Table S1. *Escherichia coli* strains used in this work.

| Strain  | Genotype or description                  | Source or reference |
|---------|-----------------------------------------|---------------------|
| MTC2179 | SM10 pEXG2-ΔrecA, GentR                 | (1)                 |
| MTC2286 | SM10 pCTX-1-P_{07990}gfP, TetR          | (1)                 |
| MTC2287 | SM10 pCTX-1-P_{07990}lux, TetR          | (1)                 |

Modes of strain construction

*P. aeruginosa* strains

**MTC2441**

MTC2327 (PA14 xerC<sub>Y272F</sub>) (1) was mated with MTC2179: 1.2 ml of an overnight LB culture of MTC2179 was mixed with 350 µl of an overnight LB culture of MTC2327, centrifuged for 2 min at 10,000 g, the supernatant was discarded, and the concentrated cell suspension was spotted on an LB-1.5% agar plate and dried. The plate was incubated at 30°C overnight, and the resulting colony was scraped up with a sterile loop and resuspended in 500 µL sterile phosphate-buffered saline (PBS) or LB. An aliquot (typically 100 µL) of the suspension was spread on VBMM agar containing 75 µg/ml gentamycin and grown overnight at 37°C to select for *P. aeruginosa* transconjugants with integrated pEXG2-ΔrecA, and several of the resulting colonies were streaked onto no-salt LB with 15% sucrose to select second crossovers (2). A number of the sucrose-resistant colonies arising were then patched on LB and LB with 20 µg/mL gentamycin. At least 2 sucrose-resistant, gent-sensitive clones were then streaked for single colonies, checked by PCR for presence of the desired deletion, and frozen at -80°C in 25% glycerol.

**MTC2444**

MTC2441 was mated with MTC2287 as described above for MTC2441, and a 10-µL aliquot of the resuspended mating colony was spread on LB plates with 75 µg/mL tetracycline and 25 µg/mL irgasan to select for *P. aeruginosa* transformants. At least 2 colonies were then re-streaked for single colonies on LB-tet (25 µg/mL), grown in LB overnight at 37°C, and stored in 25% glycerol at -80°C.

**MTC2448**

Made as for MTC2444, except MTC 2274 (PA14 ΔrecA) (1) was mated with MTC2286.

**MTC2303**

Made as for MTC2444, except MTC 2276 (PA14 ΔprtN) (1) was mated with MTC2287.
References

1. Baggett NS, Bronson AS, Cabeen MT. 2021. SOS-Independent Pyocin Production in P. aeruginosa Is Induced by XerC Recombinase Deficiency. mBio 12:e0289321.

2. Hmelo LR, Borlee BR, Almblad H, Love ME, Randall TE, Tseng BS, Lin C, Irie Y, Storek KM, Yang JJ, Siehnel RJ, Howell PL, Singh PK, Tolker-Nielsen T, Parsek MR, Schweizer HP, Harrison JJ. 2015. Precision-engineering the Pseudomonas aeruginosa genome with two-step allelic exchange. Nat Protoc 10:1820-41.