A CRISPRi-based genetic resource to study essential *Staphylococcus aureus* genes

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Abstract

We have optimized a CRISPR interference system to facilitate gene knockdown in the gram-positive bacterial pathogen *Staphylococcus aureus*. For this, we used a CRISPRi system derived from *Streptococcus pyogenes* which requires the co-expression of the *dcas9* gene encoding a catalytically inactive Cas9 protein and a customizable single guide RNA (sgRNA). In the system described in this work, *dcas9* is expressed from a single copy in the chromosome of methicillin resistant *S. aureus* (MRSA) strains COL or JE2, under the control of a tightly regulated promoter inducible by anhydrotetracycline. The sgRNAs are expressed from a replicative plasmid under the control of a constitutively active promoter. This system enables high-efficiency, inducible, knockdown of both essential and nonessential genes and was used for the construction of the Lisbon CRISPRi mutant library (LCML) of 241 strains, in the background of JE2, containing sgRNAs targeting 209 essential genes/operons. This library allows the study of the function of essential *S. aureus* genes and is complementary to the Nebraska Transposon Mutant Library which consists of nearly 2000 strains, each containing a transposon insertion within a non-essential gene. Together the two libraries should facilitate the study of *S. aureus* pathogenesis and biology.

Introduction

Staphylococcus aureus is a gram-positive bacterium that frequently colonizes the skin and nares of both humans and animals. It is also an opportunistic pathogen in community and hospital settings that can cause various clinical conditions such as skin and soft tissue infections,

bacteraemia or endocarditis (1). The emergence of multi-drug resistant strains, such as methicillin-resistant *S. aureus* (MRSA), has complicated the treatment of *S. aureus* infections and MRSA strains are currently the second most common cause of global deaths associated with bacterial antimicrobial resistance (2). The discovery of novel antibiotics, with unique modes of action, for the treatment of *S. aureus* infections is therefore vitally important in the battle against multi-drug resistant strains. One avenue that can be followed is the study of essential genes that encode core proteins required for bacterial survival and may be potential targets for new antimicrobial drugs.

Despite their importance, the function of many essential genes in *S. aureus* remains poorly studied. Gene inactivation or repression are general approaches to reveal the molecular function of genes in bacteria. Genetic tools to disrupt *S. aureus* genes or impair their transcription have become increasingly available (3). However, classical approaches such as gene deletion or transposon mutagenesis cannot be used to study essential genes due to their indispensable role in bacterial survival. Several alternative strategies have been developed including the exchange of the endogenous promoter of a gene by an exogenous inducible one, via allelic exchange, or the use of antisense RNA technology. However, the former is a labour-intensive approach that is not adequate for large scale studies, while the latter has limitations due to variable efficacy (4, 5).

In recent years, new tools based on CRISPR (clustered regularly interspaced palindromic repeats) interference systems have been developed to control gene expression. The most widely used CRISPRi system is derived from *Streptococcus pyogenes* and requires the co-expression of a gene encoding a catalytically inactive or dead (d)Cas9$_{Spy}$ protein, lacking its endonuclease activity, and a customizable single guide RNA (sgRNA) (6, 7). The dCas9-sgRNA complex binds the DNA target complementary to the sgRNA and instead of introducing double strand breaks, it causes a steric block that halts transcription by RNA polymerase, resulting in repression of the target gene/operon. Besides the complementary nucleotide sequence, the only other prerequisite for the complex to bind DNA is a three nucleotide long (NGG) recognition motif downstream of the complementary region of the targeted DNA strand, called the protospacer adjacent motif (PAM). CRISPRi systems based on *S. pyogenes* Cas9 (a type II CRISPR) have been established in several model organisms such as *Escherichia coli* (8), *Bacillus subtilis* (9) and *Streptococcus pneumoniae* (10). Type II CRISPR systems have been found in other bacteria besides *S. pyogenes*, including in *S. aureus* and, similarly to CRISPR-Cas9$_{Spy}$, they could be reprogrammed to serve as a molecular machinery for genome editing in

eukaryotic cells (11, 12). The Cas9 from *S. aureus* ($Cas9_{Sa}$) raised some interest because it is significantly smaller than the *S. pyogenes* $Cas9_{Spy}$ (1053 vs 1368 amino acids), hence allowing the use of adeno-associated virus with restrictive cargo size as vehicles for delivering Cas9 to animal cells (12). Additionally, $Cas9_{Sa}$ requires a longer PAM sequence (NNGRRT) (13), increasing specificity of DNA targeting and therefore reducing off-target effects, a potential problem while manipulating large genomes.

CRISPRi systems that can be used for genetic manipulation of *S. aureus* have already been described (14-16). These use plasmids to encode the sgRNA and the dCas9, an approach that is useful for moving the system into various strains (14-16). However, *dcas9* expression from multicopy plasmids, associated with the fact that most inducible promoters used in *S. aureus* cannot be fully repressed, leads to systems in which dCas9 production cannot be completely shut down. This is a particular problem when targeting essential genes, as basal levels of dCas9 can result in cell death. Here we report the construction of two variations of a system for gene knockdown in *S. aureus* based on the CRISPRi system established in *E. coli* by Qi and colleagues (8). The first system is composed of two shuttle vectors, one encoding the dCas9 of either *S. pyogenes* or *S. aureus* and the other encoding the corresponding version of the sgRNA, including the gene-specific target sequence. The second system allows efficient regulation of $dCas9_{Spy}$ production as the gene encoding this protein was integrated into the genome, under the control of a tightly controlled inducible promoter. This system was then used to create a knockdown library of 241 genes from 209 reported essential operons *S. aureus*, which we named the Lisbon CRISPRi mutant library (LCML). This library is complementary to the widely used Nebraska Transposon Mutant Library, which includes mutants in virtually all non-essential genes in *S. aureus* (17). The combined use of the two libraries will allow the community that studies this important pathogen to evaluate the function of any staphylococcal gene/operon.

Results

Construction of two-plasmid systems for CRISPR interference in *Staphylococcus aureus*

To establish a CRISPRi system in *S. aureus* we chose two *E. coli-S. aureus* shuttle vectors to enable initial propagation and genetic manipulation in *E. coli* followed by introduction into *S. aureus* by electroporation. *S. aureus* is not a naturally competent organism, and transformation efficiency is very low compared to other model organisms (18, 19). The genes encoding a catalytically dead Cas9 from either *S. pyogenes* ($dCas9_{Spy}$) or *S. aureus* ($dCas9_{Sa}$),

fused or not with sGFP, were cloned downstream of the cadmium-inducible promoter of the pCNX vector (20, 21), which harbors a kanamycin resistance cassette for selection in *S. aureus*, generating the plasmids pBCB40 (*dcas9*$_{Spy}$), pBCB41 (*dcas9*$_{Spy}$*-sgfp*) and pBCB42 (*dcas9*$_{Sa}$*-sgfp*). The tailor-made single guide RNAs from *S. pyogenes* (sgRNA$_{Spy}$) or *S. aureus* (sgRNA$_{Sa}$) were cloned downstream of a constitutively active promoter in the pGC2 vector (22), which contains a chloramphenicol resistance cassette for selection in *S. aureus* (Figure 1). Both sgRNA$_{Spy}$ and sgRNA$_{Sa}$ consist of two regions: the 5'end contains the variable region for targeted DNA binding (first 20 nucleotides of sgRNA$_{Spy}$ or first 22 nucleotides of sgRNA$_{Sa}$), while the downstream region contains a constant region for dCas9 binding (different for dCas9$_{Spy}$ and dCas9$_{Sa}$) and transcription termination. Targeting of specific genes of interest can be accomplished by replacing the 20 (or 22) nucleotides of the variable region, using inverse PCR, for the specific sequence of interest. In the presence of dCas9, the sgRNA forms a complex with the inactive nuclease and guides it to the complementary DNA, where the complex acts as a physical blockage for RNA polymerase, impairing transcription.

A key requirement for an efficient CRISPRi system is the ability to shut down dCas9 production, so that the system can be turned ON/OFF. Western blot analysis of dCas9$_{Spy}$ levels in strain BCBMS02 (expressing cadmium-inducible *dcas9*$_{Spy}$*-sgfp*) showed the presence of dCas9$_{Spy}$ even in samples grown in the absence of inducer, indicating that the cadmium-inducible promoter is leaky (Figure S1A). Despite this limitation, we proceeded to evaluate the efficiency of the system. For that, we constructed the reporter strains BCBMS05 (expressing *dcas9*$_{Spy}$*-sgfp*) and BCBLS01 (expressing *dcas9*$_{Sa}$*-sgfp*), in the background of strain NCTC8325-4, where the non-essential gene *rodZ,* which encodes the septal protein RodZ, was fused to the gene encoding far-red fluorescent protein eqFP650. Both strains allow easy evaluation of eqFP650-RodZ (far-red fluorescence) depletion and dCas9-sGFP (green fluorescence) production by fluorescence microscopy. We then designed four different sgRNAs for the dCas9$_{Spy}$, targeting the coding as well as the template strand, in the gene and upstream of the start-codon of *fp650-rodZ*. For the staphylococcal dCas9$_{Sa}$, we designed three sgRNAs, two targeting the coding strand and one targeting the template strand of *fp650-rodZ* (Figure 2A). To analyze the efficiency of the two systems we used pairs of strains, both expressing the same sgRNA targeting *fp650-rodZ* plus either *dcas9-gfp* expressed from a pCNX-based plasmid (test) or the empty pCNX vector (control). The two strains were mixed in the same microscopy slide for direct comparison (Figure 2A). With dCas9$_{Spy}$ system, we observed that sgRNAs targeting the promoter sequence on either strand (sg-*fp650rodZ-Spy*1 and sg-*fp650rodZ-Spy*2) or the gene sequence on the coding strand (sg-*fp650rodZ-Spy*3) were

efficient in depleting eqFP650-RodZ, leading to a strong reduction in fluorescence intensity when compared to the corresponding control strains lacking dCas9$_{Spy}$-sGFP (Figure 2B, C, E). On the contrary, the sgRNA that targeted the *fp650-rodZ* gene in the template strand (sg-*fp650rodZ-Spy*4) was not effective, in agreement with previous data for *E. coli* describing that targeting the template strand is not efficient (8) (Figure 2B, C, E). Notably, the eqFP650 signal in this strain was delocalized from the septum to the cytosol and it even increased 2.5-fold compared to control cells (expressing sg-*fp650rodZ-Spy*4 but lacking dCas9$_{Spy}$-sGFP). The sgRNA of this strain targets *rodZ*, downstream of *fp650*, so it is possible that transcription and translation of *fp650* can still occur, producing a soluble, truncated eqFP650.

We noticed that depletion of eqFP650-RodZ, resulting in loss of fluorescent signal, occured even in the absence of *dcas9* induction (Figure S1B). This indicates that the leaky *dcas9* expression results in the production of low levels of dCas9 protein, seen by western blot, that are sufficient to efficiently drive the CRISPRi system in the absence of inducer, i.e., that the system cannot be efficiently turned off.

Next, we tested an alternative version of the CRISPRi system that uses an inactive variant of a Cas9$_{Sa}$ native to *S. aureus*. Similarly to the dCas9$_{Spy}$ system, this system was also effective when the sgRNA$_{Sa}$ targeted the coding strand (sg-*fp650rodZ-Sa*1 and sg-*fp650rodZ-Sa*3) within the *fp650-rodZ* gene, as seen by the decreased fluorescence of these strains compared to the corresponding control strains lacking dCas9$_{Sa}$ (Figure 2B, D, F). No inhibition of *fp650-rodZ* expression was observed when the sgRNA targeted the template strand (sg-*fp650rodZ-Sa*2). We achieved similar knockdown efficiencies with both the dCas9$_{Spy}$ and the dCas9$_{Sa}$ system leading to a decrease in the relative fluorescence signal of eqFP650-RodZ (Figure 2D and F). Since the two systems had a similar performance, we decided to proceed with *S. pyogenes* dCas9 as it is less limited in target selection due to the shorter PAM sequence, a relevant criterion for work in the low GC content bacterium *S. aureus*.

Modified CRISPRi system enables repression of essential genes transcription in *S. aureus*

To construct an efficient knockdown system, that can be used to target essential genes in *S. aureus*, we re-designed the CRISPRi system described above, to minimize production of dCas9 in the absence of inducer. For that, we integrated *dcas9*$_{Spy}$ into the *spa* locus of the staphylococcal genome, to reduce the copy-number of the gene to one. This chromosomal copy of *dcas9*$_{Spy}$ was placed under the control of the tetracycline-inducible *xyl/tetO* promoter (23). Furthermore, we used an imperfect ribosome binding site (RBS) (24) separated from the start

codon by only 3 nucleotides to decrease translation efficiency (Figure 3A and S2). This $P_{xyl/tetO3}$-*dcas9*$_{Spy}$ construct was integrated into the *spa* locus of two MRSA strains, JE2 (generating strain BCBMS14) and COL (generating strain BCBMS15). In both backgrounds dCas9 was detected by western blot analysis in cells grown in the presence of the inducer anhydrotetracycline (aTc), but not in its absence, indicating that *dcas9*$_{Spy}$ expression was now under tight control (Figure 3B). Cells expressing *dcas9*$_{Spy}$ from the chromosome in the presence of aTc were only mildly impaired in growth (Figure 3C).

We then tested the efficiency of the improved CRISPRi system when targeting essential genes. For that we designed sgRNAs targeting the essential cell division genes *ftsZ*, *murJ* and *pbpA*. The resulting CRISPRi strains were analyzed by growth assays and microscopy (see Figure 4 and Figure S3 for strains in the background of JE2 and COL, respectively). The three strains showed severe growth inhibition upon induction of *dcas9*$_{Spy}$ expression (but not in its absence) and the expected phenotype for the depletion of each gene was observed: while depletion of MurJ did not have a visible phenotype, depletion of FtsZ led to greatly enlarged, spherical cells without detectable septa (25) and depletion of PBP1 led to enlarged, elongated cells (26). Importantly, these phenotypes were barely observed in the absence of inducer, confirming that *dcas9* is under tight regulation in this improved CRISPRi system.

Construction of a CRISPRi library of conditional mutants of essential *S. aureus* genes

The Nebraska Transposon Mutant library is an extremely useful resource for the study of *S. aureus*, as it contains transposon mutants in virtually all non-essential genes of MRSA strain JE2 (17). A complementary library containing depletion mutants for the remaining, essential, genes would be a valuable tool and therefore we decided to use the improved CRISPRi system for this purpose. Given that CRISPRi affects the expression of entire operons and not just of the target gene, we are unable to construct single mutants for each essential gene using this technology. We therefore decided to target each essential operon, which allows users to perform initial screenings that then may have to be followed by detailed studies of individual genes in operons. We first identified 209 predicted operons (Table S1) containing genes originally described in the literature as essential using antisense RNA (27) or transposon library screenings (28, 29). We designed sgRNAs targeting all essential operons (sgRNAs targeting more than one gene in an operon were designed for 32 operons resulting in a total of 241 sgRNAs tested) and cloned them into psg-*RNA*$_{Spy}$ using inverse PCR and the primers described in Table S2. We electroporated the resulting library of plasmids encoding the sgRNAs into BCBMS14, generating a library of 241 conditional mutants strains, targeting 209 operons, listed

in Table S3, which we named the Lisbon CRISPRi mutant library (LCML). Growth of these strains in the presence or absence of the aTc inducer (200 ng/ml) was followed in a 96-well plate reader, for 750 min, at 30°C. The ratio (R) of the area under the curve (AUC) of the growth curve obtained in the presence versus the absence of inducer was used to evaluate the efficiency of growth inhibition (Figure 4 and S4). 127 of the 241 strains (corresponding to 111 different operons) showed a clear growth reduction upon induction (R decrease over 50%, an arbitrary threshold chosen to decide if further optimization was done). However, 114 of the strains (corresponding to 98 different operons) had milder growth deficits (R decrease of less than 50%). For these, we designed a second sgRNA (sgRNA_B) targeting an alternative region of the gene. Of the 114 strains for which a second sgRNA was designed, 63 (corresponding to 35 operons) showed a reduction in R over 50% (Figure S4 shows R data for each strain when using the sgRNA, A or B, with highest efficiency). For 70 strains that showed a reduction in R lower or close to 50%, growth analysis was repeated at 37 ºC, given that some mutants may be may have a stronger growth defect at 37 ºC than at 30 ºC. Figure S5 shows that inhibition of the expression of a further 22 genes (corresponding to 21 operons) led to impaired growth at 37 ºC. Overall we achieved a success rate of over 80% as we observed growth inhibition (R decrease over 50%) for 197 of the 241 strains constructed (corresponding to 168 of the 209 operons targetted).

To determine if there were target regions for sgRNA that resulted in higher efficiency of the CRISPRi system, we plotted R for each sgRNA against their target position relative to the start-codon of the corresponding gene (Figure 5) and tested those targeting the coding strand for statistically relevant correlation using a Spearman correlation test. For that we split the data into two sets: the first set included the 72 sgRNAs that bind upstream of the start codon and the second set included the 160 sgRNAs targeting in the gene. sgRNAs targeting the template strand were excluded from the analyses due to small sample size (n=9). We found no statistical significant correlation between transcription repression and localization of sgRNAs targeted sequence relative to ATG, for either the first set (r: -01852; p-value: 0.1193) or the second set (r: 0.09929; p-value: 0.216).

Discussion

Construction of *S. aureus* conditional knockouts or gene deletion mutants using traditional approaches that require recombination events are labour intensive and time consuming. In contrast, use of a CRISPRi system allows the generation of conditional mutants through the simple exchange of the target sequence of the sgRNA, so that it specifically inactivates any

target gene/operon. In this study, we developed different versions of CRISPRi systems for *S. aureus*, using dCas9 proteins originated from both *S. pyogenes* and *S. aureus*. In our first approach, sgRNAs and *dcas9* from either *S. pyogenes* or *S. aureus* were expressed from two replicative vectors, allowing rapid introduction into different *S. aureus* strains and mutants. Staphylococcal Cas9$_{Sa}$ is significantly smaller than Cas9 from *S. pyogenes* (1053 vs 1368 amino acid residues) and it requires a longer PAM sequence. The smaller size of the nuclease is important when used in eukaryotes because of limited packaging size of adenovirus systems and lower transfection efficiency of very large plasmids. Additionally, a longer PAM sequence should decrease off-target effects, which is a problem in eukaryotic cells due to their large genome size. Both advantages are less important in bacteria. Nevertheless, we hypothesized that the expression of the smaller, native, nuclease, could be advantageous for *S. aureus*. However, the two CRISPRi systems, with either *S. pyogenes* or *S. aureus* dCas9, worked similarly. Given that *S. pyogenes* dCas9 is less limited in target selection due to the shorter PAM sequence, we decided to proceed with dCas9$_{Spy}$ for further optimization of the system. Optimization was required because in these two-plasmid systems low levels of dCas9 were produced even in the absence of inducer. To reduce gene transcription of *dcas9* in the absence of the aTc inducer to a minimum we (i) integrated *dcas9* into the genome of *S. aureus* MRSA strains JE2 and COL, to reduce its copy number to one, (ii) exchanged the leaky cadmium-inducible promoter with the tighter *xyl/tetO* promoter, and (iii) decreased translation efficiency by switching to an inefficient ribosome binding site. The resulting strains, BCBMS14 (JE2 background) and BCBMS15 (COL background), produced dCas9 under tight control so that it could not be detected by western blot in the absence of aTc inducer, but reached sufficient levels to silence essential genes upon addition of the inducer. Targeting of essential genes *ftsZ*, *pbpA*, or *murJ* with this improved CRISPRi system led to a severe decrease of growth, showing the efficiency of the system.

Given the efficacy of the improved CRISPRi system to silence essential genes, we constructed a library of CRISPRi conditional mutants in the strain BCBMS14, each containing a specific sgRNA targeting one of 241 genes, corresponding to 209 operons which harbour at least one essential gene. Overall, we were able to construct strains to silence 168 (80%) of these essential operons. It is possible that some of the tested genes for which expression inhibition did not result in severe growth defects are not actually essential for bacterial survival under the growth conditions tested, which would justify the lack of efficiency of some sgRNAs.

Large variation in sgRNA efficiency has been reported in several published studies (8, 30-32). In *E. coli*, the distance between transcription start site and sgRNA target was reported by Qi and colleagues to affect knockdown efficiency (8) but this effect was not observed in genome-wide screens (33, 34). We selected PAM sequences close to the start codon and all the designed sgRNAs target regions between -400 to +400 nucleotides from the ATG start codon. Given the large number of sgRNAs tested, we evaluated if there were specific locations that corresponded to more efficient sgRNAs. However, as shown in Figure 5, we could not find a correlation between the distance of the sgRNA target to the start codon of the corresponding gene and sgRNA efficiency, and therefore could not establish guiding principles for sgRNA design.

Despite these limitations, we constructed the Lisbon CRISPRi mutant library (LCML) of conditional mutants in essential genes/operons of *S. aureus*. This library is complementary to the widely used Nebraska Transposon Mutant library, which includes mutants in virtually all non-essential genes in *S. aureus* (17). The combined use of the two libraries will allow the evaluation of the function of any gene/operon of this important pathogen.

Materials and Methods

Bacterial strains and growth conditions

Plasmids and strains used in this study are described in Tables 1 and 2. *S. aureus* cells were grown at 37ºC with aeration in liquid (Tryptic Soy Broth; Difco) or on solid (Tryptic Soy Agar; VWR) medium, supplemented, when necessary, with antibiotics (chloramphenicol (Cm), Sigma-Aldrich, 10 µg/ml in liquid media, 20 µg/ml in solid medium; kanamycin (Kan), Apollo Scientific, 50 µg/ml; neomycin (Neo), Sigma-Aldrich, 50 µg/ml) and inducer (cadmium chloride, Sigma-Aldrich, 0.1 µM; or anhydrotetracycline (aTc), Sigma-Aldrich, 100-200 ng/ml). *E. coli* cells were grown at 37ºC with aeration in either LB (Luria-Bertani broth; VWR) or LA (Luria Agar; VWR), supplemented with ampicillin (Apollo Scientific, 100 µg/ml) when required. Strain growth was followed by measurement of optical density at 600 nm (OD_{600}).

DNA purification and manipulation

Plasmid DNA was extracted from *E. coli* cells using Wizard SV Plus Miniprep Kit (Promega), following the manufacturer's instructions. DNA was digested with FastDigest restriction enzymes (ThermoFisher) by incubating 0.5-1 µg DNA at 37ºC for 1 hour, with 1x FastDigest buffer, 1 µl of the selected endonuclease and 1 µl of Fast Alkaline Phosphatase (ThermoFisher), when necessary. DNA fragments were purified using Wizard SV Plus Cleanup Kit (Promega), according to manufacturer's instructions. DNA ligations were performed by standard molecular

biology techniques using T4 DNA Ligase (ThermoFisher). PCR reactions were performed using either Phusion DNA Polymerase for cloning purposes or DreamTaq Polymerase for colony screenings (both ThermoFisher), following the manufacturer's instructions. For the inverse PCR, one primer was 5' phosphorylated using a T4 Polynucleotide Kinase (ThermoFisher) using recommended reaction conditions. After PCR amplification, 1 µl DpnI (NEB) was added to the PCR mix, which was incubated for 1 h at 37°C followed by PCR purification. 5 µl of the purified PCR product were used for ligation using T4 DNA ligase with subsequent transformation.

Plasmid and strain construction

Plasmids and primers used in this study are described in Tables 1 and 3, primers used to generate the different sgRNAs for the LCML are described in Table S2. To construct the pBCB40 plasmid, *dcas9*$_{Spy}$ was amplified from plasmid pdCas9_bacteria (8, 35) using primers 5450/5451. The PCR product and the vector pCNX (21) were digested with PstI and SalI and the fragment containing *dcas9*$_{Spy}$ was ligated downstream of the cadmium-inducible promoter from pCNX. To construct pBCB41, primer 3714/5450 were used to amplify *dcas9*$_{Spy}$ with a linker insertion and *sgfp* was amplified from the plasmid pTRC99a-P7 (36) using primers 3715/5452. The two fragments were joined by overlap PCR using primers 5450/5452, digested with PstI and SalI and ligated into pCNX, digested with the same enzymes.

To construct the pBCB42, we first constructed the pCNX_sGFP plasmid by amplifying the *sgfp* gene sequence from pTRC99a-P7 plasmid using the primer pair 5602/5603 and cloning it into pCNX using EcoRI and BamHI restriction sites. The *dcas9*$_{Sa}$ gene sequence was kindly provided by F. Ann Ran (12). The sequence, comprising a ribosome binding site and the *dcas9*$_{Sa}$ gene plus restriction sites SalI and BamHI, was synthesized (NZYTech), digested with the respective restriction enzymes and cloned into the pCNX_sGFP plasmid, resulting in plasmid pBCB42.

To construct plasmid pBCB43 a fragment of the antisense *secY* expression cassette in pIMAY (37) was amplified using primers 7015/7016. The PCR product, comprising *tetR*, $P_{xyl\text{-}tetO}$ and a downstream located *tetO* site, was digested with EcoRI/NheI and ligated into similarly digested pBCB13 (38) lacking *lacI* and P_{spac}. The resulting plasmid, pBCB43, was SmaI/EagI digested to clone *dcas9*$_{Spy}$, amplified from pBCB40 with the primer pair 7268/7272, downstream of *tetO* of pBCB43, via Gibson assembly (39), generating plasmid pBCB44, verified by sequencing.

Plasmid psg-*RNA*$_{Spy}$ was constructed by cloning the sgRNA sequence including the minimal constitutive promoter and transcription terminator from plasmid pgRNA_bacteria (8, 35) into pGC2 (22). For that, the multiple cloning site of pGC2 was altered via inverse PCR with primer pair 5922/5923 (5' phosphorylated) and for a second time with primer pair 5745/5746 (5' phosphorylated), to obtain a BglBrick (40) composition with restriction sites for EcoRI, BglII; BamHI and XhoI. The vector pgRNA_bacteria was digested with EcoRI and XhoI and the fragment was ligated into the new multiple cloning site of altered pGC2 resulting in the plasmid psg-*RNA*$_{Spy}$, which was verified by sequencing.

To construct plasmids expressing different sgRNAs in the *S. pyogenes* CRISPRi systems, psg-*RNA*$_{Spy}$ was used as a template and the 20 nt target specific sequence present in psg-*RNA*$_{Spy}$ was replaced by the 20 nt sequence of interest via inverse PCR as described (35). To generate the initial test sg-*RNA*$_{Spy}$ by inverse PCR, forward primers 5887, 5849, 5890 and 5889 were used for construction of psg-*fp650rodZ-Spy*1-4 and forward primers 6423 (sg-*murJ*), 6424 (sg-*ftsZ*) and 6426 (sg-*pbpA*) were used to construct sgRNAs against selected essential genes (see Table 3). For the LCML, gene-specific base pairing regions were selected by searching for PAM sequences in/upstream of essential genes/operons and the 20 nt upstream of the first PAM sequence in the coding strand of a gene were chosen as target DNA in most cases. To generate sg-*RNA*$_{Spy}$ for the library construction, we used always 5'phosporylated reverse primer 5846 together with a gene specific forward primer (see Table S2) containing the base-pairing region as an overhang in combination with the constant part of the sgRNA gttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC.

To construct psg-*fp650rodZ-Sa1*, the *pbpb* promoter sequence (41) was amplified from NCTC8325-4 genomic DNA using primer pair 5639/5838. Primer 5838 contains the sgRNA$_{Sa}$ (42), a region targeting the eqFP650-RodZ specific gene sequence and a transcription terminator. The resulting PCR product was digested with SmaI and EcoRI and cloned into pGC2. Plasmid psg-*fp650rodZ-Sa2* was constructed by amplifying the *pbpb* promoter from NCTC8325-4 genomic DNA using primer pair 5639/5839 and the sgRNA$_{Sa}$ from *psg-fp650rodZ-Sa1* using the primer pair 5840/5837. The fragments were joined by overlap PCR with primers 5639/5837 and cloned into pCG2 using SmaI and EcoRI enzymes identical to psg-*fp650rodZ-Sa1*. For the construction of psg-*fp650rodZ-Sa3*, the *pbpb* promoter was exchanged for the synthetic minimal promoter used in psg-*RNA*$_{Spy}$ plasmids. Primer pair 6013/5837 was used to amplify the sgRNA$_{Sa}$ with primer 6013 containing the minimal promoter and target specific nucleotide sequence. The resulting PCR product was digested with SmaI and EcoRI

and cloned into pGC2. All constructed sgRNAs were confirmed by sequencing before further utilization.

All plasmids except those encoding the sgRNA for the library were initially transformed into *E. coli* DC10B (37) with ampicillin 100 (µg/ml) selection. Constructs were verified by PCR and sequencing. Plasmids were electroporated into the *S. aureus* strain RN4220 as previously described (19), followed by phage lysate preparations and transduction into other *S. aureus* strains (43). Following this protocol, the plasmids pCNX, pBCB40, pBCB41, pBCB42, psg-*fp650rodZ-Spy1-4* and psg-*fp650rodZ-Sa1-3* were electroporated into RN4220 with subsequent preparations of phage lysates. Plasmids pBCB40 and pBCB41 were transduced into JE2, generating strains BCBMS01 and BCBMS02 respectively. Strains BCBMS04, BCBMS05 and BCBLS01 were created by introducing pCNX, pBCB41 or pBCB42 respectively into the strain BCBHV200. Plasmids psg-*fp650rodZ-Spy1-4* were transduced either into BCBMS05, creating the strains BCBMS10-13, or into BCBMS04, creating control strains BCBMS06-09. Plasmids psg-*fp650rodZ-Sa1-3* were transduced either into BCBLS01, creating the strains BCBLS05-07, or into BCBMS04, creating control strains BCBLS02-04. pBCB40 and psg-*fp650rodZ-Spy2* were also transduced into BCBHV200, creating strain BCBMS26.

The plasmids for the sgRNA library based on psg-*RNA$_{Spy}$* were initially transformed into *E. coli* strain IM08B (44). IM08B has a modified DNA methylation pattern compatible with that of *S. aureus* JE2 increasing the efficiency of transformation into this strain (44). Plasmids propagated in IM08B could therefore be directly electroporated into competent BCBMS14 cells, bypassing the time-consuming steps of electroporation into RN4220 and subsequent phage transduction, frequently used to transfer plasmids into *S. aureus*. Preparation of *S. aureus* competent cells and electroporation protocol were performed as described by Johnston and colleagues (45).

We constructed a *S. aureus* strain encoding an N-terminal eqFP650-RodZ fusion as the only *rodZ* copy in the cell. For that, three DNA fragments were amplified. Fragment 1, containing a 1037 bp upstream region of *rodZ,* and fragment 3, containing a 1039 bp encompassing the *rodZ* gene and its downstream region, were amplified from NCTC8325-4 genome using the primers pairs 3627/3628 and 3382/3630, respectively. Fragment 2, encompassing the *fp650* gene without its *stop* codon and encoding a five amino acid linker (SCGAS) at the 3' end, was amplified from plasmid pFP650-F (46) using primers 3629/3381. The three fragments were then joined by overlap PCR using primers 3627/3630 and the resulting fragment was digested with SalI and NcoI and cloned into pMAD (47). The resulting plasmid was named

pMAD_*fp650rodZ* and was verified by sequencing. The pMAD_*fp650rodZ* plasmid was electroporated into RN4220 at 30°C and subsequently transduced to NCTC8325-4 (selection at 30°C with erythromycin in both steps). The replacement of the *rodZ* gene for *fp650-rodZ* was completed after a two-step homologous recombination event and was confirmed by PCR. The strain was named BCBHV200.

Plasmid pBCB44 was electroporated into RN4220 and transduced into JE2 or COL. The replacement of the *spa* gene for $P_{xyl/tetO3}$-*dcas9* was completed after a two-step homologous recombination event and the resulting strains BCBMS14 and BCBMS15 were verified by PCR and sequencing.

Fluorescence microscopy NCTC *fp650-rodZ* conditional mutant

Overnight cultures of strains expressing *dcas9-sgfp* (from *S. pyogenes* or *S. aureus*) with corresponding sgRNA targeting *fp650-rodZ* (BCBMS10-13; BCBLS05-07) and control strains (BCBMS06-09; BCBLS02-04, lacking *dcas9*) were back-diluted 1:500 in 10 ml of TSB, with 10 µg/ml Cm, 50 µg/ml of both Neo and Kan and 0.1 µM $CdCl_2$ and incubated at 37 °C, with aeration, to OD_{600nm} of 0.8. A 1 ml aliquot of culture was pelleted and re-suspended in 30 µl of phosphate buffered saline (PBS). A 5 µl sample of each dCas9-producing culture expressing a specific sgRNA, was mixed with an equal volume of the corresponding control culture lacking dCas9 but expressing the same sgRNA (see experimental setup in Figure 2A). 1 µl of the mixed culture was placed on a thin layer of 1.2% agarose in PBS and imaged using a Axio Observer.Z1 microscope equipped with a Photometrics CoolSNAP HQ2 camera (Roper Scientific), using phase contrast objective Plan Apo 100 x/1.4 oil Ph3. Cells were imaged using the ZEN software (Zeiss). The median fluorescence of sGFP and eqFP650 in each cell was determined using eHooke (48).

Microscopy analysis of conditional mutants in essential genes

Overnight cultures of derivatives of BCBMS14 (JE2 background) expressing sgRNAs against *ftsZ* (BCBMS16), *pbpA* (BCBMS17) or *murJ* (BCBMS18) were each back-diluted 1:1000 into fresh media containing 10 µg/ml Cm with (induced) or without (non-induced) 100 ng/ml aTc and grown at 37 °C. After 2 hours, a second 1:100 dilution of each culture was made and cells were grown to exponential phase (OD_{600nm} 0.8). A 1 ml aliquot of each culture was incubated with 2.5 µg/ml Nile Red (membrane stain, Invitrogen) and 1 µg/ml Hoechst 33342 (DNA stain, Invitrogen) for 5 min at 37 °C with shaking. The culture was then pelleted and resuspended in

30 µl PBS. 1 µl of cell culture was placed on a thin layer of 1.2% agarose in PBS and imaged as described above.

Western blot

S. aureus strains were grown overnight, diluted 1:500 in fresh medium and incubated at 37 °C with aeration. When necessary, cultures were supplemented with required antibiotics and aTc or $CdCl_2$. At OD_{600nm} of approximately 0.6, cells were harvested and broken with glass beads in a SpeedMill (Analytik Jena). Samples were centrifuged for 10 min at 16000 xg and resuspended in 300 µl PBS containing protease inhibitors (cOmplete, Roche). A 16 µl sample was added to 5 µl of 5x SDS sample buffer (300mM Tris-HCl, pH 6.8, 50% glycerol, 10% SDS, 0.01% Bromophenol Blue, 10% Beta-mercaptoethanol), boiled for 5 minutes and samples were separated by SDS-PAGE (12% gel) at 120V. Proteins were then transferred to Hybond-P PVDF membrane (GE Healthcare) using a BioRad Transblot Turbo transfer system, according to the manufacturer's instructions. After transfer, the membrane was cut at the 100 kDa marker band. The upper part was used for protein detection using a specific monoclonal antibody against Cas9 (1:1000 dilution, #MAC133, Sigma-Aldrich) and Alexa555 secondary antibody for fluorescence detection (iBright, ThermoFisher), while the lower part of the membrane was stained with Sypro Ruby Protein Blot Stain (Thermo Fisher), a total protein stain for western blot normalization.

Growth curves

For analysis of growth of the CRISPRi conditional mutants of BCBMS20-22 (COL with aTc-inducible CRISPRi), cells were grown overnight at 37 °C in TSB medium supplemented with 10 µg/mL Cm and diluted 1:500 into 100 ml Erlenmeyer flasks containing fresh TSB medium with 10 µg/ml Cm, with or without 100 ng/ ml aTc which induces expression of *dcas9*. After four hours, cultures were diluted a second time 1:100 in fresh medium and growth was followed by measuring the OD_{600nm} every hour. All cultures were incubated at 37 °C with agitation and the OD_{600nm} was recorded.

Analysis of the library of CRISPRi conditional mutants (derived from strain BCBMS14) was performed in a 96-well plate reader (Biotek Synergy Neo2). Overnight cultures were diluted 1:1000 in fresh media containing 10 µg/ml Cm. A 200 µl sample of each culture, in the presence and absence of 200 ng/ml aTc inducer, was added to a well in a 96-well plate. Plates were incubated at 30 °C with shaking for 120 min, the OD_{600nm} was measured every 30 minutes. After two hours, the samples were diluted 1:100 into fresh media (with 10 µg/ml Cm),

containing or not aTc and growth was followed in all cultures for a further 630 min. For CRISPRi conditional mutants showing less than 50% growth reduction at 30 °C, growth analysis was also performed at 37 °C.

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Table 1. Plasmids used and constructed in this study

Name	Description	Reference
pTRC99a-P7	Vector containing *sgfp-p7*; Amp^R	(36)
pFP60-F	pTnT with *fp650* in forward orientation	(46)
pgRNA_bacteria	Vector containing $sgRNA_{Spy}$	(8, 35)
pdCas9_bacteria	Vector containing $dcas9_{Spy}$	(8, 35)
pMAD	*E. coli* / *S. aureus* shuttle vector with a thermosensitive origin of replication for Gram positive bacteria; Amp^R; Ery^R; *lacZ*	(47)
pGC2	Replicative *E. coli* / *S. aureus* shuttle vector; Amp^R; Cm^R	(22)
pBCB13	pMAD with up- and downstream regions of *spa* and P*spac-lacI* region from pDH88	(38)
pCNX	Replicative *E. coli* / *S. aureus* shuttle vector containing the cadmium-inducible P*cad* promoter; Amp^R; Kan^R/Neo^R	(21)
pCNX_sGFP	pCNX derivative containing *sgfp* from plasmid pTRC99a-P7	This study
pBCB40	pCNX derivative containing *S. pyogenes dcas9;* Amp^R; Kan^R/Neo^R	This study
pBCB41	pCNX derivative containing *S. pyogenes dcas9-sgfp;* Amp^R; Kan^R/Neo^R	This study
pBCB42	pCNX derivative containing *S. aureus dcas9-sgfp;* Amp^R; Kan^R/Neo^R	This study
pBCB43	pBCB13 derivative lacking P_{spac} and *lacI*, containing *tetR*, $P_{xyltetO}$ and a downstream located *tetO* site, Amp^R; Ery^R; *lacZ*	This study
pBCB44	pBCB43 derivative containing $dcas9_{Spy}$ under $P_{xyl/tetO3}$ control*;* Amp^R; Ery^R; *lacZ*	This study
psg-RNA_{Spy}	pGC2 derivative containing the sgRNA cloned from pgRNA_bacteria plasmid	This study
psg-*fp650rodZ-Spy*1	psg-RNA_{Spy} derivative with sgRNA altered to sg-*fp650rodZ-Spy*1; Amp^R; Cm^r	This study
psg-*fp650rodZ-Spy*2	psg-RNA_{Spy} derivative with sgRNA altered to sg-*fp650rodZ-Spy2*; Amp^R; Cm^R	This study
psg-*fp650rodZ-Spy*3	psg-RNA_{Spy} derivative with sgRNA altered to sg-*fp650rodZ-Spy3*; Amp^R; Cm^R	This study
psg-*fp650rodZ-Spy*4	psg-RNA_{Spy} derivative with sgRNA altered to sg-*fp650rodZ-Spy4*; Amp^R; Cm^R	This study
psg-*fp650rodZ-Sa*1	pGC2 derivative containing the sg-*fp650rodZ-Sa*1 under control of a constitutive active promoter; Amp^R; Cm^R	This study
psg-*fp650rodZ-Sa*2	pGC2 derivative containing the sg-*fp650rodZ-Sa2* under control of a constitutive active promoter; Amp^R; Cm^R	This study
psg-*fp650rodZ-Sa*3	pGC2 derivative containing the sg-*fp650rodZ-Sa3* under control of a constitutive active promoter; Amp^R; Cm^R	This study
psg-*ftsZ*	psg-RNA_{Spy} derivative with sgRNA altered to sg-*ftsZ*; Amp^R; Cm^R	This study
psg-*pbpA*	psg-RNA_{Spy} derivative with sgRNA altered to sg-*pbpA*; Amp^R; Cm^R	This study
psg-*murJ*	psg-RNA_{Spy} derivative with sgRNA altered to sg-*murJ*; Amp^R; Cm^R	This study
pMAD_*fp650rodZ*	pMAD containing *rodZ* upstream region-*eqfp650–rodZ-rodZ* downstream region; Amp^r Ery^r	This study

Table 2. Strains used in this study

Name	Description	Reference
NCTC8325-4	MSSA strain NCTC8325-4	R. Novick
COL	Hospital acquired homogeneous MRSA	(49)
JE2	Derivative of community acquired MRSA	(17)
BCBMS01	JE2 with pBCB40; expressing *S. pyogenes dcas9$_{Spy}$*; Kan/Neo^R	This study
BCBMS02	JE2 with pBCB41; expressing *S. pyogenes dcas9$_{Spy}$-sgfp*; Kan/Neo^R	This study
BCBHV200	NCTC8325-4 *ΔrodZ:fp650rodZ*; expressing *rodZ* translationally fused to fluorescent protein eqFP650	This study
BCBMS04	BCBHV200 with pCNX; serves as control; Kan/Neo^R	This study
BCBMS05	BCBHV200 with pBCB41, expressing *S. pyogenes dcas9$_{Spy}$-sgfp*; Kan/Neo^R	This study
BCBMS06	BCBMS04 with psg-*fp650rodZ-Spy*1; Cm^R, Kan/Neo^R	This study
BCBMS07	BCBMS04 with psg-*fp650rodZ-Spy*2; Cm^R, Kan/Neo^R	This study
BCBMS08	BCBMS04 with psg-*fp650rodZ-Spy*3; Cm^R, Kan/Neo^R	This study
BCBMS09	BCBMS04 with psg-*fp650rodZ-Spy*4; Cm^R, Kan/Neo^R	This study
BCBMS10	BCBMS05 with psg-*fp650rodZ-Spy*1; Cm^R, Kan/Neo^R	This study
BCBMS11	BCBMS05 with psg-*fp650rodZ-Spy*2; Cm^R, Kan/Neo^R	This study
BCBMS12	BCBMS05 with psg-*fp650rodZ-Spy*3; Cm^R, Kan/Neo^R	This study
BCBMS13	BCBMS05 with psg-*fp650rodZ-Spy*4; Cm^R, Kan/Neo^R	This study
BCBLS01	BCBHV200 with pBCB42; expressing *S. aureus dcas9$_{Sa}$-sgfp*; Kan/Neo^R	This study
BCBLS02	BCBMS04 with psg-*fp650rodZ-Sa*1; Cm^R, Kan/Neo^R	This study
BCBLS03	BCBMS04 with psg-*fp650rodZ-Sa*2; Cm^R, Kan/Neo^R	This study
BCBLS04	BCBMS04 with psg-*fp650rodZ-Sa*3; Cm^R, Kan/Neo^R	This study
BCBLS05	BCBLS01 with psg-*fp650rodZ-Sa*1; Cm^R, Kan/Neo^R	This study
BCBLS06	BCBLS01 with psg-*fp650rodZ-Sa*2; Cm^R, Kan/Neo^R	This study
BCBLS07	BCBLS01 with psg-*fp650rodZ-Sa*3; Cm^R, Kan/Neo^R	This study
BCBMS14	JE2 *Δspa*: P$_{xyl/tetO3}$-*dcas9$_{Spy}$*; expressing *dcas9$_{Spy}$* under the control of aTc-inducible promoter P$_{xyl/tetO3}$ from the *spa* locus	This study
BCBMS15	COL *Δspa*: P$_{xyl/tetO3}$-*dcas9$_{Spy}$*; expressing *dcas9$_{Spy}$* under the control of an aTc-inducible promoter P$_{xyl/tetO3}$ from *spa* locus	This study
BCBMS16	BCBMS14 with psg-*ftsZ*; Cm^R	This study
BCBMS17	BCBMS14 with psg-*pbpA*; Cm^R	This study
BCBMS18	BCBMS14 with psg-*murJ*; Cm^R	This study
BCBMS20	BCBMS15 with psg-*ftsZ*; Cm^R	This study
BCBMS21	BCBMS15 with psg-*pbpA*; Cm^R	This study
BCBMS22	BCBMS15 with psg-*murJ*; Cm^R	This study
BCBMS26	BCBHV200 with pBCB40 and psg-*fp650rodZ-Spy*2; Cm^R, Kan/Neo^R	This study

Table 3. Primers used in this study

Number	Sequence
3381	CAAGGAGGCGCCGCAGGAACTATGACCTAATTTTGATGG
3382	CATAGTTCCTGCGGCGCCTCCTTGAAAACGGTCGGTGAAGCGC
3627	GCTGCGCTGTCGACGCTCATACATGCGCCTATTTATATC
3628	CTTCACCCATTTCATAGCCTCCTTACACTTACTCG
3629	GGAGGCTATGAAATGGGTGAAGATAGTGAATTAATTAG
3630	TGCATTCCATGGCCATATGAAAATGAAATCTAG
3714	TACTCCCGGGGGAGGCGCCGCAGGAGTCACCTCCTAGCTGACTC
3715	CCCGGGAGTAAAGGAGAAGAACTTTTCACTGG
5450	AACTGCAGAAAAAATAAGGAGGAAAAAAAATGGATAAGAAATACTCAA
5451	CCGACGTCGACTTAGTCACCTCCTAGCTGACTCA
5452	CCGACGTCGACTTATTTGTATAGTTCATCCA
5602	CGTGGATCCTCCTGCGGCGCCTCCAGTAAAGGAGAAGAACTTTTCACTG
5603	CGCCGAATTCTTATTTGTATAGTTCATCCATGCC
5639	CGCGCCCGGGCAACACATACTTGTACTTGCC
5745	CGAGCTCGAATCCGGTCTCCC
5746	GGGAGACCGGATTCGAGCTCG
5837	GCGGGAATTCGTCATATGTAGAGAGGTACC
5838	GCGGGAATTCGTCATATGTAGAGAGGTACCTCGAGCGGCCCAAGCTTAAAAAAATCTCGCCAACAAGTTGACGAGATAAACACGGCATTTTGCCTTGTTTTAGTAGATTCTGTAATTTTCATTACAGAGTACTAAAACTCTAATTTACGATCAACAAAATGCGAAAGACACAATACACC
5839	GTACTAAAACGATTTCTTTAAACAATCTTTTTGCGAAAGACACAATACACC
5840	GTCTTTCGCAAAAAGATTGTTTAAAGAAATCGTTTTAGTACTCTGTAATGA
5846	ACTAGTATTATACCTAGGACTGAGCTAGC
5922	AATTCTAAAGATCTCGCGGGATCCTTACTCGAGATCCCCGGGCGAGCTCGAATCCG
5923	CTCTAGAGTCGACCTGCAGCC
6013	CGCGCCCGGGTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGTCCCATTCGGCTTGGTTCGAGTTTTAGTACTCTGTAATGA
7268	TCTATCATTGATAGAGTCCCGGGAAAAAAAAATAAGGAGGAAAATGGATAAGAAATACTCAATAGGC
7272	ATTAATGCAGCGCTAGCTACGGCCGTTAGTCACCTCCTAGCTGAC
5849	TTTGGAGATCCGTTTGGTGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
5887	AAACCAAAACCACCACCAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
5889	TTCCATCGAACCAAGCCGAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
5890	TCCCATTCGGCTTGGTTCGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC

6423	CTTGGTAATTAATATACTAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6424	AAATTTCCTCCTAGTTTTATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6426	TCATATATCTTTCCTCGTTCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
7015	ATATGAATTCTTAAGACCCACTTTCACATTTAAG
7016	ATATGCTAGCTACGGCCGTACCCGGGACTCTATCAATGATAGAGAGCTTATTTTAATTATACTCTATCA
7268	TCTATCATTGATAGAGTCCCGGGAAAAAAAAAATAAGGAGGAAAATGGATAAGAAATACTCAATAGGC
7272	ATTAATGCAGCGCTAGCTACGGCCGTTAGTCACCTCCTAGCTGAC

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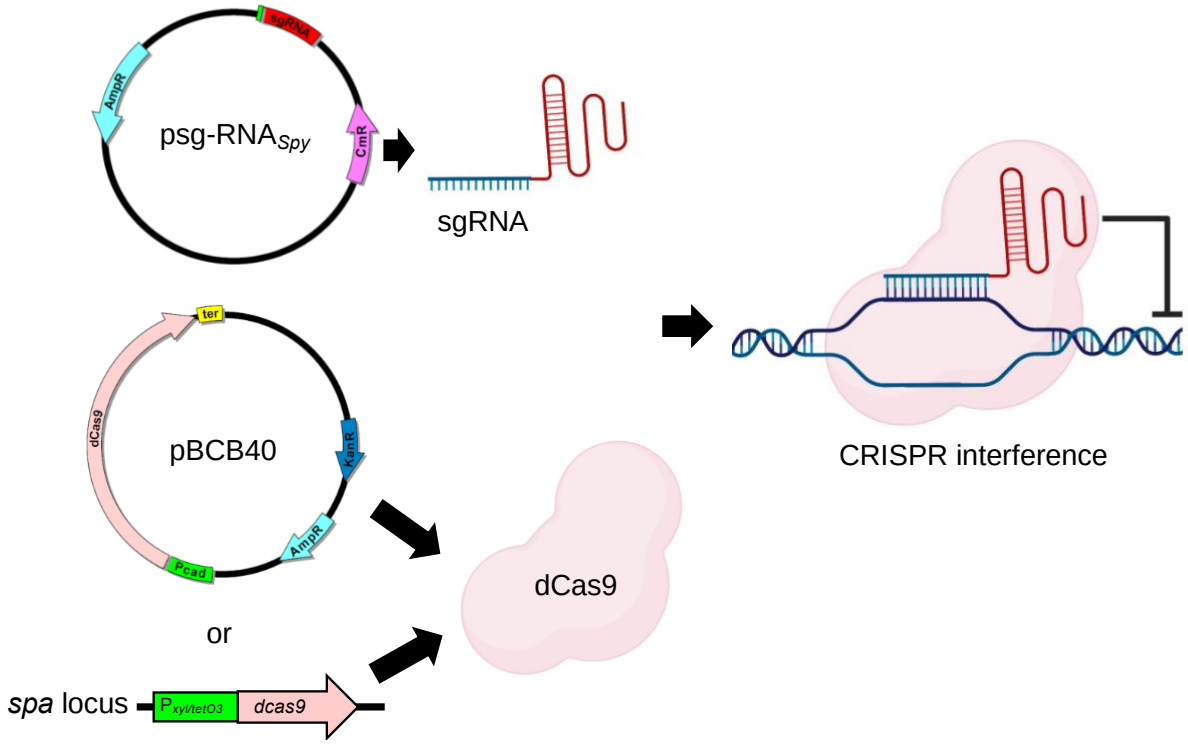

Figure 1. CRISPRi system based on *S. pyogenes* dCas9 to inhibit gene expression in *S. aureus*. The sgRNA for *S. pyogenes* Cas9 is constitutively expressed from psg-sgRNA$_{Spy}$ plasmid. dCas9$_{Spy}$ can either be expressed from a second plasmid (pBCB40), under the control of a cadmium-inducible promoter, or from the *spa* locus in the *S. aureus* chromosome, under the control of a tetracycline-inducible promoter. Transcription inhibition occurs when a complex of dCas9 and sgRNA binds specific target genes, thus blocking the RNA polymerase.

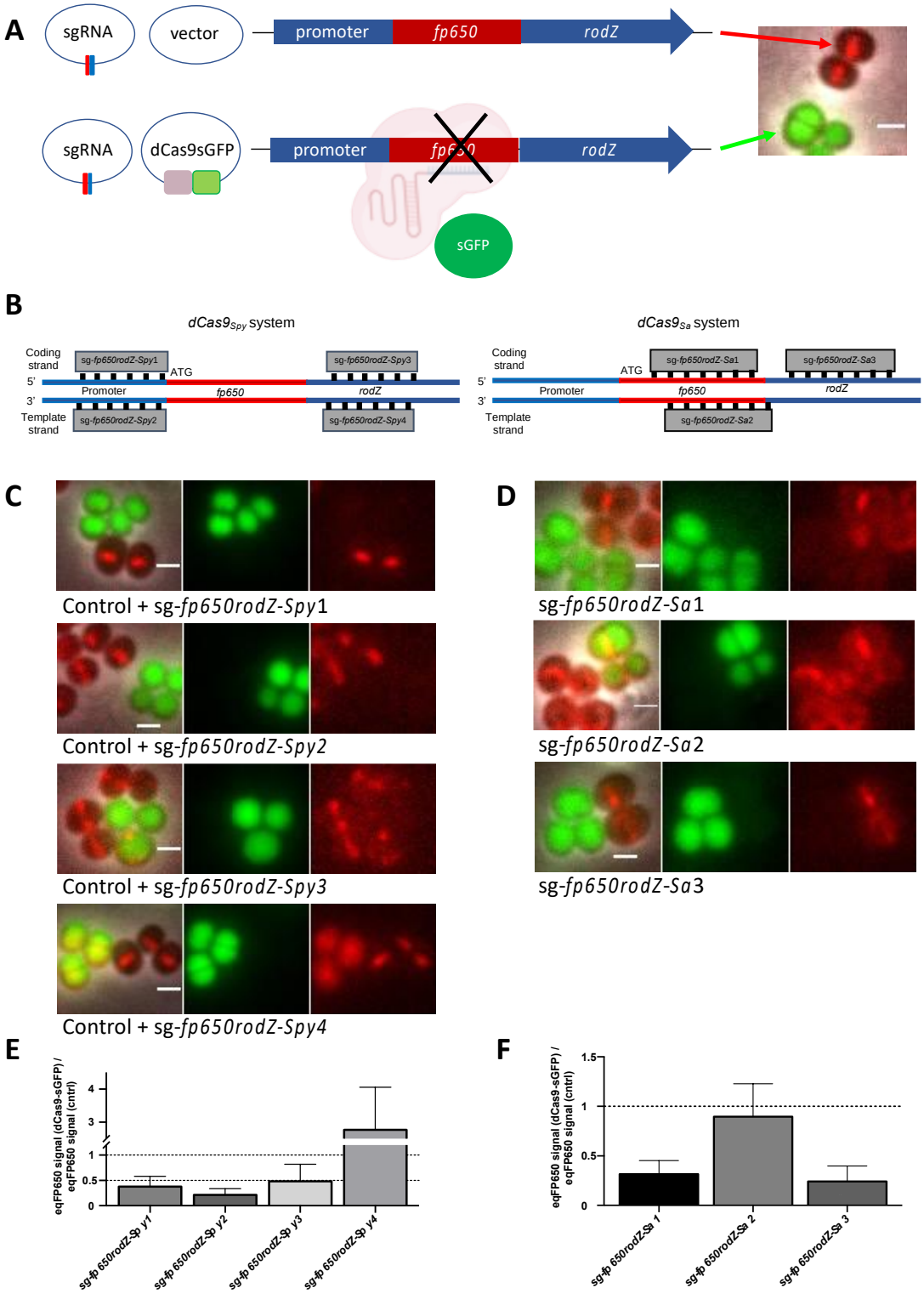

Figure 2. CRISPRi systems using either dCas9$_{Spy}$ or dCas9$_{Sa}$ inhibit gene expression in *S. aureus*. (**A**) Experimental setup to test CRISPRi systems. Genetically modified NCTC8324-5 cells producing an eqFP650 fusion to the non-essential septal protein RodZ were transformed with plasmids encoding sgRNA against *fp650-rodZ* and either an empty vector control (top, control cells) or the same vector encoding dCas9-sGFP (bottom, test cells). Strains were grown separately and subsequently mixed to perform the microscope experiments. Control cells lack dCas9 and therefore do not impair production of eqFP650-RodZ, which can be seen as a septal band (red). Test cells produce a cytoplasmic GFP fusion of dCas9 (green) which is

targeted by sgRNA to specific regions of *fp650-rodZ*, blocking its expression. (**B**) Schematic overview of sgRNA target sites in *fp650-rodZ* used to test the dCas9$_{Spy}$ (left) or dCas9$_{Sa}$ (right) CRISPRi systems. (**C**, **D**) Fluorescence microscopy images of mixtures of control cells and test cells (producing dCas9-GFP) expressing sgRNAs shown in panel B using either the dCas9$_{Spy}$ (**C**) or dCas9$_{Sa}$ (**D**) systems. Scale bars, 1 µm. (**E**, **F**) Bar charts showing the ratio of the median eqFP650 signal of cells producing dCas9-GFP versus the median eqFP650 signal of the corresponding control cells, both expressing the same sgRNA. Error bars represent standard deviation from three independent experiments.

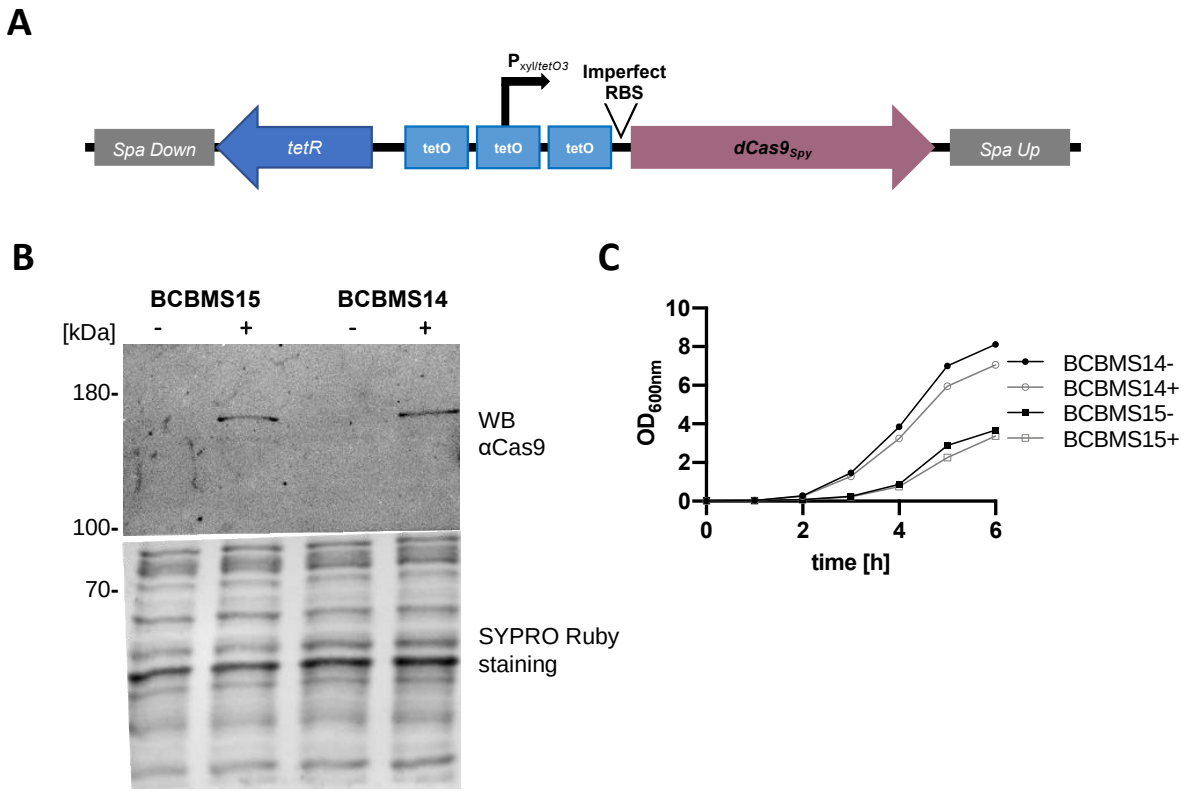

Figure 3. Optimized CRISPRi system provides tight regulation of dCas9*Spy* production. (**A**) Schematic representation of the genomic construct inserted into the *spa* locus of strains BCBMS14 (JE2 background) and BCBMS15 (COL background), which allows tight control of dCas9*Spy* production under the control of $P_{xyl/tetO3}$, using an inefficient ribosome binding site. The sgRNA is expressed from plasmid psg-RNA$_{Spy}$ (not shown) (**B**) Top: Western Blot, using anti-Cas9 antibody, of extracts from aTc induced (+) and non-induced (-) BCBMS15 and BCBMS14 cultures. Bottom: Sypro Ruby staining of proteins was used as a loading control. **(C)** Growth curves of induced (100 ng/ml aTc) and uninduced (no aTc) BCBMS14 and BCBMS15.

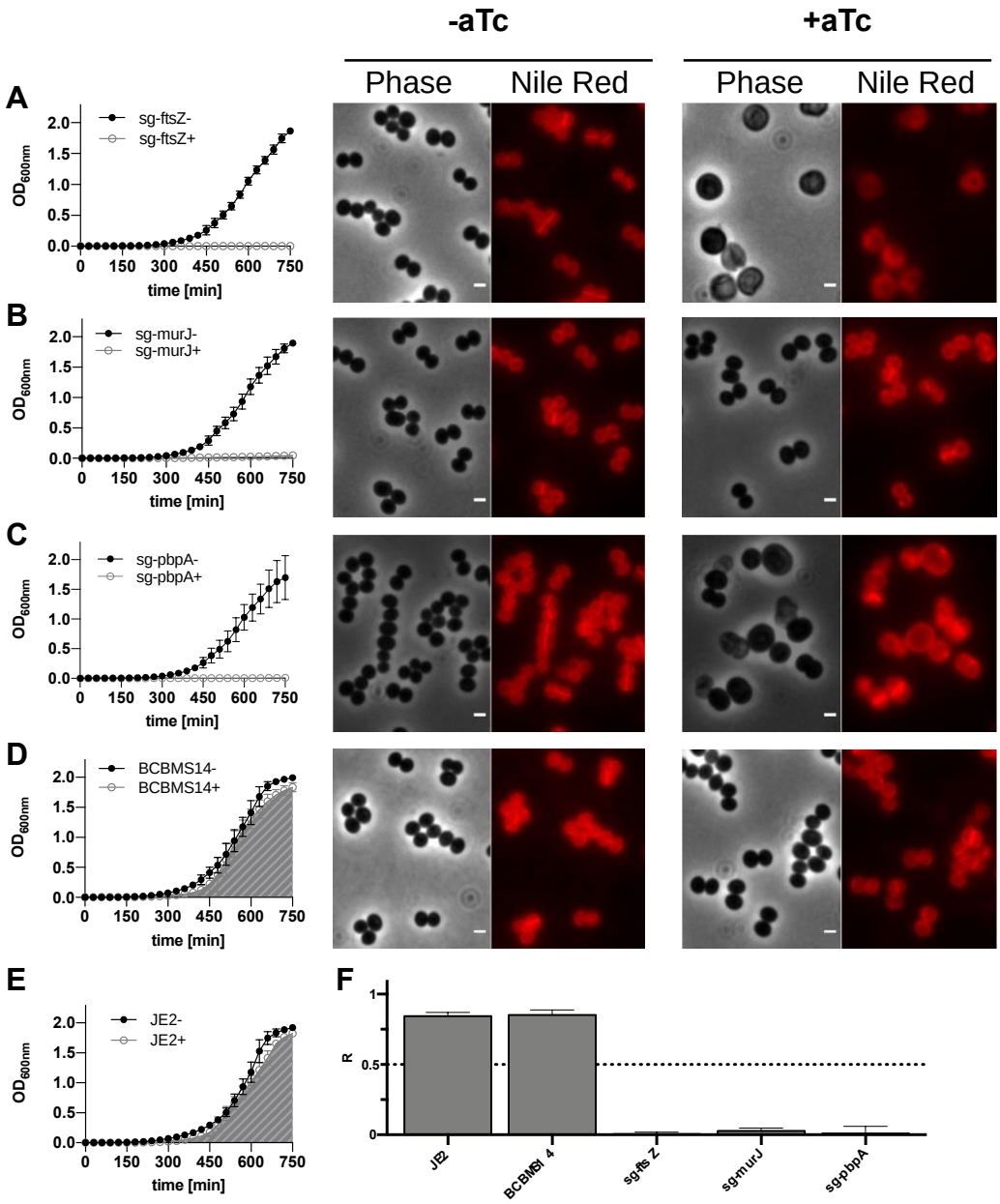

Figure 4. Optimized CRISPRi system can be used to impair transcription of essential genes. Growth assays performed in 96-well plates for 750 min at 30 °C in TSB, in the presence or absence of aTc (200 ng/ml, inducer for *dcas9* expression) of derivatives of BCBMS14 expressing sgRNAs targeting the essential genes *ftsZ* (**A**), *pbpA* (**B**) and *murJ* (**C**). Strains BCBMS14 **(D)** and wild-type JE2 **(E)** were used as controls. Mutant specific phenotypes were confirmed by fluorescence microscopy of cells grown at 37 ºC with aTc (100 ng/ml), stained with Nile Red (membrane) and Hoechst 33342 (DNA). A phase contrast image is shown on the left. Scale bars, 1 µm (**F**) The area under the curve (AUC) of growth curves obtained in the presence and absence of aTc for JE2, BCBMS14 and the strains expressing sgRNA against essential genes was measured and the ratio (R) of AUC +aTc/ AUC -aTc was plotted. This ratio varies between 0 (complete growth inhibition in the presence of the inducer) and 1 (no growth inhibition). Error bars indicate SEM calculated from data of three independent experiments.

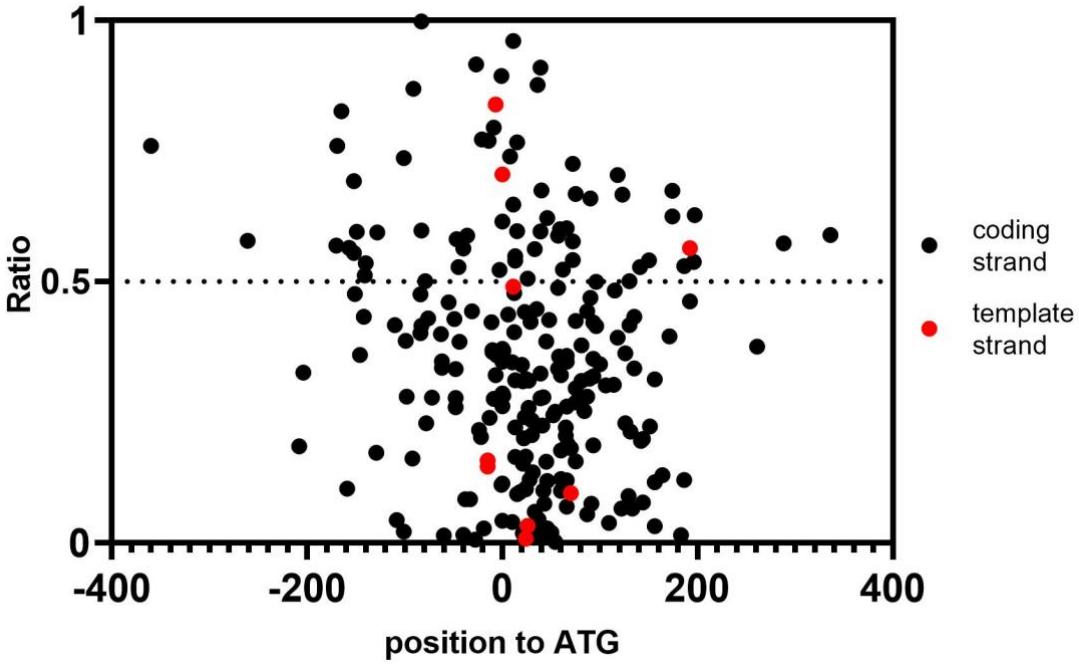

Figure 5. Localization of sgRNA target does not predict efficiency of CRISPRi. The ratio (R) of the area under the curve (AUC) of growth curves obtained in the presence vs the absence of aTc (AUC+aTc/AUC-aTc) was calculated for strains expressing sgRNAs targeting the coding (black) or template (red) strands of essential genes and plotted against the sgRNA target position relative to the start codon of the targeted gene.

SUPPLEMENTARY INFORMATION

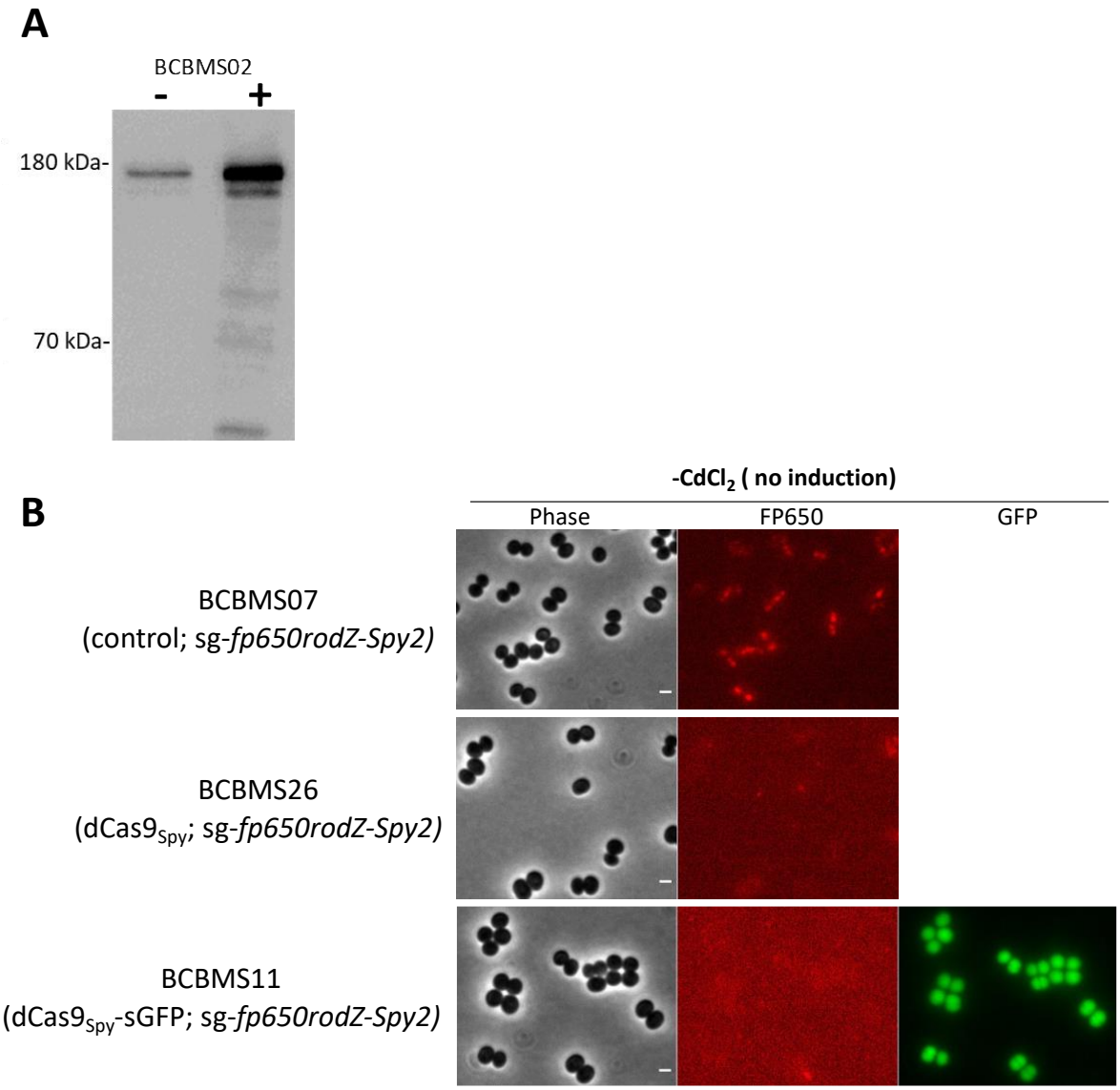

Figure S1. Two plasmid CRISPRi system has leaky dCas9 expression. (**A**) Western Blot, using anti-Cas9 antibody, of extracts from induced (+, 0.1 µM $CdCl_2$) and non-induced (-) BCBMS02 cultures shows $dCas9_{Spy}$ production even in the absence of inducer (**B**) Fluorescence microscopy images of BCBMS26 and BCBMS11 strains producing $dCas9_{Spy}$ or $dCas9_{Spy}$-GFP, respectively, from a pCNX based plasmid, and sgRNAs targeting *fp650-rodZ*, showed depletion of eqFP650-RodZ in the absence of $CdCl_2$ inducer. Control strain lacking dCas9 (BCBMS07) shows eqFP650-RodZ localized at the septum. Scale bars, 1 µm.

Figure S2. Nucleotide sequence of the dCas9 regulatory region inserted into the *spa* locus of BCBMS14/15 strains. The promoter is based on the tetracycline-inducible $P_{xyl/tetO}$ promoter with minimized promoter leakiness. In addition, an imperfect RBS was used to reduce dCas9 production. -35 and -10 regions of the xylose core promoter are indicated. Note that only a 5´end portion of the *dcas9* coding sequence is shown.

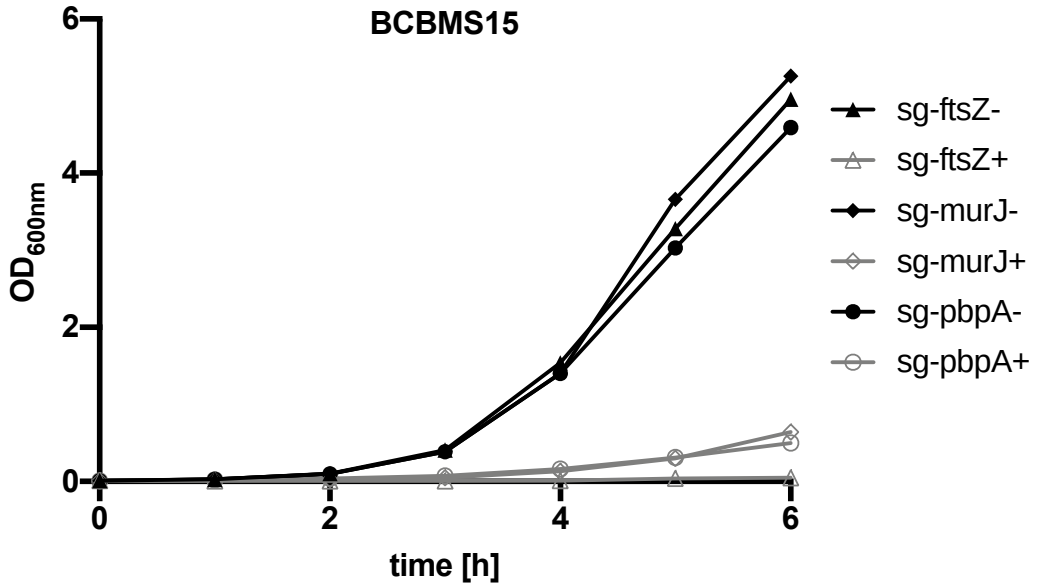

Figure S3. Optimized CRISPRi system can be used to impair transcription of essential genes in MRSA strain COL background. Derivatives of strain BCBMS15 expressing sgRNAs targeting the essential genes *ftsZ* (BCBMS20), *pbpA* (BCBMS21) and *murJ* (BCBMS22) show severe growth defects upon induction with 100 ng/ml aTc.

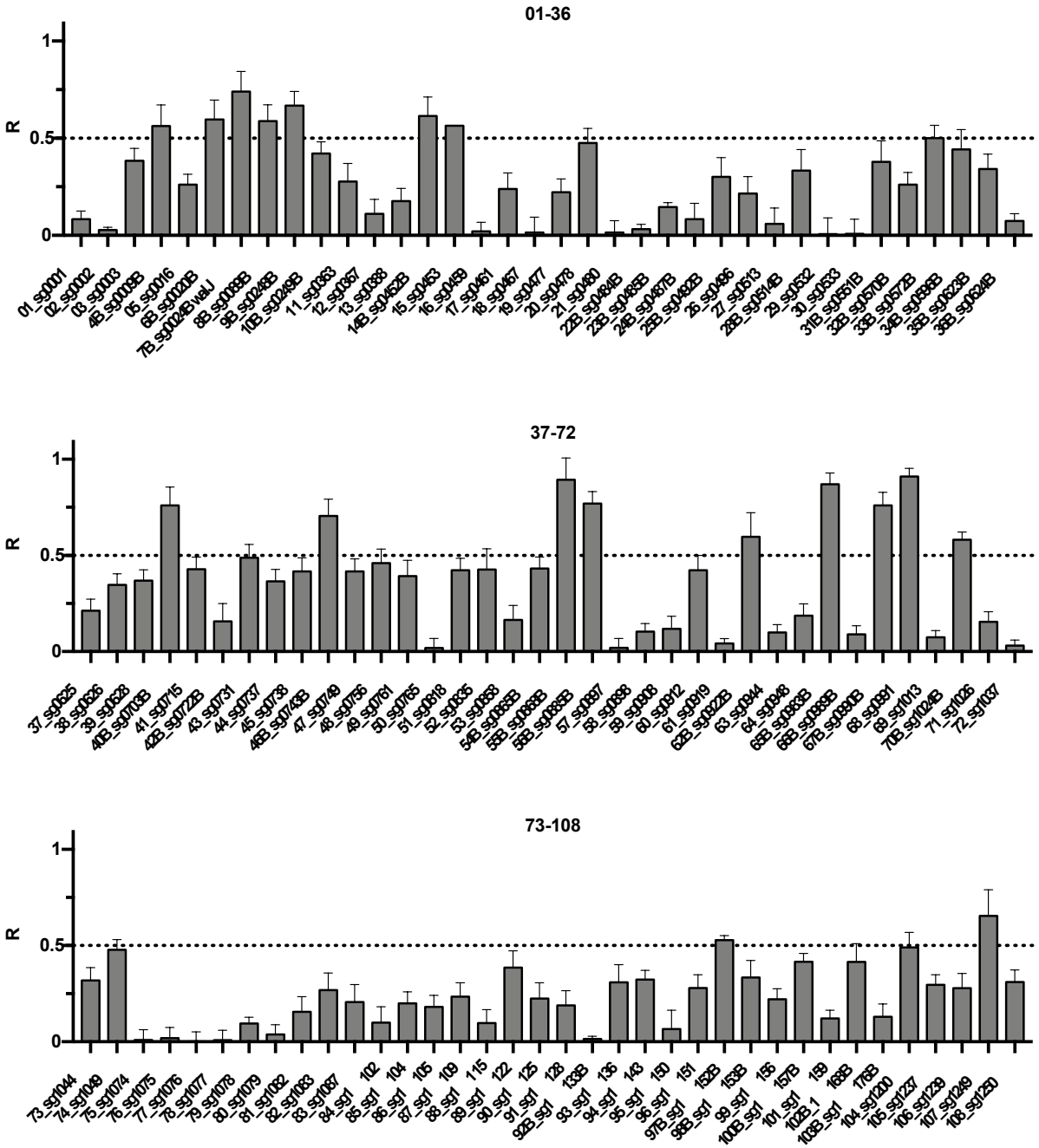

Figure S4. Growth inhibition of Lisbon CRISPRi mutant library (LCML) strains. Effect of CRISPRi on growth at 30 ºC using sgRNAs targeting 241 essential genes in the strain BCBMS14. Error bars represent SEM from three independent experiments (continues next page)

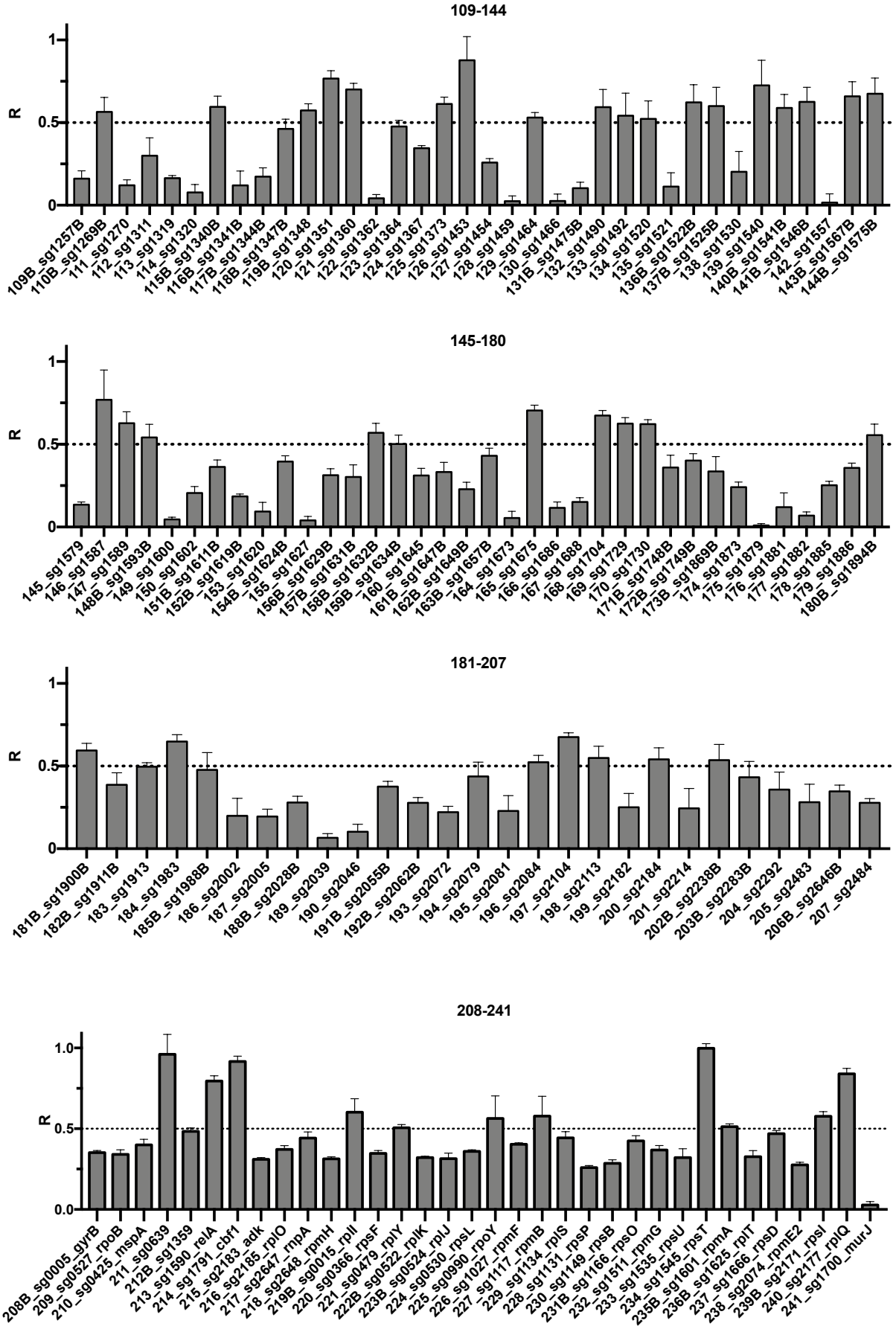

Figure S4. Growth inhibition of Lisbon CRISPRi mutant library (LCML) strains (cont).

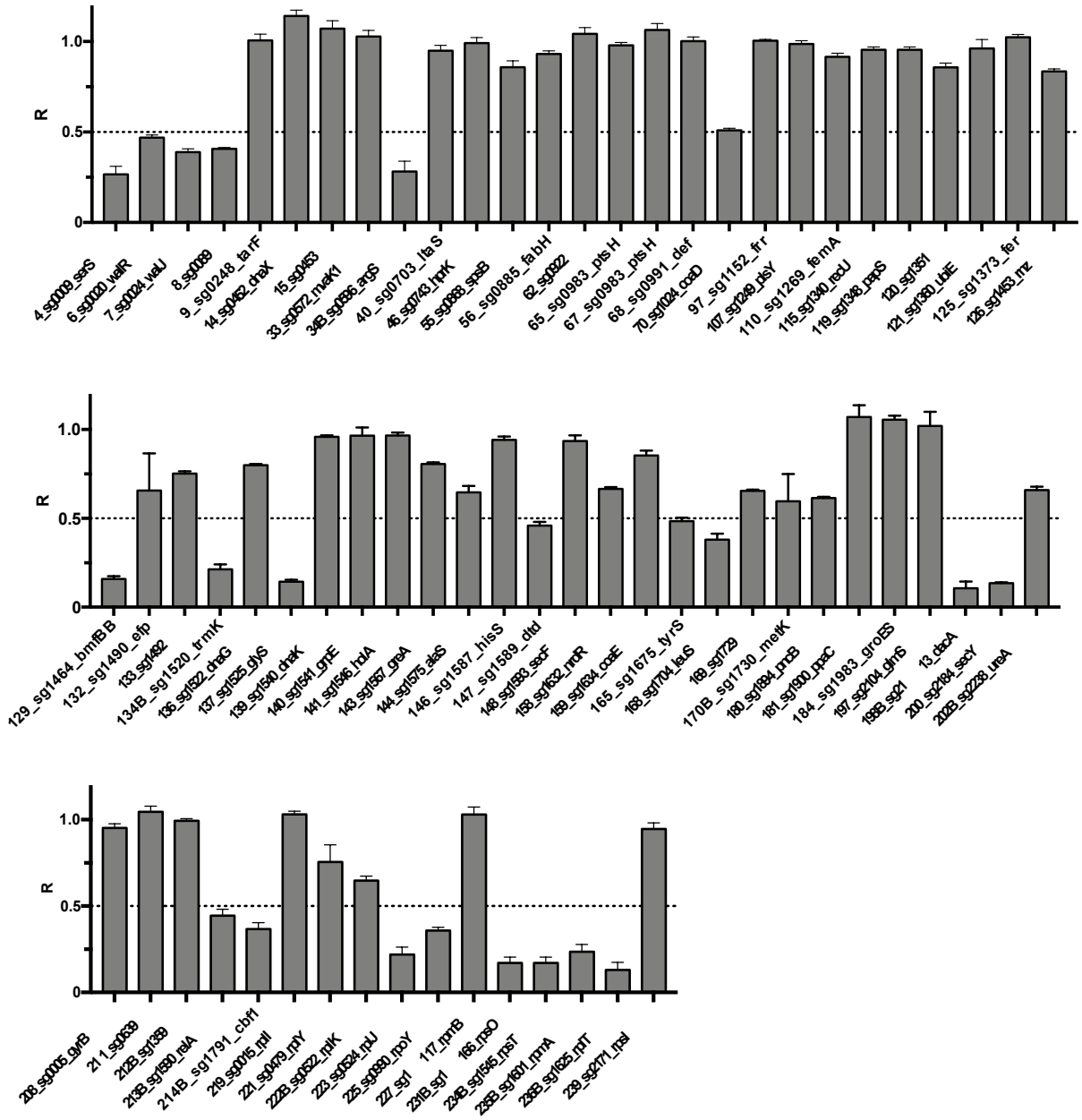

Figure S5. Growth inhibition at 37 ºC of a subset of Lisbon CRISPRi mutant library (LCML) strains. Effect of CRISPRi on growth at 37 ºC using of a subset of strains that exhibited a growth reduction of less than 50% using CRISPRi at 30 ºC. Error bars represent SEM from three independent experiments.

Supplementary Table 1. Essential operons identified in *Staphylococcus aureus*

Operon Number	Gene position in operon	Locus Tag	Gene Name
1	1	SAUSA300_0001	***dnaA***
2	1	SAUSA300_0002	***dnaN***
3	1	SAUSA300_0003	***0003***
3	2	SAUSA300_0004	*recF*
3	3	SAUSA300_0005	*gyrB*
3	4	SAUSA300_0006	*gyrA*
4	1	SAUSA300_0009	***serS***
5	1	SAUSA300_0016	***dnaB***
6	1	SAUSA300_0020	***walR***
6	2	SAUSA300_0021	*walK*
6	3	SAUSA300_0022	*walH*
6	4	SAUSA300_0023	*walI*
7	1	SAUSA300_0024	***walJ***
8	1	SAUSA300_0089	***0089***
9	1	SAUSA300_0248	***tarF***
10	1	SAUSA300_0249	***tarI / ispD***
10	2	SAUSA300_0250	*tarJ*
10	3	SAUSA300_0251	*tarL*
10	4	SAUSA300_0252	*tarS*
11	1	SAUSA300_0363	***0363***
12	1	SAUSA300_0367	***ssb***
13	1	SAUSA300_0386	*xpt*
13	2	SAUSA300_0387	*pbuX*
13	3	SAUSA300_0388	***guaB***
13	4	SAUSA300_0389	*guaA*
14	1	SAUSA300_0452	***dnaX***
15	1	SAUSA300_0453	***0453***
15	2	SAUSA300_0454	*recR*
16	1	SAUSA300_0458	*0458*
16	2	SAUSA300_0459	***tmk***
16	3	SAUSA300_0460	*pstA*
17	1	SAUSA300_0461	***holB***
17	2	SAUSA300_0462	*0462*
17	3	SAUSA300_0463	*0463*
18	1	SAUSA300_0467	***metS***
18	2	SAUSA300_0468	*0468*
19	1	SAUSA300_0477	***glmU***
20	1	SAUSA300_0478	***prs***
21	1	SAUSA300_0479	*0479*
21	2	SAUSA300_0480	***pth***
21	3	SAUSA300_0481	*mfd*
21	4	SAUSA300_0482	*0482*
21	5	SAUSA300_0483	*0483*

Operon Number	Gene position in operon	Locus Tag	Gene Name
21	6	SAUSA300_0484	*0484*
21	7	SAUSA300_0485	*0485*
22	1	SAUSA300_0484	***0484***
22	2	SAUSA300_0485	***divIC***
23	1	SAUSA300_0487	***tilS***
24	1	SAUSA300_0492	***folP***
24	2	SAUSA300_0493	*folB*
24	3	SAUSA300_0494	*folK*
25	1	SAUSA300_0496	***lysS***
26	1	SAUSA300_0513	***gltX***
27	1	SAUSA300_0514	***cysE***
27	2	SAUSA300_0515	*cysS*
27	3	SAUSA300_0516	*0516*
27	4	SAUSA300_0517	*0517*
27	5	SAUSA300_0518	*0518*
28	1	SAUSA300_0529	*0529*
28	2	SAUSA300_0530	*rpsL*
28	3	SAUSA300_0531	*0531*
28	4	SAUSA300_0532	***fusA***
29	1	SAUSA300_0533	***tuf***
30	1	SAUSA300_0553	*0553*
30	2	SAUSA300_0552	*0552*
30	3	SAUSA300_0551	***folE2***
31	1	SAUSA300_0570	***eutD***
31	2	SAUSA300_0571	*lipL*
32	1	SAUSA300_0572	***mvaK1***
32	2	SAUSA300_0573	*mvaD*
32	3	SAUSA300_0574	*mvaK2*
33	1	SAUSA300_0595	*0595*
33	2	SAUSA300_0596	***argS***
34	1	SAUSA300_0623	***tagA***
35	1	SAUSA300_0624	***tagH***
36	1	SAUSA300_0625	***tagG***
36	2	SAUSA300_0626	***tagB***
36	3	SAUSA300_0627	*tagX*
37	1	SAUSA300_0628	***tagD***
38	1	SAUSA300_0703	***ltaS***
39	1	SAUSA300_0715	***nrdI***
39	2	SAUSA300_0716	*nrdE*
39	3	SAUSA300_0717	*nrdF*
40	1	SAUSA300_0722	***murB***
41	1	SAUSA300_0731	***tagO***
42	1	SAUSA300_0737	***secA***
43	1	SAUSA300_0738	***prfB***
44	1	SAUSA300_0743	***hprK***

Operon Number	Gene position in operon	Locus Tag	Gene Name
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44	3	SAUSA300_0745	*0745*
44	4	SAUSA300_0746	*0746*
44	5	SAUSA300_0747	*trxB*
45	1	SAUSA300_0748	*0748*
45	2	SAUSA300_0749	***0749***
45	3	SAUSA300_0750	*0750*
46	1	SAUSA300_0755	*gapR*
46	2	SAUSA300_0756	***gapA***
46	3	SAUSA300_0757	*pgk*
46	4	SAUSA300_0758	*tpiA*
46	5	SAUSA300_0760	*eno*
47	1	SAUSA300_0761	*0761*
48	1	SAUSA300_0763	*est*
48	2	SAUSA300_0764	*rnr*
48	3	SAUSA300_0765	***smpB***
49	1	SAUSA300_0818	***sufC***
49	2	SAUSA300_0819	*sufD*
49	3	SAUSA300_0820	*sufS*
49	4	SAUSA300_0821	*sufU*
49	5	SAUSA300_0822	*sufB*
50	1	SAUSA300_0835	***dltA***
50	2	SAUSA300_0836	*dltB*
50	3	SAUSA300_0837	*dltC*
50	4	SAUSA300_0838	*dltD*
51	1	SAUSA300_0858	***0858***
52	1	SAUSA300_0865	***pgi***
53	1	SAUSA300_0866	*0866*
53	2	SAUSA300_0867	*spsA*
53	3	SAUSA300_0868	***spsB***
54	1	SAUSA300_0885	***fabH***
54	2	SAUSA300_0886	*fabF*
55	1	SAUSA300_0897	***trpS***
56	1	SAUSA300_0898	***spxA***
57	1	SAUSA300_0906	*0906*
57	2	SAUSA300_0907	*relQ*
57	3	SAUSA300_0908	***ppnK***
57	4	SAUSA300_0909	*0909*
57	5	SAUSA300_0910	*mgtE*
57	6	SAUSA300_0911	*cpaA*
58	1	SAUSA300_0912	***fabI***
59	1	SAUSA300_0919	***murE***
59	2	SAUSA300_0920	*0920*
59	3	SAUSA300_0921	*prfC*
60	1	SAUSA300_0922	***0922***

Operon Number	Gene position in operon	Locus Tag	Gene Name
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62	1	SAUSA300_0945	*0945*
62	2	SAUSA300_0946	*menD*
62	3	SAUSA300_0947	*0947*
62	4	SAUSA300_0948	***menB***
63	1	SAUSA300_0983	***ptsH***
63	2	SAUSA300_0984	*ptsI*
64	1	SAUSA300_0989	***rnjA***
64	2	SAUSA300_0990	***rpoY***
65	1	SAUSA300_0991	***def***
66	1	SAUSA300_1013	***ftsW***
67	1	SAUSA300_1023	*rsmD*
67	2	SAUSA300_1024	***coaD***
68	1	SAUSA300_1026	***1026***
68	2	SAUSA300_1027	***rpmF***
69	1	SAUSA300_1037	*pheS*
69	2	SAUSA300_1038	*pheT*
70	1	SAUSA300_1044	***trxA***
71	1	SAUSA300_1049	***murI***
71	2	SAUSA300_1050	*1050*
71	3	SAUSA300_1051	*1051*
72	1	SAUSA300_1072	*mraZ*
72	2	SAUSA300_1073	*mraW*
72	3	SAUSA300_1074	***ftsL***
72	4	SAUSA300_1075	***pbp1***
73	1	SAUSA300_1076	***mraY***
73	2	SAUSA300_1077	***murD***
73	3	SAUSA300_1078	***divIB***
73	4	SAUSA300_1079	***ftsA***
73	5	SAUSA300_1080	***ftsZ***
74	1	SAUSA300_1081	*1081*
74	2	SAUSA300_1082	***1082***
74	3	SAUSA300_1083	***sepF***
74	4	SAUSA300_1084	*1084*
74	5	SAUSA300_1085	*1085*
74	6	SAUSA300_1086	*1086*
75	1	SAUSA300_1087	***ileS***
76	1	SAUSA300_1102	***gmk***
76	2	SAUSA300_1103	*rpoZ*
77	1	SAUSA300_1104	***coaBC***
77	2	SAUSA300_1105	***priA***
78	1	SAUSA300_1108	*def*
78	2	SAUSA300_1109	***fmt***
78	3	SAUSA300_1110	*sun*
78	4	SAUSA300_1111	*rlmN*

Operon Number	Gene position in operon	Locus Tag	Gene Name
78	**5**	SAUSA300_1112	*stpI*
78	**6**	SAUSA300_1113	*pknB*
79	**1**	SAUSA300_1114	*rsgA*
79	**2**	SAUSA300_1115	***rpe***
79	**3**	SAUSA300_1116	*thiN*
80	**1**	SAUSA300_1121	*fapR*
80	**2**	SAUSA300_1122	***plsX***
80	**3**	SAUSA300_1123	*fabD*
80	**4**	SAUSA300_1124	*fabG*
81	**1**	SAUSA300_1125	***acpP***
82	**1**	SAUSA300_1127	*smc*
82	**2**	SAUSA300_1128	***ftsY***
82	**3**	SAUSA300_1129	*1129*
82	**4**	SAUSA300_1130	*ffh*
83	**1**	SAUSA300_1131	*rpsP*
83	**2**	SAUSA300_1132	*rimM*
83	**3**	SAUSA300_1133	***trmD***
83	**4**	SAUSA300_1134	*rplS*
84	**1**	SAUSA300_1136	***rbgA***
84	**2**	SAUSA300_1137	*rnhB*
85	**1**	SAUSA300_1142	*dprA*
85	**2**	SAUSA300_1143	***topA***
86	**1**	SAUSA300_1149	*rpsB*
86	**2**	SAUSA300_1150	***tsf***
87	**1**	SAUSA300_1151	***pyrH***
87	**2**	SAUSA300_1152	***frr***
88	**1**	SAUSA300_1153	***uppS***
88	**2**	SAUSA300_1154	*cdsA*
89	**1**	SAUSA300_1155	*rasP*
89	**2**	SAUSA300_1156	***proS***
90	**1**	SAUSA300_1157	***polC***
91	**1**	SAUSA300_1158	*rimP*
91	**2**	SAUSA300_1159	***nusA***
91	**3**	SAUSA300_1160	*1160*
91	**4**	SAUSA300_1161	*1161*
91	**5**	SAUSA300_1162	*infB*
92	**1**	SAUSA300_1168	***rnjB***
93	**1**	SAUSA300_1169	*ftsK*
93	**2**	SAUSA300_1170	*1170*
93	**3**	SAUSA300_1171	*1171*
93	**4**	SAUSA300_1172	*1172*
93	**5**	SAUSA300_1173	*1173*
93	**6**	SAUSA300_1174	*1174*
93	**7**	SAUSA300_1175	*1175*
93	**8**	SAUSA300_1176	***pgsA***

Operon Number	Gene position in operon	Locus Tag	Gene Name
94	**1**	SAUSA300_1200	***glnR***
94	**2**	SAUSA300_1201	*glnA*
95	**1**	SAUSA300_1237	***lexA***
96	**1**	SAUSA300_1239	***tkt***
97	**1**	SAUSA300_1249	***plsY***
98	**1**	SAUSA300_1250	***parE***
98	**2**	SAUSA300_1251	*parC*
99	**1**	SAUSA300_1257	***msrR***
100	**1**	SAUSA300_1269	***femA***
100	**2**	SAUSA300_1270	***femB***
101	**1**	SAUSA300_1312	*1312*
101	**2**	SAUSA300_1311	***murG***
101	**3**	SAUSA300_1310	*1310*
102	**1**	SAUSA300_1319	*folA*
102	**2**	SAUSA300_1318	*1318*
103	**1**	SAUSA300_1320	***thyA***
104	**1**	SAUSA300_1340	***recU***
104	**2**	SAUSA300_1341	***pbp2***
105	**1**	SAUSA300_1344	***dnaD***
105	**2**	SAUSA300_1343	*nth*
105	**3**	SAUSA300_1342	*1342*
106	**1**	SAUSA300_1349	*1349*
106	**2**	SAUSA300_1348	***papS***
106	**3**	SAUSA300_1347	***birA***
106	**4**	SAUSA300_1346	*1346*
107	**1**	SAUSA300_1354	*1354*
107	**2**	SAUSA300_1353	*1353*
107	**3**	SAUSA300_1352	*1352*
107	**4**	SAUSA300_1351	***1351***
108	**1**	SAUSA300_1360	***ubiE***
108	**2**	SAUSA300_1359	*1359*
109	**1**	SAUSA300_1362	***hup***
110	**1**	SAUSA300_1364	***engA***
110	**2**	SAUSA300_1363	*gpsA*
111	**1**	SAUSA300_1367	***cmk***
111	**2**	SAUSA300_1366	*1366*
112	**1**	SAUSA300_1373	***fer***
113	**1**	SAUSA300_1453	***rnz***
114	**1**	SAUSA300_1454	***zwf***
115	**1**	SAUSA300_1460	*1460*
115	**2**	SAUSA300_1459	***gnd***
116	**1**	SAUSA300_1467	*ipdA*
116	**2**	SAUSA300_1466	***bfmBAA***
116	**3**	SAUSA300_1465	*1465*
116	**4**	SAUSA300_1464	***bmfBB***

Operon Number	Gene position in operon	Locus Tag	Gene Name
117	**1**	SAUSA300_1476	***accB***
117	**2**	SAUSA300_1475	***accC***
117	**3**	SAUSA300_1474	*1474*
117	**4**	SAUSA300_1473	*nusB*
117	**5**	SAUSA300_1472	*xseA*
117	**6**	SAUSA300_1471	*xseB*
117	**7**	SAUSA300_1470	*1470*
118	**1**	SAUSA300_1491	*pepQ2*
118	**2**	SAUSA300_1490	***efp***
119	**1**	SAUSA300_1492	***1492***
119	**2**	SAUSA300_1493	*1493*
120	**1**	SAUSA300_1520	***trmK***
120	**2**	SAUSA300_1519	*1519*
121	**1**	SAUSA300_1521	***rpoD***
122	**1**	SAUSA300_1522	***dnaG***
123	**1**	SAUSA300_1525	***glyS***
124	**1**	SAUSA300_1531	*phoH*
124	**2**	SAUSA300_1530	***ybeY***
124	**3**	SAUSA300_1529	*dgcA*
124	**4**	SAUSA300_1528	*cdd*
124	**5**	SAUSA300_1527	*era*
124	**6**	SAUSA300_1526	*recO*
125	**1**	SAUSA300_1542	*hrcA*
125	**2**	SAUSA300_1541	***grpE***
125	**3**	SAUSA300_1540	***dnaK***
125	**4**	SAUSA300_1539	*dnaJ*
125	**5**	SAUSA300_1538	*prmA*
125	**6**	SAUSA300_1537	*rsmE*
125	**7**	SAUSA300_1536	*mtaB*
126	**1**	SAUSA300_1548	*comEB*
126	**2**	SAUSA300_1547	*comEC*
126	**3**	SAUSA300_1546	***holA***
127	**1**	SAUSA300_1558	*mtnN*
127	**2**	SAUSA300_1557	*1557*
127	**3**	SAUSA300_1556	*1556*
127	**4**	SAUSA300_1555	*aroE*
127	**5**	SAUSA300_1554	*1554*
127	**6**	SAUSA300_1553	*nadD*
127	**7**	SAUSA300_1552	*1552*
127	**8**	SAUSA300_1551	*1551*
127	**9**	SAUSA300_1550	*1550*
127	**10**	SAUSA300_1549	*1549*
128	**1**	SAUSA300_1571	*1571*
128	**2**	SAUSA300_1570	*1570*

Operon Number	Gene position in operon	Locus Tag	Gene Name
128	**3**	SAUSA300_1569	*1569*
128	**4**	SAUSA300_1568	*udk*
128	**5**	SAUSA300_1567	***greA***
129	**1**	SAUSA300_1575	***alaS***
129	**2**	SAUSA300_1574	*1574*
129	**3**	SAUSA300_1573	*1573*
129	**4**	SAUSA300_1572	*1572*
130	**2**	SAUSA300_1579	***iscS***
130	**1**	SAUSA300_1578	*mnmA*
131	**1**	SAUSA300_1587	***hisS***
131	**2**	SAUSA300_1586	*aspS*
132	**1**	SAUSA300_1590	***relA***
132	**2**	SAUSA300_1589	***dtd***
132	**3**	SAUSA300_1588	*lytH*
133	**1**	SAUSA300_1593	***secF***
134	**1**	SAUSA300_1600	***obgE***
134	**2**	SAUSA300_1599	*1599*
134	**3**	SAUSA300_1598	*ruvA*
134	**4**	SAUSA300_1597	*ruvB*
134	**5**	SAUSA300_1596	*queA*
134	**6**	SAUSA300_1595	*tgt*
134	**7**	SAUSA300_1594	*yajC*
135	**1**	SAUSA300_1611	***valS***
135	**2**	SAUSA300_1610	*folC*
136	**1**	SAUSA300_1619	***hemA***
136	**2**	SAUSA300_1618	*hemX*
136	**3**	SAUSA300_1617	*hemC*
136	**4**	SAUSA300_1616	*hemD*
136	**5**	SAUSA300_1615	*hemB*
136	**6**	SAUSA300_1614	*hemL*
137	**1**	SAUSA300_1620	***engB***
138	**1**	SAUSA300_1624	***1624***
138	**2**	SAUSA300_1623	*1623*
139	**1**	SAUSA300_1627	***infC***
139	**2**	SAUSA300_1626	*rpmI*
139	**3**	SAUSA300_1625	*rplT*
140	**1**	SAUSA300_1629	***thrS***
141	**1**	SAUSA300_1632	***nrdR***
141	**2**	SAUSA300_1631	***dnaB***
141	**3**	SAUSA300_1630	*dnaI*
142	**1**	SAUSA300_1636	*polA*
142	**2**	SAUSA300_1635	*mutM*
142	**3**	SAUSA300_1634	***coaE***
143	**1**	SAUSA300_1645	***pfkA***

Operon Number	Gene position in operon	Locus Tag	Gene Name
143	2	SAUSA300_1644	*pykA*
144	1	SAUSA300_1647	***accD***
144	2	SAUSA300_1646	*accA*
145	1	SAUSA300_1650	*1650*
145	2	SAUSA300_1649	***dnaE***
146	1	SAUSA300_1658	*1658*
146	2	SAUSA300_1657	***ackA***
147	1	SAUSA300_1673	***plsC***
148	1	SAUSA300_1675	***tyrS***
149	1	SAUSA300_1691	*1691*
149	2	SAUSA300_1690	*1690*
149	3	SAUSA300_1689	*1689*
149	4	SAUSA300_1688	***1688***
149	5	SAUSA300_1687	*1687*
149	6	SAUSA300_1686	***murC***
150	1	SAUSA300_1700	*1700*
150	2	SAUSA300_1699	*1699*
150	3	SAUSA300_1698	*1698*
151	1	SAUSA300_1704	***leuS***
151	2	SAUSA300_1703	*1703*
152	1	SAUSA300_1729	***1729***
153	1	SAUSA300_1730	***metK***
154	1	SAUSA300_1748	***1748***
155	1	SAUSA300_1749	***1749***
156	1	SAUSA300_1869	***map***
157	1	SAUSA300_1873	***murT***
157	2	SAUSA300_1872	*gatD*
158	1	SAUSA300_1879	***dgkB***
158	2	SAUSA300_1878	*rumA*
159	1	SAUSA300_1882	***gatC***
159	2	SAUSA300_1881	***gatA***
159	3	SAUSA300_1880	*gatB*
160	1	SAUSA300_1887	*pcrB*
160	2	SAUSA300_1886	***pcrA***
160	3	SAUSA300_1885	***ligA***
160	4	SAUSA300_1884	*1884*
161	1	SAUSA300_1894	***pncB***
161	2	SAUSA300_1893	*nadE*
162	1	SAUSA300_1899	***pncA***
162	2	SAUSA300_1900	***ppaC***
163	1	SAUSA300_1914	*pmtR*
163	2	SAUSA300_1913	***pmtA***
163	3	SAUSA300_1912	*pmtB*
163	4	SAUSA300_1911	***pmtC***
163	5	SAUSA300_1910	*pmtD*

Operon Number	Gene position in operon	Locus Tag	Gene Name
164	**1**	SAUSA300_1983	***groES***
164	**2**	SAUSA300_1982	*groEL*
165	**1**	SAUSA300_1988	***hld***
166	**1**	SAUSA300_2005	***tsaE***
166	**2**	SAUSA300_2004	*tsaB*
166	**3**	SAUSA300_2003	*rimI*
166	**4**	SAUSA300_2002	***gcp***
167	**1**	SAUSA300_2031	*2031*
167	**2**	SAUSA300_2030	*2030*
167	**3**	SAUSA300_2029	*2029*
167	**4**	SAUSA300_2028	***acpS***
167	**5**	SAUSA300_2027	*alr*
168	**1**	SAUSA300_2039	***ddl***
168	**2**	SAUSA300_2038	*murF*
169	**1**	SAUSA300_2046	***oxaA***
170	**1**	SAUSA300_2056	*2056*
170	**2**	SAUSA300_2055	***murA***
170	**3**	SAUSA300_2054	*fabZ*
171	**1**	SAUSA300_2062	***atpF***
171	**2**	SAUSA300_2061	*atpH*
171	**3**	SAUSA300_2060	*atpA*
171	**4**	SAUSA300_2059	*atpG*
171	**5**	SAUSA300_2058	*atpD*
171	**6**	SAUSA300_2057	*atpC*
172	**1**	SAUSA300_2073	*tdk*
172	**2**	SAUSA300_2072	***prfA***
172	**3**	SAUSA300_2071	*prmC*
172	**4**	SAUSA300_2070	*2070*
172	**5**	SAUSA300_2069	*ptpB*
173	**1**	SAUSA300_2079	***fbaA***
174	**1**	SAUSA300_2081	***pyrG***
175	**1**	SAUSA300_2084	***coaA***
176	**1**	SAUSA300_2104	***glmS***
177	**1**	SAUSA300_2113	***dacA***
177	**2**	SAUSA300_2112	*ybbR*
177	**3**	SAUSA300_2111	*glmM*
178	**1**	SAUSA300_2182	***infA***
178	**2**	SAUSA300_2181	*rpmJ*
178	**3**	SAUSA300_2180	*rpsM*
178	**4**	SAUSA300_2179	*rpsK*
178	**5**	SAUSA300_2178	*rpoA*
178	**6**	SAUSA300_2177	*rplQ*
179	**1**	SAUSA300_2205	*rpsJ*
179	**2**	SAUSA300_2204	*rplC*
179	**3**	SAUSA300_2203	*rplD*

Operon Number	Gene position in operon	Locus Tag	Gene Name
179	**4**	SAUSA300_2202	*rplW*
179	**5**	SAUSA300_2201	*rplB*
179	**6**	SAUSA300_2200	*rpsS*
179	**7**	SAUSA300_2199	*rplV*
179	**8**	SAUSA300_2198	*rpsC*
179	**9**	SAUSA300_2197	*rplP*
179	**10**	SAUSA300_2196	*rpmC*
179	**11**	SAUSA300_2195	*rpsQ*
179	**12**	SAUSA300_2194	*rplN*
179	**13**	SAUSA300_2193	*rplX*
179	**14**	SAUSA300_2192	*rplE*
179	**15**	SAUSA300_2191	*rpsN*
179	**16**	SAUSA300_2190	*rpsH*
179	**17**	SAUSA300_2189	*rplF*
179	**18**	SAUSA300_2188	*rplR*
179	**19**	SAUSA300_2187	*rpsE*
179	**20**	SAUSA300_2186	*rpmD*
179	**21**	SAUSA300_2185	*rplO*
179	**22**	SAUSA300_2184	***secY***
179	**23**	SAUSA300_2183	*adK*
180	**1**	SAUSA300_2214	***femX***
180	**2**	SAUSA300_2213	*2213*
181	**1**	SAUSA300_2238	***ureA***
181	**2**	SAUSA300_2239	*ureB*
181	**3**	SAUSA300_2240	*ureC*
181	**4**	SAUSA300_2241	*ureE*
181	**5**	SAUSA300_2242	*ureF*
181	**6**	SAUSA300_2243	*ureG*
181	**7**	SAUSA300_2244	*ureD*
182	**1**	SAUSA300_2283	***rpiA***
183	**1**	SAUSA300_2293	*corA*
183	**2**	SAUSA300_2292	***fni***
184	**1**	SAUSA300_2483	***mvaA***
185	**1**	SAUSA300_2484	***mvaS***
186	**1**	SAUSA300_2646	***trmE***
186	**2**	SAUSA300_2645	*gidA*
186	**3**	SAUSA300_2644	*gidB*
186	**4**	SAUSA300_2643	*2643*

	Ribosomal	Proteins	*Operons*
Operon Number	Gene position in operon	Locus Tag	Gene Name
1	1	SAUSA300_0013	*0013*
1	2	SAUSA300_0014	*gdpP*
1	3	SAUSA300_0015	***rplI***
1	4	SAUSA300_0016	***dnaB***
2	1	SAUSA300_0366	***rpsF***
2	2	SAUSA300_0367	***ssb***
2	3	SAUSA300_0368	*rpsR*
3	1	SAUSA300_0479	***rplY***
3	2	SAUSA300_0480	*pth*
3	3	SAUSA300_0481	*mfd*
3	4	SAUSA300_0482	*0482*
3	5	SAUSA300_0483	*0483*
3	6	SAUSA300_0484	*0484*
3	7	SAUSA300_0485	***divIC***
4	1	SAUSA300_0522	***rplK***
4	2	SAUSA300_0523	*rplA*
5	1	SAUSA300_0524	***rplJ***
5	2	SAUSA300_0525	*rplL*
6	1	SAUSA300_0529	*rplGB*
6	2	SAUSA300_0530	***rpsL***
6	3	SAUSA300_0531	*rpsG*
6	4	SAUSA300_0532	*fusA*
6	5	SAUSA300_0533	*tuf*
7	1	SAUSA300_0990	***rpoY***
7	2	SAUSA300_0989	***rnjA***
8	1	SAUSA300_1026	*1026*
8	2	SAUSA300_1027	***rpmF***
9	1	SAUSA300_1117	***rpmB***
10	1	SAUSA300_1131	***rpsP***
10	2	SAUSA300_1132	*rimM*
10	3	SAUSA300_1133	***trmD***
10	4	SAUSA300_1134	***rplS***
11	1	SAUSA300_1149	***rpsB***
11	2	SAUSA300_1150	***tsf***
12	1	SAUSA300_1166	***rpsO***
13	1	SAUSA300_1511	***rpmG***
14	1	SAUSA300_1535	***rpsU***
15	1	SAUSA300_1545	***rpsT***
16	1	SAUSA300_1603	*rplU*
16	2	SAUSA300_1602	***1602***
16	3	SAUSA300_1601	***rpmA***
17	1	SAUSA300_1627	***infC***
17	2	SAUSA300_1626	*rpmI*
17	3	SAUSA300_1625	***rplT***

Operon Number	Gene position in operon	Locus Tag	Gene Name
18	1	SAUSA300_1666	***rpsD***
19	1	SAUSA300_2074	***rpmE2***
20	1	SAUSA300_2172	*rplM*
20	2	SAUSA300_2171	***rpsI***
21	1	SAUSA300_2182	***infA***
21	2	SAUSA300_2181	*rpmJ*
21	3	SAUSA300_2180	*rpsM*
21	4	SAUSA300_2179	*rpsK*
21	5	SAUSA300_2178	*rpoA*
21	6	SAUSA300_2177	*rplQ*
22	1	SAUSA300_2183	***adk***
22	2	SAUSA300_2184	***secY***
22	3	SAUSA300_2185	***rplO***
22	4	SAUSA300_2186	*rpmD*
22	5	SAUSA300_2187	*rpsE*
22	6	SAUSA300_2188	*rplR*
22	7	SAUSA300_2189	*rplF*
22	8	SAUSA300_2190	*rpsH*
22	9	SAUSA300_2191	*rpsN*
22	10	SAUSA300_2192	*rplE*
22	11	SAUSA300_2193	*rplX*
22	12	SAUSA300_2194	*rplN*
22	13	SAUSA300_2195	*rpsQ*
22	14	SAUSA300_2196	*rpmC*
22	15	SAUSA300_2197	*rplP*
22	16	SAUSA300_2198	*rpsC*
22	17	SAUSA300_2199	*rplV*
22	18	SAUSA300_2200	*rpsS*
22	19	SAUSA300_2201	*rplB*
22	20	SAUSA300_2202	*rplW*
22	21	SAUSA300_2203	*rplD*
22	22	SAUSA300_2204	*rplC*
22	23	SAUSA300_2205	*rpsJ*
23	1	SAUSA300_2647	***rnpA***
23	2	SAUSA300_2648	***rpmH***

Genes listed in bold were targeted with specific sgRNAs in the LCML library. Those appearing in the Nebraska transposon mutant library (and therefore likely non-essential) are underlined.

Supplementary Table 2. Lisbon CRISPRi *mutant* library (LCML) Primer List

Plasmid	Primer Number	Primer Sequence
	5846 - EcR (reverse)	ACTAGTATTATACCTAGGACTGAGCTAGC
psg0001_dnaA	8276	ATACACAAAAATAACAGCCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0002_dnaN	8277	TCGTTTATATATAATTATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0003	8278	ACAAATTTCATTTAAAATAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0009_serS	8279	CAAATAATTATCATTTATTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0016_dnaB	8280	TCATACATTCTATCCATGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0020_walR	8281	ATGTTTGTGTAAAAAATCACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0024_walJ	8282	TGTAACTTTAGTTCATCGACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0089	8283	GCCAAAATAAAAAATGGACGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0248_tarF	8284	ATATAATTACAAAAACACGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0249_ispD	8285	ATATAGATATAGTTGAATGGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0363	8286	TGATTATAAGCAGTCATAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0367_ssb	8287	TTTATATTTGCACCTCCTTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0388_guaB	8288	GGTAAAATATCAGATTGTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0452_dnaX	8289	GGAAATTTTACGATTCCGTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0453	8290	ATTGATTTTCAAGGAGGAAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg00459_tmk	8291	GTTTCTCCTTTGAAAATAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0461_holB	8292	CCTTTTGCTTTTATATAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0467_metS	8303	AAGATTTCTATGCATTTCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0477_glmU	8304	TTCAGCACCATGTCCTACGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0478_prs	8305	AGATTACATTAATATTACATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0480_pth	8306	GACAAATTCATCATTGTCATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0484	8398	TTCTAGCCACTGCTTTAACGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0485_divIC	8399	CAATATCATTGCGATGTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0487_tilS	8400	ACTTTTACAATTTTCAAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0492_folP	8494	TTTATTATCTTATCATTCACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0496_lysS	8495	TAATCCTTTAGTATTCCAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0513_gltX	8496	TGAAGATACCCAGTTGGACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0514_cysE	8497	CATACAAGTTGTATTGTTAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0532_fusA	8498	CTGAATAAATACGATAGATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0533 _tuf	8499	GTGACCGATAGTACCGATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0551_folE2	8500	TTAAAGTGATATGTCCAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0570_eutD	8501	TAAATCTTATTAATCATTCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0572_mvaK1	8502	AAAAATAATGAATCAAGTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0596_argS	8503	ATATCAAGATAATGAAAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0623_tagA	8504	CTTTCTTCAACAGTCATAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0624_tagH	8505	AATCTAGATAAATGTGAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0625_tagG	8674	AAACCATAATTTGCATAACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0626_tagB	8675	TGCGGTTTATCAATCACTTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0628_tagD	8676	TCCTCATATTGTCACATCATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0703_ltaS	8677	CTTCAAGGTAATCGTTATTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0715_nrdI	8678	TAAAAAATGCTTTAACAACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0722_murB	8679	TCAACTTTAATTTTTTCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg0731_tagO	8680	TTCGATATTGCAATAACAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0737_secA	8681	CCTTTAGCTAAAAAACTGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0738_prfB	8682	GTTTGGTTATCCCAAAAATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0743_hprK	8683	TATTGCTTGCTTAATTTACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0749	8684	TTGTCCACCCGTACAACCGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0756_gapA	8685	TTCAAGTATTATCTTTGCTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0761	8755	CGTTAAATTAAGTAATGCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0765_smpB	8756	CGATTTTCCGCTAATGTACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0818_sufC	8757	TATCCTCAATAGACACATGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0835_dltA	8758	TGTCTAACAGCAATGCTTTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0858	8759	GTCTCAACAAACGCACCGTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0865_pgi	8760	TTTCAACAAACATTTCAAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0868_spsB	8761	CGCTCGCCATCTTTCAAAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0885_fabH	8762	GCATTGTCAATAATCTTTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0897_trpS	8763	TAGTAGGAATTCCACTAGGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0898_spxA	8764	TTACGGCAAGATGTGCAACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0908_ppnK	8765	GTTCATCATTTTATGCTTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0912_fabI	8766	CCTTATAATAATTAATTTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0919_murE	9735	ACTATTAAAAATAAAACATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0922	9220	ACGACAAGTACCCATAAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0944_menA	9736	ACGGAAGCAGTTAATGTATGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0948_menB	9737	GTAAACGCATTGCGTACTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0983_ptsH	9221	GTTTGTACTAACATTGTTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0989_rnjA	9286	GCATATACACCTACTTCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0990_rpoY	9287	AAACTTTAAATACTGCCATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0991_def	9288	GCTGCTTTTTGACGCAAAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1013_ftsW	9738	CGGATAATCAATAAACTTTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1024_coaD	9289	GTAATGGGGTCAAAACTACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1026	9739	ACCGTTTGATCAAATTCAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1037_pheS	9740	AACGCAGGTTTATCTTCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1044_trxA	8877	ACCGGAGCGATCATTTTACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1049_murI	8878	CCAGAGTCTATTACACCTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1074_ftsL	8879	AAACTTGTTCGTCATATGGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1075_pbp1_b	8880	GTCCGAATAAACCAACAAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1076_mraY	8881	TTTAATGTAGGTATTAAAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1077_murD	8882	AATAAAAATGTATTAGTTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1078_divIB	8883	GGAATACATCTAAGAAAAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1079_ftsA	8884	ATTTTTTATACCGCTCGTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1082	8885	TTTGTAACTGCAATCACGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1083_sepF	8886	TTTACCTGTTGTTGTTTGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1087_ileS	8887	CGCATTGGGAAATCTGTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1102_gmk	8888	GTACCTTTACCTACTCCAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1104_coaBC	8889	TGCCGCAATGCCACCTGTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1105_priA	8890	GATGACAGATTCGAGTTGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1109_fmt	8891	AAAACAGTTGTTGAAAAGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1115_rpe	8892	AAATCAACAGATAATAATGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1122_plsX	8893	GATATCGTATTAGAAGCCGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1125_acpP	8894	TATCAGCGTCTACACCTAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1128_ftsY	8895	TTGACCTTGTTCTTCTGTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1133_trmD	8896	AAAACACCATCAAACATTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1136_rbgA	8897	TTGGCTTTCGCCATATGTCCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1143_topA	8898	TCAATGGTTTTTGCTTTTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1150_tsf	8899	TAGCAATACCTTTTTCACGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1151_pyrH	8900	GCAACACTTTTAATAATTACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1152_frr	8981	AATTAGCTAACATCAGTGCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1153_uppS	8982	ATTTATTAGCTTTTTAAACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1156_proS	8983	CATCGTTGGTATAAAAACTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1157_polC	8984	TCTTCATGAGCTAAGAATTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1159_nusA	8985	GCATCAATTAATACTGCTCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1168_rnjB	8986	TGAATAGTGAGTTTATATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1176_pgsA	8987	CTAAAAACCGTAATCTGGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1200_glnR	8988	TGATGCAATCAGACGAAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1237_lexA	8989	CCAATTTCGCGAACACTAGGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1239_tkt	8990	TTGAAGTAATCTTTAGATTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1249_plsY	8991	TTTCCAATTACGAATCCACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1250_parE	8992	GTTGATCCAATATACATACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1257_msrR	9018	TTCTTCTTCTTTTTTCGCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
ppsg1269_femA	9019	TTACAGATAGCATGCCATACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1270_femB	9020	TTGTACAAAGTTGTCAAATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1311_murG	9021	TGTCCAACTGTTCCCCCTCCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1319_folA	9022	CAAGTCATGTGCAACTAGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1320_thyA	9023	ACTTTCTTTGTCGTTAATAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1340_recU	9024	TTACGATATGGTTTACCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1341_pbp2	9025	CCATTATTACCGTTTTTCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1344_dnaD	9026	AATTCTCTTCGTATCACTACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1347_birA	9027	CTTTGTCCAGATATATAATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1348_papS	9028	TCTTGAATTTGTTCTAATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1351	9029	ACTTTGTGTTGTGCCCATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1360_ubiE	9030	AAAACGCGTCATGAAAGACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1362_hup	9031	CATTAGACATTCACCTCCTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1364_engA	9032	ATTGTAGATTTACCTACATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1367_cmk	9033	ACCATCTAATGCAATATTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1373_fer	9034	TCGTCGTAATCATATATATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1453_rnz	9035	TGTGTATTTCTCTCTTTTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1454_zwf	9036	CCAAAGATTGTGATTAAACAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1459_gnd	9037	AGCTAGGTTTTTACCCATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1464_bmfBB	9038	TGAACACTCTCACCTAACTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1466_bmfBAA	9039	CTTTTAGGTCTTCTTCGCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1475_accC	9040	TCCTAACTGCGATTTCACCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1490_efp	9041	CTTTACCAGGCTTTACATGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1492	8530	TCTGTTGTTTTTTCTTCTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1520_trmK	8531	ATTCTCCTTTATGAAAAAAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1521_rpoD	8532	CTGTGTTATCAGACATGAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1522_dnaG	8533	ATTTATTTTGTTCAATATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1525_glyS	8534	TCATACATGAAAACGCCCCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1530_ybeY	8535	TCAATGATCTTACTTACCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1540_dnaK	8536	TCAGGGTTTTGAATTACTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1541_grpE	8537	TCATTAATTTTTTGATCTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1546_holA	8538	CTTTGTTTTTCAACCAATTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1557	8539	ATTGATTGAACATATGAATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1567_greA	8540	TCAAAACCTTCTTGAGTCATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1575_alaS	8541	ATTGGCACTAATGGTGCAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1579_iscS	8738	TACTTCAGGTTTTACTGGTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1587_hisS	8739	AAAATATCCTGCGTCCCTCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1589_dtd	8740	GTATTTCACCATTCATTTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1593_secF	8741	TTTATAAGTTGCAGCCATTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1600_obgE	8742	AATACCATTACCACCATCACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1602	8743	CATATTCACCATGGTCAGCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1611_valS	8744	ACTTCACGAGGATCATATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1619_hemA	8745	ATCTTCATGGGCAATTCGTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1620_engB	8746	ATGATTAATTCTATATTATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1624	8747	TTATTAAAAATAATTTCTCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1627_infC	8748	TTGAGTTTGATCTTTTGCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1629_thrS	8749	AACGCCTTTTTATTACCATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1631_dnaB	8792	TGGTCTTAAGCCGAATTCGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1632_nrdR	8793	ATTGTGTAGAATTACATTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1634_coaE	8794	CCTGTTAGACCAATAACTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1645_pfkA	8795	TGTACGAACAACTGCTCTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1647_accD	8796	CACTTAGTCATAATACCTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1649_dnaE	8797	TTCAGACACAGCAAGTCTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1657_ackA	8798	TTTGTTACTAATTCCTCTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1673_plsC	8799	ACGACATATTTACTATCCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1675_tyrS	8800	TAAACTATCTGCCGTTGGATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1704_leuS	8803	ATTTCTTTTCAATTTGATTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1729	8804	ATAATGAGTCCAATGACTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1730_metK	8805	CTTGGTCAGCGATTTTATCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1748	8806	TCGCGTATAATTTCATTCCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1749	8807	TAATAGAATAACCATCCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1869_map	8808	TTTCGTAGTGATACCTGGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1873_murT	8809	TACGCGCCAATTTCGCTAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1879_dgkB	8810	ATAGCTCTTTACCTGATGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1881_gatA	8811	ATATCTTTAACAACATCAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1882_gatC	8812	GCCATTTCTTCCGTTTCTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1885_ligA	8813	TATTCACTATCTGGTACAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1886_pcrA	8814	GCACCTGCCATAATTAACAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1894_pncB	8815	TTAAACTGTCGTCTTCTAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1900_ppaC	8970	GATGAAATTGCATCAGTGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1911_pmtC	8971	ACCATTCATTACTTTCATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1913_pmtA	8972	ATTAACATTACTTAATTCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1983_groES	8973	ATAATCACACGATTTCCAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1988_hld	8974	ACTAGATCACAGAGATGTGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2002_gcp	8975	TGATGTCTACTTGCCACTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2005_tsaE	8976	TTTAATGATGTTAAATGTCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2028_acpS	8977	AAAATCCGCTCAACCAATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2055_murA	8980	AATAAAGATGCTGTCAATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2062_atpF	8981	TAAGTTAGCTGTTTCAGTCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2072_prfA	9003	CTGAATCATTTACAACATCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2079_fbaA	9004	ATTTCTTTCATTGAAACTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2081_pyrG	9005	CCTGGGTCAACATTTAAGTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg2113_dacA	9008	ACTGAGGTTTTGAAAAAAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2182_infA	9009	AACGCAATGTTTAAAGTAGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg2214_femX	9011	TAATAAATCTCCATTTGGGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2238_ureA	9012	CTGATTAAAGCTAATGCCTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2283_rpiA	9013	TTAATTAGTTGCGCCATTTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2646_trmE	9017	CCAATTGCCCCTTCACCCATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2484_mvaS	9016	CCATGTCTACATAGTACTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg0639	9487	TCAATACATGTTTTTTTATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1359	9488	TTTGCCACGTTAATCACCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1590_relA	9489	TTCGCCTTAAACAATGATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1791_cbf1	9490	AATGTAATCACTTCTTTTGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2183_adk	9491	TGAGTTCCTTTACCTGCGCCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2185_rplO	9492	TAACTCATGTAATTTCATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg2648_rpmH	9494	TTACTATGTTTACGTTTATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0015_rplI	9495	AAGTTATTTGCATAACCTACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg0522_rplK	9498	TGATATCGTGATGTGGTCACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
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psg0530_rpsL	9500	CGTACTAATTGGTTAATAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0990_rpoY	9501	TAACCCTAGTAAAATCGTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1027_rpmF	9502	GTTTTAGAAGTTCTTCTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1117_rpmB	9503	CAGTTTACTCAAAATATAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1131_rpsP	9504	GCTACTACGATACGATAGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1134_rplS	9505	CCTCGACAAATATATAGCAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1149_rpsB	9506	CCATTATAAATTCCTCCTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1166_rpsO	9507	AATACTCCTTAATCCGAGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1511_rpmG	9508	TAAAGTTACGTTTACGCGCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1535_rpsU	9509	TCCCTCCCTCCAAATATCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1545_rpsT	9510	TTGTGCAATCATTTATTTGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1601_rpmA	9511	TGTAAGTTTAATTTTAACATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1625_rplT	9512	GGCATAAAAAATTCCTCCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1666_rpsD	9513	CCATGTTGTCCTGGTGCGTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2074_rpmE2	9514	CTCCTTTGCCCTGAACCATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2171_rpsI	9515	CCACGTAATTCGTAGTTTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2177_rplQ	9516	GAAAAGAAGATTGATAAAGGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1700_murJ	6423	CTTGGTAATTAATATACTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
B Clones		
psg0003_B	9275	TATTAAAATTTTAAATTTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0009_serS_B	9062	ATTTTGCTCTTAACTGTGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0020_walR_B	9063	AATTCTAAAATATCAGCAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0024_walJ_B	9064	ATACACTCATGCGTATCAAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0089_B	9065	AATTCAGTGAAAAACACATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0248_tarF_B	9066	AAATAAATTAGATTCTTGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0249_ispD_B	9276	TCTAATGTATGGATTAAAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0452_dnaX_B	8542	GGGTCTGTACATACGATATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0453_B	9546	GTTTGTTGTGCTTGTTGCTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0478_prs_B	8546	TAATTGCGTAAGTAAATAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0484_B	8543	ATGAGGCCGCGTTAAAGCAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0485_divIC_B	8544	CGACTTGAAGAAGCGTAAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0487_tilS_B	8545	TAGAAACAGCGACAACAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0492_folP_B	8695	GTCAGCACCTTCATCTATCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0514_cysE_B	8696	TCTAATGTTGAACGTGCCGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0551_folE2_B	8697	GTCATTTCGTTCTTAGTAGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0570_eutD_B	8698	ACACGTTCGTCCTCTCCTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0572_mvaK1_B	9277	CGAATAGTTCCCGCTCTCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0596_argS_B	8699	GGAACTTCAATTTTAATATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0623_tagA_B	8700	CGTTGATTGATTTGCAAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0624_tagH_B	8701	AAATGTTTTGTTTTTATGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0703_ltaS_B	9278	CTTTGATTTAACAAGTATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0715_nrdI_B	9279	CTGTTCTCTTAATAAAACGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0722_murB_B	9210	TGATATTAAAGACATGAGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0731_tagO_B	9547	TTTTTTCATCATATCATTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0737_secA_B	9211	AGCTAAAGGAGCGAACGAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0738_prfB_B	9212	ATAAATAGGAGCGAATGCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0743_hprK_B	9213	AGTTTTTCTGTCGTTAACATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0749_B	9214	CAATCGCCGTAATATCAATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0756_gapA_B	9215	TACTTTTACTGCCATTATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0818_sufC_B	9280	AAGTGCACATAATTTCTCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0835_dltA_B	9281	GCTTCAACATATTTATTAGGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0865_pgi_B	9283	TCTTCTTTGTCGTAATCAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0868_spsB_B	9216	GATTCACCTTTAATTGTATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0885_fabH_B	9217	TTAATAAAATTTTTAATACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0908_ppnK_B	9218	AAACGATGTTGAATTTCATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg0912_fabI_B	9219	CTTTACGGCTACGTTCTTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0922_B	9220	AAATAAGGTAAGATCAAACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0983_ptsH_B	9221	AAATTCAATTCGTTTAAAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0989_rnjA_B	9286	ATCCCTAATAAGTTATCATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0990_rpoY_B	9287	GGATAAAAAGTTTGGTAGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0991_def _B	9288	TTAAAAAATGATTAAAGTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1024_coaD_B	9289	TAACGTTTAATCATATTAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1049_murI_B	9290	CCATTCAATTTAATTTTGAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1133_trmD_B	9291	TTTACAACATCAGCAATATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1152_frr_B	8999	GCTAATTGTTGTACAGGTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1153_uppS_B	9292	TCGTAATGACCTTTAATTCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1157_polC_B	9293	AAAATTAAATGCTTTATATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1168_rnjB_B	9000	ATTTCGCCAACACCGCCTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1176_pgsA_B	9294	AACCATCAACAAAATCGCTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1200_glnR_B	9001	TCATAGTAACGTATTTGCCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1249_plsY_B	9295	TATCATATTCATTTAAATTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1257_msrR_B	9296	TTTCTTAATAAATCGTACTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1269_femA_B	9297	AAAAAAGTAATATGAACGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1311_murG_B	9298	GGTTCACCTTTATCATATGGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1340_recU_B	9299	AATACATTTACCGTTAATGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1341_pbp2_B	9300	AATTTAGCTTCGGTAAAAGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1344_dnaD_B	9301	GATTTTAACAATGAACTTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1347_birA_B	9302	ATACCTTGATACCAAATATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1348_papS_B	9303	CGAACAAATGTAACACCACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1351_B	9304	GTAGTTATAAAATCATAATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1360_ubiE_B	9305	ATACCAGTAACTTCACCTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1373_fer_B	9306	ATTATCCGTTTTACAGAGTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1453_rnz_B	9307	GAACGACTAGAAAGTAATCCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1475-_accC_B	9308	TGCACATATGCTTCTTCGTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1490_efp_B	9309	ACGCTTATGTTAAAACTATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1492_B	9552	AAATGCACAAACGTTTCACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1520_trmK_B	9553	TTCAAACGTTTACTACGACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1522_dnaG_B	9067	ACTTACCAAGTCTAAAATGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1525_glyS_B	9068	CACCGTAAATATCACTACCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
p sg1540_dnaK_B	9554	TTTCGCCAAATTTAAGTTATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1541_grpE_B	9069	CGTTCTATATTGTCTATTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1546_holA_B	9070	GTTAATGTTTCTTCAACAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1567_greA_B	9071	TTAATTTTCTCTACAACTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1575_alaS_B	9072	TTTACAATTCTTGGCTTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
p sg1589_dtd_B	9555	TGAAACTTTAATTAAATTGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1593_secF_B	9073	ATTTTGTCGCCTTTATTTAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1611_valS_B	9323	TACCAGTTACATTTGGTGGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1619_hemA_B	9324	TACAAAAACTATGAAATATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1624_B	9325	AAATGAGTTGTTTATATGAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1629_thrS_B	9326	ATTGATCCATCAGTTTCAAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1631_dnaB_B	9327	ACTGCTTGCGTTCCAATTAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1632_nrdR_B	9328	CTAACTCCGAAGTCAGAGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1634_coaE_B	9329	CTTCCACACACTTTGCATACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1647_accD_B	9330	TGAACTTGACCAGTTAAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1649_dnaE_B	9331	ACTGAAACTCCTGACGCATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1657_ackA_B	9332	ACTATTACAGATTATTTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1675_tyrS_B	9333	ACCCTTACAACAAATATGTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1704_leuS_B	9334	AAACCAGCACCTGATGGATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1729_B	9335	ACTTTGTCTAATTTTTCCCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1730_metK_B	9336	TGTTGTTGTAGAAATTTCGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1748_B	9337	AGTAGAGTCGCCTATCTCTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1749_B	9338	GTTACAACCCATATGATTGTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1869_map_B	9339	AAGATGTTACTTTAGTATTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1894_pncB_B	9340	GTAGACTATAATATAAAGCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1900_ppaC_B	9341	AATGAAGCCACTCCCTCAGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1911_pmtC_B	9342	AAAATATATGAATATAAATCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1913_pmtA_B	9343	TTCTTTAACCAAATTTTCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1983_groES_B	9344	ATAAATTATAAACAAATATTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1988_hld_B	9345	AGTATTTATTTCCTACAGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2028_acpS_B	9346	CTTGACCACCCGCTGTATAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2055_murA_B	9347	ATTTTACTAACATATTCATAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2062_atpF_B	9348	CTGAATTTATGCGATAGGCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2079_fbaA_B	9556	ACAATATTTTATAAGTAGATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2084_coaA_B	9557	CAATTAATGTTTTACAAACGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2104_glmS_B	9558	TCTGCTTTCATAATATAAATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2113_dacA_B	9559	TGATTATTACTGTCAATCAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2184_secY_B	9560	CCAGATTCTACTAATAAAGCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2238_ureA_B	9349	ATCTAATCGAAAACAAATAGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2283_rpiA_B	9350	ATTTTAAATAATACTCGTTAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2646_trmE_B	9351	GTCAGTCTTACGTTTGTCTGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg005_gyrB_B	9608	GTCGATCCTATATACATACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0527_rpoB_B	9609	ATAAAAAGACAAAAAGAAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0425_mspA_B	9610	ATATATCTCATTGGCATAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0639_B	9611	ATATATCTCATTGGCATAACgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1359_B	9612	TACTCAGAATAACAAATGCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1590_relA_B	9613	TGTATGGTAATCCGTTTTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1791_cbf1_B	9614	TTAACATGTACAATTTCTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0015_rplL_B	9615	TTTAAGTTGTGTTGCCGCATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0479_rplY_B	9675	ACGAAACTATTATACACGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0522_rplK_B	9676	ACGAAACTATTATACACGTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0524rplJ_B	9616	CTTCAGCTACTGTTAATCCAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg0990_rpoY_B	9287	TAACGATTTGGTTAATAACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1027_rpmF_B	9617	TGTTGTAATTTTTGAAGCCTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1117_rpmB_B	9618	ATTAAAGGAGGTACTCATATgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1134_rplS_B	9619	GTATCACCAGGACGGAAACTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1166_rpsO_B	9677	AGTACAGCGATTTGTACTTCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1545_rpsT_B	9620	CTTTTAGGAGGTGACAGAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

psg1601_rpmA_B	9621	CTTTTAGGAGGTGACAGAAAgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1625_rplT_B	9622	TAACACGTTTAGCTGCTCCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg1666_rpsD_B	9623	TAACACGTTTAGCTGCTCCGgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2171_rpsI_B	9624	ACTGTGATGTTACCTTCACCgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC
psg2177_rplQ_B	9625	GTTGAGAAATTAATCACTTTgttttAGAGCTAGAAATAGCAAGTTAAAATAAGGC

Supplementary Table 3. Lisbon CRISPRi *mutant* library (LCML) strain List

Name	Description
LCML1	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0001 (*dnaA*)
LCML2	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containingsg0002 (*dnaN*)
LCML3	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0003
LCML4	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0009 (*serS*)
LCML5	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0016 (*dnaB*)
LCML6	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0020 (*walR*)
LCML7	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0024 (*walJ*)
LCML8	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0089
LCML9	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0248 (*tarF*)
LCML10	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0249 (ispD)
LCML11	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0363
LCML12	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0367 (ssb)
LCML13	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0388 (guaB)
LCML14	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0452 (dnaX)
LCML15	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0453
LCML16	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg00459 (tmk)
LCML17	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0461 (holB)
LCML18	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0467 (metS)
LCML19	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0477 (glmU)
LCML20	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0478 (prs)
LCML21	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0480 (pth)
LCML22	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0484
LCML23	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0485 (divIC)
LCML24	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0487 (tilS)
LCML25	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0492 (folP)
LCML26	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0496 (lysS)
LCML27	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0513 (gltX)
LCML28	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0514 (cysE)
LCML29	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0532 (fusA)
LCML30	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0533 (tuf)
LCML31	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0551 (folE2)
LCML32	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0570 (eutD)
LCML33	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0572 (mvaK1)
LCML34	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0596 (argS)
LCML35	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0623 (tagA)
LCML36	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0624 (tagH)
LCML37	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0625 (tagG)
LCML38	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0626 (tagB)
LCML39	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0628 (tagD)
LCML40	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0703 (ltaS)
LCML41	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0715 (nrdI)

LCML42	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0722 (murB)
LCML43	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0731 (tagO)
LCML44	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0737 (secA)
LCML45	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0738 (prfB)
LCML46	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0743 (hprK)
LCML47	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0749
LCML48	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0756 (gapA)
LCML49	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0761
LCML50	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0765 (smpB)
LCML51	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0818 (sufC)
LCML52	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0835 (dltA)
LCML53	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0858
LCML54	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0865 (pgi)
LCML55	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0868 (spsB)
LCML56	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0885 (fabH)
LCML57	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0897 (trpS)
LCML58	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0898 (spxA)
LCML59	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0908 (ppnK)
LCML60	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0912 (fabI)
LCML61	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0919 (murE)
LCML62	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0922
LCML63	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0944 (menA)
LCML64	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0948 (menB)
LCML65	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0983 (ptsH)
LCML66	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0989 (rnjA)
LCML67	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0990 (rpoY)
LCML68	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0991 (def)
LCML69	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1013 (ftsW)
LCML70	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1024 (coaD)
LCML71	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1026
LCML72	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1037 (phe)
LCML73	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1044 (trxA)
LCML74	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1049 (murI)
LCML75	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1074 (ftsL)
LCML76	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1075 (pbp1)
LCML77	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1076 (mraY)
LCML78	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1077 (murD)
LCML79	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1078 (divIB)
LCML80	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1079 (ftsA)
LCML81	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1082
LCML82	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1083 (sepF)
LCML83	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1087 (ileS)
LCML84	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1102 (gmk)
LCML85	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1104 (coaBC)
LCML86	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1105 (priA)
LCML87	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1109 (fmt)

LCML88	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1115 (rpe)
LCML89	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1122 (plsX)
LCML90	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1125 (acpP)
LCML91	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1128 (ftsY)
LCML92	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1133 (trmD)
LCML93	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1136 (rbgA)
LCML94	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1143 (topA)
LCML95	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1150 (tsf)
LCML96	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1151 (pyrH)
LCML97	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1152 (frr)
LCML98	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1153 (uppS)
LCML99	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1156 (proS)
LCML100	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1157 (polC)
LCML101	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1159 (nusA)
LCML102	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1168 (rnjB)
LCML103	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1176 (pgsA)
LCML104	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1200 (glnR)
LCML105	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1237 (lexA)
LCML106	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1239 (tkt)
LCML107	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1249 (plsY)
LCML108	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1250 (parE)
LCML109	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1257 (msrR)
LCML110	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1269 (femA)
LCML111	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1270 (femB)
LCML112	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1311 (murG)
LCML113	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1319 (folA)
LCML114	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1320 (thyA)
LCML115	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1340 (recU)
LCML116	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1341 (pbp2)
LCML117	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1344 (dnaD)
LCML118	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1347 (birA)
LCML119	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1348 (papS)
LCML120	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1351
LCML121	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1360 (ubiE)
LCML122	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1362 (hup)
LCML123	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1364 (engA)
LCML124	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1367 (cmk)
LCML125	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1373 (fer)
LCML126	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1453 (rnz)
LCML127	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1454 (zwf)
LCML128	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1459 (gnd)
LCML129	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1464 (bmfBB)
LCML130	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1466 (bmfBAA)
LCML131	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1475 (accC)
LCML132	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1490 (efp)
LCML133	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1492

LCML134	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1520 (trmK)
LCML135	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1521(rpoD)
LCML136	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1522 (dnaG)
LCML137	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1525 (glyS)
LCML138	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1530 (ybeY)
LCML139	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1540 (dnaK)
LCML140	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1541 (grpE)
LCML141	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1546 (holA)
LCML142	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1557
LCML143	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1567 (greA)
LCML144	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1575 (alaS)
LCML145	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1579 (iscS)
LCML146	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1587 (hisS)
LCML147	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1589 (dtd)
LCML148	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1593 (secF)
LCML149	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1600 (obgE)
LCML150	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1602
LCML151	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1611 (valS)
LCML152	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1619 (hemA)
LCML153	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1620 (engB)
LCML154	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1624
LCML155	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1627 (infC)
LCML156	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1629 (thrS)
LCML157	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1631 (dnaB)
LCML158	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1632 (nrdR)
LCML159	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1634 (coaE)
LCML160	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1645 (pfkA)
LCML161	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1647 (accD)
LCML162	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1649 (dnaE)
LCML163	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1657 (ackA)
LCML164	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1673 (plsC)
LCML165	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1675 (tyrS)
LCML166	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1686 (murC)
LCML167	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1688
LCML168	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1704 (leuS)
LCML169	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1729
LCML170	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1730 (metK)
LCML171	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1748
LCML172	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1749
LCML173	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1869 (map)
LCML174	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1873 (murT)
LCML175	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1879 (dgkB)
LCML176	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1881 (gatA)
LCML177	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1882 (gatC)
LCML178	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1885 (ligA)
LCML179	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1886 (pcrA)

LCML180	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1894 (pncB)
LCML181	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1900 (ppaC)
LCML182	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1911 (pmtC)
LCML183	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1913 (pmtA)
LCML184	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1983 (groES)
LCML185	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1988 (hld)
LCML186	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2002 (gcp)
LCML187	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2005 (tsaE)
LCML188	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2028 (acpS)
LCML189	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2039 (ddl)
LCML190	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2046 (oxaA)
LCML191	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2055 (murA)
LCML192	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2062 (atpF)
LCML193	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2072 (prfA)
LCML194	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2079 (fbaA)
LCML195	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2081 (pyrG)
LCML196	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2084 (coaA)
LCML197	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2104 (glmS)
LCML198	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2113 (dacA)
LCML199	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2182 (infA)
LCML200	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2184 (secY)
LCML201	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2214 (femX)
LCML202	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2238 (ureA)
LCML203	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2283 (rpiA)
LCML204	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2292 (fni)
LCML205	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2483 (mvaA)
LCML206	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2646 (trmE)
LCML207	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2484 (mvaS)
LCML208	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0005 (gyrB)
LCML209	JE2 *Δspa:*$P_{xyl/tetO33}$-*dcas9*$_{Spy}$ containing psg0527 (rpoB)
LCML210	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0425 (mspA)
LCML211	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0639
LCML212	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1359
LCML213	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1590 (relA)
LCML214	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1791 (cbf1)
LCML215	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2183 (adk)
LCML216	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2185 (rplO)
LCML217	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2647 (rnpA)
LCML218	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2648 (rpmH)
LCML219	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0015 (rplI)
LCML220	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0366 (rpsF)
LCML221	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0479 (rplY)
LCML222	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0522 (rplK)
LCML223	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0524 (rplJ)
LCML224	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0530 (rpsL)
LCML225	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0990 (rpoY)

LCML226	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1027 (rpmF)
LCML227	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1117 (rpmB)
LCML228	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1131 (rpsP)
LCML229	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1134 (rplS)
LCML230	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1149 (rpsB)
LCML231	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1166 (rpsO)
LCML232	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1511 (rpmG)
LCML233	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1535 (rpsU)
LCML234	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1545 (rpsT)
LCML235	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1601 (rpmA)
LCML236	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1625 (rplT)
LCML237	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1666 (rpsD)
LCML238	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2074 (rpmE2)
LCML239	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2171 (rpsI)
LCML240	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2177 (rplQ)
LCML241	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1700 (murJ)
B Clones	
LCML3_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0003_B
LCML4_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0009 (serS_B)
LCML6_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0020 (walR_B)
LCML7_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0024 (walJ_B)
LCML8_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0089_B
LCML9_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0248 (tarF_B)
LCML10_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0249 (ispD_B)
LCML14_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0452 (dnaX_B)
LCML15_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0453_B
LCML20_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0478 (prs_B)
LCML22_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0484_B
LCML23_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0485_(divIC_B)
LCML24_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0487 (tilS_B)
LCML25_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0492 (folP_B)
LCML28_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0514 (cysE_B)
LCML31_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0551 (folE2_B)
LCML32_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0570 (eutD_B)
LCML33_ B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0572 (mvaK1_B)
LCML34_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0596 (argS_B)
LCML35_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0623 (tagA_B)
LCML36 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0624 (tagH_B)
LCML40 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0703 (ltaS_B)
LCML41 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0715 (nrdI_B)
LCML42 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0722 (murB_B)
LCML43 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0737 (secA_B)
LCML45 _B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0738 (prfB_B)
LCML46_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg0743 (hprK_B)

LCML47_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0749_B
LCML48 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0756 (gapA_B)
LCML51 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0818 (sufC_B)
LCML52 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0835 (dltA_B)
LCML54 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0865 (pgi_B)
LCML55 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0868 (spsB_B)
LCML56 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0885 (fabH_B)
LCML59 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0908 (ppnK_B)
LCML60 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0912 (fabI_B)
LCML62_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0922_B
LCML65 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0983 (ptsH_B)
LCML66 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0989 (rnjA_B)
LCML67_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0990 (rpoY_B)
LCML68 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg0991 (def _B)
LCML70 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1024 (coaD_B)
LCML75 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1049 (murI_B)
LCML92_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1133 (trmD_B)
LCML97_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1152 (frr_B)
LCML98_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1153 (uppS_B)
LCML100_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1157 (polC_B)
LCML102_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1168 (rnjB_B)
LCML103_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1176 (pgsA_B)
LCML104_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1200 (glnR_B)
LCML107_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1249 (plsY_B)
LCML109_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1257(msrR_B)
LCML110_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1269 (femA_B)
LCML112_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1311 (murG_B)
LCML115 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1340 (recU_B)
LCML116_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1341 (pbp2_B)
LCML117_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1344 (DnaD_B)
LCML118_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1347 (birA_B)
LCML119 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1348 (papS_B)
LCML120_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1351_B
LCML121 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1360 (ubiE_B)
LCML125 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1373 (fer_B)
LCML126 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1453 (rnz_B)
LCML131 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1475 (accC_B)
LCML132_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1490 (efp_B)
LCML133_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1492_B
LCML134_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1520 (trmK_B)
LCML136 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1522 (dnaG_B)
LCML137_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1525 (glyS_B)
LCML139_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1540 (dnaK_B)
LCML140 _B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1541 (grpE_B)
LCML141_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1546 (holA_B)
LCML143_B	JE2 *Δspa:*$P_{xyl/tetO3}$*-dcas9*$_{Spy}$ containing psg1567 (greA_B)

LCML144_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1575 (alaS_B)
LCML147_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1589 (dtd_B)
LCML148_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1593 (secF_B)
LCML151_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1611 (valS_B)
LCML152_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1619 (hemA_B)
LCML154_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1624_B
LCML156_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1629 (thrS_B)
LCML157_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1631 (dnaB_B)
LCML158_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1632 (nrdR_B)
LCML159_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1634 (coaE_B)
LCML161_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1647 (accD_B)
LCML162_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1649 (dnaE_B)
LCML163_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1657 (ackA_B)
LCML165_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1675 (tyrS_B)
LCML168_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1704 (leuS_B)
LCML169_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1729_B
LCML170_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1730 (metK_B)
LCML171_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1748_B
LCML172_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1749_B
LCML173_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1869 (map_B)
LCML180_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1894 (pncB_B)
LCML181_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1900 (ppaC_B)
LCML182_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1911 (pmtC_B)
LCML183_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1913 (pmtA_B)
LCML184_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1983 (groES_B)
LCML185_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg1988 (hld_B)
LCML188_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2028 (acpS_B)
LCML191_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2055 (murA_B)
LCML192_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2062 (atpF_B)
LCML194_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2079 (fbaA_B)
LCML196_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2084 (coaA_B)
LCML197_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2104 (glmS_B)
LCML198_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2113 (dacA_B)
LCML200_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2184 (secY_B)
LCML202_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2238 (ureA_B)
LCML203_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2283 (rpiA_B)
LCML206_B	JE2 *Δspa:*$P_{xyl/tetO3}$-*dcas9*$_{Spy}$ containing psg2646 (trmE_B)