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Single-copy vectors for integration at the SaPI1 attachment site for *Staphylococcus aureus*

John Chen^{a,*}, Pauline Yoong^b, Geeta Ram^a, Victor J. Torres^b, and Richard P. Novick^{a,**}

^aSkirball Institute Program in Molecular Pathogenesis and Departments of Microbiology and Medicine, New York University Medical Center, New York, NY 10016, USA

^bDepartment of Microbiology, New York University School of Medicine, New York, NY 10016, USA

Abstract

We have previously reported the construction of *Staphylococcus aureus* integration vectors based on the staphylococcal pathogenicity island 1 (SaPI1) site-specific recombination system. These are shuttle vectors that can be propagated in *Escherichia coli*, which allows for standard DNA manipulations. In *S. aureus*, these vectors are temperature-sensitive and can only be maintained at non-permissive (42 °C) temperatures by integrating into the chromosome. However, most *S. aureus* strains are sensitive to prolonged incubations at higher temperatures and will rapidly accumulate mutations, making the use of temperature-sensitive integration vectors impractical for single-copy applications. Here we describe improved versions of these vectors, which are maintained only in single-copy at the SaPI1 attachment site. In addition, we introduce several additional cassettes containing resistance markers, expanding the versatility of integrant selection, especially in strains that are resistant to multiple antibiotics.

Keywords

Single-copy; Integration; SaPI; Complementation

1. Introduction

Staphylococcus aureus is a leading cause of bacterial infections and mortality worldwide (Klein et al., 2007; Naimi et al., 2003). The construction and study of isogenic genetic mutants in this, or any pathogen, is key to the delineation of its virulence and physiological mechanisms. However, secondary mutations can arise during the process of gene inactivation, hence interpretation of data garnered from such strains can potentially lead researchers to aberrant conclusions with regard to the true function of their gene of interest (Labandeira-Rey et al., 2007; Sun et al., 2010; Villaruz et al., 2009; Wyatt et al., 2010). Hence, upon inactivation of any gene, complementation of that same locus is necessary. The complemented mutant should regain a phenotype comparable to that of the wildtype strain, affirming that any phenotypes observed in the genetic knockout were not attributed to secondary mutations.

*Corresponding author. john.chen@med.nyu.edu (J. Chen). **Corresponding author. richard.novick@med.nyu.edu (R.P. Novick).

Complementation with the wildtype gene on an autonomously replicating plasmid is a strategy that is commonly used in *S. aureus* (Bubeck Wardenburg et al., 2006; Yoong and Pier, 2012). The wildtype locus and its native promoter region (or that of a different promoter fused upstream of the gene) can be cloned into the plasmid vector, and transformed into the knockout strain. While complementation in the knockout strain can be achieved from a plasmid, the amount of wildtype proteins expressed can differ significantly from that of the parental strain, commonly higher than wildtype, due in part to the multi-copy nature of most plasmid vectors. The maintenance of plasmids can also be an issue should selective pressure be removed, say in experiments involving animal infections. A more stable and accurate system of complementation would be the integration of the wildtype gene coupled with its native regulatory sequences. Two such complementation strategies have been used in *S. aureus*, involving integration into the $\phi 11$ or L54 phage attachment sites (Luong and Lee, 2007). While the basis of these complementation systems is sound, there are limitations, depending on the strain of study. Many *S. aureus* strains are lysogenic with a resident prophage at the $\phi 11$ or L54 attachment sites, and integrating a vector at these attachment sites will cure the strain of the respective phage. The deletion of a phage may significantly alter a strain, since many *S. aureus* phages are important contributors to pathogenesis (Bae et al., 2006; Novick et al., 2001; van Wamel et al., 2006). In strains that do not carry a prophage at the L54 attachment site, integration of a plasmid disrupts the major lipase gene, *geh*, which has been implicated in virulence (Hu et al., 2012). Hence, genetic complementation in the lipase gene can alter the virulence of the strain, a trait that is undesirable, especially if the pathogenesis of *S. aureus* is being studied.

Here, we describe an additional system that enables the integration of genes into the *S. aureus* chromosome. This system is based on integration into the chromosomal attachment site (*attC*) of the *S. aureus* pathogenicity island 1 (SaPI1) (Lindsay et al., 1998; Ubeda et al., 2009). There are five known SaPI *attC* sites, and all five are present in every *S. aureus* genome sequenced to date. Each insertion site is located at the 3' end of a gene, such that integration does not disrupt that gene (Novick et al., 2010). We also developed several cassettes containing resistance markers that are selectable in single copy, expanding the versatility of integrant selection, especially in strains that are resistant to multiple antibiotics. In this report, we highlight the stability of the SaPI1 site-specific integrated vectors. Additionally, we present a comparison of gene function restoration using the SaPI1 integrated vectors with that of an extrachromosomal plasmid vector. Taken together, these features of the SaPI1 integration vectors greatly advance the set of genetic tools available for the study of *S. aureus* physiology and pathogenesis.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The *S. aureus* strains and plasmids used in this study are listed in Table 1.

Cloning was performed with *Escherichia coli* strains DH5 α and Top Ten. All clones were transformed into *S. aureus* strain RN4220, our standard recipient for *E. coli* DNA, or its derivative containing the site-specific SaPI1 integrase (RN9011) before phage transduction to other *S. aureus* strains. *S. aureus* cells from overnight plates containing the appropriate

selective antibiotics (tetracycline 10 µg/mL, chloramphenicol 10 µg/mL, erythromycin 5 µg/mL cadmium chloride [CdCl₂] 0.1 mM and/or sodium arsenite [NaAsO₂] 0.5–1.0 mM) were used as base inocula for all experiments. Clones selected on sodium arsenite were transferred to non-selective media for maintenance and storage within 1 day.

2.2. Plasmid construction

The plasmids used in this study were constructed by cloning PCR products amplified with oligonucleotide primers purchased from Integrated DNA Technologies (Coralville, IA) (see Table 1). Clones were sequenced by Macrogen (Rockville, MD). Blunt PCR products were cloned into the HincII site of pUC18. A SaPII integration vector with a temperature sensitive pT181 replicon (Novick et al., 1982), pJC1079, was digested with HpaI and self-ligated to delete the pT181 replicon to generate pJC1110. The cadmium chloride resistance *cadCA* cassette from pJC1071 (AvrII-SacII), with the same restriction sites as the resistance cassettes described by Charpentier et al. (2004), replaced the chloramphenicol resistance *cat194* gene on pJC1110 to generate pJC1111. The erythromycin resistance *ermC* cassette from pCN33 (AvrII-SacII) (Charpentier et al., 2004) replaced *cat194* on pJC1110 to generate pJC1112. The sodium arsenite resistance *arsABC* cassette from pJC1300 (AvrII-SacII) replaced *cat194* on pJC1110 to generate pJC1302. The tetracycline resistance *tetM* cassette from pJC1305 (AvrII-SacII) replaced *cat194* on pJC1110 to generate pJC1306. While the base vector originated with multiple cloning sites from pUC19, some sites are no longer unique, depending on the vector. The available cloning sites in all SaPII integration vectors are summarized in Fig. 1.

Plasmid pJC1111, which contains a cadmium resistance cassette and the SaPII *attS* sequence was used as the backbone vector for β-lactamase (*blaZ*) fusion constructs using the polylinker of pUC18. pJC1111 derivatives were integrated into the *S. aureus* genome at the SaPII *attC* site by electroporation into RN9011. An *agr*₃ β-lactamase reporter was constructed by moving the *agr*₃-*blaZ* fragment from pJC1349 (containing promoter *agr*₃ cloned from RN6734) (Geisinger et al., 2012).

2.3. Reporter assays

Bacteria containing β-lactamase fusions were transferred from overnight plates and washed once, then inoculated into casamino acids–yeast extract–glycerophosphate (CYGP) without glucose at OD₆₀₀ of 0.005. Cells were grown at 37 °C with shaking to OD₆₀₀ of 0.05 and were diluted with fresh media to OD₆₀₀ of 0.01, representing T₀ and corresponding to approximately 10⁷ CFU/mL. This dilution step permitted further reduction of residual *agr* and reporter transcripts, cell density normalization, and synchronization in early exponential phase. Subsequent growth in CYGP broth without glucose or antibiotics was performed at 37 °C with shaking. Cell density was determined by using a ThermoMax microplate reader (Molecular Devices) to measure the optical density at 600 nm (OD₆₀₀) of 100 µL samples in 96-well round-bottom assay plates. Samples were collected at the indicated time points and stored at –80 °C. Samples were thawed on ice and diluted with media to appropriate densities for turbidity measurements and assays. Assay of β-lactamase activity was performed by the nitrocefin method as described previously (Ji et al., 1995). Assay data were normalized to β-lactamase units (V_{max}/OD₆₀₀).

2.4. Plasmid stability assay

Strain JCSA1155 was grown overnight on tryptic soy agar (TSA) plates with selection (0.1 mM CdCl₂) at 37 °C. Cells were resuspended in tryptic soy broth (TSB) without antibiotics and adjusted to OD₆₀₀ = 0.5 and diluted 10⁻⁶ in TSB without antibiotics and shaken at 37 °C. Every 12 hours, cultures were readjusted to OD₆₀₀ = 0.5, diluted 10⁻⁶ in TSB, and this cycle was repeated to maintain exponential growth until the strains had grown approximately 70–80 generations. Serial dilutions of the cultures were plated on TSA without selection for single colonies, and plasmid retention was measured by picking 100 colonies from the non-selective plates onto plates with selection (0.1 mM CdCl₂).

3. Results and discussion

3.1. Plasmid backbone

A modular shuttle vector built previously in this laboratory (Charpentier et al., 2004) was used as the basis of this system of gene complementation. The ColE1 replicon for plasmid replication in *E. coli*, and the multiple cloning site (MCS), both derived from pUC19, were retained from the pCN vectors. However, the replicon for *S. aureus* was deleted here, making this a suicide vector in *S. aureus*.

3.2. Selection markers

We employ four different markers for the selection of this integration vector. The erythromycin resistance cassette was retained from the pCN plasmids (Charpentier et al., 2004). In addition, we are introducing cassettes containing genes coding for resistance to tetracycline, cadmium chloride and sodium arsenite. Distinct pairs of restriction sites flank the genes encoding for proteins that confer resistance, thus making the selection marker modules interchangeable.

3.3. Site-specific integration into the *S. aureus* chromosome

The attachment sites of SaPII (*attS*) on the plasmids are necessary for recombination of the plasmid into the complementary SaPII attachment site within the *S. aureus* genome (*attC*). Also required is the integrase specific to SaPII that catalyses the crossover. The SaPII integrase was cloned into a second plasmid (pRN7023) that is selectable by chloramphenicol.

3.4. Strategy

The gene to be complemented, and its promoter elements, can be cloned into the MCS and propagated in *E. coli*. Purified plasmids are then transformed into the restriction deficient *S. aureus* strain carrying the appropriate integrase plasmid (RN9011). Integrative recombination of the plasmid is carried out on media containing the appropriate selective chemical(s), depending on the marker that is being used. Plasmids that fail to undergo recombination will be lost due to lack of a *S. aureus* replicon. Once integrated, the plasmid can be moved by phage-mediated generalized transduction to any strain of choice. However, similarly to the *phi*11 or L54 phage attachment sites systems, the SaPII integration vectors

will cure a strain of a resident SaPI element at the SaPI1 *attC* site when introduced by transduction.

3.5. Integration and excision at the SaPI1 *attC* site

3.5.1. Frequency of integration into the SaPI1 *attC* site—Previously, we reported that SaPIs readily integrate into alternative sites in the absence of the cognate *attC* site (Chen and Novick, 2009). To determine the frequency of integration at the SaPI1 *attC* site, we performed PCR analysis on 50 independent transformants of strain RN9011 with pJC1111, using one primer (JCO717) specific to the chromosome and another primer (JCO719) specific to a common region in the integration vectors to amplify an integration junction (Fig. 2A). All 50 transformants gave an amplicon of the expected size of 1.1 kb (20 representative transformants are shown in Fig. 2B), showing that all of the transformants had pJC1111 integrated at the SaPI1 *attC* site in the correct orientation, indicating that the integration vectors will insert into the SaPI1 *attC* site most of the time when it is present.

3.5.2. Frequency of spontaneous excision from the SaPI1 *attC* site—SaPI elements have been observed to excise spontaneously at low frequencies (Novick et al., 2010); however, because the integration vectors do not carry genes for excision, they are not predicted to excise spontaneously. To determine the stability of the SaPI1 integration vectors, pJC1111 was moved to a phage and SaPI-free strain (RN450) by transduction and the spontaneous excision rates of pJC1111 in three independent transductants (JCSA1155) were measured by testing for loss of cadmium resistance during growth without antibiotic selection. Since the integration vectors are unable to replicate episomally in *S. aureus*, they can only be maintained as integrants in the chromosome. As shown in Fig. 3, cadmium resistance was not detectably lost in the course of many generations, indicating that the integrated vectors are highly stable without selection and rarely excise spontaneously.

3.6. Comparing gene expression from integrated and extrachromosomal plasmids

3.6.1. Single and multi-copy *agrp3-blaZ* reporter fusions— β -Lactamase transcriptional reporters were fused to a promoter of the *S. aureus agr* regulon (*agrp3*). This fusion construct was cloned into the SaPI1 integration vector pJC1111 (giving rise to pJC1359) followed by subsequent integration into the *S. aureus* chromosome, in addition to the autonomously replicating vector pJC1280 (giving rise to pJC1349). Ten independent clones of each were selected at random for measurement of β -lactamase activity. Figure 4 shows comparable β -lactamase activity from 10 independent integrated *agrp3-blaZ* constructs of pJC1359. β -Lactamase activity from the SaPI1 *attC* integrated reporter vector was highly consistent from all clones, at or near 3000 units. In contrast, 10 clones of *S. aureus* carrying the *agrp3-blaZ* reporter on the independently replicating plasmid pJC1349 yielded highly variable β -lactamase readings ranging from ~400 to 1100 units (Fig. 5).

3.6.2. Complementation of PVL in a *pvl* strain—The genes encoding for PVL, with their native regulatory elements, were cloned into pJC1111 and into the multicopy plasmid pOS1, giving rise to pJC1111-*ppvI* and pOS1-*ppvI* (Yoong and Pier, 2012), respectively. The pJC1111-*ppvI* construct was integrated into the SaPI1 *attC* site in the chromosome, while pOS1-*ppvI* replicated autonomously. Western blots were conducted to

detect relative levels of PVL in culture supernatants from the different strains. The blots showed pJC1111-*ppv*/restoring PVL levels similar to the WT strain (Fig. 6). The multicopy plasmid pOS1-*ppv*/produced PVL at levels that were significantly higher than the WT strain.

4. Conclusions

Taken together, these experiments show that gene expression from autonomously replicating plasmids can vary greatly, as seen with the *agr*₃ transcriptional fusions, and differ significantly from chromosomal gene expression. Single copy maintenance on the *S. aureus* chromosome eliminated genetic instability and expression variability. In addition, genetic complementation from the SaPI1 *attC* site can greatly advance the study of *S. aureus* pathogenesis by restoring gene expression in mutant strains that approximate physiological wildtype levels. The advantages offered by the SaPI1 integration make these the next generation of *S. aureus* genetic tools.

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References

- Bae T, et al. Prophages of *Staphylococcus aureus* Newman and their contribution to virulence. Mol Microbiol. 2006; 62:1035–1047. [PubMed: 17078814]
- Bubeck Wardenburg J, et al. Host defenses against *Staphylococcus aureus* infection require recognition of bacterial lipoproteins. Proc Natl Acad Sci U S A. 2006; 103:13831–13836. [PubMed: 16954184]
- Charpentier E, et al. Novel cassette-based shuttle vector system for gram-positive bacteria. Appl Environ Microbiol. 2004; 70:6076–6085. [PubMed: 15466553]
- Chen J, Novick RP. Phage-mediated intergeneric transfer of toxin genes. Science. 2009; 323:139–141. [PubMed: 19119236]
- Geisinger E, et al. Identification of ligand specificity determinants in AgrC, the *Staphylococcus aureus* quorum-sensing receptor. J Biol Chem. 2008; 283:8930–8938. [PubMed: 18222919]
- Geisinger E, et al. Allele-dependent differences in quorum-sensing dynamics result in variant expression of virulence genes in *Staphylococcus aureus*. J Bacteriol. 2012; 194:2854–2864. [PubMed: 22467783]
- Hu C, et al. Functional characterization of lipase in the pathogenesis of *Staphylococcus aureus*. Biochem Biophys Res Commun. 2012; 419:617–620. [PubMed: 22369949]
- Ji G, et al. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci U S A. 1995; 92:12055–12059. [PubMed: 8618843]
- Klein E, et al. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg Infect Dis. 2007; 13:1840–1846. [PubMed: 18258033]
- Labandeira-Rey M, et al. *Staphylococcus aureus* Pantón-Valentine leukocidin causes necrotizing pneumonia. Science. 2007; 315:1130–1133. [PubMed: 17234914]
- Lindsay JA, et al. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol. 1998; 29:527–543. [PubMed: 9720870]
- Luong TT, Lee CY. Improved single-copy integration vectors for *Staphylococcus aureus*. J Microbiol Methods. 2007; 70:186–190. [PubMed: 17512993]
- Naimi TS, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 2003; 290:2976–2984. [PubMed: 14665659]
- Novick RP, et al. Coding sequence for the pT181 repC product: a plasmid-coded protein uniquely required for replication. Proc Natl Acad Sci U S A. 1982; 79:4108–4112. [PubMed: 6287465]

- Novick RP, et al. Pathogenicity and resistance islands of staphylococci. *Microbes Infect.* 2001; 3:585–594. [PubMed: 11418332]
- Novick RP, et al. The phage-related chromosomal islands of Gram-positive bacteria. *Nat Rev Microbiol.* 2010; 8:541–551. [PubMed: 20634809]
- Ruzin A, et al. Molecular genetics of SaPII – a mobile pathogenicity island in *Staphylococcus aureus*. *Mol Microbiol.* 2001; 41:365–377. [PubMed: 11489124]
- Sun F, et al. Aureusimines in *Staphylococcus aureus* are not involved in virulence. *PLoS ONE.* 2010; 5:e15703. [PubMed: 21209955]
- Ubeda C, et al. Specificity of staphylococcal phage and SaPI DNA packaging as revealed by integrase and terminase mutations. *Mol Microbiol.* 2009; 72:98–108. [PubMed: 19347993]
- van Wamel WJ, et al. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. *J Bacteriol.* 2006; 188:1310–1315. [PubMed: 16452413]
- Villaruz AE, et al. A point mutation in the *agr* locus rather than expression of the Pantone-Valentine leukocidin caused previously reported phenotypes in *Staphylococcus aureus* pneumonia and gene regulation. *J Infect Dis.* 2009; 200:724–734. [PubMed: 19604047]
- Wyatt MA, et al. *Staphylococcus aureus* nonribosomal peptide secondary metabolites regulate virulence. *Science.* 2010; 329:294–296. [PubMed: 20522739]
- Yoong P, Pier GB. Immune-activating properties of panton-valentine leukocidin improve the outcome in a model of methicillin-resistant *Staphylococcus aureus* pneumonia. *Infect Immun.* 2012; 80:2894–2904. [PubMed: 22665379]

Fig. 1.
Map of multi-cloning sites of the SaPII integration vectors.

Fig. 2.

(A) *S. aureus* NCTC 8325 chromosomal region containing the SaPII *attC* site. A generic integrant is shown, which results in the duplication of the SaPII *attC* site. Large arrows represent ORFs and small arrows indicate the annealing positions and directions of primers JCO717 and JCO719. (B) PCR analysis of 20 independent transformants of strain RN9011 with pJC1111. Lane 1 = 1 kb ladder (NEB) in both gels. 1–20 = 20 independent transformants. The expected size of the amplicon with primers JCO 717 and JCO 719 is approximately 1.1 kb.

Fig. 3.

Vectors integrated at the SaPI1 *attC* site are stably maintained without antibiotic selection. Plasmid loss in strain JCSA1155 was assayed by growing cells in the absence of selection for several generations. Cells were plated at regular intervals on TSA without selection for single colonies, and plasmid retention was measured by picking colonies from the non-selective plates onto plates with selection. Values are means $\pm$ SD (n = 3 independent samples).

Fig. 4.

Beta-lactamase activity from single copy *blaZ* fusion vectors chromosomally integrated at the SaPII attachment site. V = promoterless *blaZ* transcriptional reporter (pJC1358). 1–10 = 10 independent clones of *agr₃* promoter-*blaZ* fusion vector (pJC1359).

Fig. 5. Beta-lactamase activity from *blaZ* fusion vectors that replicate independently of the chromosome (episomal plasmids). V = promoterless *blaZ* transcriptional reporter (pJC1280). 1–10 = 10 independent clones of *agr*₃ promoter-*blaZ* fusion vector (pJC1349).

Table 1

Strains, plasmids, and primers used in this study.

<i>S. aureus</i> strains		
Strain	Genotype or description	Reference
JCSA359	<i>S. aureus</i> strain RN6734/pJC1280	This work
JCSA362	<i>S. aureus</i> strain RN6734/pJC1349	This work
JCSA367	<i>S. aureus</i> strain RN6734 SaPI1 <i>attC</i> :pJC1358	This work
JCSA370	<i>S. aureus</i> strain RN6734 SaPI1 <i>attC</i> :pJC1359	This work
RN9011	<i>S. aureus</i> strain RN4220/pRN7023 (SaPI1 integrase, <i>cat194</i>)	(Ruzin et al., 2001)
JCSA1155	<i>S. aureus</i> strain RN450 SaPI1 <i>attC</i> :pJC1111	This work
Plasmids		
Integration vectors		
Plasmid	Description	Reference
pJC1110	SaPI1 <i>attS</i> suicide vector, chloramphenicol resistant (<i>cat194</i>)	This work
pJC1111	SaPI1 <i>attS</i> suicide vector, cadmium resistant (<i>cadCA</i>)	(Geisinger et al., 2008)
pJC1112	SaPI1 <i>attS</i> suicide vector, erythromycin resistant (<i>ermC</i>)	This work
pJC1302	SaPI1 <i>attS</i> suicide vector, arsenite resistant (<i>arsABC</i>)	This work
pJC1306	SaPI1 <i>attS</i> suicide vector, tetracycline resistant (<i>tetM</i>)	This work
Cloning of antibiotic resistance cassettes		
Plasmid	Description	Reference
pJC1071	pUC18 PCR JCO 124 + JCO 125 on pI524, amplifies <i>cadCA</i>	This work
pJC1300	pUC18 PCR JCO 212 + JCO 213 on pI258, amplifies <i>arsABC</i>	This work
pJC1305	pUC18 PCR JCO 389 + JCO 398 on SaPI1 <i>tsstI</i> : <i>tetM</i> , amplifies <i>tetM</i>	This work
Reporter fusions		
Plasmid	Description	Reference
pJC1280	<i>blaZ</i> transcriptional reporter, pT181 replicon (<i>cat194</i>)	(Geisinger et al., 2012)
pJC1349	P3- <i>blaZ</i> transcriptional fusion. P3 promoter cloned into pJC1280 (<i>cat194</i>)	(Geisinger et al., 2012)
pJC1358	<i>blaZ</i> transcriptional reporter, cloned into pJC1111 (<i>cadCA</i>)	This work
pJC1359	P3- <i>blaZ</i> transcriptional fusion. P3 promoter cloned into pJC1358 (<i>cadCA</i>)	This work
PVL complementation constructs		
Plasmid	Description	Reference
pJC1111- <i>ppvI</i>	<i>ppvI</i> genes with its native promoter cloned into pJC1111	This work
pOS1- <i>ppvI</i>	<i>ppvI</i> genes with its native promoter cloned into pOS1	(Yoong and Pier, 2012)
Primers		
	Sequence (5'-3')	Reference
JCO 124	5'-CCTAGGGTCATACCCTGGTCAAAACCGTTTCG-3'	This work
JCO 125	5'-CCGCGGCCGCAGCTGCTGTAAGTATCG-3'	This work

<i>S. aureus</i> strains		
Strain	Genotype or description	Reference
JCO 212	5'-CCTAGCTCAAGGGATTCAAGTCGCC-3'	This work
JCO 213	5'-CCGCGGAGCGTTTCTCACAGCG-3'	This work
JCO 389	5'-CCTAGGATCAAGAAACAAAGGCAACCC-3'	This work
JCO 398	5'-CCGCGGATCTTGATCATTACTCCATGTATC-3'	This work
JCO 717	5'-GTGCTTCACCAGCACCACATGCTG-3'	This work
JCO 719	5'-GGTATTAGTTTGAGCTGTCTTGGTTCATTGATTGC-3'	This work