

Supplementary Table 1. CRISPR/Cas9 killing and mutation frequencies, using ABD2003 with strain RN1

Sample	TU*/cell (x)	Expected survival ($S_e=e^{-x}$)	Observed survival (S_o)	IP/cell(y) $y=\ln(1/S_o)$	IP/TU (y/x)	CRISPR-resistant mutants		
						%infected	No.	Freq.
1								
1	.4	0.67	.031±.003	3.5±.17	8.8±.43	97	101	1.0x10 ⁻⁴
2	.2	0.82	.16±.05	1.9±.40	11±2.1	84	72	8.4x10 ⁻⁵
3	.1	0.90	.33±.11	1.25±.43	13±4.3	67	50	7.9x10 ⁻⁵
4	.05	0.95	.60±.01	.52±.006	10.3±.33	40	22	6.9x10 ⁻⁵
5	.025	0.98	.77±.07	.28±.087	11±3.4	23	19	8.6x10 ⁻⁵
6	0	100			10.8±.67			

*TU=transduction units; x=TU/cell; S_e =expected survival; S_o = observed survival; IP=infective particles; y=IP/cell

Supplementary Table 2. Bacterial strains

Strains	Description	Reference
<i>E. coli</i>		
Top10	<i>E. coli</i> cloning strain	Invitrogen
<i>L. monocytogenes</i>		
SK1442	Serotype 3B	1
<i>S. aureus</i>		
USA300 LAC	CA-MRSA	2
Newman		3
Newman 7B4	(phage-cured)	4
N315	MRSA	5
RN8703	RN4282 (SaPI1 <i>tst::tetM</i>)	6
RN8465	(agr group III)	7
RN4850	(agr group IV)	8
RN9130	502 Δ TcR plasmid (agr group II)	7
17855	Penicillin resistant clinical isolate	9
RN1	NCTC8325	10
RN25	NCTC8325 (cured of phages 11 and 12)	11
RN450	NCTC8325 (cured of phages 11, 12 and 13)	11
RN4220	Restriction-defective derivative of RN450	12
RN10616	RN4220(80 α)	13
RN10359	RN450(80 α)	14
JCSA777	RN10359- Δ <i>agr::cadCA</i>	15
JCSA778	JCSA777 (80 α Δ <i>terS</i>)	15
RN408	8331 (PS29)	
RN5006	<i>S. aureus</i> clinical isolate	
RN5007	<i>S. aureus</i> clinical isolate	
GRSA828	RN10616- Δ <i>agr::cadCA</i>	(This work)
GRSA830	GRSA828 (ABD2001)	(This work)
RN12134	RN1- Δ <i>agr::cadCA</i>	(This work)
GRSA845	GRSA828 (ABD2003)	(This work)
GRSA891	GRSA828 (ABD2002)	(This work)
GRSA891	GRSA828 (ABD2004)	(This work)
GRSA906	GRSA828 (ABD2005)	(This work)
GRSA908	GRSA828 (ABD2006)	(This work)
RN12154	JCSA778 (ABD2001)	(This work)
RN12065	JCSA778 (ABD2002)	(This work)
RN12066	JCSA778 (ABD2003)	(This work)
RN12153	JCSA778 (ABD2004)	(This work)
RN12143	JCSA778 (ABD2005)	(This work)
RN12148	GRSA778 (ABD2006)	(This work)
RN12133	RN25 (80 α , pRN7141)	(This work)
GRSA1005	RN12133 (ABD2001)	(This work)
GRSA1008	RN12133 (ABD2005)	(This work)
GRSA1011	RN12133 (ABD2006)	(This work)

Supplementary Table 3. Plasmids

Plasmid	Description	Reference
pUC18	<i>E. coli</i> vector	16
pDB114	Plasmid carrying CRISPR/ <i>cas9</i>	17
Pdcas9	Plasmid carrying CRISPR/ <i>dcas9</i>	18
pMAD	Vector for allelic replacement	19
pET28b	<i>E. coli</i> vector	Novagen #69865-3
pET28b-S2Up:CRISPR/ <i>cas9</i> :S2Dn*	pET28b with flanked CRISPR/ <i>cas9</i> module – used for spacer insertion	This work
pMAD-S2Up:CRISPR/ <i>cas9</i> /spacer:S2Dn	pMAD with flanked CRISPR/ <i>cas9</i> module + spacer, used for allelic exchange	This work
pACYC184	<i>E. coli</i> vector	20
pACYC184-S2Up:CRISPR/ <i>dcas9</i> :S2Dn	pACYC184 with flanked CRISPR/ <i>dcas9</i> module- used for spacer insertion	This work
pMAD-S2Up:CRISPR/ <i>dcas9</i> /spacer:S2Dn	pMAD with flanked CRISPR/ <i>dcas9</i> module + spacer used for allelic exchange	This work
pRN7141	pMK4:: <i>agrP₃-lux</i>	21

*S2Up and S2Dn are the SaPI2 flanking sequences up and downstream of the projected insertion site, which participate in allelic exchange with ABD2001.

Supplementary Table 4. Primer and spacer oligonucleotides

5'- AATT CTGAG TACAAATAGTGAAGTTTTAGATAATTCC-3'
5'- AATT CCCGGG CAAGGATTATATGGTTGGAATG-3'
5'-AATT CTGCAG TTACGAAATCATCCTGTGGAGC-3'
5-AATT CTGAG GAACTCAACAAGTCTCAGTGTGCTG-3'
5'-AAACATTTAACATTTGTCTACAAAGTTGCAGCGAG-3'
5'-AAAACCTGCTGCAACTTTGTAGACAAATGTTAAAT-3'
5'-AAACAAGATTGTAATAAATCGTATAATGACAGTGG-3'
5'-AAAACCACTGTCATTATACGATTTAGTACAATCTT-3'
5'-AAACGTCCTAAGACGCCAATCGAAAAGAAACACGG-3'
5'-AAAACCGTGTTCCTTTTCGATTGGCGTCTTAGGAC-3'
5'-AATT CTGCAG CTGTTTCCTTTTCTTTATGATAGTTTAC-3'
5-AATT CTGAG CCTTTTTTAAAAGTCAATATACTGTAAC-3'
5'-AATT AGATCT ATTATCGATAATTAATCCGAACATATC-3'
5'-AATT CTGCAG ATAAGCATTATATTGCAATGAAAAAG-3'
5'-AATT CTGCAG CTGTTTCCTTTTCTTTATGATAGTTTAC-3'

Shading represents the actual spacer sequence

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