Supplementary Information

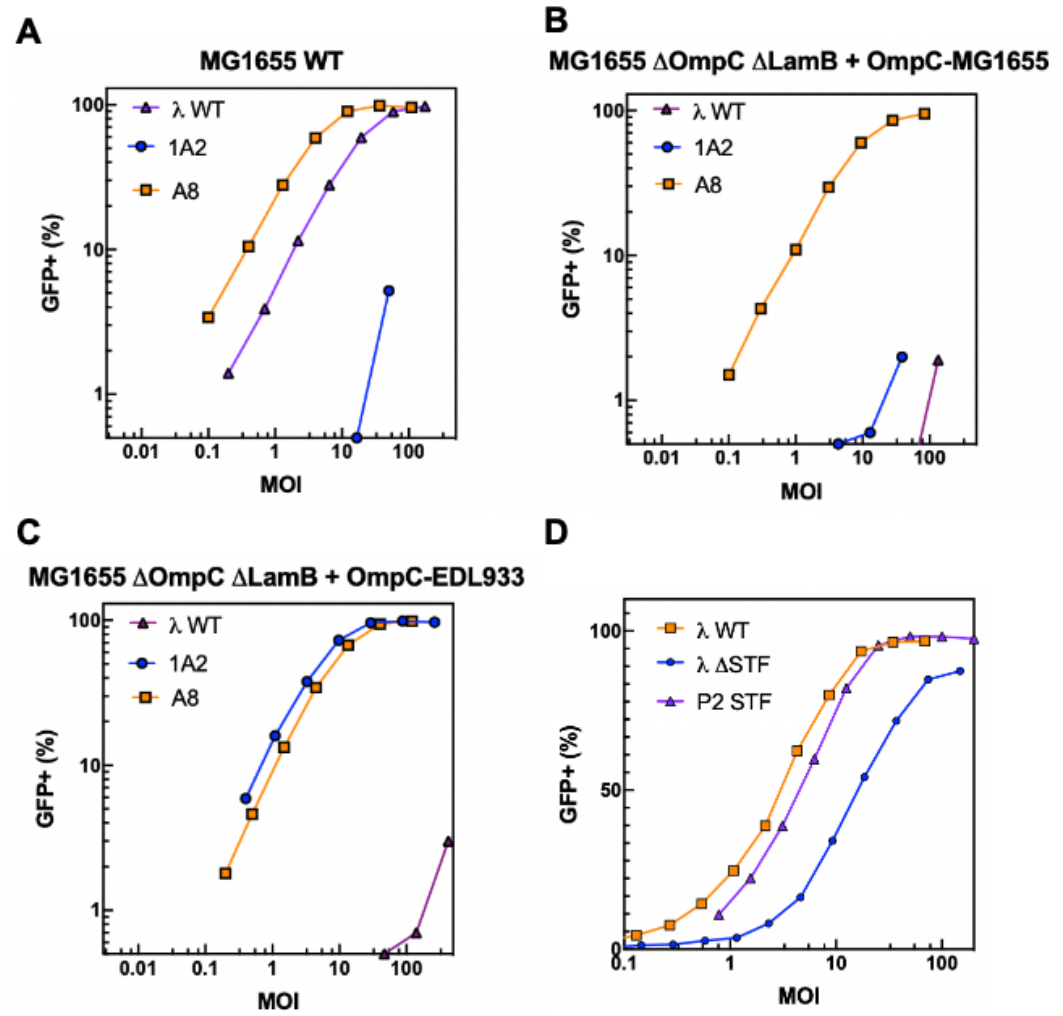

Figure S1: Cell receptor analysis for efficient cosmid delivery. Delivery efficiency assays of cosmids harboring Ur-λ wild-type (WT) gpJ and stf, or harboring gpJ chimeras and P2 STF. Transduction of a payload encoding a *sfGFP* fluorescent protein gene was measured in a flow cytometer (excitation: 488 nm, emission: 530/30 BP; Attune NxT Thermo Scientific) at different MOIs. **A)** Delivery efficiency into MG1655 WT, carrying the endogenous OmpC variant in the genome. **B)** and **C)** Delivery efficiency into a MG1655 strain deleted for both *lamB* and *ompC* genes, complemented with two different *ompC* variants on a plasmid (p1471 or p1472). **D)** Delivery efficiencies in *E. coli* MG1655 of λ particles carrying the WT λ STF (orange line), no STF (blue line) and λ-P2 STF chimera (p938) (purple line).

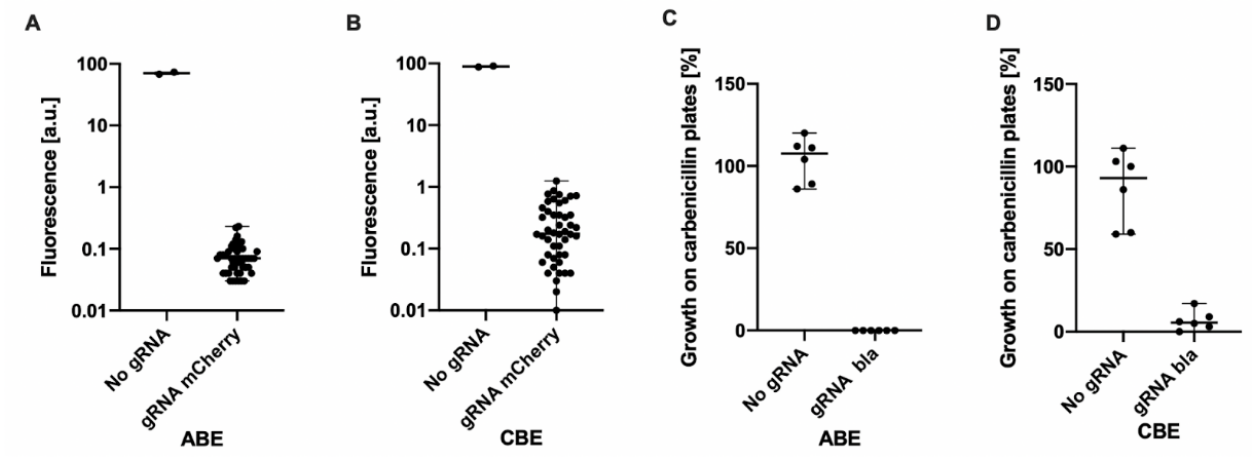

Figure S2: Targeted base editing of the *E. coli* genome *in vitro* after DNA payload transformation and antibiotic selection. A) Adenine base editing (ABE = ABE8e, plasmid p2325) of MG1655-*mCherry*. The experiment was performed in the presence (48 colonies analyzed) or absence (2 colonies analyzed) of a guide RNA targeting the active site of mCherry (tripeptide: M71, Y72, G73). **B)** Cytosine base editing (CBE = evoAPOBEC1-nCas9-UGI, plasmid p2326) of mCherry on MG1655-*mCherry* genome. The experiment was performed in presence (48 colonies analyzed) or absence (2 colonies analyzed) of a guide RNA inserting a stop codon at position Q114* into mCherry. Fluorescence of individual colonies was measured by flow cytometry (excitation: 561 nm, emission: 620/15 BP; Attune NxT Thermo Scientific). Dots represent single colonies after overnight incubation on chloramphenicol plates. **C)** Adenine base editing of β-lactamase (*bla*) in MG1655-*bla in vitro* (plasmid p1396). The experiment was performed in presence or absence of a guide RNA targeting the active site of β-lactamase (K73E or K73R). **D)** Cytosine base editing of β-lactamase in MG1655-*bla in vitro* (plasmid p2327). The CBE inserts a premature stop codon (Q37*) into the target gene, resulting in the re-sensitization of the bacterial population to β-lactam carbenicillin. Adenine and cytosine base editing of β-lactamase was analyzed by colony counting after overnight incubation on chloramphenicol (CA) and CA/carbenicillin agar plates at 30°C. The percentage growth was obtained by dividing the number of colonies on CA/carbenicillin plates by the number on CA plates. One dot represents one transformation after overnight incubation on plates.

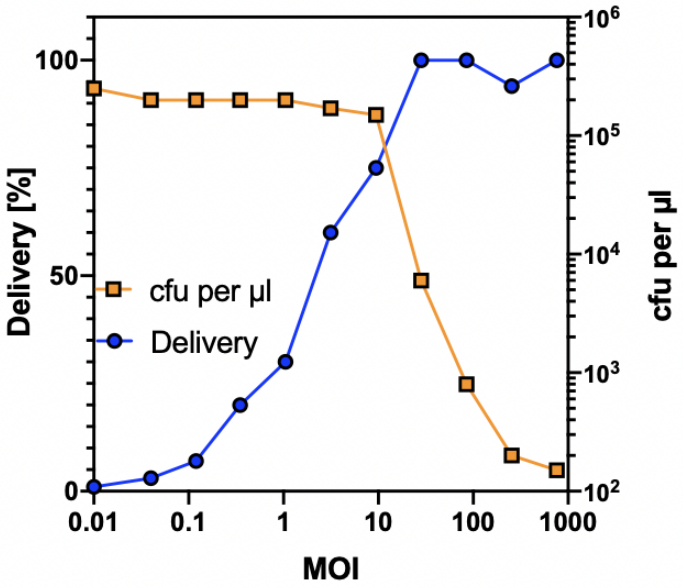

Figure S3: Multiplicity of injection (MOI)-dependent delivery efficiency and corresponding adenine base editing (ABE). Delivery efficiency was obtained by colony counting after overnight incubation on LB and LB (chloramphenicol) agar plates. Left y axis: The percentage delivery was obtained by dividing the number of colonies on chloramphenicol plates by the number on LB plates. Right y axis: Base editing of β-lactamase in strain MG1655-*bla* diminishes cell growth on carbenicillin plates (colony-forming units cfu per µl).

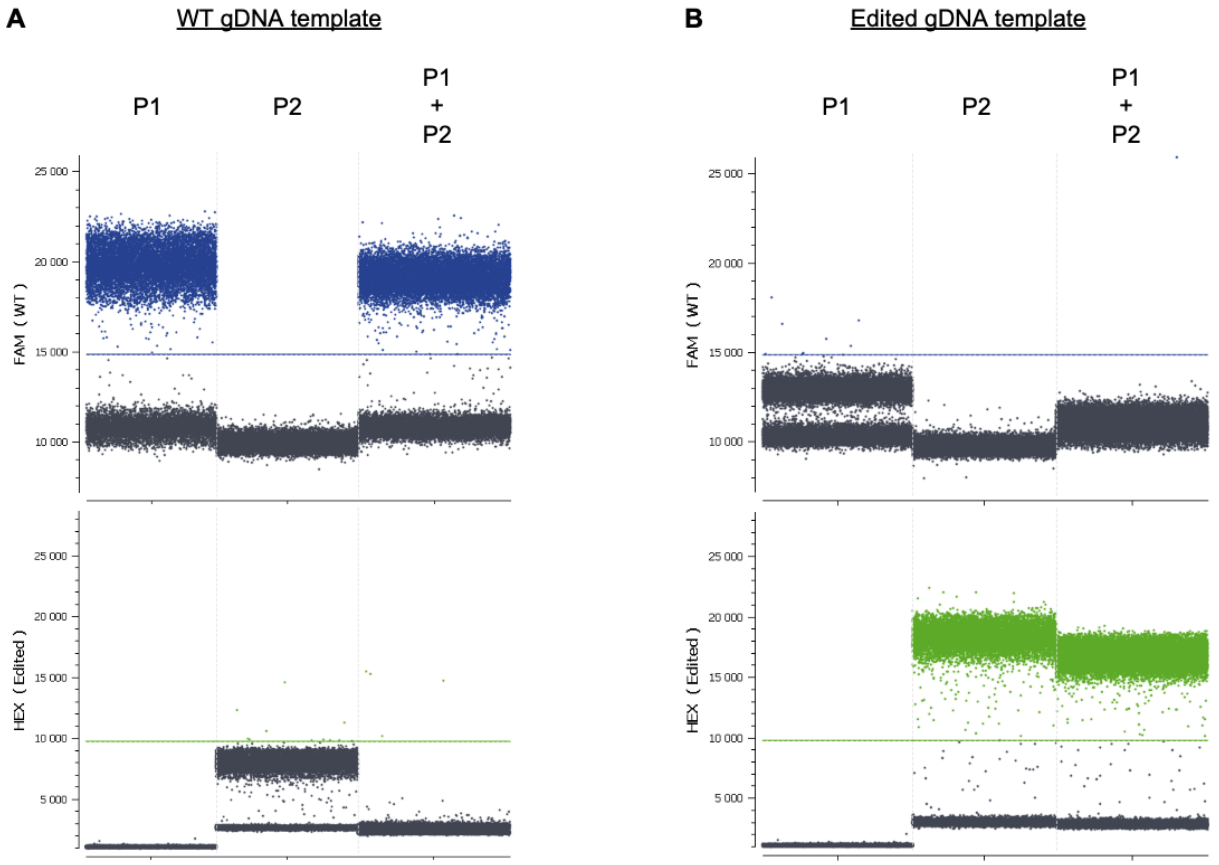

Figure S4: Validation of ddPCR Taqman assay. Probes P1 (wild-type WT target) and P2 (base-edited target) were assessed for their specificity, either individually or when mixed together at a 1:1 molar ratio, using purified genomic DNA (gDNA) from wild-type WT (**panel A**) and *in vitro*-edited (**panel B**) MG1655-*bla* as template.

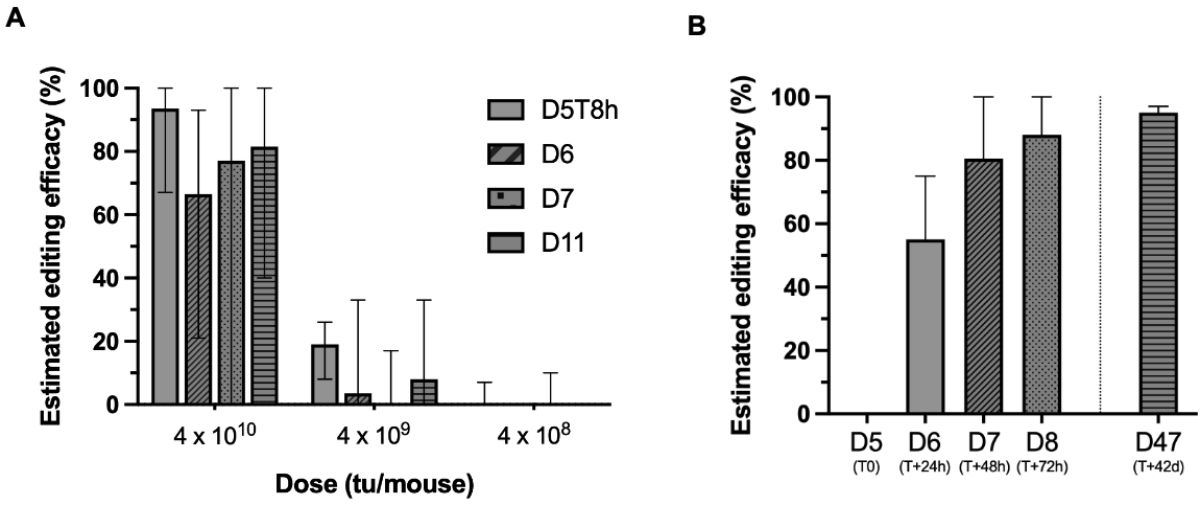

Figure S5: Estimated efficacy of targeted adenine base editing on the *E. coli* genome in gut of BALB/c mice after cosmid treatment using a non-replicative payload. Estimation of the editing efficacy as measured by repatching individual colonies onto agar plate with or without carbenicillin, for the initial dose-response experiment (**panel A**), and for the experiment looking at cumulative effects (**panel B**). Bars represent the group median, with 95% confidence interval.

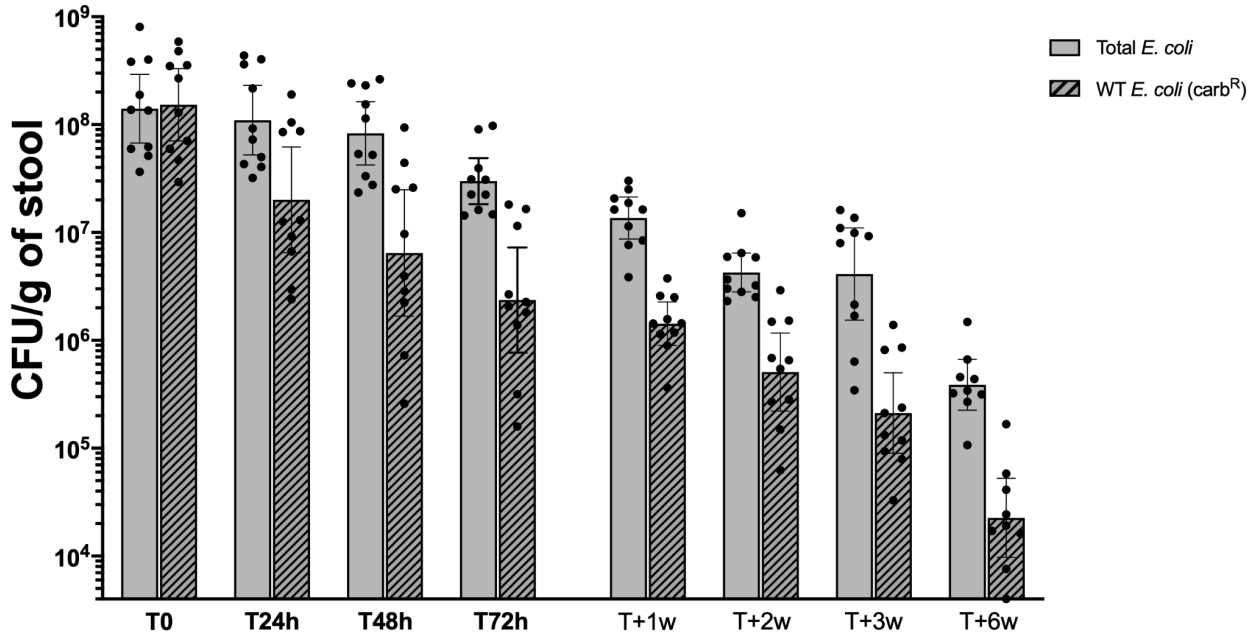

Figure S6: *E. coli* s21052 colonization levels over time in the streptomycin-treated BALB/c model during treatment with base-editing cosmid particles. Total *E. coli* bacteria were quantified by plating resuspended stool samples into Drigalski plates supplemented with streptomycin. Wild-type WT (ie, non-edited) *E. coli* s21052 were quantified by plating samples onto Drigalski plates with carbenicillin.

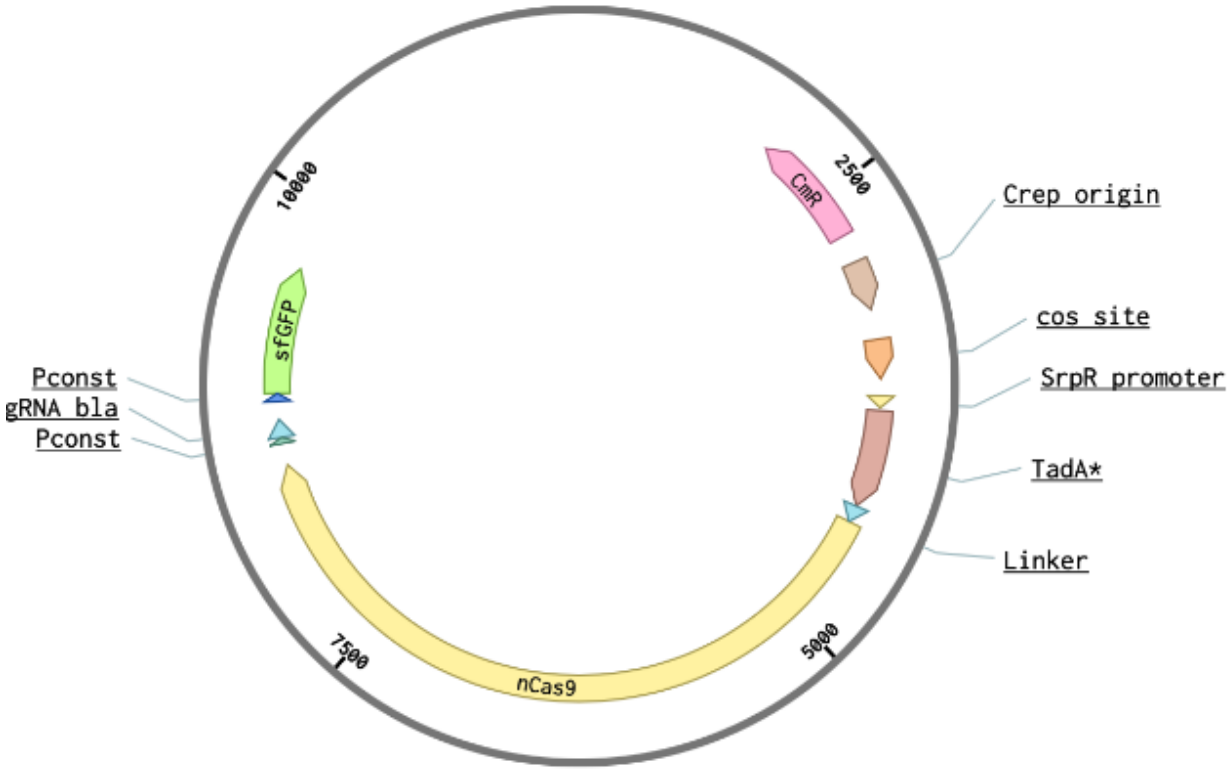

Figure S7: Plasmid map of the non-replicative cosmid encoding the adenine base editor and a guide RNA targeting the active site of β-lactamase (*bla*). Packaging into λ phage particles is enabled by the cohesive end site (*cos)* of the λ genome. The adenine base editor is a fusion gene of *TadA** and *nCas9* under the SrpR promoter. The guide RNA targeting the *bla* gene is under a constitutive promoter. The plasmid carries a constitutively-expressed chloramphenicol resistance gene (CmR) and the non-replicative primase origin of replication (Crep). In addition, the plasmid carries the *sfGFP* gene under a constitutive promoter to investigate cosmid delivery efficiencies. The total plasmid size is 10,747 bp.

Table S1: Genotypes of *E. coli* strains used in this study.

Strain	Genotype	Supplier
DH10B	*F– mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ (ara-leu)7697 galU galK λ– rpsL(StrR) nupG*	Thermo Scientific
CY2120	*BW25113 Δ9 λcI857 PaPa Sam7 Δcos lamB-*	University of Illinois
CY-1A2	*BW25113 Δ9 λcI857 Sam7 Δcos gpJ-1A2 ∆stf lacZ::SrpR lamB-*	This study
CY-A8	*BW25113 Δ9 λcI857 Sam7 Δcos gpJ-A8 ∆stf lacZ::SrpR lamB-∆OmpC*	This study
CY-Ur-λ	*BW25113 Δ9 λcI857 orf401::orf314 Sam7 Δcos lamB-*	This study
MG1655	*K-12 F- λ- ilvG- rfb-50 rph-1*	Pasteur Institute
MG1655-*ΔLamB*	*K-12 F- λ- ilvG- rfb-50 rph-1 ΔLamB*	This study
MG1655-*ΔLamB*-ΔompC	*K-12 F- λ- ilvG- rfb-50 rph-1 ΔLamB ΔompC*	This study
MG1655-*mCherry*	*K-12 F- λ- ilvG- rfb-50 rph-1 mCherry*	Pasteur Institute
MG1655-*bla*	*K-12 F- λ- ilvG- rfb-50 rph-1 rpsLK42R wbbL::bla-rfp*	This study
s14269	*K-12 F- λ- ilvG- rfb-50 rph-1 rpsLK42R ompC-EDL933*	This study
s14269-bla	*K-12 F- λ- ilvG- rfb-50 rph-1 rpsLK42R wbbL::bla-rfp ompC-EDL933*	This study
s21052	*K-12 F- λ- ilvG- rfb-50 rph-1 rpsLK42R wbbL::bla-rfp ompC-EDL933 rpslK42R*	This study
E. coli CFT073	*O6:K2:H1*	Pasteur Institute

Table S2: List of plasmids used in this study.

Name	Plasmid	Class	Resistance	Source	Notes
p2325	p15A-cos-pSrpR-RBS-ABE8e gRNA1(mCherry)	Cosmid	CamR	This study	Encodes ABE targeting mCherry in a constitutive origin of replication
p2326	p15A-cos-pSrpR-RBS-CBE gRNA2(mCherry)	Cosmid	CamR	This study	Encodes CBE targeting mCherry in a constitutive origin of replication
p1396	p15A-cos-pSrpR-RBS-ABE8e gRNA4(*bla*)	Cosmid	CamR	This study	Encodes ABE targeting bla in a constitutive origin of replication
p2327	p15A-cos-pSrpR-RBS-CBE gRNA5(*bla*)	Cosmid	CamR	This study	Encodes CBE targeting bla in a constitutive origin of replication
p2328	pCrep-cos-pSrpR-RBS-ABE8e gRNA4(*bla*)	Cosmid	CamR	This study	Encodes ABE targeting bla in a conditional origin of replication
p1324	pCrep-cos-sfGFP	Cosmid	CamR	This study	Encodes sfGFP in a conditional origin of replication
p513	p15A-cos-sfGFP	Cosmid	CamR	This study	Encodes sfGFP in a constitutive origin of replication
p938	pSC101-pPhlF-RBS-stf P2	Stf	KanR	This study	Encodes an inducible λ-P2 STF chimera
p1471	pEco-OmpC MG1655 G1	Receptor	KanR	This study	Encodes OmpC from MG1655
p1472	pEco-OmpC G17	Receptor	KanR	This study	Encodes OmpC from EDL933
p2076	pIncW-RARE7-RBS-primase	Crep system	TpR	This study	Encodes a constitutive primase gene
p1321	pSC101-pPhlF-RBS-primase	Crep system	KanR	This study	Encodes an inducible primase gene

Table S3: List of gRNAs used in this study.

Name	Editor	gRNA	Position	Target
gRNA1	ABE	CGTACATAAATTGCGGGCTC	4A, 6A, 8A	mCherry (M71T, Y72H)
gRNA2	CBE	CACTCAGGACTCCTCCCTGC	5C	mCherry (Q114*)
gRNA3	CBE	TAGAACCGTACATAAATTGC	6C, 7C	mCherry (G73N or G73D)
gRNA4	ABE	ACTTTTAAAGTTCTGCTATG	1A, 7A, 8A	β-lactamase (K71E or K71R)
gRNA5	CBE	GATCAGTTGGGAGCCCGTGT	4C	β-lactamase (Q37*)

Table S4: Selection of oligonucleotides used for plasmid sequencing and ddPCR. Probes P1 and P2 contained a different fluorophore (FAM or HEX), as well as carefully positioned Locked Nucleic Acid bases (LNA; symbolized by the base A, T, C, or G preceded by a "+" sign in the sequences above).

Name	Oligonucleotide sequence	Gene
F1	ATGGTTTCCAAGGGCGAGG	mCherry
R1	TTATTTGTACAGCTCATCCATGCC	mCherry
F2	ATGAGTATTCAACATTTCCGTGTCGC	*Bla*
R2	TTACCAATGCTTAATCAGTGATGC	*Bla*
F3	GGATCTCAACAGCGGTAAG	*Bla* (ddPCR, primer)
R3	GGCATCAACACGGGATAATA	*Bla* (ddPCR, primer)
P1	FAM-CT+TT+T+A+AA+GTT+C+T+GC	*Bla* (ddPCR, probe 1)
P2	HEX-CT+TT+T+G+AAGTT+CT+GC	*Bla* (ddPCR, probe 2)