Supporting Information

Elimination of transforming activity and gene degradation during UV and UV/H_2O_2 treatment of plasmid-encoded antibiotic resistance genes

Younggun Yoon[1], Michael C. Dodd[2], Yunho Lee[1]*

[1]School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea

[2]Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98195, USA

*Corresponding Author: Yunho Lee; Phone: 82-62-715-2468; Fax: 82-62-715-2434; Email: yhlee42@gist.ac.kr

Submitted to Environmental Science: Water Research & Technology

SI-Text-1. Standards and reagents

The following chemicals were purchased from various suppliers and used as received: acetonitrile (A998SK, Fisher), agar powder (A1296, Sigma), atrazine (45330, Sigma), ampicillin sodium salt (A0166, Sigma), calcium chloride dihydrate (C7902, Sigma), copper sulfate pentahydrate (209198, Sigma), ethylenediaminetetracetic acid (E6758, sigma), glycerol (G5516, Sigma), hydrogen peroxide solution (216763, Sigma), magnesium chloride (M8266, Sigma), methanol (A452SK, Fisher), phosphoric acid solution (W290017, Sigma), peroxidase from horseradish (P6782, Sigma), potassium iodide (28624, Duksan), sodium chloride (S7653, Sigma), sodium hydroxide solution (415413, Sigma), sodium phosphate monobasic dihydrate (71505, Sigma), sodium phosphate dibasic dihydrate (30435, Sigma), sulfuric acid (320001, Sigma), tryptone (1612, Conda), and yeast extract (1702, Conda). Stock solutions of hydrogen peroxide (H_2O_2) were prepared by diluting H_2O_2 commercial solution (30%) and standardized spectrophotometrically using the molar absorption coefficient, $\varepsilon = 40\ M^{-1}\ cm^{-1}$ at 240 nm for H_2O_2 (1).

SI-Text-2. Preparation of antibiotic-resistant *E. coli*

E. coli DH5α was inoculated from freshly streaked overnight LB agar plates into 5 mL of LB broth containing 50 μg/mL of ampicillin, which was grown at 37°C for 12 h to the stationary phase. One-hundred μL of the cultured solution were transferred into 5 mL of LB broth containing 50 μg/mL of ampicillin, and incubated for 6 h to reach mid-exponential phase as determined by monitoring the solution absorbance at λ of 600 nm. The *E. coli* cells were then washed by centrifuging at 8,000 rpm for 2 min, discarding the broth and rinsing three times with 1 mM of phosphate-buffered solution (PB, pH 7), and re-suspended in 1 mM of the PB solution. The *E. coli* cell concentration in these primary stocks was in the range of ~10^9 CFU/ml, which was determined

by a plate count method (LB agar) with serial dilution of samples.

SI-Text-3. Preparation of competent *E. coli*

Non-resistant *E. coli* DH5α was inoculated from freshly streaked overnight LB agar plates into 5 mL of LB broth, and grown at 37°C for 12 h to the stationary phase. One-hundred μL of the cultured solution were transferred into 10 mL of LB broth in a test tube, and incubated for 4 hrs to reach mid-exponential phase as determined by monitoring solution absorbance at λ of 600 nm. When the OD_{600nm} reached 0.7 – 0.8 (~7×10^8 CFU/ml), the culture tube was stored on ice for 30 min. The *E. coli* cells were then centrifuged at 8,000 rpm for 5 min at 4°C. The supernatant was discarded and pellets were gently resuspended in 250 μL of ice-cold Buffer I solution (80 mM $MgCl_2$ and 20 mM $CaCl_2(H_2O)$) and stored on ice for 30 min. The pellets were recovered by centrifugation at 8,000 rpm for 5 min at 4°C, after which the supernatant was discarded. This step was repeated two times. After that, the pellets were gently resuspended in 250 μL of Buffer II solution (100 mM $CaCl_2$ with 15 % glycerol). One-hundred μL of the resulting competent cell suspension was dispensed into 1.5 ml tubes and stored at -80°C until use in transformation assays.

Table S1. DNA sequence information for *amp*^{R} and *ori* amplicons located in pUC19.[a]

amp^R (192 bp)	*Sequence*	GTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGG CATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGA AAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGA GTTTTCGCCCCGAAGAACGTTTTCCAA
	Base number	A = 44, T = 59, G = 44, C = 45
	Base pair content (%)	A-T base pairs = 103 (53.6%), G-C base pairs = 89 (46.4%)
amp^R (400 bp)	*Sequence*	GTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGG CATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGA AAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGA GTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGC CGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAG AATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC ATAACCATGAGTGATAACACTGCGGCCAACTT
	Base number	A = 102, T = 111, G = 94, C = 93
	Base pair content (%)	A-T base pairs = 213 (53.3%), G-C base pairs = 187 (46.7%)
amp^R (603 bp)	*Sequence*	GTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGG CATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGA AAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGA GTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGC CGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAG AATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAC AACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACC GGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACC ACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTAT TAACTGGCGAACTACTTACTCTAGCTTCCCGGC
	Base number	A = 159, T = 153, G = 144, C = 147
	Base pair content (%)	A-T base pairs = 312 (51.7%), G-C base pairs = 291 (48.3%)
amp^R (851 bp)	*Sequence*	GTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGG CATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGA AAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGA GTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT

		AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGC CGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAG AATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAC AACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACC GGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACC ACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTAT TAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTA ATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTC TGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAA TCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGC ACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCT ACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCAT
	Base number	A = 220, T = 211, G = 218, C = 202
	Base pair content (%)	A-T base pairs = 431 (50.6%), G-C base pairs = 420 (49.4%)
ori (190bp)	*Sequence*	GCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC AGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTT TTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA TACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA AGAACTCTGTAGCACCGCCTACATACCT
	Base number	A = 51, T = 47, G = 40, C = 54
	Base pair content (%)	A-T base pairs = 98 (51.6%), G-C base pairs = 94 (48.4%)
ori (390bp)	*Sequence*	GCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC AGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTT TTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA TACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG CAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCA GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACA GCGTGAGCTATGAGAAAGCGCCACGC
	Base number	A = 97, T = 85, G = 101, C = 107
	Base pair content (%)	A-T base pairs = 182 (46.6%), G-C base pairs = 208 (53.4%)
ori (530bp)	*Sequence*	GCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC AGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTT TTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA TACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT

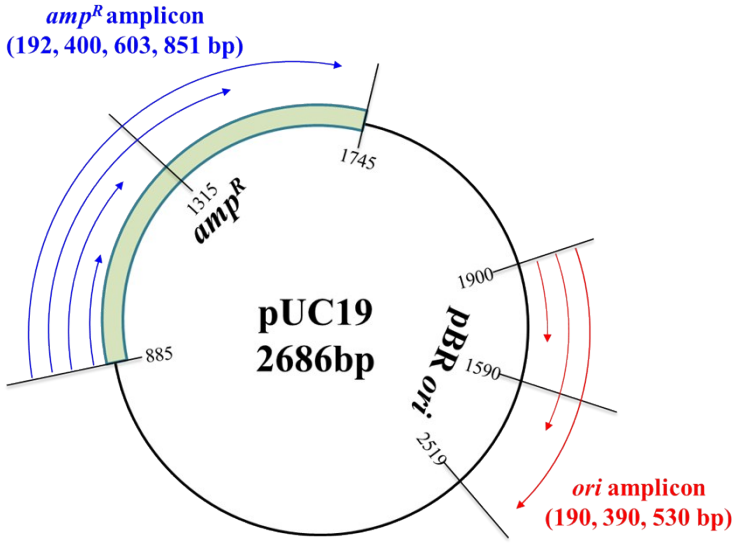
		TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG CAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCA GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACA GCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGA AAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAG GAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCG TCGA
	Base number	A = 128, T = 111, G = 151, C = 140
	Base pair content (%)	A-T base pairs = 239 (45.1%), G-C base pairs = 291 (54.9%)

[a]The complete genome sequence of pUC19 is available at https://www.addgene.org/browse/sequence/74677/

Table S2. Primers used for qPCR analysis of target *amp*^{R} and *ori* amplicons.[a,b]

Primer	Start-end positions in pUC19 plasmid	Target amplicon length (bp)	Sequence
amp^R-FP	889	-	5’ GTA TTC AAC ATT TCC GTG TCG C
amp^R-RPa	1080	192	5’ TTG GAA AAC GTT CTT CGG GG
	1288	400	5’ AAG TTG GCC GCA GTG TTA TC
	1491	603	5’ GCC GGG AAG CTA GAG TAA GT
	1738	851	5’ ATG CTT AAT CAG TGA GGC ACC
ori-FP	1936	-	5’ GCG TAA TCT GCT GCT TGC A
ori-RP	2125	190	5’ AGG TAT GTA GGC GGT GCT AC
	2325	390	5’ GCG TGG CGC TTT CTC ATA G
	2465	530	5’ TCG ACG CTC AAG TCA GAG G

[a]FP = forward primer; RP = reverse primer, [b]Primers were designed using the NCBI Primer-BLAST tool.



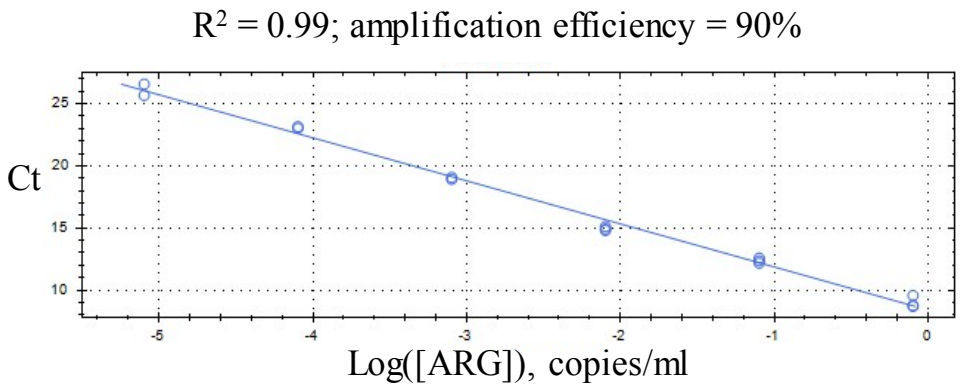
86

87 **Figure S1**. The pUC19 plasmid and the position of the target qPCR amplicons (i.e., 192, 400, 603,

88 and 851 bps of *amp*^{R} gene and 190, 390, and 530 bps of *ori* region).

89

90

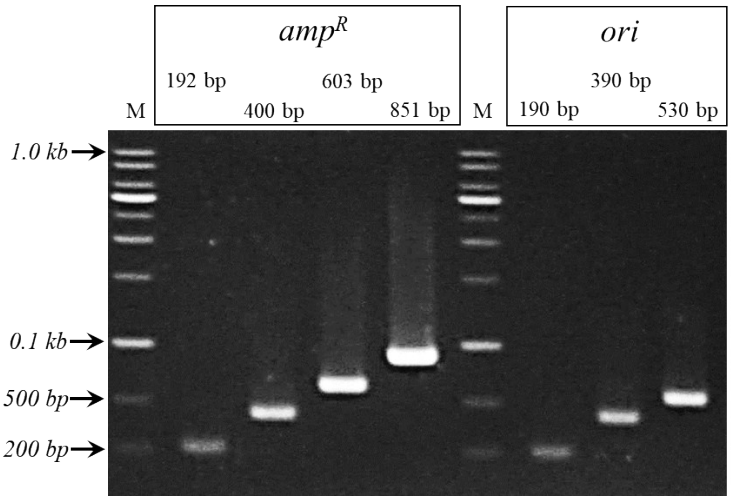


91

92 **Figure S2**. The representative standard curve for a qPCR run with *amp*^{R} amplicon (851 bp).

93 Different concentrations of plasmid DNA were prepared by diluting the prepared primary standards

94 (~10^{12} copies/mL) that were quantified by the NanoDrop ND-2000 spectrophotometer.

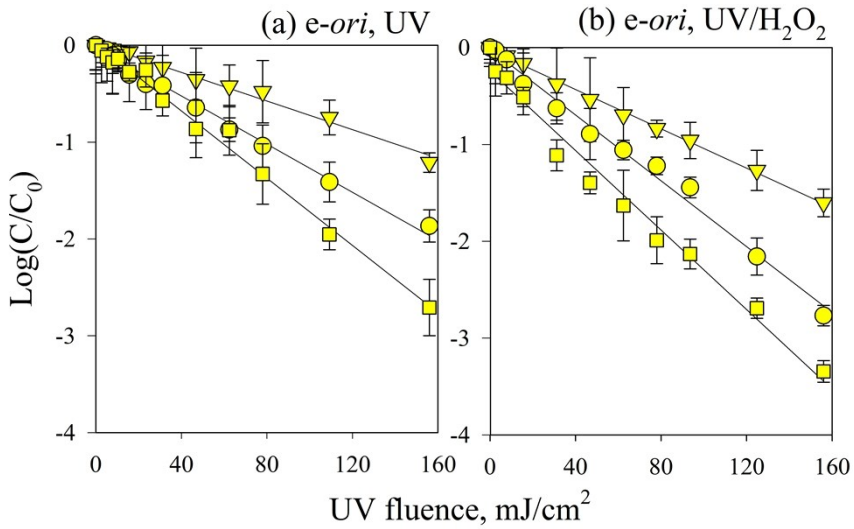


95

96 **Figure S3**. Images of agarose gel electrophoresis for qPCR amplification products of *amp*^{R} (192,
97 400, 603, and 851 bp) and *ori* (190, 390, and 530 bp). The lane 'M' shows the standard MW markers.

98

99



100

101 **Figure S4**. Logarithmic relative concentrations of *ori* amplicons as a function of UV fluence,
102 measured by qPCR (190 (▽), 390 (○), and 530 (□) base pair amplicons) during treatment of
103 extracellular pUC19 at pH 7 with (a) UV and (b) UV/H_2O_2 ($[H_2O_2]_0$ = 10 mg/L). The symbols
104 represent the measured data and the error bars represent one standard deviation from triplicate
105 experiments. The lines are linear regressions of the data.

106

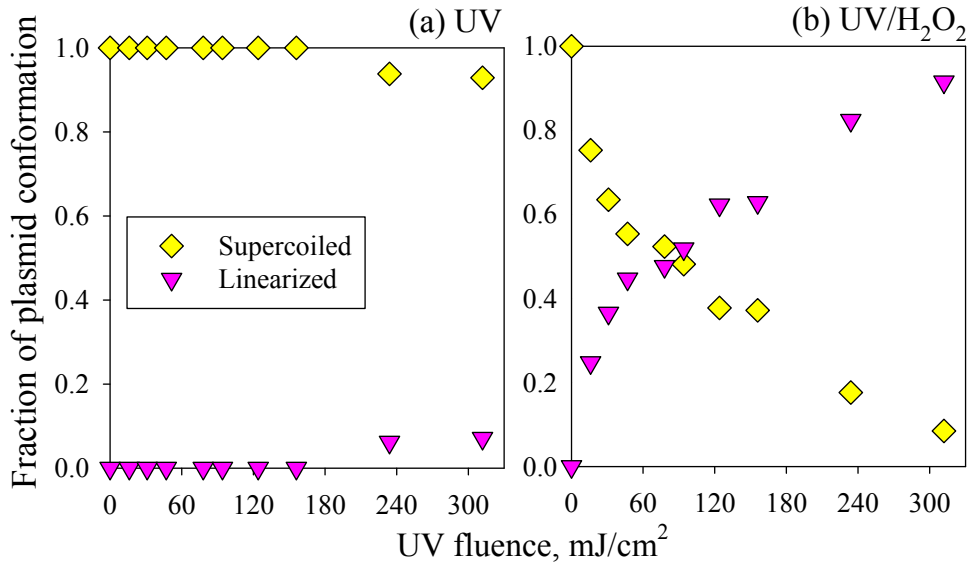


Figure S5. Conformational changes of extracellular plasmid pUC19 during UV and UV/H_2O_2 ($[H_2O_2]_0$ = 10 mg/L) treatment. The fractions of supercoiled and linearized forms of pUC19 were quantified from image analysis of the agarose gel electrophoresis data (Figure 3), using the method of described in (2).

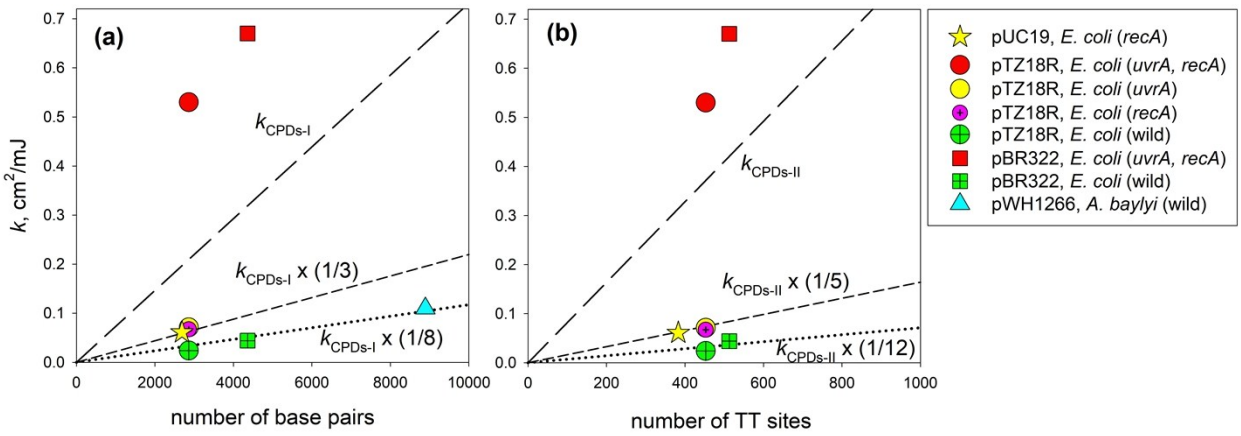


Figure S6. UV fluence-based rate constants (k) for elimination of transforming activity of plasmids as a function of number of (a) base pairs (k_{CPDs-I}) and (b) TT sites ($k_{CPDs-II}$). These figures are the same

as Figure 4 in the main manuscript except for that a Φ_{CPD} value (3) of 1.0×10^{-3} was used to calculate the $k_{CPDs\text{-}I}$ for Figure S6(a).

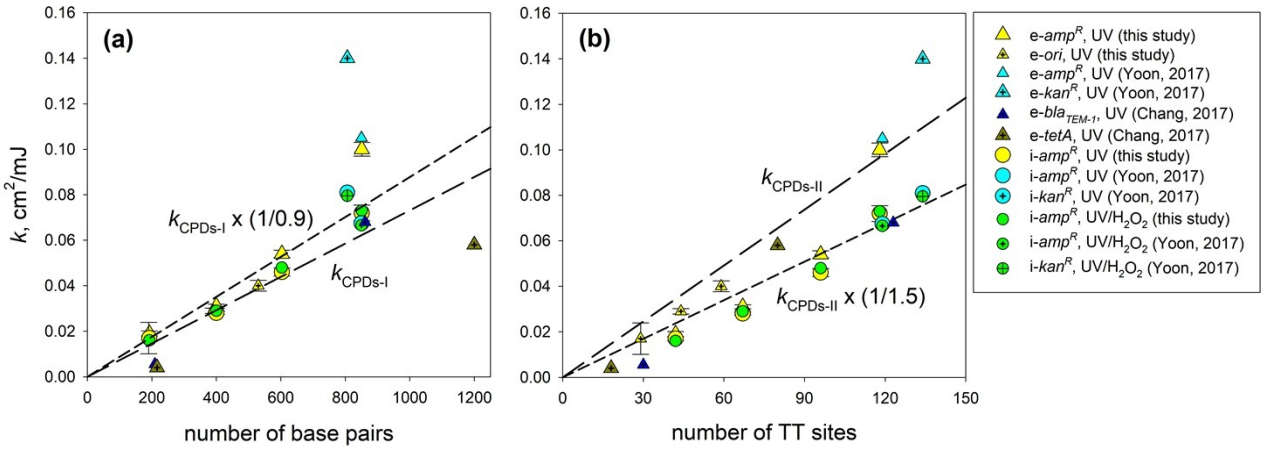


Figure S7. UV fluence-based rate constants (k) for gene damage of extracellular (e-ARGs, triangles) and intracellular (i-ARGs, circles) plasmid-encoded genes as a function of number of (a) base pairs ($k_{CPD\text{-}I}$) and (b) TT sites ($k_{CPD\text{-}II}$). These figures are the same as Figure 5 in the main manuscript except for that a Φ_{CPD} value (3) of 1.0×10^{-3} was used to calculate the $k_{CPDs\text{-}I}$ for Figure S7(a).

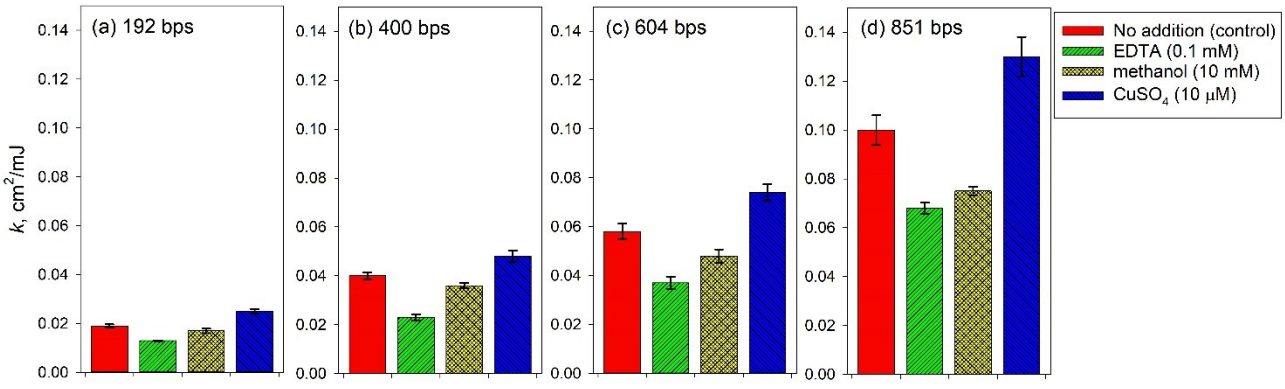


Figure S8. Effect of EDTA (chelator, 0.1 mM), methanol (•OH scavenger, 10 mM), and Cu(II) (10 μM) on the degradation rate of e-amp^R amplicons during UV treatment of pUC19 at pH 7 (2 mM phosphate buffer).

References

1. Bader H, Sturzenegger V, Hoigne J. Photometric method for the determination of low concentrations of hydrogen peroxide by the peroxidase catalyzed oxidation of N, N-diethyl-p-phenylenediamine (DPD). Water Research. 1988;22(9):1109-15.

2. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012;9:671.

3. Döuki T. Low ionic strength reduces cytosine photoreactivity in UVC-irradiated isolated DNA. Photochemical & Photobiological Sciences. 2006;5(11):1045-51.