Supporting information – Misiura et al.,

"Roles of two large serine recombinases in mobilizing the methicillin-resistance cassette SCC*mec*"

Primer	Sequence	Description
CcrA-923-F	CACCATGAAACAAGTCATAGGCTAT	Clone *ccrA* gene from 923 (USA300) for overexpression in *E. coli* in pET101/D-TOPO
CcrA-923-R	TTATGCAATCGATGATTGCATG	Clone *ccrA* gene from 923 (USA300) for overexpression in *E. coli* in pET101/D-TOPO
CcrB-923-F	CACCATGCAACAACTTAAAACAAAACGTG	Clone *ccrB* gene from 923 (USA300) for overexpression in *E. coli* in pET101/D-TOPO
CcrB-923-R	CTAAATAGTAAGATATAGTGTTGG	Clone *ccrB* gene from 923 (USA300) for overexpression in *E. coli* in pET101/D-TOPO
attB top	CTAGTACAGAGCATTTAAGATTATGCGAGGAGAGGCGT ATCATAAGTAAAACTAAAAAATTCTGTA	Synthetic oligo to clone attB site into pMS183
attB bottom	CATGTACAGAATTTTTTAGTTTTACTTATGATACGCCT CTCCTCGCATAATCTTAAATGCTCTGTA	Synthetic oligo to clone attB site into pMS183
attSCC top	GATCTTAATTTTTCAAAAACCGCATCATTAACTGATAA GCAGAAGCTTATCATAAATGATGCGGTTTTTTCA	Synthetic oligo to clone attSCC site into pMS183
attSCC bottom	GTACTGAAAAAACCGCATCATTTATGATAAGCTTCTGC TTATCAGTTAATGATGCGGTTTTTGAAAAATTAA	Synthetic oligo to clone attSCC site into pMS183
attL top	CTAGTACAGAGCATTTAAGATTATGCGAGGAGAAGCTT ATCATAAATGATGCGGTTTTTTCA	Synthetic oligo to clone attL site into pMS183
attL bottom	CATGTGAAAAAACCGCATCATTTATGATAAGCTTCTCC TCGCATAATCTTAAATGCTCTGTA	Synthetic oligo to clone attL site into pMS183
attR top	GATCTTAATTTTTCAAAAACCGCATCATTAACTGATAA GCAGAGGCGTATCATAAGTAAAACTAAAAAATTCTGTA	Synthetic oligo to clone attR site into pMS183
attR bottom	GTACTACAGAATTTTTTAGTTTTACTTATGATACGCCT CTGCTTATCAGTTAATGATGCGGTTTTTGAAAAATTAA	Synthetic oligo to clone attR site into pMS183
attB to attL forward	GATTATGCGAGGAGAAGCTTATCATAAATGATGCGGTT TTTTCACATGGCGGTGAACAG	QuikChange primer to exchange half site to transform attB into attL
attB to attL reverse	CTGTTCACCGCCATGTGAAAAAACCGCATCATTTATGA TAAGCTTCTCCTCGCATAATC	QuikChange primer to exchange half site to transform attB into attL
attSCC to attR forward	AACTGATAAGCAGAGGCGTATCATAAGTAAAACTAAAA AATTCTGTAGTACATTGCATGC	QuikChange primer to exchange half site to transform attSCC into attR
attSCC to attR reverse	GCATGCAATGTACTACAGAATTTTTTAGTTTTACTTAT GATACGCCTCTGCTTATCAGTT	QuikChange primer to exchange half site to transform attSCC into attR
attB noA tract froward	GCGTATCATAAGTATCTGTACATGGCGG	QuikChange primer to delete the A-tract from attB right half site
attB noA tract reverse	CCGCCATGTACAGATACTTATGATACGC	QuikChange primer to delete the A-tract from attB right half site
Inverted attSCC top	CTAGTGAAAAAACCGCATCATTTATGATAAGCTTCTGC TTATCAGTTAATGATGCGGTTTTTGAAAAATTAACATG	Synthetic oligo to clone inversely oriented attSCC site into pMS183
Inverted attSCC bot	TTAATTTTTCAAAAACCGCATCATTAACTGATAAGCAG AAGCTTATCATAAATGATGCGGTTTTTTCA	Synthetic oligo to clone inversely oriented attSCC site into pMS183

Table S1. Primers used in this work.

For QuikChange mutagenesis a set of 3 primers are listed as examples. The others are available on request.

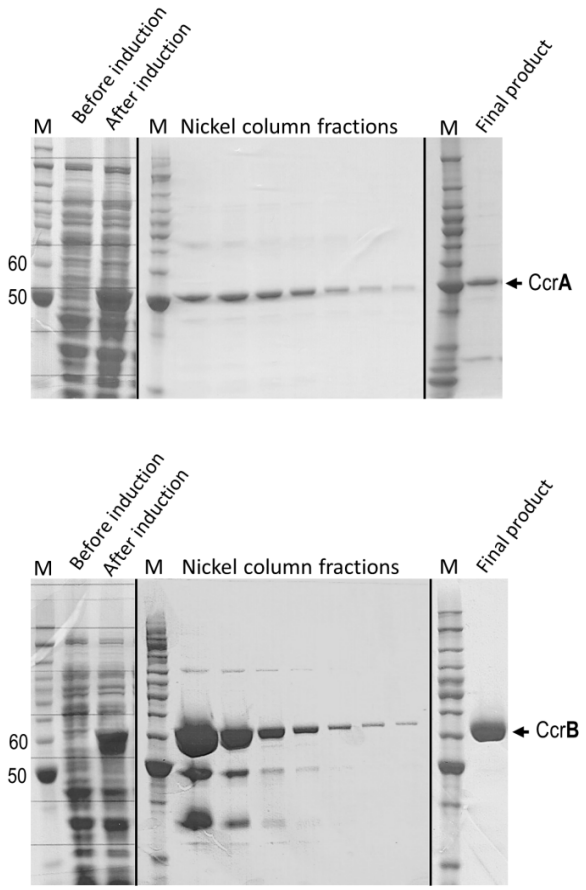

Figure S1. Expression and purification of CcrA and CcrB proteins.

SDS-PAGE gel of CcrA (top panel) and CcrB (bottom panel). Lanes labeled (M) are the molecular weight markers (BenchMark Protein Ladder, Invitrogen). The two following lanes show uninduced and induced *Rosetta (DE3)pLysS* cells in which the Ccr proteins were expressed. C-terminally His_6-tagged CcrA and CcrB were initially isolated via affinity chromatography (Nickel column fractions). For CcrA, the cleanest fractions were pooled, re-run on the same column, then pooled again (rightmost lane). For CcrB, fractions showing multiple bands underwent additional purifications steps including Heparin and MonoS columns. A final sample is shown in the rightmost lane. As CcrA was poorly soluble, its further concentration was not possible and its storage required 1 M NaCl. In contrast, CcrB is soluble and can be easily concentrated.

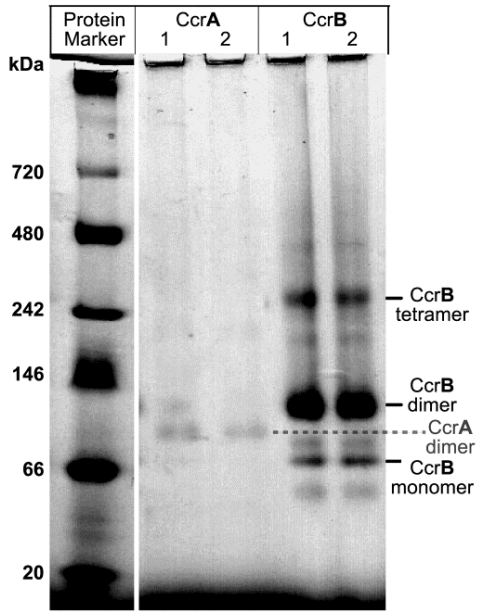

Figure S2. "Blue" Native Protein PAGE.

NativePAGE Novex Bis-Tris Gel System (Invitrogen) showing oligomerization state of CcrA and CcrB proteins. In such gels native proteins run according to their molecular weights. Purified His_6-tagged CcrA and CcrB were prepared with NativePAGE Sample Buffer (4x) (Invitrogen) according to the manufacturer's protocol and kept on ice for 20 min before loading on the gel. Optional NativePAGE 5% G-250 Sample Additive (Invitrogen) was added to a final concentration of 0.5% in sample lanes numbered 2 immediately prior to electrophoresis (no NativePAGE G-250 Sample Additive was present in sample lanes 1). Native-Mark Unstained Protein Standard (Invitrogen) was used as a molecular weight standard. The anode and dark cathode buffers were prepared as described in the user manual for NativePAGE System (Invitrogen). 20 µl protein samples, corresponding to about *7* µg of total protein, were loaded on a precast gradient 4-16% NativePAGE Novex Bis-Tris gel (Invitrogen). Due to CcrA's insolubility the prepared sample had more salt than recommended by the manufacturer protocol (500mM instead of recommended 50mM), which caused poor staining of the CcrA bands. Electrophoresis was performed in a cold room (4° C) at a constant voltage of 100 V for the first hour after which the voltage was set to 250 V (15 mA limiting). Electrophoresis was stopped when the G-250 dye front reached bottom of the gel. Once electrophoresis was completed, the staining was carried using the protocol from Invitrogen. Briefly, the gel was placed in fix solution (40% methanol, 10% acetic acid) and microwaved for 45 seconds on high (950-1100 watts). Next, the gel was gently shaken for 15 min, fix solution was discarded and the gel was placed in destain solution (8% acetic acid), microwaved again. The gel was shaken in destain solution until the desired background was obtained. Lastly, the gel was scanned using HP Photosmart C4480 scanner.

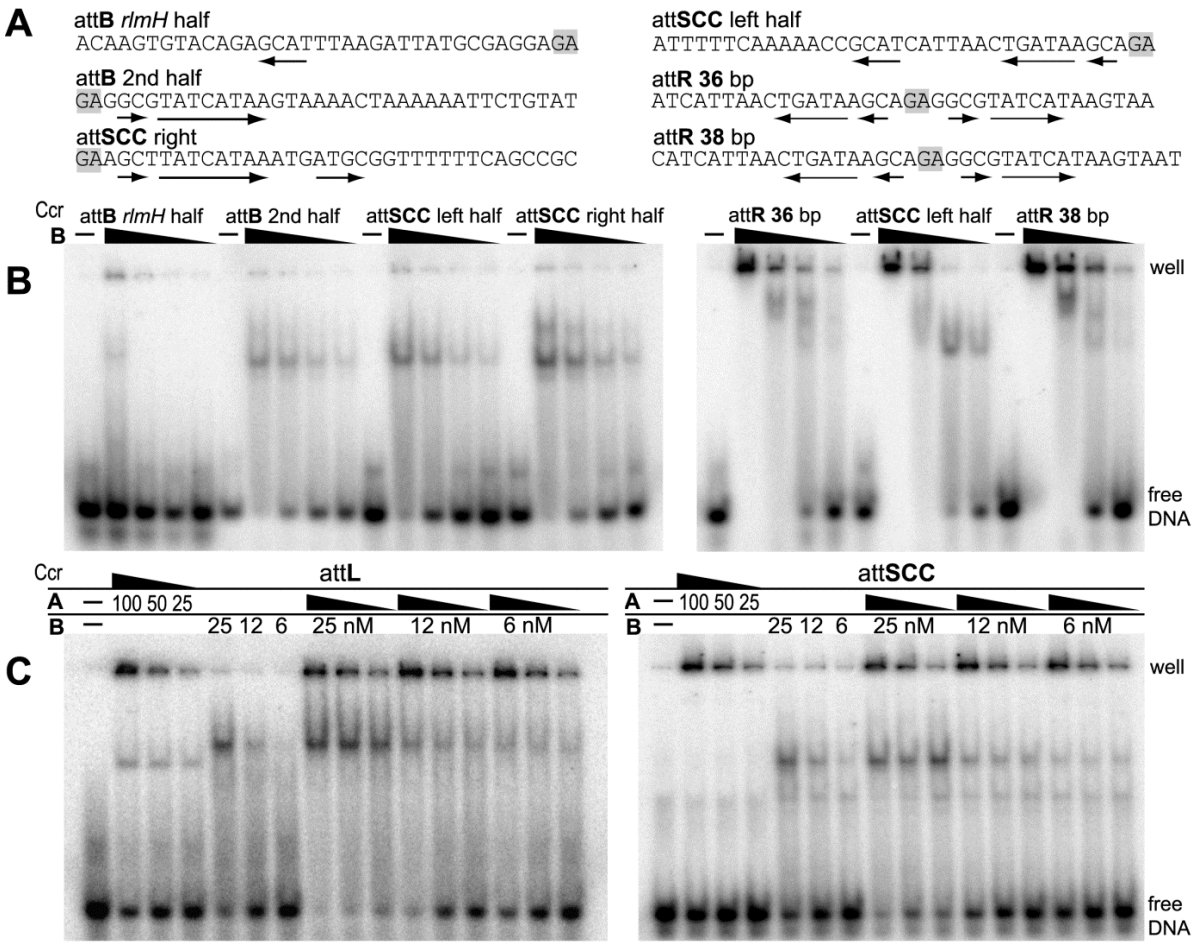

Figure S3. Ccr proteins binding to their cognate attachment sites, related to Figure 2.

(A) Sequences of the duplexes tested. The four half sites that constitute all cognate attachment sites were carried on 36 bp duplexes with the central dinucleotide GA (grey box) at one end. 36 bp and 38 bp probes containing the center of the full attR site were also used for comparison. Horizontal arrows represent conserved inverted repeats.

(B) Binding of CcrB to all four half-sites and short attR sites. Left gel: CcrB (500, 250, 125, 63 nM) binds with roughly equal strength to the three half sites of similar sequence but weakly to the RlmH half site. Right gel: Comparison of CcrB (2 μM, 1 μM, 500 nM, 250 nM) binding to same-size duplexes containing half vs. full sites. Complexes of CcrB formed with attR site run predominantly slower than complexes of CcrB with the half site suggesting that a higher oligomerization state of CcrB is bound to the attR site: presumably a dimer on attR and a monomer on the half site. For both experiments, radiolabeled DNA duplexes were incubated for 20 min with titrations of CcrB and run on a 5% nondenaturing polyacrylamide gel.

(C) CcrA does not inhibit DNA binding by CcrB to attL (left gel) or attSCC (right gel). First three lanes: titration of CcrA alone (100, 50, 25 nM). Next, three lanes: titration of CcrB alone (25, 12, 6 nM). The following lanes contain both CcrA and CcrB at all combinations of the above concentrations as indicated above the lanes. CcrA was pre-incubated with the DNA for 10 minutes, then CcrB was added and the samples were incubated an additional 10 minutes. DNA probes used here were the same as in Fig. 2 for attL and attSCC sites.

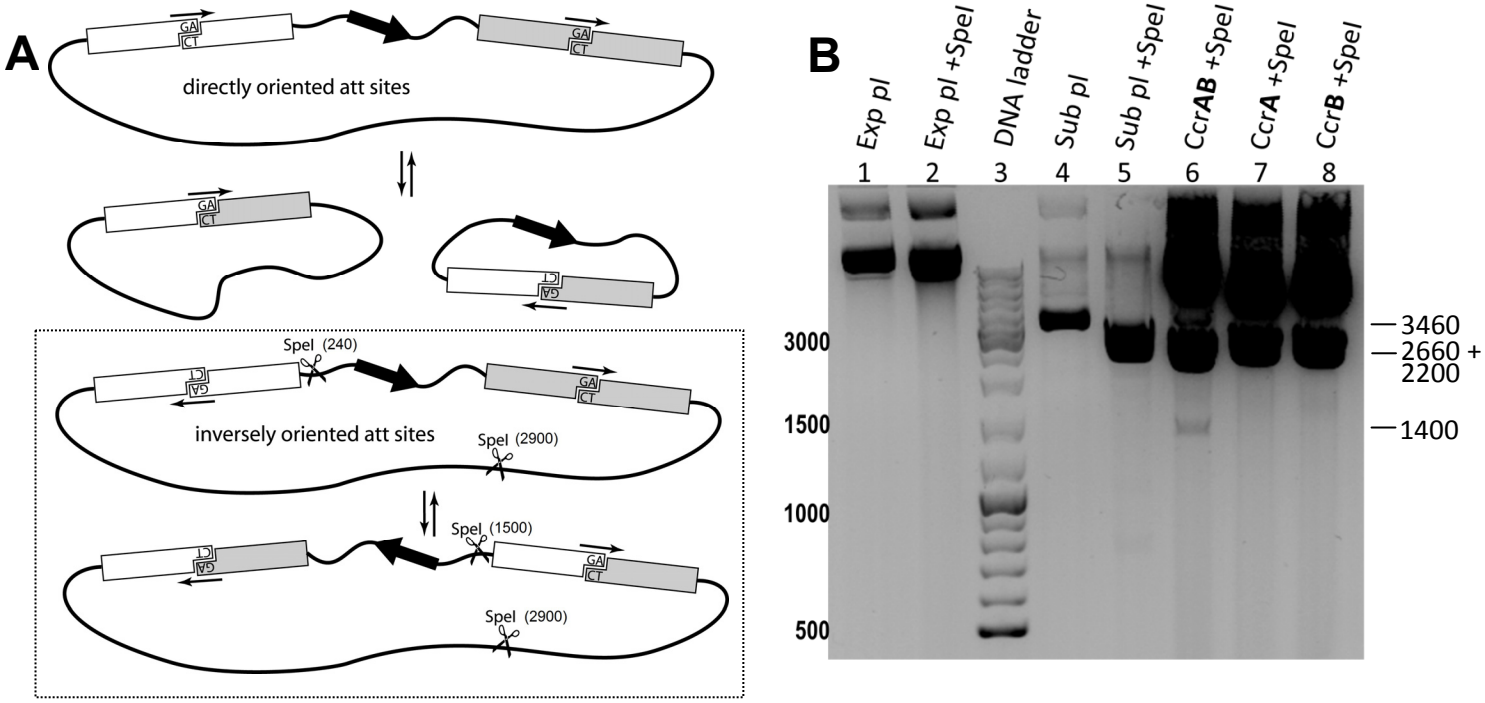

Figure S4. *In vivo* inversion by CcrA and CcrB, related to Figure 3.

(A) Scheme for assaying inversion. Top cartoon: When two att site are in direct repeat (with respect to the central dinucleotides), as they were for the substrates in Fig 3, recombination deletes the intervening segment. Bottom cartoon: When one of the sites is flipped over, recombination inverts the intervening DNA segment. Inversion can be detected by SpeI digestion (the relative positions of the SpeI sites are shown). Note that the Ccr-expressing plasmids contain no SpeI sites.

(B) Restriction enzyme analysis of CcrAB's activity on inversely oriented attB and attSCC sites. To generate a test plasmid with oppositely oriented attB and attSCC sites, the substrate with directly oriented attB and attSCC sites was treated with NheI and SphI enzymes to cut out attSCC, then synthetic oligos carrying the inverted attSCC sequence and appropriate overhangs (see Table 1) were ligated in. The changes were confirmed by sequencing. The new substrate plasmid was transformed into *E.coli* strain DS941, after which a Ccr-encoding plasmid was introduced. After 48 h liquid culture incubation at 37°C the total plasmid DNA was extracted. The purified DNA was digested with SpeI, which should yield two fragments of 2660 bp and 2200 bp before inversion and two fragments of 1400 bp and 3460 bp after inversion. The total plasmid DNA was run on an agarose gel and stained with ethidium bromide. The lowest band of 1400 bp size was readily visible, however the higher molecular weight bands were harder to separate and overlap with one another.

Lane 1:Expression plasmid,
lane 2: Expression plasmid treated with SpeI,
lane 3: DNA ladder,
lane 4: untreated substrate plasmid containing inversely oriented attB and attSCC sites,
lane 5: the substrate plasmid treated with SpeI,
lane 6: SpeI- digested total plasmid from cells co-expressing CcrA and CcrB,
lane 7: SpeI- digested total plasmid from cells expressing CcrA alone,
lane 8: SpeI- digested total plasmid from cells expressing CcrB alone.

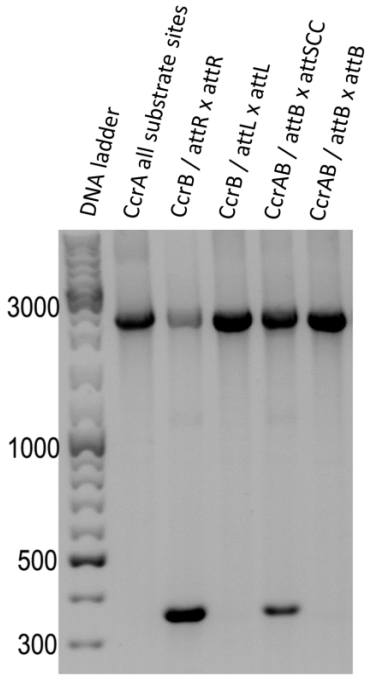

Figure S5. PCR analysis of total plasmid DNA extracted from *in vivo* reactions from Figure 3. The forward and reverse primers were designed to amplify the intervening sequence between the att sites on the substrate plasmids starting ~ 150 bp from each att site outside the reporter gene. If recombination deletes the intervening *galK* gene a PCR product of ~300 bp is expected; otherwise a PCR product of ~2000 bp is expected. Lane 1: DNA ladder, lane 2: combined total DNA from all reactions with CcrA alone, lane 3: total DNA from a reaction with CcrB and substrate plasmid containing two attR sites, lane 4: total DNA from a reaction with CcrB and substrate plasmid containing two attL sites, lane 5: total DNA from a reaction with CcrAB and substrate plasmid containing attB and attSCC sites, lane 6: total DNA from a reaction with CcrAB and substrate plasmid containing two attB sites. The recombination pattern agrees with that in Figure 3.