Sister chromatid interactions in bacteria revealed by a site-specific recombination assay

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Supplementary material

Figure S1. Flow cytometry analysis of the cell cycle synchronization

Cells were grown under slow growth conditions (minimal medium A, casamino acids, 0.2% succinate), where *E. coli* undergoes a eukaryote-like cell cycle consisting of a G1 like-phase (called B period), followed by the S like-phase of replication (C period) and finally the post-replication G2 like-phase (D period). Flow cytometry analysis of the MG1655*dnaC2* strain containing the *Laclox* tool at the Ori-3 locus during the cell cycle synchronization process. The flow cytometry was performed on aliquots harvested and fixed before each Cre induction pulse. The DNA content was followed by propidium iodide fluorescence.

Figure S2. SCI can be quantified according to the frequency of Lac+ colonies formed on X-gal plates.

A) Measure of the frequency of Lac+ colonies for the Ori-3 locus with the intramolecular cassette (intra SC). Histograms present the frequencies of *Laclox* recombination obtained following a 5 or 10 min Cre induction. After the induction, colonies were diluted in LB and kept in liquid culture with shaking for indicated delay time before plating **B)** Measure of the frequency of SCI by Lac+ colonies counting (inter SC). Histograms present the frequencies of *Laclox* recombination obtained for two *Laclox* insertions at Ori-1 and Ter-1 loci following a 10 or 20 min Cre induction. After the induction, colonies were diluted in LB and kept in liquid culture with shaking for indicated delay time before plating before plating (ON, overnight).**C)** Southern blot analysis of the recombination products observed for the Ori-3 locus in the same induction conditions than on Figure 2C. Genomic DNA was extracted immediately after induction or after a 60 min of dilution. The amount of recombined products (% of 1 + 3 *loxP*) was quantified with a Typhoon scanner. **D)** Cre recombination follows single exponential decay kinetics for the Ori_{SNAP} and Ter_{dif} inter and the intra molecular recombination reactions. The data were plotted according to the disappearing of Lac- colonies versus the length of the induction. Single exponential fits are represented. To avoid taking into consideration the lag observed during the first 5 minutes, the fits exclude the time 0 and 2.5 min of induction.

Figure S3. Absolute recombination rate observed in the wt and *matP* strain

Measurement of the frequency of SCI (inter-SC) and intramolecular recombination (intra-SC) according to the extent of Cre induction in the wt and the *matP* strains for the Ori-1, Ori-3, Ter-1, Ter-6 and Ter_{dif} loci.. The histograms represent the average of 3 experiments. The induction of Cre recombinase is mediated by arabinose addition for the indicated time. NI (not induced).

Figure S4. Topo-IV influences SCI and colocalization of the sister chromatids.

A) Absolute recombination rate observed in the wt and *parC1215* strains for the Ori-1, Ori-3, Ter-1, Ter-6 and Ter_{dif} loci. The histograms represent the average of 3 experiments. The induction of Cre recombinase is mediated by arabinose addition for the indicated time. The experiments were performed at 30°C or after a 20 min shift to 42°C. NI (not induced).

B) The inactivation of Topo-IV provokes a reduction in the number of MG1655*parC1215* cells segregating the sister Ter-1 *parS*/ParBP1 foci. A representative picture of the Ter-1 *parS*/ParBP1-GFP tag in the MG1655 and MG1655*parC1215* cells after a 60 min shift at 42°C in the absence or presence of cephalexin to inhibit cell division.

Figure S5. Recombination rates in the *gyrBts* and *mukB* strains.

A) Absolute recombination rate observed in the *mukB* strain. Absolute recombination rate observed in the wt and *mukB* strain. The histograms represent the average of 3 experiments. The induction of Cre recombinase is mediated by arabinose addition for the indicated time.

The experiments were performed at 22°C. NI (not induced).

B) Absolute recombination rate observed in the *gyrBts* strain. Absolute recombination rate observed in the wt and *gyrB*^{203} strain. The histograms represent the average of 3 experiments. The induction of Cre recombinase is mediated by arabinose addition for the indicated time. The experiments were performed at 30°C or after a 20 min shift to 42°C. NI (not induced).

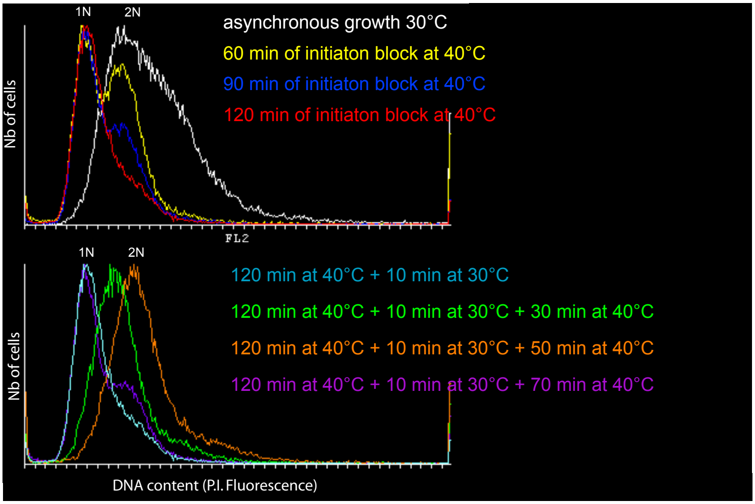

1N
2N
asynchronous growth 30°C
60 min of initiaton block at 40°C
90 min of initiaton block at 40°C
120 min of initiaton block at 40°C
Nb of cells
FL2
1N
2N
120 min at 40°C + 10 min at 30°C
120 min at 40°C + 10 min at 30°C + 30 min at 40°C
120 min at 40°C + 10 min at 30°C + 50 min at 40°C
120 min at 40°C + 10 min at 30°C + 70 min at 40°C
Nb of cells
DNA content (P.I. Fluorescence)

Figure S1

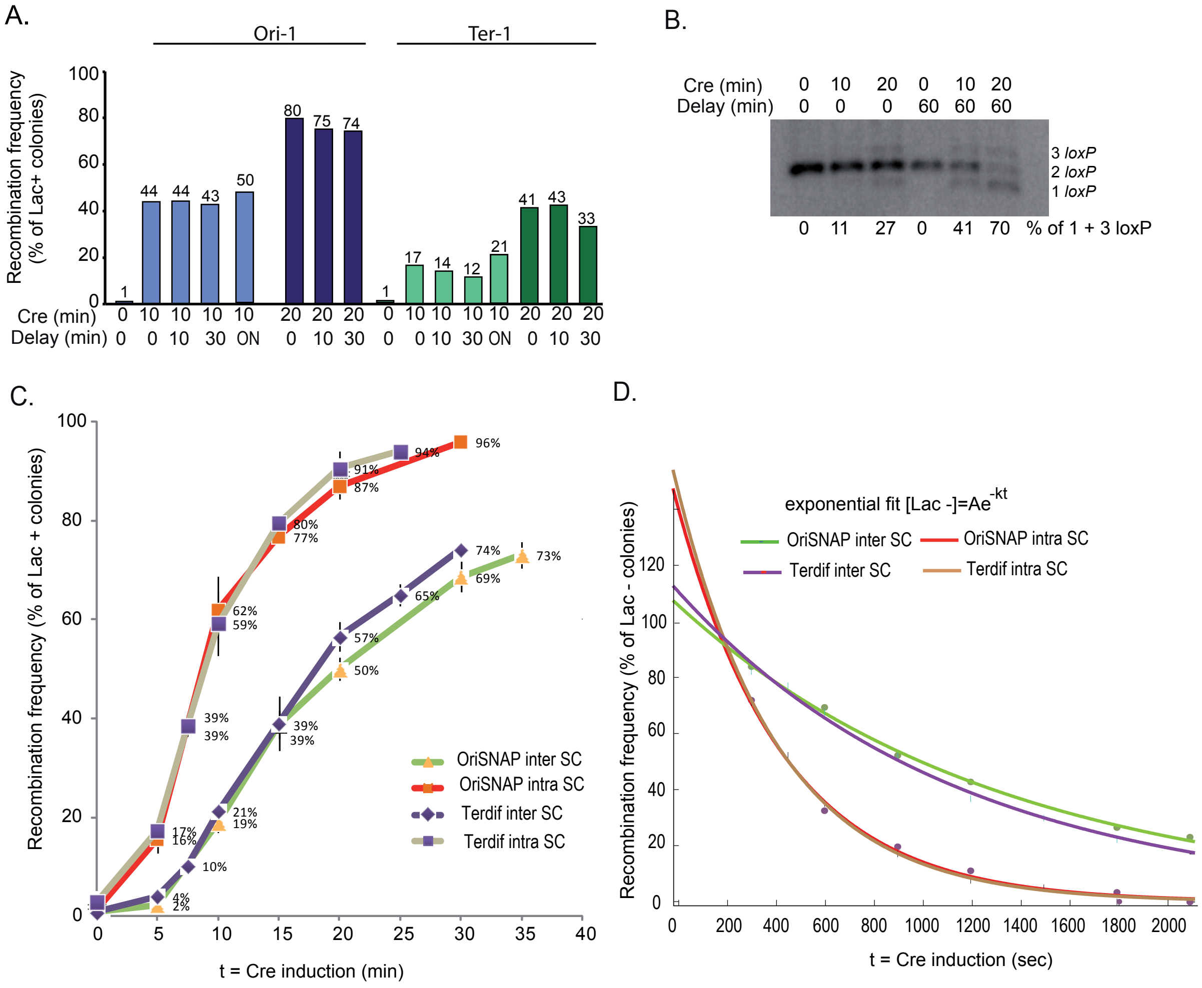

A.
Ori-1
Ter-1
Recombination frequency (% of Lac+ colonies)
Cre (min)
Delay (min)
B.
Cre (min) 0 10 20 0 10 20
Delay (min) 0 0 0 60 60 60
3 loxP
2 loxP
1 loxP
0 11 27 0 41 70 % of 1 + 3 loxP
C.
Recombination frequency (% of Lac + colonies)
t = Cre induction (min)
OriSNAP inter SC
OriSNAP intra SC
Terdif inter SC
Terdif intra SC
D.
exponential fit [Lac -]=Ae-kt
OriSNAP inter SC
OriSNAP intra SC
Terdif inter SC
Terdif intra SC
Recombination frequency (% of Lac - colonies)
t = Cre induction (sec)

Figure S2

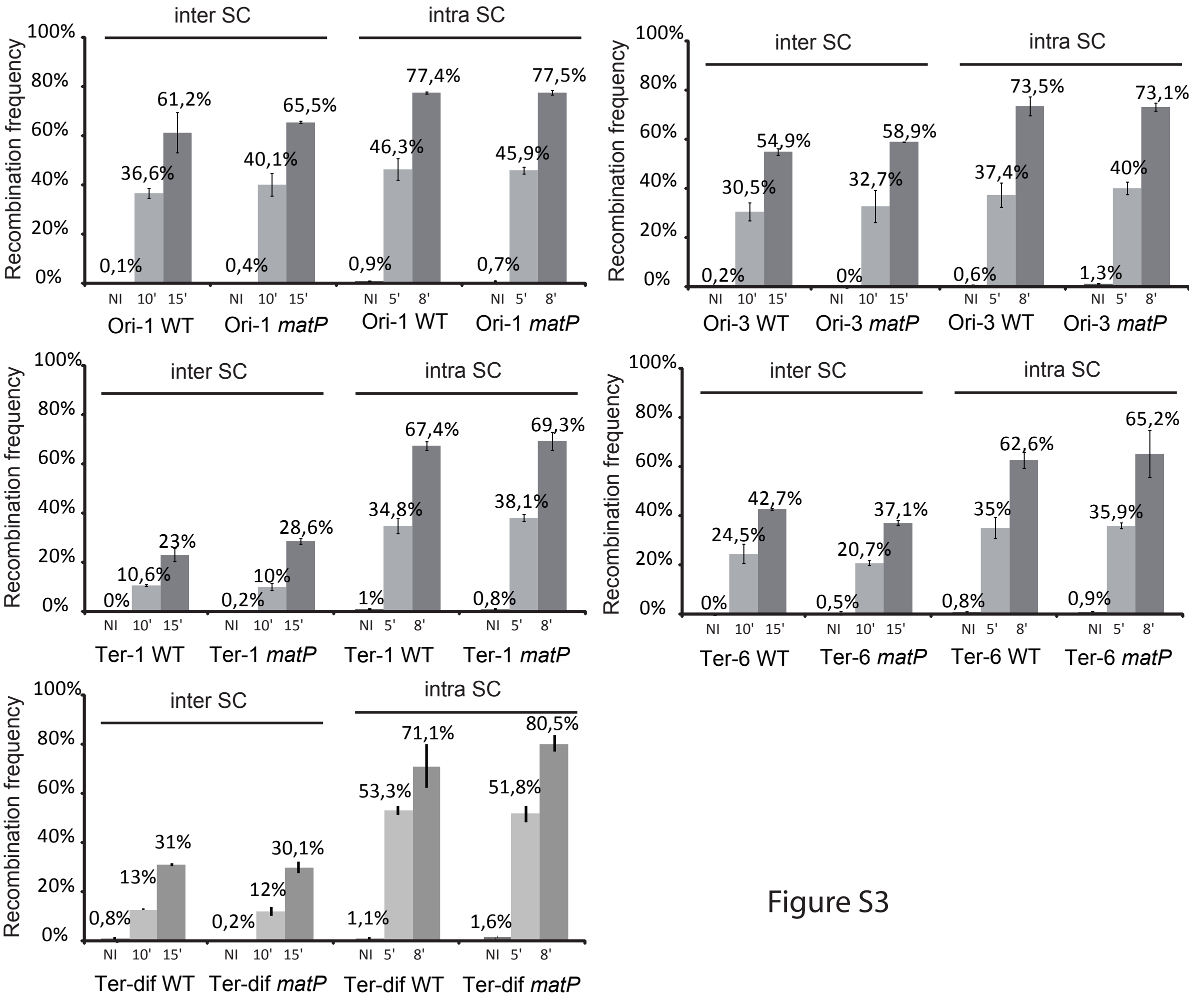
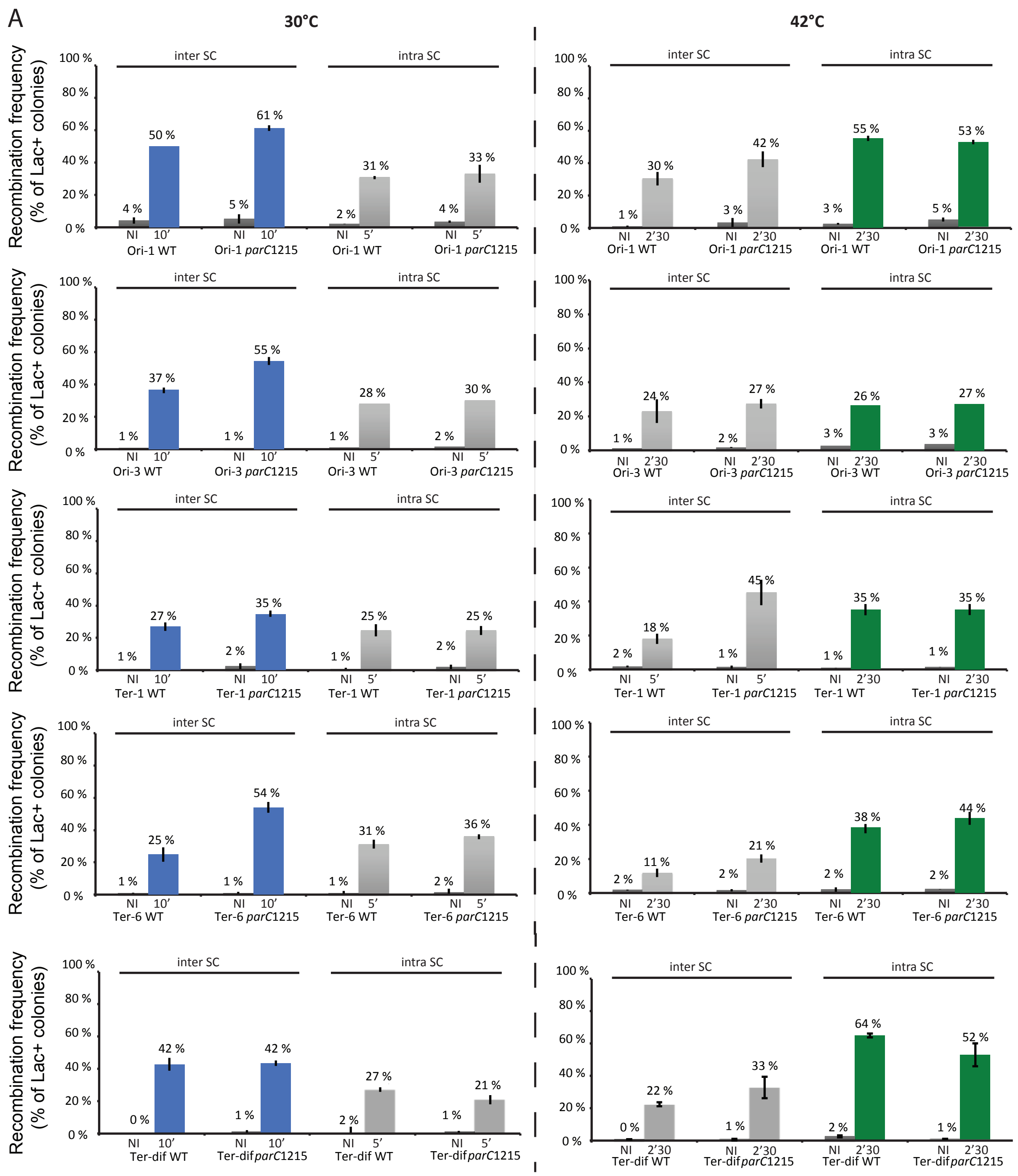

inter SC
intra SC
Recombination frequency
100%
80%
60%
40%
20%
0%
0,1%
36,6%
61,2%
0,4%
40,1%
65,5%
0,9%
46,3%
77,4%
0,7%
45,9%
77,5%
NI 10' 15'
NI 5' 8'
Ori-1 WT
Ori-1 matP
0,2%
30,5%
54,9%
0%
32,7%
58,9%
0,6%
37,4%
73,5%
1,3%
40%
73,1%
Ori-3 WT
Ori-3 matP
0%
10,6%
23%
0,2%
10%
28,6%
1%
34,8%
67,4%
0,8%
38,1%
69,3%
Ter-1 WT
Ter-1 matP
0%
24,5%
42,7%
0,5%
20,7%
37,1%
0,8%
35%
62,6%
0,9%
35,9%
65,2%
Ter-6 WT
Ter-6 matP
0,8%
13%
31%
0,2%
12%
30,1%
1,1%
53,3%
71,1%
1,6%
51,8%
80,5%
Ter-dif WT
Ter-dif matP

Figure S3


A
30°C
42°C
Recombination frequency (% of Lac+ colonies)
100 %
80 %
60 %
40 %
20 %
0 %
inter SC
intra SC
4 % 50 % 5 % 61 % 2 % 31 % 4 % 33 %
NI 10′ Ori-1 WT
NI 10′ Ori-1 parC1215
NI 5′ Ori-1 WT
NI 5′ Ori-1 parC1215
1 % 30 % 3 % 42 % 3 % 55 % 5 % 53 %
NI 2′30 Ori-1 WT
NI 2′30 Ori-1 parC1215
1 % 37 % 1 % 55 % 1 % 28 % 2 % 30 %
NI 10′ Ori-3 WT
NI 10′ Ori-3 parC1215
NI 5′ Ori-3 WT
NI 5′ Ori-3 parC1215
1 % 24 % 2 % 27 % 3 % 26 % 3 % 27 %
NI 2′30 Ori-3 WT
NI 2′30 Ori-3 parC1215
1 % 27 % 2 % 35 % 1 % 25 % 2 % 25 %
NI 10′ Ter-1 WT
NI 10′ Ter-1 parC1215
NI 5′ Ter-1 WT
NI 5′ Ter-1 parC1215
2 % 18 % 1 % 45 % 1 % 35 % 1 % 35 %
NI 5′ Ter-1 WT
NI 5′ Ter-1 parC1215
NI 2′30 Ter-1 WT
NI 2′30 Ter-1 parC1215
1 % 25 % 1 % 54 % 1 % 31 % 2 % 36 %
NI 10′ Ter-6 WT
NI 10′ Ter-6 parC1215
NI 5′ Ter-6 WT
NI 5′ Ter-6 parC1215
2 % 11 % 2 % 21 % 2 % 38 % 2 % 44 %
NI 2′30 Ter-6 WT
NI 2′30 Ter-6 parC1215
0 % 42 % 1 % 42 % 2 % 27 % 1 % 21 %
NI 10′ Ter-dif WT
NI 10′ Ter-dif parC1215
NI 5′ Ter-dif WT
NI 5′ Ter-dif parC1215
0 % 22 % 1 % 33 % 2 % 64 % 1 % 52 %
NI 2′30 Ter-dif WT
NI 2′30 Ter-dif parC1215

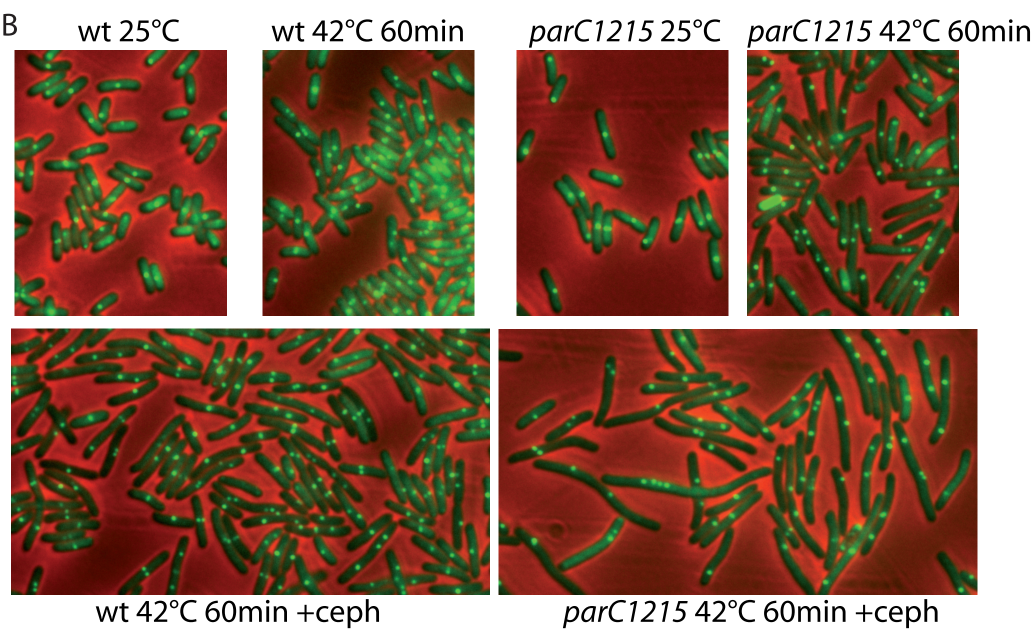

B
wt 25°C
wt 42°C 60min
parC1215 25°C
parC1215 42°C 60min
wt 42°C 60min +ceph
parC1215 42°C 60min +ceph

Figure S4

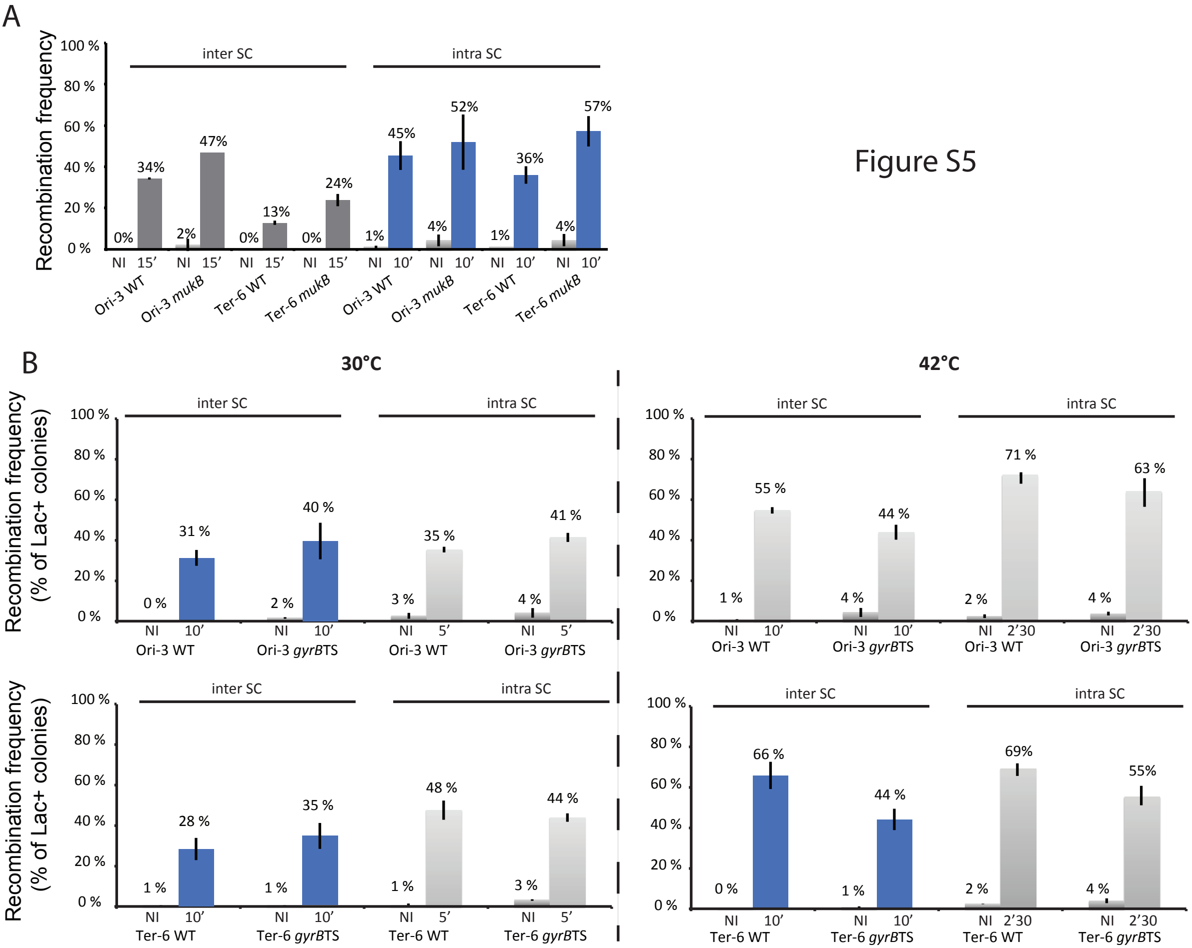

A
Recombination frequency
100 %
80 %
60 %
40 %
20 %
0 %
inter SC
intra SC
0%
34%
2%
47%
0%
13%
0%
24%
1%
45%
4%
52%
1%
36%
4%
57%
NI 15'
NI 10'
Ori-3 WT
Ori-3 mukB
Ter-6 WT
Ter-6 mukB
B
30°C
42°C
Recombination frequency (% of Lac+ colonies)
0 %
31 %
2 %
40 %
3 %
35 %
4 %
41 %
NI 10'
NI 5'
Ori-3 WT
Ori-3 gyrBTS
1 %
55 %
4 %
44 %
2 %
71 %
4 %
63 %
NI 2'30
1 %
28 %
1 %
35 %
1 %
48 %
3 %
44 %
Ter-6 WT
Ter-6 gyrBTS
0 %
66 %
1 %
44 %
2 %
69 %
4 %
55%

Figure S5

Table S1 Strains and plasmids

Strain	**Genotype**	**Reference**
MG1656	MG1655 Δ*lac*MluI	Espeli et al., 2001
Ori-1 inter SC	MG1656 Ori-1 *lacZ*::*loxP*::20bp::*loxP* frt::*cat*::frt	this work
Ori-3 inter SC	MG1656 Ori-3 *lacZ*::*loxP*::20bp::*loxP* frt::*cat*::frt	this work
Ter-1 inter SC	MG1656 Ter-1 *lacZ*::*loxP*::20bp::*loxP* frt::*cat*::frt	this work
Ter-6 inter SC	MG1656 Ter-6 *lacZ*::*loxP*::20bp::*loxP* frt::*cat*::frt	this work
Ori-1 intra SC	MG1656 Ori-1 *lacZ*::*loxP*::*Rif*::*loxP* frt::*cat*::frt	this work
Ori-3 intra SC	MG1656 Ori-3 *lacZ*::*loxP*::*Rif*::*loxP* frt::*cat*::frt	this work
Ter-1 intra SC	MG1656 Ter-1 *lacZ*::*loxP*::*Rif*::*loxP* frt::*cat*::frt	this work
Ter-6 intra SC	MG1656 Ter-6 *lacZ*::*loxP*::*Rif*::*loxP* frt::*cat*::frt	this work
EG01	MG1656 Ori-1 *lacZ*::*loxP*::20bp::*loxP*	this work
EG02	MG1656 Ori-3 *lacZ*::*loxP*::20bp::*loxP*	this work
EG03	MG1656 Ter-1 *lacZ*::*loxP*::20bp::*loxP*	this work
EG04	MG1656 Ter-6 *lacZ*::*loxP*::20bp::*loxP*	this work
EG05	MG1656 Ori-1 *lacZ*::*loxP*::*Rif*::*loxP*	this work
EG06	MG1656 Ori-3 *lacZ*::*loxP*::*Rif*::*loxP*	this work
EG07	MG1656 Ter-1 *lacZ*::*loxP*::*Rif*::*loxP*	this work
EG08	MG1656 Ter-6 *lacZ*::*loxP*::*Rif*::*loxP*	this work
EG09	MG1656 Ori-1 *parS P1* frt::*Trim*::frt	this work
EG10	MG1656 Ori-3 *parS P1* frt::*Trim*::frt	this work
EG11	MG1656 Ter-1 *parS P1* frt::*Trim*::frt	this work
EG12	MG1656 Ter-6 *parS P1* frt::*Trim*::frt	this work
EG13	MG1656 *ssb-yfp*::frt::*cat*::frt	Mercier et al 2008
EG14	EG01 *dnaC*::Tc	from J.L. Ferrat
EG15	EG02 *dnaC*::Tc	this work
EG16	EG03 *dnaC*::Tc	this work
EG17	EG04 *dnaC*::Tc	this work
EG18	EG05 *dnaC*::Tc	this work
EG19	EG06 *dnaC*::Tc	this work
EG20	EG07 *dnaC*::Tc	this work
EG21	EG08 *dnaC*::Tc	this work
RM1	MG1655 *matP* frt::*cat*::frt	Mercier et al., 2008
EG22	EG01 *matP* frt::*cat*::frt	this work
EG23	EG02 *matP* frt::*cat*::frt	this work
EG24	EG03 *matP* frt::*cat*::frt	this work
EG25	EG04 *matP* frt::*cat*::frt	this work
EG26	EG05 *matP* frt::*cat*::frt	this work
EG27	EG06 *matP* frt::*cat*::frt	this work
EG28	EG07 *matP* frt::*cat*::frt	this work
EG29	EG08 *matP* frt::*cat*::frt	this work
REP990	MG1655 *mukB::kn*	from J.L. Ferrat

EG30	EG01 *mukB*::*kn*	this work
EG31	EG02 *mukB*::*kn*	this work
EG32	EG03 *mukB*::*kn*	this work
EG33	EG04 *mukB*::*kn*	this work
EG34	EG05 *mukB*::*kn*	this work
EG35	EG06 *mukB*::*kn*	this work
EG36	EG07 *mukB*::*kn*	this work
EG37	EG08 *mukB*::*kn*	this work
MG1656 *parCts*	MG1656 parC1215 ::Tc	Lab collection
EG38	EG01 *parC1215*::*Tc*	this work
EG39	EG02 *parC1215*::*Tc*	this work
EG40	EG03 *parC1215*::*Tc*	this work
EG41	EG04 *parC1215*::*Tc*	this work
EG42	EG05 *parC1215*::*Tc*	this work
EG43	EG06 *parC1215*::*Tc*	this work
EG44	EG07 *parC1215*::*Tc*	this work
EG45	EG08 *parC1215*::*Tc*	this work
REP990	*mukB*::*kn*	J.L. Ferat
EG46	EG01 *xerC*::*kn*	this work
EG47	EG02 *xerC*::*kn*	this work
EG48	EG03 *xerC*::*kn*	this work
EG49	EG04 *xerC*::*kn*	this work
EG50	EG05 *xerC*::*kn*	this work
EG51	EG06 *xerC*::*kn*	this work
EG52	EG07 *xerC*::*kn*	this work
EG53	EG08 *xerC*::*kn*	this work
EG54	EG01 *recA*::*cat*	this work
EG55	EG02 *recA*::*cat*	this work
EG56	EG03 *recA*::*cat*	this work
EG57	EG04 *recA*::*cat*	this work
EG58	EG05 *recA*::*cat*	this work
EG59	EG06 *recA*::*cat*	this work
EG60	EG07 *recA*::*cat*	this work
EG61	EG08 *recA*::*cat*	this work
EG62	EG01 *xerC*::*kn recA*::*cat*	this work
EG63	EG02 *xerC*::*kn recA*::*cat*	this work
EG64	EG03 *xerC*::*kn recA*::*cat*	this work
EG65	EG04 *xerC*::*kn recA*::*cat*	this work
EG66	EG05 *xerC*::*kn recA*::*cat*	this work
EG67	EG06 *xerC*::*kn recA*::*cat*	this work
EG68	EG07 *xerC*::*kn recA*::*cat*	this work
EG69	EG08 *xerC*::*kn recA*::*cat*	this work
JJC1603	AB1157 *gyrB203*ts::*tc*	from B. Michel
EG70	EG01 *gyrB*::*Tc*	this work

EG71	EG02 *gyrB*::*Tc*	this work
EG72	EG03 *gyrB*::*Tc*	this work
EG73	EG04 *gyrB*::*Tc*	this work
EG74	EG05 *gyrB*::*Tc*	this work
EG75	EG06 *gyrB*::*Tc*	this work
EG76	EG07 *gyrB*::*Tc*	this work
EG77	EG08 *gyrB*::*Tc*	this work
Ter-dif inter SC	MG1656 Ter-dif *lacZ*::*loxP*::20bp::*loxP* frt::*cat*::frt	this work
Ter-dif intra SC	MG1656 Ter-dif *lacZ*::*loxP*::*Rif*::*loxP* frt::*cat*::frt	this work
EG78	Ter-dif inter SC *dnaC*::Tc	this work
EG79	Ter-dif inter SC *parC1215*::*Tc*	this work
EG80	Ter-dif intra SC *parC1215*::*Tc*	this work
EG81	Ter-dif inter SC *matP frt*::*rif*::*fr*t	this work
EG82	Ter-dif intra SC *matP frt*::*rif*::*frt*	this work
EG83	EG01 *migS*::*cat*	From Niki
EG84	EG02 *migS*::*cat*	this work
EG85	EG05 *migS*::*cat*	this work
EG86	EG06 *migS*::*cat*	this work

Plasmid	**Description**	**Reference**
pKD46		Datsenko and Wanner 2000
pCP20	FLP expression vector, pSC101ts replicon	Datsenko and Wanner 2000
pGBKD3-*parS*	derived from pKD4	Espeli et al., 2008
pGBKDLacloxP::20bp::loxP		this work
pGBKDLacloxP::rif::loxP		this work
pFX465	pSC101 with P*BAD* and P*Lac* promoters flanking the MSC	Gift from FX Barre
pCre	*cre* cloned under *PBAD* promoter into pFX465	this work
pGBKD3-2*parS*::*Trim*	matrix for *parSP1*::trimethoprim resistant insertion	Thiel et al in press
pALA2705	GFP-Δ30ParBP1 expression vector	Li et al., 2002
pKD3	template plasmid *frt-cat-frt*	Datsenko and Wanner, 2000
pLEX5BAparE-parC	overexpression of TopoIV	Mossessova et al., 2000

Table S2

Oligonucleotide	Sequence	Description	Reference
rif_bamHI_up	CCCGCGGATCCGCGCGGAATAGGAACTTCACAATT	Integration of the *rif* gene at BamHI site into the 2 *loxP* sites of pGBKDLacloxP ::20bp ::loxP	this work
rif_bamHI_do	CCCGCGGATCCGCGAACTTCAGGATCTAAAACCGG		this work
lacloxpup	CTGCTTCAATCAGCGTGCCGTC	PCR to monitor recombination of the 2 *loxP* sites	this work
lacloxpdo	CAGGATATGTGGCGGATGAGCGG		this work
sondelacZup	TATCGCTCGCCACTTCAACA	Southern blot probe	this work
sondelacZdo	TTTAACCGCCAGTCAGGCTT		this work
Ori-1 up	TTTCAGCAATAAGACCAGAAAACGTGATTTAACGCC TGATTGTGTAGGCTGGAGCTGCTTCG	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	Espeli et al., 2008
Ori-1 do	ACCAGACGGGGGGCGAAAGGGAAGACTCCAGGTAC GACAATGGTCGCTATGTGGTGCTATCT		Espeli et al., 2008
Ori-3 up	GTATACGACTGATGCGACGCTGGTTTCGATTAACTAA ATGTGTGTAGGCTGGAGCTGCTTCG	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	Espeli et al., 2008
Ori-3 do	GGCGCGAGCGCCTTGTCCGGCCTACAATTTTCACATA TTTTGGTCGCTATGTGGTGCTATCT		Espeli et al., 2008
Ter-1 up	CGCGTGAATGTGTTAAACAATAGTAGATGACTTTATC CGCTGTGTAGGCTGGAGCTGCTTCGCGCGT	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	Espeli et al., 2008
Ter-1 do	TTCCTGAGGTACAGTTGCTCGCTCAAATCCTTTGATT ACTGGTCTGCTATGTGGTGCTATCT		Espeli et al., 2008
Ter-6 up	TTTTACGCTGGCCTACAATTCTGTACTGGCATTGTAG GCGGTCTGCTATGTGGTGCTATCT	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	this work
Ter-6 do	GCATCAATTGCCAGATGTGCCACTGACGTGTTTTATT TGGATTGTGTAGGCTGGAGCTGC		this work
Ter-dif up	TAGCAAAACCGCAATATCGGGATAACGCTACAGGAG AAGGTGTGTAGGCTGGAGCTGCTTCG	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	Espeli et al., 2008
Ter-dif do	TTTTGAATATTCACCTTGTTTCAGGAAAATAGATCAA TCGTG GTCGCTATGTGGTGCTATCT		Espeli et al., 2008
Right-2 up	GGCAGCTATGGTTAGAAACTACCTGACGTCAGTCCTT GCGTGTGTAGGCTGGAGCTGCTTCG	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	Espeli et al., 2008
Right-2 do	ATCCTGTTGTAGCAGGACAAATTTACGAAAGCCTGC TCCCGGTCTGCTATGTGGTGCTATCT		Espeli et al., 2008
glnA up	ATGTTGCACCAATATAGTGCTTCAATGGAAACATTA AGCAGATTGTGTAGGCTGGAGCTGC	Chromosome integration of the *lacloxP* and the *parS*/ParB systems	this work
glnA do	GCATAAAAAGGGTTATCCAAAGGTCATTGCACCAAC ATGGGGTCTGCTATGTGGTGCTATCT		this work