Supporting Information for

Rapid and Efficient Genome Editing in *Staphylococcus aureus* by Using an Engineered CRISPR/Cas9 System

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Detailed protocol for genome editing in *S. aureus*

All the pCasSA plasmids for gene deletion, single-base substitution mutation and gene insertion were assembled using the following protocol:

(1). Oligo Design

Select a 20 bp-spacer sequence before NGG (NGG is not included in the spacer) in the target gene of *S. aureus* (40%~60% GC ratio is the best). Synthesize the two oligos in the following form:

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5′- GAAANNNNNNNNNNNNNNNNNNNN -3′
     3′-NNNNNNNNNNNNNNNNNNNNCAAA -5′
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(2). Phosphorylation

2 μl	oligo I (50 μM)
2 μl	oligo II (50 μM)
5 μl	10x T4 DNA ligase buffer (NEB)
1 μl	T4 polynucleotide kinase (Takara)
40 μl	ddH_2O
50 μl	

Incubate at 37 ^{o}C for 1 hour.

(3). Annealing

Add 2.5 μl of 1 M NaCl to the phosphorylated oligo pairs.

Incubate at 95 ^{o}C for 3 min and slowly cool down to room temperature (use a thermocycler).

(Alternatively, use a heat block and take the block out of the heater and let it cool naturally for 2 hours.)

Dilute the annealed oligos 20 times using ddH_2O.

(4). Golden gate assembly

xx μl	20 fmol pCasSA plasmid
1 μl	diluted annealed oligos (100 fmol)
1 μl	10x T4 DNA ligase buffer (NEB)
0.5 μl	T4 DNA ligase (NEB)
0.5 μl	BsaI-HF (NEB)
xx μl	ddH_2O to 10 μl
10 μl	

37 ^{o}C	2 min	} 25 cycles
16 ^{o}C	5 min	
50 ^{o}C	5 min	
80 ^{o}C	15 min	
10 ^{o}C	for ever	

(5).Transformation

10 μl product of Golden Gate assembly was transformed into 100 μl *E.coli* DH10B competent cells. The successful colonies were selected on a LB agar plate containing 50 μg/ml kanamycin. The success for the construction of the pCasSA-NN_spacer plasmid was verified by PCR or sequencing.

(6). Digest the constructed plasmid with XbaI and XhoI

5 μl	10x M buffer (Takara)
xx μl	2 μg constructed plasmid pCasSA-NN_spacer
5 μl	10x BSA (Takara)
2 μl	XbaI (Takara)
xx μl	ddH_2O to 50 μl
50 μl	

Incubate the digestion solution at 37 ^{o}C for 2 hours. Add 5 μl 10x H buffer (Takara) and 2 μl XhoI (Takara) in the digestion solution and incubate at 37 ^{o}C for another 2~3 hours. The digested plasmid was purified by using the SanPrep PCR purification kit (Sangon Biotech, Shanghai, China).

(7). Amplify the upstream and downstream of the target gene

Select ~1 kb DNA sequence of the upstream and downstream of the target gene, respectively. Design the primers as following:

5’ primer of the upstream is in this form:

5’- tttgagatctgtccatacccatggTCTAGANNNNNNNNNNNNNNNNNNNN -3’

3’ primer of the downstream is in this form:

5’- AAGATACAGGTATATTTTTCTGACTCGAGNNNNNNNNNNNNNNNNNNNNN -3’

3’ primer of the upstream and 5’ primer of the downstream should have 30~40 bp overlap.

(For example, the primers to amplify the upstream and downstream fragements of the target gene are:

Upstream 5’F: 5’- NNNNNNNNNNNNNNNNNNN -3’

Upstream 3’R: 5’- TTTTTTTTTTTTTTTTTTTTTTT -3’

Downstream 5’F: 5’- GGGGGGGGGGGGGGGGGGG -3’

Downstream 3’R: 5’- DDDDDDDDDDDDDDDDDDD -3’

Then the primers should be designed as following:

Upstream 5’F: 5’- **tttgagatctgtccatacccatggTCTAGA**NNNNNNNNNNNNNNNNNNN -3’

Upstream 3’R: 5’- **CCCCCCCCCCCCCCCCCCCCC**TTTTTTTTTTTTTTTTTTTTTTT -3’

Downstream 5’F: 5’- **AAAAAAAAAAAAAAAAAAA**GGGGGGGGGGGGGGGGGGG -3’

Downstream 3’R: 5’- **AAGATACAGGTATATTTTTCTGACTCGAG**DDDDDDDDDDDDDDDDDDD -3’)

Amplify the upstream and downstream of the target gene from the *S.aureus* genomic DNA by PCR. Purify the PCR products with the SanPrep PCR purification kit (Sangon Biotech, Shanghai, China).

(For the gene insertion, the *rfp* gene was amplified from the pET28a(gg)-His-TEV-RFP plasmid. 3’ primer and 5’ primer of the *rfp* gene have 20~30 bp overlap with the upstream and the downstream of the target gene, respectively.)

(8). Gibson assembly

15 μl	Gibson assembly mixture
xx μl	20 fmol XbaI/XhoI digested pCasSA-NN_spacer plasmid
xx μl	20 fmol upstream of the target gene
xx μl	20 fmol downstream of the target gene
xx μl	ddH_2O to 20 μl
20 μl	

Incubate at 50 ^{o}C for 1 hour.

(For the gene insertion, external 20 fmol DNA fragment of the *rfp* gene was added into the reaction solution.)

20 μl Gibson assembly product was transformed into 100 μl DH10B competent cells. The successful colonies were selected on a LB agar plate containing 50 μg/ml kanamycin. The success for the construction of the plasmid pCasSA-NN was verified by PCR or sequencing.

(9). Genome editing in the RN4220 strain

Transform the pCasSA-NN plasmid into the RN4220 strain using electroporation. The colonies were selected on a TSB agar plate containing 5 μg/ml chloramphenicol. Pick colonies from the plate and extract their genomic DNA using the Ezup column bacteria genomic DNA purification kit (Sangon Biotech, Shanghai, China). The successful colonies were verified by PCR screening using the genomic DNA as template, and further confirmed by sequencing. If the target gene does not exist in the RN4220 genome, the plasmids could be extracted directly from the RN4220 strain without verification.

(10.1). Genome editing in the Newman strain

Extract the plasmid from the RN4220 strain that contains the desired mutation, and transform it into the Newman strain by electroporation (it is better to use fresh competent cells). The cells were plated on a TSB agar plate containing 10 μg/ml chloramphenicol. After incubated at 30 ^{o}C overnight, the colonies were grown in TSB containing 10 μg/ml chloramphenicol for genomic DNA extraction. The genome editing efficiency was verified by PCR or sequencing using the genomic DNAs as the PCR templates.

(10.2). Genome editing in the USA300 strain

Because plasmid transformation efficiency by electroporation in the USA300 strain is very low, we used phage transduction to transduce the pCasSA plasmid into the USA300 strain. The colony from the transformed and confirmed RN4220 mutant was inoculated in TSB containing 10 μg/ml chloramphenicol and grown at 30 °C overnight. The overnight cultured cells were transferred into fresh HIB (heart infusion media) containing 5 mM $CaCl_2$ and incubated at 30 °C until cell OD_{600}=0.5, followed by the addition of phage 85. The cell lysates were collected and filtrated to isolate the recombinant phage 85. Then, the plasmids were transduced into the USA300 strain using the phage 85 and the resulting cells were plated on a TSB agar plate containing 10 μg/ml of chloramphenicol at 30 °C. After two days, 10 random colonies were incubated in TSB containing 10 μg/ml of chloramphenicol at 30 °C and 50 μl of the cells was resuspended in TE buffer and genomic DNA was extracted by the addition of lysostaphin (0.1 μg/ml), followed by 10 min incubation at a 37 °C heat block. Finally, the genome editing was confirmed by PCR using the isolated genomic DNA and further verified by DNA sequencing.

(11). Plasmid curing

Pick a colony of the confirmed *S. aureus* mutant that containis the pCasSA plasmid and incubate it in TSB at 30 ^{o}C overnight. The next day, 3 µl culture was diluted 1:1000 in 3 ml TSB, and incubated at 42 ^{o}C until the culture was evident. Then a fraction of the culture was streaked onto a TSB agar plate and incubated at 37 ^{o}C overnight. The curing of the pCasSA plasmid was confirmed by streaking the colonies on TSB agar plates in the presence or absence of chloramphenicol (5 µg/ml).

Figure S1. *agrA*-spacer introduced pCasSA (pCasSA-*agrA*_spacer) efficiently killed the *S. aureus* RN4220 strain. An empty pCasSA plasmid was transformed into the RN4220 strain as a control (left). The *agrA*-spacer introduced pCasSA plasmid was transformed into the RN4220 strain (right).

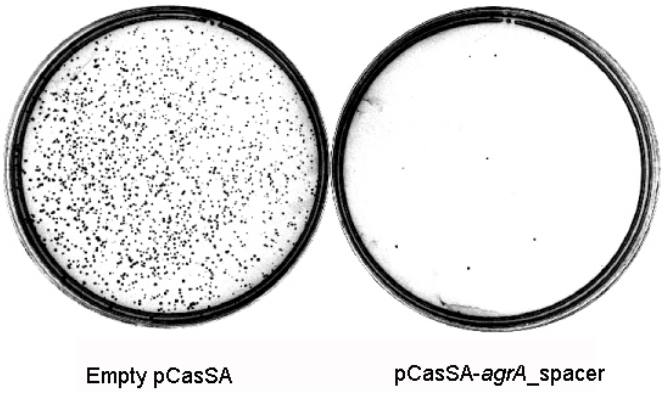

Figure S2. pCasSA-mediated disruption of *cymR* gene in the RN4220 strain. The editing efficiency is 4/12. The lane of ck is the PCR product from the wild-type strain as a control.

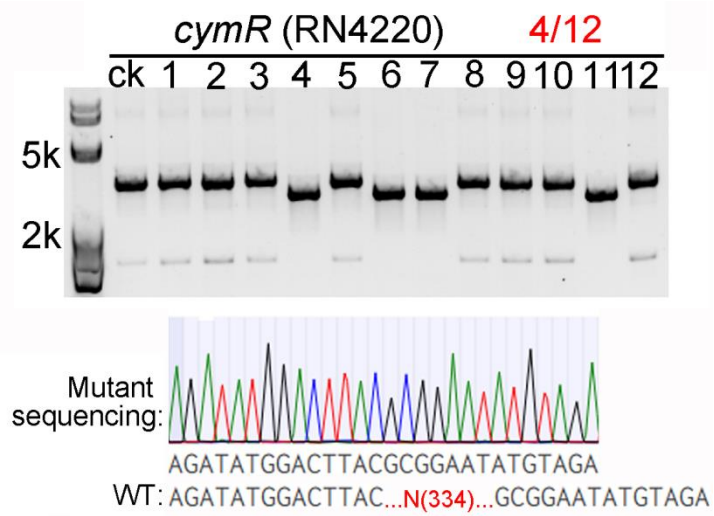

Figure S3. The pCasSA plasmid can be easily cured after editing. Four individual colonies obtained after the curing steps were streaked onto the TSB agar plates in the presence (right) or absence (left) of chloramphenicol (Cm) to test the effectiveness of curing.

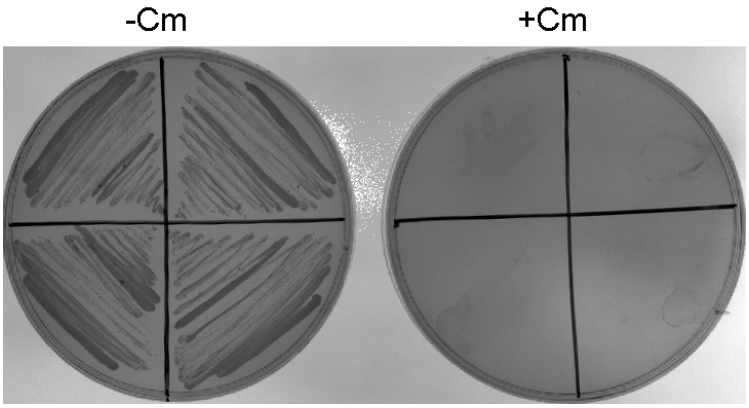

Figure S4. A representative sequencing chromatogram of the *sae* (*saeR* and *saeS*) sites of five RN4220 *agrA* mutants. No additional mutations were observed for all the samples tested.

Figure S5. pCasSA-mediated gene deletion in the *S. aureus* Newman strain. (a) pCasSA-mediated disruption of *spa* gene in the Newman strain. The editing efficiency is 7/10. The lane of ck is the PCR product from the wild-type strain as a control. (b) pCasSA-mediated disruption of *murR* gene in the Newman strain. The editing efficiency is 4/10. (c) pCasSA-mediated disruption of *cymR* gene in the USA300 strain. The editing efficiency is 10/10.

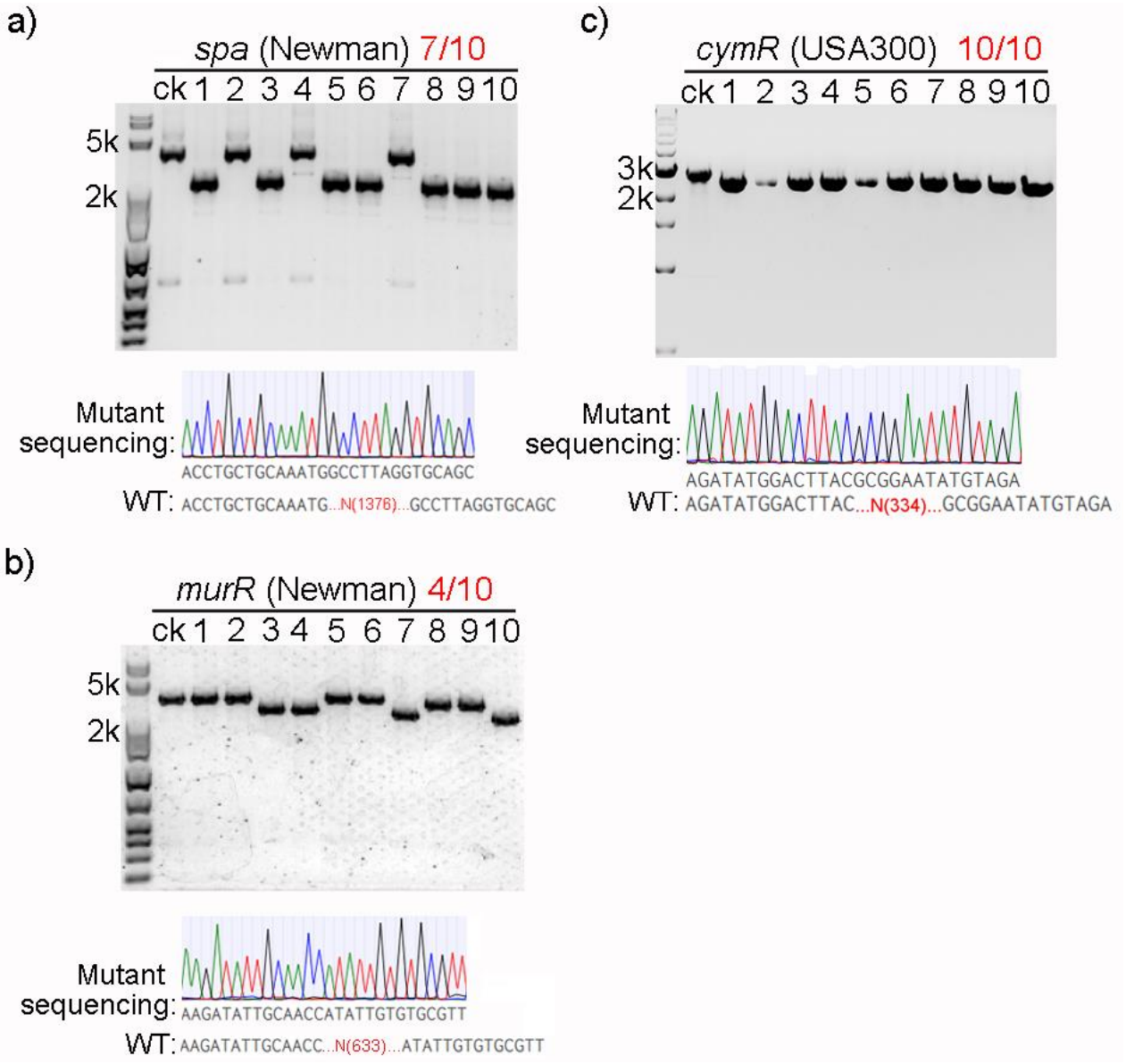

Figure S6. CRISPR/Cas9-mediated single-base substitution mutation and gene insertion in the *S. aureus* RN4220 strain. (a) pCasSA enables highly efficient single-base substitution mutation in the RN4220 strain. A pre-mature stop codon is introduced the *agrA* gene. The mutation site is colored red. The mutation efficiency is 12/12, confirmed by sequencing. (b) pCasSA enables efficient gene insertion in the RN4220 strain. The blue arrows are the primers used for PCR validation. The editing efficiency is 5/9.

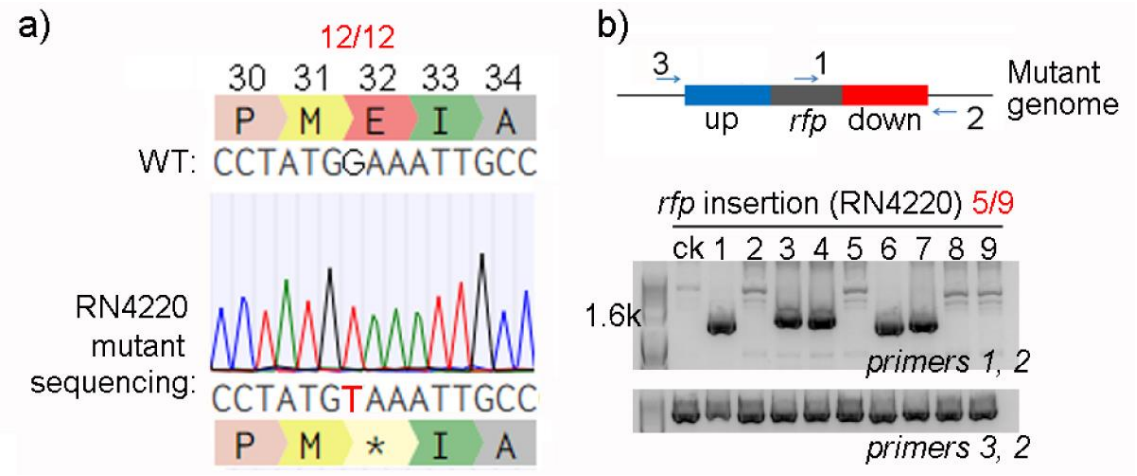

Table S1. Bacterial strains and plasmids used in this study

Plasmids or strains	Description	Reference
Plasmids		
pKOR1	Allelic replacement vector, Ap^r, Cm^r	[1]
pCas9	Bacterial expression of Cas9 nuclease, tracrRNA and crRNA guide	[2]
pCRISPR	A crRNA expression plasmid for targeting a specific sequence	[2]
pET28a(gg)-His-TEV-RFP	Plasmid carrying the *rfp* gene, Km^r	Lab stock
pCasSA	*S.aureus* genome editing vector, Km^r, Cm^r	This study
pCasSA-*agrA*_spacer	pCasSA derivative with *agrA* spacer	This study
pCasSA-*agrA*	pCasSA derivative for *agrA* deletion	This study
pCasSA-*cntA*	pCasSA derivative for *cntA* deletion	This study
pCasSA-*cymR*	pCasSA derivative for *cymR* deletion	This study
pCasSA-*murR*	pCasSA derivative for *murR* deletion	This study
pCasSA-*spa*	pCasSA derivative for *spa* deletion	This study
pCasSA-*agr*Am	pCasSA derivative for *agra* 32E mutation to stop codon	This study
pCasSA-*agrA*-RFP	pCasSA derivative for *agrA*::*rfp*	This study
pCasiSA	*S.aureus* transcription inhibition vector, Km^r, Cm^r	This study
pCasiSA-*agrA*	pCasiSA derivative with *agrA* spacer	This study
pCasiSA-*sasE*	pCasiSA derivative with *sasE* spacer	This study
Strains		
E.coli		
DH10B	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
S. aureus		
RN4220	Restriction-deficient transformation recipient	Lab stock
RN4220*agrA*	RN4220Δ*agrA*	This study
RN4220*cntA*	RN4220Δ*cntA*	This study
RN4220*cymR*	RN4220Δ*cymR*	This study
RN4220*murR*	RN4220Δ*murR*	This study
RN4220*agr*Am	RN4220 *agra* 32E mutation to stop codon	This study
RN4220*agra*RFP	RN4220 *agra*::*rfp*	This study
Newman	Wild type	Lab stock
Newman*agrA*	NewmanΔ*agrA*	This study
Newman*cntA*	NewmanΔ*cntA*	This study
Newman*cymR*	NewmanΔ*cymR*	This study
Newman*murR*	NewmanΔ*murR*	This study
Newman*spa*	NewmanΔ*spa*	This study
Newman*agr*Am	Newman *agra* 32E mutation to stop codon	This study
Newman*agra*RFP	Newman *agra*::*rfp*	This study
NewmanpCasi	Newman carrying pCasiSA empty plasmid	This study
NewmanpCasi-*agrA*	Newman carrying pCasiSA-*agrA*	This study
NewmanpCasi-*sasE*	Newman carrying pCasiSA-*sasE*	This study
USA300	Wild type	Lab stock
USA300*agrA*	USA300Δ*agrA*	This study
USA300*cymR*	USA300Δ*cymR*	This study
USA300*murR*	USA300Δ*murR*	This study

Ap^r, ampicillin resistant; Km^r, kanamycin resistant; Cm^r, chloramphenicol resistant.

Table S2. Primers used in this study

	Name	Sequence (5'-3')	Description
pCasSA plasmid construction	pCasrpslF	GACCAGAGGTCTCCACATACCCATGGTCTAGAATGC TCGAGtcagaaaaatatacctgtatctttttttcaaaagc	amplification of the *rpsL* promoter from Newman genome
	pCasrpslR	GACCAGAGGTCTCCgtgatatgtcctcctctcttc	amplification of the *rpsL* promoter from Newman genome
	pCasCas9F	GACCAGAGGTCTCGTCACatggataagaaatactcaataggc	amplification of the *Cas9* gene from pCas9 plasmid
	pCasCas9R	GACCAGAGGTCTCGCGTCGGGAAAACTGTCCATAC CCATGGGTATTTACCACAACAGTACGCCAACCAGCC Atcagtcacctcctagctgactc	amplification of the *Cas9* gene from plasmid pCas9
	pCasSARepF	GACCAGAGGTCTCGgacgtctaagaaaccattattatca	amplification of *S. aureus repF* origin from plasmid pKOR1
	pCasSARepR	GACCAGAGGTCTCGggtaaccaagataacaaagaataca	amplification of *S. aureus repF* origin from plasmid pKOR1
	pCasSACmF	GACCAGAGGTCTCGTACCcgtcttcttaatatgcgtaattg	amplification of chloramphenicol -resistance marker from plasmid pKOR1
	pCasSACmR	GACCAGAGGTCTCGCgatatccccgtatagtgagtc	amplification of chloramphenicol -resistance marker from plasmid pKOR1
	pCasKmColEF	GACCAGAGGTCTCCATCgtcggaattgccagctgg	amplification of the origin ColE1 and kanamycin-resistance marker from plasmid pCRISPR
	pCasKmColER	GACCAGAGGTCTCCATGTCGACaacgcaggaaagaacat gtgagc	amplification of the origin ColE1 and kanamycin-resistance marker from plasmid pCRISPR
agrA gene deletion	agrAspacerF	GAAAtgtctacaaagttgcagcga	*agrA* spacer for gene deletion
	agrAspacerR	AAACtcgctgcaactttgtagaca	*agrA* spacer for gene deletion
	agrAUPF	TTTGAGATCTGTCCATACCCATGGTCTAGAcacaaataa actcggatgaagc	amplification of ~1kb *agrA* upstream for gene deletion
	agrAUPR	gttaactgactttattatcttaACATTCACATCCTTATGGCT	amplification of ~1kb *agrA* upstream for gene deletion
	agrADNF	AGCCATAAGGATGTGAATGTtaagataataaagtcagttaacg gc	amplification of ~1kb *agrA* downstream for gene deletion
	agrADNR	AAGATACAGGTATATTTTTCTGACTCGAGccattatgggat aacgctgaa	amplification of ~1kb *agrA* downstream for gene deletion
	agrAVF	ATGTTTGATAGCGCGTCCTT	amplification of *agrA* locus from genome
	agrAVR	AGTCCGATGAGAGATGCACA	amplification of *agrA* locus from genome
	agrAsqF	GGTGAAGGTCGTGGTTTAGG	sequencing of *agrA* locus
cntA gene deletion	cntAspacerF	GAAAcaagcaaaagtaatgcctgc	*cntA* spacer for gene deletion
	cntAspacerR	AAACgcaggcattacttttgcttg	*cntA* spacer for gene deletion
	cntAUPF	TTTGAGATCTGTCCATACCCATGGTCTAGAacatgggat cgacacattca	amplification of ~1kb *cntA* upstream for gene deletion
	cntAUPR	ttctcaatgcctgatgttgcTTGCTTTTCCTCTTTCTAAATTGAT AAGTTG	amplification of ~1kb *cntA* upstream for gene deletion
	cntADNF	CAACTTATCAATTTAGAAAGAGGAAAAGCAAgcaacat caggcattgagaa	amplification of ~1kb *cntA* downstream for gene deletion
	cntADNR	AAGATACAGGTATATTTTTCTGACTCGAGctagtccaggc catgcaaag	amplification of ~1kb *cntA* downstream for gene deletion
	cntAVF	TTGGTTCGCAAATGATTGTC	amplification of *cntA* locus from genome
	cntAVR	GCCCTCCCTTAATCTTGGAT	amplification of *cntA* locus from genome
	cntAsqF	GCCAGGCGTACAAGGATATG	sequencing of *cntA* locus
cymR gene deletion	cymRspacerF	GAAAcgaagtgtacgcggtgctaa	*cymR* spacer for gene deletion
	cymRspacerR	AAACttagcaccgcgtacacttcg	*cymR* spacer for gene deletion
	cymRUPF	TTTGAGATCTGTCCATACCCATGGTCTAGAatcaatggc actcattgctg	amplification of ~1kb *cymR* upstream for gene deletion
	cymRUPR	tcttcacttgtatctacatattccgcGTAAGTCCATATCTCCCTTTA GTAG	amplification of ~1kb *cymR* upstream for gene deletion
	cymRDNF	CTACTAAAGGGAGATATGGACTTACgcggaatatgtagata caagtgaaga	amplification of ~1kb *cymR* downstream for gene deletion
	cymRDNR	AAGATACAGGTATATTTTTCTGACTCGAGagggcttccat atgtgttcg	amplification of ~1kb *cymR* downstream for gene deletion
	cymRVF	CGGCCTAGATCTTTAGCACCT	amplification of *cymR* locus from genome
	cymRVR	TGTTCGTGCTTATCCAGCAG	amplification of *cymR* locus from genome
	cymRsqF	ATATTGCGTACTGCCCGAAA	sequencing of *cymR* locus
murR gene deletion	murRspacerF	GAAAattgcagtcaattgcacagg	*murR* spacer for gene deletion
	murRspacerR	AAACcctgtgcaattgactgcaat	*murR* spacer for gene deletion
	murRUPF	TTTGAGATCTGTCCATACCCATGGTCTAGAagaaccatt gcaacctggac	amplification of ~1kb *murR* upstream for gene deletion
	murRUPR	ctaactttatgttagaacgcacacaatatGGTTGCAATATCTTCG GGTAAG	amplification of ~1kb *murR* upstream for gene deletion
	murRDNF	CTTACCCGAAGATATTGCAACCatattgtgtgcgttctaacata aagttag	amplification of ~1kb *murR* downstream for gene deletion
	murRDNR	AAGATACAGGTATATTTTTCTGACTCGAGgacaaggggt agtccgttga	amplification of ~1kb *murR* downstream for gene deletion
	murRVF	GGTGCTTGGATTACGCAACT	amplification of *murR* locus from genome
	murRVR	TCGCGTTTCAACACCATTAC	amplification of *murR* locus from genome

	murRsqF	AAAAGGTGCATTGCCAGTTG	sequencing of *murR* locus
spa gene deletion	spaspacerF	GAAAtgaatctcaagcaccgaaag	*spa* spacer for gene deletion
	spaspacerR	AAACctttcggtgcttgagattca	*spa* spacer for gene deletion
	spaUPF	TTTGAGATCTGTCCATACCCATGGTCTAGAcagcaagaaaacacacttcca	amplification of ~1kb *spa* upstream for gene deletion
	spaUPR	aataacgctgcacctaaggcCATTTGCAGCAGGTGTTACG	amplification of ~1kb *spa* upstream for gene deletion
	spaDNF	CGTAACACCTGCTGCAAATGgccttaggtgcagcgttatt	amplification of ~1kb *spa* downstream for gene deletion
	spaDNR	AAGATACAGGTATATTTTTCTGACTCGAGtggagtgcaccattcttcaa	amplification of ~1kb *spa* downstream for gene deletion
	spaVF	AAAAAGTCAAGCCTGAAGTCG	amplification of *spa* locus from genome
	spaVR	TCTTTGCTTGGAGTCCGTTC	amplification of *spa* locus from genome
	spasqF	CACATTCAAAGCCCCACTTT	sequencing of *spa* locus
agrA gene single-base mutation	agrAmspacerF	GAAAaatgatagaagaaaagccta	*agrA* spacer for single-base mutation
	agrAmspacerR	AAACtaggctttttcttctatcatt	*agrA* spacer for single-base mutation
	agrAUPF	TTTGAGATCTGTCCATACCCATGGTCTAGA cacaaataaactcggatgaagc	amplification of ~1kb *agrA* upstream for single-base mutation
	agrAmUPR	GATTATCAGTTGCGAGGGCAATTTACATAGGCTTTTCTTCTATCATTATATAATTTTTA	amplification of ~1kb *agrA* upstream for single-base mutation
	agrAmDNF	TAAAAATTATATAATGATAGAAGAAAAGCCTATGTAAATTGCCCTCGCAACTGATAATC	amplification of ~1kb *agrA* downstream for single-base mutation
	agrADNR	AAGATACAGGTATATTTTTCTGACTCGAGccattatgggataacgctgaa	amplification of ~1kb *agrA* downstream for single-base mutation
	agrAVF	ATGTTTGATAGCGCGTCCTT	amplification of *agrA* locus from genome
	agrAVR	AGTCCGATGAGAGATGCACA	amplification of *agrA* locus from genome
	agrAmsqF	GGTGAAGGTCGTGGTTTAGG	sequencing of *agrA* mutation site
rfp gene insertion in *agra* locus	agrAspacerF	GAAAtgtctacaaagttgcagcga	*agrA* spacer for gene insertion
	agrA spacerR	AAACtcgctgcaactttgtagaca	*agrA* spacer for gene insertion
	agrAUPF	TTTGAGATCTGTCCATACCCATGGTCTAGA cacaaataaactcggatgaagc	amplification of ~1kb *agrA* upstream for gene insertion
	agrAUpR2	aacgtcttcgctactcgccatACATTCACATCCTTATGGCT	amplification of ~1kb *agrA* upstream for gene insertion
	agrARFPF	AGCCATAAGGATGTGAATGTatggcgagtagcgaagacgtt	amplification o fthe *rfp* gene
	agrARFPR	GCCGTTAACTGACTTTATTATCTTAttaagcaccggtggagtgacgaccttc	amplification of the *rfp* gene
	agrADNF2	gaaggtcgtcactccaccggtgcttaaTAAGATAATAAAGTCAGTTAACGGC	amplification of ~1kb *agrA* downstream for gene insertion
	agrADNR	AAGATACAGGTATATTTTTCTGACTCGAGccattatgggataacgctgaa	amplification of ~1kb *agrA* downstream for gene insertion
	agrAVF	ATGTTTGATAGCGCGTCCTT	amplification of *agrA* locus from genome
	agrAVR	AGTCCGATGAGAGATGCACA	amplification of *agrA* locus from genome
	agrAsqVF	GGTGAAGGTCGTGGTTTAGG	sequencing of *agrA* locus
	agrAsqVR	AAAATTGCGCCATAGGATTG	sequencing of *agrA* locus
	agrARFPVF	actgcgtggtaccaacttcc	PCR verification of the *rfp* gene insertion
pCasiSA plasmid construction	pCasiF1	CATGGATAAGAAATACTCAATAGGCTTAgctATCGGCACAAATAGCGTCGG	amplification of pCasSA fragment with mutation site
	pCasiR1	GTCTTTAAGGAAACTTTGTGGAACAATggcATCGACATCATAATCACTTAAACG	amplification of pCasSA fragment with mutation site
	pCasiF2	CGTTTAAGTGATTATGATGTCGATgccATTGTTCCACAAAGTTTCCTTAAAGAC	amplification of pCasSA fragment with mutation site
	pCasiR2	cgattatgtcttttgcgcagtc	amplification of pCasSA fragment
	pCasiF3	cgcaccagcgaaaactggt	amplification of pCasSA fragment
	pCasiR3	CCGACGCTATTTGTGCCGATagcTAAGCCTATTGAGTATTTCTTATCCATG	amplification of pCasSA fragment with mutation site
qRT-PCR	agrAspacaF	GAAAtgtctacaaagttgcagcga	*agrA* spacer for pCasiSA
	agrAspacerR	AAACtcgctgcaactttgtagaca	*agrA* spacer for pCasiSA
	sasEpcasiPRF	GAAAgtcatgttgtttttcctccta	*sasE* spacer for pCasiSA
	sasEpcasiPRR	AAACtaggaggaaaacaacatgac	*sasE* spacer for pCasiSA
	rRNAF	acgtggataacctacctataagactgggat	amplification of 16S rRNA in qRT-PCR as reference gene
	rRNAR	taccttaccaactagctaatgcagcg	amplification of 16S rRNA in qRT-PCR as reference gene
	agrARTF	tcacagactcattgcccatt	amplification of *agrA* in qRT-PCR
	agrARTR	caccgatgcatagcagtgtt	amplification of *agrA* in qRT-PCR
	sasERTF	gcagacagccaacaagtcaa	amplification of *sasE* in qRT-PCR
	sasERTR	gcattgtttaacacggtttgg	amplification of *sasE* in qRT-PCR

References

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