Table 1. Bacterial strains.

Strain	Relevant Genotype	Plasmids	Source
AB1157	*thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 str-31 tsx-33 sup-37*		Bachmann (1987)
BB078	AB1157	pKD46	Wanner (2000)
JC9941	*recA200*(Ts)		A.J. Clark
SK129	*recB270*(Ts) *recC271*(Ts)		Kushner (1974)
JB29	AB1157 Δ*rdgB62*		Bradshaw and Kuzminov (2003)
JB30	JC9941 Δ*rdgB62*		Bradshaw and Kuzminov (2003)
JB36	SK129 Δ*rdgB62*		Bradshaw and Kuzminov (2003)
AK107	SK129 *dut-1*		Kouzminova (2004)
BB006	AB1157 Δ*deoB*::*cat*		Deletion-replacement
BB324	JC9941 ΔdeoB		JC9941 x P1 BB006, cat FLPed
BB007	JB29 Δ*deoB*	pK96	JB29 x P1 BB006, *cat* FLPed
BB004	JB30 Δ*deoB*		JB30 x P1 BB006, *cat* FLPed
BB005	JB36 Δ*deoB*		JB36 x P1 BB006, *cat* FLPed
BB014	AB1157 Δ*deoD*::*cat*		Deletion-replacement
BB325	JC9941 ΔdeoD		JC9941 x P1 BB014, *cat* FLPed
BB015	JB29 Δ*deoD*	pK96	JB29 x P1 BB014, *cat* FLPed
BB012	JB30 Δ*deoD*		JB30 x P1 BB014, *cat* FLPed
BB013	JB36 Δ*deoD*		JB36 x P1 BB014, *cat* FLPed
BB022	AB1157 Δ*dusB*::*cat*		Deletion-replacement
BB322	JC9941 ΔdusB		JC9941 x P1 BB022, cat FLPed
BB023	JB29 Δ*dusB*	pK96	JB29 x P1 BB022, *cat* FLPed
BB020	JB30 Δ*dusB*		JB30 x P1 BB022, *cat* FLPed
BB021	JB36 Δ*dusB*		JB36 x P1 BB022, *cat* FLPed
BB030	AB1157 Δ*hpt*::*cat*		Deletion-replacement
BB327	JC9941 Δhpt		JC9941 x P1 BB030, cat FLPed
BB031	JB29 Δ*hpt*	pK96	JB29 x P1 BB030, *cat* FLPed
BB028	JB30 Δ*hpt*		JB30 x P1 BB030, *cat* FLPed
BB029	JB36 Δ*hpt*		JB36 x P1 BB030, *cat* FLPed
BB038	AB1157 Δ*dgt*::*cat*		Deletion-replacement
BB333	JC9941 Δdgt		JC9941 x P1 BB038, cat FLPed
BB039	JB29 Δ*dgt*	pK96	JB29 x P1 BB038, *cat* FLPed
BB036	JB30 Δ*dgt*		JB30 x P1 BB038, *cat* FLPed
BB037	JB36 Δ*dgt*		JB36 x P1 BB038, *cat* FLPed
BB046	AB1157 Δ*tdk*::*cat*		Deletion-replacement
BB329	JC9941 Δtdk		JC9941 x P1 BB046, *cat* FLPed
BB047	JB29 Δ*tdk*	pK96	JB29 x P1 BB046, *cat* FLPed
BB044	JB30 Δ*tdk*		JB30 x P1 BB046, *cat* FLPed
BB045	JB36 Δ*tdk*		JB36 x P1 BB046, *cat* FLPed
BB090	AB1157 *gmk*::*cat*		Deletion-replacement
BB341	JC9941 Δgmk::cat		JC9941 x P1 BB090
BB094	JB29 *gmk*::*cat*	pK96	JB29 x P1 BB090
BB091	JB30 *gmk*::*cat*		JB30 x P1 BB090
BB092	JB36 *gmk*::*cat*		JB36 x P1 BB090
BB101	AB1157 Δ*purR*::*cat*		Deletion-replacement
BB332	JC9941 ΔpurR		JC9941 x P1 BB101, cat FLPed
BB106	JB29 Δ*purR*	pK96	JB29 x P1 BB101, *cat* FLPed
BB104	JB30 Δ*purR*		JB30 x P1 BB101, *cat* FLPed

BB105	JB36 Δ*purR*		JB36 x P1 BB101, *cat* FLPed
BB107	AB1157 Δ*nfi*::*cat*		Deletion-replacement
BB326	JC9941 Δnfi		JC9941 x P1 BB107, cat FLPed
BB112	JB29 Δ*nfi*	pK96	JB29 x P1 BB107, *cat* FLPed
BB110	JB30 Δ*nfi*		JB30 x P1 BB107, *cat* FLPed
BB111	JB36 Δ*nfi*		JB36 x P1 BB107, *cat* FLPed
BB095	AB1157 Δ*rpoC*::*cat*		Deletion-replacement
BB323	JC9941 ΔrpoC		JC9941 x P1 BB095, cat FLPEd
BB120	JB29 Δ*rpoC*::*cat*	pK96	JB29 x P1 BB095
BB118	JB30 Δ*rpoC*::*cat*		JB30 x P1 BB095
BB119	JB36 Δ*rpoC*::cat		JB36 x P1 BB095
BB099	JB36 Δ*rpoC*		JB36 x P1 BB095, *cat* FLPed
b3283	MG1655 Δ*yrdD*::*aph*		ECK3270 from Keio Collection
BB328	JC9941 ΔyrdD		JC9941 x P1 b3283, aph FLPed
BB156	JB29 Δ*yrdD*	pK96	JB29 x P1 b3283, *aph* FLPed
BB150	JB30 Δ*yrdD*		JB30 x P1 b3283, *aph* FLPed
BB151	JB36 Δ*yrdD*		JB36 x P1 b3283, *aph* FLPed
b1014	MG1655 Δ*putA*::*aph*		ECK1005 from Keio Collection
BB330	JC9941 ΔputA		JC9941 x P1 b1014, aph FLPed
BB129	JB29 Δ*putA*	pK96	JB29 x P1 b1014, *aph* FLPed
BB127	JB30 Δ*putA*		JB30 x P1 b1014, *aph* FLPed
BB128	JB36 Δ*putA*		JB36 x P1 b1014, *aph* FLPed
b1015	MG1655 Δ*putP*::*aph*		ECK1006 from Keio Collection
BB138	JB29 Δ*putP*	pK96	JB29 x P1 b1015, *aph* FLPed
BB136	JB30 Δ*putP*		JB30 x P1 b1015, *aph* FLPed
BB137	JB36 Δ*putP*		JB36 x P1 b1015, *aph* FLPed
BB337	JB30 ΔputP::aph		pRL27 insertion
BB335	JC9941 ΔputP::aph		pRL27 insertion
b1877	MG1655 Δ*yecT*::*aph*		ECK1878 from Keio Collection
BB147	JB29 Δ*yecT*	pK96	JB29 x P1 b1877, *aph* FLPed
BB145	JB30 Δ*yecT*		JB30 x P1 b1877, *aph* FLPed
BB146	JB36 Δ*yecT*		JB36 x P1 b1877, *aph* FLPed
BB336	JB30 ΔyecT::aph		pRL27 insertion
BB334	JC9941 ΔyecT::aph		pRL27 insertion
BB257	JB29, contains high copy *purA* plasmid	pK96, pBBMC1	
BB258	JB29, contains high copy *yjjX* plasmid	pK96, pBBMC2	
BB260	JB29, contains high copy *rnb* plasmid	pK96, pBBMC4	
BB261	JB29, contains high copy *nepI* plasmid	pK96, pBBMC5	
BB263	JB30, contains high copy *purA* plasmid	pBBMC1	
BB264	JB30, contains high copy *yjjX* plasmid	pBBMC2	
BB266	JB30, contains high copy *rnb* plasmid	pBBMC4	
BB267	JB30, contains high copy *nepI* plasmid	pBBMC5	
BB269	JB36, contains high copy *purA* plasmid	pBBMC1	
BB270	JB36, contains high copy *yjjX* plasmid	pBBMC2	
BB272	JB36, contains high copy *rnb* plasmid	pBBMC4	
BB273	JB36, contains high copy *nepI* plasmid	pBBMC5	

Table 2. Primer sequences used to amplify *deoBD*, *dgt, hpt, nfi, purR,* and *tdk* from the *E. coli* AB1157 genome.

Primer Name	Sequence (5' to 3')	Engineered Restriction Site	5' end of Primer Binding Site
deoBD-L	acatgcatgcatgtgcacaccaactgtctatcgc	SphI	4617559
deoBD-R	catgccatggcatgaagccggagcagtctccc	NcoI	4619684
dgt-L	catgccatggcatggtatagatttcgcaaccgcc	NcoI	179049
dgt-R	ccccccgggggtgttcttcagattcgttttatagcc	XmaI	180845
hpt-L	ccgctcgagcgggagtaatcgtcgcgagcc	XhoI	141190
hpt-R	ccatcgatggaacctcaagctgaaacacgc	ClaI	142040
nfi-L2	cccatcgatgggaggaggacagctgtgattatggatctcgcg	ClaI	4196807
nfi-R	acatgcatgcatgtcagacgcagatgaattggg	SphI	4197556
purR-L	ccccccgggggccacacaaaaagtgatattacgc	XmaI	1735592
purR-R	gctctagagcgacgctgaataaggagtggc	XbaI	1736947
tdk-L	gctctagagctatgcaaggcttcgtaaggg	XbaI	1292626
tdk-R	gctctagagctaagagccctgtgaggcg	XbaI	1293496

Table 3. Plasmids used in this study.

Plasmid	Description	Source
pMTL22	High copy pUC8-based cloning vector	[1]
pK80	Low copy plasmid vector	[2]
pK96	22.5 kbp plasmid used for EndoV study	[2]
pBB01	*deoBD* PCR product inserted into SphI and NcoI of pMTL22	This study
pBB02	*dgt* PCR product inserted into NcoI and XmaI of pMTL22	This study
pBB03	*hpt* PCR product inserted into XhoI and ClaI of pMTL22	This study
pBB04	*nfi* PCR product inserted into ClaI and SphI of pMTL22	This study
pBB05	*purR* PCR product inserted into XmaI and XbaI of pMTL22	This study
pBB06	*tdk* PCR product inserted into XbaI of pMTL22, co-directional with *bla*	This study
pBB07	*hpt* from pBB03 cloned into pBB01 using XbaI and ClaI	This study
pBB08	*purR* from pBB05 cloned into pBB07 using XmaI and XbaI	This study
pBB09	*dgt* from pBB02 cloned into pBB08 using NcoI and XmaI	This study
pBB10	*nfi* from pBB04 cloned into pBB09 using ClaI and SphI	This study
pBB11	*tdk* from pBB06 cloned into pBB10 using XbaI	This study
pBB13	7909 bp BamHI-XhoI fragment from pBB11 ligated to pK80 cut with BamHI and XhoI	This study
pBBMC1	pMTL22 containing MluI fragment of E. coli K-12 containing *yjeT* and the first 1297 of 1299 bp of *purA*	This study
pBBMC2	pMTL22 containing MluI fragment of E. coli K-12 containing *yjjX*, *ytjC*, the last 81 of 327 bp of *trpR*, and the last 321 of 870 bp of *rob*	This study
pBBMC4	pMTL22 containing MluI fragment of E. coli K-12 containing *rnb*, the last 216 of 2676 bp of *acnA*, the last 92 bp of 591 bp of *ribA*, the first 280 of 1986 bp of *gmr*, and the last 527 of 1128 bp of *yciW*.	This study
pBBMC5	pMTL22 containing MluI fragment of E. coli K-12 containing *nepI*, *nlpA*, *yicS,* the last 802 of 924 bp of *yicL*, the last 44 of 453 bp of *yicN*, the last 181 of 762 bp of *ydeO*, and the first 606 of 1683 bp of *ydeN*.	This study
pBBMC1i	pBBMC1 with *yjeT* and *purA* inactivated by removal of 1205 bp HindIII fragment	This study
pBBMC2i	pBBMC2 with *yjjX* inactivated by removal of the 670 bp BamHI fragment	This study
pBBMC4i	pBBMC4 with *rnb* inactivated by removal of the 709 bp SalI fragment	This study
pBBMC5i-1	pBBMC5 with *nepI* inactivated by removal of the 2149 bp Bpu10I fragment	This study
pBBMC5i-2	pBBMC5 with *yicS* and *nepI* inactivated by removal of the 2902 bp AccI fragment	This study
pBBMC5i-3	pBBMC5 with *nlpA* inactivated by removal of the 1620 bp BglII – BspMI fragment	This study

Table 4. Suppressors of *rdgB recA* inviability. Inactivational suppressors which had two or more independent hits were selected for further analysis as in-frame deletion mutants.

Insertion Suppressors Cumulative Hits	Gene	Function
18	nfi	Endonuclease V
15	dgt	dGTP triphosphohydrolase
9	tdk	Thymidine / deoxyuridine kinase
5	hpt	Guanine / hypoxantine phosphoribosyltransferase
3	purR	Transcriptional repressor
3	yecT	Hypothetical protein
3	yrdD	Putative DNA topoisomerase
2	deoB	Phosphopentose mutase
2	deoD	Purine nucleoside phosphorylase
2	dusB	Dihydrouridine synthase / (fis transcriptional regulator)
2	glnG	NtrC transcriptional regulator of glutamine biosynthesis
2	gmk	Deoxyguanylate kinase
2	hdfR	Negative regulator of flagellar master operon
2	putA	Transcriptional repressor of put operon / proline dehydrogenase
2	putP	Transmembrane proline transporter
2	rpoC	RNA polymerase ß' subunit
1	dcd	Deoxycytosine deaminase
1	hupA	HU transcription regulator
1	nudC	NADH pyrophosphatase
1	nupC	Nucleoside (cytosol) to periplasm transporter
1	orn	Oligoribonuclease
1	polB	DNA polymerase II
1	rrlA	23S Ribosomal RNA
1	sapA	Peptide uptake ABC transporter
1	sapB	Peptide uptake ABC transporter
1	sapF	Peptide uptake ABC transporter
1	seqA	Negative modulator of initiation of DNA replication

1	thrU	Threonine tRNA U
1	tufB	Elongation factor Tu
1	waaG	LPS glucosyltransferase I (Generates UDP)
1	yacF	Conserved protein
1	yaeH	Conserved protein
1	ydeH	Conserved protein
1	ygfK	Putative oxidoreductase Fe-S subunit, possible selenate reductase
1	yifE	Conserved protein
1	ypjB	Predicted protein

94 total hits

Multicopy suppressors

Cumulative Hits	Gene	Function
29	yjjX	ITPase/XTPase
16	purA	Subunit of adenylosuccinate synthetase
7	rnb	Ribonuclease II
1	nepI	Nucleoside efflux permease-inosine

53 total hits

References

1. Chambers, S.P., et al., *The pMTL nic- cloning vectors. I. Improved pUC polylinker regions to facilitate the use of sonicated DNA for nucleotide sequencing.* Gene, 1988. **68**(1): p. 139-49.
2. Kuzminov, A. and F.W. Stahl, *Stability of linear DNA in recA mutant Escherichia coli cells reflects ongoing chromosomal DNA degradation.* J Bacteriol, 1997. **179**(3): p. 880-8.