Supplement for the paper by **Pritha Rao and Andrei Kuzminov**

Sources of thymidine and analogs fueling futile damage-repair cycles and ss-gap accumulation during thymine starvation in *Escherichia coli*

Supplemental figures with legends

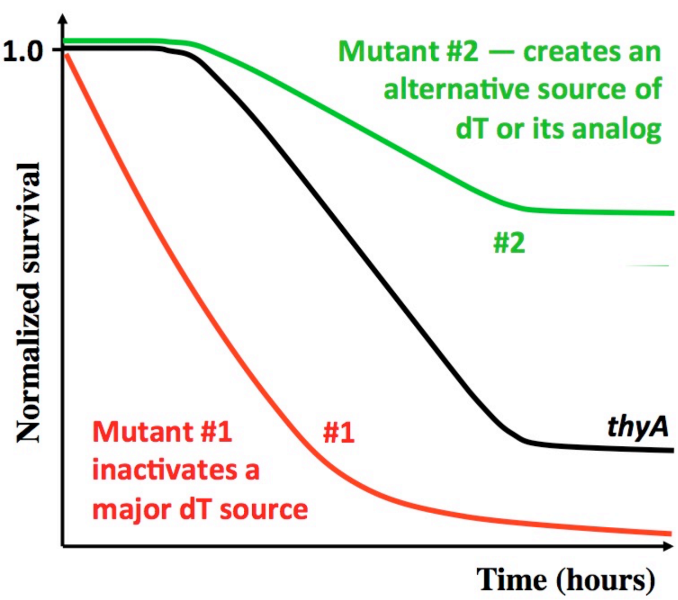

Fig. S1. The two major expected types of TLD-affected mutants in nucleotide metabolism and their interpretations. A stylized TLD kinetics of a *thyA* mutant is shown in black.

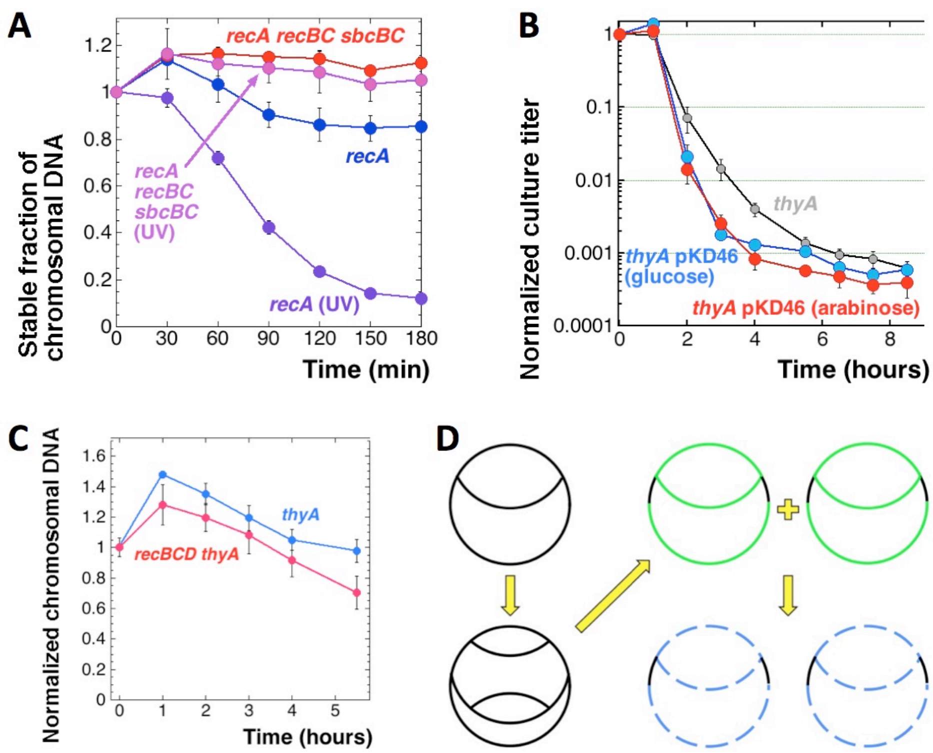

Fig. S2. The role of linear DNA degradation in thymine starvation phenomena and suggested patterns of chromosomal DNA instability.

A. Stabilization of prelabeled chromosomal DNA (TCA-precipitable counts) in UV-irradiated *recA* mutant cells by *recBC sbcBC* defect. The strains are (all ThyA+): *recA*, RA50; *recA recBC sbcBC*, RA49. The UV-dose was 50J/m^2.

B. Time course of TLD in the *thyA* pKD46 strain (KKW58). Glucose suppresses expression of the lambda Red proteins (inhibit RecBCD), arabinose induces them.

C. Evolution of the total amount of chromosomal DNA in the *thyA recBCD* mutant (KJK63) during T-starvation. The *thyA* mutant curve from Fig. 2E is shown for comparison.

D. A possible explanation for the observed pattern of the chromosomal DNA evolution. The accumulation during the resistance phase at first reflects the completion of the ongoing replication rounds with no new initiations, whereas the subsequent DNA instability, mostly during the RED phase reflects unknown defects in the nascent DNA (solid green), which eventually make this DNA unstable (dashed blue).

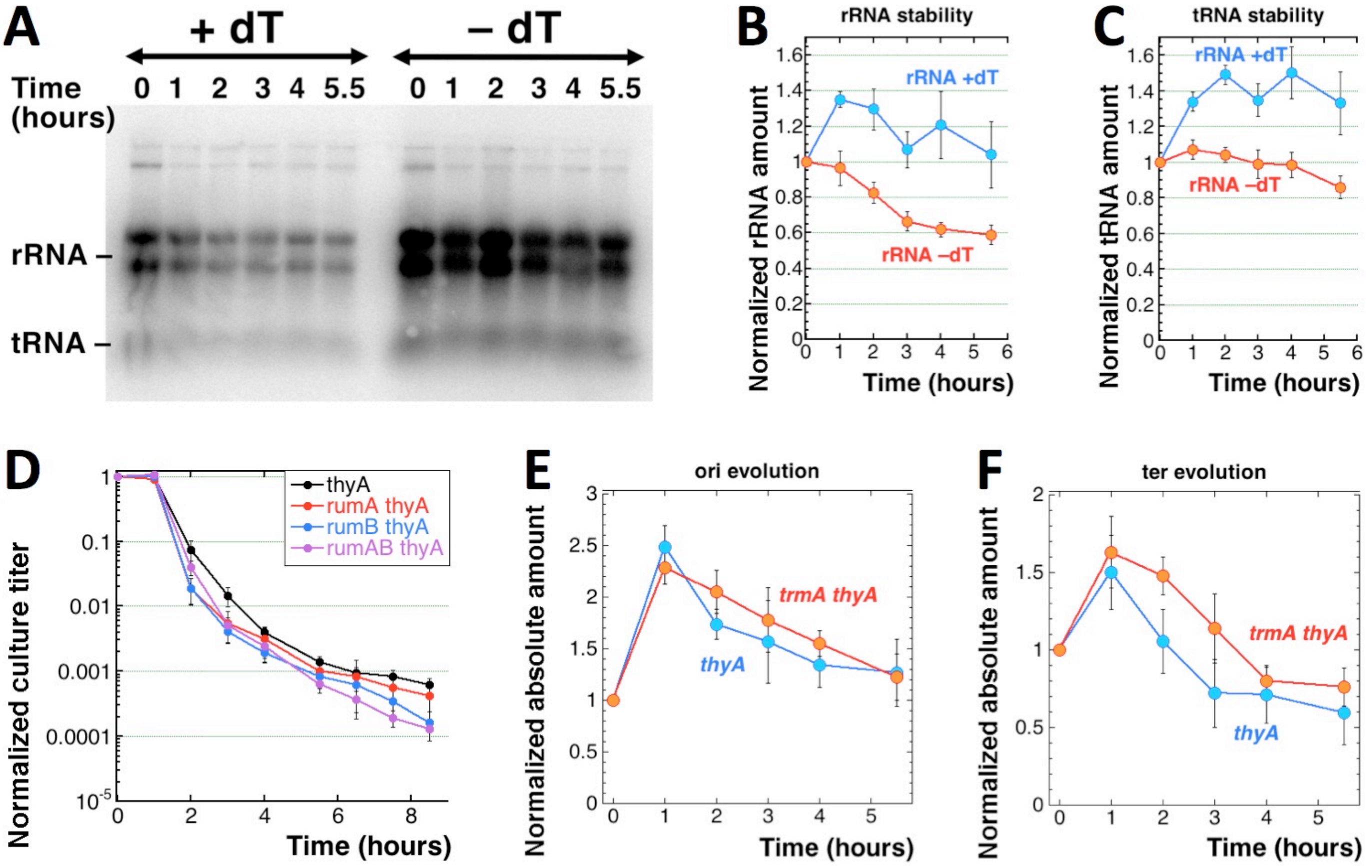

Fig. S3. (A lack of) stable RNA contribution to resistance phase of TLD.

A. The gel to determine stability rRNA and tRNA during growth. The *thyA* mutant (strain #) was labeled with 32P-orthophosphate during growth in the presence of dT, and then the medium was changed to one with or without dT, but having no label, and shaking of the culture continued at the same temperature. The culture grown in the presence of dT was gradually diluted several fold during growth, unlike the dT-less culture, — this is why the "+ dT" signal decreases substantially.

B. Stability of rRNA during thymine starvation, determined from several gels like in "B". The exogenous ^{32}P-orthophosphoric acid keeps incorporating for at least one hour under these conditions (Kuong and Kuzminov 2012). At the indicated time points, the label in the rRNA bands was normalized to the label at the = 0. The strain is KKW58.

C. The same as in "C", but done for tRNA.

D. Time course of TLD in the *rumA* and *rumB* mutants. The strains are: *thyA*, KKW58; *rumA thyA*, RA10; *rumB thyA*, RA11; *rumAB thyA*, RA13.

E. Evolution of the replication origin absolute amount in the *thyA* and *trmA thyA* mutants (strains like in Fig. 3D) during T-starvation.

F. Same as in "F", but for the terminus.

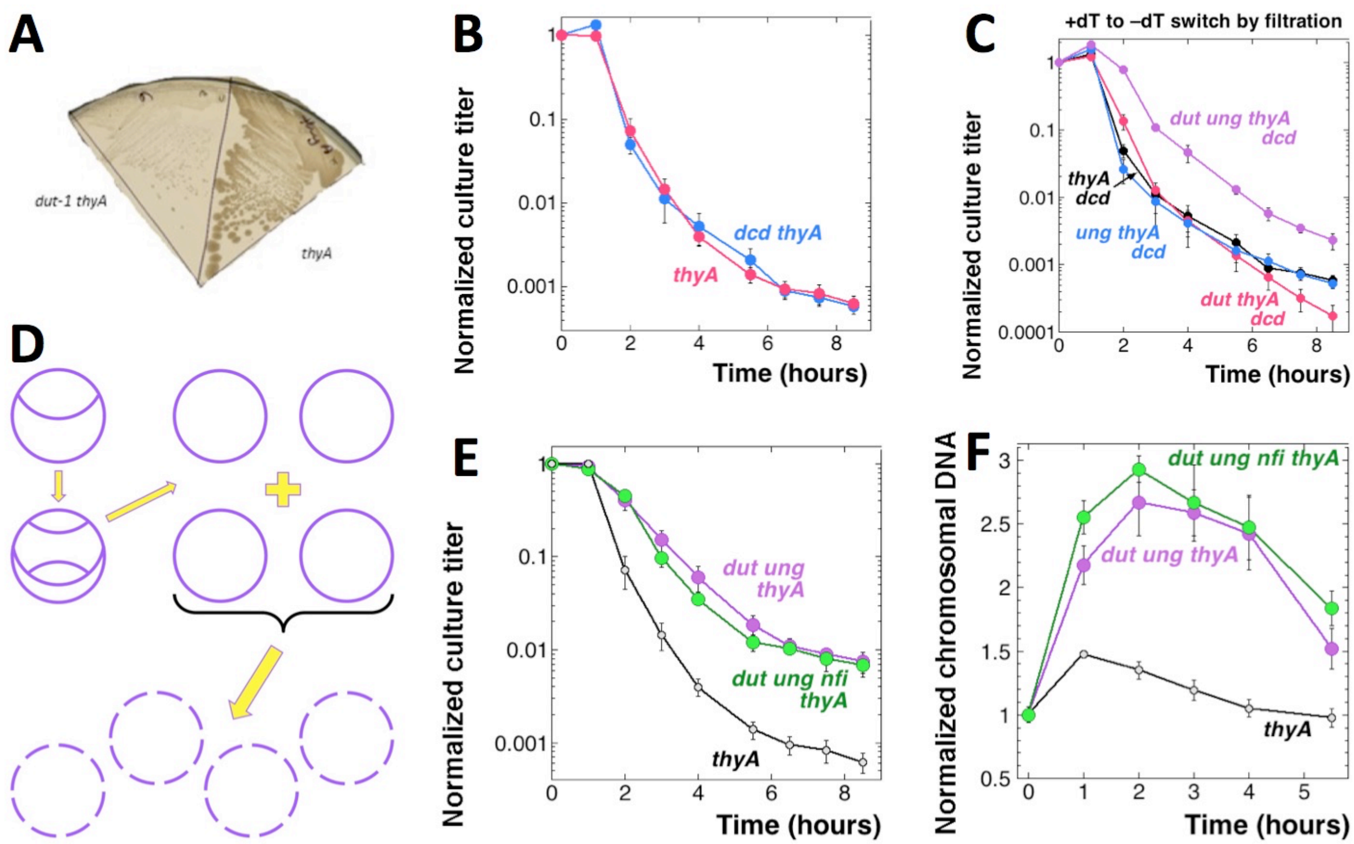

Fig. S4. Testing uracil incorporation-excision as the cause of TLD and analysis of TLD in the *dut ung thyA* mutants.

A. The extremely slow growth of the *dut thyA* mutant, even on rich medium supplemented with 3 μg/ml dT (optimal concentration). The strains are: *thyA*, KKW58; *thyA dut-1*, RA16.

B. Time course of TLD in the *dcd thyA* mutant. The strains are: *thyA*, KKW58; *dcd thyA*, RA25.

C. Time course of TLD in the *dcd dut thyA* and *dcd ung thyA* mutants. The +dT/-dT medium switch is by filtration (standard procedure). Strains are: *dcd thyA*, RA25; *dcd dut thyA*, RA27; *dcd ung thyA*, RA26; *dcd dut ung thyA*, RA28.

D. A scheme of the chromosome behavior during T-starvation of the *thyA dut ung* mutant. The elevated pool of dUTP, in combination with DNA-dU stability, facilitates chromosomal replication to the extent that both the existing and the newly-initiated rounds are finished, yielding four complete chromosomes. However, most cells still die while their chromosomes are degraded, by unclear mechanisms.

E. TLD kinetics of the *dut ung thyA* versus *dut ung nfi thyA* mutants. The *thyA* mutant is shown as a control. Strains are: *thyA*, KKW58; *dut ung thyA*, RA18; *dut ung nfi thyA*, RA30.

F. Evolution of the chromosomal DNA amount during thymine starvation. The strains are like in "E".

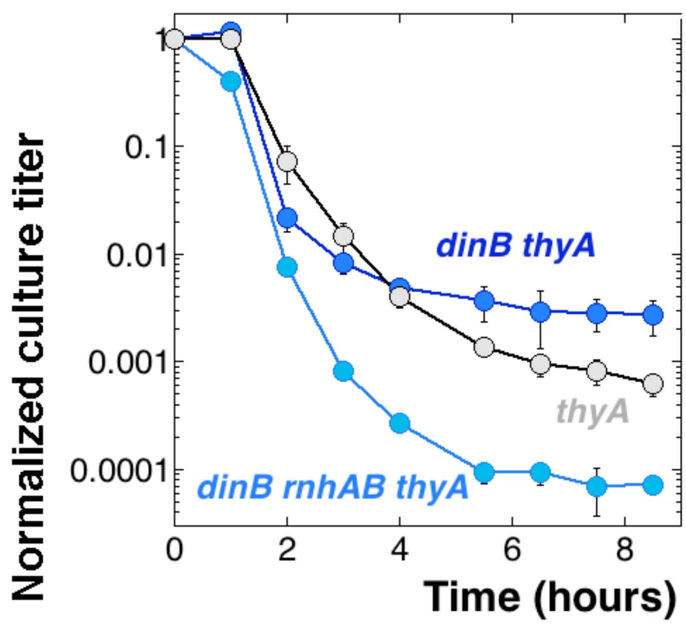

Fig. S5. TLD kinetics of the *dinB thyA* mutant and its *dinB rnhAB thyA* variant. Strains are: *dinB thyA*, KJK90; *dinB rnhAB thyA*, RA39.

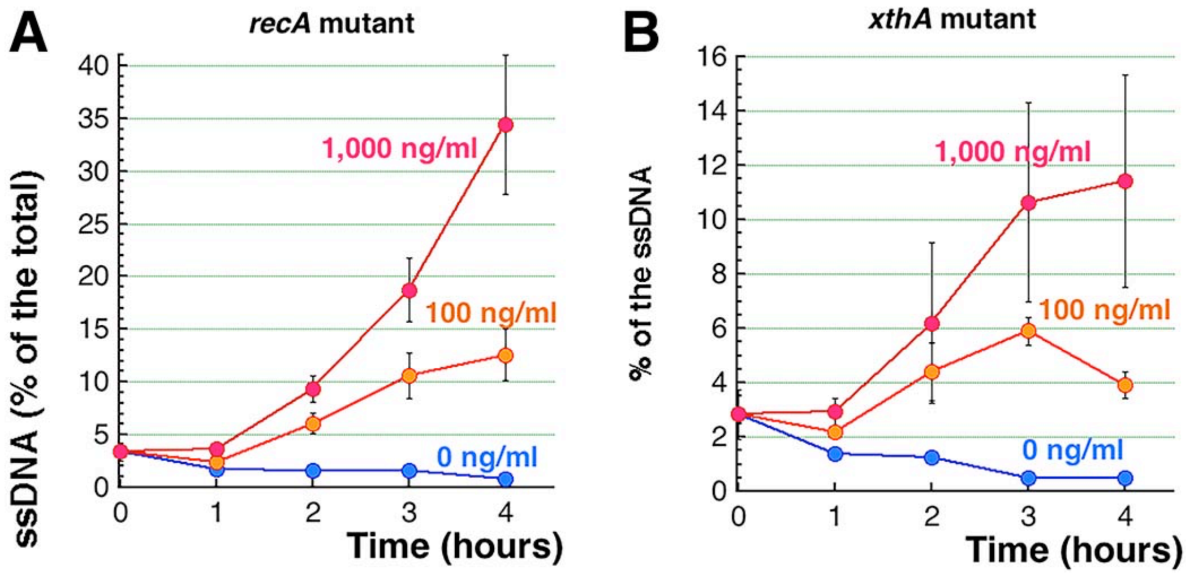

Fig. S6. Single-stranded DNA accumulation in genomic DNA of growing cultures treated with 0, 100 or 1,000 ng/ml AZT.

A. The *recA* mutant (RA50).

B. The *xthA* mutant (HT50).

Table S1. *E. coli* strains and plasmids used in this study.

Published Strains

Strain Name	Relevant Genotype[a]	Source
AAM1	Δ*thyA71::cat*	(Kuong and Kuzminov 2009)
AM3	*recF20::cat*	(Miranda and Kuzminov 2003)
BW1161	*nfi::cat*	(Guo and Weiss 1998)
ER131	Δ*rnhA::cat*	(Kouzminova, Kadyrov et al. *2017)*
GY9701*	*recA938::cam* miniF-kan *recA*+	R. Devoret via Benedicte Michel
HT50	*xthA::cat*	(Ting, Kouzminova et al. 2008)
JB1 pSA122	Δ*recBCD3*::*kan* pRecBC+	Lab collection
JC7623	*recB21 recC22 sbcB15 sbcC201*	(Kushner, Nagaishi et al. 1971)
JW0178-1*	Δ*rnhB782::kan*	CGSC#8427 (Baba, Ara et al. 2006)
JW0221-1*	Δ*dinB749::kan*	CGSC#8456 (Baba, Ara et al. 2006)
JW0843-1*	Δ*rumB760::kan*	CGSC#8879 (Baba, Ara et al. 2006)
JW2756-1*	Δ*rumA783::kan*	CGSC#11916 (Baba, Ara et al. 2006)
JW3937-1*	Δ*trmA753::kan*	CGSC#12049 (Baba, Ara et al. 2006)
KJK63	Δ*thyA72* Δ*deoCABD2* Δ*recBCD3::kan*	(Kuong and Kuzminov 2010)
KJK78	Δ*thyA72* Δ*deoCABD2* Δ*ung::cat*	(Kuong and Kuzminov 2010)
KJK87	Δ*thyA72* Δ*deoCABD2* Δ*umuCD595::cat*	Kawai Jessica Kuong, this lab
KJK90	Δ*thyA72* Δ*deoCABD2* Δ*dinB749::kan*	Kawai Jessica Kuong, this lab
KJK183	Δ*deoCABD2 dut1 zic 4901::*Tn*10*	Kawai Jessica Kuong, this lab
KKW47	Δ*deoCABD1::cat*	(Kuong and Kuzminov 2009)
KKW58	Δ*thyA72* Δ*deoCABD2*	(Kuong and Kuzminov 2009)
KKW59	Δ*deoCABD2*	(Kuong and Kuzminov 2009)
L76	AB1157 *dcd329::kan*	Elena Kouzminova, this lab
L418	Δ*rnhA::cat* Δ*rnhB782::kan*	(Kouzminova, Kadyrov et al. 2017)
RPC501	AB1157 *nfo-1::kan*	(Cunningham, Saporito et al. 1986)
RW82	*uvrA6 umuCD595::cat*	(Woodgate 1992)

This Study

Strain Name	Relevant Genotype[a]	Construction
RA9	Δ*thyA72* Δ*deoCABD2* Δ*trmA753::kan*	KKW58 x P1 JW3937-1
RA10	Δ*thyA72* Δ*deoCABD2* Δ*rumA783::kan*	KKW58 x P1 JW11916
RA11	Δ*thyA72* Δ*deoCABD2* Δ*rumB760::kan*	KKW58 x P1 JW8879
RA12	Δ*thyA72* Δ*deoCABD2* Δ*rumA*	RA10 treated with pCP20
RA13	Δ*thyA72* Δ*deoCABD2* Δ*rumA* Δ*rumB760::kan*	RA12 x P1 JW8879
RA14	Δ*thyA72* Δ*deoCABD2* Δ*trmA753::kan* Δ*rumB*	RA11 treated with pCP20 and transduced with P1 JW3937-1
RA15	Δ*thyA72* Δ*deoCABD2* Δ*rumA* Δ*rumB* Δ*trmA753::kan*	RA13 treated with pCP20 and transduced with P1 JW3937-1
RA16	Δ*thyA71::cat* Δ*deoCABD2 dut-1 zic 4901::*Tn*10*	KJK183 x P1 AAM1
RA17	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10*	RA16 treated with pCP20
RA18	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10* Δ*ung::cat*	RA17 x P1 KJK78
RA22	Δ*thyA72* Δ*deoCABD2* Δ*xthA::cat*	KKW58 x P1 HT50
RA23	Δ*thyA72* Δ*deoCABD2* Δ*nfo-1::kan*	KKW58 x P1 RPC501
RA24	Δ*thyA72* Δ*deoCABD2* Δ*xthA::cat* Δ*nfo-1::kan*	RA22 x P1 HT50
RA25	Δ*thyA72* Δ*deoCABD2 dcd329::kan*	KKW58 x P1 L76
RA26	Δ*thyA72* Δ*deoCABD2* Δ*ung::cat dcd329::kan*	KJK78 x P1 L76
RA27	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10 dcd329::kan*	RA16 x P1 L76
RA28	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10* Δ*ung::cat dcd329::kan*	RA17 x P1 L76
RA29	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10* Δ*ung*	RA18 treated with pCP20
RA30	Δ*thyA72* Δ*deoCABD2 dut-1 zic 4901::*Tn*10* Δ*ung Δnfi::cat*	RA29 x P1 BW1161

RA31	*ΔthyA72 ΔdeoCABD2 ΔrecF20::cat*	KJK78 x P1 AM3
RA32	*ΔthyA72 ΔdeoCABD2 dut-1 zic 4901::*Tn*10 Δung ΔrecF20::cat*	RA29 x P1 AM3
RA33	*ΔthyA72 ΔdeoCABD2 ΔrnhA::cat*	KKW58 x P1 ER131
RA34	*ΔthyA72 ΔdeoCABD2 ΔrnhB782::kan*	KKW58 x P1 JW0178-1
RA35	*ΔthyA72 ΔdeoCABD2 ΔrnhA::cat ΔrnhB782::kan*	KKW58 x P1 L418
RA36	*ΔthyA72 ΔdeoCABD2 ΔdinB749::kan umuCD595::cat*	KJK87 x P1JW0221-1
RA37	*ΔthyA72 ΔdeoCABD2 ΔrnhA::cat ΔrnhB782::kan* pEAK86	
RA38	*ΔthyA72 ΔdeoCABD2 ΔrnhA2 ΔrnhB2*	RA35 treated with pCP20
RA39	*ΔthyA72 ΔdeoCABD2 ΔrnhA2 ΔrnhB2 ΔdinB749::kan*	RA38 x P1 JW0221-1
RA40	*ΔthyA72 ΔdeoCABD2 ΔrnhA2 ΔrnhB2 umuCD595::cat*	RA38 x P1 RW82
RA41	*ΔthyA72 ΔdeoCABD2 ΔrnhA2 ΔrnhB2 ΔdinB749::kan umuCD595::cat*	RA39 x P1 RW82
RA42	*ΔdeoCABD::cat recB21 recC22 sbcB15 sbcC201*	JC7623 x P1 KKW47
RA43	*ΔdeoCABD2 recB21 recC22 sbcB15 sbcC201*	RA42 treated with pCP20
RA44	*ΔthyA71::cat ΔdeoCABD2 sbcB15 sbcC201*	RA43 x AAM1
RA45	*ΔthyA71::cat ΔdeoCABD2 sbcB15 sbcC201 ΔrecBCD::kan*	RA44 x P1 JB1 pSA122
RA46	*recA938::cat ΔthyA72 ΔdeoCABD2*	KKW58 x P1 GY9701
RA47	*ΔrecBCD sbcB15 sbcC201 recA938::cat ΔthyA72 ΔdeoCABD2*	RA45 treated with pCP20 and transduced with P1 GY9701
RA48	*ΔdeoCABD2 recF20::cat*	KKW59 x P1 AM3
RA49	*recA938::cat recB21 recC22 sbcB15 sbcC201*	JC7623 x P1 GY9701
RA50	*recA938::cat*	AB1157 x P1 GY9701

a — Background for all strains, except those marked with * is AB1157, which also includes: F– lambda– rac- *thi-1 hisG4 D(gpt-proA)62 argE3 thr-1 leuB6 kdgK51 rfbD1 araC14 lacY1 galK2 xylA5 mtl-1 tsx-33 glnV44 rpsL31*

Plasmids

Plasmid	Replicon/drug resistance/other genes	Reference
pBR322	cloning vector / *bla tet*	(Bolivar, Rodriguez et al. 1977)
pMTL20	pBR322 / *bla* / *lacZ alpha*	(Chambers, Prior et al. 1988)
pEAK86	pSC101* / *bla* / *csdA*	(Kouzminova, Kadyrov et al. 2017)
pCP20	Rep^{ts} / *bla cat* / *FLP+*	(Datsenko and Wanner 2000)
pKD46	Rep^{ts}/ *bla* / *exo gam bet araC*	(Datsenko and Wanner 2000)
pSA122	p15A / *cat* / *recB+ recC+*	(Amundsen, Taylor et al. 2000)

Primers

Δ ***trmA***
To verify deletion
PR01 CCGTCATTATGGTGTTCTGG
PR02 CAAGTGGATGCTACAGGTTG

Δ ***rumA***
To verify deletion

PR06   CCAGGATCAGAATCATCATGCGTG
PR07   GTGCACTTCTTACCGCAACC

Δ *rumB*
To verify deletion
PR08   CCAGTCGGCGGTTCTTCCAG
PR09   CAATCTGAAGCCGCACATGC

dcd
To verify *dcd329* insertion
PR10   GTGATAACCTAGTATGCCCTTGACG
PR11   CAGGAGTATCATCAGCGTCG

Δ *nfi*
To verify deletion
PR14   TTGCAGGTTTCGGTCACGGC
PR15   CGGTAAATTTGCCCATCACC

Δ *nfo*
To verify deletion
PR23   AAAGCTGGGGCGAAACCTCTG
PR24   GAGCAATGAATTGTACCGGG

Δ *deoCABD*
To verify deletion
PR42   TTGATCCTGATGCGTTTGCC
PR43   ATCCAGAGGAATTTCCGCAG

recB
To verify *recB21* insertion
PR44   AAGAATGAGTGATGTCGCCG
PR45   GCATCATGACTTAACAGTGCCG

Δ *rnhA*

#167   GCACCAATCTGGTTCATACC

#168   CAATGTCGTAAACCACAGGC

Δ *rnhB*

To verify deletion

#269   GAACTGCATCAGCAGATCCG
#270   CAGACATCTTCAGATTCCGG

Δ *dinB*

To verify deletion
dinB(Exp)F   CGCGAATTCCGCAGCGAACGCGTTAAATG
dinB(PCR)B   AACGCTTCGAATGCGCTGGC

Δ *umuDC*
To verify deletion
umuCD(Exp)F   CGCGAATTCCAGTCATAATCATTCGCCTC
umuCD (PCR)B   GATCTGTTCGGTCGCTAATC

Δ *xthA*
To verify deletion

xthA(F)   CGGTAAGCAACGCGAAATTC

xthA(B)   GTATAACAAAGGACGGCAGG

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