Electronic Supplementary Material (ESI) for Chemical Science

Programmable Adenine Deamination in Bacteria Using a Cas9-Adenine-Deaminase Fusion

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1 Detailed protocol for adenine base editing in *S. aureus* and *E. coli*

1.1 All the editing plasmids (except for the multiplex genome editing) were constructed using the following protocol:

(1) Design of the Oligos

Select a 20 bp-spacer sequence before a NGG PAM and two oligos were designed in the following form:

Forward oligo: 5'-gaaaNNNNNNNNNNNNNNNNNNNN-3'

Reverse oligo: 3'-NNNNNNNNNNNNNNNNNNNNcaaa-5'

(2) Phosphorylation of the oligos

10X T4 DNA ligase buffer (NEB)	5 μL
Oligo F (50 μM)	2 μL
Oligo R (50 μM)	2 μL
T4 polynucleotide kinase (Takara)	1 μL
ddH_2O	40 μL
	50 μL

Incubate the solution at 37 °C for one hour.

(3). Annealing of the oligos

2.5 μL of 1 M NaCl solution was added to the phosphorylated oligos. The solution was incubated at 95 °C for 3 min. The solution was slowly cooled down to room temperature using a thermo cycler (BIO-RAD, C1000 Touch). Next, the solution containing the annealed oligos was diluted by 20 times.

(4). Golden gate assembly

10X T4 DNA ligase buffer (NEB)	1 μL
pABE plasmid (100 fmol/μL)	0.2 μL
annealed oligos (20 fmol/μL)	1 μL
T4 DNA ligase (NEB)	0.5 μL
BsaI-HF (NEB)	0.5 μL
ddH_2O	6.8 μL
	10 μL

Run the above reaction to insert the annealed oligos into the pABE plasmid using the following protocol

Temperature (°C)	Time (min)	
37	3	25 cycles
16	4	
50	5	
80	15	
10	∞	

1.2 Multiplex genome editing plasmid was constructed using the following protocol:

(1). Amplification of sgRNA cassette from pABE-murR3.

(2). Restriction enzyme digestion (XbaI/ XhoI) of pABE-C46-4 and PCR products of sgRNA cassette.

10X CutSmart buffer (NEB)	5 μL
DNA	40 μL
XbaI Restriction enzyme (NEB)	2.5 μL
XhoI Restriction enzyme (NEB)	2.5 μL
	50 μL

The enzyme-digested products were purified by gel extraction.

(5). T4 DNA ligase reaction.

10X T4 DNA ligase buffer (NEB)	1 μL
pABE-C46-4 plasmid (10 fmol/μL)	1 μL
sgRNA (50 fmol/μL)	1 μL
T4 DNA ligase (NEB)	0.5 μL
ddH_2O	6.5 μL
	10 μL

The reaction mixture was placed at room temperature for 1 h.

1.2 Transformation

The product was transformed into 100 μL *E. coli* DH5α competent cells and the cells were plated onto a LB agar plate containing 50 μg/ml kanamycin. The plate was incubated at 30 ^{o}C for 24 h. The successful construction of the plasmid was confirmed by PCR, enzyme digestion, and sequencing.

1.3 Adenine base editing

See the detailed procedures in the “Adenine base editing” section of the Methods part.

2 Supporting Figures

Figure S1. pABE enabled highly efficient adenine to guanine conversion in the *S. aureus* RN4220 strain. (A) A36 of the *agrA* gene was synonymously mutated with an efficiency of 3/4 using pABE. (B) M37 of the *murR* gene was mutated to V with an efficiency of 4/8 using pABE.

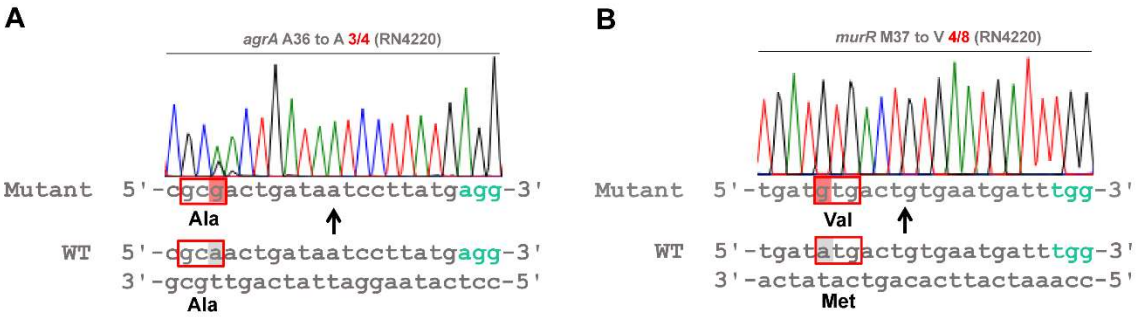


Figure S2. pABE enabled highly efficient adenine to guanine conversion in clinically isolated *S. aureus* strains Newman and N315. (A) S55 of the *cntA* gene in the Newman strain was mutated to P with an efficiency of 8/8. (B) L77 of the *cntA* gene in the Newman strain was synonymously mutated with an efficiency of 6/7. (C) S55 of the *cntA* gene in the N315 strain was mutated to P with an efficiency of 7/8. (D) N83 of the *murR* gene in the N315 strain was synonymously mutated with an efficiency of 8/8.

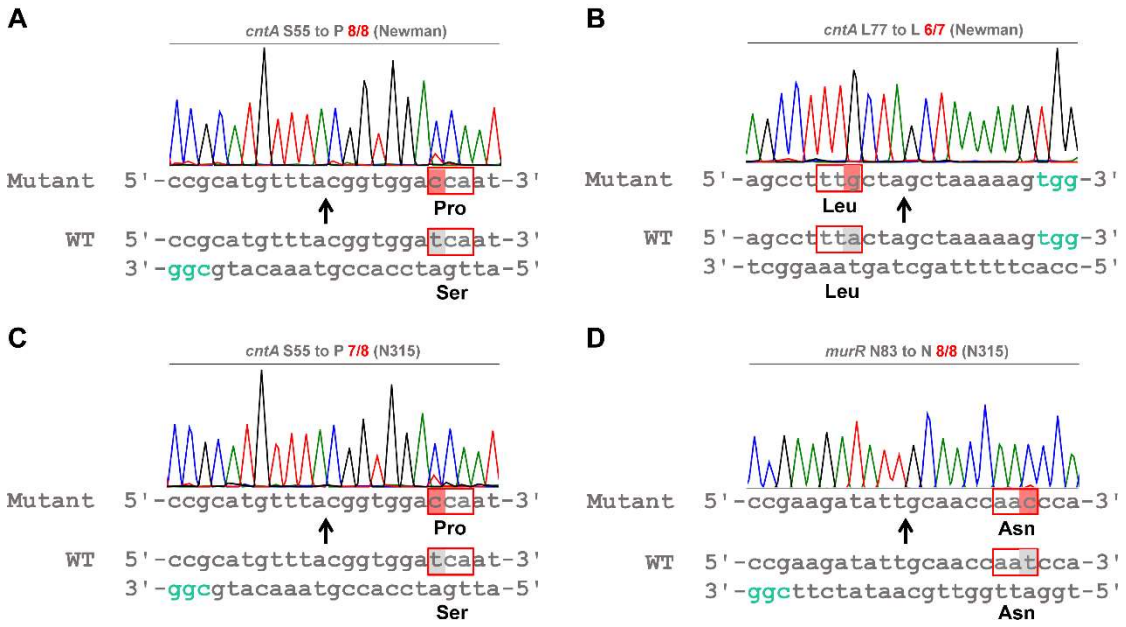


Figure S3. The editable window of pABE in *S. aureus*. The As at the positions from 4 to 8 were edited with the efficiencies of 5/8, 5/7, 8/8, 4/8 and 8/8, respectively. The As at the positions of 2, 3, 9, and 10 could not be edited by pABE.

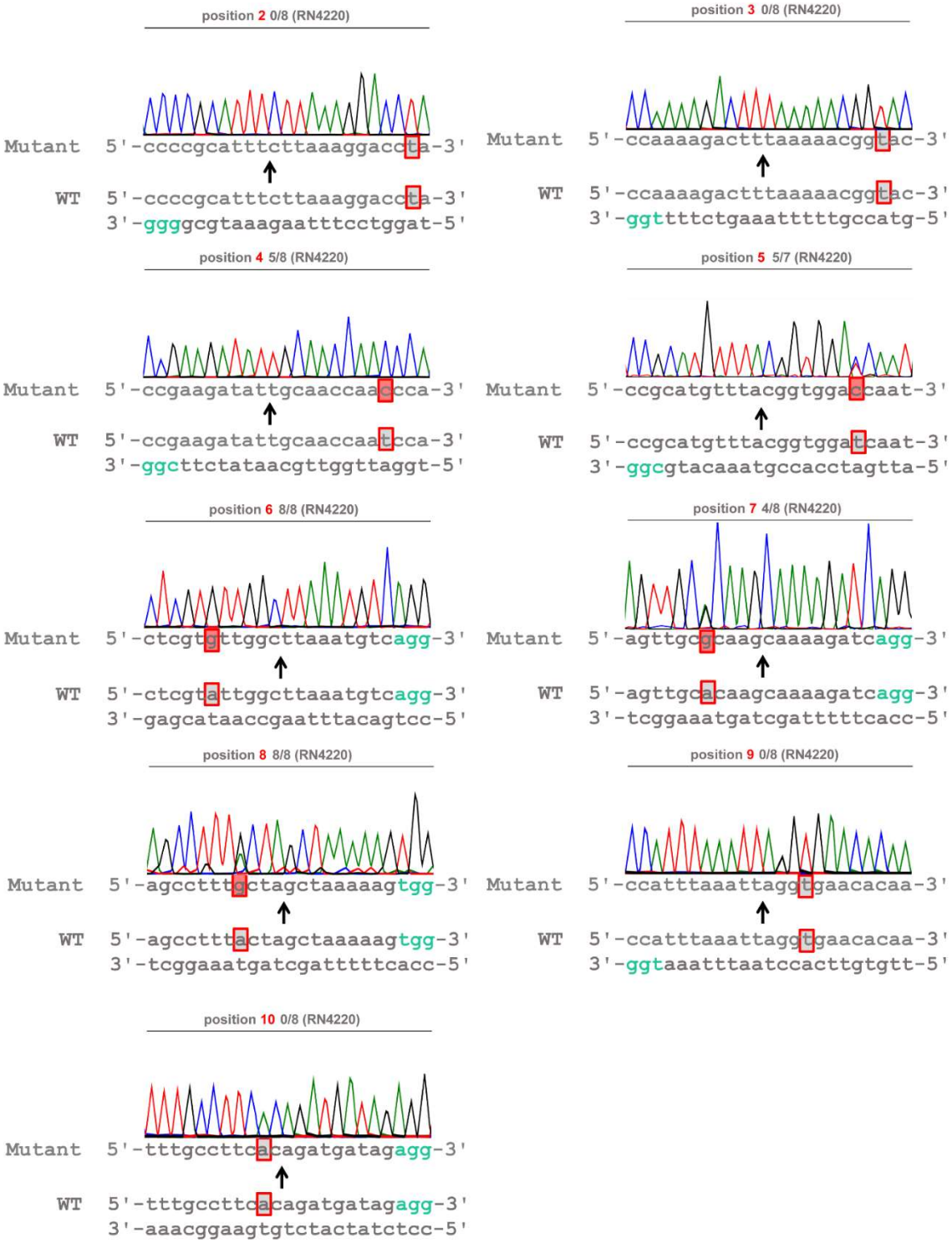


Figure S4. pABE enabled highly efficient adenine to guanine conversion in *E. coli* MG1655 strain. R38 of the *lacZ* gene in the MG1655 strain was synonymously mutated to R with an efficiency of 8/8.

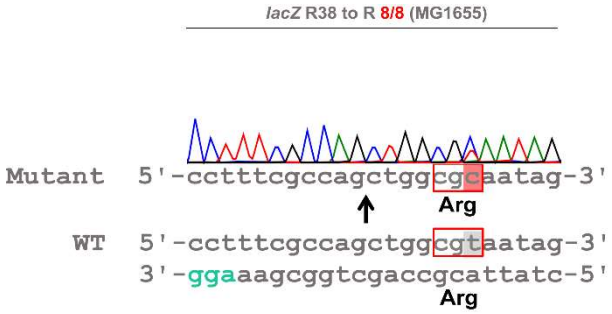


Figure S5. pABE enabled highly efficient adenine to guanine conversion in multiplex gene editing in the *S. aureus* RN4220 strain. D193 of *cntC* gene and A141 of *murR* gene were simultaneously mutated.

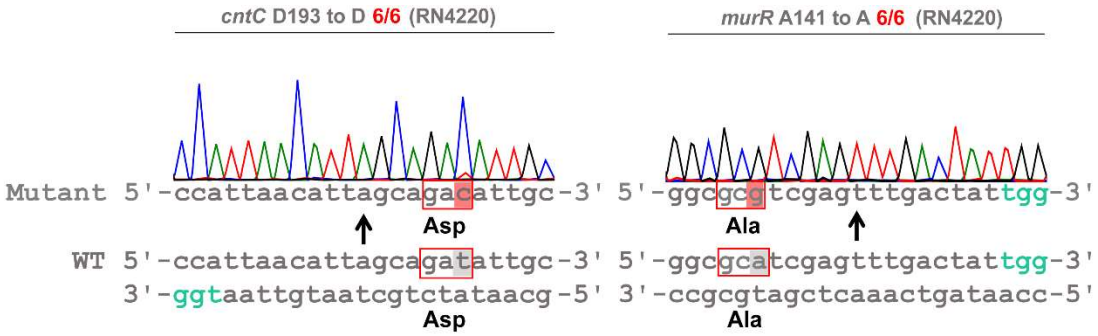


Figure S6. Five editable sites from 4 spacers in the *S. aureus* RN4220 strain were chosen to evaluate the unbiased editing efficiency of pABE via deep sequencing. Each site was read 50 thousand times.

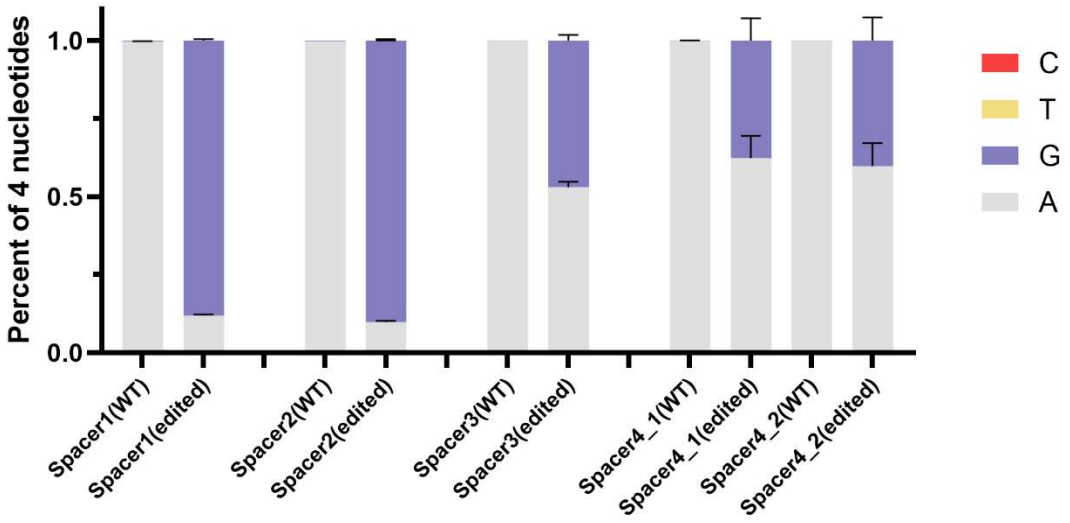
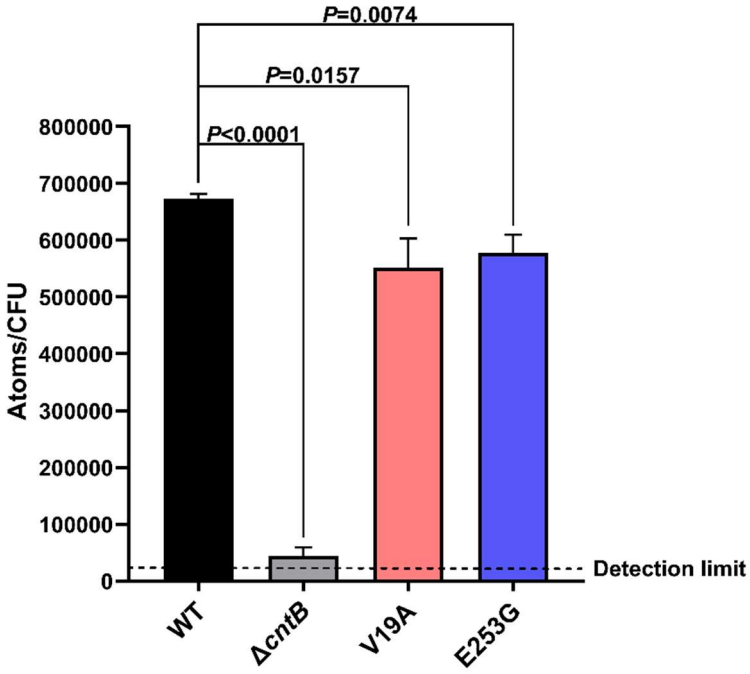


Figure S7. Thirty-eight spacers targeting 42 different sites of *cntBC* using pABE. The editable spacers were shown in boxes. The four key residues were highlighted.

Figure S8. Co^{2+} accumulation measurements of various RN4220 strains by ICP-OES. Assays were performed in the presence of 0.5 μM Co^{2+}. The detection limit of ICP-OES is 26260 atoms/CFU. The *P* values are calculated from Student's *t* test.



3 Supporting Tables

Table S1. Proportion of 4 nucleotides in edited sites measured via high-throughput sequencing. Each site was read 50 thousand times. Sequencing was repeated three times.

	Edited Site	Strain Type	Nucleotide Type	Average Percentage	Standard Deviation
SPACER1	6th	WT	A	0.99726	0.00011
			T	0.00007	0.00004
			G	0.00265	0.00011
			C	0.00002	0.00002
		Edited	A	0.11846	0.00425
			T	0.00010	0.00002
			G	0.88144	0.00423
			C	0.00000	0.00000
SPACER2	6th	WT	A	0.99775	0.00015
			T	0.00006	0.00004
			G	0.00215	0.00016
			C	0.00004	0.00003
		Edited	A	0.09805	0.00416
			T	0.00005	0.00001
			G	0.90177	0.00402
			C	0.00012	0.00016
SPACER3	8th	WT	A	0.99882	0.00006
			T	0.00002	0.00002
			G	0.00112	0.00001
			C	0.00004	0.00007
		Edited	A	0.53012	0.01772
			T	0.00005	0.00003
			G	0.46980	0.01772
			C	0.00003	0.00003
SPACER4	5th	WT	A	0.99887	0.00018
			T	0.00001	0.00001
			G	0.00109	0.00015
			C	0.00003	0.00004
		Edited	A	0.62313	0.07100
			T	0.00005	0.00003
			G	0.37678	0.07098
			C	0.00004	0.00005
	7th	WT	A	0.99852	0.00016
			T	0.00004	0.00002
			G	0.00129	0.00014
			C	0.00015	0.00004
		Edited	A	0.59754	0.07395
			T	0.00011	0.00010
			G	0.40229	0.07388
			C	0.00007	0.00002

Table S2. List of possible off-target sites.

Target	Mismatch	Sequence	PAM
cntA-S5-2	0	ATTGATCCACCGTAAACATG	CGG
Off_1	9	**TCAT**A**CAG**AC**GT**TAAACATG	TGG
Off_2	12	**CAAAGAATGATT**TAAACATG	TGG
Off_3	10	A**AATTATATTT**GTAAACATG	TGG
Off_4	10	**TA**TCCAAC**TGTC**TAAACATG	AGG
Off_5	9	**CACATG**C**A**AT**C**ATAAACATG	TGG
Off_6	12	**TGCCTCTGTTAA**TAAACATG	CAG
Off_7	10	**TT**G**TTCAAGAT**GTAAACATG	GAG
Off_8	7	**TT**CCATC**TT**C**TC**TAAACATG	CAG
Off_9	8	A**AT**A**AT**A**AGTAT**TAAACATG	CAG
Off_10	8	**TTTATT**G**AA**A**GA**TAAACATG	TAG
Off_11	10	A**GATGC**C**ATTGC**TAAACATG	TAG
Off_12	9	A**AGA**A**CT**C**GTGC**TAAACATG	AAG
Off_13	11	**GGAT**A**AGGTTAT**TAAACATG	CAG
Off_14	7	**GA**TGA**AGGCA**CGTAAACATG	AAG
Off_15	8	A**GCAGC**C**A**A**T**C**C**TAAACATG	GAG
Off_16	10	A**CATGCTTTGG**GTAAACATG	TAG
Off_17	8	**TTTA**A**CAA**A**AAT**TAAACATG	CAG
Off_18	10	**CGCAT**TC**GTTAA**TAAACATG	TAG
Off_19	11	A**ACACAATTTGT**TAAACATG	GAG
Off_20	8	**G**TTG**CATGCTG**GTAAACATG	TGA
Off_21	9	A**CAA**A**A**C**TTTAA**TAAACATG	AGA
Off_22	10	**TA**TTA**ATATTAT**TAAACATG	AGA
Off_23	8	A**AA**GAT**TGTGAT**TAAACATG	GGA
Off_24	10	**CACT**A**CAA**A**TGT**TAAACATG	CGA
Off_25	9	A**AGTT**TC**GGTTT**TAAACATG	GGA
Off_26	10	**TA**TC**A****AATCAT**TAAACATG	GGA
Off_27	10	**TAATGAT**CA**TTT**TAAACATG	TGA
Target	**Mismatch**	**Sequence**	**PAM**
murR-S6	0	CTCGTATTGGCTTAAATGTC	AGG
Off_1	11	**GGTCCGGC**G**ATG**TAAATGTC	GGG
Off_2	9	**TCAACC**TT**TAG**TTAAATGTC	AGG
Off_3	8	**A**TT**TA**AT**GATG**TTAAATGTC	GGG
Off_4	11	**TT****ACCTAAAATA**TAAATGTC	TGG
Off_5	9	**AAAAATCG**GGC**A**TAAATGTC	AGG
Off_6	9	**GGGTAGG**TG**CA**TTAAATGTC	GAG
Off_7	9	C**A**C**TCGG**T**TCAG**TAAATGTC	CAG
Off_8	9	**TC**C**T**TA**AACAAA**TAAATGTC	CGA
Off_9	9	**TAT**G**CTG**TG**TAA**TAAATGTC	TGA
Off_10	8	**GA**C**A**T**C**T**CATT**TTAAATGTC	AGA
Off_11	10	**TGTAAGGG**G**CT**TTAAATGTC	AGA

Table S3. Bacterial strain used in this study

Bacterial strain	Description	Reference
E.coli		
DH5α	F-ϕ80*lacZ* ΔM15 Δ(*lacZ*YA-argF) U169 *endA*1 *recA*1 *hsdR*17(rk-,mk+) *supE*44λ-thi-1 *gyrA*96 relA1 *phoA*	Lab stock
DH5α-Δ*lacZ*	DH5α Δ*lacZ*	Lab stock
DH5α-sp*murR*-SA	DH5α *murR* N266 mutation to N and D267 mutation to D	This study
DC10B	F- *endA*1 *recA*1 *galU galK deoR nupG rpsL* Δ*lacX*74 Φ80*lacZ*ΔM15 *araD*139 Δ(*ara,leu*)7697 *mcrA* Δ(*mrr-hsdRMS-mcrBC*) λ	Lab stock
MG1655	K12 F- lambda- *ilvG*- rfb-50 rph-1	Lab stock
MG1655-*lacZ*-S6	MG1655 *lacZ* R38 mutation to R	This study
MG1655-*yagI*-S6	MG1655 *yagI* D15 mutation to G	This study
MG1655-*lacZ*-SC	MG1655 *lacZ* Q51 mutation to stop codon	This study
S. aureus		
RN4220	Restriction-deficient transformation recipient	Lab stock
RN4220-*agrA*1	RN4220 *agrA* I26 mutation to V	This study
RN4220-*agrA*2	RN4220 *agrA* A36 mutation to A	This study
RN4220-*agrA*3	RN4220 *agrA* K77 mutation to E	This study
RN4220-*murR*1	RN4220 *murR* M37 mutation to V	This study
RN4220-*murR*3	RN4220 *murR* A141 mutation to A	This study
RN4220-*murR*4	RN4220 *murR* S52 mutation to S	This study
RN4220-*murR*-S4	RN4220 *murR* mutation for editing window 4	This study
RN4220-*cntA*-S5-2	RN4220 *cntA* mutation for editing window 5	This study
RN4220-*murR*-S6	RN4220 *murR* mutation for editing window 6	This study
RN4220-*sasC*-S7	RN4220 *sasC* mutation for editing window 7	This study
RN4220-*cntA*-S8	RN4220 *cntA* mutation for editing window 8	This study
RN4220-Δ*cntB*	RN4220 *cntB* Q68 mutation to stop codon	This study
RN4220-Δ*cntC*	RN4220 *cntC* R6 mutation to stop codon	This study
RN4220-B56-3	RN4220 *cntB* N229 mutation to D	This study
RN4220-B56-2	RN4220 *cntB* S153 mutation to P	This study
RN4220-B4-2	RN4220 *cntB* L249 mutation to P	This study
RN4220-B8-1	RN4220 *cntB* R197 mutation to G	This study
RN4220-B4578-1-1	RN4220 *cntB* I107 mutation to M	This study
RN4220-B4578-1-2	RN4220 *cntB* I107 mutation to V	This study
RN4220-B7-6	RN4220 *cntB* E253 mutation to G	This study
RN4220-B7-6(10)	RN4220 *cntB* Y254 mutation to C	This study
RN4220-B467-1	RN4220 *cntB* T37 mutation to T	This study
RN4220-B7-1	RN4220 *cntB* V19 mutation to A	This study
RN4220-B47-1	RN4220 *cntB* T116 mutation to A	This study
RN4220-B7-4	RN4220 *cntB* S124 mutation to G	This study
RN4220-B7-5	RN4220 *cntB* T168 mutation to A	This study

RN4220-B7-2	RN4220 *cntB* M77 mutation to V	This study
RN4220-B7-3	RN4220 *cntB* T83 mutation to A	This study
RN4220-B78-3	RN4220 *cntB* L264 mutation to S	This study
RN4220-B46-2	RN4220 *cntB* Y280 mutation to C	This study
RN4220-B568-1-1	RN4220 *cntB* I95 mutation to I	This study
RN4220-B568-1-3	RN4220 *cntB* R94 mutation to R	This study
RN4220-B78-2	RN4220 *cntB* L179 mutation to S	This study
RN4220-B456-1	RN4220 *cntB* F193 mutation to S	This study
RN4220-B46-1	RN4220 *cntB* I242 mutation to V	This study
RN4220-B47-2	RN4220 *cntB* I184 mutation to V	This study
RN4220-B57-1	RN4220 *cntB* Y254 mutation to C	This study
RN4220-B468-1	RN4220 *cntB* Y280 mutation to H and V281 mutation to A	This study
RN4220-B56-1	RN4220 *cntB* L66 mutation to L	This study
RN4220-B567-1	RN4220 *cntB* E54 mutation to G	This study
RN4220-C48-1	RN4220 *cntC* I144 mutation to V	This study
RN4220-C4-2	RN4220 *cntC* I93 mutation to V	This study
RN4220-C46-1	RN4220 *cntC* T43 mutation to T	This study
RN4220-C8-1-4	RN4220 *cntC* M141 mutation to T	This study
RN4220-C8-1-3	RN4220 *cntC* F143 mutation to L	This study
RN4220-C7-1	RN4220 *cntC* I90 mutation to V	This study
RN4220-C47-2	RN4220 *cntC* V252 mutation to A	This study
RN4220-C46-4	RN4220 *cntC* D193 mutation to D	This study
RN4220-C57-1	RN4220 *cntC* I251 mutation to V	This study
RN4220-C46-2	RN4220 *cntC* C113 mutation to R	This study
RN4220-C47-1	RN4220 *cntC* I206 mutation to V	This study
RN4220-C68-2	RN4220 *cntC* R63 mutation to G	This study
RN4220-C457-1	RN4220 *cntC* F81 mutation to L	This study
RN4220-C58-1	RN4220 *cntC* M256 mutation to T	This study
RN4220-C68-1	RN4220 *cntC* Y36 mutation to H	This study
RN4220-V19S	RN4220 *cntB* V19 mutation to S	This study
RN4220-E253A	RN4220 *cntB* E253 mutation to A	This study
RN4220-C113A	RN4220 *cntC* C113 mutation to A	This study
RN4220-F143A	RN4220 *cntC* F143 mutation to A	This study
RN4220-C46-4-murR3	RN4220 *cntC* D193 mutation to D and *murR* A141 mutation to A	This study
Newman	Wild type	Lab stock
Newman-*cntA*-S5	Newman *cntA* S55 mutation to P	This study
Newman-*cntA*-S8	Newman *cntA* L77 mutation to L	This study
N315	Wild type	Lab stock
N315-*cntA*-S5	N315 *cntA* S55 mutation to P	This study
N315-*murR*-S4	N315 *murR* N83 mutation to N	This study

Table S4. Plasmids used in this study

Plasmids	Description	Reference
pnCasSA-BEC	S.aureus base editing vector, Kmr, Cmr	1
pUC57-ABE7.10	pUC57 derivative containing codon optimized ABE7.10 gene, Ampr	This study
pABE	genome editing vector, Kmr, Cmr	This study
pABE-*agrA*1	pABE derivative with *agrA* spacer, I26 mutation to V	This study
pABE-*agrA*2	pABE derivative with *agrA* spacer, A36 mutation to A	This study
pABE-*agrA*3	pABE derivative with *agrA* spacer, K77 mutation to E	This study
pABE-*murR*1	pABE derivative with *murR* spacer, M37 mutation to V	This study
pABE-*murR*3	pABE derivative with *murR* spacer, A141 mutation to A	This study
pABE-*murR*4	pABE derivative with *murR* spacer, S52 mutation to S	This study
pABE-*cymR*-S2	pABE derivative with *cymR*-S2 spacer	This study
pABE-*cntA*-S3	pABE derivative with *cntA*-S3 spacer	This study
pABE-*murR*-S4	pABE derivative with *murR*-S4 spacer	This study
pABE-*cntA*-S5-2	pABE derivative with *cntA*-S5 spacer	This study
pABE-*murR*-S6	pABE derivative with *murR*-S6 spacer	This study
pABE-*sasC*-S7	pABE derivative with *sasC*-S7 spacer	This study
pABE-*cntA*-S8	pABE derivative with *cntA*-S8 spacer	This study
pABE-*cntA*-S9	pABE derivative with *cntA*-S9 spacer	This study
pABE-*cntA*-S10	pABE derivative with *cntA*-S10 spacer	This study
pABE-*lacZ*-S6	pABE derivative with *lacZ* spacer, R38 mutation to R	This study
pABE-*yagI*-S6	pABE derivative with *yagI* spacer, D15 mutation to G	This study
pABE-sp*murR*-SA	pABE derivative with *murR* spacer, N266 mutation to N and D267 mutation to D	This study
pBECKP	K.peneumoniae base editing vector, Kmr	4
pBECKP-*lacZ*-sc	pBECKP derivative with *lacZ* spacer, Q51 mutation to stop codon	This study
pABE-*lacZ*-cv	pABE derivative with *lacZ* spacer, stop codon mutation to Q51	This study
pnCasSA-BEC-Δ*cntB*	pnCasSA-BEC derivative with *cntB* spacer, Q68 mutation to stop codon	This study
pnCasSA-BEC-Δ*cntC*	pnCasSA-BEC derivative with *cntC* spacer, R6 mutation to stop codon	This study
pABE-B56-3	pABE derivative with *cntB* spacer	This study
pABE-B56-2	pABE derivative with *cntB* spacer	This study
pABE-B4-2	pABE derivative with *cntB* spacer	This study
pABE-B8-1	pABE derivative with *cntB* spacer	This study
pABE-B4578-1	pABE derivative with *cntB* spacer	This study
pABE-B7-6	pABE derivative with *cntB* spacer	This study
pABE-B467-1	pABE derivative with *cntB* spacer	This study
pABE-B7-1	pABE derivative with *cntB* spacer	This study
pABE-B47-1	pABE derivative with *cntB* spacer	This study

pABE-B7-4	pABE derivative with *cntB* spacer	This study
pABE-B7-5	pABE derivative with *cntB* spacer	This study
pABE-B7-2	pABE derivative with *cntB* spacer	This study
pABE-B7-3	pABE derivative with *cntB* spacer	This study
pABE-B78-3	pABE derivative with *cntB* spacer	This study
pABE-B46-2	pABE derivative with *cntB* spacer	This study
pABE-B568-1	pABE derivative with *cntB* spacer	This study
pABE-B78-2	pABE derivative with *cntB* spacer	This study
pABE-B456-1	pABE derivative with *cntB* spacer	This study
pABE-B46-1	pABE derivative with *cntB* spacer	This study
pABE-B47-2	pABE derivative with *cntB* spacer	This study
pABE-B57-1	pABE derivative with *cntB* spacer	This study
pABE-B468-1	pABE derivative with *cntB* spacer	This study
pABE-B56-1	pABE derivative with *cntB* spacer	This study
pABE-B567-1	pABE derivative with *cntB* spacer	This study
pABE-C48-1	pABE derivative with *cntC* spacer	This study
pABE-C4-2	pABE derivative with *cntC* spacer	This study
pABE-C46-1	pABE derivative with *cntC* spacer	This study
pABE-C8-1	pABE derivative with *cntC* spacer	This study
pABE-C7-1	pABE derivative with *cntC* spacer	This study
pABE-C47-2	pABE derivative with *cntC* spacer	This study
pABE-C46-4	pABE derivative with *cntC* spacer	This study
pABE-C57-1	pABE derivative with *cntC* spacer	This study
pABE-C46-2	pABE derivative with *cntC* spacer	This study
pABE-C47-1	pABE derivative with *cntC* spacer	This study
pABE-C68-2	pABE derivative with *cntC* spacer	This study
pABE-C457-1	pABE derivative with *cntC* spacer	This study
pABE-C58-1	pABE derivative with *cntC* spacer	This study
pABE-C68-1	pABE derivative with *cntC* spacer	This study
pCasSA	S.aureus genome editing vector, Kmr, Cmr	6
pCasSA-spB7-1	pCasSA derivative with B7-1 spacer	This study
pCasSA-spB7-6	pCasSA derivative with B7-6 spacer	This study
pCasSA-spC46-2	pCasSA derivative with C46-2 spacer	This study
pCasSA-spC8-1	pCasSA derivative with C8-1 spacer	This study
pCasSA-V19S	pCasSA derivative for V19S mutation	This study
pKOR1-E253A	pKOR1 derivative for E253A mutation	This study
pCasSA-C113A	pCasSA derivative for C113A mutation	This study
pCasSA-F143A	pCasSA derivative for F143A mutation	This study
pABE-C46-4-murR3	pABE derivative with *cntC* and *murR* spacers	This study

Table S5. Primers used in this study

	Name	Sequence (5'-3')	Description
pABE plasmid construction	T7	TAATACGACTCACTATAGGG	amplification of the ABE gene from plasmid pUC57-ABE7.10
	T7ter	TGCTAGTTATTGCTCAGCGG	amplification of the ABE gene from plasmid pUC57-ABE7.10
	PCasABEF1	TAGCGGTGGTAGCAGCGGTGGCAGCGATAAGAAATACTCAATAGGCTTAGCTATCGG	amplification of the nCas9 gene and part of *S. aureus* repF origin from pnCasSA-BEC plasmid
	PCasABER1	ctgtacgctgtttctcacgctttct	amplification of the nCas9 gene and part *S. aureus* repF origin from pnCasSA-BEC plasmid
	PCasABEF2	agaaagcgtgagaaacagcgtacag	amplification of part *S. aureus* repF origin, chloramphenicol-resistance marker, the origin ColE1 and kanamycin-resistance Marker, sgRNA and the rpsL promoter from pnCasSA-BEC plasmid
	PCasABER2	CATGGCTGAACTCCACTTCGCTCATGTGATATGTCCTCCTCTCTTCACT	amplification of part *S. aureus* repF origin, chloramphenicol-resistance marker, the origin ColE1 and kanamycin-resistance Marker, sgRNA and the rpsL promoter from pnCasSA-BEC plasmid
pABE plasmid construction for multiplex genome editing	sgRNA-F	catggTCTAGAAGAGTTTGCAAAATATACAG	amplification of sgRNA from pABE-murR3 plasmid
	sgRNA-R	TCTGACTCGAGCATTCATGCGGCCGCccatgggtatggacagatct	amplification of sgRNA from pABE-murR3 plasmid
agrA gene base editing	*agrA*1F	gaaaAATGATAGAAGAAAAGCCTA	*agrA* spacer for its I26 mutation to V
	*agrA*1R	aaacTAGGCTTTTCTTCTATCATT	*agrA* spacer for its I26 mutation to V
	*agrA*2F	gaaaCGCAACTGATAATCCTTATG	*agrA* spacer for its A36 mutation to A

	*agrA*2R	aaacCATAAGGATTATCAGTTGCG	*agrA* spacer for its A36 mutation to A
	*agrA*3F	gaaaATTCGTAAGCATGACCCAGT	*agrA* spacer for its K77 mutation to E
	*agrA*3R	aaacACTGGGTCATGCTTACGAAT	*agrA* spacer for its K77 mutation to E
	*agrA*vF	ggtgaaggtcgtggtttagg	amplification of the verified *agrA* DNA from genome
	*agrA*vR	gccagctatacagtgcatttg	amplification of the verified *agrA* DNA from genome
murR gene base editing	*murR*1F	gaaaTGATATGACTGTGAATGATT	*murR* spacer for its M37 mutation to V
	*murR*1R	aaacAATCATTCACAGTCATATCA	*murR* spacer for its M37 mutation to V
	*murR*3F	gaaaGGCGCATCGAGTTTGACTAT	*murR* spacer for its A141 mutation to A
	*murR*3R	aaacATAGTCAAACTCGATGCGCC	*murR* spacer for its A141 mutation to A
	*murR*4F	gaaaCATCAATTGTTAGATTTAGT	*murR* spacer for its S52 mutation to S
	*murR*4R	aaacACTAAATCTAACAATTGATG	*murR* spacer for its S52 mutation to S
	*murR*vF	ATTGGTGGCGCTGTAATAGG	amplification of the verified *murR* DNA from genome
	*murR*vR	CTGTAATGTGTGGCCACCTG	amplification of the verified *murR* DNA from genome
targeting An at different positions	*cymR*-S2F	gaaataggtcctttaagaaatgcg	A2 spacer for base editing
	cymR-S2R	aaaccgcatttcttaaaggaccta	A2 spacer for base editing
	cntA-S3F	gaaaGTACCGTTTTTAAAGTCTTT	A3 spacer for base editing
	cntA-S3R	aaacAAAGACTTTAAAAACGGTAC	A3 spacer for base editing
	murR-S4F	gaaaTGGATTGGTTGCAATATCTT	A4 spacer for base editing
	murR-S4R	aaacAAGATATTGCAACCAATCCA	A4 spacer for base editing
	cntA-S5-2F	gaaaATTGATCCACCGTAAACATG	A5 spacer for base editing

	cntA-S5-2R	aaacCATGTTTACGGTGGATCAAT	A5 spacer for base editing
	murR-S6F	gaaaCTCGTATTGGCTTAAATGTC	A6 spacer for base editing
	murR-S6R	aaacGACATTTAAGCCAATACGAG	A6 spacer for base editing
	sasC-S7-6F	gaaaagttgcacaagcaaaagatc	A7 spacer for base editing
	sasC-S7-6R	aaacgatcttttgcttgtgcaact	A7 spacer for base editing
	cntA-S8F	gaaaAGCCTTTACTAGCTAAAAAG	A8 spacer for base editing
	cntA-S8R	aaacCTTTTTAGCTAGTAAAGGCT	A8 spacer for base editing
	cntA-S9F	gaaaTTGTGTTCACCTAATTTTAAA	A9 spacer for base editing
	cntA-S9R	aaacTTTAAATTAGGTGAACACAA	A9 spacer for base editing
	cntA-S10F	gaaaTTTGCCTTCACAGATGATAG	A10 spacer for base editing
	cntA-S10R	aaacCTATCATCTGTGAAGGCAAA	A10 spacer for base editing
	*cymR*vF	ATATTGCGTACTGCCCGAAA	amplification of the verified *cymR* DNA from genome
	*cymR*vR	TGCCATAGTGAAACCTCCTTG	amplification of the verified *cymR* DNA from genome
	*cntA*vF	GCCAGGCGTACAAGGATATG	amplification of the verified *cntA* DNA from genome
	*cntA*vR	TGGAAACATGAGCGCAATAC	amplification of the verified *cntA* DNA from genome
	*sasC*vF	tttcttgttgcgcttgtttg	amplification of the verified *sasC* DNA from genome
	*sasC*vR	gtcaggtgaatcaagcagca	amplification of the verified *sasC* DNA from genome
lacZ gene base editing	m-*lacZ*-S6F	GAAActattacgccagctggcgaa	*lacZ* spacer for its R38 mutation to R
	m-*lacZ*-S6R	AAACttcgccagctggcgtaatag	*lacZ* spacer for its R38 mutation to R
	Kp-*lacZ*-SCvF	tagtCAACAGTTGCGCAG	*lacZ* spacer for its Q51 mutation to

		CCTGAA	stop codon
	Kp-*lacZ*-SCvR	aaacTTCAGGCTGCGCAACTGTTG	*lacZ* spacer for its Q51 mutation to stop codon
	lacZ-CVvF	gaaaCAACTATTGGGAAGGGCGAT	*lacZ* spacer for its stop codon mutation to Q51
	lacZ-CVvR	aaacATCGCCCTTCCCAATAGTTG	*lacZ* spacer for its stop codon mutation to Q51
	*lacZ*vF	attaatgcagctggcacgac	amplification of the verified *lacZ* DNA from genome
	*lacZ*vR	ccgtaatgggataggtcacg	amplification of the verified *lacZ* DNA from genome
yagI gene base editing	m-*yagI*-S6F	GAAAcctcgacctgttcaacgagc	*yagI* spacer for its D15 mutation to G
	m-*yagI*-S6R	AAACgctcgttgaacaggtcgagg	*yagI* spacer for its D15 mutation to G
	*yagI*vF	cggctccatttcattgattt	amplification of the verified *yagI* DNA from genome
	*yagI*vR	tttgccttctgccgaatatc	amplification of the verified *yagI* DNA from genome
cntB gene base editing	*cntB*-SC-s9F	gaaaTATTAATTCAATATAAAAAT	*cntB* spacer for its Q68 mutation to stop codon
	cntB-SC-s9R	aaacATTTTTATATTGAATTAATA	*cntB* spacer for its Q68 mutation to stop codon
	B7-1F	gaaaAAACTTACTACAATCATCAA	*cntB* spacer for editing
	B7-1R	aaacTTGATGATTGTAGTAAGTTT	*cntB* spacer for editing
	B56-3F	gaaaGCGTAATGCTTTACAAGTTG	*cntB* spacer for editing
	B56-3R	aaacCAACTTGTAAAGCATTACGC	*cntB* spacer for editing
	B56-2F	gaaaATTGAAGCTATCCAATATGA	*cntB* spacer for editing
	B56-2R	aaacTCATATTGGATAGCTTCAAT	*cntB* spacer for editing
	B4-2F	gaaaACTAGTCCACCCATTATCAT	*cntB* spacer for editing
	B4-2R	aaacATGATAATGGGTGGACTAGT	*cntB* spacer for editing
	B8-1F	gaaaAAATGTTAGACGCTCGATGG	*cntB* spacer for editing
	B8-1R	aaacCCATCGAGCGTCTAACATTT	*cntB* spacer for editing

	B4578-1F	gaaaAACAATAATTTCAAGTGTTA	*cntB* spacer for editing
	B4578-1R	aaacTAACACTTGAAATTATTGTT	*cntB* spacer for editing
	B7-6F	gaaaTTATCGAGTATATCTTTGCA	*cntB* spacer for editing
	B7-6R	aaacTGCAAAGATATACTCGATAA	*cntB* spacer for editing
	B467-1F	gaaaGTGACAATTTTACATGCACA	*cntB* spacer for editing
	B467-1R	aaacTGTGCATGTAAAATTGTCAC	*cntB* spacer for editing
	B47-1F	gaaaATGATTACATCAATTATTTT	*cntB* spacer for editing
	B47-1R	aaacAAAATAATTGATGTAATCAT	*cntB* spacer for editing
	B7-4F	gaaaGTAGTTAGTGCATTAAAAAG	*cntB* spacer for editing
	B7-4R	aaacCTTTTTAATGCACTAACTAC	*cntB* spacer for editing
	B7-5F	gaaaTTGCCGACTTCTGGATTAAC	*cntB* spacer for editing
	B7-5R	aaacGTTAATCCAGAAGTCGGCAA	*cntB* spacer for editing
	B7-2F	gaaaGAAGCGATGCAATTTAATTT	*cntB* spacer for editing
	B7-2R	aaacAAATTAAATTGCATCGCTTC	*cntB* spacer for editing
	B7-3F	gaaaTTTGGTACAAGCTACATTAC	*cntB* spacer for editing
	B7-3R	aaacGTAATGTAGCTTGTACCAAA	*cntB* spacer for editing
	B78-3F	gaaaAAACTTAATTGACCTAGTCC	*cntB* spacer for editing
	B78-3R	aaacGGACTAGGTCAATTAAGTTT	*cntB* spacer for editing
	B46-2F	gaaaAGCATATGTATTAATTGTAG	*cntB* spacer for editing
	B46-2R	aaacCTACAATTAATACATATGCT	*cntB* spacer for editing
	B568-1F	gaaaGACCAATACGTTCAGCAACT	*cntB* spacer for editing
	B568-1R	aaacAGTTGCTGAACGTATTGGTC	*cntB* spacer for editing

	B78-2F	gaaaACTGGCAATATGTAACTTTC	*cntB* spacer for editing
	B78-2R	aaacGAAAGTTACATATTGCCAGT	*cntB* spacer for editing
	B456-1F	gaaaTCTAAAGTAAATACCAGCAT	*cntB* spacer for editing
	B456-1R	aaacATGCTGGTATTTACTTTAGA	*cntB* spacer for editing
	B46-1F	gaaaTCTATACCAATGATAATGGG	*cntB* spacer for editing
	B46-1R	aaacCCCATTATCATTGGTATAGA	*cntB* spacer for editing
	B47-2F	gaaaGTTATTACGATTGCCTATGC	*cntB* spacer for editing
	B47-2R	aaacGCATAGGCAATCGTAATAAC	*cntB* spacer for editing
	B57-1F	gaaaGAGTATATCTTTGCATGGCC	*cntB* spacer for editing
	B57-1R	aaacGGCCATGCAAAGATATACTC	*cntB* spacer for editing
	B468-1F	gaaaAATACATATGCTTGAATGAC	*cntB* spacer for editing
	B468-1R	aaacGTCATTCAAGCATATGTATT	*cntB* spacer for editing
	B56-1F	gaaaTATTAATTCAATATAAAAAT	*cntB* spacer for editing
	B56-1R	aaacATTTTTATATTGAATTAATA	*cntB* spacer for editing
	B567-1F	gaaaGCAGAAACGAATGAGAAGTA	*cntB* spacer for editing
	B567-1R	aaacTACTTCTCATTCGTTTCTGC	*cntB* spacer for editing
	*cntB*vF	GTATTGCGCTCATGTTTCCA	amplification of the verified *cntB* DNA from genome
	*cntB*vR	TCGCACGATCAGTGAACTTT	amplification of the verified *cntB* DNA from genome
	*cntB*vF(T)	CGGTAAAGAGCGTTCAGACG	amplification of the verified *cntB* DNA from genome
cntC gene base editing	*cntC*-SC-s4F	gaaaAAACGATTATTACAAGATAA	*cntC* spacer for its R6 mutation to stop codon
	cntC-SC-s4R	aaacTTATCTTGTAATAATCGTTT	*cntC* spacer for its R6 mutation to stop codon
	C48-1F	gaaaGGCATTTATTTTGACGCGTT	*cntC* spacer for editing

	C48-1R	aaacAACGCGTCAAAATAAATGCC	*cntC* spacer for editing
	C4-2F	gaaaTCTATTTTAGGATTCTTATC	*cntC* spacer for editing
	C4-2R	aaacGATAAGAATCCTAAAATAGA	*cntC* spacer for editing
	C46-1F	gaaaGATACAGCAAACAAATTTGC	*cntC* spacer for editing
	C46-1R	aaacGCAAATTTGTTTGCTGTATC	*cntC* spacer for editing
	C8-1F	gaaaAAATGCCATGATAATATTTT	*cntC* spacer for editing
	C8-1R	aaacAAAATATTATCATGGCATTT	*cntC* spacer for editing
	C7-1F	gaaaGTACTTATTGGATCTATTTT	*cntC* spacer for editing
	C7-1R	aaacAAAATAGATCCAATAAGTAC	*cntC* spacer for editing
	C47-2F	gaaaATAATCACTATGGCAATACC	*cntC* spacer for editing
	C47-2R	aaacGGTATTGCCATAGTGATTAT	*cntC* spacer for editing
	C46-4F	gaaaGCAATATCTGCTAATGTTAA	*cntC* spacer for editing
	C46-4R	aaacTTAACATTAGCAGATATTGC	*cntC* spacer for editing
	C57-1F	gaaaTGCCATAGTGATTATAGTGA	*cntC* spacer for editing
	C57-1R	aaacTCACTATAATCACTATGGCA	*cntC* spacer for editing
	C46-2F	gaaaATCACACGCACGCATGATTA	*cntC* spacer for editing
	C46-2R	aaacTAATCATGCGTGCGTGTGAT	*cntC* spacer for editing
	C47-1F	gaaaTCAATGATCTTGCAAATATC	*cntC* spacer for editing
	C47-1R	aaacGATATTTGCAAGATCATTGA	*cntC* spacer for editing
	C68-2F	gaaaTAGGTAGAGATATTTTAACT	*cntC* spacer for editing
	C68-2R	aaacAGTTAAAATATCTCTACCTA	*cntC* spacer for editing
	C457-1F	gaaaACAAAGACATATAACAAACT	*cntC* spacer for editing

	C457-1R	aaacAGTTTGTTATATGTCTTTGT	*cntC* spacer for editing
	C58-1F	gaaaTGCCATCACTATAATCACTA	*cntC* spacer for editing
	C58-1R	aaacTAGTGATTATAGTGATGGCA	*cntC* spacer for editing
	C68-1F	gaaaGGATCATAAAATGTCACAAG	*cntC* spacer for editing
	C68-1R	aaacCTTGTGACATTTTATGATCC	*cntC* spacer for editing
	*CntC*vF	TATCTTTGCATGGCCTGGAC	amplification of the verified *cntC* DNA from genome
	*CntC*vR	TGAAAACTCATGCCAGCAAA	amplification of the verified *cntC* DNA from genome
	*CntC*vR(T)	ACGAGTGGTTGATCTGTCCA	amplification of the verified *cntC* DNA from genome
CntB V19 mutation to S	sp-B7-1-2F	gaaaCTGAACGCTCTTTACCGTTT	*cntB* spacer for point mutation
	sp-B7-1-2R	aaacAAACGGTAAAGAGCGTTCAG	*cntB* spacer for point mutation
	SAupB71F	tttgagatctgtccatacccatggTCTAGACACGGTTGAATTGAATTTGAAAGAAGC	amplification of ~1kb *cntB* upstream for V19 mutation to S
	B71upR	TTTACCGTTTTGTATTTTAAATGCGT	amplification of ~1kb *cntB* upstream for V19 mutation to S
	B71dnF	ACGCATTTAAAATACAAAACGGTAAA	amplification of ~1kb *cntB* downstream for V19 mutation to S
	SAdnB71R	aagatacaggtatatttttctgaCTCGAGAATCGTGTTCAAGTATTGCTTTTAAACTT	amplification of ~1kb *cntB* downstream for V19 mutation to S
	V19SF	TGTTTCCATTGATGATTGTATCAAGTTTTATGACATTTCTAT	primer for point mutation V19 mutation to S
	V19SR	AACTTGATACAATCATCAATGGAAACATGAGCGCAAT	primer for point mutation V19 mutation to S

CntB E253 mutation to A	B76tempF	CAACTTTGTACAAAAAA GCAGGCTCATTTTGAAAC AAATTGATGATGAAGGTA	amplification of ~1kb *cntB* upstream for E253 mutation to A
	B76upR-neo	GTCCACCCATTATCATTG GTATAGACATACAAAAGA TTGATACCGCAACTTGTA AAGCATTACGC	amplification of ~1kb *cntB* upstream for E253 mutation to A
	B76dnF-neo	CCAATGATAATGGGTGGA CTAGTTGTTATCGAGTAT	amplification of ~1kb *cntB* downstream for E253 mutation to A
	B76tempR	TGCCAACTTTGTACAAAA AAGCTGCCAGGTATCTGT AATCGTCAAATGTT	amplification of ~1kb *cntB* downstream for E253 mutation to A
	pKOR1F	AACATTTGACGATTACAG ATACCTGGCAGCTTTTTT GTACAAAGTTGGCA	amplification of ~8kb pKOR1 backbone
	pKOR1R	TACCTTCATCATCAATTTG TTTCAAAATGAGCCTGCT TTTTTGTACAAAGTTG	amplification of ~8kb pKOR1 backbone
CntC C113 mutation to A	C46-2F	gaaaATCACACGCACGCA TGATTA	*cntC* spacer for point mutation
	C46-2R	aaacTAATCATGCGTGCGT GTGAT	*cntC* spacer for point mutation
	SAupC462F	tttgagatctgtccatacccatgg TCTAGAGACCCAGTTGCT GAACGTATTG	amplification of ~1kb *cntC* upstream for C113 mutation to A
	C462upR	ACATCAGCCGCACGCATG ATTAATGCGTCAA	amplification of ~1kb *cntC* upstream for C113 mutation to A
	C462dnF	TTGACGCATTAATCATGC GTGCGGCTGATGT	amplification of ~1kb *cntC* downstream for C113 mutation to A
	SAdnC462R	aagatacaggtatatttttctga CTCGAGACATTCCTCCTG ATAACATGTAAGGG	amplification of ~1kb *cntC* downstream for C113 mutation to A
CntC F143 mutation to A	C8-1F	gaaaAAATGCCATGATAAT ATTTT	*cntC* spacer for point mutation
	C8-1R	aaacAAAATATTATCATGG CATTT	*cntC* spacer for point mutation
	SAupC81F	tttgagatctgtccatacccatgg TCTAGACATCAATTATTTT AGGTGTAGTTAGTGCA	amplification of ~1kb *cntC* upstream for F143 mutation to A
	C81upR	TCAAAATAGCTGCCATGA TAATATTTTCCGCACC	amplification of ~1kb *cntC* upstream for F143 mutation to A
	C81dnF	GGTGCGGAAAATATTATC	amplification of ~1kb *cntC*

		ATGGCAGCTATTTTGA	downstream for F143 mutation to A
	SAdnC81R	aagatacaggtatatttttctga CTCGAGTAATTGTATCTA AAGCCGTTGTCGG	amplification of ~1kb *cntC* downstream for F143 mutation to A
High-throughput sequencing	C1-cntA8WT-F	AGCAGATACGGTGGATC AATGTCTGC	amplification of 183bp DNA fragment of *cntA* (WT) for high-throughput sequencing
	C2-cntA8WT-F	AGCAGGTACGGTGGATC AATGTCTGC	amplification of 183bp DNA fragment of *cntA* (WT) for high-throughput sequencing
	C3-cntA8WT-F	AGCAGCTACGGTGGATC AATGTCTGC	amplification of 183bp DNA fragment of *cntA* (WT) for high-throughput sequencing
	C-cntA8WT-R	AACTGCGTCAGCATCAAA TG	amplification of 183bp DNA fragment of *cntA* (WT) for high-throughput sequencing
	D1-cntA8-F	CATTCATACGGTGGATCA ATGTCTGC	amplification of 183bp DNA fragment of *cntA* (edited) for high-throughput sequencing
	D2-cntA8-F	CATTCGTACGGTGGATCA ATGTCTGC	amplification of 183bp DNA fragment of *cntA* (edited) for high-throughput sequencing
	D3-cntA8-F	CATTCCTACGGTGGATCA ATGTCTGC	amplification of 183bp DNA fragment of *cntA* (edited) for high-throughput sequencing
	D-cntA8-R	AACTGCGTCAGCATCAAA TG	amplification of 183bp DNA fragment of *cntA* (edited) for high-throughput sequencing
	G1-murR6WT-F	GCATCATGTTGAAAAATG CCAGGACA	amplification of 235bp DNA fragment of *murR* (WT) for high-throughput sequencing
	G2-murR6WT-F	GCATCGTGTTGAAAAATG CCAGGACA	amplification of 235bp DNA fragment of *murR* (WT) for high-throughput sequencing
	G3-murR6WT-F	GCATCCTGTTGAAAAATG CCAGGACA	amplification of 235bp DNA fragment of *murR* (WT) for high-throughput sequencing
	G-murR6WT-R	CTGTAATGTGTGGCCACC TG	amplification of 235bp DNA fragment of *murR* (WT) for high-throughput sequencing
	H1-murR6-F	ATTGGATGTTGAAAAATG CCAGGACA	amplification of 235bp DNA fragment of *murR* (edited) for high-throughput sequencing
	H2-murR6-F	ATTGGGTGTTGAAAAAT	amplification of 235bp DNA

		GCCAGGACA	fragment of *murR* (edited) for high-throughput sequencing
	H3-murR6-F	ATTGGCTGTTGAAAAATGCCAGGACA	amplification of 235bp DNA fragment of *murR* (edited) for high-throughput sequencing
	H-murR6-R	CTGTAATGTGTGGCCACCTG	amplification of 235bp DNA fragment of *murR* (edited) for high-throughput sequencing
	I1-murR3WT-F	CACATATATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (WT) for high-throughput sequencing
	I2-murR3WT-F	CACATGTATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (WT) for high-throughput sequencing
	I3-murR3WT-F	CACATCTATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (WT) for high-throughput sequencing
	I-murR3WT-R	CTATCATCATGCGTCGCAAA	amplification of 189bp DNA fragment of *murR* (WT) for high-throughput sequencing
	J1-murR3-F	GTTCAATATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (edited) for high-throughput sequencing
	J2-murR3-F	GTTCAGTATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (edited) for high-throughput sequencing
	J3-murR3-F	GTTCACTATGGATGCGCAAATTGATG	amplification of 189bp DNA fragment of *murR* (edited) for high-throughput sequencing
	J-MurR3-R	CTATCATCATGCGTCGCAAA	amplification of 189bp DNA fragment of *murR* (edited) for high-throughput sequencing
	K1-cntC57WT-F	AGATTAAGGTGTCAAAGCGCCTACTG	amplification of 220bp DNA fragment of *cntC* (WT) for high-throughput sequencing
	K2-cntC57WT-F	AGATTGAGGTGTCAAAGCGCCTACTG	amplification of 220bp DNA fragment of *cntC* (WT) for high-throughput sequencing
	K3-cntC57WT-F	AGATTCAGGTGTCAAAGCGCCTACTG	amplification of 220bp DNA fragment of *cntC* (WT) for high-throughput sequencing
	K-cntC57WT-R	TCATGATTGCACCACTCCTT	amplification of 220bp DNA fragment of *cntC* (WT) for high-throughput sequencing

	K1-cntC57-F	CCAATAAGGTGTCAAAG CGCCTACTG	amplification of 220bp DNA fragment of *cntC* (edited) for high-throughput sequencing
	K2-cntC57-F	CCAATGAGGTGTCAAAG CGCCTACTG	amplification of 220bp DNA fragment of *cntC* (edited) for high-throughput sequencing
	K3-cntC57-F	CCAATCAGGTGTCAAAG CGCCTACTG	amplification of 220bp DNA fragment of *cntC* (edited) for high-throughput sequencing
	K-cntC57-R	TCATGATTGCACCACTCC TT	amplification of 220bp DNA fragment of *cntC* (edited) for high-throughput sequencing

4 Supporting References

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