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Supplementary Information for:

Genome editing using the endogenous Type I CRISPR-Cas system in *Lactobacillus crispatus*

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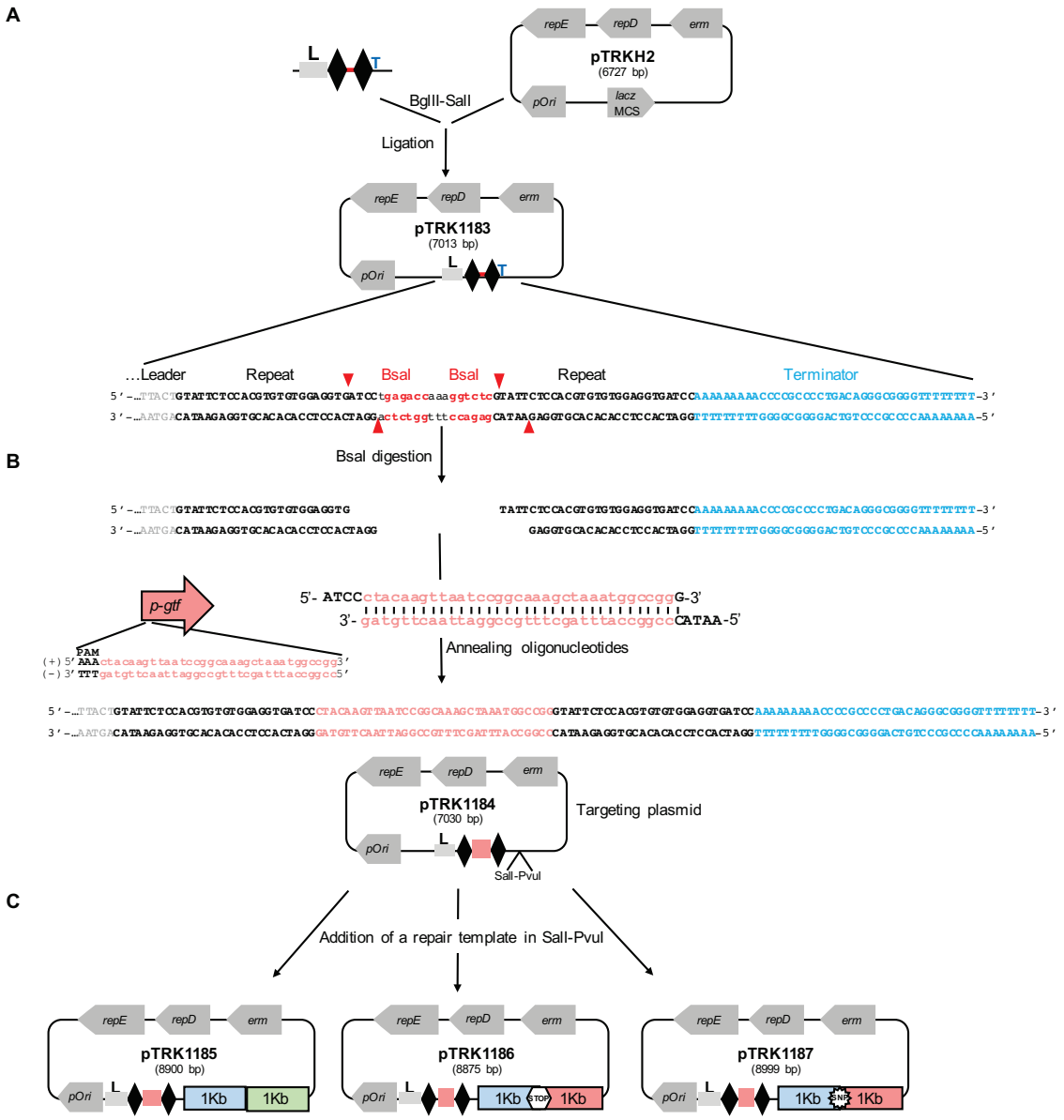

Fig. S1. Cloning strategy to generate the plasmid-based technology to repurpose the endogenous CRISPR system Type I-E in *L. crispatus* NCK1350. (*A*) An artificial crRNA containing the native leader (L) of the CRISPR-3 of *L. crispatus* NCK1350 as promoter, together with two repeats (native repeat sequence of NCK1350) and a Rho-terminator were synthesized as a gene block and cloned into BglII-SalI digested pTRKH2 to generate the plasmid-based technology pTRK1183. (*B*) The pTRK1183 plasmid allows cloning a spacer (target) using annealing oligonucleotides with overhand ends to the BsaI-digested pTRK1183 generating the targeting plasmid pTRK1184, that will express the crRNA to repurpose the endogenous CRISPR systems I-E against the desire target. (*C*) The generated targeting plasmid contains SalI-PvuI restriction sites for convenient and easy cloning of different repair templates to perform different genome editing outcomes as deletion (pTRK1185), insertion (pTRK1186) or single base editing (pTRK1187).

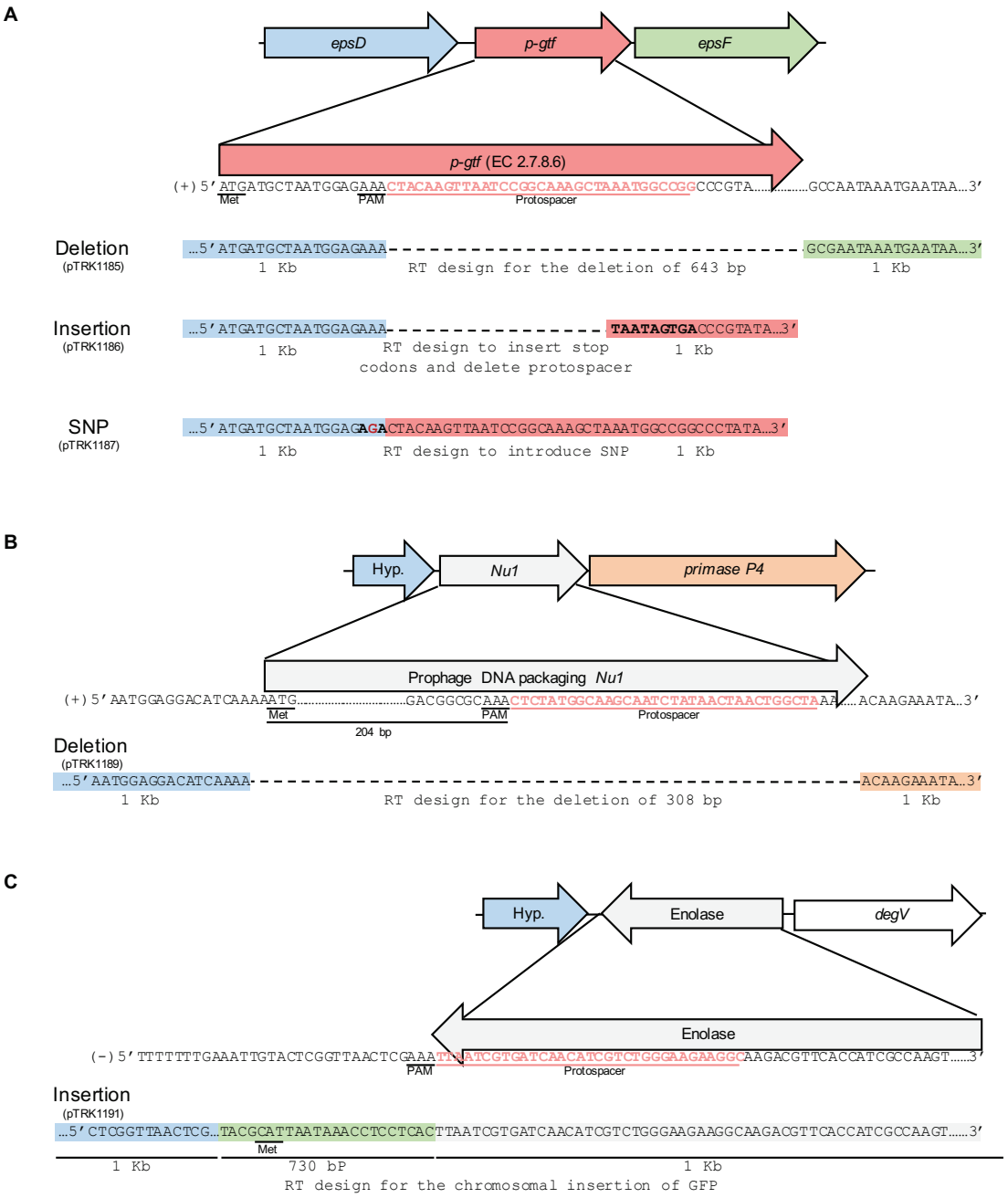

Fig. S2. Cloning strategy to design the repair templates for the different genome editing outcomes. A total of five different edits were performed in three different chromosomal targets with different designs associated with the homologous repair template (RT). For each design, the homologous arms were designed with an average length of 1 kb each. For each target, the chromosomal architecture, the gene of interest and the nucleotide sequence is displayed, with the protospacer targeted (T) region in red. (*A*) Design for the deletion, insertion of stop codons or single base substitution is shown for the exopolysaccharide priming-glycosyl transferase *p-gtf* (EC 2.7.8.6). Each template was cloned into the targeting plasmid pTRK1184 to generate pTRK1185, pTRK1186 and pTRK1187 respectively (see SI Appendix, Table S6). The homologous arm for the upstream region (blue) was designed until the PAM (5'-AAA-3') sequence, while the

downstream arm was designed according to the desire mutation to be introduced for the deletion or the insertion of stop codons. To perform single base editing, the upstream homologous arm contains the single base substitution in the PAM sequence, while the downstream region remains as the chromosomal sequence, including the protospacer sequence. (*B*) Repair template designed to delete the prophage DNA packaging *Nu1* gene. The PAM motif detected in the prophage DNA packaging *Nu1* gene is located closer to the 3' end of the gene. In this scenario the upstream arm was designed until the start codon of the *Nu1* gene, located 204 bp upstream from PAM motif. This designed repair template was cloned into pTRK1188 to generate pTRK1189. (*C*) Repair template designed to perform a chromosomal insertion of the GFP in the downstream region of the highly expressed enolase gene. The upstream arm was designed until the PAM but without including the PAM sequence, followed by the GFP gene to be inserted (730 bp) carrying its own ribosomal binding site followed by the downstream arm that includes the protospacer region. The designed repair template was cloned into pTRK1190 to generate pTRK1191.

Table S1. *Lactobacillus crispatus* genomes available at NCBI.

Source	Strain	Isolation source	GenBank genome
Human isolates	125-2-CHN	Vaginal isolate	ACPV00000000
	214-1	Vaginal isolate	ADGR00000000
	2029	Healthy women genital tract	AVFH00000000
	C037	Adult female bladder	MAKH00000000
	CTV-05	Vaginal isolate	ADML00000000
	FB049-03	Vaginal isolate	AGZF00000000
	FB077-07	Vaginal isolate	AGZG00000000
	JV-V01	Normal human vaginal flora	ACKR00000000
	MV-1A-US	Vaginal isolate	ACOG00000000
	MV-3A-US	Vaginal isolate	ACQC00000000
	OAB24-B	Human urine	MAMR00000000
	PSS7772C	Human urine	LSQY00000000
	SJ-3C-US	Vaginal isolate	ADDT00000000
	VMC1	Mid-vaginal wall from BV	LJCZ00000000
	VMC2	Mid-vaginal wall from BV	LJDA00000000
	VMC3	Mid-vaginal wall from BV	LJGP00000000
	VMC4	Mid-vaginal wall from BV	LJGQ00000000
	VMC5	Mid-vaginal wall healthy women	LJOK00000000
	VMC6	Mid-vaginal wall healthy women	LJOL00000000
	VMC7	Mid-vaginal wall healthy women	LJOM00000000
	VMC8	Mid-vaginal wall healthy women	LJON00000000
	DSM 20584*	Human Eye	AZCW00000000
	EM-LC1	Human fecal sample	AXLM00000000
	DISK12	Human oral cavity	MKXG01
	NCK1350	Human endoscopy	SGWL00000000
Chicken/Turkey isolates	C25	Chicken cecum	MCJG00000000
	JCM 5810	Chicken feces	LSVK00000000
	ST1	Chicken crop isolate	NC-014106
	UMNLC1	Turkey Ileum	LYQR00000000
	UMNLC2	Turkey Ileum	LYQS00000000
	UMNLC3	Turkey Ileum	LYQT00000000
	UMNLC4	Turkey Ileum	LYQU00000000
	UMNLC5	Turkey Ileum	LYQV00000000
	UMNLC6	Turkey Ileum	LYQW00000000
	UMNLC7	Turkey Ileum	LYQX00000000
	UMNLC8	Turkey Ileum	LYQY00000000
	UMNLC9	Turkey Ileum	LYQZ00000000

	UMNLC10	Turkey Ileum	LYRA00000000
	UMNLC11	Turkey Ileum	LYRB00000000
	UMNLC12	Turkey Ileum	LYRC00000000
	UMNLC13	Turkey Ileum	LYRD00000000
	UMNLC14	Turkey Ileum	LYRE00000000
	UMNLC15	Turkey Ileum	LYRF00000000
	UMNLC16	Turkey Ileum	LYRG00000000
	UMNLC18	Turkey Ileum	LYRH00000000
	UMNLC19	Turkey Ileum	LYRI00000000
	UMNLC20	Turkey Ileum	LYRK00000000
	UMNLC21	Turkey Ileum	LYRK00000000
	UMNLC22	Turkey Ileum	LYRL00000000
	UMNLC23	Turkey Ileum	LYRM00000000
	UMNLC24	Turkey Ileum	LYRN00000000
	UMNCL25	Turkey Ileum	LYRO00000000

Table S2. CRISPR-Cas systems in *Lactobacillus crispatus* genomes available at NCBI

Isolation source	Strain	CRISPR Subtype	Repeat sequence*	Repeat Length	No. spacers	*cas1*	*cas3*	*cas9*
Human isolates	125-2-CHN	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	3	-	-	-
	214-1	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	6	Y	-	Y
	2029	II-A	GTTTTAGATGGTTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
	C037	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	3	Y	-	Y
		I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	2	-	-	-
	CTV-05	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	5	Y	-	Y
	FB049-03	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
		I-E	GTATTCTCCACACATGTGGAGGTGATCC	28	4	-	-	-
	FB077-07	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
	JV-V01	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	4	Y	-	Y
	MV-1A-US	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	3	Y	-	Y
	MV-3A-US	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	4	Y	-	Y
	OAB24-B	II-A	–	-	-	Y	-	Y
		I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	2	-	-	-
	PSS7772C	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	6	Y	-	Y
	SJ-3C-US	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
	VMC1	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
		I-E	GTATTCTCCACACATGTGGAGGTGATCC	28	4	-	-	-
	VMC2	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	6	Y	-	Y
	VMC3	I-B	GTATTTATTTATCTTAAGAGAAATGTAAAT	30	13	Y	Y	-
		I-E	GTATTCTCCACGCATGTGGAGGTGATCC GTATTCTCCACGAGTGTGGGGATCCTAT	28 28	35	Y	Y	-
	VMC4	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	4	Y	-	Y
	VMC5	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	4	Y	-	Y
		I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	4	-	-	-
	VMC6	II-A	GTTTTAGATGATTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
		I-E	GTATTCTCCACACATGTGGAGGTGATCC	28	4	-	-	-
	VMC7	II-A	GTTTTAGATGGTTGTTAGATCAATGAGGTTTAGATC	36	5	Y	-	Y
	VMC8	II-A	GTTTTAGATGGTTGTTAGATCAATGAGGTTTAGATC	36	7	Y	-	Y
	DSM 20584	I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	5	Y	Y	-
	EM-LC1	-	–	-	-	-	-	-
	DISK12	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	10	-	-	-
	NCK1350	I-E	GTATTCTCCACGTATGTGGAGGTCATCC GTATTCTCCACGTATGTGGAGGTGATCC GTATTCTCCACACATGTGGAGGTGATCC GTATTCTCCACGTGTGTGGAGGTGATCC	28	53	Y	Y	-
Chicken / Turkey isolates	C25	I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	37	Y	Y	-
	JCM 5810	I-E	GTATTCTCCACGCGTGTGGAGGTGATCC	28	55	Y	Y	-
	ST1	I-E	GTATTCTCCACGTGTGTGGAGGTGATCC GTATTCTCCACGTATGTGGAGGTGATCCC	28 29	38	Y	Y	-
	UMNLC1	I-E	GTATTCTCCACGTGTGTGGAGGTGATCC	28	49	Y	Y	-
	UMNLC2	I-E	GTATTCTCCACACATGTGGAGGTGATCC	28	40	Y	Y	-

	UMNLC3	I-E	GTATTCTCCACACATGTGGAGGTGATCC	28	40	Y	Y	-
	UMNLC4	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	40	Y	Y	-
	UMNLC5	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	39	Y	Y	-
	UMNLC6	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	64	Y	Y	-
	UMNLC7	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	36	Y	Y	-
	UMNLC8	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	46	Y	Y	-
	UMNLC9	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	76	Y	Y	-
	UMNLC10	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	56	Y	Y	-
	UMNLC11	I-E	GTATTCTCCACGTGTGTGGAGGTGATCC GTATTCTCCACGTGTGTGGAGGTGATCCT	28 29	47	Y	Y	-
	UMNLC12	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	56	Y	Y	-
	UMNLC13	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	43	Y	Y	-
	UMNLC14	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	63	Y	Y	-
	UMNLC15	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	40	Y	Y	-
	UMNLC16	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	50	Y	Y	-
	UMNLC18	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	66	Y	Y	-
	UMNLC19	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	66	Y	Y	-
	UMNLC20	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	62	Y	Y	-
	UMNLC21	I-E	GTATTCTCCACGTATGTGGAGGTGATCC GTATTCTCCACGTATGTGGAGGTGATCCT	28 29	62	Y	Y	-
	UMNLC22	I-E	GTATTCTCCACGTATGTGGAGGTGATCC	28	62	Y	Y	-
	UMNLC23	I-E	GTATTCTCCACGTATGTGGAGGTGATCC GTATTCTCCACGTATGTGGAGGTGATCCT	28 29	62	Y	Y	-
	UMNLC24	I-E	GTATTCTCCACGTATGTGGAGGTGATCC GTATTCTCCACGTATGTGGAGGTGATCCT	28 29	62	Y	Y	-
	UMNCL25	I-E	GTATTCTCCACGTATGTGGAGGTGATCC GTATTCTCCACGTATGTGGAGGTGATCCT	28 29	64	Y	Y	-

Table S3. Protospacers targeted by *L. crispatus* spacers from CRISPR subtype II-A

Isolation Source	Strain	Spacer - Contig	PAM protospacers	Plasmid / Phage	Strain
Human	125	3-	TTCGTGATTAGTTTGATCTCGTTGTTGTAAGCGACGAA	rudivirus	Sulfolobales Mexican rudivirus
	214	5-82	AAATTAACACCTCTATTATTTTTTTCTGTAAGATACTT	pDF308	*Deferribacter desulfuricans* SSM1
	CTV-05	1-49	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*
	JVVO1	4-84	AAAAAAAGGATTATCTGTACCATCATCTAACGGCGTA	pXNC1	Xenorhabdus nematophila ATCC 19061
		4-84	CAGAAAATGGTTTATTTGTCATTTCTTCATGGCGGGCT	phage vB-PmiM-Pm5461	
	MV-1A-US	1-65	CAGAAAATGGTTTATTTGTCATTTCTTCATGGCGGGCT	phage vB-PmiM-Pm5461	
	MV-3A-US	4-60	TAAAAAAAGGATTATCTGTACCATCATCTAACGGCGTA	pXNC1	Xenorhabdus nematophila ATCC 19061
				pXNC2	Xenorhabdus nematophila AN61
			CAGAAAATGGTTTATTTGTCATTTCTTCATGGCGGGCT	phage vB-PmiM-Pm5461	Proteus phage
	PSS7772	1-21	TAAAAAAAGGATTATCTGTACCATCATCTAACGGCGTA	pXNC1	Xenorhabdus nematophila ATCC 19061
				pXNC2	Xenorhabdus nematophila AN61
	SJ-3C-US	5-67	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*
	VMC1	3-15	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*
	VMC2	5-153	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*
	VMC4	4-76	TAAAAAAAGGATTATCTGTACCATCATCTAACGGCGTA	pXNC1	Xenorhabdus nematophila ATCC 19061
				pXNC2	Xenorhabdus nematophila AN61
			CAGAAAATGGTTTATTTGTCATTTCTTCATGGCGGGCT	phage vB-PmiM-Pm5461	Proteus phage
	VMC5	3-117	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*
		4-117	AAATTAACACCTCTATTATTTTTTTCTGTAAGATACTT	pDF308	*Deferribacter desulfuricans* SSM1
	VMC6	5-50	CCCACGTTGGTACCTTCGCAAAAGCTATTGGGCGCCAC	Phage EFDG1	*Enterococcus faecalis*

*Underlined nucleotides indicate the putative PAM

Table S4. Protospacers targeted by *L. crispatus* spacers from CRISPR subtype I-B

Isolation Source	Strain	Spacer - Contig	PAM_protospacers	Plasmid / Phage	Strain
Human	VMC3	1	GTCCACCGTAACTAAGAACGACAGGATCTTTTTCTAGGTCAA	Phage KC5a	*Lactobacillus*
		1	TTTATGGTGTATCAAGAACAACAGATTCAGTTTTTAGTTCAA	pLM1	*L. mucosae* LM1
		2	GTTGATGGGTTATGGGAAAATGCCCGTTCAAAAAATCTTTATAA	Phage e112	*E.coli* O157:H7
		2	GTTGATGGGAAAATGCCCGTTCAAAAAATCTCTATAA	phage vB_EcoM_ACG-C40	
		4	ACCTGGTGCAACAGCAACTACTCCTGTAACTCTGCCTGCAAAC	phage vB_CsaM_GAP31	
		5	CCTGCCGGGGATGGTGAATCCCTCGGCAGGGCGCATTTACAGTCG	Phage Job42	
		6	GATTTACCGTTAATAGAATCTGGCGATAAAGTCAACATTGTTCTGC	Phage 0507-KN2-1	*Klebsiella*

* Underlined nucleotides indicate the predicted PAM

Table S5. Protospacers targeted by *L. crispatus* spacers from CRISPR subtype I-E

Isolation Source	Strain	Spacer - Contig	PAM protospacers	Plasmid / Phage	Strain
Human	VMC3	2-36	GCTTCAAACATGGGTGAGATTATCCGGAAAGGATAAGATATG	pUMNLJ22	*L. johnsonii* UMNLJ22
		16-36	AGCCTTAACAGATGGATTAAACAATTTTTAACGGCTGGTTT	pL11995-5	*L. paracollinoides* TMW1.1995
				pR2	*L. salivarius* Ren
				pPC892-4	*P. pentosaceus* SRCM100892
	NCK1350	1-18	AATCGAAAGTCCGCATGACTTCGTTGACAATAGCTCTCA	pL1481-4	*L. lindneri* TMW1.481
				pL11991-8	*L. backii* TMW1.1991
				plca36 (repA)	*L. casei* Zhang
Poultry	C25	1-18	TCAATTAACTAACAATGCTCAAACGTTAAATATGGTTGATA	plasmid1	*L. amylovorus* GRL1112
		1-18	AAAATTAACTAACAACGCACAAACGTTAAATTTGGTTGATA	pLH1	*L. helveticus* DSM20075
	JCM5810	3-4	AAGCACAAACCTTGCATAAATCGAGCGATCCGACCAGCATA	pUMNLJ22	*L. johnsonii* UMNLJ22
		3-4	AAGCACAAACCTTGCATAAATCGAGCGATCCGACCAGCATA	pUMNLJ21	*L. johnsonii* UMNLJ21
		15-4	TGCCGTAACAATTGACATGGCAAAAGAGCTTTGCATGATGT	phiJB	*L. delbrueckii bulgaricus*
	ST1	8-2	TTAACTAACAATGCTCAAACGTTAAATATGGTTGATAAAGA	plasmid1	*L. amylovorus* GRL1112
	UMNLC1	13-19	ATAAAAAATAGGCGATTCCGCAATACTTGCGAACCTATCG	phage AQ113	*L. helveticus*
	UMNLC6	13-32	TTAACTAACAATGCTCAAACGTTAAATATGGTTGATAAAGA	plasmid1	*L. amylovorus* GRL1112
		11-32	GGGCTTAATTGTATCAATGCTAATAAGAATGTTCTGCCCGG	phage phi hlb1	*L. gasseri*
		12-38	CATGAAAATAATCTGCTACTTTTGCTAAATCTTCAGCTTTT	Phage PLgT-1	*Lactococcus*
	UMNLC9	22-09	GAAATTAATGTTGGTGCATTAATGGAAGATGCATATTTAGA	phage AQ113	*L. helveticus*
		6-50	CTGCTCAATTAGTTAAAGGTTTTGGTGGTTTGGCTTCTGCG	phage AQ113	*L. helveticus*
		17-09	TTAACTAACAATGCTCAAACGTTAAATATGGTTGATAAAGA	plasmid1	*L. amylovorus* GRL1112

* Underlined nucleotides indicate the predicted PAM

Table S6. Spacers, annealing oligonucleotides and oligonucleotides used in this study

Protospacers	**Sequence 5' - 3'**
S6-CRISPR1	tatataagatctCAGTTTAGGTACCATTTTTTGACGATCAAAATCgtcgactatata
PS6-CRISPR1	tatataagatct**AAA**CAGTTTAGGTACCATTTTTTGACGATCAAAATCgtcgactatata
S21-CRISPR2	tatataagatctCAGTTCAAATGTTACTTGGCCACGCAAATATAAgtcgactatata
PS21-CRISPR2	tatataagatct**AAA**CAGTTCAAATGTTACTTGGCCACGCAAATATAAgtcgactatata
S26-CRISPR3	tatataagatctCGTGTTGTTTCCATATTCATTAGATAAAACATCgtcgactatata
PS26-CRISPR3	tatataagatct**AAA**CGTGTTGTTTCCATATTCATTAGATAAAACATCgtcgactatata
Annealing Oligos (ssDNA)	**Sequence 5` - 3`**
*p-gtf*_A	atccCTACAAGTTAATCCGGCAAAGCTAAATGGCCGGg
*p-gtf*_B	aatacCCGGCCATTTAGCTTTGCCGGATTAACTTGTAG
*Nu1*_A	atccCTCTATGGCAAGCAATCTATAACTAACTGGCTAg
*Nu1*_B	aatacTAGCCAGTTAGTTATAGATTGCTTGCCATAGAG
*enolase*_A	atccTTAATCGTGATCAACATCGTCTGGGAAGAAGGCg
*enolase*_B	aatacGCCTTCTTCCCAGACGATGTTGATCACGATTAA
Oligonucleotides	**Sequence 5`-3`**
*p-gtf*_F	ATGGCGACCCAGTTCAGATG
*p-gtf*_R	ATCTCTCTACACCACCGGCA
*p-gtf*_RT$_{KO}$_SalI_F	gtaatagtcgacTTAGAGCTGAAAACGGTGGC
*p-gtf*_RT$_{KO}$_PvuI_R	ttagtacgatcgCAACTAATATACCATTCACG
*p-gtf*_RT$_{STOP}$_SalI_F	tatatagtcgacTTAGAGCTGAAAACGGTGGC
*p-gtf*_RT$_{STOP}$_PvuI_R	tatatacgatcgGCCATGTGGATTATAGATAA
*p-gtf*_RT$_{SNP}$_Up_SalI_F	tatatagtcgacTTAGAGCTGAAAACGGTGGC
*p-gtf*_RT$_{SNP}$_Up_Rev	TCTCTCCATTAGCATCATTGCC
*p-gtf*_RT$_{SNP}$_Dw_SOE-PCR_F	caatgatgctaatggagagacTACAAGTTAATCCGGCAAA
*p-gtf*_RT$_{SNP}$_Dw_PvuI_R	tatatacgatcgGCCATGTGGATTATAGATAA
KO_*p-gtf*_F	CACCTTGACGGCGTAGTCTT
KO_*p-gtf*_R	TCATGCCACACCCTTTTAGCA
*Nu1*_F	GGTAGCGGCAGAACAAGGAA
*Nu1*__R	GCGCCTTTGAATATTGGGTCA
*Nu1*_RT$_{KO}$_SalI_F	tatatagtcgacAAGTTTATTATTTTCTTTAC
*Nu1*_RT$_{KO}$_PvuI_R	tatatacgatcgAATTATTATCAGTTAAA
KO_*Nu1*_F	CCACCGCCCATTATTTGACC
KO_*Nu1*_R	AGCGCCTAGTACCCGTCTTA
*GFP*_F	CCAACGGGCAAGGCAAAATA
*GFP*_R	CTGGTGACACCTTCATCGCT
*enolase*_RT$_{GFP}$_Dw_SalI_F	tatatagtcgacGCGCAGCTAGATTAAATGAT
*enolase*_RT$_{GFP}$_Dw_R	CGAGTTAACCGAGTACAAT
*enolase*_RT$_{GFP}$_Up_F	TTAATCGTGATCAACATCGT
*enolase*_RT$_{GFP}$_Up_PvuI_R	tatatacgatcgAGACGGTACTCCAAACAAG

RT_{GFP}_GFP_SOE-PCR_F	aattgtactcggttaactcgTTATTTGTATAGTTCATCCATGC
RT_{GFP}_GFP_SOE-PCR_R	acgatgttgatcacgattaaGTGAGGAGGTTTATTAATGCGTA
GFP_Insertion_F	GCATTGCGCGACAAGATTGA
GFP_Insertion_R	CGACTCACGTGGTAACCCAA
M13_F	GCTCGTATGTTGTGTGGAAT
M13_R	GGATGTGCTGCAAGGCGAT
*lacz*_R	CATTCAGGCTGCGCAACTGT
253_R	GAATGATCGACCAGGCAATG
*erm*_F	CAGGTAAAGGGCATTTAAC
*erm*_R	TATTCTCGATTGACCCATTT

* The PAM sequence cloned with the protospacers is showed in bold.

** The overhand ends of the annealing oligonucleotides to BsaI site are in lowercase

*** Restriction sites present on the spacers and the oligonucleotides are underlined

**** Overlap region of SOE-PCR oligonucleotides are showed in lower case