**Depurination of colibactin-derived interstrand cross-links**

Mengzhao Xue,[1] Kevin Wernke,[1] and Seth B. Herzon[*,1,2]

[1]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States.

[2]Department of Pharmacology, Yale School of Medicine, New Haven, Connecticut 06520, United States

*Biochemistry*

Supporting Information

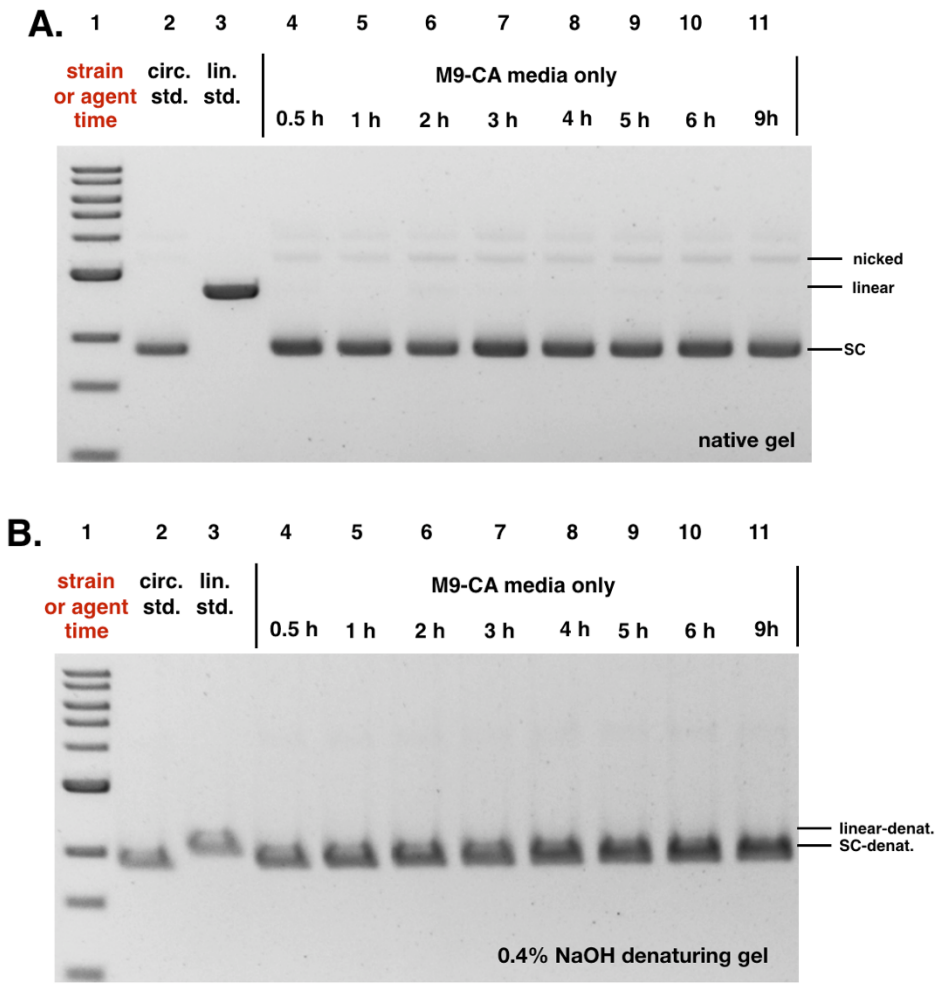


**Fig. S1. DNA plasmid cleavage assay employing circular pUC19 DNA and M9-cas amino acid media (M9-CA media) only**. **A. Native gel; B. 0.4% NaOH denaturing gel.** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); M9-CA media 0.5 h (Lane #4), 1 h (Lane #5), 2 h (Lane #6), 3 h (Lane #7) 4 h (Lane #8), 5 h (Lane #9), 6 h (Lane #10), and 9 h (Lane #11). Conditions (Lane #4–#11): circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 0.5 h,1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed either (**Fig. S1A**) by native agarose gel electrophoresis, or (**Fig. S1B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 2 h). SC-denat. = supercoiled DNA in denaturing form, linear-denat. = linearized DNA in denaturing form.

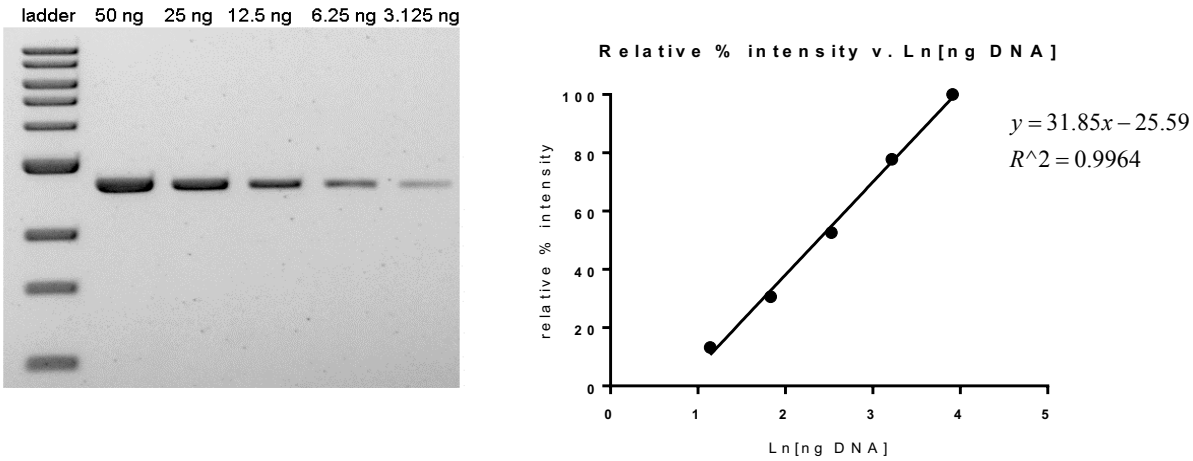


**Fig. S2. Calibration curve correlating the intensity of the gel band with the amount of DNA present.** DNA ladder (Lane #1); 50 ng DNA (Lane #2); 25 ng DNA (Lane #3); 12.5 ng DNA (Lane #3); 6.25 ng DNA (Lane #4); 3.125 ng DNA (Lane #5). Gel band intensity is expressed as a ratio vs. Lane #2. The data show that the gel band intensity increases as the ln (DNA quantity in ng) increases. $R^2 = 0.9964$.

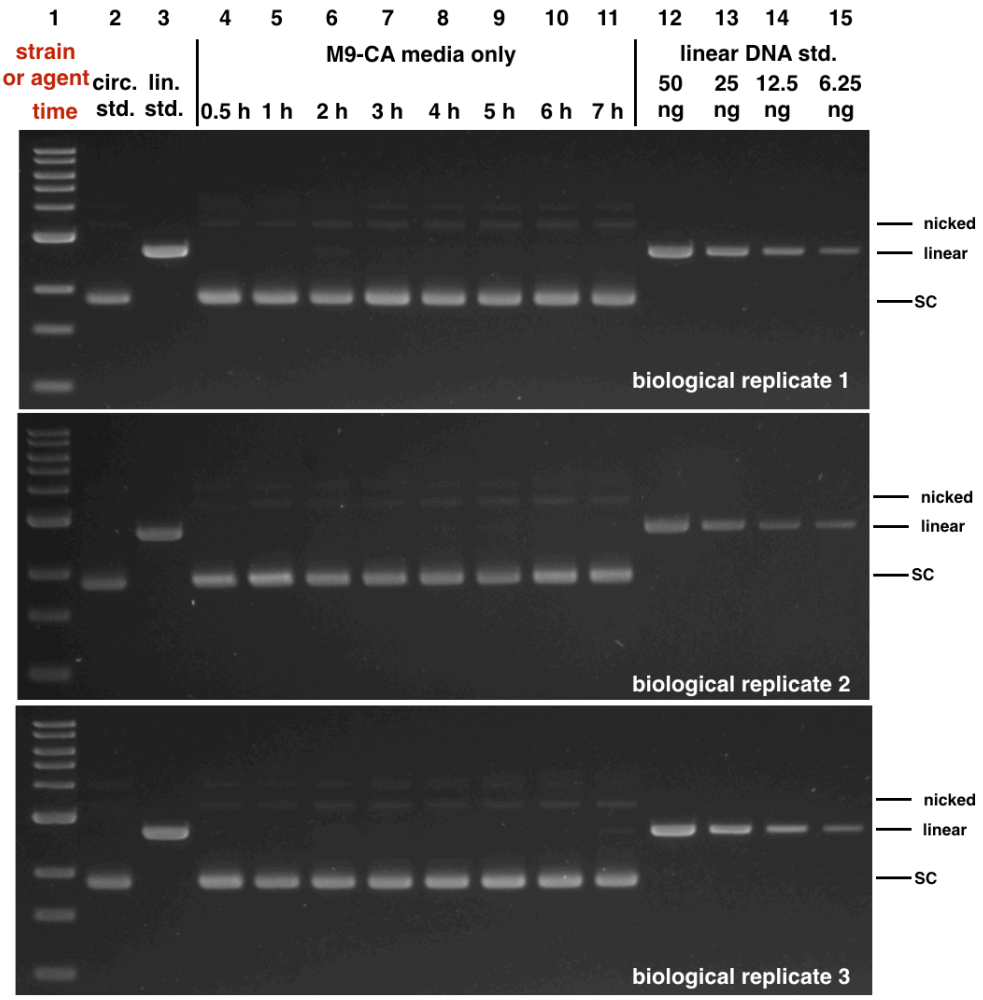


**Fig. S3. DNA plasmid cleavage assay employing circular pUC19 DNA and M9-CA media.** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); M9-CA media 0.5 h (Lane #4), 1 h (Lane #5), 2 h (Lane #6), 3 h (Lane #7), 4 h (Lane #8), 5 h (Lane #9), 6 h (Lane #10), and 9 h (Lane #11); linearized pUC19 DNA standard 50 ng (Lane #12), 25 ng (Lane #13), 12.5 ng (Lane #14), and 6.25 ng (Lane #15). Conditions (Lane #4–#11): circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 0.5 h,1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

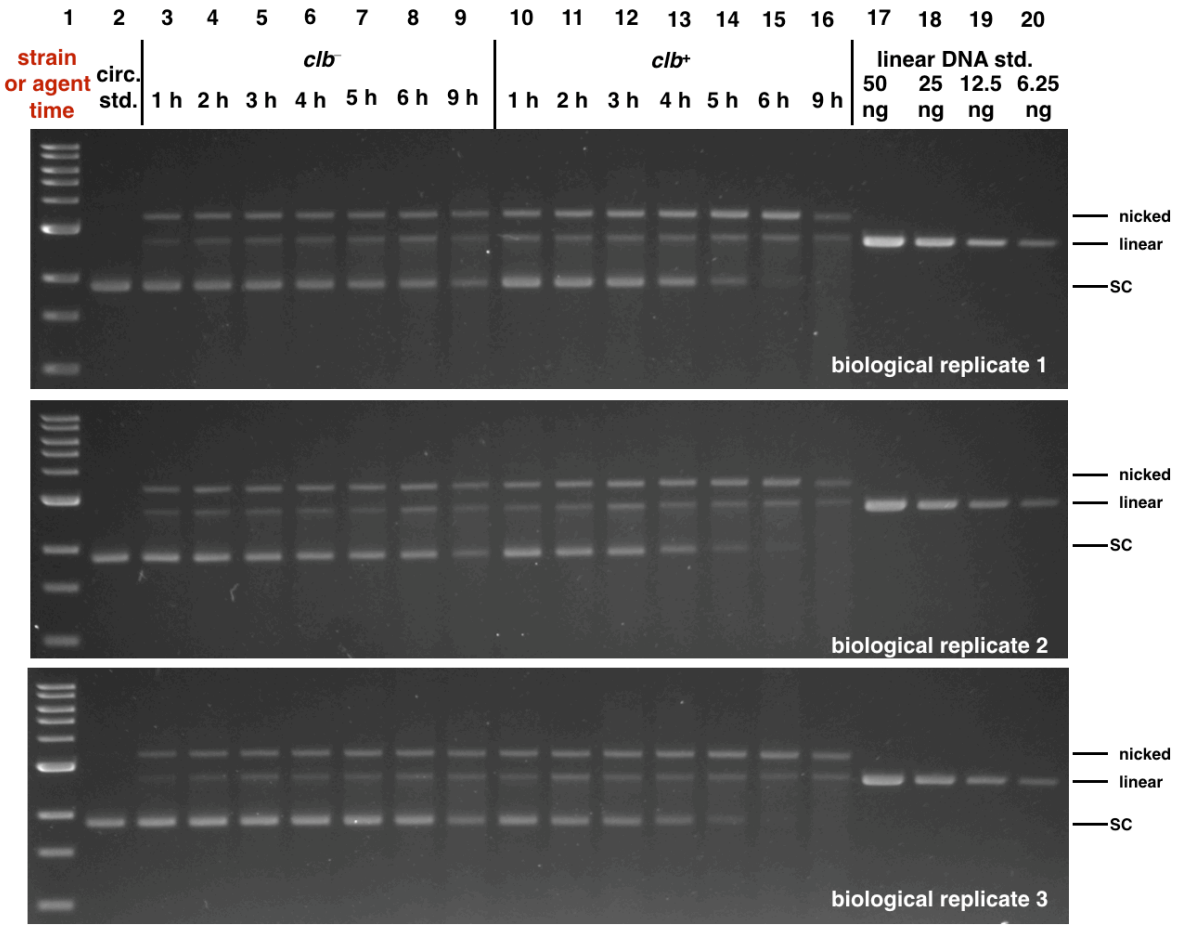


**Fig. S4. DNA plasmid cleavage assay employing circular pUC19 DNA and *clb*⁻ (or *clb*⁺) BW25113 *E. coli*.** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clb*⁻ BW25113 *E. coli.* 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); *clb*⁺ BW25113 *E. coli.* 1 h (Lane #10), 2 h (Lane #11), 3 h (Lane #12), 4 h (Lane #13), 5 h (Lane #14), 6 h (Lane #15), and 9 h (Lane #16); linearized pUC19 DNA standard 50 ng (Lane #17), 25 ng (Lane #18), 12.5 ng (Lane #19), and 6.25 ng (Lane #20). Conditions (Lane #3–#16): *clb*⁻ BW25113 *E. coli.* (Lane #3–#9) or *clb*⁺ BW25113 *E. coli.* (Lane #10–#16), circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 1 h, 2 h, 4 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

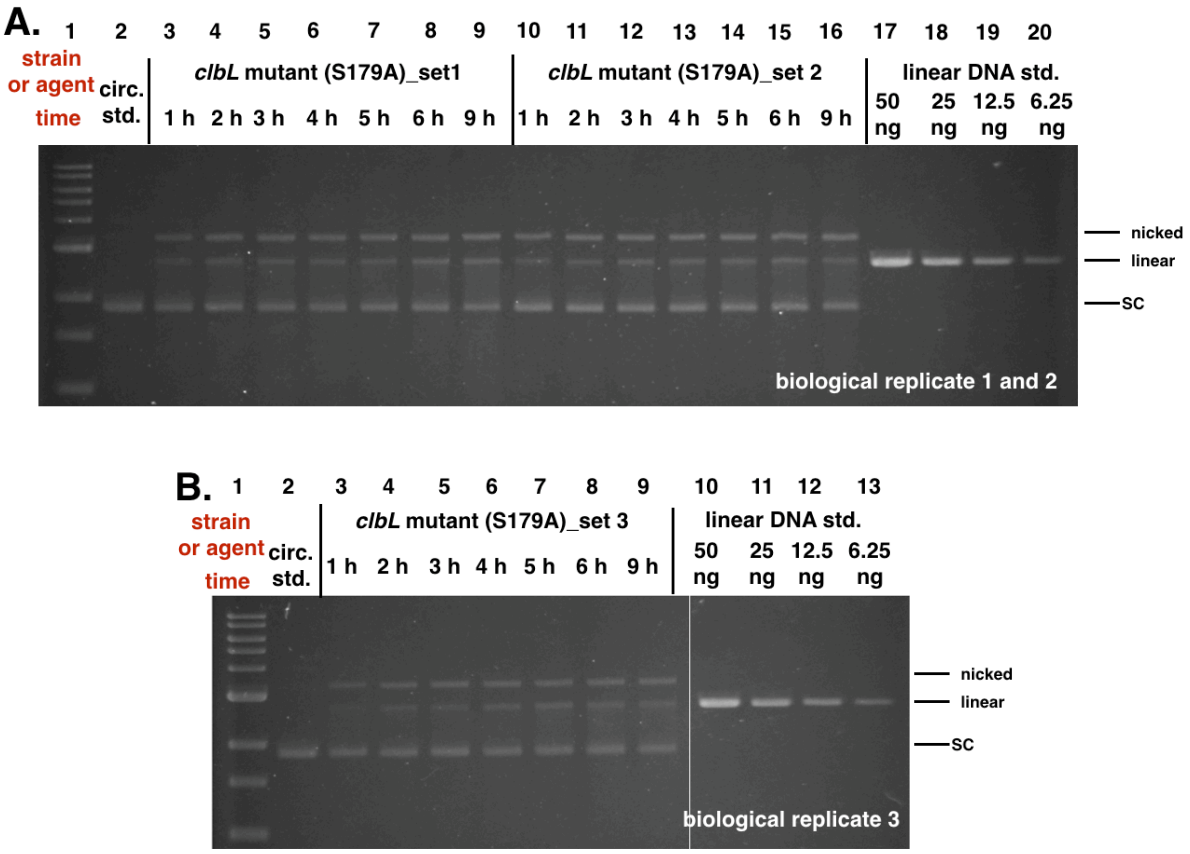


**Fig. S5. DNA plasmid cleavage assay employing circular pUC19 DNA and *clbL* mutant (S179A) BW25113 *E. coli*. A. Biological replicate 1 and 2; B. Biological replicate 3. (Fig. S5A.)** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clbL* point mutant (S179A) BW25113 *E. coli* set #1, 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); *clbL* point mutant (S179A) set #2, 1 h (Lane #10), 2 h (Lane #11), 3 h (Lane #12), 4 h (Lane #13), 5 h (Lane #14), 6 h (Lane #15), and 9 h (Lane #16); linearized pUC19 DNA standard 50 ng(Lane #17), 25 ng (Lane #18), 12.5 ng (Lane #19), and 6.25 ng (Lane #20). **(Fig. S5B.)** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clbL* point mutant (S179A) BW25113 *E. coli* set #3, 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); linearized pUC19 DNA standard 50 ng (Lane #10), 25 ng (Lane #11), 12.5 ng (Lane #12), and 6.25 ng (Lane #13). Conditions (**Fig. S5A.** Lane #3–#16, and **Fig. S5B.** Lane #3–#9): *clbL* point mutant (S179A) BW25113 *E. coli*, circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 1 h, 2 h, 4 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

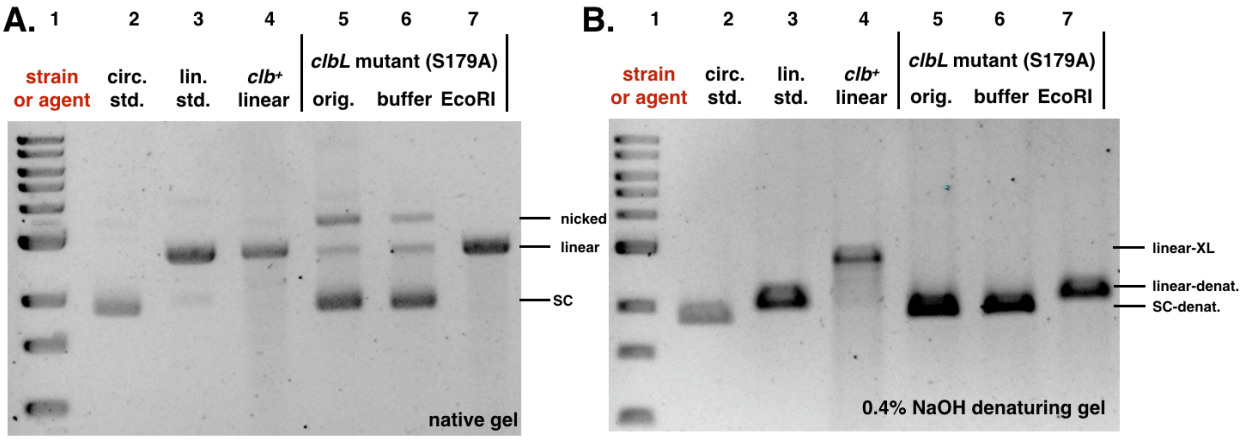


**Fig. S6. Plasmid DNA linearization test employing circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli*. A. Native gel. B. 0.4% NaOH denaturing gel**. The cross-linked linearized pUC19 isolated from co-culture with *clb*$^+$ BW25113 *E. coli* was used as a positive control. DNA ladder (Lane #1); circular pUC19 DNA (Lane #2); linearized pUC19 DNA (Lane #3); linearized pUC19 DNA co-cultured with *clb*$^+$ BW25113 *E. coli* (Lane #4); circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli* (Lane #5), reacted with buffer (Lane #6), reacted with EcoRI restriction enzyme (Lane #7). Conditions (Lane #4): linearized pUC19 DNA, *clb*$^+$ BW25113 *E. coli*, M9-CA media, 4 h at 37 °C. Conditions (Lanes #5–#7): circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane # 5); the DNA (15.4 μM base pair) was reacted with CutSmart Buffer® (New England Biolabs®), pH 7.9, at 37 °C for 30 min (Lane #6); the DNA (15.4 μM base pair) was reacted with 20 units of EcoRI-HF restriction enzyme in CutSmart Buffer® (New England Biolabs®), pH 7.9, at 37 °C for 30 min (Lane #7). The DNA was isolated and analyzed by either (**Fig. S6A**) 0% NaOH native agarose gel electrophoresis, or (**Fig. S6B**) 0.4% NaOH denature agarose gel electrophoresis (90 V, 1.5 h). SC = supercoiled, nicked = SSB, linear = DSB, SC-denat. = supercoiled DNA in denaturing form, linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin.

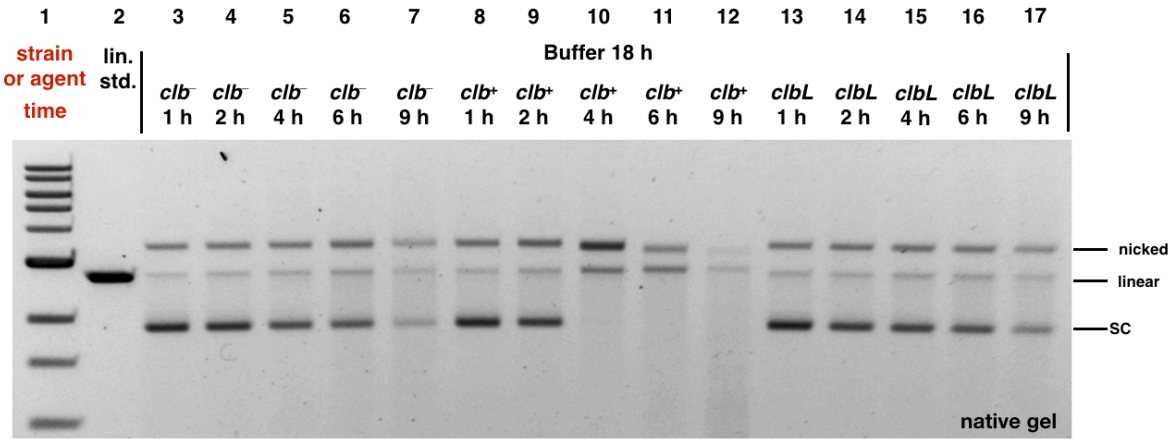


**Fig. S7. Plasmid DNA stability test employing circular pUC19 DNA isolated from co-culture with *clb*⍰, *clb*⁺, or *clbL* mutant (S179A) BW25113 *E. coli.*** DNA ladder (Lane #1); linearized pUC19 DNA standard (Lane # 2); post buffer-reacted with DNA isolated from *clb*⁻ BW25113 *E. coli* 1 h (Lane #3), 2 h (Lane #4), 4 h (Lane #5), 6 h (Lane #6), and 9 h (Lane #7); post buffer-reacted with DNA isolated from *clb*⁺ BW25113 *E. coli* 1 h (Lane #8), 2 h (Lane #9), 4 h (Lane #10), 6 h (Lane #11), and 9 h (Lane #12); post buffer-reacted with DNA isolated from *clbL* point mutant (S179A) BW25113 *E. coli* 1 h (Lane #13), 2 h (Lane #14), 4 h (Lane #15), 6 h (Lane #16), and 9 h (Lane #17). Conditions (Lane #3–#17): NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h, 50 µg of DNA isolated from reacting with *clb*⁻ BW25113 *E. coli* for 1 to 9 hours (Lane #3–#7), reacting with *clb*⁺ BW25113 *E. coli* for 1 to 9 hours (Lane #8–#12), and reacting with *clbL* point mutant (S179A) BW25113 *E. coli* for 1 to 9 hours (Lane #13–#17). The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

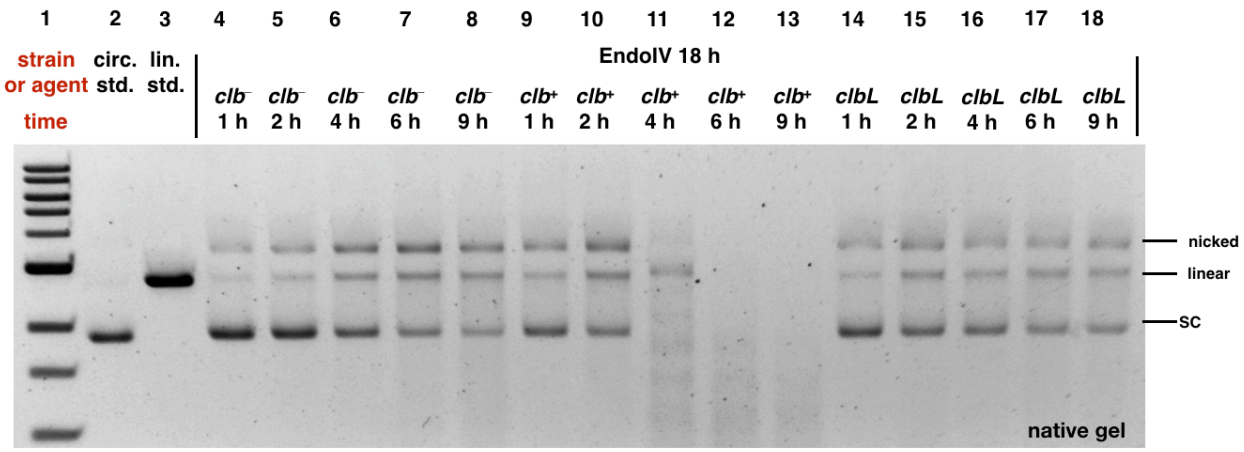


**Fig. S8. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA isolated from co-culture with *clb*⍰, *clb*⁺, or *clbL* mutant (S179A) BW25113 *E. coli*.** The Endonuclease IV selectively cleaves DNA 5' apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); EndoIV-reacted with DNA isolated from *clb*⁻ BW25113 *E. coli* 1 h (Lane #4), 2 h (Lane #5), 4 h (Lane #6), 6 h (Lane #7), and 9 h (Lane #8); EndoIV-reacted with DNA isolated from *clb*⁺ BW25113 *E. coli* 1 h (Lane #9), 2 h (Lane #10), 4 h (Lane #11), 6 h (Lane #12), and 9 h (Lane #13); post buffer-reacted with DNA isolated from *clbL* point mutant (S179A) BW25113 *E. coli* 1 h (Lane #14), 2 h (Lane #15), 4 h (Lane #16), 6 h (Lane #17), and 9 h (Lane #18). Conditions (Lane #4–#18): 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h, 50 µg of DNA isolated from reacting with *clb*⁻ BW25113 *E. coli* for 1 to 9 hours (Lane #4–#8), reacting with *clb*⁺ BW25113 *E. coli* from 1 to 9 hours (Lane #9–#13), and reacting with *clbL* point mutant (S179A) BW25113 *E. coli* for 1 to 9 hours (Lane #14–#18). The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

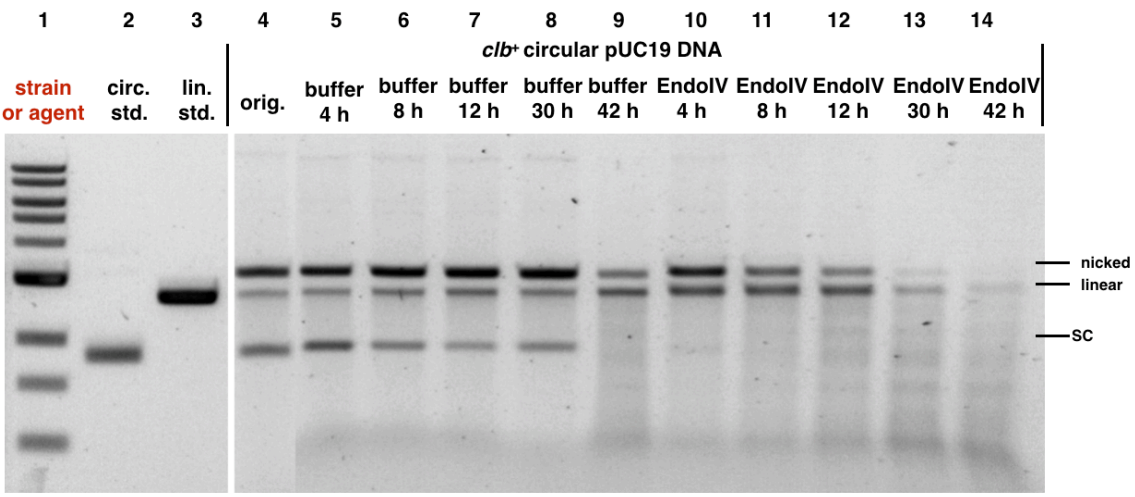


**Fig. S9. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli*.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane #4); the DNA (3.9 μM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

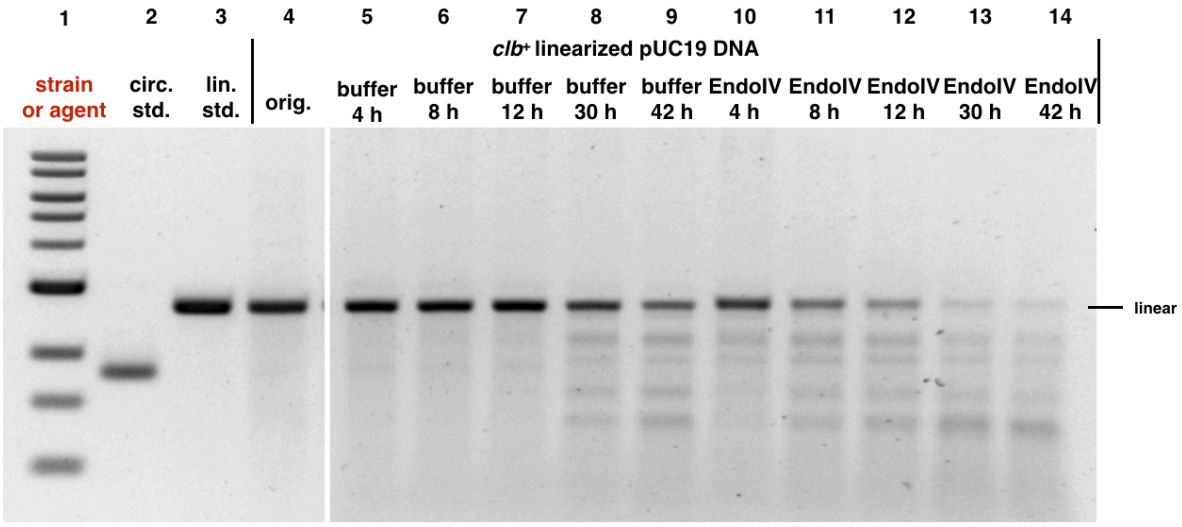


**Fig. S10. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli*.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane #4); the DNA (3.9 μM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). Linear = linearized DNA cross-linked by colibactin

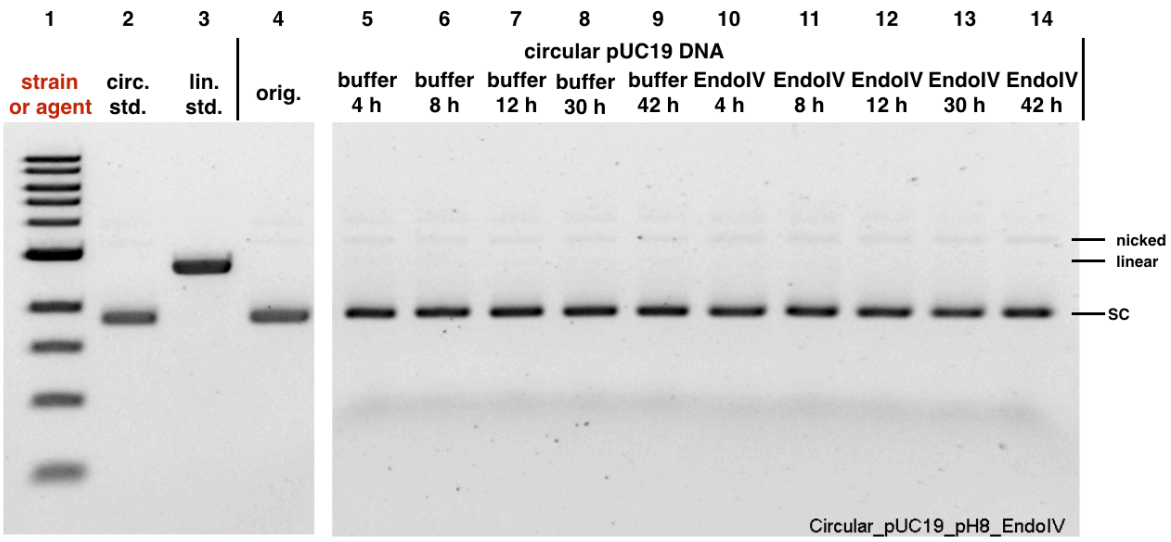


**Fig. S11. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA original sample (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #5–#14): circular pUC19 DNA (3.9 μM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

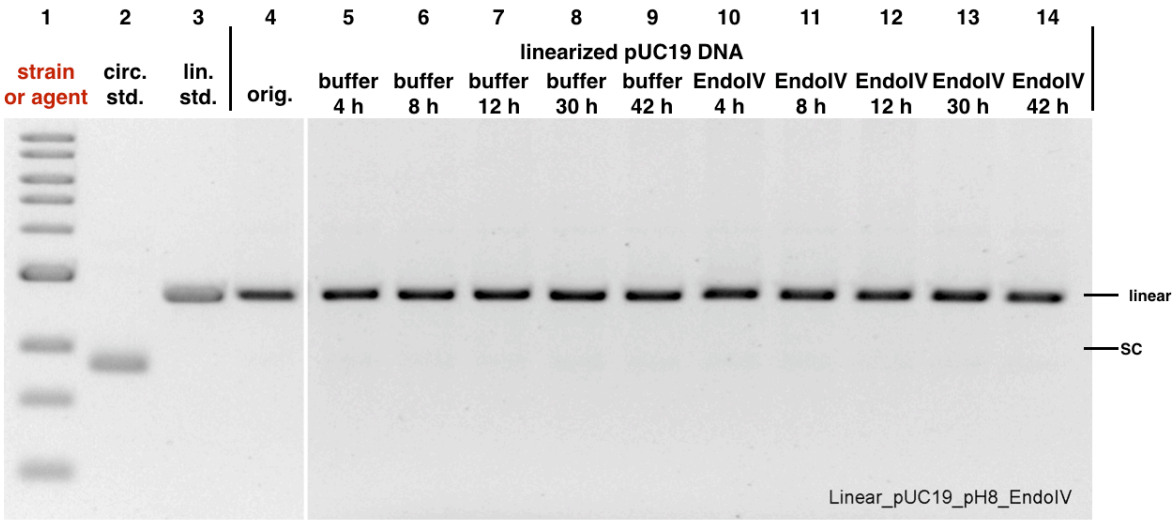


**Fig. S12. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); linearized pUC19 DNA control (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #5–#14): linearized pUC19 DNA (3.9 μM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, linear = linearized DNA.

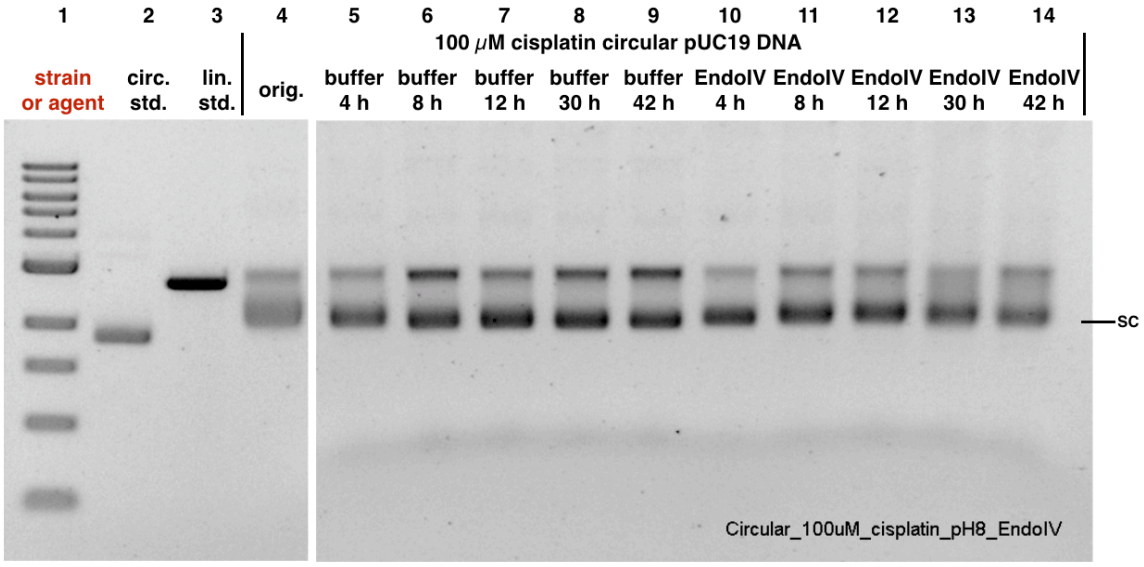


**Fig. S13. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA thathas been previously treated with 100 μM cisplatin.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA control (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA 100 μM cisplatin, pH 5.0, 3 h (Lane #4), 100 μM cisplatin-treated circular pUC10 DNA reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): circular pUC19 DNA (15.4 μM base pair) that has been reacted with 100 μM cisplatin in 10 mM sodium citrate buffer, pH 5.0 for 4 h at 37 °C (Lane #4); the DNA (3.9 μM base pair) was further reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was further reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled DNA cross-linked by cisplatin.

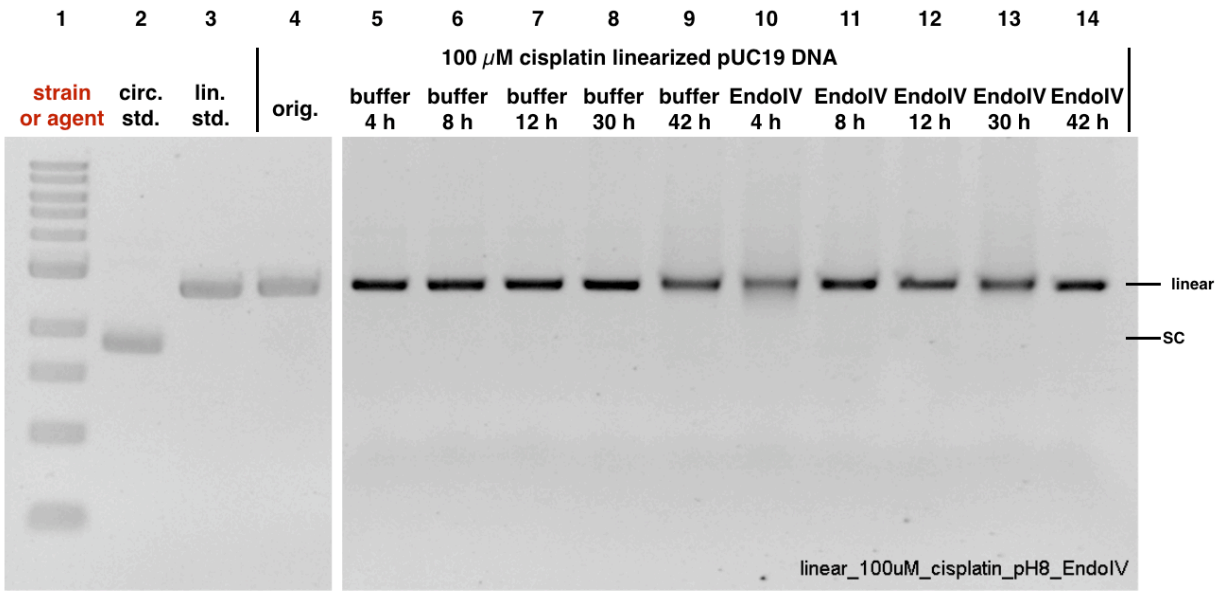


**Fig. S14. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA which has been previously treated with 100 µM cisplatin.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA control (Lane #2); linearized pUC19 DNA standard (Lane # 3); linearized pUC19 DNA 100 µM cisplatin, pH 5.0, 3 h (Lane #4), 100 µM cisplatin-treated linearized pUC10 DNA reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); linearized pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): linearized pUC19 DNA (15.4 µM base pair) that has been reacted with 100 µM cisplatin in 10 mM sodium citrate buffer, pH 5.0 for 4 hour at 37 °C (Lane #4); the DNA (3.9 µM base pair) was further reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was further reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, linear = linearized DNA cross-linked by cisplatin.

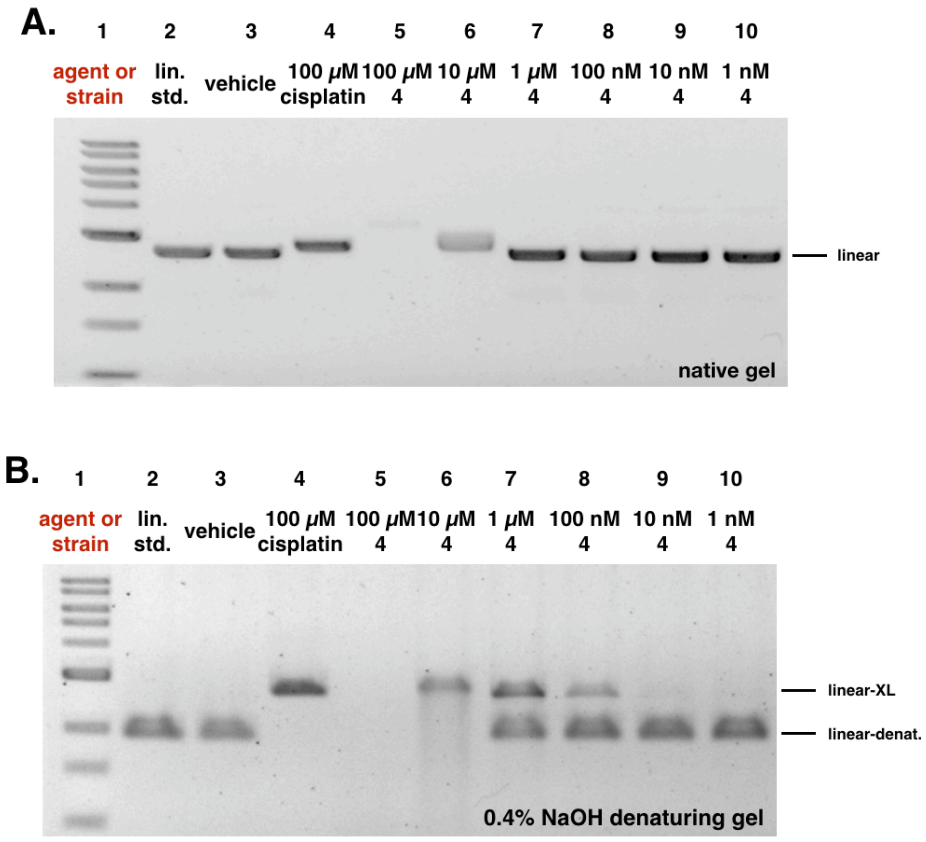


**Fig. S15. DNA cross-linking assay employing linear pUC19 DNA and synthetic colibactin linear precursor 4. A. Native gel. B. 0.4% NaOH denaturing gel.** 5% DMSO was used as vehicle (negative control), and 100 μM cisplatin was used as positive control. DNA ladder (Lane #1); linearized pUC19 DNA standard (Lane #2); 5% DMSO (Lane #3); 100 μM cisplatin (Lane #4); 100 μM **4** (Lane #5); 10 μM **4** (Lane #6); 1 μM **4** (Lane #7); 100 nM **4** (Lane #8); 10 nM **4** (Lane #9); 1 nM **4** (Lane #10). Conditions (Lane #3): linearized pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lane #4): linearized pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 100 μM cisplatin, 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lanes #5–#10): circular pUC19 DNA (15.4 μM in base pairs), **4** (100 μM–1 nM), 5% DMSO, 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. The DNA was analyzed either (**Fig. S15A**) by native agarose gel electrophoresis, or (**Fig. S15B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 1.5 h). Linear = linearized DNA, Linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin or cisplatin.

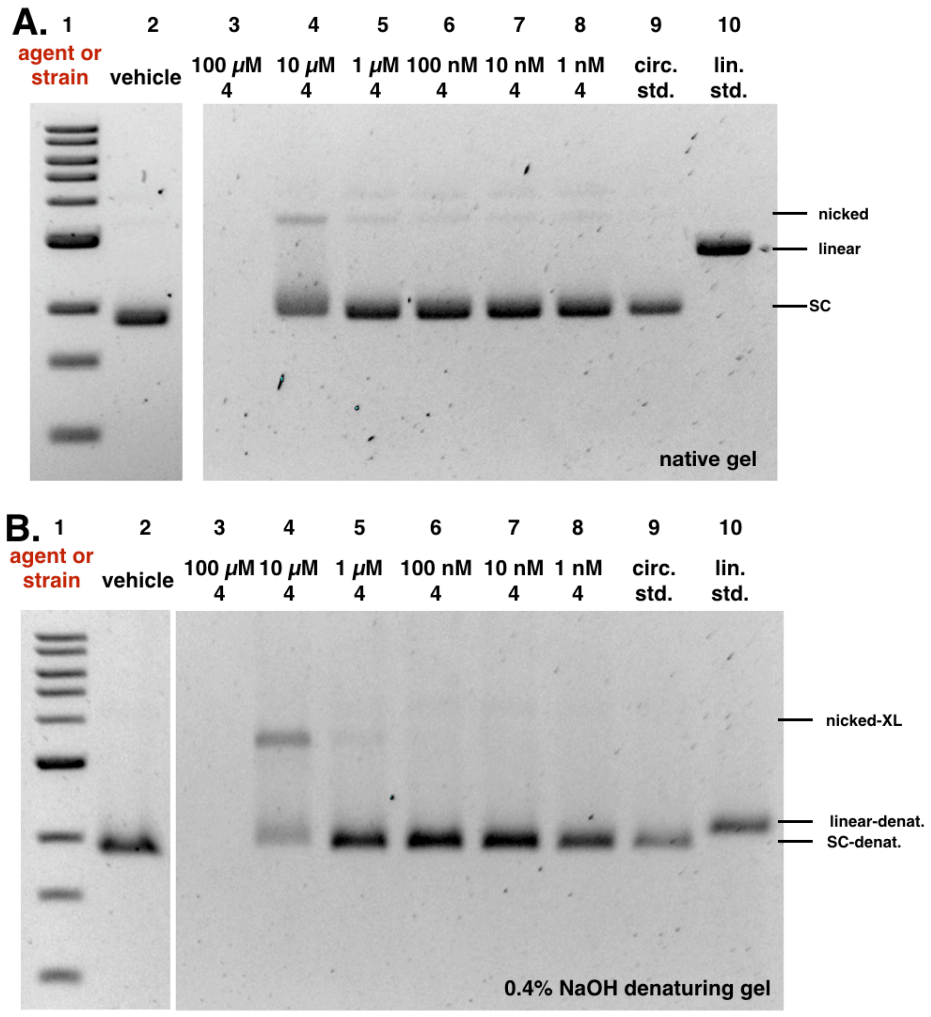


**Fig. S16. DNA plasmid cleavage assay employing circular pUC19 DNA and synthetic colibactin linear precursor 4**. **A. Native gel; B. 0.4% NaOH denaturing gel.** 5% DMSO was used as vehicle. DNA ladder (Lane #1); 5% DMSO (Lane #2); 100 μM **4** (Lane #3); 10 μM **4** (Lane #4); 1 μM **4** (Lane #5); 100 nM **4** (Lane #6); 10 nM **4** (Lane #7); 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): circular pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lanes #3–#8): circular pUC19 DNA (15.4 μM in base pairs), **4** (100 μM–1 nM), 5% DMSO, 10 mM citric buffer, pH 5.0, 3 h, 37 ºC. The DNA was analyzed either (**Fig. S16A**) by native agarose gel electrophoresis, or (**Fig. S16B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 1.5 h). SC = supercoiled, nicked = SSB, linear = DSB, Linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin or cisplatin.

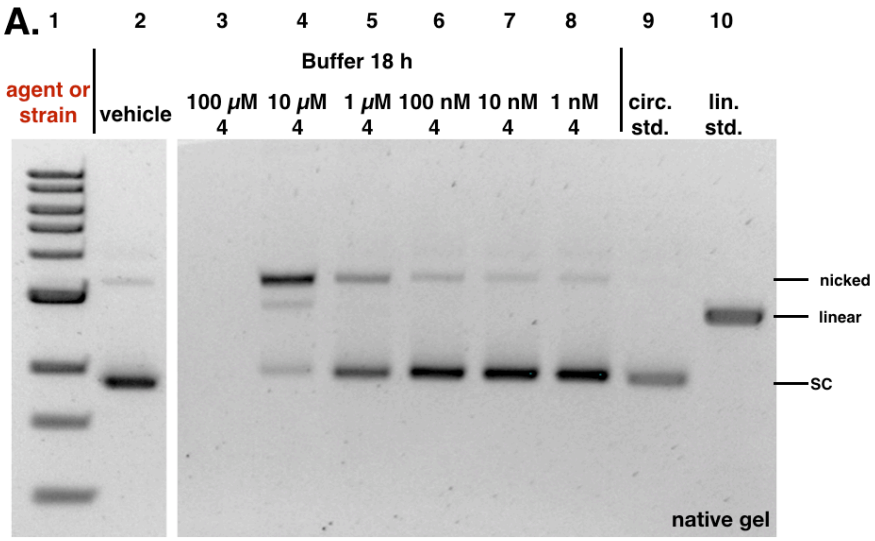


**Fig. S17A. Plasmid DNA buffer stability test employing circular pUC19 DNA that had been previously treated with synthetic colibactin linear precursor 4 at different concentrations.** 5% DMSO was used as vehicle when preparing the DNA samples for testing. DNA ladder (Lane #1); post buffer-reacted after 5% DMSO (Lane #2); post buffer-reacted after 100 μM **4** (Lane #3); post buffer-reacted after 10 μM **4** (Lane #4); post buffer-reacted after 1 μM **4** (Lane #5); post buffer-reacted after 100 nM **4** (Lane #6); post buffer-reacted after 10 nM **4** (Lane #7); post buffer-reacted after 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): the 5% DMSO-treated circular pUC19 DNA (3.9 μM in base pairs), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. Conditions (Lanes #3–#8): **4** (100 μM–1 nM)-treated circular pUC19 DNA (3.9 μM in base pairs), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

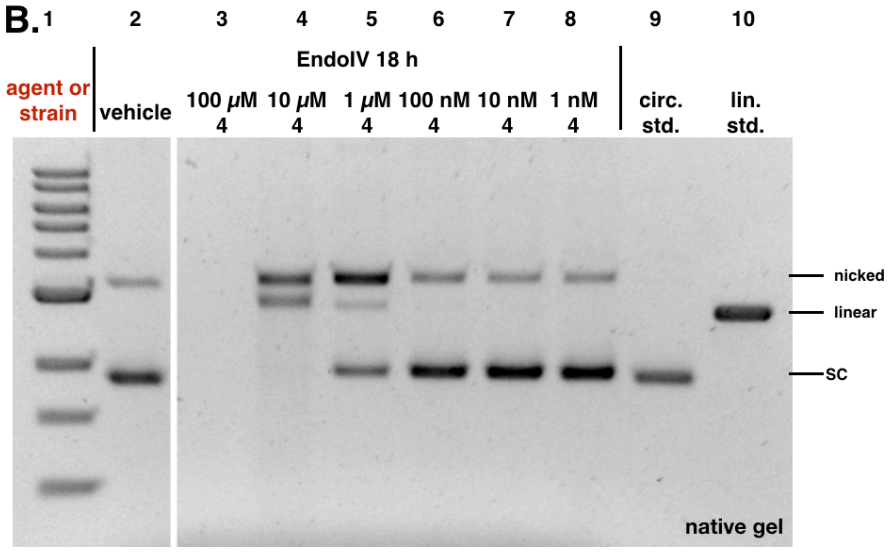


**Fig. S17B. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA that had been previously treated with synthetic colibactin linear precursor 4 at different concentrations.** Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. 5% DMSO was used as vehicle when preparing the DNA samples for testing. DNA ladder (Lane #1); post EndoIV-reacted after 5% DMSO (Lane #2); post EndoIV-reacted after 100 μM **4** (Lane #3); post EndoIV-reacted after 10 μM **4** (Lane #4); post EndoIV-reacted after 1 μM **4** (Lane #5); post EndoIV-reacted after 100 nM **4** (Lane #6); post EndoIV-reacted after 10 nM **4** (Lane #7); post EndoIV-reacted after 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): the 5% DMSO-treated circular pUC19 DNA (3.9 μM in base pairs), 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. Conditions (Lanes #3–#8): **4** (100 μM–1 nM)-treated circular pUC19 DNA (3.9 μM in base pairs), 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.