Supplementary Methods, Figures, Tables

Methods

Cloning and protein purification of MuGam and *E. coli* LigA, LigB

The Gam gene was PCR amplified from genomic DNA of a Mu lysogen (HM8305), and cloned into pHAT4 expression vector (1), which contains TEV protease cleavable region leaving six His residues (His_6) upstream of the cloned site. The resulting plasmid Gam-$pHAT_4$ was electroporated into BL21(DE3) host for protein overexpression and purification. N-FLAG-Gam, N-$(HA)_2$-LigA and N-$(HA)_2$-LigB were amplified from the MG1655 genomic DNA and cloned in a similar way, but with different primer pairs (Table S3) to introduce the FLAG (2) or HA (3) N-terminal epitopes.

Purification of all the cloned proteins was basically similar. Strains for expression were grown in LB broth supplemented with 100 μg/mL ampicillin at 37ºC until the OD_{600} reached 0.6. The culture was then induced with 100 μM IPTG for 4 hours, cells were harvested by centrifugation and flash frozen by liquid nitrogen before storing at -80ºC until further use. For protein purification, quickly thawed cell pellets from 1L culture was resuspended in 25 mL buffer A (10 mM Tris-HCl pH 8.0, 300 mM NaCl and 10 mM imidazole), which also contains a complete protease inhibitor cocktail (EDTA free; from Sigma), 5 mM $MgCl_2$ and 5 units of DNaseI (NEB). The cell suspension was lysed by ultrasonication (Sonics) on ice for 3 minutes with 9.9/9.9 sec pulses, and the lysate was centrifuged at 14,000 rpm for 40 minutes at 4°C to remove cellular debris. The supernatant was loaded onto Ni-Sepharose High Performance affinity matrix (Qiagen) pre-equilibrated with buffer A. The matrix was then extensively washed with buffer A followed by buffer B (10 mM Tris-HCl pH 8.0, 300 mM NaCl and 50 mM imidazole) in order to remove any non-specific protein contaminants. For tagless MuGam and N-FLAG-Gam, two additional

wash steps with buffer C (10 mM Tris-HCl pH 8.0, 150 mM NaCl) followed by buffer D (10 mM Tris-HCl pH 8.0, 1 M NaCl) were included to remove Gam-bound DNA. After the washing steps the matrix bound protein was finally eluted with buffer E (10 mM Tris-HCl pH 8.0, 300 mM NaCl and 300 mM imidazole).

The eluted protein in buffer E was mixed with $(His)_6$-TEV protease and dialyzed overnight against buffer A at 4ºC to complete TEV-mediated removal of the N-terminal $(His)_6$ tag on target constructs. The dialyzed protein was subjected to Ni-Sepharose High Performance affinity matrix pre-equilibrated with buffer A to remove the $(His)_6$-TEV protease as well as undigested $(His)_6$-tagged target proteins. The flow-through containing proteins without the $(His)_6$ tag were finally dialyzed against the storage buffer. For tagless Gam and N-FLAG-Gam the final storage buffer was 20 mM Tris pH 8.0, 500 mM KCl, 5 mM DTT, 10% (v/v) glycerol, whereas for $(HA)_2$-LigA and $(HA)_2$-LigB the final storage buffer was 20 mM Tris pH 7.5, 300 mM NaCl, 5 mM DTT, 10% (v/v) glycerol. The purified proteins in final storage buffer were concentrated by Amicon ultra concentrators (Millipore; 10kDa cutoff) and flash frozen in liquid nitrogen before storing at -80ºC until used. TEV protease was a gift from Makkuni Jayaram's lab and RecBCD was a gift from the Finkelstein lab (4). Before storage the purity of the protein samples was analyzed by 12% SDS-PAGE and the concentration was calculated by Bradford method (5).

Bulk assays for MuGam DNA binding, RecBCD protection and Ligation

EMSA experiments were performed using 300 ng of PCR- amplified and purified 100 bp linear 16S dsDNA substrate (using primer pair GamSub-F and GamSub-R), incubated with increasing concentrations of MuGam or N-FLAG-Gam proteins (0 to 30 nM), at 30ºC for 20 minutes in 10 μL of Gam reaction buffer (40 mM Tris pH 8.0, 150 mM KCl, 0.1% Triton X100, 10% glycerol).

The reaction mixtures were then directly loaded into 5% (v/v) native PAGE gels. After electrophoresis, gels were stained with EtBr and photographed with UV transilluminator Geldoc system (Biorad). The intensity of the unreacted substrate DNA bands as a function of increasing Gam/N-FLAG-Gam concentrations were measured and compared with the intensity of the control DNA band by ImageJ software (https://imagej.nih.gov/ij/)

For RecBCD protection assays, purified Gam (10 μM) was allowed to interact with 150 ng linearized pHAT4 plasmid DNA (4.5 kb, digested with *Eco*RI and *Hind*III) in a 10 μL reaction containing 40 mM Tris pH 8.0, 150 mM KCl, 0.1% Triton X100, 10% glycerol, 10 mM $MgCl_2$, 1 mM ATP, 1 mM DTT for 20 minutes at 30ºC. 0.25 μM purified RecBCD exonuclease or 125 μg of crude cell extracts from BW25113 or its Δ*recB* derivative (6) was added to the reaction mixtures and incubated at 37ºC for different times. The reaction was stopped by the addition of phenol-chloroform before analysis by electrophoresis through 1% (w/v) agarose gel, and visualized under UV after staining with EtBr.

Activity of *E coli* DNA ligaseA (NEB), T_4DNA ligase (NEB), purified *E. coli* $(HA)_2$-LigA and $(HA)_2$-LigB in the presence and absence of MuGam was determined as follows. PCR amplified and purified 550bp linear DNA (template with *gam* ORF) with non-complementary sticky ends (*Eco*RI and *Sal*I) was incubated with increasing amounts of Gam at 30ºC for 30 minutes in buffer consisting 40 mM Tris pH 8.0, 150 mM KCl, 0.1% Triton X100, 4 mM $MgCl_2$, 26 μM NAD^+, 1 mM DTT, 10 % glycerol (for *E coli* DNA ligase assays) or 40 mM Tris pH 8.0, 150 mM KCl, 0.1 % Triton X100, 5 mM $MgCl_2$, 1 mM ATP 10 mM DTT (for T_4 DNA ligase assays) before the addition of DNA ligase. After the addition of DNA ligase enzyme (4 units of commercial *E coli* DNA LigA, 4 units of T_4 DNA ligase, 25pmol $(HA)_2$-LigA and 300 pmol

$(HA)_2$-LigB), the reaction mixtures were further incubated overnight at 30ºC. The reaction products were deproteinized by the treatment of phenol-chloroform, separated by electrophoresis in 1% (v/v) agarose gel and visualized under UV after staining with EtBr.

Repair of linear plasmid DNA *in vivo*

Linear plasmid DNA substrate was prepared by digesting pYTK050 plasmid, carrying superfolder GFP and chloramphenicol (Cm) resistance (7) with *Nde*I to remove portion of GFP ORF followed by enzymatic removal of the 5´PO_4 group by alkaline phosphatase (NEB). The resulting linear plasmid DNA (GFP^-/Cm^R) was gel-purified (QIAquick Gel Extraction kit) and digested again with *Nde*I to minimize the remaining uncut plasmids from the first *Nde*I digestion, followed by re-gel purification of the linear plasmid DNA band. 250ng of linear plasmid DNA substrate was electroporated into 100µL of electro-competent host strains (8), revived with 900µL LB media for 6 hours at 30ºC, and were plated on chloramphenicol (25µg/ml). Recovered plasmid DNAs from GFP^-Cm^R colonies were isolated and sequenced. The types of repair junctions were identified by pair wise sequence alignment (http://www.genome.jp/tools-bin/clustalw) with the control uncut pYTK050 sequence.

Repair of I-*Sce*I mediated chromosomal dsDNA breaks

E. coli strain SMR14353 and its *recA*- derivative SB08 were transformed with $(HA)_2$-LigA-pTRC99a. Cultures were grown to OD_{600}~0.3 in LB media supplemented with 0.2% glucose, and then induced for Gam and LigaseA with 200 ng/mL tetracycline and 10 µM IPTG, respectively. The cells were further grown for 1 hour (for exponential phase experiments) or 12 hours (stationary phase experiments) at 37 ºC, washed and resuspended in M9 media supplemented with 10 µg/mL thiamine, 0.2% glycerol and 0.02 % arabinose to sustain continued induction of I-*Sce*I (or no

arabinose, for the uninduced control), and further grown at 37 ºC for 2 hours with continued induction for Gam and Ligase as well. Cells were then washed and resupended again in 10 µg/mL thiamine containing M9 media with no usable carbon source (in order to allow repair without cell growth) and supplemented with (+/-) 0.02 % arabinose with continued induction for Gam and Ligase, grown for another 6 hours in the same growth condition before plating on LB media. 20 ml culture aliquots (>10^7 cells) of controls with no I-*Sce*I induction, as well as those from exponential and stationary phase induction experiments, were fixed by the addition of 40 mL -20 ºC 95% ethanol, spun down and resuspended in 5mL of -20 ºC 95% ethanol. Samples were stored at -20 until the genomic DNA (gDNA) was purified using a kit (Wizard, promega). gDNA samples were submitted for sequencing in 50 µL, 100 ng/µL aliquots for each perspective experiment. Colony forming units (cfu) counts were obtained for each step in the experiment for both the RecA+ and RecA- strains with induced or uninduced levels of Gam/LigA. These cfu counts were then normalized to the cfu totals of uninduced I-*Sce*I under similar conditions of Gam/LigA induction.

The in-house core facility (GSAF) prepared sequencing libraries of the gDNA using a low-cost, high-throughput library preparation method. Sequencing was performed on the Illumina Next-Seq platform using 2X150 paired end reads with a target of 25 million reads. This target provides a 161x coverage for each basepair in the genome. The sequence was aligned to a modified *E. coli* MG1655 genome (genbank, U00096.3) with the Kan^R:I-*Sce*I artificially inserted at position 3939251. The Burrogh-Wheelers alignment (BWA) was used to align the samples with the modified reference genome. Individual reads that aligned within 10 kb of the Kan^R:I-*Sce*I insertion were analyzed for the presence of the I-*Sce*I site. Only reads with either a perfect repair of the 18 bp I-*Sce*I site (indicating accurate repair), or exhibiting clipped junction between sites flanking the

I-*Sce*I site (indicating a deletion) were considered for analysis. If the I-*Sce*I site was inferred in the unsequenced region of a long template, the data was discarded. Since the average template length from library prep was only 300 bp long, the majority of reads near the I-*Sce*I site were suitable for analysis. The type of junction repairs were analyzed similarly to the plasmid repair method. Since only ~1% of cells survived I-*Sce*I, this number would be an absolute maximum of cells that did not experience I-*Sce*I cleavage. Therefore, sequences recovered after I-*Sce*I induction would have a 1% error in representing the population of cells that did not undergo I-*Sce*I cleavage.

Mu lytic growth curves and phage titers

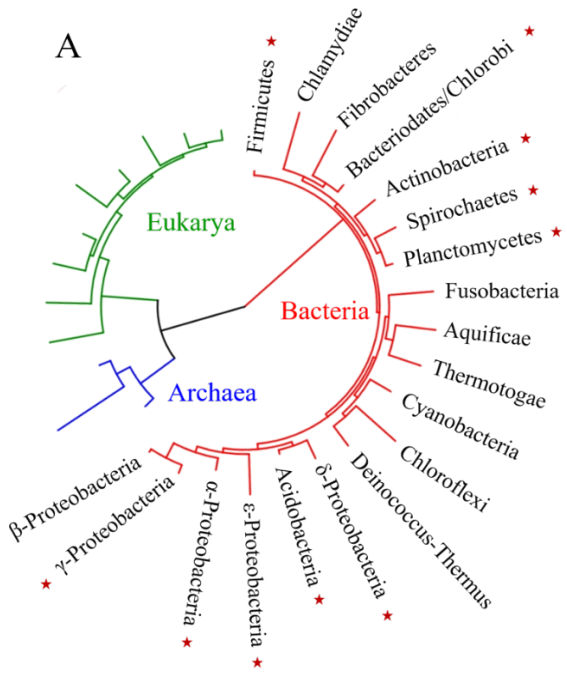
100μL of an overnight culture of a Mu lysogen containing a thermosensitive Mu repressor were transferred to 10 mL of fresh LB media containing 2.5 mM $CaCl_2$ and 5 mM $MgSO_4$ and grown at 30°C to an OD_{600} of ~0.4. Replication was induced by transferring the culture to 42°C until lysis. To obtain phage titers, 10 μL of an appropriate dilution of the lysate were mixed with 100 μL of a sensitive host (*E. coli* MG1655) grown to $OD_{600}$of~0.5–0.6 in LB including 2.5 mM $CaCl_2$ and 5 mM $MgSO_4$. The mixture was added to 3 mL of 0.5% molten soft agar at 42°C, and poured on top of an LB agar plate containing 2.5 mM $CaCl_2$ and 5 mM $MgSO_4$. Plates were incubated overnight at 37°C.

Quantitative real-time PCR analysis

This assay was conducted as described previously (9). The number of Mu DNA copies (estimated by using primers Mu-F and Mu-R) were normalized to a chromosomal locus *hipA* using primers hipA-F and hipA-R (Table S3).

Figures

A



B

γ-proteobacterial order	Gam	Ku/LigD
Alteromonadeles	+	+
Vibrionales	-	-
Enterobacteriales	+	-
Pasteurellales	+	-
Thiotrichales	+	-
Leigionellales	-	+
Oceanaospirallales	-	+
Xanthomonadeles	-	+
Chromatiales	+	+
Psuedomonadeles	+	+

Fig. S1. Phylogenetic distribution of Gam and Ku proteins. A. MuGam homologs are found only in Bacteria, the specific phyla in which they occur indicated by red stars. These Gams are linked to either partial or complete Mu-like sequences. **B**. Occurrence of MuGam, *Mycobacterium tuberculosis* Ku and LigD homologs in γ-proteobacterial orders. This representative distribution shows that Ku and LigD homologs always occur together, but the presence of Gam is independent of LigD.

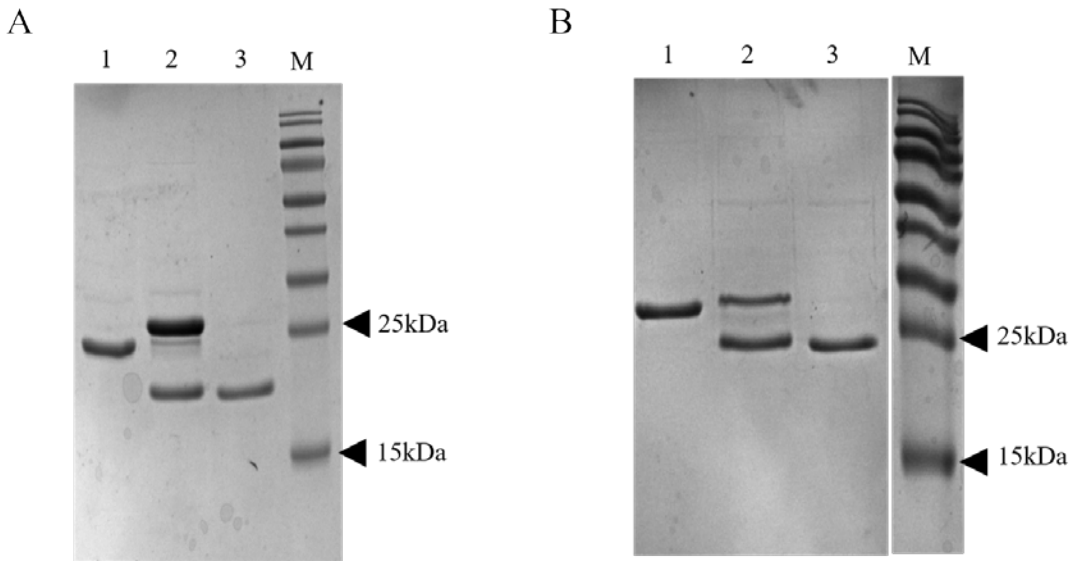


Figure S2. Purified MuGam proteins. Purification details are found in Methods. Briefly, Gam proteins without (**A**) or with (**B**) an N-terminal FLAG epitope were expressed with six His residues (His_6) followed by a TEV protease-cleavable site on their N-termini, and purified over a Ni column (lanes 1). The proteins were cleaved with (His_6)-TEV (lanes 2), and re-purified on Ni columns, where the tagless Gam and FLAG-Gam eluted in the flow-through (lanes 3). M, molecular size markers. Arrows point to MW of markers in the range of the MuGam (21 kDa) and FLAG-Gam (24 kDa). Proteins were fractionated on 12% SDS-PAGE gels and visualized with Coomassie blue.

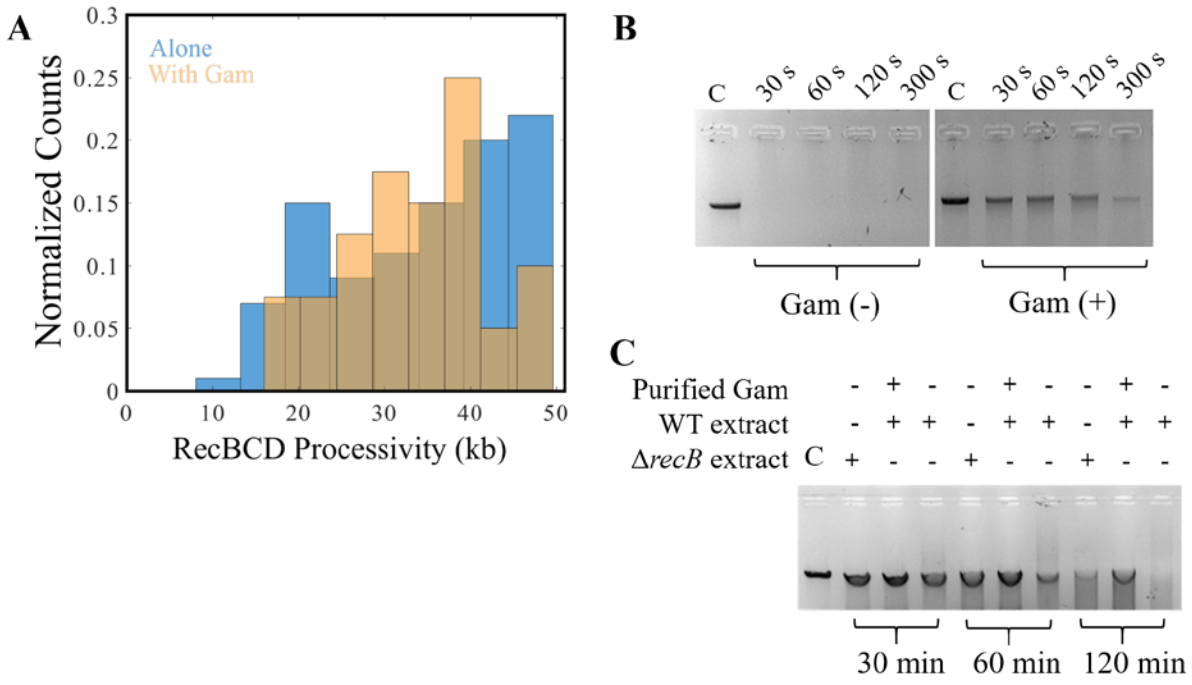


Figure S3. **Gam slows RecBCD digestion of linear DNA. A.** Normalized distribution of RecBCD processivities without (blue) and with Gam (orange). RecBCD processivity on naked DNA skews towards the very end of the distribution, indicating that the length of DNA limits the true RecBCD processivity. In contrast, the peaked distribution of Gam-bound DNA indicates that Gam reduces RecBCD processivity to a value below the length of our DNA substrate. **B.** Linear DNA (4.5kb; C) was first incubated without (Gam-) or with (Gam+) purified MuGam, and next with purified RecBCD for 30 sec to 300 sec as indicated. **C.** As in B, except crude cell extracts from wild-type ($RecBCD^+$) or Δ*recB* ($RecBCD^-$) strains were used as the source of exonuclease, and incubation times were from 30 min to 120 min. The samples were run on a 1% agarose gel and DNA visualized with EtBr staining. C, control no-protein DNA lane. See Methods for experimental details.

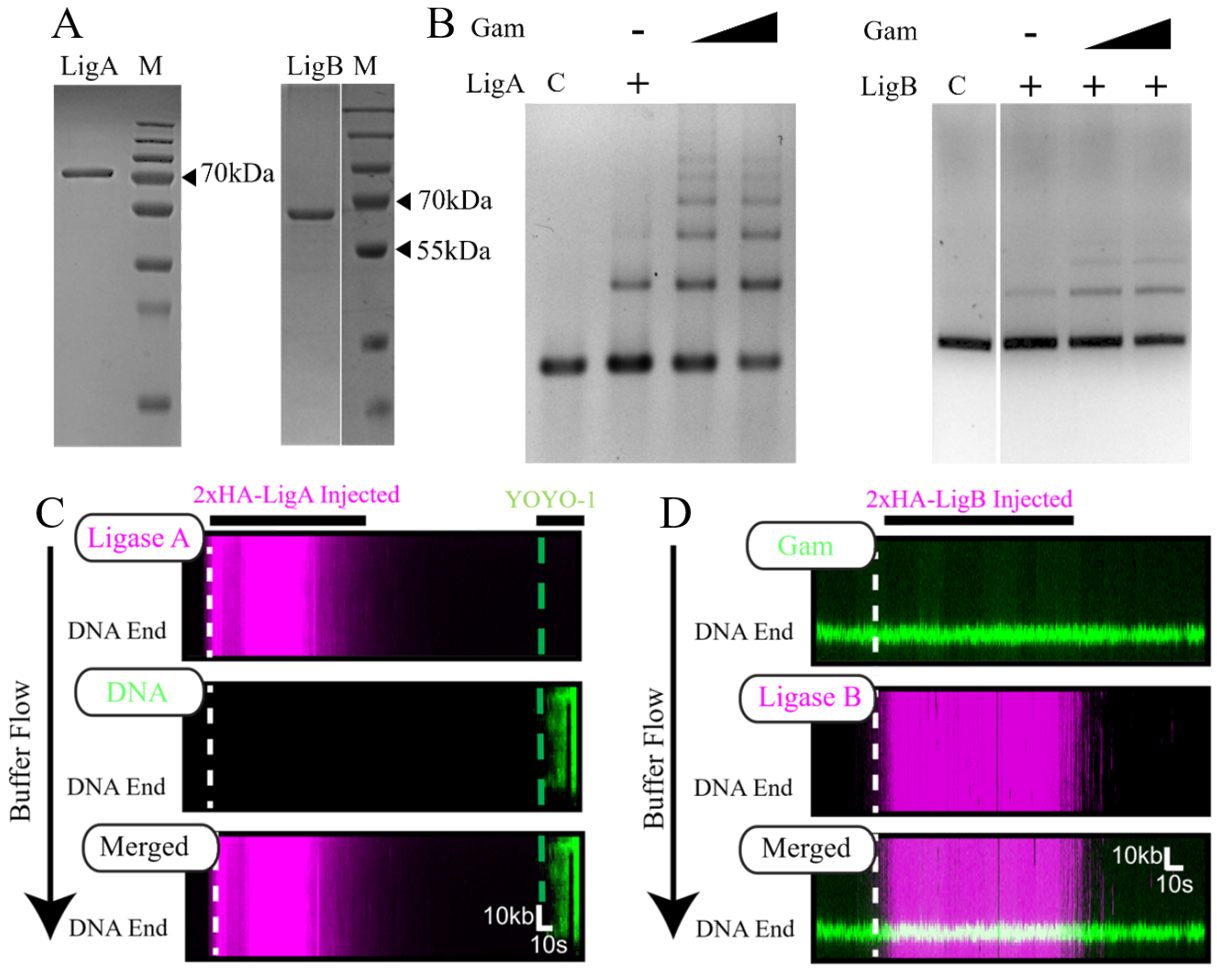


Fig. S4. *E. coli* DNA ligases and their activity in the presence of MuGam. $(HA)_2$-tagged *E. coli* ligases were cloned and the proteins purified and analyzed as described under Methods and in Fig. S2. **A.** Left, purified $(HA)_2$-Ligase A**.** Right, purified $(HA)_2$-Ligase B. Arrows point to MW of markers in the range of Ligase A (73.6 kDa) and Ligase B (63.1 kDa). **B.** Activity of $(HA)_2$-tagged ligases in absence and presence of increasing concentrations of Gam. 550 bp substrate DNA with non-complementary sticky ends *(Eco*RI/*Sal*I) was incubated with increasing DNA:Gam molar ratios (1:20, 1:40), and analyzed as in Fig. 4A. C, control DNA lane. **C.** Kymograph showing that $(HA)_2$-LigA (magenta) does not interact with DNA in the absence of Gam. The DNA was labeled with a fluorescent intercalating dye (YOYO-1, green) after the $(HA)_2$-LigA was injected into the flowcell. Note that ligase should interact with DNA at some point, but we cannot see very fast (<300 ms) or weak (kD > 100 nM) transient interactions. **D**. Kymograph showing that fluorescent Ligase B does not interact with Gam-bound DNA ends. Here, FLAG-Gam was labeled with an anti-FLAG antibody (green) and $(HA)_2$-LigB was labeled with an anti-HA antibody (magenta). Dashed line indicates when fluorescent LigB was introduced into the flowcell.

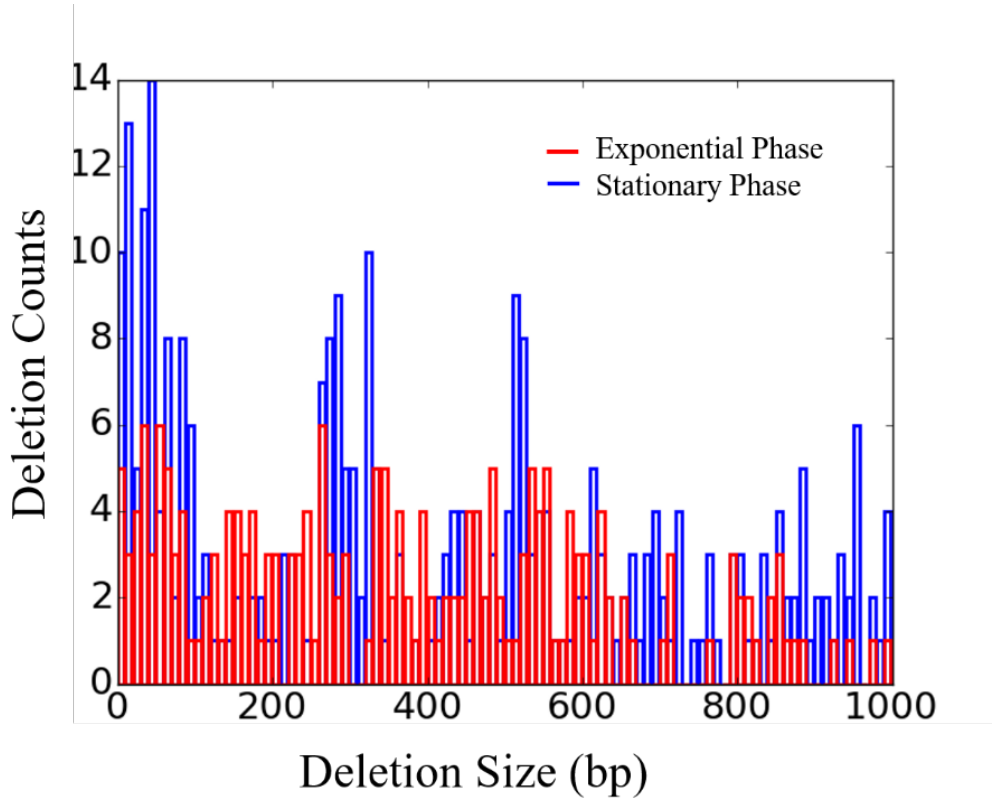


Figure S5. Pattern of chromosomal deletions recovered after I-*Sce*I cleavage. Genomic DNA sequence of cells after I-*Sce*I cleavage in the presence of Gam and LigA in the experiment shown in Fig. 6. Deletion sizes across I-*Sce*I ranged from 10 to 1000 bp. Over 99% of chromosomal repairs are captured within this window, with only a handful of deletions larger than 1 kb observed. Deletions are preferentially of smaller lengths for both the stationary and exponential phases. Deletions in the exponential phase shows a wider distribution between 0 and 1000 bp compared to the stationary phase.

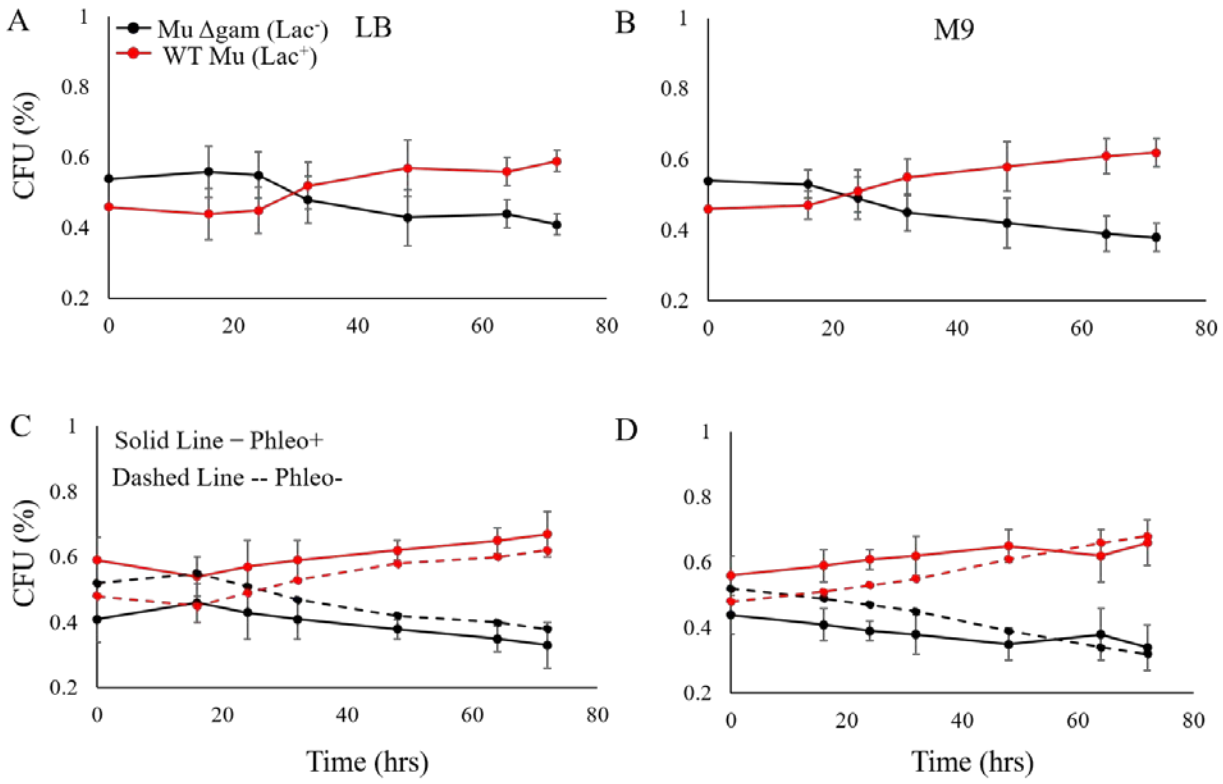


Figure S6. Presence of Gam increases host fitness. A, B. The experiments were similar to those shown in Fig. 7, except that the strains carrying the Lac^+ and Lac^- alleles were reversed. Thus, the strain with WT Mu is Lac^- (DMW153) and the isogenic Mu*Δgam* strain is Lac^+ (DMW152). Relative fitness values for Gam^+ over Gam^- were 1.04 (CI_{95} = ±0.004) in LB and 1.08 (CI_{95} = ±0.012) in M9. There is a drop in relative fitness for the strains in the experiment shown in B when compared to a similar experiment in Fig. 7B, where the strains carrying the Lac alleles are reversed. However, the fitness for Gam^+ is still significant enough to provide a competitive advantage. **C, D**. Relative fitness for Gam^+ over Gam^- in the phleomycin-treated strains was 1.04 (CI_{95} = ±0.02) in LB and 1.06 (CI_{95} = ± 0.007) in M9. These data are similar to those in Fig. 7C,D.

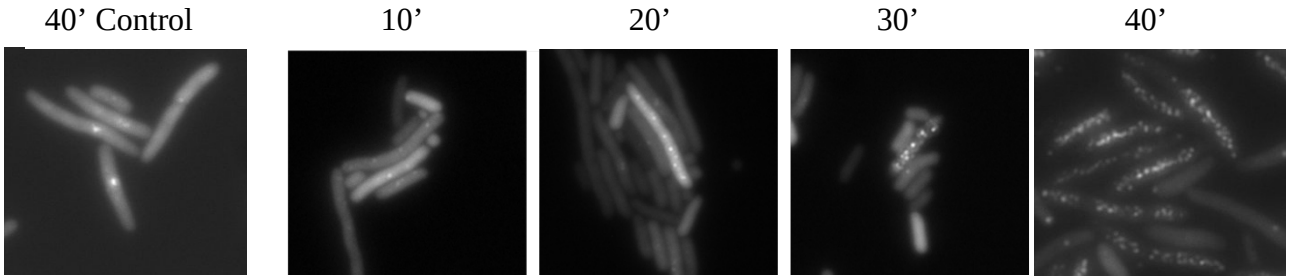


Fig. S7. **Time course of appearance of Gam-GFP foci during Mu infection.** In this experiment, Gam-GFP is expressed at a chromosomal location from lambda P_R promoter, under control of a thermo-sensitive repressor (SMR16470). The time course of appearance of the puncta was similar to that in Fig. 8E, with no puncta above background in control cells held at for 40 minutes at 42°C (40’ control). In the infected strain, no foci above background were detected at 10’ and 20’; foci began to appear around 30-35 minutes (30’ shown), with the majority of cells displaying multiple puncta at 40’.

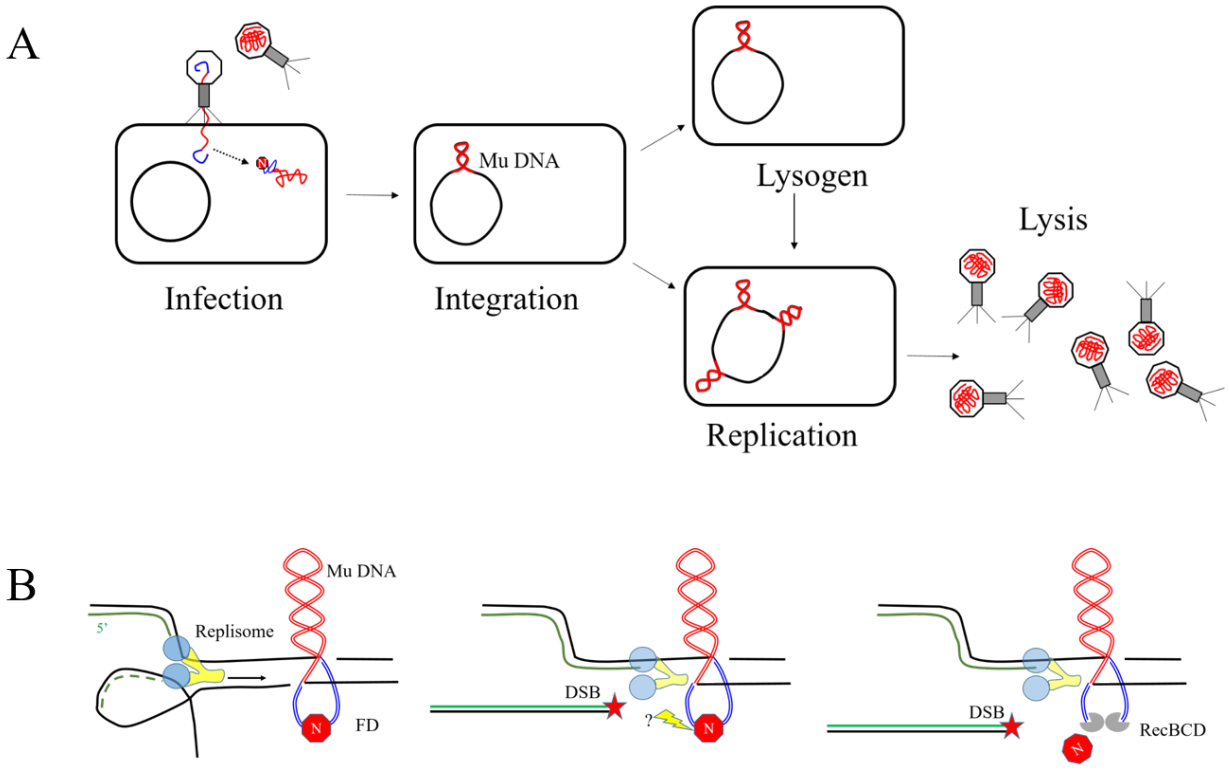


Figure S8. **Repair of a Mu insertion upon infection. A**. Schematic of the Mu infection cycle. **B.** Sequence of repair events after integration of infecting Mu. After integration into the *E. coli* chromosome, Mu waits for the arrival of the Pol III fork (left). N, injected Mu protein that protects linear Mu ends; FD, flanking DNA derived from packaging *E. coli* DNA from either side of the Mu insertion (see Fig. 8G). When the fork runs into the nick at the Mu end, a DSB ensues (middle). Interaction between Pol III and the Mu transpososome (not shown in the interest of clarity) signals removal of the N protein (thunderbolt), allowing RecBCD entry (right). The trimmed FD ends are eventually sealed to generate a prophage. The chromosomal DSB is repaired by HR (10).

Tables

Table S1. Strains and Phage

Bacterial Strain/Phage	**Relevant genotypes**	**Source (ref.)**
Phage		
P1	C1-100	(11)
Escherichia coli		
MG1655	$F^- \lambda^-$ *rph* -1	(12)
AB1157	*thr-1 araC14 leuB6(Am), Δ(gpt-proA)62 lacY1 tsx-33 qsr'-0 glnV44(AS) galK2(Oc)λ- Rac-0 hisG4(Oc) rfbC1 mgl-51 rpoS396(Am) rpsL31(strR) kdgK51 xylA5 mtl-1 argE3(Oc) thi-1*	(13)
MP1999	AB1157 *recB recC sbcB malF*::Mu *cts62*	(13)
CW45	M1999 with Cm cassette inserted at nt 35040 of Mu	(14)
HM8305	F' *pro lac*::Mu*cts62/Δpro lac his met rpsL* Mu^r	(15)
BW25113	*Δ(araB–D)567 ΔlacZ4787(::rrnB-3) rph-1 Δ(rhaD–B)568 hsdR514*	(16)
JW2788	BW25113 *ΔrecB::kan*	(16)
BL21(DE3)	*E coli* B with a λ prophage carrying the T7 RNA polymerase gene and $lacI^q$	(17)
SB01	HM8305 *Δgam::kan* (*Δ*5801- 6325 bp of Mu)	λ-Red oligo (primers *gam*F/*gam*R) pKD13 in HM8305

SB02	HM8305*Δgam::frt*	SB1 x pCP20
SB03	HM8305*Δgam::frt, recB::kan*	SB2x P1 JW2788 to Km
SMR14353	MG1655 Δ*araBAD567* Δ*attλ*::P_{BAD} I-*Sce* I *zfd2509.2*::P_{N25}*tetR* FRT Δ*attTn7*::FRT*cat*FRT $P_{N25tetO}$*gam* I-site C	(18)
SB06	SMR14353 + $(HA)_2$LigA/pTRC99a	$(HA)_2$LigA/pTRC99a plasmid in SMR14353
SB07	HM8305 Δ*rec*A::*gen* (Δ2822708 to 2823769 bp of *E coli* genome)	λ-Red oligo (primers *rec*F/*rec*R) pKD46 GenR (19) in HM8305
SB08	SMR14353 Δ*rec*A::*gen*	SMR14353 x P1 SB07 to Gen
SB09	SB86 + $(HA)_2$LigA/pTRC99a	$(HA)_2$LigA/pTRC99a plasmid in SB08
SB77	MP1999 Δ*gam::kan* + pGam-GFP	pGam-GFP plasmid in SB04
JP2063	MG1655 Δ*lac::kan*	λ-Red oligo (primers JDP855/JDP856) pKD4 in MG1655
DMW 61	MG1655 with Mu:Cm at nt 995741	MG1655 infected with phage from CW45
DMW152	DMW61 Δ*gam::frt*	DMW61 x P1 SB77, pCP20
DMW153	DMW61 Δ*lac::frt*	DMW61 x P1 JP2063, pCP20
DMW154	DMW152 Δ*lac::frt*	DMW152 x P1 DMW153, pCP20
SMR16470	MG1655 Δ*araBAD567* Δ*attλ*::P_{BAD} *zfd2509.2*::P_{N25}*tetR*::FRT Δ*attTn7*::FRT*cat*FRT λ*c*I*ts857* P_R*gam-gfp*	(18)

Table S2. Plasmids

Name	Construction/ usage	Source (ref.)
pGam-GFP	GamGFP expressed from pRHA-113	(10)
pGFP	GFP expressed from pRHA-113	(10)
pKD13	Source of *kan*	(20)
pKD46	Plasmid for λ-Red recombination	(20)
pKD46 (genR)	Source of *gen*	(19)
pCP20	Plasmid expressing Flp recombinase for antibiotics removal	(20)
pHAT4	pET based *E coli* protein expression vector	(1)
pTRC99a	*E coli* protein expression vector with inducible *lac*I promoter	(21)
pYTK050	Plasmid carrying superfolder GFP	(7)
Gam-pHAT4	Plasmid expressing TEV protease cleavable N-$(His)_6$-TEV-Gam construct	This study
N-FLAG-Gam-pHAT4	Plasmid expressing N-$(His)_6$-TEV-FLAG-Gam construct	This study
N-$(HA)_2$-LigA-pHAT4	Plasmid expressing N-$(His)_6$-TEV-$(HA)_2$-ligA construct	This study

N-$(HA)_2$-LigB-pHAT4	Plasmid expressing N-$(His)_6$-TEV-$(HA)_2$-ligB construct	This study
N-$(HA)_2$-LigA-pTRC99a	Plasmid expressing N-$(HA)_2$-ligA construct	This study
pIF53	RecBCD expression vector	(4)

Table S3. ***Oligonucleotides***

Name	**Sequence (5´ to 3´)**
Substituting Mu *gam* gene with *kan*	
*gam*F	GTATCCAGCATTGTATACAGCGGATATTAATTAACAGGAGCTTTAATTTG TGTAGGCTGGAGCTGCTTCG
*gam*R	ATCGCGCCATAATTAAAAGAATGTGAATTAATTAAATATTAATGGTGGT AATTCCGGGGATCCGTCGACC
Substituting *E. coli* gene *lacZ* with *kan*	
JPD855	GCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGCTGGGTGTAGGCTG GAGCTGCTTC
JPD866	CCAGGAGTCGTCGCCACCAATCCCCATATGGAAACCGTCCATATGAATA TCCTCCTTAG
Substituting *E. coli rec*A gene with *gen*	
*rec*F	CAACAGAACATATTGACTATCCGGTATTACCCGGCATGACAGGAGTAAA A
*rec*R	AAAAAAGCAAAAGGGCCGCAGATGCGACCCTTGTGTATCAAACAAGAC GA
Cloning of Mu Gam in pHAT4 vector	
GamF	ATATATGAATTCATGGCTAAACCAGCAAAACGTATCAAGAG
GamR	ATATATGTCGACTTAAATACCGGCTTCCTGTTCAAATGG
Cloning of N-FLAG-Gam in pHAT4 vector	
FLAG-GamF	ATATATGAATTCGACTACAAAGACGATGACGACAAGTCTGGTGGTGGTG GCGGCATGGCTAAACCAGCAAAACGTATC
Cloning of *E coli* N-$(HA)_2$ LigA in pHAT4	
LigA-F	ATATATGAATTCTACCCATACGATGTTCCAGATTACGCTTACCCATACGA TGTTCCAGATTACGCTGGTGGTATGGAATCAATCGAACAACAACTG
LigA-R	ATATATGTCGACTCAGCTACCCAGCAAACGCAGCATTTCCGCTTCGTC
Cloning of *E coli* N-$(HA)_2$ LigB in pHAT4	
LigB-F	ATATATGTCGACTACCCATACGATGTTCCAGATTACGCTTACCCATACGA TGTTCCAGATTACGCTGGTGGTATGAAAGTATGGATGGCGATATTA
LigB-R	ATATATAAGCTTCTAAGGTTCAAAACCTGTGATCTGCTGG

Sequencing of pYTK050 repair junctions.	
pYTK-F	CACGAGGGAGCTTCCAGGGGGAAACGC
pYTK-R	GCTGGCGATTCAGGTTCATCATGCCGTTTG
PCR primers to amplify Gam substrate DNA (100bp) for EMSA	
GamSub-F	GTGGCGGACGGGTGAGTAATGT
GamSub-R	CCCCTCTTTGGTCTTGCGACGTTA
Real-time PCR primers	
Mu-F	CTTCATCTGGTTCCAGCTCTT
Mu-R	GTGCTCAAGCCAATGAGAAAC
hipA-F	CCGACGACGCACATCATTA
hipA-R	GCCAGCAGCAGACAGTAATA
Single-Molecule Imaging	
IF007	[p]AGG TCG CCG CCC[Bio]
LM031	GGG CGG CGA CCT

References

1. Peranen J, Rikkonen M, Hyvonen M, & Kaariainen L (1996) T7 vectors with modified T7lac promoter for expression of proteins in Escherichia coli. *Anal Biochem* 236(2):371-373.
2. Einhauer A & Jungbauer A (2001) The FLAG peptide, a versatile fusion tag for the purification of recombinant proteins. *J Biochem Biophys Methods* 49(1-3):455-465.
3. Wagner E, Plank C, Zatloukal K, Cotten M, & Birnstiel ML (1992) Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. *Proc Natl Acad Sci U S A* 89(17):7934-7938.
4. Finkelstein IJ, Visnapuu ML, & Greene EC (2010) Single-molecule imaging reveals mechanisms of protein disruption by a DNA translocase. *Nature* 468:983-987.
5. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254.
6. Choi W, Jang S, & Harshey RM (2014) Mu transpososome and RecBCD nuclease collaborate in the repair of simple Mu insertions. *Proc Natl Acad Sci U S A* 111(39):14112-14117.
7. Lee ME, DeLoache WC, Cervantes B, & Dueber JE (2015) A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly. *ACS Synth Biol* 4(9):975-986.
8. Dower WJ, Miller JF, & Ragsdale CW (1988) High efficiency transformation of E. coli by high voltage electroporation. *Nucleic Acids Res* 16(13):6127-6145.
9. Jang S, Sandler SJ, & Harshey RM (2012) Mu insertions are repaired by the double-strand break repair pathway of *Escherichia coli. PLoS genetics* 8:e1002642.
10. Jang S & Harshey RM (2015) Repair of transposable phage Mu DNA insertions begins only when the E. coli replisome collides with the transpososome. *Mol Microbiol* 97(4):746-758.
11. Lobocka MB*, et al.* (2004) Genome of bacteriophage P1. *J Bacteriol* 186(21):7032-7068.
12. Bachmann BJ (1972) Pedigrees of some mutant strains of Escherichia coli K-12. *Bacteriol Rev* 36(4):525-557.
13. Saha RP, Lou Z, Meng L, & Harshey RM (2013) Transposable prophage Mu is organized as a stable chromosomal domain of *E. coli*. *PLoS Genet* 9:e1003902.
14. Choi W & Harshey RM (2010) DNA repair by the cryptic endonuclease activity of Mu transposase. *Proc Natl Acad Sci U S A* 107:10014-10019.
15. Bukhari AI (1975) Reversal of mutator phage Mu integration. *J Mol Biol* 96:87-99.
16. Baba T*, et al.* (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:2006.0008.
17. Studier FW & Moffatt BA (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J Mol Biol* 189(1):113-130.
18. Shee C*, et al.* (2013) Engineered proteins detect spontaneous DNA breakage in human and bacterial cells. *Elife* 2:e01222.
19. Doublet B*, et al.* (2008) Antibiotic marker modifications of lambda Red and FLP helper plasmids, pKD46 and pCP20, for inactivation of chromosomal genes using PCR products in multidrug-resistant strains. *J Microbiol Methods* 75(2):359-361.
20. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* 97(12):6640-6645.
21. Amann E, Ochs B, & Abel KJ (1988) Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in Escherichia coli. *Gene* 69(2):301-315.