Supplementary Table S1. R-loop-promoted mutagenesis requires TraI ssDNA endonuclease.

	Mutation Rate (Lac$^+$ colonies per 10^8 viable cells per day, days 2 to 7)				**Effect of Δ*rnhA* in *traI* cells** (Δ*traI* Δ*rnhA* /Δ*traI*)	**Requirement for TraI in Δ*rnh* cells** (mutation rate Δ*rnhA* / Δ*traI* Δ*rnhA*)	**Requirement for TraI in WT cells** (mutation rate WT / Δ*traI*)	**Effect of Δ*rnhA* in WT cells** (Δ*rnhA* / WT)
Genotype:	Wild-type	Δ_{rnhA}	Δ*traI*	Δ*traI* Δ*rnhA*				
Strain:	SMR 4562	PJH 683	PJH 234	PJH 963				
Exp 1	136	271	2.36	5.28	2.24	51.3	57.6	1.99
Exp 2	230	679	2.74	3.51	1.28	193	83.9	2.95
Exp 3	75.8	261	2.06	3.76	1.81	69.4	36.8	3.44
Mean	147	404	2.4	4.2	1.8	**108**	**60**	2.8
SEM	45	138	0.2	0.6	0.3	32	14	0.4

Mutagenesis promoted by R-loops in RNase H-defective cells requires TraI ssDNA endonuclease.

Supplementary Table S2. R-loop-promoted GamGFP foci in F require TraI ssDNA endonuclease.

Genotype	Number of cells counted	% of cells showing one or more GamGFP foci
F^-	2117	6.3 ± 0.9
F’	2970	24 ± 2
F’Δ*traI*	3095	8 ± 2
Δ*rnhA* F^-	1182	38 ± 4
Δ*rnhA* F’	1785	73 ± 2
Δ*rnhA* F’Δ*traI*	2317	50 ± 2

Supplementary Table S3. *Escherichia coli* K12 strains used in this study.

Strain	Relevant genotype	Source
FC29	Δ(*lac-proB*)XIII *ara thi* [F'Δ(*lacI-lacZ*)]	Ref. 29
FC36	Δ*lac-proB*)XIII *ara thi* Rif^R	Ref. 29
SMR4562	Δ*lac-proB*)XIII *ara thi* Rif^R [F' *lacI33*Ω*lacZ proAB*$^+$]	Ref 61. Independent construction of FC40, Ref. 29
SMR5830	SMR4562 *dinB10* [F' *dinB10*]	Ref. 43
SMR5975	SMR4562 [F' *codA21*miniTn*7*Kan(I-*Sce*I cutsite)]	Ref. 21
SMR6272	SMR4562 Δ*araBAD567*	Ref. 21
SMR6276	SMR4562 Δ*araBAD567* Δ*attλ*::P_{BAD}I-*Sce*I	Ref. 21
SMR6280	SMR4562 Δ*araBAD567* [F' *mhpA32*miniTn*7*Kan(I-*Sce*I cutsite)]	Ref. 21
SMR14015	FC36 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gamGFP*	This work
SMR16387	SMR4562 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gam-gfp*	This work
SMR16389	SMR4562 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gam-gfp* Δ*rnhA*::FRTKanFRT	This work
SMR16475	SMR4562 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gam-gfp* [F'Δ*traI::dhfr*]	This work
SMR16477	SMR4562 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gam-gfp* Δ*rnhA*::FRTKanFRT [F'Δ*traI*::*dhfr*]	This work
SMR16379	FC36 Δ*araBAD567* Δ*zfd2509.2*::P_{N25}*tetR*FRT Δ*attTn7*::FRT*cat*FRT$P_{N25tetO}$*gam-gfp* Δ*rnhA*::FRTKanFRT	This work
PJH234	SMR4562 Δ*traI*::*dhfr*	This work
PJH683	SMR4562 Δ*rnhA*::FRTKanFRT	This work
PJH791	SMR4562 *dinB10* Δ*rnhA*::FRTKanFRT	This work
PJH795	SMR4562 *dinB10* Δ*mfd*::FRTKanFRT	This work
PJH813	SMR4562 Δ*mfd*::FRTKanFRT	This work

PJH946	SMR4562 Δ*mfd*::FRTKanFRT Δ*rnhA*::FRTKanFRT	This work
PJH963	SMR4562 Δ*traI*::*dhfr* Δ*rnhA*::FRTKanFRT	
PJH1089	SMR4562 Δ*araBAD567* Δ*rnhA*::FRTKanFRT	This work
PJH1091	SMR6272 [pBAD18]	SMR6272 x pBAD18
PJH1093	SMR6272 [pBAD18*rnhA*]	SMR6272 x pBAD18*rnhA*
PJH1237	SMR6276 [pBAD18]	SMR6276 x pBAD18
PJH1239	SMR6276 [pBAD18*rnhA*]	SMR6276 x pBAD18*rnhA*
PJH1319	SMR6280 [pBAD18]	SMR6280 x pBAD18
PJH1321	SMR6280 [pBAD18*rnhA*]	SMR6280 x pBAD18*rnhA*
SMR5383	FC29 Δ*ara714* [pRDK35]	Rosenberg collection
PJH2023	SMR4562 Δ*attTn7*::$P_{N25tetO}$*gam-gfp*FRT Δ*zfd2509.2*::P_{N25}*tetR*FRT	This work
PJH2439	SMR4562 Δ*rnhA*::FRTKanFRT Δ*zfd2509.2*::P_{N25}*tetR*FRT	This work
PJH2443	SMR4562 Δ*attTn7*::$P_{N25tetO}$*gam-gfp*FRT Δ*rnhA*::FRTKanFRT Δ*zfd2509.2*::P_{N25}*tetR*FRT	This work

Supplementary Reference

61 McKenzie, G. J., Harris, R. S., Lee, P. L. & Rosenberg, S. M. The SOS response regulates adaptive mutation. *Proc Natl Acad Sci U S A* **97**, 6646-6651, (2000).