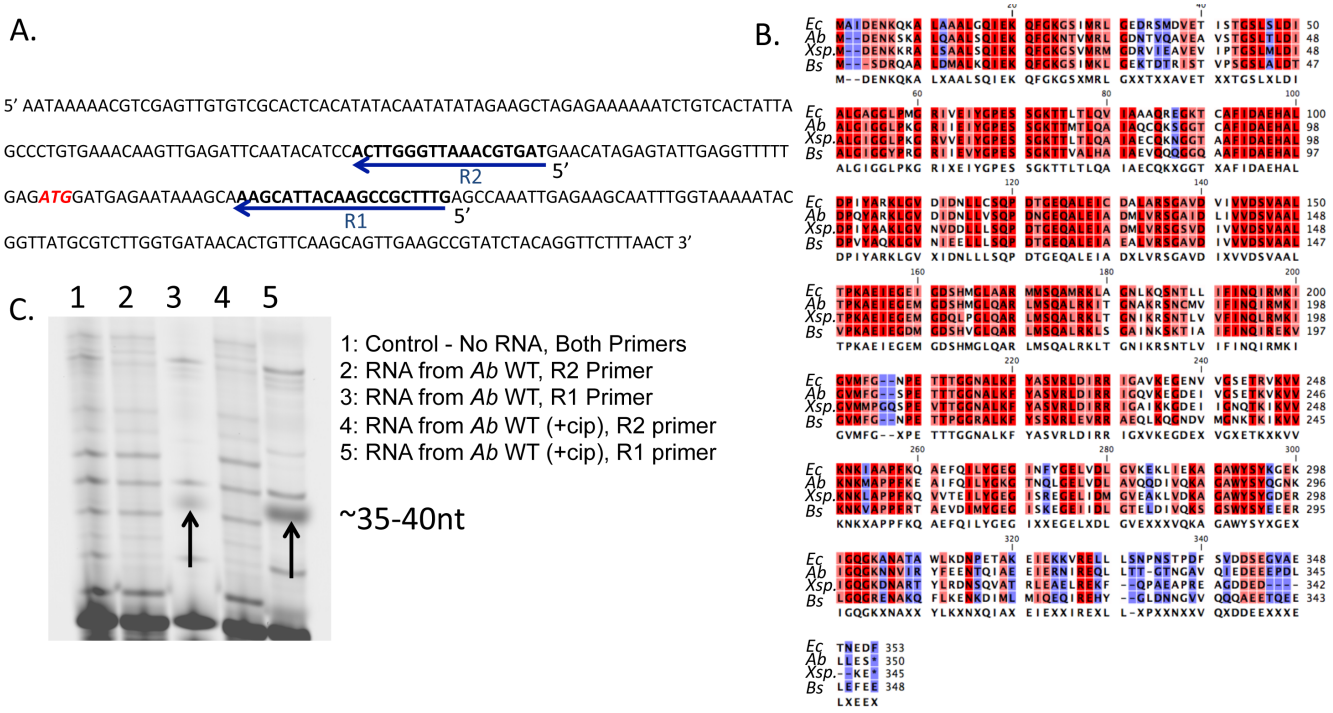


Fig S1. Primer extension assay suggests mRNA structure. (A) Schematic of the *recA* gene and upstream region. The ATG is indicated in red, and primers used for primer extension assay are shown in as blue arrows. **(B)** Multiple sequence alignment (CLC Genomics) showing the RecA coding sequence conservation among different bacteria to determine starting methionine. *Ec*, *E. coli*; *Ab*, *A. baumannii*; *X sp*., *Xanthomonas* species; *Bs*, *Bacillus subtilis*. Red boxes indicate conservation. The consensus RecA sequence is shown underneath the compound sequences. **(C)** Separated products of extension assay on a 10% denaturing acrylamide gel containing urea and visualized using the Typhoon 8600 Imager (General Electric Hartford, CT). Using fluorescently labeled primers R1 downstream of the ATG and R2 upstream of it, first strand cDNA synthesis was performed at 65°C using the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Fisher) on RNA extracted from *A. baumannii* cells with or without cip treatment as described in the main Methods section. This experiment should result in a size

product encompassing the reverse primer up to the +1 transcriptional start including the ribosomal binding site. Control reactions did not contain RNA template. Unique bands were only readily observed in lanes 3 and 5, which are estimated to be 35-40nt in length based on control primers of known sizes run on the same gel. The increase in product in lane 5 indicates that there was a higher amount of transcript template, as would be expected in cells that were treated with DNA damage. The short product from Primer R1 is within the coding region of the *recA* gene, indicating that the mRNA structure may be occluding further extension. No product observed when using Primer R2 may indicate structural occlusion of the mRNA template.

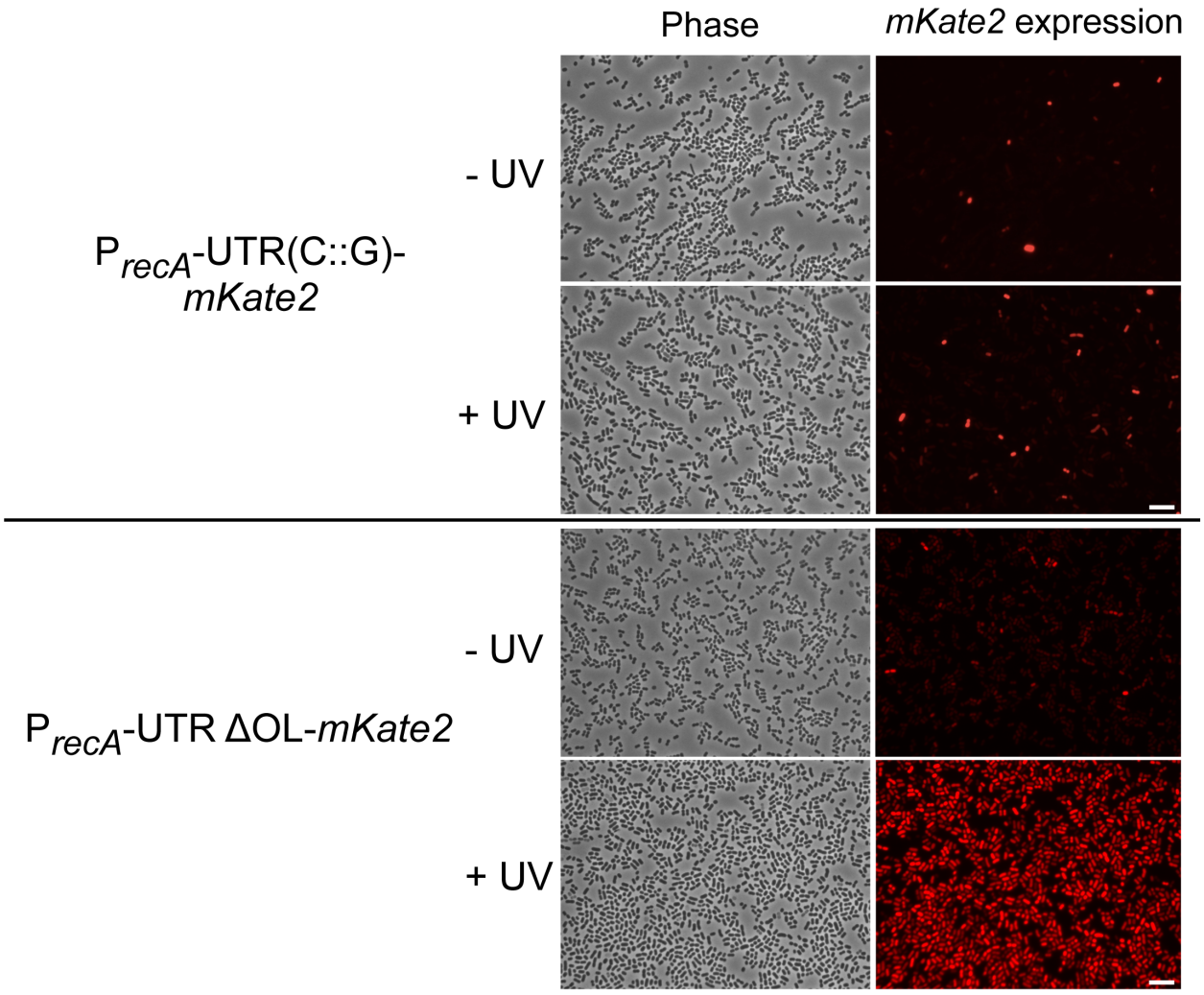
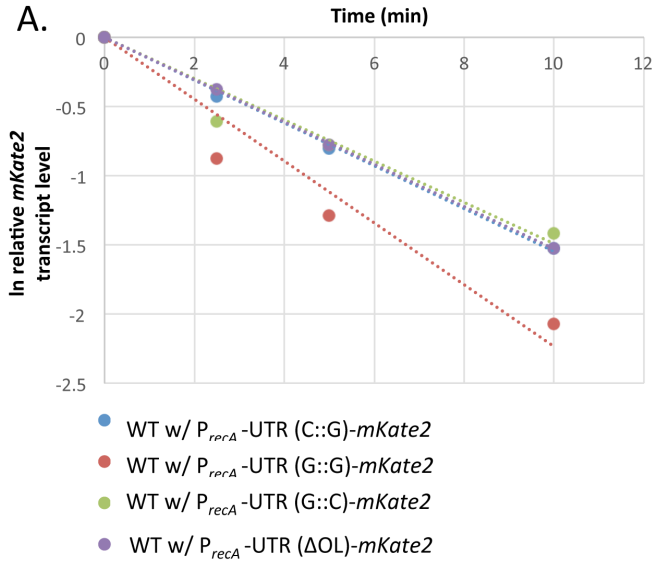


Fig S2. Strain with the plasmid borne *mKate2* reporter responds to UV radiation treatment. Representative fluorescent microscopy images of *A. baumannii* cells with the native P_{recA}-UTR(C::G)-*mKate2* and with the variant P_{recA}-UTR(ΔOL)-*mKate2* reporters without (-) or with (+) UV treatment. Scale bar represents 10μm. For treatment saturated liquid cultures were diluted 1:100 in LB and grown for 3 hours to exponential phase. Cells were then washed in SMO (100mM NaCl, 20mM Tris-HCl, pH 7.5) and spread in a glass petri dish. Plates were irradiated in the dark under a UV germicidal lamp with 54 J/m2. Cells were recovered in 5mL of LB medium for 3 hours before imaging.

A.



B.

	k	R^2	Half-life (min, ± SD)
UTR(C::G)-*mkate2*	-0.155	0.99	4.5 (3.8-5.6)
UTR(G::G)-*mkate2*	-0.252	0.79	2.8 (2.5-3.2)
UTR(G::C)-*mkate2*	-0.144	0.83	4.8 (4.4-5.3)
UTR(ΔOL)-*mkate2*	-0.153	0.99	4.5(4.0-5.1)

Fig S3. *mKate2* mRNA decay curves and half-life. (**A**) Plot of the natural logarithm (ln) of relative *mKate2* mRNA expression for each time point post rifampicin treatment. Half-life was calculated as described in the methods. (**B**) Table summary of rate constants, R^2, and half-life [± standard deviation (SD)] for each variant.

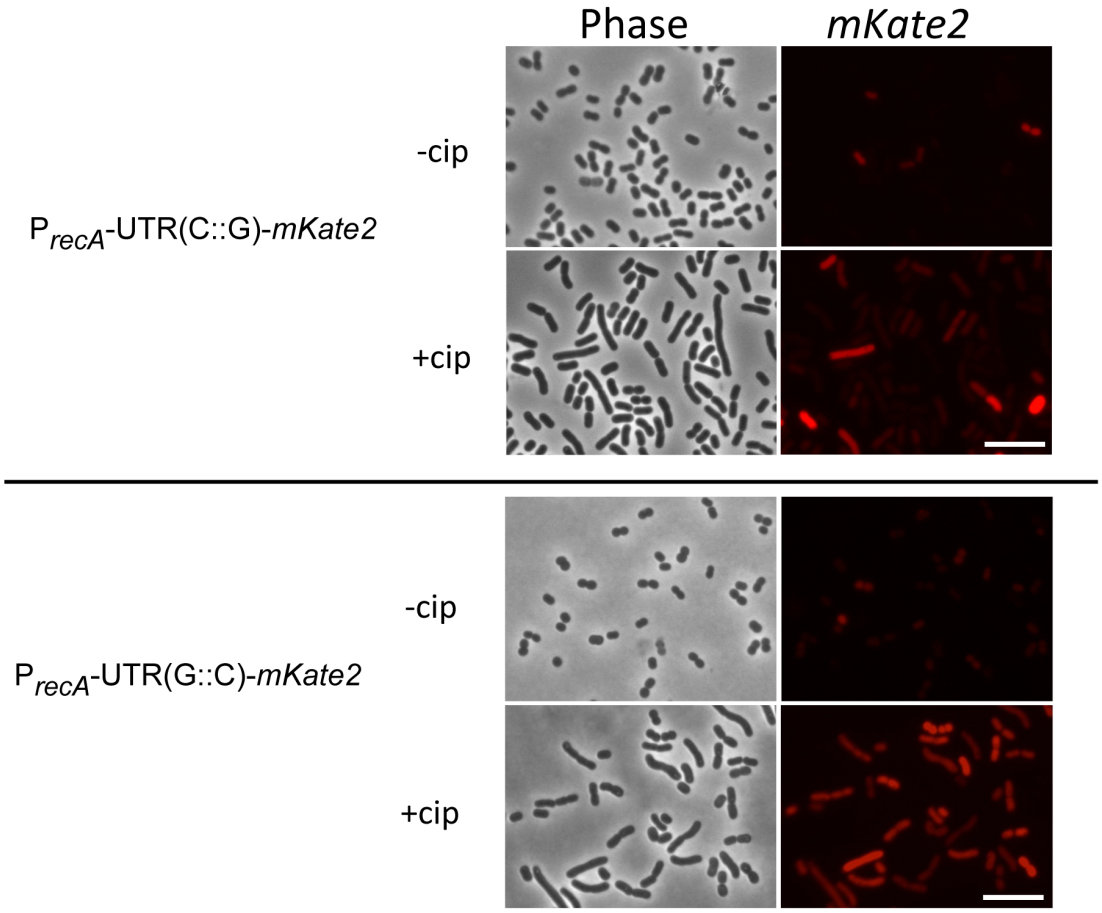


Fig S4. Cells with the native P_{recA}-UTR(G::C)-*mKate2* reporter have increased expression upon DNA Damage treatment. Representative phase and fluorescent microscopy images of the *A. baumannii* WT cells with the P_{recA}-UTR(C::G)-*mKate2* reporter compared to the P_{recA}-UTR(G::C)-*mKate2* reporter without (-) or with 10X MIC cip (+).

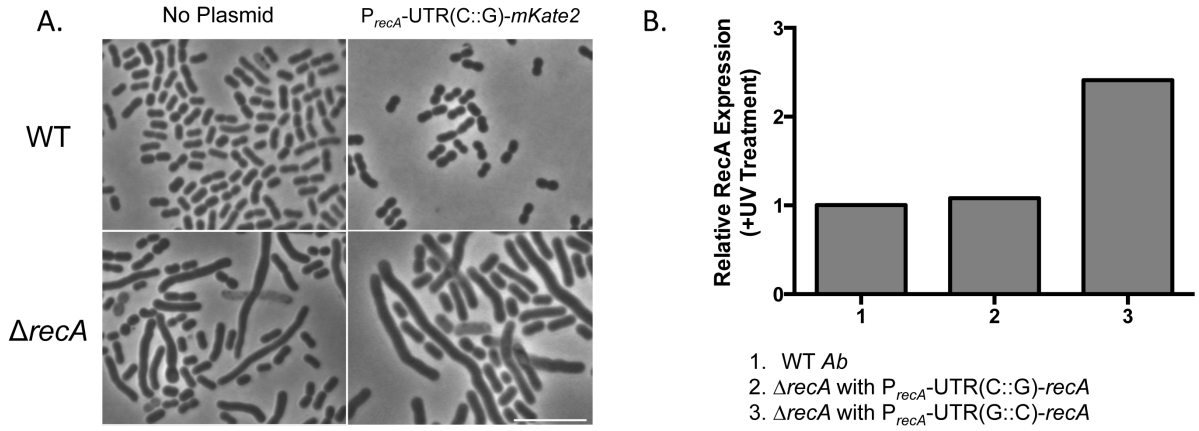


Fig S5. The elongation phenotype of *A. baumannii* Δ*recA* is not rescued by pNLAC1 bearing *mKate2* in place of *recA*, while DNA damage treatment alters RecA protein levels. **(A)** The plasmid borne P_{recA}-UTR(C::G)-*mKate2* reporter with the native UTR does not affect cell physiology. Representative contrast phase images of WT *A. baumannii* cells with no plasmid and with the plasmid containing the native P_{recA}-UTR(C::G)-*mKate2* reporter. Also shown are images of *A. baumannii* Δ*recA* cells with no plasmid and with the native reporter. Cells shown are in the absence of DNA damage treatment. Scale bar represents 10μm. **(B)** Immunoblot to detect RecA from UV treated (27 J/m^2) WT cells and Δ*recA* cells with plasmid borne, P_{recA}-UTR(C::G)-*recA* compared to P_{recA}-UTR(G::C)-*recA*. Total protein loaded per lane was standardized to the RpoB loading control as shown in Fig 5B. Graph shows relative protein expression with WT cells set as 1. A comparative experiment using the isogenic Δ*recA* strain and Δ*recA* with P_{recA}-UTR(G::G)-*recA* could not be performed due to their sensitivity to UV irradiation.

Table S1. Variant *A. baumannii recA* UTR Sequences

Name	Sequence
UTR(C::G) (native)	GAGAAAAAATCTGTCACTATTAGCCCTGTGAAACAAGTTGAGATTCAATACATCCACTTGGGTT AAACGTGATGAACATAGAGTATTGAGGTTTTTGAG
UTR(G::G)	GAGAAAAAATCTGTCACTATTAGGGGTGTGAAACAAGTTGAGATTCAATACATCCACTTGGGTT AAACGTGATGAACATAGAGTATTGAGGTTTTTGAG
UTR(G::C)	GAGAAAAAATCTGTCACTATTAGGGGTGTGAAACAAGTTGAGATTCAATACATCCACTTCCCTT AAACGTGATGAACATAGAGTATTGAGGTTTTTGAG
UTR(ΔOL)	GAGAAAAAATCTGTCACTATTAGCCCTGTGAAACAAGTTGAGATTCAATACATCCACTTGGGTT AAACGTGATGAACATGTTTTTGAG

Table S2. Plasmids and Strains used in this study

Plasmid	Description	Reference
pNLAC1	*ori-repM (A.baumannii)*, *oripMB1(E. coli),* Amp^R Tet^R	Gift of T. Russo ([1])
pCC1 (P_{recA}-UTR(C::G)–*mKate2*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(C::G)–*mKate2*, with, Tet^R	This work
pCC2(P_{recA}-UTR(G::G)–*mKate2*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(G::G)–*mKate2,* Tet^R	This work
pCC3(P_{recA}-UTR(ΔOL)–*mKate2*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(ΔOL)–*mKate2*, Tet^R	This work
pCC4 (P_{recA}-UTR(C::G)–*recA*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(C::G)–*recA*, Tet^R	This work
pCC5 (P_{recA}-UTR(G::G)–*recA*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(G::G)–*recA,* Tet^R	This work
pCC6 (P_{recA}-UTR(G::C)–*recA*)	pNLAC1 containing *recA* (A1S_1962) promoter–UTR(G::C)–*recA*, Tet^R	This work
pCC7 (P_{trpB}-UTR(C::G)–*mkate2*)	pNLAC1 containing *trpB* (A1S_1692) promoter–UTR(C::G)–*mKate2*, Tet^R	This work
pCC8 (Promoterless *mKate2*)	pNLAC1 containing promoterless *mKate2*, Tet^R	This work
pCC9 (P_{recA}-NO UTR–*mKate2*)	pNLAC1 containing *recA* (A1S_1962) promoter–*mKate2*, Tet^R	This work
pCC10 (P_{trpB}-NO UTR–*mKate2*)	pNLAC1 containing *trpB* (A1S_1692) promoter–*mKate2***,** Tet^R	This work
pYC251	contains *mKate2* coding sequence	Gift of Y. Chai
Strain	**Description**	**Reference**
A. baumannii	ATCC17978 Parental strain	American Type Culture Collection
A. baumannii Δ*recA*	ATCC17978 *recA::km*	Gift of G. Bou ([2])
CC002	ATCC17978 with pCC1	This work
CC034	ATCC17978 *ΔrecA* with pCC1	This work
CC068	ATCC17978 with pCC3	This work
CC302	ATCC17978 with pCC2	This work
CC314	ATCC17978 with pCC4	This work
CC315	ATCC17978 *ΔrecA* with pCC4	This work
CC334	ATCC17978 *ΔrecA* with pCC5	This work
CC357	ATCC17978 *ΔrecA* with pCC6	This work
CC364	ATCC17978 with pCC7	This work
CC382	ATCC17978 with pCC10	This work
CC401	ATCC17978 with pCC9	This work
E. coli P_{recA}-*gfp*	*Escherichia coli* P90C, F− *ara* Δ(*lac-proB*)XIII *thi*, with pUA66-*recA*–GFP, kan^R	([3])

References:

1. Luke NR, Sauberan SL, Russo TA, Beanan JM, Olson R, Loehfelm TW, Cox AD, Michael FS, Vinogradov E V., Campagnari AA (2010) Identification and characterization of a glycosyltransferase involved in *Acinetobacter baumannii* lipopolysaccharide core biosynthesis. *Infect Immun* **78**: 2017–2023.
2. Aranda J, Bardina C, Beceiro A, Rumbo S, Cabral MP, Barbé J, Bou G (2011) *Acinetobacter baumannii* RecA protein in repair of DNA damage, antimicrobial resistance, general stress response, and virulence. *J Bacteriol* **193**: 3740–3747.
3. Benson RW, Norton MD, Lin I, Du Comb WS, Godoy VG (2011) An active site aromatic triad in *Escherichia coli* DNA Pol IV coordinates cell survival and mutagenesis in different DNA damaging agents. *PLoS One* **6**: e19944.

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Name	Sequence (5' to 3')
16SRNAF	GACTCCTACGGGAGGCWGCAG
16SRNAR	GTATTACCGCGGCTGCTGG
F1	GTTGAGATTCAATACATCCACTTGG
F2	CCTGTGAAACAAGTTGAGATTCAA
F3	GTCACTATTAGCCCTGTGAAAC
F4	AGAAAAAATCTGTCACTATTAGCC
F5	AGAAGCTAGAGAAAAAATCTGTC
F6	CATATACAATATATAGAAGCTAGAG
MCSmKate2F	GTACCTGCAGGAGCTCTCTAGATTGAGGTTTTTGAGATGGATTCAATAG
MCSmKate2R	GTACCTGCAGGGTACCATCGGATCCTTATCTGTGCCCCAG
mKate2F	TGATGGATTCAATAGAAAAGGTAAGCGAGCTGATTAAG
mKate2qPCRF	GGTAAGCGAGCTGAATTAAGGA
mKate2qPCRR	GCTTGCCTTCGCCCTCG
mKate2R	ACCACGATGCCTGCAGATCGGATCCTTATCT
PNLACF	AGTTTGCGCAACGTTGTTGCCA
PNLACR	AACGACGAGCGTGACACCAC
PrecAF	GTTGCCATTGCTGCACGACCTGACCATAGA
PrecAF2	GTACGAGCTCCGACCTGACCATAGACCAAG
PrecAR	GTACTCTAGATCTAGCTTCTATATATTGTATATGTGAGTG
PtrpF	GTACGAGCTCGTCGTAGGTGATGCCAGTAT
PtrpR	GTACTCTAGAATCATCCCTCTTTTTAAATTATTAAAG
R1	Cy5-CAAAGCATTACAAGCCGCTT
R2	Cy5-ATCACGTTTAACCCAAGT
recAmKate2R	CTATTGAATCCATCAGTGTTATCACCAAGACG
recAmidF	GCACGCAAACTTGGTGTAGA
recAmidR	GCGCCTGAACGGACAAG
recAR	ACCACGATGCCTGCAAATTACGATTCTAATAAAAGATCT
SD1F	ATCTGTCACTATTAGGGGTGTGAAACAAGTTGAGATTC
SD1R	CTAATAGTGACAGATTTTTTCTCTAGCTTCTATATATTGT
SD2F	GGGTTAAACGTGATGAACATGTTTTTGAGATGGATGAGAA
SD2R	ATGTTCATCACGTTTAACCCAAGTGGATGTATTGAATCTC
SD3F	TCAATACATCCACTTCCCTTAAACGTGATGAACATAGAG
SD3R	AAGTGGATGTATTGAATCTCAACTTGTTTCACAGGGCTAA
P_{trp}-UTR(C::G)-*mKate2*	GTTGCCATTGCTGCAGAGCTCGTCGTAGGTGATGCCAGTATGGCACCGTATGAGCTGA AATCGGTAGGTGGCTCAGTTGAATATATGAATGATGAAGCTGGTGAAGTTTGGTTACGT CGCCTACGCCAGCATTTTGATAAAACAGCATGGTTAAACCCTGAAACAGAAGGGTATT GGCATTACACTCAAACAATTGGTTGGATTAAAGAGATTTTTGAAAACCATATGTATCCAA TGACCCTAAAAGGCATTGAAGATTTGACGCGTTACCTTTCTCGATAAACTTGATAACTTT AATAATTTAAAAAGAGGGATGATTCTAGAAAAAAATCTGTCACTATTAGCCCTGTGAAAC AAGTTGAGATTCAATACATCCACTTGGGTTAAACGTGATGAACATAGAGTATTGAGGTT TTTGAGATGGATTCAATAGAAAAGGTAAGCGAGCTGATTAAGGAGAACATGCACATGA AGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCG AAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGC CCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCT TCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTT CACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGA CACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTT CCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGA GACGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAGC

	TCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACAGATCCAAGAAAC CCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGAAGACTGGAAAGAAT CAAGGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATA CTGCGACCTCCCTAGCAAACTGGGGCACAGATAAGGATCCGATCGCGGCCGCCTGCA GGCATCGTGGT

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