

# Supporting Information

Jain et al. 10.1073/pnas.1211144109

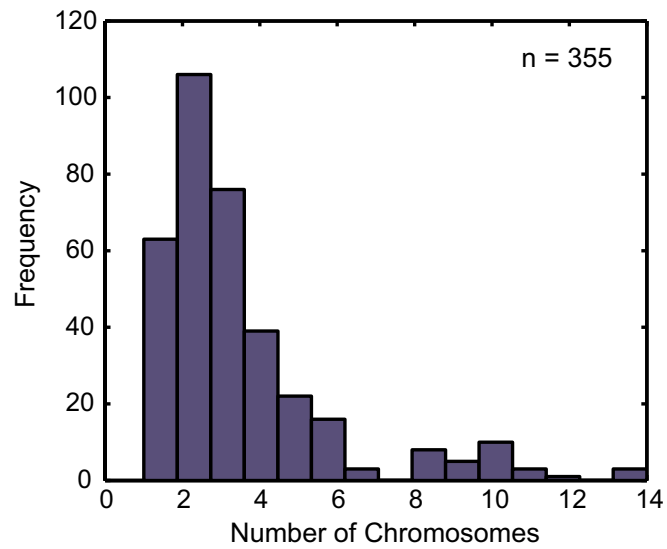
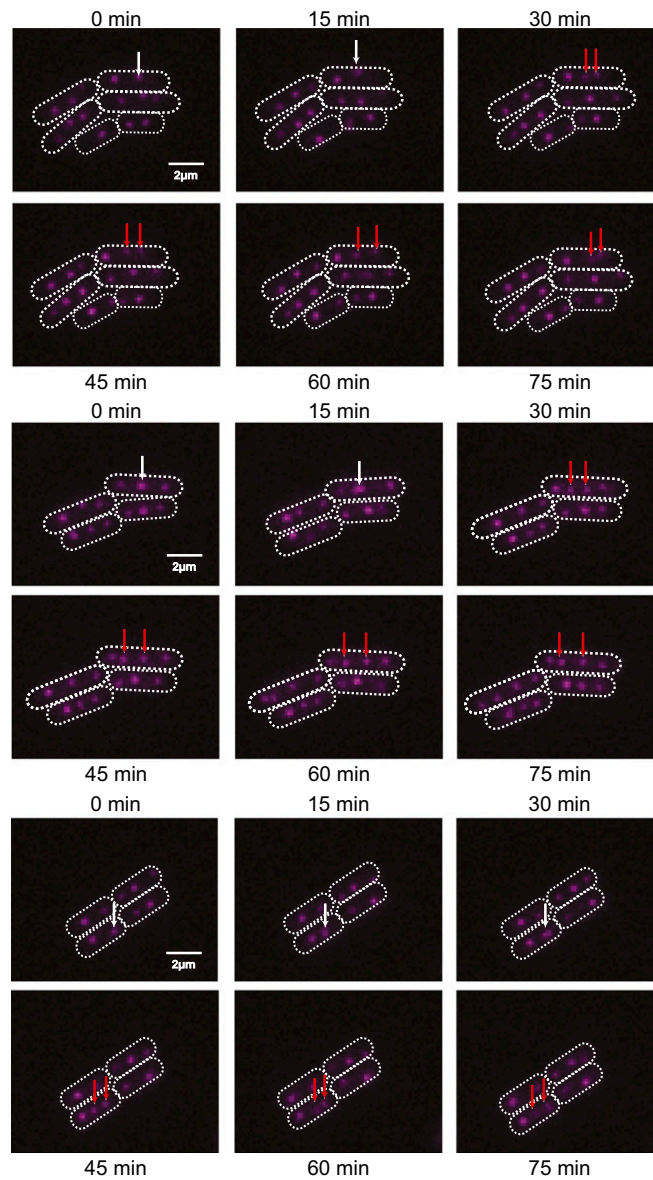


Fig. S1. Histogram of the number of chromosomes per cell in an exponentially growing wild-type population.







**Fig. S4.** Three time courses of wild-type cells. A single genomic locus proximal to the origin is labeled by using *tet* operator arrays (pink dots). White arrows point to a replicating chromosome and red arrows point to the resulting, replicated chromosomes. A single z-section is shown for each time course.

**Table S1. Table of plasmids**

Plasmid	Description	Resistance	Source
pAM1303	Neutral site 1 integration vector	Sp/Sm	(1)
pAM1579	Neutral site 2.1 integration vector	Kan (Amp)	(1)
pAM1573	Neutral site 2.1 integration vector	Cm (Amp)	(1)
EB2065	Neutral site 2.2 integration vector	Cm (Amp)	This work
EB2066	Site A integration vector (between chromosomal position 1595934 and 1595935 relative to GenBank CP000100)	Cm (Amp)	This work
EB2067	Site B integration vector (between chromosomal position 1758400 and 1758401 relative to GenBank CP000100)	Cm (Amp)	This work
eBB110	Source of 120 Tet operator repeats	Amp	(2)
pLAU43	Source of 120 Lac operator repeats	Amp	(3)
EB2068	120 Tet operators PCRed using primers pBBSall and pBBXbal from eBB110 cloned between Sall and Xbal of pAM1579	Kan (Amp)	This work
EB2069	120 Lac operators from Xbal, SmaI fragment of pLAU43 cloned between Xbal and SmaI of pAM1573	Cm (Amp)	This work
EB2070	120 Lac operators from SmaI, Sall fragment of pLAU43 cloned between SmaI and XhoI of EB2066	Cm (Amp)	This work
EB2071	120 Lac operators from SmaI, Sall fragment of pLAU43 cloned between SmaI and XhoI of EB2067	Cm (Amp)	This work
pJRC23	Source of ECFP	Sp/Sm	(4)
EB2072	<i>PkaiBC::tetR::ECFP PkaiBC::EYFP::lacI</i> cloned between NotI and SacI of pAM1303	Sp/Sm	This work
EB2073	<i>PkaiBC::tetR::EYFP</i> cloned between NotI and SacI of pAM1303	Sp/Sm	This work
EB2074	<i>PapA::rbcl::ECFP</i> cloned between SmaI and XhoI of EB2065	Cm (Amp)	This work
pAM2055	Source of gentamycin cassette for deletion vectors	Gm (Amp)	(1)
EB2075	<i>mreB (Synpcc7942_0300)</i> deletion vector with gentamycin cassette inserted between BamHI and SacI of pUC18	Gm (Amp)	This work
EB2076	<i>ftsZ (Synpcc7942_2378)</i> deletion vector with gentamycin cassette inserted between HindIII and SacI of pUC18	Gm (Amp)	This work
EB2077	<i>parA1 (Synpcc7942_0220)</i> deletion vector with gentamycin cassette inserted between BamHI and SacI of pUC18	Gm (Amp)	This work
EB2078	<i>minD (Synpcc7942_0896)</i> deletion vector with gentamycin cassette inserted between BamHI and SacI of pUC18	Gm (Amp)	This work
EB2079	<i>parA2 (Synpcc7942_1833)</i> deletion vector with gentamycin cassette inserted between BamHI and SacI of pUC18	Gm (Amp)	This work

Markers shown in parenthesis are additional markers used for selection of plasmids in *E. coli*. Cm, chloramphenicol; Gm, gentamycin; Kan, kanamycin; Sp/Sm, spectinomycin/streptomycin.

1. Mackey SR, Ditty JL, Clerico EM, Golden SS (2007) Detection of rhythmic bioluminescence from luciferase reporters in cyanobacteria. *Methods Mol Biol* 362:115–129.
2. Marquis KA, et al. (2008) SpoIIIE strips proteins off the DNA during chromosome translocation. *Genes Dev* 22:1786–1795.
3. Lau IF, et al. (2003) Spatial and temporal organization of replicating *Escherichia coli* chromosomes. *Mol Microbiol* 49:731–743.
4. Chabot JR, Pedraza JM, Luitel P, van Oudenaarden A (2007) Stochastic gene expression out-of-steady-state in the cyanobacterial circadian clock. *Nature* 450:1249–1252.

**Table S2. Table of *S. elongatus* strains**

Strain	Description	Plasmids used to create this strain from WT	Resistance	Source
EOC200	TetO (NS 2.1)	EB2068	Kan	This work
EOC201	TetO (NS 2.1), LacO (A)	EB2068, EB2070	Kan, Cm	This work
EOC202	TetO (NS 2.1), LacO (B)	EB2068, EB2071	Kan, Cm	This work
EOC203	TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1)	EB2068, EB2073	Kan, Sp/Sm	This work
EOC204	TetO (NS 2.1), LacO (A), <i>tetR::ECFP EYFP::lacI</i> (NS 1)	EB2068, EB2070, EB2072	Kan, Cm, Sp/Sm	This work
EOC205	TetO (NS 2.1), LacO (B), <i>tetR::ECFP EYFP::lacI</i> (NS 1)	EB2068, EB2071, EB2072	Kan, Cm, Sp/Sm	This work
EOC206	TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2068, EB2073, EB2074	Kan, Cm, Sp/Sm	This work
EOC207	$\Delta$ <i>mreB</i> incomplete segregation	EB2075	Gm	This work
EOC208	$\Delta$ <i>ftsZ</i> incomplete segregation	EB2076	Gm	This work
EOC209	$\Delta$ <i>parA1</i>	EB2077	Gm	This work
EOC210	$\Delta$ <i>minD</i>	EB2078	Gm	This work
EOC211	$\Delta$ <i>parA2</i>	EB2079	Gm	This work
EOC212	$\Delta$ <i>mreB</i> , TetO (NS 2.1)	EB2075, EB2068	Gm, Kan	This work
EOC213	$\Delta$ <i>ftsZ</i> , TetO (NS 2.1)	EB2076, EB2068	Gm, Kan	This work
EOC214	$\Delta$ <i>parA1</i> , TetO (NS 2.1)	EB2077, EB2068	Gm, Kan	This work
EOC215	$\Delta$ <i>minD</i> , TetO (NS 2.1)	EB2078, EB2068	Gm, Kan	This work
EOC216	$\Delta$ <i>parA2</i> , TetO (NS 2.1)	EB2079, EB2068	Gm, Kan	This work
EOC217	$\Delta$ <i>mreB</i> incomplete segregation, TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2075, EB2068, EB2073, EB2074	Gm, Kan, Sp/Sm, Cm	This work
EOC218	$\Delta$ <i>ftsZ</i> incomplete segregation, TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2076, EB2068, EB2073, EB2074	Gm, Kan, Sp/Sm, Cm	This work
EOC219	$\Delta$ <i>parA1</i> , TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2077, EB2068, EB2073, EB2074	Gm, Kan, Sp/Sm, Cm	This work
EOC220	$\Delta$ <i>minD</i> , TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2078, EB2068, EB2073, EB2074	Gm, Kan, Sp/Sm, Cm	This work
EOC221	$\Delta$ <i>parA2</i> , TetO (NS 2.1), <i>tetR::EYFP</i> (NS 1), <i>rbcl::ECFP</i> (NS 2.2)	EB2079, EB2068, EB2073, EB2074	Gm, Kan, Sp/Sm, Cm	This work

**Table S3. Table of primers**

Primer	Sequence
<b>Primers for site A (EB2066), B (EB2067), and NS 2.2 (EB2065) integration vectors</b>	
MCS_F	5' GTGCTTCTGGCTATAGCTGACTcgtcacgggtaaccga 3'
MCS_R	5' ccgcgctggggttaccag 3'
A-Up-F	5' GACCACACCCGTCTGTGGATCCTGCAACTATCCCTCGATC 3'
A-Up-R	5' AGTCAGCTATAGCCAGAAGCACTGGGAGGCATTAGAAGC 3'
A-Dn-F	5' ctggtaaccccagcgcggATGCGGATTGAAATTGTTTC 3'
A-Dn-R	5' TCGTCCGGCGTAGAGGATCCGTTCTCGACCACCCATTTCAG 3'
B-Up-F	5' GACCACACCCGTCTGTGGATCCGTTTGTGTAGGCCACGAT 3'
B-Up-R	5' AGTCAGCTATAGCCAGAAGCACTGGGAGGCATTAGAAGC 3'
B-Dn-F	5' ctggtaaccccagcgcggGACTGTGATGAATTACCTTCAG 3'
B-Dn-R	5' TCGTCCGGCGTAGAGGATCCAGTAAGCAACGTGGCC 3'
NS22-Up-F	5' GACCACACCCGTCTGTGCAGGCTCAGTGTGGTTCG 3'
NS22-Up-R	5' AGTCAGCTATAGCCAGAAGCAC 3'
NS22-Dn-F	5' ctggtaaccccagcgcggAGATCTCGCAGCGTAAAGCCGT 3'
NS22-Dn-R	5' TCGTCCGGCGTAGAGGATCCGATCCCATCCACGCAC 3'
<b>Primers for tet operator vector (EB2068)</b>	
pBBSall	5' TAAACTATGTGACCTTCTTATCTTGATAATAAGGGTAAC 3'
pBBXbal	5' ATAGTTTATCTAGAccgtccttgaacatgact 3'
<b>Primers for PapcA::Rbcl::ECFP vector (EB2074)</b>	
Smal_ApcA	5' CATGCCCGGGTACGAGCGCTATATCACCCC 3'
ApcA_RBS	5' TAATGGATTCCTCCAAGACTAGATTGAAACCAGACTGGCCTCCACC 3'
RBS_Rbcl	5' TCTAGTCTTGGAGGAATCCATTAATGCCCAAGACGCAATCTG 3'
Rbcl_linker	5' ACTAGAACCAGAACTACCAGTACTAGAGGCTTGTCCATCGTTTCGAATTC 3'
Linker_ECFP	5' TCTAGTGGTAGTCTTGGTCTAGTATGGTGAAGGCGGAGGAG 3'
ECFP_XhoI	5' CATGCTCGAGTTACTTGTACAGCTCGTCCATGC 3'
<b>Primers for deletion vectors (EB2075 to EB2079)</b>	
Gent_F	5' gacgcacaccgtgga 3'
Gent_R	5' gcggcgttgtagcaaa 3'
1833KO-Up-F	5' GATCGATCGGATCCACAAAAAGGGGCTGGTTAG 3'
1833KO-Up-R	5' GtttccacggtgtgctcACTAGT CGACAACCTCCCAAAGCG 3'
1833KO-Dn-F	5' AaattgtcacaacgcgcTGACTGACGCCTTTGACC 3'
1833KO-Dn-R_Sac	5' GATCGATCGAGTCCAAACAAAATGCCCAAAGT 3'
0300KO-Up-F	5' GATCGATCGGATCCGGTAGCCGAATCACTCCGA 3'
0300KO-Up-R	5' GtttccacggtgtgctcACTAGTTCGCCTTGATGACGTG 3'
0300KO-Dn-F	5' AaattgtcacaacgcgcAGCGGTGCGGAGCAG 3'
0300KO-Dn-R_Sac	5' GATCGATCGAGCTCAGAGTGTACTAGCGCTAGAAGTG 3'
0220KO-Up-F	5' GATCGATCGGATCCACCAGATATGCTCGATTGC 3'
0220KO-Up-R	5' GtttccacggtgtgctcACTAGTGGTGGATCTCAAGTTCAGGG 3'
0220KO-Dn-F	5' AaattgtcacaacgcgcCCATGACCCGCAAATCTG 3'
0220KO-Dn-R_Sac	5' GATCGATCGAGTCCAACTGAGCTTTGGCTTGCT 3'
2378KO-Up-F_HindIII	5' GATCGATCAAGCTTAACGGCTGCAGCGCTT 3'
2378KO-Up-R	5' GtttccacggtgtgctcACTAGTGGGGTGAGTAGTAGCA 3'
2378KO-Dn-F	5' AaattgtcacaacgcgcTGGCTGTCCGATCGCC 3'
2378KO-Dn-R_Sac	5' GATCGATCGAGTCTCTGTCAATGGTCCCCT 3'
0896KO-Up-F	5' GATCGATCGGATCCCGCCAGTAACCGTACAGAT 3'
0896KO-Up-R	5' GtttccacggtgtgctcACTAGTAGGGTCCGAAGAGCAGGAG 3'
0896KO-Dn-F	5' AaattgtcacaacgcgcGGTCTTTGCAGCAATGCT 3'
0896KO-Dn-R_Sac	5' GATCGATCGAGTCTGACCACCTAGCAAATGTTCC 3'

Table S4. Sequence of TetR and LacI fluorescent fusion protein constructs

EB2072: *PkaiBC*::**RBS**::*tetR*::**ECFP** *PkaiBC*::**RBS**::**EYFP**::**linker**::**lacI** cloned between *NotI* and *SacI* of pAM1303. Note that the beginning of *PkaiBC* contains the *kaiA* terminator which will serve to decouple TetR and LacI expression.

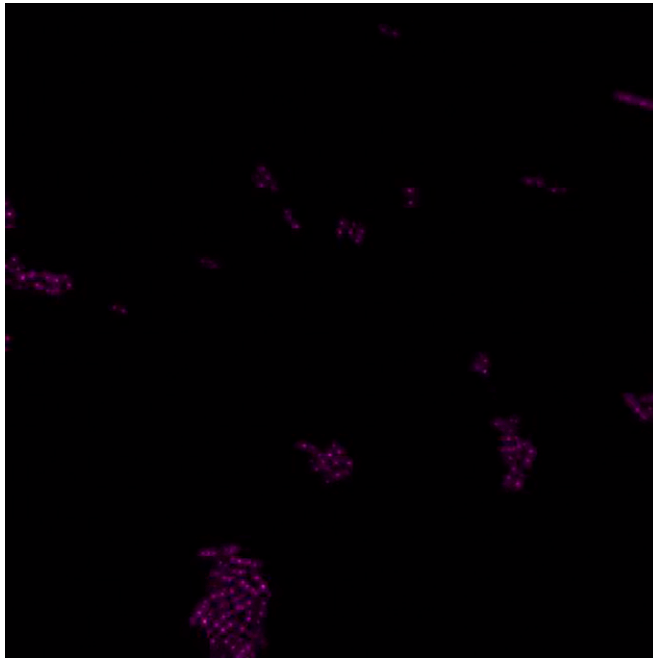
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Table S4. Cont.

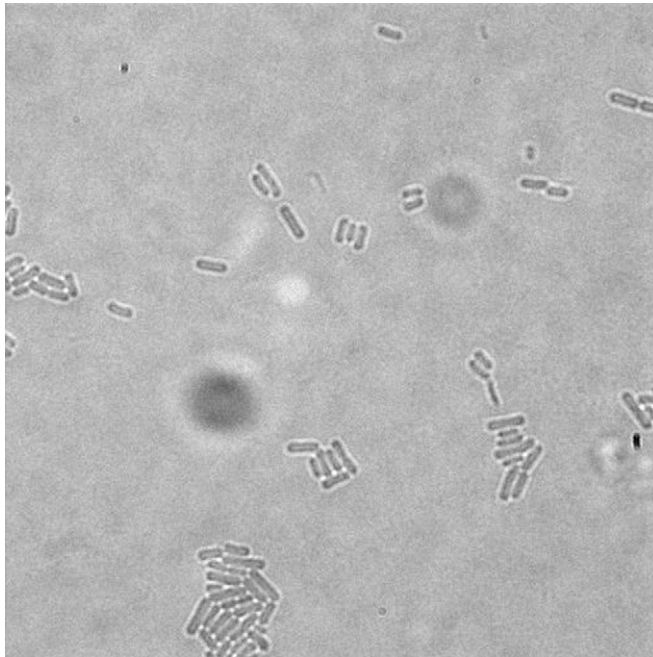
EB2073: *PkaiBC*::*RB3*::*tetR*::*linker*::*EYFP* cloned between *NotI* and *SacI* of pAM1303

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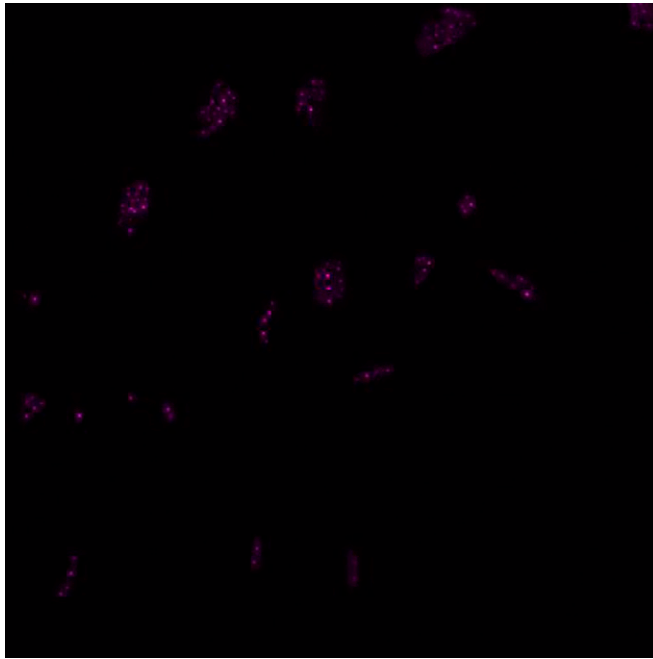
**Movie S1.** Time-lapse movies of wild-type cells with labeled chromosomes (TetR-EYFP). Individual frames are separated by 1 h. A single z-plane is shown.

[Movie S1](#)



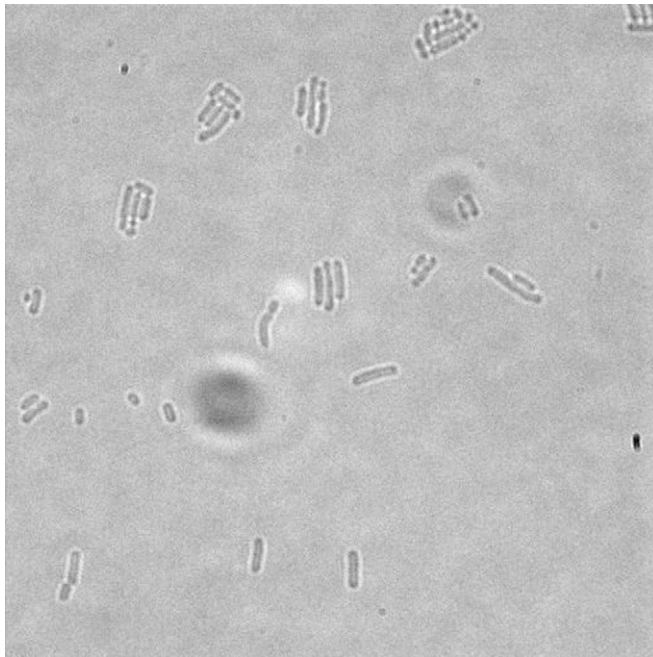
**Movie S2.** Corresponding DIC images for [Movie S1](#).

[Movie S2](#)



**Movie S3.** Time-lapse movies of  $\Delta minD$  cells with labeled chromosomes (TetR-EYFP). Individual frames are separated by 1 h. A single z-plane is shown.

[Movie S3](#)



**Movie S4.** Corresponding DIC images for [Movie S3](#).

[Movie S4](#)