Supplementary Materials

Recombinase and translesion DNA polymerase decrease the speed of replication fork progression during the DNA damage response in *Escherichia coli* cells.

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Table S1. Bacterial strains used in this study

Name	Bacterial genotype	Reference or source
MG1655	sequenced wild-type *E. coli* K12	(1) [a]
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	(2)
eCOMB	MG1655 except Δ*thyA* Δ(*yjjG-deoB*)	(3)
JW0059	BW25113 except Δ*polB*::*kan*	Keio Collection: (4) [b]
JW0221	BW25113 except Δ*dinB*::*kan*	Keio Collection: (4) [b]
JW0941	BW25113 except Δ*sulA*::*kan*	Keio Collection: (4) [b]
JW1850	BW25113 except Δ*ruvA*::*kan*	Keio Collection: (4) [b]
JW2549	BW25113 except Δ*recO*::*kan*	Keio Collection: (4) [b]
JW2669	BW25113 except Δ*recA*::*kan*	Keio Collection: (4) [b]
JW3786	BW25113 except Δ*uvrD*:*kan*	Keio Collection: (4) [b]
MK7004	BW25113 except Δ*umuDC*::*kan*	this work [c]
MK7451	eCOMB except Δ*dinB*	this work: P1(JW0221) × eCOMB [d]
MK7452	eCOMB except Δ*sulA*::*kan*	this work: P1(JW0941) × eCOMB
MK7453	eCOMB except Δ*sulA*	this work: P1(JW0941) × eCOMB [d]
MK7455	eCOMB except Δ*sulA* Δ*dinB*	this work: P1(JW0941) × MK7451 [d]
MK7456	eCOMB except Δ*sulA* Δ*lexA*::*kan*	this work [c]
MK7460	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*dinB*	this work: P1(MK7456) × MK7455
MK7462	eCOMB except Δ*sulA* Δ*polB*	this work: P1(JW0059) × MK7453 [d]
MK7463	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*polB*	this work: P1(MK7456) × MK7462
MK7465	eCOMB except Δ*sulA* Δ*umuDC*	this work: P1(MK7004) × MK7453 [d]
MK7466	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*umuDC*	this work: P1(MK7456) × MK7465
MK7485	eCOMB except Δ*sulA* Δ*recA*	this work: P1(JW2669) × MK7453 [d]
MK7486	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*recA*	this work: P1(MK7456) × MK7485/pRECA1 [e]
MK7488	eCOMB except Δ*sulA* Δ*uvrD*	this work: P1(JW3786) × MK7453 [d]
MK7489	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*uvrD*	this work: P1(MK7456) × MK7488
MK7496	eCOMB except Δ*sulA* Δ*dinB* Δ*recA*	this work: P1(JW0221) × MK7485/pRECA1 [f]
MK7498	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*dinB* Δ*recA*	this work: P1(MK7456) × MK7496/pRECA1 [e]
MK7913	eCOMB except Δ*sulA* Δ*ruvA*	this work: P1(JW1850) × MK7453 [d]
MK7916	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*ruvA*	this work: P1(MK7456) × MK7913
MK7922	eCOMB except *sulA*::*lacZ'YA*::*kan*	this work: P1(SY2) × eCOMB
MK7925	SMR7467 except *sulA*::*lacZ'YA*::*kan*	this work: P1(SY2) × SMR7467
MK7926	SMR7623 except *sulA*::*lacZ'YA*::*kan*	this work: P1(SY2) × SMR7623
MK7930	eCOMB except Δ*sulA* Δ*recO*	this work: 1(JW2549) × MK7453 [d]
MK7933	MK7922 except *recA441*(Ts) *srl300*::Tn*10*	this work: P1(RM112) × MK7922
MK7934	MK7922 except *srl300*::Tn*10*	this work: P1(RM112) × MK7922
MK7954	eCOMB except Δ*sulA* Δ*lexA*::*kan* Δ*recO*	this work: P1(MK7456) × MK7930
MK7961	MK7933 except *lexA3*(Ind^{-}) *malB*::Tn*9*	this work: P1(SMR7467) × MK7933
RM112	*lexA51*(Def) *uvrA6 recA441*(Ts) *srlC300*::Tn*10*	(5) [g]
SMR7467	MG1655 except *lexA3*(Ind^{-}) *malB*::Tn*9* Δ*attλ*::P$_{sulA}$Ω*gfp-mut2*	(6) [g]
SMR7623	MG1655 except *lexA51*(Def) *malB*::Tn*9* Δ*attλ*::P$_{sulA}$Ω*gfp-mut2 sulA211*	(6) [g]
SY2	F^{-} Δ*lacX74 strA araD139* Δ(*ara lue*)7697 *galU*$^{-}$ *galK*$^{-}$ *hsr*$^{-}$ *hsm*$^{+}$ *sulA*::*lacZ'YA*::*kan*	(7) [b]

All strains are derivatives of *E. coli* K12. For P1 transduction, "P1(A) × B" means that strain B was

infected by P1 phage grown on strain A. MK7485/pRECA1 and MK7496/pRECA1 represent MK7485 and MK7496, respectively, carrying the *recA*-expressing plasmid pRECA1 (Table S2).

[a] MG1655 was purchased from the Coli Genetic Stock Center at Yale University, USA.

[b] Keio Collection and SY2 strains were obtained from the National BioResource Project: *E. coli* (National Institute of Genetics, Japan).

[c] MK7004 and MK7456 were constructed by disrupting *umuDC* and *lexA*, respectively, with the kanamycin resistance gene (*kan*) using the chromosomal gene disruption method with Red recombinase (2). The MK7004 strain was constructed by Daichi Ogawara (Nara Institute of Science and Technology, Japan).

[d] After P1(*vir*) transduction, *kan* was deleted by flippase-mediated recombination (2).

[e] After P1 transduction, pRECA1 was eliminated by incubating cells at 42 °C.

[f] After P1(*vir*) transduction, the temperature-sensitive plasmid pRECA1 was removed by incubating transductants at 42 °C, and then *kan* was deleted by flippase-mediated recombination.

[g] RM112 and SMR strains were obtained from Dr. Mark D. Sutton (University at Buffalo, State University of New York, USA) and Dr. Susan M. Rosenberg (Baylor College of Medicine, USA), respectively.

References for Table S1

1. Guyer, M.S., Reed, R.R., Steitz, J.A. and Low, K.B. (1981) Identification of a sex-factor-affinity site in *E. coli* as γδ. *Cold Spring Harb. Symp. Quant. Biol.,* **45 Pt 1**, 135-140.
2. Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA.*, **97**, 6640-6645.
3. Pham, T.M., Tan, K.W., Sakumura, Y., Okumura, K., Maki, H. and Akiyama, M.T. (2013) A single-molecule approach to DNA replication in *Escherichia coli* cells demonstrated that DNA polymerase III is a major determinant of fork speed. *Mol. Microbiol.*, **90**, 584-596.
4. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L. and Mori, H. (2006) Construction of *Escherichia col*i K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.*, **2**, 2006 0008.
5. Maul, R.W. and Sutton, M.D. (2005) Roles of the *Escherichia coli* RecA protein and the global SOS response in effecting DNA polymerase selection *in vivo*. *J. Bacteriol.*, **187**, 7607-7618.
6. Pennington, J.M. and Rosenberg, S.M. (2007) Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nature Genet.*, **39**, 797-802.
7. Ohmori, H., Saito, M., Yasuda, T., Nagata, T., Fujii, T., Wachi, M. and Nagai, K. (1995) The *pcsA* gene is identical to *dinD* in *Escherichia coli*. *J. Bacteriol.*, **177**, 156-165.

Table S2. Plasmids used in this study

Name	Plasmid properties	Reference or source
pCP20	flippase helper plasmid	(1)
pKD13	template plasmid with FRP-flanked *kan*	(1)
pKD46	Red recombinase-expressing plasmid	(1)
pNTR-*thrA*	Mobile plasmid clone with P_{tac}-*thrA* (*amp*)	(2) [a]
pNTR-*lexA*	Mobile plasmid clone with P_{tac}-*lexA* (*amp*)	(2) [a]
pRECA1	a temperature-sensitive pSC101 plasmid (*cam*) carrying *recA*$^+$	K.O. Yoshiyama [b]
pCC1BAC	a single-copy vector (*cam*)	Epicentre, USA
pSCG3	pCC1BAC carrying *dinBo-21* (operator-constitutive *dinB*$^+$)	this work; (3)
pSCG11	pCC1BAC carrying *recAo* (operator-constitutive *recA*$^+$)	this work

[a] Mobile plasmids were obtained from the National BioResource Project: *E. coli* (National Institute of Genetics, Japan).

[b] Plasmid pRECA1 was a kind gift from Dr. Kaoru O. Yoshiyama (Kyoto Sangyo University, Japan).

Abbreviations are as follows: *amp*, ampicillin resistance gene; *cam*, chloramphenicol resistance gene; FRP, flippase recognition target; *kan*, kanamycin resistance gene; P_{tac}, Tac promoter.

References for Table S2

1. Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA.*, **97**, 6640-6645.
2. Saka, K., Tadenuma, M., Nakade, S., Tanaka, N., Sugawara, H., Nishikawa, K., Ichiyoshi, N., Kitagawa, M., Mori, H., Ogasawara, N. and Nishimura, A. (2005) A complete set of *Escherichia coli* open reading frames in mobile plasmids facilitating genetic studies. *DNA Res.*, **12**, 63-68.
3. Galhardo, R.S., Do, R., Yamada, M., Friedberg, E.C., Hastings, P.J., Nohmi, T. and Rosenberg, S.M. (2009) DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in *Escherichia coli*. *Genetics*, **182**, 55-68.

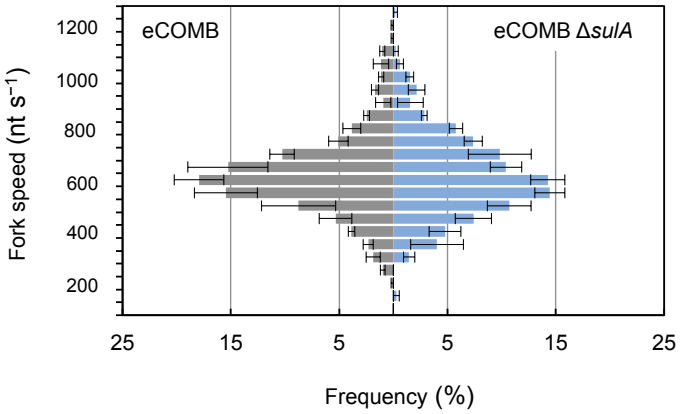
Table S3. Oligonucleotides used in this study

Name	DNA sequences of oligonucleotides (5' → 3')	Purpose
dKm-F2	CAAGAACAGA CTACTGTATA TAAAA ACAGT ATAACTTCAG GCAGATTATT GTGTAGGCTG GAGCTGCTTC	amplification of FRT-flanked *kan* with pKD13 as template to disrupt *umuDC*
dKm-R2	GCTAATCCAT TCGGCGCTCC TGCGG GAGCG CTTTTTTCCT GCCGCTATAT CTGTCAAACA TGAGAATTAA	amplification of FRT-flanked *kan* with pKD13 as template to disrupt *umuDC*
JW4003-KN	TGCTGTATAT ACTCACAGCA TAACT GTATA TACACCCAGG GGGCGGAATG ATTCCGGGGA TCCGTCGACC	amplification of FRT-flanked *kan* with pKD13 as template to disrupt *lexA*
JW4003-KC	CCAGGCGGCA TCGCGGTCTC AGAGA TATGT TACAGCCAGT CGCCGTTGCG TGTAGGCTGG AGCTGCTTCG	amplification of FRT-flanked *kan* with pKD13 as template to disrupt *lexA*
recA-BF1	**ACTATAGGGC GAATT**ACGCG TAGAT GATCGGCGTACGCGAAG	amplification of *recA* containing an operator-constitutive mutation
recA-MR1	GACATTGCTC ATACAGTATC AAGTG TTTTGTAG	amplification of *recA* containing an operator-constitutive mutation
recA-MF1	TGTATGAGCA ATGTCTATAA TTGCT TCAACAGAAC ATATTGACTA TCCG	amplification of *recA* containing an operator-constitutive mutation
recA-BR1	**TACCGAGCTC GAATT**GCTAG CGGGA TGTTGATTCT GTCATGGC	amplification of *recA* containing an operator-constitutive mutation
dinB-BF2X	atccgGAATT CACAACAACT GGAAC CTTTCGGGTG	amplification of *dinBo-21* (operator-constitutive *dinB*)
dinB-MR2	GTATACCTTG ATTTCAGGGT TTGAG AAATGCGTAAAG	amplification of *dinBo-21* (operator-constitutive *dinB*)
dinB-BR3X	atacgGGATC CCGATGCATA CAGTG ATACCCTC	amplification of *dinBo-21* (operator-constitutive *dinB*)
dinB-MF2	CTCAAACCCT GAAATCAAGG TATAC TTTACCAGTG TTGAGAGGTG AG	amplification of *dinBo-21* (operator-constitutive *dinB*)

Nucleotides complementary to their genomic target sequences are shown in normal upper-case letters without underline. Single-underlined and bold nucleotides are complementary sequence to, respectively, pKD13 (see Table S2) and pCC1BAC (Epicentre). Nucleotides underlined with double, broken, dotted and wavy lines are *Eco*RI, *Bam*HI, *Nhe*I and *Mlu*I sites, respectively. Highlighted nucleotides are mutations introduced into amplified DNA fragments. The lower-case sequences are extra nucleotides for efficient digestion with restriction enzymes. *kan* denotes the kanamycin resistance gene.

Fig. S1.

A

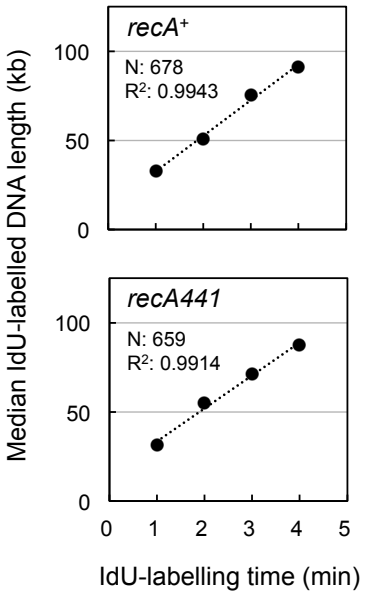


B

Strain	Total number of DNAs observed	Median length of IdU (kb)		Fork speed (nt sec^{-1})	
		2 min	3 min	Mean	SEM
MK7456 /pNTR-*lexA*	342	71.8	105.0	602	56
	338	69.2	101.6		
	304	69.8	112.6		
MK7456 /pNTR-*thrA*	271	51.1	68.7	322	16
	255	51.4	71.0		
	269	50.4	71.2		

Fig. S2.

A



B

Strain	Slope (kb min^{-1})	Fork speed (nt s^{-1})
recA^{+}	19.98	333
recA441	18.49	308

C

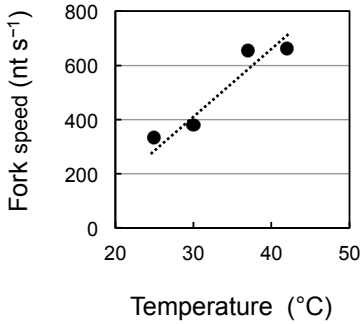
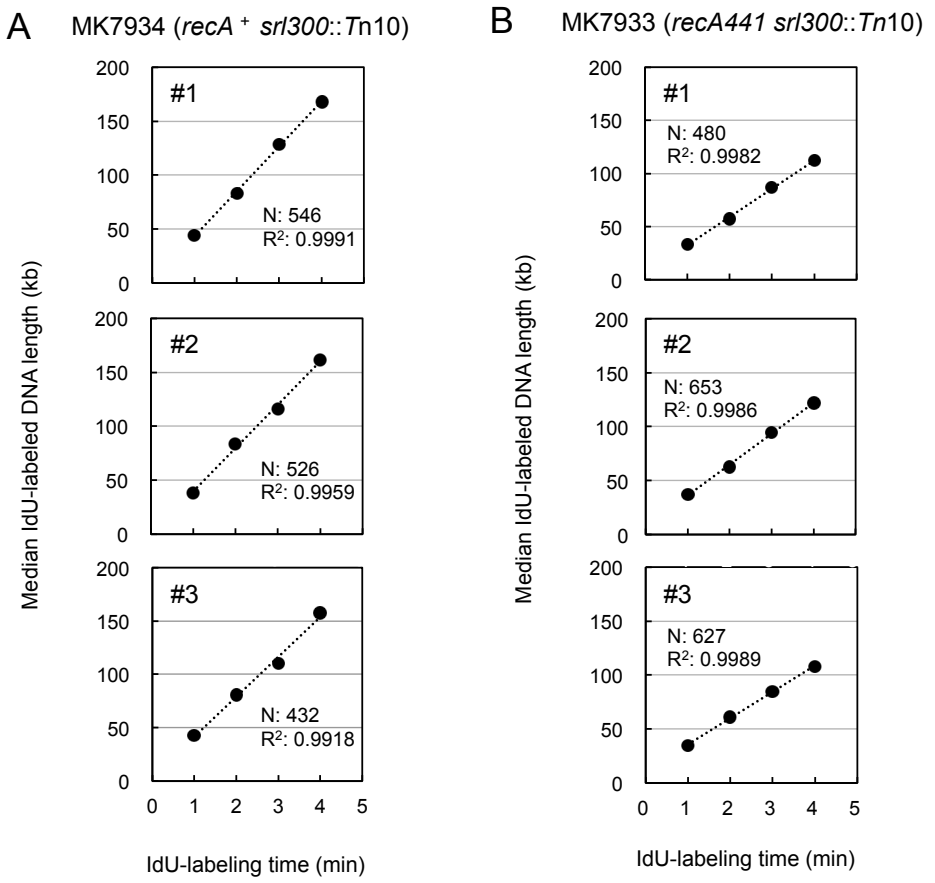


Fig. S3.



C

MK7934 (*recA* + *srl300*::*Tn*10)			
Time-course	Slope (kb min^{-1})	Fork speed (nt s^{-1})	
		Mean	SEM
(A) #1	41.63	662	21
(A) #2	40.18		
(A) #3	37.35		

MK7933 (*recA441 srl300*::*Tn*10)			
Time-course	Slope (kb min^{-1})	Fork speed (nt s^{-1})	
		Mean	SEM
(B) #1	26.55	442	21
(B) #2	28.60		
(B) #3	24.37		

D

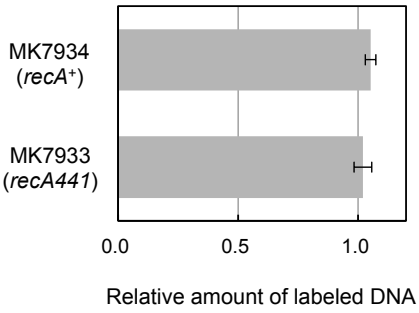
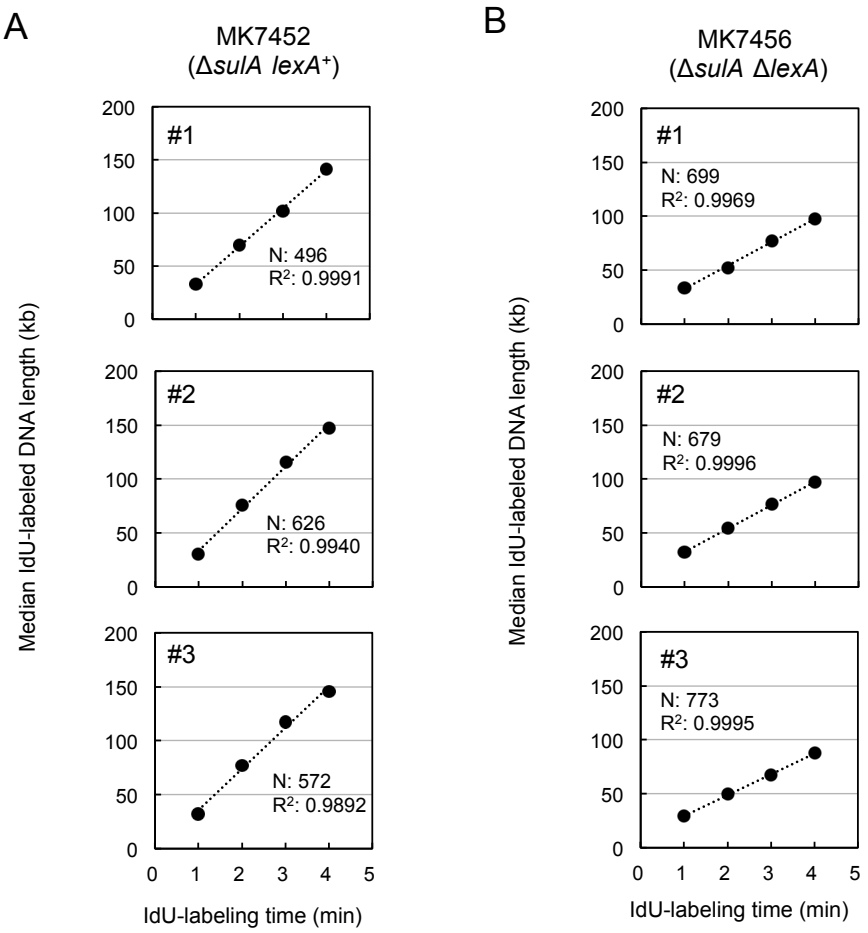


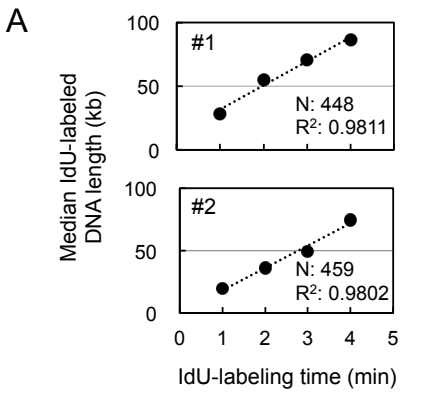
Fig. S4.

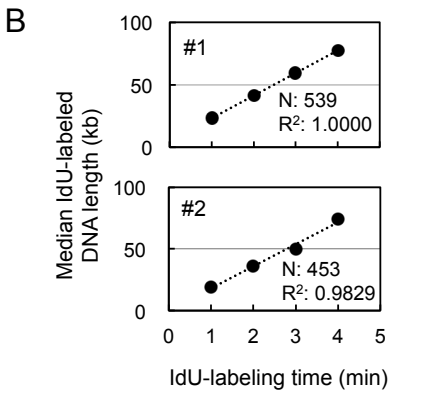


C

MK7452 (Δ*sulA*)				MK7456 (Δ*sulA* Δ*lexA*)			
Time-course	Slope (kb min^{-1})	Fork speed (nt s^{-1})		Time-course	Slope (kb min^{-1})	Fork speed (nt s^{-1})	
		Mean	SEM			Mean	SEM
(A) #1	35.68	627	16	(B) #1	21.58	347	13
(A) #2	39.01			(B) #2	21.66		
(A) #3	38.12			(B) #3	19.29		

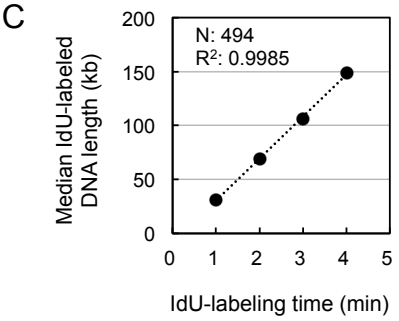
Fig. S5.

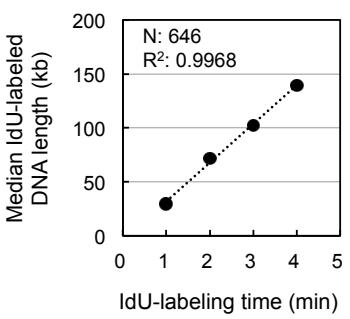


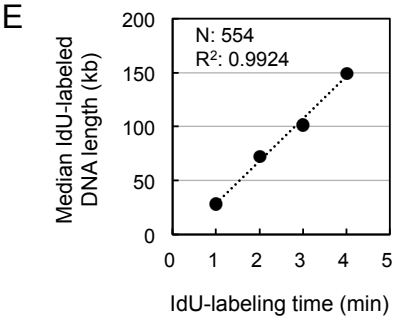


MK7463 (Δ*lexA* Δ*polB*)		
Time-course experiment	Slope (kb min^{-1})	Mean fork speed (nt s^{-1})
#1	18.88	307
#2	17.91	

MK7489 (Δ*lexA* Δ*uvrD*)		
Time-course experiment	Slope (kb min^{-1})	Mean fork speed (nt s^{-1})
#1	18.08	299
#2	17.85	







F

MK7498 (Δ*lexA* Δ*dinB* Δ*recA*)			
Time-course experiment	Slope (kb min^{-1})	Fork speed (nt s^{-1})	
		Mean	SEM
(C) #1	38.86	633	18
(D) #2	35.88		
(E) #3	39.26		

Fig. S6.

Strain	Time-course experiment	Total number of DNAs observed	Coefficient of determination (R^2)	Slope (kb min^{-1})	Speed (nt s^{-1})	
					Mean	SEM
MK7460 (Δ*lexA* Δ*dinB*)	#1	515	0.9920	26.79	424	16
	#2	505	0.9941	26.07		
	#3	430	0.9713	23.52		
MK7466 (Δ*lexA* Δ*umuDC*)	#1	450	0.9999	19.60	347	13
	#2	575	0.9989	22.29		
	#3	696	0.9965	20.59		
MK7486 (Δ*lexA* Δ*recA*)	#1	760	0.9936	29.19	524	24
	#2	637	0.9830	30.89		
	#3	601	0.9997	34.22		
MK7916 (Δ*lexA* Δ*ruvA*)	#1	556	0.9967	21.14	338	11
	#2	556	0.9960	20.68		
	#3	541	0.9990	18.94		
MK7954 (Δ*lexA* Δ*recO*)	#1	462	0.9713	22.68	374	2
	#2	598	0.9921	22.24		
	#3	535	0.9947	22.33		
MK7961 (*recA441 lexA3*)	#1	573	0.9953	34.39	658	46
	#2	475	0.9910	40.17		
	#3	462	0.9965	43.80		
MK7922 / pCC1BAC	#1	573	0.9995	33.38	519	19
	#2	453	0.9976	29.83		
	#3	454	0.9917	30.27		
MK7922 / pSCG3 (*dinBo*)	#1	554	0.9848	21.53	363	11
	#2	520	0.9886	20.76		
	#3	545	0.9881	23.00		
MK7922 / pSCG11 (*recAo*)	#1	662	0.9863	24.93	369	23
	#2	466	0.9989	20.65		
	#3	467	0.9969	20.90		

Supplementary Materials

Figure Legends

Fig. S1. Analysis of replication fork speed in Δ*sulA* and Δ*lexA* Δ*sulA* cells.
(A) Comparable distribution of fork speed in *sulA*$^+$ and Δ*sulA* cells. Cells were pulse-labelled with 50 μg ml^{-1} CldU for 2 min and then with 50 μg ml^{-1} IdU for 2 min at 37 °C, in triplicate as shown in Fig. 2A. Fork speed was calculated from the length of IdU tracks in combed CldU-IdU DNA. The grey and blue bars represent eCOMB and MK7452 (eCOMB Δ*sulA*), respectively. The fork-speed distribution of eCOMB is the same profile as that previously reported by Pham *et al*. (*Mol*. *Microbiol*., **90**, 584-596). The distribution profile of fork speed in MK7452 was calculated from the same data as those used for the top graph of Fig. 4A. The median values of the speed distributions were 630 nt s^{-1} for eCOMB and 621 nt s^{-1} for MK7452. (B) Fork speed determination in MK7456 (eCOMB Δ*lexA* Δ*sulA*) cells carrying either pNTR-*thrA* (MK7456/pNTR-*thrA*) or pNTR-*lexA* (MK7456/pNTR-*lexA*). Cells were exponentially grown in 56/2 medium containing 0.5 mM IPTG to induce expression of plasmid-borne *thrA* or *lexA*. The cells were pulse-labelled at 37 °C with 50 μg ml^{-1} CldU for 2 min, and then with 50 μg ml^{-1} IdU for either 2 or 3 min, in triplicate. The median value of IdU track length in CldU-IdU DNA was determined at each time point. Fork speed was calculated from the difference between the values of the time points. The total number of DNA fibres observed was 795 for MK7456/pNTR-*thrA* and 984 for MK7456/pNTR-*lexA*.

Fig. S2. Analysis of replication fork speed in cells incubated at various temperatures.
(A) Fork speed determination in MK7934 (*recA*$^+$, upper) and MK7933 (*recA441*, lower) cells. The cells were pulse-labelled at 25 °C as described in Fig. 2B. The median value of IdU-labelled chromosomal DNA length is plotted at each time point. N shows the total number of DNA fibers observed in each experiment. The broken lines are linear regression lines determined by the least-squares method; R^2 values are coefficients of determination. (B) Mean fork speed at 25 °C. The mean fork speed value was determined using the slope value of the linear regression line from the corresponding

single time-course experiment shown in (A). The speed value for MK7934 was also used in (C). (C) Mean fork speed in cells growing at various temperatures (25, 30, 37 and 42 °C). The mean fork speed at 30 °C was determined with a single time-course experiment as described in Fig. 2B. The fork speed at 37 °C was previously determined for eCOMB by Pham *et al*. (*Mol. Microbiol*., **90**, 584-596). The speed values at the other temperatures were for MK7934 (*recA*$^+$); the speed at 42 °C is the same data as in Fig. 2D.

Fig. S3. Analysis of SOS-induced *recA441* cells for replication fork speed and DNA degradation.

(A) Fork speed determination in MK7934 (*recA*$^+$). The cells were pulse-labelled at 42 °C with CldU and then with IdU in three individual time-course experiments as described in Fig. 2B. Median value of IdU-labelled chromosomal DNA length in combed CldU-IdU DNA is plotted at each time point. The numbers (N) are the total numbers of DNA fibres observed in each experiment. The broken lines are linear regression lines determined by the least-squares method, and the R^2 values are coefficients of determination. (B) Fork speed determination in MK7933 (*recA441*). Cells were labeled with the analogues at 42 °C as in (A). (C) Mean fork speed. The mean fork speed and SEM were calculated from the three slope values of (A) for MK7934 and (B) for MK7933. The total number of DNA fibres observed was 1504 for MK7934 and 1760 for MK7933. (D) DNA degradation during the labelling period. MK7934 and MK7933 cells were cultured in 56/2 medium with [^{14}C] thymidine from OD_{600}=0.02 to 0.5 (time zero) and then further incubated in the absence of [^{14}C] thymidine for 5 and 15 min at 42 °C. To determine the relative amount of labelled DNA, radioactivity of ^{14}C in cells at 15 min was divided by that at 5 min. The radioactivity values were the same data as those used for normalizing the DNA synthesis rate in Fig. 6A. Error bars indicate the SEMs from three independent experiments. The change in the amount of labelled DNA during the 10-min incubation was not significantly different between MK7934 and MK7933 ($P > 0.05$, Student's *t*-test).

Fig. S4. Fork speed determination in constitutively SOS-inducing Δ*lexA* cells.

(A, B) Fork speed determination. (A) MK7452 (Δ*sulA lexA*$^+$) and (B) MK7456 (Δ*sulA* Δ*lexA*) cells were pulse-labelled with the analogues at 37 °C in triplicate as shown in

Fig. 2B. IdU-labelled DNA length was plotted as described in the legend for Fig. S3. N, the broken lines and R^2 are the same as in Fig. S3. (C) Mean fork speed with SEM. The data were analyzed and presented as described in the legend for Fig. S3. The total number of DNA fibers observed was 1694 for MK7452 and 2151 for MK7456.

Fig. S5. Fork speed determination in Δ*lexA* cells lacking various genes.
Cells were pulse-labelled with the analogues at 37 °C as shown in Fig. 2B. IdU-labelled DNA length was plotted as described in the legend for Fig. S3. N, the broken lines and R^2 are the same as in Fig. S3. (A, B) Mean fork speed of Δ*lexA* cells lacking either *polB* or *uvrD*. Mean fork speed of (A) MK7463 (Δ*lexA* Δ*polB*) and (B) MK7489 (Δ*lexA* Δ*uvrD*) was determined using the slope values of the linear regression lines from the two time-course experiments shown in the upper part of (A) and (B), respectively. (C-F) Rates of fork progression in Δ*lexA* cells lacking both *dinB* and *recA*. The time-course experiments were performed with MK7498 (Δ*sulA* Δ*lexA* Δ*dinB* Δ*recA*) cells in triplicate: (C) Experiment #1, (D) Experiment #2, and (E) Experiment #3. (F) Mean fork speed with SEM. The data were analyzed and presented as described in the legend for Fig. S3. The total number of DNA fibres observed was 1694.

Fig. S6. Summary of fork speed determination for various strains.
Exponentially growing cells were pulse-labelled with the analogues at 37 °C in three independent labelling experiments (#1, #2 and #3) as shown in Fig. 2B, except MK7961 (at 42 °C). The total number of CldU-IdU DNA fibres observed is shown for each time-course experiment. To calculate slope values and coefficients of determination R^2 from linear regression lines, IdU-labelled DNA length was plotted as described in the legend for Fig. S3. Mean fork speed and SEM were calculated from three slope values. MK7922/pCC1BAC, MK7922/pSCG3 and MK7922/pSCG11 represent MK7922 cells carrying single-copy pCC1BAC, pSCG3 (*dinBo*) and pSCG11 (*recAo*) plasmids, respectively.