD<sub>550nm</sub> of 0.4 were diluted 10-fold in C+Y medium (pH 6.8-7.0) and incubated at 37°C in a 96 wells plate, OD<sub>595nm</sub> measurements were taken every 15 minutes using a microtiterplate reader. Error bars indicate the standard deviation of the mean, calculated from three independent replicates. For improved visualisation of the lag phase, the Y-axis is in a linear scale.

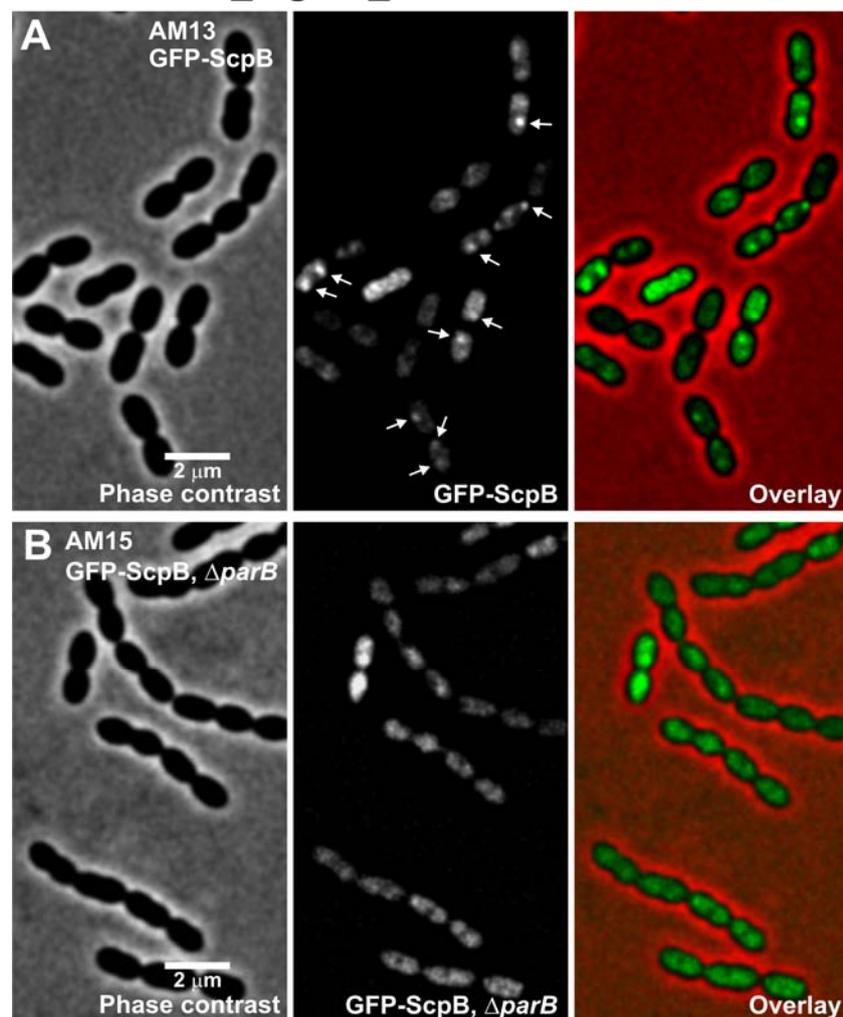
### Minnen *et al.*\_Figure\_S2



**Fig. S2. The GFP-SMC fusion. (A)** Schematic representation of the genetic layout of strain AM14 ( $P_{Zn}$ -*gfp-smc*). Strain AM14 was generated by a double crossover integration event of plasmid pAM6 into the *bgaA* locus of strain D39. **(B)** Western blot analysis: Exponentially growing cells of strains AM14 (GFP-SMC) and AM16 (GFP-SMC,  $\Delta parB$ ) were harvested for Western blot analysis using antibodies against GFP, after 1h of induction with 0.15 mM  $ZnSO_4$ . **(C-D)** Growth curves: The strains used in this study were D39 (WT; black / grey) and its derivatives: AM14 ( $P_{Zn}$ -*gfp-smc*; red / orange) and AM38 ( $\Delta smc::trmp$ ,  $P_{Zn}$ -*gfp-smc*; violet / pink). For inoculation, stocks of bacteria grown in C+Y medium, or in C+Y medium +75 $\mu$ M  $ZnSO_4$ , to an  $OD_{550}$  of 0.4 were diluted 100-fold in C+Y medium or in C+Y medium +75 $\mu$ M  $ZnSO_4$

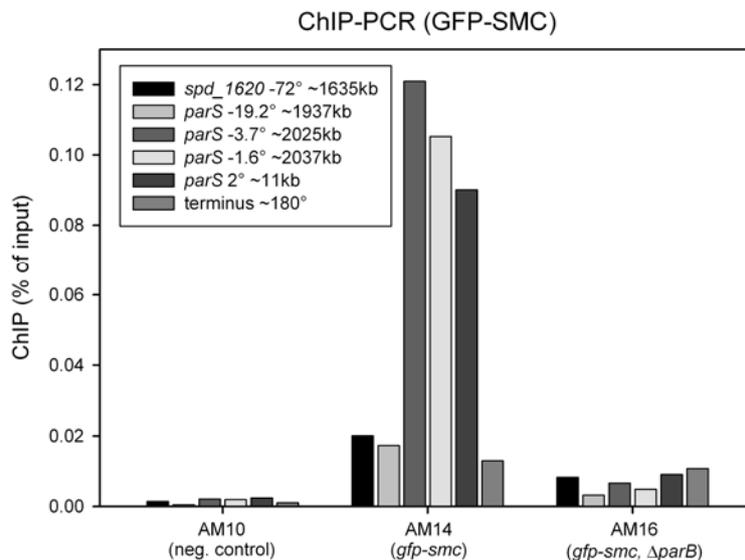
(pH 6.8-7.0) and incubated at 37°C in a 96 wells plate,  $OD_{595nm}$  measurements were taken every 10 minutes. Error bars indicate the standard deviation of the mean, calculated from six independent replicates. The difference between the C and D experiment is that cells for panel D were first precultured with  $ZnSO_4$  whereas this was not the case for cells of panel C. For improved visualisation of the lag phase, the Y-axis is in a linear scale.

### Minnen *et al.*\_Figure\_S3



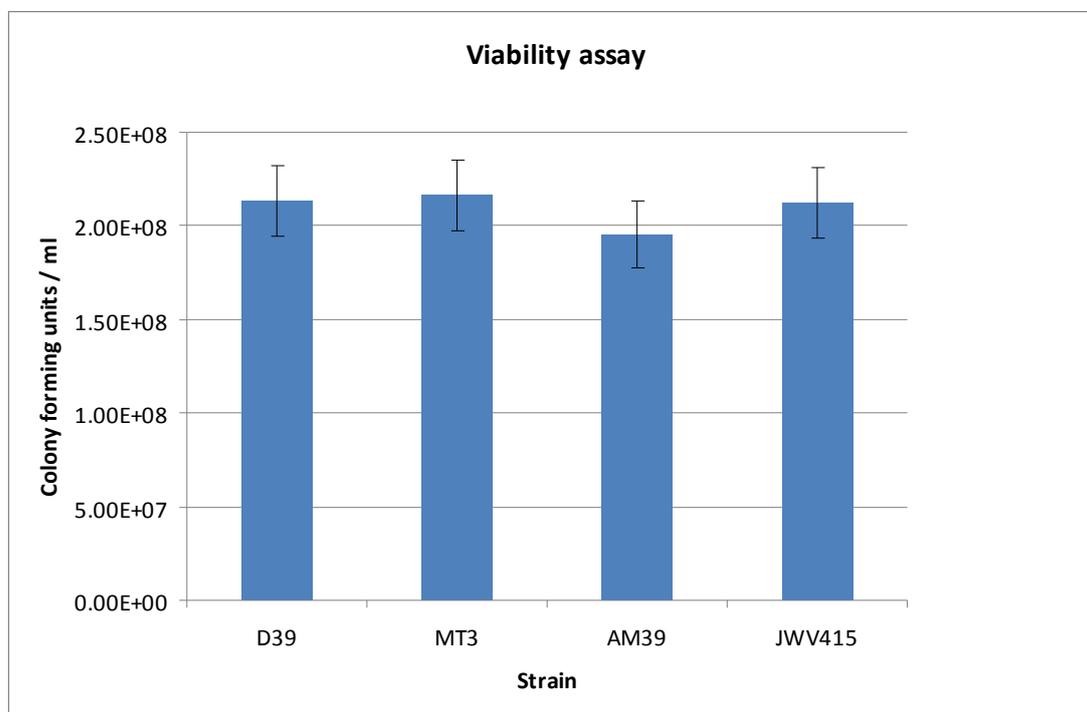
**Fig. S3. Localization of ScpB depends on ParB.** Cells were analyzed by fluorescence microscopy as described in the Experimental Procedures. Micrographs of strains AM13 ( $P_{Zn-gfp-scpB}$ ) (A) and AM15 ( $P_{Zn-gfp-scpB}, \Delta parB$ ) (B) are shown. Arrows indicate GFP-ScpB foci.

### Minnen *et al*\_Figure\_S4



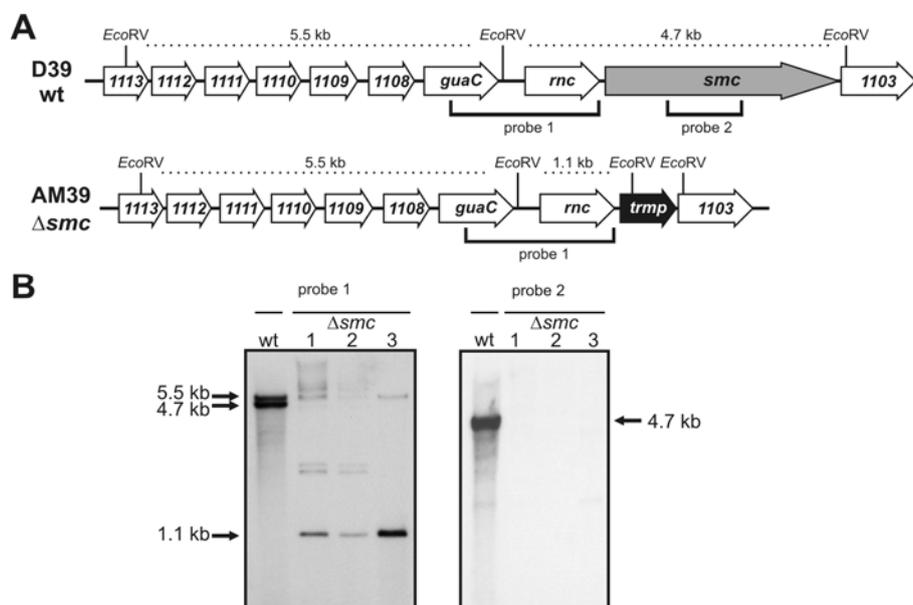
**Fig. S4. Enrichment of SMC at *oriC* depends on ParB.** Exponentially growing cells of strains AM10 ( $P_{Zn}$ -*gfp*), AM14 ( $P_{Zn}$ -*gfp-smc*) and AM16 ( $P_{Zn}$ -*gfp-smc*,  $\Delta$ *parB*) were collected for ChIP analysis using anti-GFP antibodies. Input and eluate DNA samples were analyzed by real-time PCR. Pull-down efficiency (ChIP-DNA/input-DNA\*100) is plotted for each primer pair. A typical outcome of a ChIP-PCR experiment is shown.

### Minnen *et al.*\_Figure\_S5



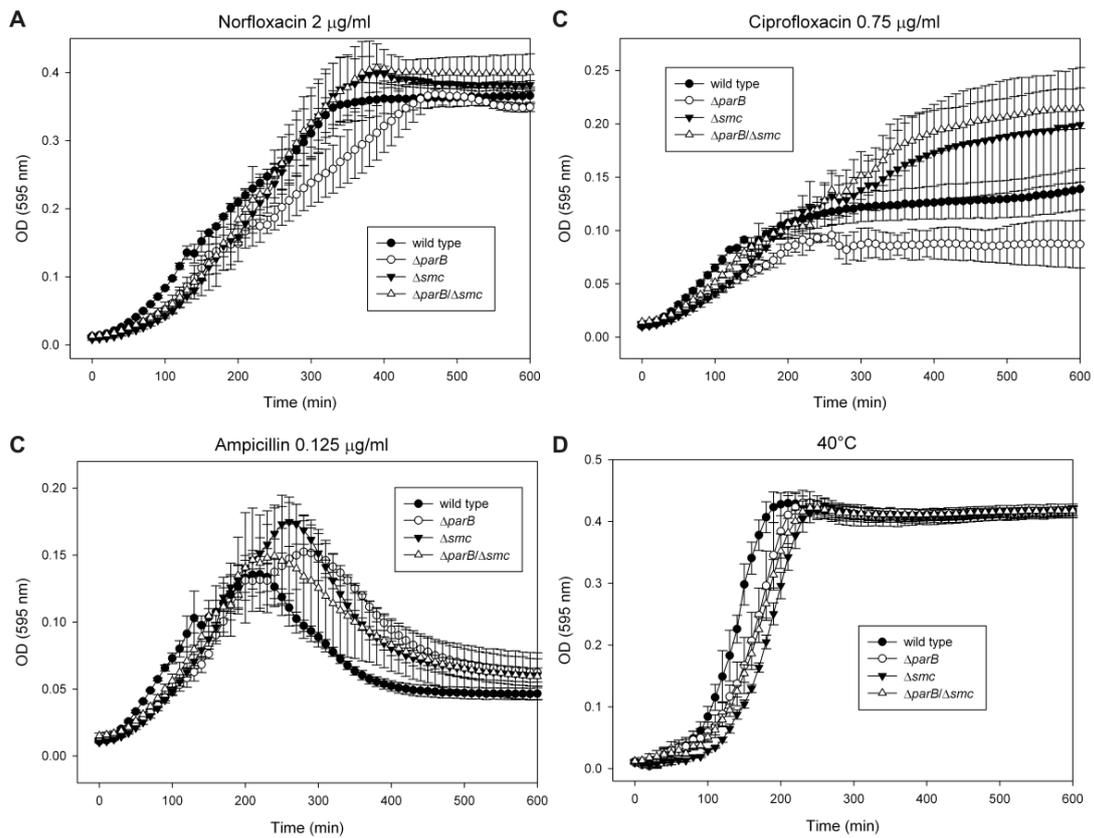
**Figure S5. Viability assay on the *parB* and *smc* mutants.** Cells of strains D39 (wild type), MT3 ( $\Delta(parB)$ -spec), AM39 ( $\Delta smc::trmp$ ) and JWV415 ( $\Delta(parB)$ -spec,  $\Delta smc::trmp$ ) were grown to an optical density (600nm) of 0.2 and plated in Columbia blood agar. After overnight incubation at 37°C, the number of colony forming units (CFU) were counted and plotted. Error bars indicate the standard deviation of the mean, calculated from three independent replicates.

### Minnen *et al.*\_Figure\_S6



**Fig. S6. Southern blot analysis verified the successful construction of a *smc* replacement mutant. (A)** Schematic representation of the *smc* locus in D39 and of strain AM39. SPD numbers are shown for genes lacking annotation. **(B)** Genomic DNA of the wild type (D39) and three individual transformants in which *smc* was successfully replaced by *trmp* as indicated by PCR was digested with EcoRV and hybridized using two separate probes as indicated in the figure. Each transformant lacked the *smc* gene (probe 2) and displayed the anticipated restriction pattern for probe 1.

Minnen *et al.*\_Figure\_S7



**Fig. S7. *parB* and *smc* mutants are not hypersensitive to antibiotics or heat stress.** Cells were grown in C+Y medium at 37°C or 40°C in microtiterplates in the presence of the indicated concentration of antibiotic and the OD600nm was measured every 10 minutes. Error bars show the standard deviation between at least 3 independent growth curves. For improved visualisation of the lag phase, the Y-axis is in a linear scale.

## References

Arai, R., Ueda, H., Kitayama, A., Kamiya, N., and Nagamune, T. 2001. Design of the linkers which effectively separate domains of a bifunctional fusion protein. *Protein Eng* **14**: 529-532.

Caymaris, S., Bootsma, H.J., Martin, B., Hermans, P.W., Prudhomme, M., and Claverys, J.P. 2010. The global nutritional regulator CodY is an essential protein in the human pathogen *Streptococcus pneumoniae*. *Molecular microbiology* **78**: 344-360.

Eberhardt, A., Wu, L.J., Errington, J., Vollmer, W., and Veening, J.W. 2009. Cellular localization of choline-utilization proteins in *Streptococcus pneumoniae* using novel fluorescent reporter systems. *Mol. Microbiol.* **74**: 395-408.

Hendriksen, W.T., Bootsma, H.J., Estevao, S., Hoogenboezem, T., de Jong, A., de Groot, R., Kuipers, O.P., and Hermans, P.W. 2008. CodY of *Streptococcus pneumoniae*: link between nutritional gene regulation and colonization. *J. Bacteriol.* **190**: 590-601.

Leenhouts, K., Buist, G., Bolhuis, A., ten Berge, A., Kiel, J., Mierau, I., Dabrowska, M., Venema, G., and Kok, J. 1996. A general system for generating unlabelled gene replacements in bacterial chromosomes. *Mol Gen. Genet.* **253**: 217-224.

Martin, B., Garcia, P., Castanie, M.P., and Claverys, J.P. 1995. The *recA* gene of *Streptococcus pneumoniae* is part of a competence-induced operon and controls lysogenic induction. *Molecular microbiology* **15**: 367-379.

Terzaghi, B.E. and Sandine, W.E. 1975. Improved medium for lactic streptococci and their bacteriophages. *Appl. Microbiol* **29**: 807-813.

Supplementary information, Minnen *et al.* SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*.

**Table S1. Oligonucleotides<sup>a</sup>**

Primer	Sequence (5' to 3')	Restriction site
1 parB-up-F	TGCCATCGGTAGCCCGTTAG	
2 parB-up-R+Ascl	CGTAT <b>GGCGCGCC</b> CGAAAAACATAGTGGATAACCTT GTGAGA	Ascl
3 parB-down-F+NotI	GCTAA <b>GCGGCCGC</b> TCTCACAAGGTTATCCACTATGT TTTTCG	NotI
4 parB-down-R	TTGGCATGGGCATGAATGAC	
5 parB-F-down+EcoRI	GCGC <b>GAATTC</b> GCCTGAAATAAGGCTGTTCTTTTA	EcoRI
6 parB-R+linker+BamHI	GCTC <b>GGATCC</b> TTTAGCTGCAGCTTCTCCACCAGATC CTCTAGATTTCAAGGCTGTTGATAATTCTACTATATTC	BamHI
7 parB-R+EcoRI-KO	GCGC <b>GAATTC</b> GATTCTACACTAACACATCTTTTCTCT TATGTAAAGC	EcoRI
8 parB-check-F	CAGCGAATCTCTAGTGAAGG	
9 parB-check-R	ACCTTCGTTGGTCACGTCAA	
10 smc-up-F	ACAACACCCTCGTCTGAAAG	
11 smc-up-R+NotI	CGATT <b>GCGGCCGC</b> TTATTCTAAGCGACCATCTTGAC GATAGG	NotI
12 smc-down-F+Ascl	GCATA <b>GGCGCGCC</b> AAAAATTTCTGCAAGTTTATGC CTTGCAGG	Ascl
13 smc-down-R	CAGGTCTTGGTGTGCTATG	
14 smc_check-F	TCGGCATGGTATTCCAACAC	
15 smc_check-R	CTTGATGCCATCTCAGAAGG	
16 smc-up-F-KO	TGGTGCAGATGCTACTAAGG	
17 smc-R+KpnI-KO	GCGC <b>GGTACC</b> TAAATACATGAATCCAGCCCTTTCT C	KpnI
18 Smc-down-F+KpnI	GCGC <b>GGTACC</b> AGATTTAGAAAGTATTGAAGGATGAC	KpnI
19 smc-check-F-KO	AGCCGTGCAGAAGCGGATAC	
20 smc-UP-R+BamHI	GCTC <b>GGATCC</b> ATCCTTCAATACTTTCTAAATCTTTTA ACTT	BamHI
21 smc-GFP-UP-F	GAATCAACAGGAGGCACAAG	
22 smc-GFP-check-F	GTCCGTCTGCGTGATATTCATGACCTCTTC	
38 spec-F-Ascl	GCATA <b>GGCGCGCC</b> CTAATCAAATAGTGAGGAGGAT AT	Ascl
39 spec-R-NotI	CGATT <b>GCGGCCGC</b> ACTAAACGAAATAAACGCTAAAA CG	NotI
44 trmp-F+Ascl	GCAT <b>GGCGCGCC</b> GGATTTTTGTGAGCTTGGA	Ascl
45 trmp-R+NotI	GCAT <b>GCGGCCGC</b> GTTACGACGCGCATAGACGG	NotI
46 gfp_sf-F+BamHI	CGAC <b>GGATCC</b> TCAAAGGAGAAGAAGACTTTTTACAGG TG	BamHI
47 gfp_sf-R+EcoRI	GCGC <b>GAATTC</b> GCTCATTATTATTTATAAAGTTCGTCC	EcoRI
60 trmp F seq	ACGGCGTCTTTCTATCTGAG	
63 smc-F+XbaI pJWV025	GCGC <b>TCTAGA</b> TATTTAAAGGAAATCGAAATTCAGGG G	XbaI
64 smc-R+NotI pJWV025	GCATT <b>GCGGCCGC</b> TTATCATCCTTCAATACTTTCTAA ATCTTTTAAC	NotI
69 scpB-F+SpeI	GCGC <b>ACTAGTAGT</b> ACTTTAGCAAAAATAGAAGCGC	SpeI
70 scpB-R+NotI	GCATT <b>GCGGCCGC</b> TTATTATTGATTCTCATCTTCTTC TATCC	NotI
71 gfp-F seq pJWV25	GGGATTACACATGGCATGGATGAGC	
83 trmp-F+KpnI	GCGC <b>GGTACC</b> AGGTAAAAATATTCGGAGGAATTTT CATATGAACCCG	KpnI
84 trmp-R+KpnI	GCGC <b>GGTACC</b> CGTTACGACGCGCATAGACGG	KpnI
88 smc in seq F	TTGCCAAGGAGCAAGAGGAG	
89 smc in seq2 F	TCGCTGTAAGCCCAGGTTTC	
90 smc in seq R	AAGCGTTCCTTGACCTCATC	
91 smc in seq2 R	GCGAGCTTGATCCAAGTGAC	
93 parS 11357 Sp forward	CGAAAAACAGGAGATGTTTTGA	
94 parS 11357 Sp reverse	TCGCAATTCCCAAATTGA	

Supplementary information, Minnen *et al.* SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*.

95 parS 2025200 Sp forward	CTTCAACAGTCGCTCCAACA
96 parS 2025200 Sp reverse	AGCAGAATCCGGAGAGATTG
97 parS 2036431 Sp forward	CAGACACATGGGGAAAAATTG
98 parS 2036431 Sp reverse	GATGGCCCCATACTTTGTTG
99 parS 1936956 Sp forward	TATTGTTCTGGCGACGTTCC
100 parS 1936956 Sp reverse	GTGATGAATGGCGTTTTGAA
101 ter Sp forward	TTTTCAGCTGGGTTTTGGAG
102 ter Sp reverse	ATTCAGCAAGGGACGTTTAC
103 pPP2-R	CGAAATACGGGCAGACATGG
112 BgaA_down check R	AGCTAGAGTTCCGCAATTGG
113 TetM-check F	CATATGTCCTGGCGTGTCTATG
114 SPD_1620 F	AAATTTTCATCCGACGTGTCC
115 SPD_1620 R	AAGGTTGCCATCATTTCAGG
116 ter Sp forward 1056kb	GAAAAGTACCATCCCCAGCA
117 ter Sp reverse 1056kb	AGCCTTGGTGCCTATCATTG
118 pepS forward 250kb	CCTTTGGGACCAAATCTTCA
119 pepS reverse 250kb	TAAGCATATCGGCCTTGCTC
120 valS forward 500kb	GGATGGAAAATGTCCACGAC
121 valS reverse 500kb	AGTCCATCCGTCACCTTCTG
122 deoC forward 750kb	CTTTGCCAGTGTTCGCTTA
123 deoC reverse 750kb	TTTCACGGCTGAAGTTGTTG
124 ter Sp forward 1030kb rexB	AAATGGCTGAACCTGTCCAA
125 ter Sp reverse 1030kb rexB	TCCAAGAAGAGCCCTTGGTA
126 def forward 1250kb	CAAGATCCTGTCATGGCTGA
127 def reverse 1250kb	TCCAAATCGTAGGCTTCCTG
128 sun forward 1500kb	TGGGAGAGAATAGGCAATGG
129 sun reverse 1500kb	ATTGGTCAACGCTGTCCTTC
130 wrbA forward 1750kb	TTGCTGAGAGTAACGGCTCTG
131 wrbA reverse 1750kb	CATCAACTGAGCCTCTGCAA
132 parS 1936956 Sp forward	TCCATGAAAATGCATGAGC
133 parS 1936956 Sp reverse	AGCTTGGATCATTTCGTC
134 ter Sp forward 1048kb	AAACAATCAGGACAATCAAATCAA
135 ter Sp reverse	AAGGAGCTATGAGATACAAAACACA
136 comCDE promoter reverse	GAAGGCGAATGCTCTATCCA
137 comCDE promoter forward	TATCGCGCTTTTACGATTCA
138 parB prote forward	ACGGTCTATCCCAGCTGTTG
139 parB prote reverse	ATAGGCGCGTGCTTCTTCTA
140 comE end reverse	CCAAAAATGACTTGTGAGGAGA
141 comE end forward	TTGAAACAACAGGGGTATCTCA
142 rplB forward	TTCTGCAAACATCGCTCTTG
143 rplB reverse	AAGAGCGTTTCCGACTTTGA
144 rpoB forward	TTCACGGACATTACGTTCCA
145 rpoB reverse	TTCCTCAACTGGCTGAAGG
146 ftsH near rrlA forward	ACGACTTTGAACAAGCGACA
147 ftsH near rrlA reverse	CTCTGTGCACCAAGCATAGC
148 CDS1192	TTAAACTAGGCGCTGGAGGA
149 CDS1192 reverse	ATAGCAATGCCAGCCTGTTC

---

<sup>a</sup>Relevant restriction sites are underlined.