

Supplementary Material

Fig. S1 Chromosome segregation pattern is replication fork-dependent. Relative positioning of L6 (green)-R6 (red) in WT (left panel) and ectopic *terB'* (100kb from *dif* and 100kb from R6, right panel) strains was analysed using FROS in τ 100min cells after 60min cephalixin treatment. Four patterns of informative sister nucleoids were observed and their number and proportion were listed. Informative nucleoid sister pairs are those in which both foci had duplicated and at least one sister derived from a given locus had segregated away from midcell.

Fig. S2. Snapshot analysis. In snapshot analysis, the frequency of a pattern is a reflection both of the fraction of a cell cycle spent with that pattern and probability that that pattern will arise in a normal cell cycle. For example, in Fig. 2A, the fall in frequency of pattern A, as one goes from R4 to R6 may reflect differences in organization-segregation. This could arise because of positional restraints in the *ter* region (main text; Fig 1; Fig. 4). Alternatively, the fall could arise when the loci have exactly the same segregation behavior, yet R6 spends 40% of time in that pattern as compared to R4.

A. Relative positioning of L3 (red)-R3 (green) in WT and RecA⁻ strains in exponential culture (left panel, τ 100min) and after 60min cephalixin treatment (right panel) was analyzed using FROS. The proportion and number of each of the three informative patterns are listed.

B. Relative positioning of locus pairs on opposite chromosome arms (B1) and on the same chromosome arm (B2). For each locus pair, ~ 250 4-focus cells (B1), or ~400 4-focus cells (B2) were analyzed by FISH and the fraction of cells having either of the two patterns shown in the inset was calculated. The % for each locus pair varies as the frequency of the asymmetric pattern; it being most frequent for markers away from *ori* and *ter* (Fig. 2A). The actual numbers for L6-R6 are low and not statistically significant.

C. Sister locus position patterns of L3-R3 and L4-R4 pairs. For each locus pair, ~ 240 4-focus cells were analyzed by FISH. Every pattern that constituted a fraction larger than > 1% of the whole 4-focus cell population was selected and classified into five groups. The L3/L4 locus is shown in red and R3/R4 in green. For the L3-R3 pair, there are 51 4-focus position patterns in addition to the 18 patterns shown. The cells having these 51 patterns

constitute 22% of the total 4-focus cell population. For the L4-R4 pair, there are 48 4-focus position patterns in addition to the 20 patterns shown, and the cells having these 48 patterns constitute 23% of the total 4-focus cell population.

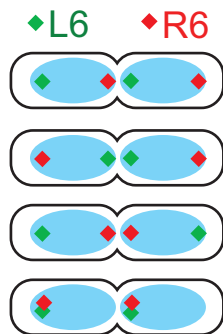
Fig. S3. Timelapse FROS analysis of L3 (red)-R3 (green) shows an imprinting mechanism that directs a given polar locus in a daughter cell to the position occupied by its mother in the previous generation. The fate of L3 and R3 was followed (10min time intervals) for one generation (τ 100min). The frequencies of the 4 patterns shown are listed. Examples are shown in the lower panel. White arrows show closely separated sister foci that may have duplicated recently close to midcell.

Fig.S4. Analysis of the *dif* locus.

A. Relative positioning of the *dif* locus (8kb from *dif* and 11 kb from Ter) and L3 or R3 was analyzed by FROS snapshots in an exponential culture (exp) and after 60min cephalixin treatment (cpx). In the cells where *dif* has duplicated (194/2145 in exp, and 711/1397 in cpx), the percentages of all the patterns are listed. The background is labeled green when *dif* co-localizes with L3, and pink with R3. After 60min cephalixin treatment, the fraction of sister *dif* loci at midcell decreased from 88% to 78%, while the asymmetry positioning of sister *dif* loci increased from 12% to 22%, showing that after division, a sister *dif* may move from the new to the old pole, before re-migration back to midcell for re-replication

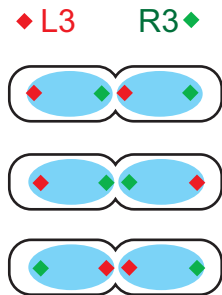
B. Illustration of timelapse analysis of L3 (green)-*dif* (red) with 10min time intervals. The fate of *dif* is listed separately with L3-*dif* pairs in brackets. *dif* segregates with left and right replichores with comparable frequency.

Fig.S1



 R6 replicated by either fork		 R6 prefers the right fork	
128	67%	261	85%
15	8%	11	4%
4	2%	16	5%
45	23%	19	6%
Non-info 291/483		Non-info 614/921	

Fig.S2A



Exponential		Cephalexin for 1h	
WT	RecA ⁻	WT	RecA ⁻
76%(217)	81%(202)	85%(300)	88%(733)
12%(36)	10% (24)	8%(28)	6%(52)
12%(36)	9%(22)	7%(26)	6%(53)

Fig.S2B

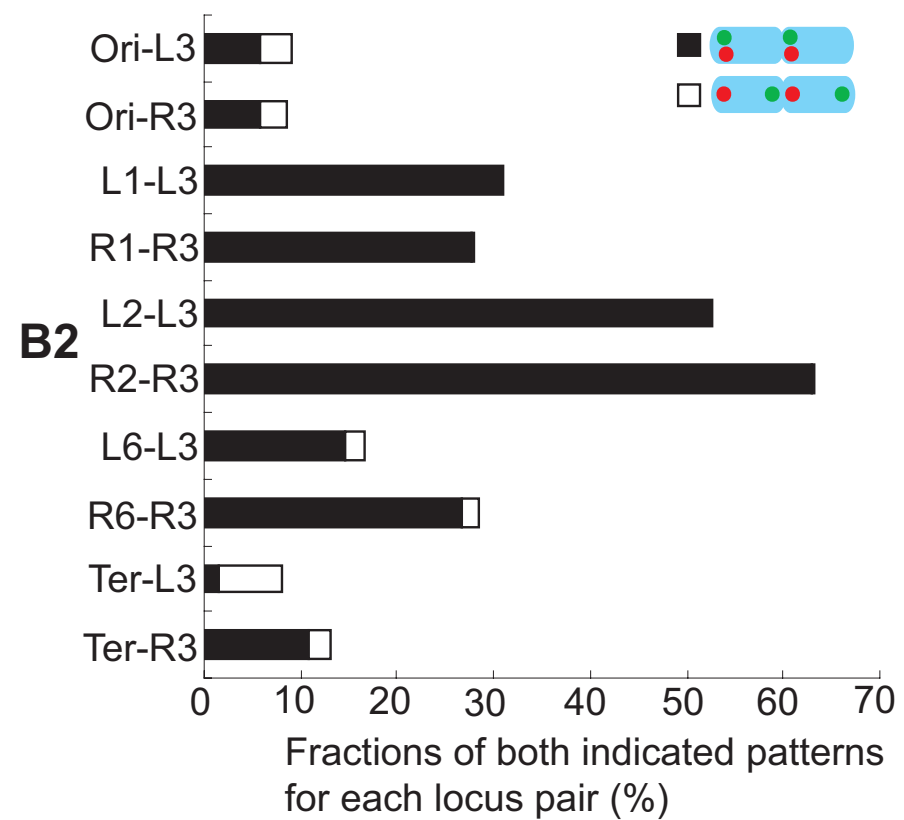
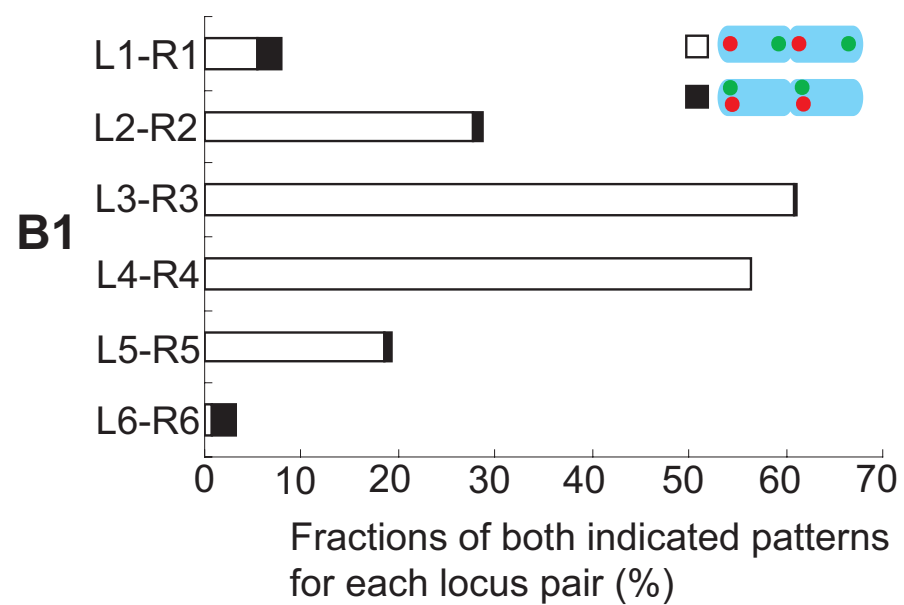


Fig.S2C







































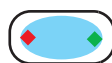
L3-R3		L4-R4	
Pattern	%	Pattern	%
	30.9		26.4
	5.6		7.5
	5.6		5.5
	5.0		4.3
	3.3		3.9
	3.3		2.7
	2.4		1.6
	1.5		1.2
	1.5		
	58.8		53.1
	2.7		4.7
	1.8		2.4
	1.2		2.0
	1.2		1.6
	6.9		1.2
			11.9
	4.1		2.7
	1.2		2.0
	3.3		1.2
	3.0		2.0
	11.6		1.2
			9.1
	1.2		1.6
			1.2

Fig.S3

Timelapse

L3 R3



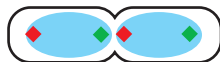
17

a

b

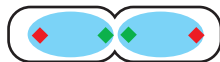
c

d



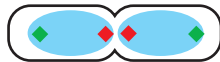
12

L R L R



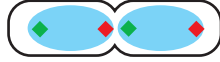
3

L R R L



1

R L L R



1

R L R L

L3 R3

d

c

a

a

a

min
0

10

20

30

40

50

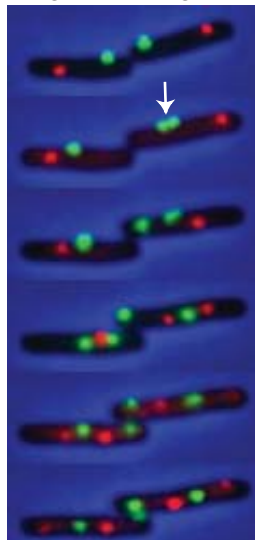
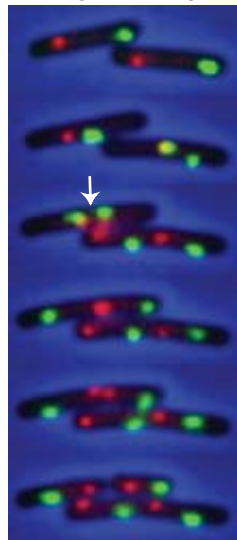
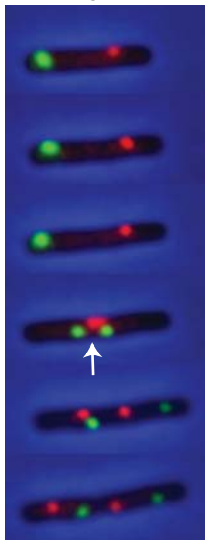

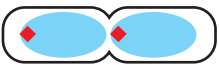



Fig.S4

A

L3 R3				
LRLR	exp 76%	cpx 71	6 10	0 0
RLRL			5 10	
LRRL	6 3	0.5 1	0 0	
RLLR	6 4	0.5 1	0 0.1	
Σ	88 78	12 22	0 0.1	

B

