Supplemental Information

Table S1

RLG number	Sequence	Purpose	Genom Position*
A			
5312	TTCTGCCTTTGCGTCGGTTGTTGCGACATTGAA TAACCTGCGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *rrnA* (used with 5313)	4034452
5313	AATCAGGATCCATTTAGCCACCGGGTTCATACT GGTAAAGCACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *rrnA* (used with 5312)	4034452
5300	TTGGTGTCGTGCCGGCGATTAAACCTGCTGCA CGTCTGACGGCCGATAAAAAGCCGAAGCCTTA AAC	Insert *parS* P1 at *rrnB* (used with 5301)	4165804
5301	CGAAACGCGCGATCAGCTCATGAGTATAAGAA CGTTTAACTGCACAGCTTTAGAGCGTTTTGCGA T	Insert *parS* P1 at *rrnB* (used with 5300)	4165804
5925	CACCGGGCAAAATGGTGCCGGGTTCATATTCA CCTTTTAAGATCCACAGCTTTAGAGCGTTTTGC GATGAC	Insert *parS* P1 at *rrnC* (used with 5926)	3921253
5926	GCTGCACAGAAAAACCTTTCGTATGTTCTGGCT GAGAAGCTGGCGCAACGCGATAAAAAGCCGAA GCCTTAAACTTTC	Insert *parS* P1 at *rrnC* (used with 5925)	3921253
5396	TTATTGCTGAGGTAAGTATGTCTGATGTTTTAC GCCCATACCGCGCGATAAAAAGCCGAAGCCTT AAAC	Insert *parS* P1 at *rrnD* (used with 5397)	3429263
5397	TGCTATCGTCGATCATTACGCGCTGACCGATTT GTGGAAAAAGATCACAGCTTTAGAGCGTTTTG CGAT	Insert *parS* P1 at *rrnD* (used with 5396)	3429263
4442	GCGAATTTTTCTCTTTCAATGGTGATCACAATTT TGACTGTGGTTCACAGCTTTAGAGCGTTTTGC GAT	Insert *parS* P1 at *rrnE* (used with 4443)	4207707
4443	AGTTATCCACAATCATCAATGTAATTTCTGTATT TTGCCCACGGTCGATAAAAAGCCGAAGCCTTA AAC	Insert *parS* P1 at *rrnE* (used with 4423)	4207707
5275	TTCCGCCGCCGGAAATGAATATTCACAGCATC AGGGGATGCGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *rrnG* (used with 5276)	2724420
5276	CCGGTGCTTGCACCGGCGACATCCCAGGCCA AATCCTTCCCACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *rrnG* (used with 5275)	2724420
5578	GATGTTAAGGCATCCAGACGTCTAAATCAATCA GGTTTATGCGAACACAGCTTTAGAGCGTTTTGC GAT	Insert *parS* P1 at *rrnH* (used with 5279)	222765
5579	CATGTGACGCTAGTATCGCATGTTTCGACCTG CAAGAAAGTGCTCCGATAAAAAGCCGAAGCCT TAAAC	Insert *parS* P1 at *rrnH* (used with 5278)	222765
5308	GGCGGTGAAATATCCCTGCGTAGTGCAGAAAA ATAAGAAAAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *rrnB* (used with 5309)	4165804
5309	AATCATGCGGTAGAAGAGGTCAGACTACGCAA ATAATTTGCTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *rrnB* (used with 5308)	4165804

5927	CACCGGGCAAAATGGTGCCGGGTTCATATTCACCTTTTAAGATCCGCCAAAAAAACCCGCCGAAGC	Insert *parS* PMT at *rrnC* (used with 5928)	3921253
5928	GCTGCACAGAAAAACCTTTCGTATGTTCTGGCTGAGAAGCTGGCGCAACGAGGAAGCGGAATTCCGGACCGG	Insert *parS* PMT at *rrnC* (used with 5927)	3921253
5398	TTATTGCTGAGGTAAGTATGTCTGATGTTTTACGCCCATACCGCGAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *rrnD* (used with 5399)	3429263
5399	TGCTATCGTCGATCATTACGCGCTGACCGATTTGTGGAAAAAGATCTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *rrnD* (used with 5398)	3429263
4444	GCGAATTTTTCTCTTTCAATGGTGATCACAATTTTGACTGTGGTTAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *rrnE* (used with 4445)	4207707
4445	AGTTATCCACAATCATCAATGTAATTTCTGTATTTTGCCCACGGTCTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *rrnE* (used with 4444)	4207707
5475	TTCCGCCGCCGGAAATGAATATTCACAGCATCAGGGGATGAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *rrnG* (used with 5476)	2724420
5476	GATGTTAAGGCATCCAGACGTCTAAATCAATCAGGTTTATGCGAACTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *rrnG* (used with 5475)	2724420
5580	GAGTTAACGCTCGAGGTTTTTTTTCGTCTGTATATCTATTATTGCAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *rrnH* (used with 5581)	222765
5581	GTTGAGGAAAAAGAGCGTAAAATGCAGAGGATTTTTGCGATTCTGCTCGAGATGCAGAAGACGCA	Insert parS PMT at *rrnH* (used with 5580)	222765
5775	CAAAATGATCCCCTGCTGCCGGGATACTCGTTTAACGCGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *araC* (used with 5776)	70390
5776	ACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *araC* (used with 5775)	71406
5406	AAGCGGAGATCGCCTAGTGATTTTAAACTATTGCTGGCAGCATTCTTGAGCGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *λatt* (used with 5407)	807328
5407	CTAAAGAACAACCTGACCCAGCAAAAGGTACACAATACTTTTATATTGGACACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *λatt* (used with 5406)	807328
5408	AAGCGGAGATCGCCTAGTGATTTTAAACTATTGCTGGCAGCATTCTTGAGAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *λatt* (used with 5409)	807328
5409	CTAAAGAACAACCTGACCCAGCAAAAGGTACACAATACTTTTATATTGGACTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *λatt* (used with 5408)	807328
6913	GCACTGGCGCACGTCGCCAGAAAGTATTGTTAATAAAGCGTAGTGAAACTCGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *serT* (used with 6914)	1039083
6914	TTTTTATCGCTAAAAGATAAATCCACACAGTTTGTATTGTTTTGTGCAAACACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *serT* (used with 6913)	1039083
6915	GCACTGGCGCACGTCGCCAGAAAGTATTGTTAATAAAGCGTAGTGAAACTAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *serT* (used with 6916)	1039083
6916	TTTTTATCGCTAAAAGATAAATCCACACAGTTTGTATTGTTTTGTGCAAACTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *serT* (used with 6915)	1039083

5268	GCAGGGTATTGCCCAACAGAACAGCTTTAAAC ATACCTGACGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *ter* (used with 5269)	1570489
5269	TGAGCTGCTTAGCTTTACCTGTCTGTACTGATG TAGCCATCACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *ter* (used with 5268)	1570489
5324	TAGGCCCGATAAGCAGGCGCATCGGGCAAAT GTGTTAACCAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *mocA* (used with 5325)	3015910
5325	TTAATCAGGTTACCGAGCATAAGATGTCTGCC GAGTAAGCCTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *mocA* (used with 5324)	3015910
5775	CCCGACGGCATCTACGCCCAGGTGGCGGTTTA CTCCGACCATTTCAGCCACGATAAAAAGCCGA AGCCTTAAAC	Insert *parS* P1 *yghJ* (used with 5776)	3114493
5779	CCCGACGGCATCTACGCCCAGGTGGCGGTTTA CTCCGACCATTTCAGCCAGTTATCGATTACGCC CCGCCCTGC	Insert *parS* PMT at *yghJ* (used with 5780)	3114493
5776	AATGCATCCAGCTGGTCATTCTTAGCAGCACTT TCCGCATCACCGAAACACACAGCTTTAGAGCG TTTTGCGAT	Insert *parS* P1 *yghJ* (used with 5775)	3114548
5780	AATGCATCCAGCTGGTCATTCTTAGCAGCACTT TCCGCATCACCGAAACAAGGAAGCGGAATTCC GGACCGG	Insert *parS* PMT at *yghJ* (used with 5779)	3114548
4856	GCAAAACGCACGCCTTTGACAAGTGCTACAGT CAATACACGAAGAAAAAAAGGAAGCGGAATTC CGGACC	Insert *parS* PMT at *ispB* (used with 4857)	3333580
4857	GATTGTACTGAAAAAATGGCACAGATAAACGTT ACCGTACAAGTTGTGTTCTCGAGATGCAGAAG ACGCA	Insert *parS* PMT at *ispB* (used with 4856)	3333581
5451	CAGATTTTGCGACCATTGTGGTGAGTCGATGC CGGAAATGGGGAAAAAGAAGGAAGCGGAATTC CGGACC	Insert *parS* PMT at *rpsL* (used with 5452)	3377629
5452	CCACATCGCCAATAAGGGACTAAGTCAACTATT TCAGACTAAAGCGCATCCTCGAGATGCAGAAG ACGCA	Insert *parS* PMT at *rpsL* (used with 5451)	3377630
5445	AGACGCACAGCGTCGCATCAGGCAACGGCTG TCGGATGCGGCGTAAACGCAGGAAGCGGAATT CCGGACC	Insert *parS* PMT at *rep245* (used with 5446)	3392187
5446	TGTGCGTCTTATCAGGCCTACAAACGGAACAT AACCGTAGGTCGGATAAGCTCGAGATGCAGAA GACGCA	Insert *parS* PMT at *rep245* (used with 5445)	3392188
5437	ATTCACTACATCAATATATATTTCAATTTACGAG GTTTTAATTCTGCCTCAGGAAGCGGAATTCCG GACC	Insert *parS* PMT at *envR* (used with 5446)	3413664
5438	ACACAAAAAAAGATTAATATTCTACTGTTTTATT TTGACGCGGGTTGAAACTCGAGATGCAGAAGA CGCA	Insert *parS* PMT at *envR* (used with 5437)	3413665
5349	ATTCACTACATCAATATATATTTCAATTTACGAG GTTTTAATTCTGCCTCAGGAAGCGGAATTCCG GACC	Insert *parS* PMT at *arcF* (used with 5350)	3418266
5350	ACACAAAAAAAGATTAATATTCTACTGTTTTATT TTGACGCGGGTTGAAACTCGAGATGCAGAAGA CGCA	Insert *parS* PMT at *arcF* (used with 5349)	3418267
4850	TTTCGGATGGCCTTTCGCTTGATTTGATGTCTG GCAGTTTATGGCGGGCGAGGAAGCGGAATTC CGGACC	Insert *parS* PMT at *yhdZ* (used with 4851)	3423277

4851	GTTGTTTGTCGGTGAACACTCTCCCGAGTAGG ACAAATCCGCCGGGAGCGCTCGAGATGCAGA AGACGCA	Insert *parS* PMT at *yhdZ* (used with 4850)	3423324
5285	CAAAACTGTTCCGGGGACAGTAAGAACATTTG CAGTTAACGATAAAAAGCCGAAGCCTTAAAC	Insert *parS* P1 at *arsR* (used with 5286)	3648881
5286	TAAAACACATCTGAAAATTCATATGTGTTTAGCT AAATTTCACAGCTTTAGAGCGTTTTGCGAT	Insert *parS* P1 at *arsR* (used with 5285)	3648881
5272	GCAGGATGTTTGATTAAAAACATAACAGGAAGA AAAATGCAGGAAGCGGAATTCCGGACC	Insert *parS* PMT at *oriC* (used with 5272)	3911813
5271	AATCGGTTACGGTTGAGTAATAAATGGATGCC CTGCGTAACTCGAGATGCAGAAGACGCA	Insert *parS* PMT at *oriC* (used with 5271)	3911819
5402	CTCATTAACGTTGGTTGTCAGTTCCGGTGCCAT CGAGAGCGCATGCTCCACCAAGGAAGCGGAA TTCCGGACC	Insert *parS* PMT *yjaA* (used with 5343)	4214112
5343	AGCCGGTTGGATTTATGTTGCTAAGTGGGCAG CATATGGATGCTCGAGATGCAGAAGACGCA	Insert *parS* PMT *yjaA* (used with 5402)	4214112
6095	AACGCAAACCAGAGCTGTACAGGCTTGGGCGC GGCTTTCAAACCAGTCGTCTCGAGATGCAGAA GACGCATCTG	Insert *parS* PMT at *yjhE* (used with 6096)	4506513
6096	GTTATACAGAAATGTCCGTTAAGCAGAGTTCAA AATTGATTGCCGTGATCAGGAAGCGGAATTCC GGACCGGC	Insert *parS* PMT at *yjhE* (used with 6095)	4506513
B			
7052	GTACGAGGGCATTTTTATCGCAGGTAATCCATT AATTGAATGTTAGTTCGCGATAAAAAGCCGAAG CCTTAAACTTTC	Delete *rrnD* operon (RLG13970) (used with 7105)	3430018
7105	GACAAACAACAGATAAAACAAAAGGCCCAGTC TTCCGACTGAGCCTTTTGTCACAGCTTTAGAGC GTTTTGCGATGAC	Delete *rrnD* operon (RLG13970) (used with 7052)	3423897
7106	CGCGACAGTGAGCTGAAAGCCGCGTCGCAAC TGCTCTTTAACAATTTATCCGATAAAAAGCCGA AGCCTTAAACTTTC	Delete *rrnD* structural Gene (RLG14083) (used with 7105)	3429362
7055	GATAAATTGTTAAAGAGCAGTTGCGACGCGGC TTTCAGCTCACTGTCGCGCGATAAAAAGCCGA AGCCTTAAACTTTC	Delete *rrnD* Fis sites, P1P2 (RLG13995) (used with 7052)	3429541
7326	AAGCACCGTTTTGTGTGCGATTGCAGCAAAAA GGGTGAAAAAACAACCGATAAAAAGCCGAAGC CTTAAAC	Delete *rrnD* Fis sites, P1 promoter (RLG14187) (used with 7327)	3429707
7327	ATTGTTAAAGAGCAGTTGCGACGCGGCTTTCA GCTCACTGTCGCGAGGTGCACAGCTTTAGAGC GTTTTGCGAT	Delete *rrnD* Fis sites, P1 promoter (RLG14187) (used with 7326)	3429647
7328	TTCGTTGTTCCGACCATCCTGTGAAGTGTTTCA CGTTGTCGTCTCAACGGAGCACAGCTTTAGAG CGTTTTGCGAT	Delete *rrnD* P2 promoter (used with 7055) (RLG14524)	3429648
7282	GTTCCGACCATCCTGTGAAGTGTTTCACGTTGT CGTCTCAAAAAATGAGACGTTGATCGGCACG	Insert *cat-sacB* to *rrnD* P1 (used with 7283)	3429647
7283	GAAAAAAAGATCAAAAAAATACTTGTGCAAAAA ATTGGGATCATCAAAGGGAAAACTGTCCA	Insert *cat-sacB* to *rrnD* P1 (used with 7282)	3429687
7330	AAGGGTGAAAAAACAACAAACAGAAAAAAAGAT CAAAAAAATACTTGTGCAAAAAATTGGGATCCC TCTAACGCGCCTCCG	Create tCtaaC mutation in *rrnD* P1 (used with 7331)	3429668

7331	GTTCCGACCATCCTGTGAAGTGTTTCACGTTGT CGTCTCAACGGAGGCGCGTTAGAGGGATCCC AATTTTTTGCACAAGTA	Create tCtaaC mutation in *rrnD* P1 (used with 7330)	3429586
C			
4679	GGCATACTTCGAAAATTTTGCGTAAACAGAAAT AAAGAGCTGACAGAACTTGCCCTCTTGGGTTA TCAAG	Delete *fis* (used with 4680)	3411271
4680	TCCCCATGCCGAGTAGCGCCTTTTTAATCAAG CATTTAGCTAACCTGAATGTACGTTGGAGCCG CATTAT	Delete *fis* (used with 4679)	3411567
6080	TCTATTATTACCTCAACAAACCACCCCAATATA AGTTTGAGATTACTACATGCCCTCTTGGGTTAT CAAG	Delete *hns* (used with 6081)	1292922
6081	CAATAAAAAATCCCGCCGCTGGCGGGATTTTA AGCAAGTGCAATCTACAAAAGAGTACGTTGGA GCCGCATTAT	Delete *hns* (used with 6080)	1292509
6211	CAACAGAACATATTGACTATCCGGTATTACCCG GCATGACAGGAGTAAAATGCCCTCTTGGGTTA TCAAG	Delete r*ecA* (used with 6212)	2823769
6212	TAAAAAAGCAAAAGGGCCGCAGATGCGACCCT TGTGTATCAAACAAGACGAGTACGTTGGAGCC GCATTAT	Delete *recA* (used with 6211)	2822708
7192	AAATGATGAATAAACGCCCCTGTTAATGAATAT CTGGCATGTTGTACTAATGCCCTCTTGGGTTAT CAAG	Delete *lrp* (used with 7195)	932595
7195	TAAGCAGCGCCGTATAGCCCTGAATAAACCCT TGTCTTTCCAGCCGACGCACGTACGTTGGAGC CGCATTAT	Delete *lrp* (used with 7192)	933089
D			
4442	CGATAAAAAGCCGAAGCCTTAAAC	Amplify parS P1 kan (used with 4443)	n/a
4443	CACAGCTTTAGAGCGTTTTGCGAT	Amplify parS P1 kan (used with 4442)	n/a
4444	AGGAAGCGGAATTCCGGACC	Amplify parS PMT Cm (used with 4445)	n/a
4445	CTCGAGATGCAGAAGACGCA	Amplify *parS* PMT Cm (used with 4444)	n/a

Table S1. Oligonucleotide Primers Used for Strain Constructions

Sequences are always written 5' to 3'. The primers used together are indicated in the "Purpose" column. The numerical designation of Genome Position is from EcoCyc.

(A) Primers used for creation of *parS* sites at different positions in the *E. coli* chromosome. *parS* sites from phage P1 or plasmid PMT, as indicated, bind the ParB-CFP or ParB-YFP fusions, respectively.

(B) Primers used for deletions or mutations in *rrnD*. Mutation endpoints/positions are described in the legend of Figure 3.

(C) Gene deletions. In each case, the deletion starts precisely at the translation initiation codon and ends with the stop codon (see Supplemental Materials and Methods).

(D) Primers for amplifying *parS* sites.

Table S2.

	ParB-YFP			ParB-CFP		
Medium	β-gal activity (M.U.)		percent	β-gal activity (M.U.)		percent
LB	10700	9300	87%	9900	7600	76%
MOPS glu, aa, nucl	10000	9300	94%	8800	6800	76%
MOPS gly, aa, nucl	6600	6300	95%	7200	4300	61%
MOPS glu	3900	3600	92%	4100	2900	73%
MOPS gly	3400	2400	70%	3000	2300	79%

Table S2. Effect of ParB Binding on rRNA Promoter Activity

ParB has been reported to oligomerize and spread from its site of initial binding (Nielson et al. 2006). The effect of a *parS* site on the bacterial chromosome relatively close to the rRNA promoter region was tested for interference with expression of an adjacent *rrnB* P1-*lacZ* fusion in media resulting in a range of growth rates during log phase. The *parS* site was 80 bp upstream of the transcription start site. Activities are in Miller Units (M.U.). Effects of two different ParB fusion proteins were analyzed, ParB-YFP (RLG7730) and ParB-CFP (RLG7728) bound to their cognate *parS* sites. The *parS* sites inserted just upstream of an rRNA operon affected promoter activity only very slightly.

Supplemental Figure Legends

Figure S1. Positions of all loci analyzed

The *parS* insertions are named by the nearest gene (see also Table 1). The sequence coordinate (in parenthesis) refers to the position of the beginning of the coding region of that gene. The colored regions indicate the limits of proposed macrodomains on the *E. coli* chromosome (Espeli and Boccard 2006). Names of macrodomains are in upper case inside the circle. All loci examined and their genome coordinates are indicated on the outside of the circle. The sequence coordinates (and the sequences) of the primers used to create the *parS* site insertions are in Table S1.

Figure S2. Additional rRNA operon pairs examined

Cells were examined in EZ-rich glucose defined medium. Genetic distance (kb) and physical distance (nm) separating pairs are shown along with number of cells examined (n). The first member of the pair has the *parS* site with the bound ParB-CFP, and the second member of the pair has the *parS* site with the bound ParB-YFP. Strain names are in parentheses.

(A) *rrnA*, *rrnB* (RLG11531)

(B) *rrnA, rrnD* (RLG10665)

(C) *rrnE, rrnH* (RLG10669)

(D) *rrnD, rrnE* (RLG10673)

(E) *rrnA, rrnH* (RLG10654)

(F) *rrnB, rrnG* (RLG10974)

(G) *rrnD*, *rrnB* (RLG11507)

(H) *rrnG*, *rrnD* (RLG11980)

Figure S3. Movement of foci with time

(A) A representative cell containing *rrnD*-CFP viewed at one sec intervals. Sequential displacement of the CFP focus is represented by lines illustrating movement at 1 sec intervals.

(B) Close-up of the ParB-CFP focus at *rrnD* shown in (A).

(C) Distribution of distances traveled by the *rrnD*-CFP focus in 1 sec (69 nm ± 14 nm; n=1000). Measurements were collected from foci in multiple cells.

(D) Close-up of a representative ParB-YFP focus at *rrnE*. Sequential displacement of the YFP focus is represented by lines illustrating movement at 1 sec intervals.

(E) Distribution of distances traveled by the *rrnE*-YFP focus in 1 sec (84 nm ± 24 nm; n=2000). Measurements were collected from foci in multiple cells.

Figure S4. Distances between rRNA-rRNA operon pairs are the same in cells with one focus pair or two focus pairs

Cells shown in (A) and (B) were from the same culture.containing *rrnD*-CFP and *rrnE*-YFP (RLG10673) grown in EZ–rich glucose medium at 30°C. Medians and distributions are shown next to images.

(A) Cells with 1 focus pair.

(B) Cells with 2 focus pairs.

(C) A cell containing *rrnD*-CFP *and rrnA*-YFP (RLG10672) with an uneven number of foci (indicated by red arrows). The extra focus is from the *parS* site adjacent to *rrnA*, which is closer to the origin of replication than *rrnD*.and thus replicated first.

Figure S5. Additional examples of loci that do not co-localize

(A) *rrnC*, *rrnB* (RLG12002)

(B) *rrnC*, *rrnG* (RLG12005)

(C) *rrnD, yhdZ* (RLG10922)

(D) *rpsL*, *rrnD* (RLG11990)

(E) *rrnD, ispB* (RLG10928)

(F) *rrnD*, *yghJ* (RLG10996)

(G) *rrnD, mocA* (RLG11511)

(H) *rrnD, yjhE* (RLG11519)