Supplemental material

Table S4.1. List of bacterial strains used in this study.

Strain	Characteristics	Source
E. coli MG1655 (wild type)	K-12; F^- λ^- *rph-1*	ATCC
E. coli Δ3D	MG1655; *strR* Δ*DARS1* Δ*DARS2* Δ*datA*	[318]
E. coli DH5α	MG1655; *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG* ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF)U169 hsdR17* (r_K^- m_K^+)	NEB
E. coli DH5α x pCas9	DH5α harbouring the pCas9 plasmid	This study
E. coli DH5α x pCas9_ampR	DH5α harbouring the pCas9_ampR plasmid	This study
E. coli DH5α x pTarget_dnaA-PAFP	DH5α harbouring one of the 9 plasmids of the pTarget_dnaA-PAFP series	This study
E. coli MG1655 x pCas9, pTarget_dnaA-PAFP	MG1655 harbouring the pCas9 plasmid and one of the ten plasmids of the pTarget_dnaA-PAFP series	This study
E. coli MG1655 *dnaA-PAFP* x pCas9, pTarget_dnaA-PAFP	MG1655 harbouring the pCas9 plasmid and one of the ten plasmids of the pTarget_dnaA-PAFP series; *dnaAΔ(259-312)::PAFP*	This study
E. coli dnaA-PAmCherry2.1	MG1655; *dnaAΔ(259-312)::PAmCherry2.1*	This study
E. coli dnaA-dronpa2	MG1655; *dnaAΔ(259-312)::dronpa2*	This study
E. coli dnaA-mEos4b	MG1655; *dnaAΔ(259-312)::mEos4b*	This study
E. coli dnaA-mMaple3	MG1655; *dnaAΔ(259-312)::mMaple3*	This study
E. coli Δ3D x pCas9_ampR, pTarget_dnaA-PAmCherry2.1	MG1655 harbouring the pCas9_ampR and the pTarget_dnaA-PAmCherry2.1 plasmids; *strR* Δ*DARS1* Δ*DARS2* Δ*datA*	This study
E. coli Δ3D *dnaA-PAmCherry2.1* x pCas9_ampR, pTarget_dnaA-PAmCherry2.1	MG1655 harbouring the pCas9_ampR and the pTarget_dnaA-PAmCherry2.1 plasmids; *strR* Δ*DARS1* Δ*DARS2* Δ*datA dnaAΔ(259-312)::PAmCherry2.1*	This study
E. coli Δ3D *dnaA-PAmCherry2.1*	MG1655; *strR* Δ*DARS1* Δ*DARS2* Δ*datA dnaAΔ(259-312)::PAmCherry2.1*	This study

Table S4.2. List of plasmids used in this study. *DnaA(n1-n2)* stands for the sequence of the *dnaA* gene comprised between base pair n1 and base pair n2, counting from the G at position 1.

Plasmid	Characteristics	Source
pCas9	*repA101*(Ts) *kanR cas9* P_{araB}*-Red lacI*^{q} P_{trc}-sgRNA-*pMB1*	[319]
pCas9_ampR	pCas9; Δ*kanR ampR*	This study
pTarget	pMB1; *aadA pJ23119*	[319]
pTarget_dnaA-PAFP series		
pTarget_dnaA-PAmCherry2.1	pTarget; *pJ23119*-sgRNA(*dnaA*) *dnaA(209-258)-PAmCherry2-dnaA(313-362)*	This study
pTarget_dnaA-Dronpa2	pTarget; *pJ23119*-sgRNA(*dnaA*) *dnaA(209-258)-Dronpa2-dnaA(313-362)*	This study
pTarget_dnaA-mEos4b	pTarget; *pJ23119*-sgRNA(*dnaA*) *dnaA(209-258)-mEos4b-dnaA(313-362)*	This study
pTarget_dnaA-mMaple3	pTarget; *pJ23119*-sgRNA(*dnaA*) *dnaA(209-258)-mMaple3-dnaA(313-362)*	This study

Table S4.3. List of oligonucleotides and chemically synthesised DNA fragments and their use in the study.

Identifier	Sequence (5′-3′)	Used for
BG25452	ATGTCGGTGATCAAACCAGATATGAAGATCAAACTTCGTATGGAGGGAGCGGT CAATGGGCATCCTTTCGCGATCGAGGGTGTCGGGCTGGGCAAACCCTTCGAA GGGAAGCAAAGTATGGACTTAAAAGTCAAAGAGGGAGGCCCGTTACCCTTTG CGTATGATATTTTGACCACAGTTTTTTGCTACGGGAATCGCGTATTTGCTAAGTA CCCGGAAAACATCGTCGATTACTTCAAACAGAGCTTCCCAGAAGGATATAGTT GGGAGCGCTCAATGAACTACGAGGACGGTGGCATTTGTAATGCGACGAACGA CATTACATTAGACGGCGACTGCTACATTTATGAGATCCGTTTCGACGGTGTCAA CTTTCCAGCGAATGGACCTGTGATGCAAAAGCGCACTGTAAAATGGGAACCTT CAACCGAGAAGTTATATGTGCGTGACGGGGTCTTAAAAGGCGATGTAAATACC GCTTTGAGTTTAGAGGGAGGTGGCCATTACCGTTGCGATTTCAAAACGACTTAT AAGGCAAAAAAAGTGGTACAATTACCTGATTATCACTTCGTCGATCATCACATC GAGATTAAATCACATGATAAGGACTATTCTAATGTGAATCTTCACGAACATGCC GAGGCCCATAGTGAGTTACCGCGTCAAGCAAAGTAA	*E. coli* codon-optimised *dronpa2.*
BG28278	ATGGTTAGTGCGATTAAACCAGATATGCGTATCAAGTTGCGTATGGAGGGAAA TGTTAATGGACATCACTTCGTCATTGATGGAGACGGAACTGGCAAGCCGTACG AGGGTAAGCAGACCATGGACCTGGAGGTCAAGGAGGGTGGCCCATTGCCGTT CGCGTTTGATATCCTGACGACAGCTTTCCACTATGGGAATCGTGTCTTCGTAAA ATATCCAGATAACATCCAGGACTATTTCAAGCAGTCATTTCCCAAAGGTTACTCT TGGGAACGCAGCTTAACCTTCGAAGACGGGGGGATTTGCAACGCCCGCAACG ACATTACTATGGAGGGAGACACGTTTTACAACAAAGTGCGTTTTTATGGAACAA ACTTCCCGGCGAACGGGCCTGTTATGCAGAAGAAGACTCTGAAGTGGGAGCC GTCCACGGAAAAGATGTACGTGCGCGATGGGGTCTTAACTGGGGATATTGAG ATGGCCTTGCTGCTTGAAGGTAATGCTCACTACCGCTGCGATTTCCGTACGACC TATAAGGCAAAAGAAAAGGGGGTCAAGTTGCCAGGTGCTCATTTTGTTGATCA CGCGATTGAAATTTTGTCGCATGATAAGGATTACAATAAGGTTAAACTTTACGA GCATGCGGTGGCTCATAGCGGTCTTCCCGACAATGCCCGTCGTTAA	*E. coli* codon-optimised *mEos4b.*
BG30037	ATGGTCTCTAAGGGAGAAGAGACGATCATGTCGGTAATCAAACCGGATATGAA AATCAAGCTTCGCATGGAAGGTAACGTCAACGGTCACGCCTTTGTCATCGAAG GTGAAGGCTCAGGTAAGCCATTTGAAGGTATCCAAACCATCGACTTGGAAGTA AAGGAGGGTGCGCCTTTACCATTCGCATACGACATTCTTACCACCGCTTTCCAT TACGGAAACCGCGTGTTCACCAAGTACCCTCGCAAGATCCCTGACTACTTCAAA CAGAGCTTCCCAGAGGGATATTCTTGGGAACGTAGTATGACGTACGAGGACG GGGGTATCTGCAATGCGACTAATGATATTACAATGGAAGAAGATTCGTTTATCA ATAAAATTCACTTCAAAGGTACGAATTTTCCGCCCAACGGCCCGGTAATGCAGA AACGTACTGTAGGTTGGGAGGTCTCGACTGAAAAGATGTATGTTCGTGACGGC GTGCTGAAGGGAGATGTTAAAATGAAGCTGCTTCTTAAGGGTGGCTCCCATTAT CGCTGTGATTTTCGTACCACATACAAGGTGAAGCAAAAAGCAGTGAAATTGCC CAAAGCACACTTTGTCGACCATCGTATCGAGATCCTTTCTCACGATAAAGATTA CAACAAGGTTAAGTTGTATGAACATGCTGTAGCTCGCAATTCCACAGATAGTAT GGACGAGCTGTATAAATAA	*E. coli* codon-optimised *mMaple3.*
BG21364	TCAGCCTTAGTCATTATCGAC	Amplified the *dnaA* gene in *E. coli* chromosome
BG21365	GGTTTACGATGACAATGTTCTG	Amplified the *dnaA* gene in *E. coli* chromosome

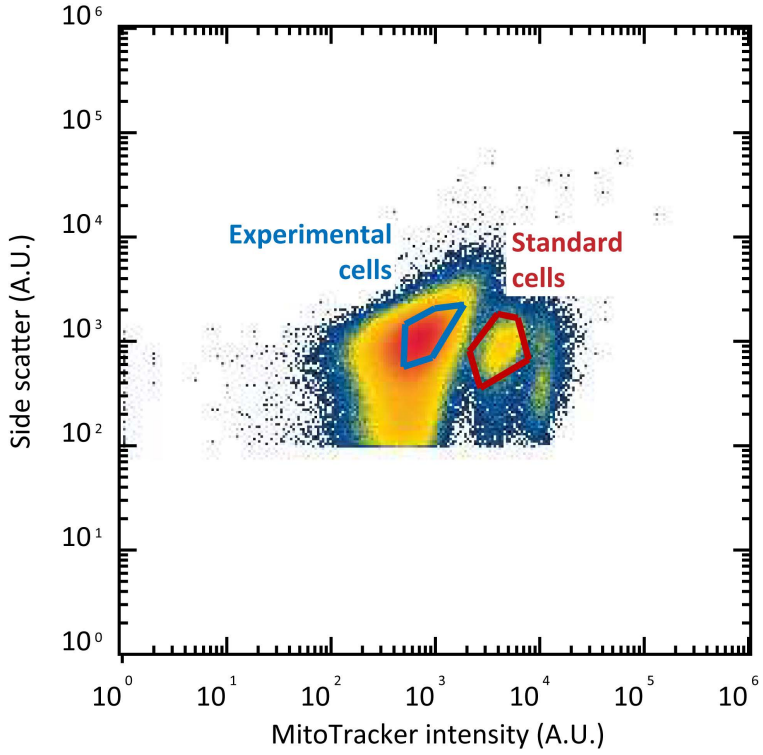

Figure S4.1. Separation of standard and experimental cell by YL3-H gating. Cells with a higher red emission, derived from the MitoTracker™ dye, were gated as standard cells, whereas the other population was gated as experimental cells.

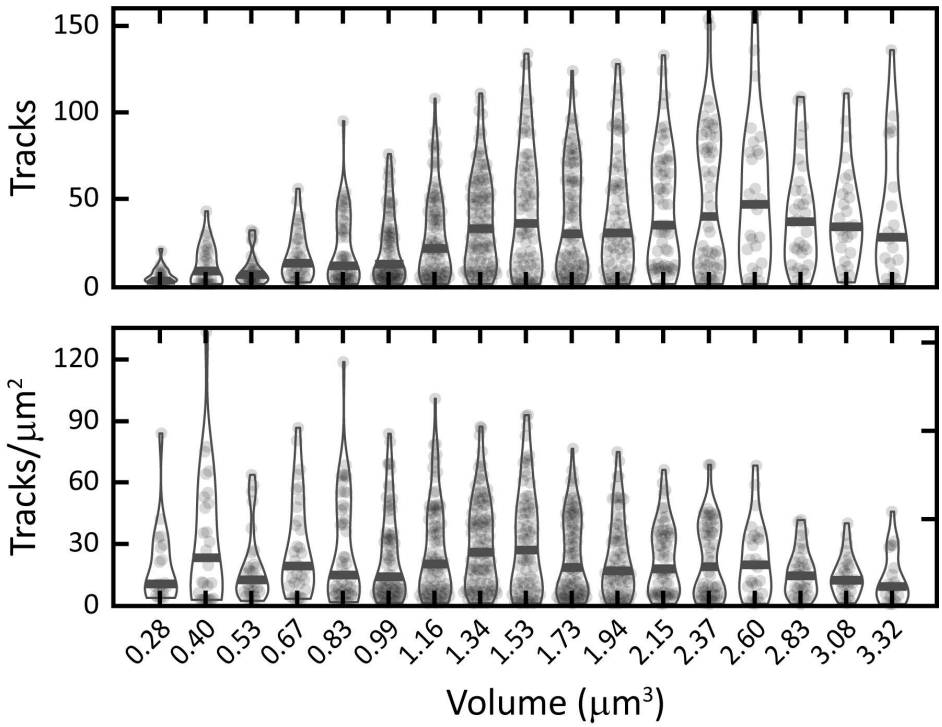

Figure S4.2. Tracks and concentration of *Lb*dCas12a under the control of simple repression, mediated by the *pLtetO-1* promoter and the related TetR repressor. On the contrary of negative autoregulation, simple repression causes accumulation of new protein only until a certain level. After this level, the track count stabilises and the concentration decreases.

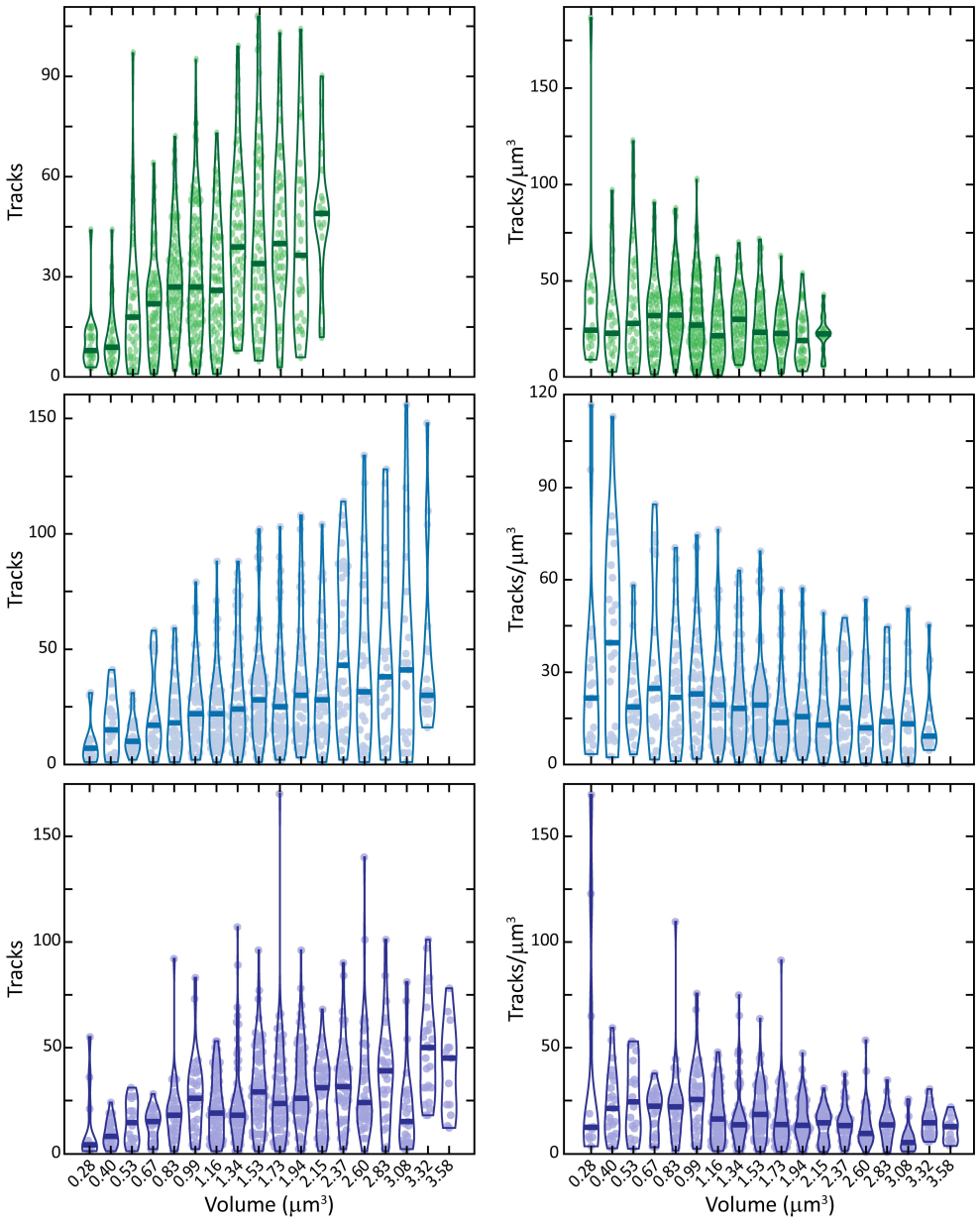

Figure S4.3. Track number and concentration for DnaA in *E. coli* Δ3D. For each growth condition and cellular volume range, the number of recorded tracks per cell (left) and the concentrations of recorded tracks per cell (tracks/µm³, right) are plotted as violin plots. On the x-axis, the number indicates the middle value of the range. Dots represent individual values of cells in the indicated volume range, whereas bold lines represent the median. Green indicates values of cells grown in slow growth regime, blue values of cells grown in intermediate growth regime and purple values of cells grown in fast growth regime.

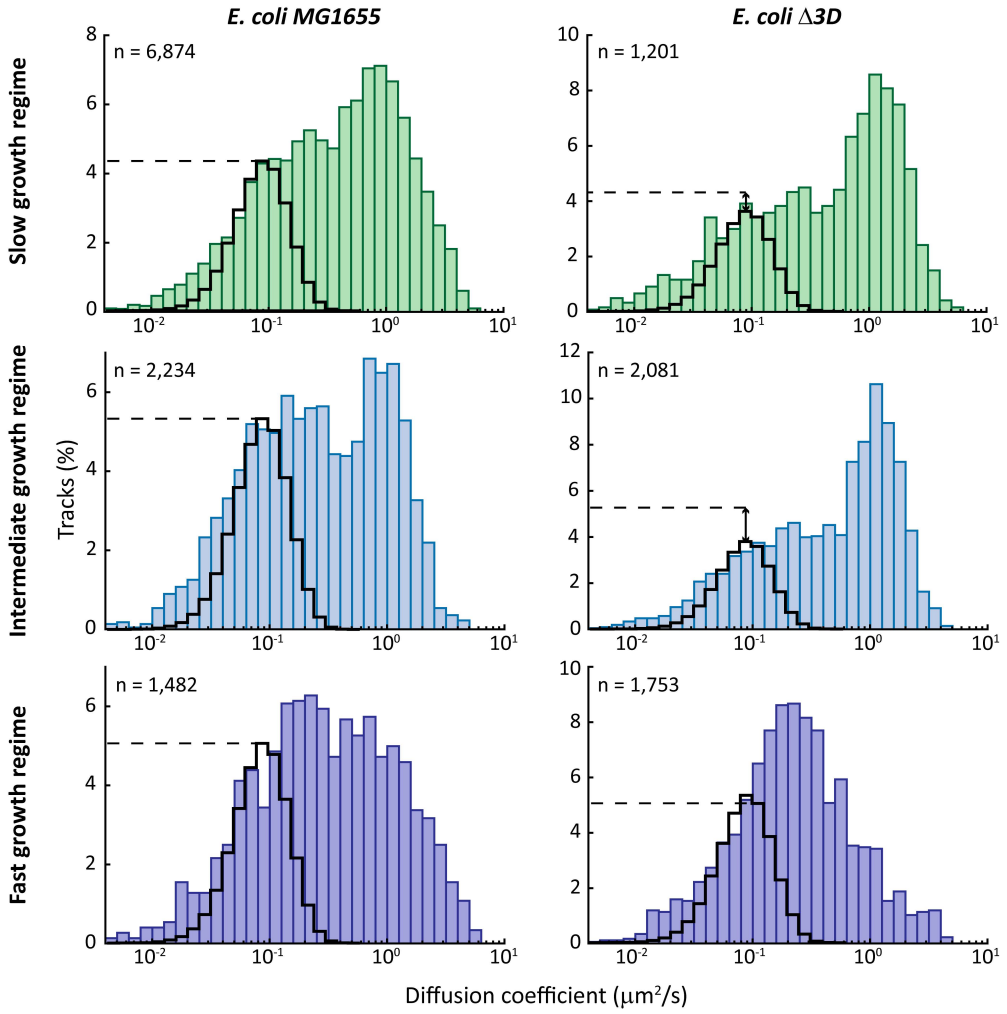

Figure S4.4. Persistent binding of DnaA across growth conditions and *E. coli* genotype. Diffusional coefficients of DnaA tracks longer than 5 steps (> 6 localisations, > 50 ms long), collected into 36 logarithmic-divided bins from D* = 0.04 μm²/s to D* = 10 μm²/s. Green histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* Δ3D cells grown in slow growth regime. Blue histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* Δ3D cells grown in intermediate growth regime. Purple histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* Δ3D cells grown in fast growth regime. Black lines represent an hypothetical immobile and static species with a track length of 5 and was used as a qualitative measure for persistent binding in each condition. The dashed line represent the percentage of DnaA tracks in the wild type genotype at the peak of the hypothetical immobile species. The line was used as a comparison between the two strains to qualitative assess a reduction in persistent binding upon deletion of *DARS1, DARS2* and *datA*.

Source data

Source Data Table 4.1. Source data of kinetic rates of DnaA in either slow, intermediate or fast growth regime, used for the scatter plot in Figure 4.2C.

	$k_{Free \rightarrow bound}$ (s^{-1})		$k_{Bound \rightarrow free}$ (s^{-1})	
Growth regime	Value	St. dev.	Value	St. dev.
Slow	110	5	46	4
Intermediate	107	5	57	3
Fast	95	9	75	7

Source Data Table 4.2. Source data of kinetic rates of DnaA in the wild type *E. coli* MG1655, in either slow, intermediate or fast growth regime, used for the scatter plot in Figure 4.2C.

Growth regime	Bound fraction (%)	St. dev.
Slow	70.5	4.3
Intermediate	65.2	3.8
Fast	55.8	6.5

Source Data Table 4.3. Extremes of each volume range used for the violin plots in Figure 4.3 and Figure 4.4D.

Pixel range	Volume range (μm^3)	Middle point (μm^3)
50-65	0.23-0.34	0.28
66-80	0.34-0.46	0.40
81-95	0.46-0.6	0.53
96-110	0.6-0.75	0.67
111-125	0.75-0.9	0.83
126-140	0.9-1.07	0.99
141-155	1.07-1.25	1.16
156-170	1.25-1.44	1.34
171-185	1.44-1.64	1.53
186-200	1.64-1.83	1.73
201-215	1.83-2.04	1.94
216-230	2.04-2.26	2.15
231-245	2.26-2.46	2.37
246-260	2.46-2.71	2.60
261-275	2.71-2.95	2.83
276-290	2.95-3.2	3.08
291-305	3.2-3.45	3.32
306-320	3.45-3.71	3.58

Source Data Table 4.4. Source data of kinetic rates and derived bound fractions of DnaA in the mutant *E. coli* Δ3D, in either slow, intermediate or fast growth regime, used for the bar plot of bound fractions in Figure 4.4E.

	$k_{Free-->Bound}$		$k_{Bound-->Free}$		Bound fraction	
Regime	Value	St. dev.	Value	St. dev.	Value	St. dev.
Slow	80	5	58	4	0.57971	0.045125
Intermediate	109	5	63	7	0.633721	0.043007
Fast	116	7	68	8	0.630435	0.052667

Source Data Table 4.5. Source data of volumes of initiation of either the wild type *E. coli* MG1655 or the mutant *E. coli* Δ3D, in either slow, intermediate or fast growth regime, used for the bar plot in Figure 4.4F.

Strain	Growth regime	Volume initiation	St. dev.
E. coli MG1655	Slow	0.527963242	0.128615451
	Intermediate	0.366663738	0.068312876
	Fast	0.377714632	0.032840001
E. coli Δ3D	Slow	0.606818985	0.07243655
	Intermediate	0.686966969	0.057704679
	Fast	0.534894582	0.050480645

Source Data Table 4.6. Cellular volume and number of origins of either *E. coli* MG1655 or *E. coli* Δ3D in either slow, intermediate or fast growth regime, used to estimate the volume of initiation plotted in the bar graph of Figure 4.4F.

		Average volume (μm³)		Number of origins	
Strain	Growth regime	Value	St. dev.	Value	St. dev.
E. coli MG1655	Slow	0.678465868	0.16522444	1.853953582	0.011595011
	Intermediate	1.010858586	0.187395417	3.977379052	0.073825525
	Fast	1.286390533	0.107618857	4.91341633	0.116306801
E. coli Δ3D	Slow	1.217928	0.144470366	2.895589507	0.038714931
	Intermediate	1.846496063	0.154868456	3.877814851	0.017952345
	Fast	1.797699029	0.132968393	4.848677779	0.284203588