Fatal Necrotizing Enterocolitis in Neonate Caused by *Cronobacter sakazakii* Sequence Type 64 Strain of CRISPR Sublineage b

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We report fatal neonatal necrotizing enterocolitis in China caused by *Cronobacter sakazakii* capsular profile K1:CA1, sequence type 64, and CRISPR type 197. Phylodynamic analyses indicated that the strain originated from the ancient, widespread, and antimicrobial drug–sensitive CRISPR sublineage b. Enhanced surveillance and pathogenesis research on this organism are required.

*C*ronobacter sakazakii is a major foodborne pathogen that is associated with outbreaks of life-threatening necrotizing enterocolitis, meningitis, and sepsis in neonates and infants. Although the incidence of this pathogen is low, the case-fatality rate is high in premature and immunocompromised infants (*1*,*2*). Multilocus sequence typing (MLST) is a powerful tool for effectively identifying and discriminating different *Cronobacter* strains. Specific sequence types (STs) and clonal complexes are closely related to infections (*3*).

Compared with MLST, CRISPR (clustered regularly interspaced short palindromic repeats) typing is superior for distinguishing similar strains (*4*). *C. sakazakii* ST64, the major ST in food samples, was further divided into 2 sublineages based on CRISPR diversity (*5*). We report a *C. sakazakii* ST64 strain that caused necrotizing enterocolitis in a neonate in China and further examine its origin and phylogenetic relationship with ST64 strains based on CRISPR diversity and whole-genome single-nucleotide polymorphism (wgSNP).

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The Study

This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (Guangzhou, China; no. 2016081029). Experiments were performed at the Institute of Microbiology, Guangdong Academy of Sciences, and analyses and manuscript preparation were completed at Guangdong University of Technology.

On April 28, 2019, a 17-day-old male neonate born with severe congenital heart disease and perioral cyanosis for 2 hours was hospitalized in a children's hospital in Guangzhou, China. The patient had necrotizing enterocolitis symptoms develop on May 6 and was given meropenem and metronidazole as antiinfection therapy. However, his symptoms did not improve, and intestinal perforation and peripheral hydrocephalus developed a few days later. Despite the efforts of the doctor, the patient died.

We identified a *Cronobacter* species isolated from ascites by using an automated VITEK 2 Compact system (bioMérieux, https://www.biomerieux. com). An isolate, GZfs, was identified as *C. sakazakii* ST64 of serotype O2. This ST has not previously been reported to cause neonatal necrotizing enterocolitis (*6*). *C. sakazakii* GZfs were susceptible to almost all antimicrobial drugs tested, except cephalothin (Appendix Table, https://wwwnc.cdc. gov/EID/article/29/9/23-0537-App1.pdf). We sequenced genomic and plasmid DNA by using the PacBio RS II (Pacific Biosciences, https://www. pacb.com) and HiSeq (Illumina, https://www .illumina.com) platforms, assembled, and annotated as described (*1*,*7*). *C. sakazakii* GZfs had a single circular chromosome, 4.2 Mb, 57.11% GC content, and 2 plasmids (denoted as pFS1, 115,925
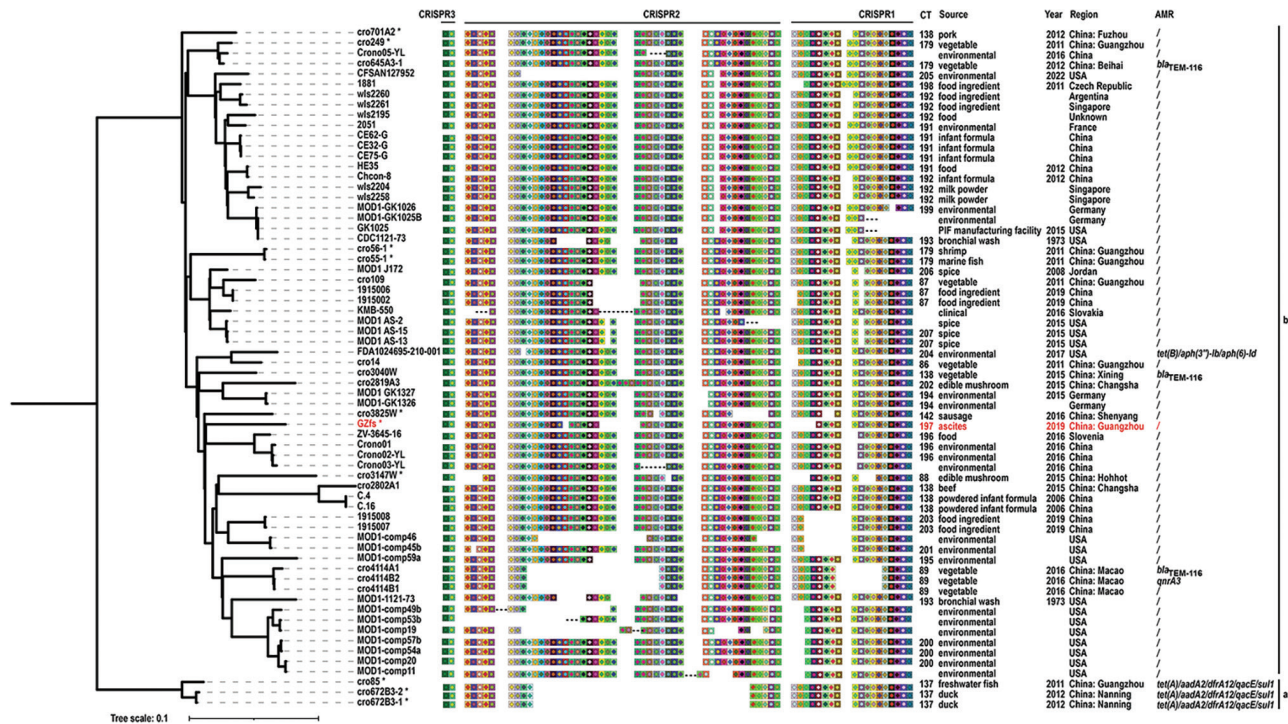
Figure 1. Multilocus sequence typing phylogenetic tree based on whole-genome sequencing single-nucleotide polymorphisms of a Cronobacter sakazakii ST64 strain from a fatal case of necrotizing enterocolitis in a 17-day-old male neonate, China, compared with reference strains. Asterisks indicate newly sequenced strains in this study; red text indicates isolate from the neonate. CRISPR spacers arrangement, CT, source, year, region and genes are listed next to corresponding strains. Color schemes in CRISPR arrays are provided at the spacer level to illustrate differences among strains by using CRISPRStudio software (https://www.semanticscholar.org). Ellipsis in spacers indicate partial CRISPR arrays without determined end (incomplete CRISPR arrays). Scale bar indicates nucleotide substitutions per site. AMR, antimicrobial resistance; CR, CRISPR type; CRISPR, clustered regularly interspaced short palindromic repeats; ST, sequence type.

bp, 57.09% GC; and pFS2, 110,391 bp, 50.10% GC) (GenBank accession nos. CP123201–3).

In our previous study, we divided *C. sakazakii* ST64 strains into 2 CRISPR sublineages, a and b, and compared antimicrobial drug resistance profile strains in sublineage a with strains in sublineage b (*5*). To explore the origin of this pathogenic strain and its phylogenetic relationship with other *C. sakazakii* ST64 strains, we performed whole-genome sequencing of 9 ST64 strains (GenBank accession nos. JARUQD000000000–L000000000) and downloaded all ST64 strains with whole-genome sequences from the *Cronobacter* PubMLST database (https://pubmlst.org/organisms/cronobacter-spp) and GenBank genome databases. We provide antimicrobial drug resistance results of 14 food-source ST64 strains (Appendix Table).

After deleting all poor-quality sequences and duplicate strains, we used 66 whole-genome sequences for further analyses. We extracted CRISPR arrays and spacers from those sequences and assigned CRISPR type (CT) numbers to 55 strains with intact CRISPR arrays,

according to methods from our previous study (*4*). All ST64 strains had the same 2 spacers: CRISPR3, which was not detected in our previous study because of the lack of *cas* genes (*7*), and CRISPR3, which was not useful for CT in this ST. There were 25 CTs, including 17 new, and we identified *C. sakazakii* GZfs as a new type of CT197 (Appendix). Based on spacer composition, GZfs belonged to CRISPR sublineage b. However, no other strain was found to have an identical spacer profile.

We calculated the wgSNP of ST64 strains by using Harvest software (*8*) and extracted those strains by using SNP-sites software (*9*). We constructed a maximum-likelihood phylogenetic tree by using FastTree software (*10*) and edited in iTOL (*11*). We used a Bayesian phylogenetic approach to estimate the nucleotide substitution rates and divergence times of *C. sakazakii* ST64 according to a previous study (*7*). The maximum-likelihood tree based on the wgSNPs of ST64 strains also showed 2 distinct phylogenetic clusters in accordance with the CRISPR sublineages (Figure 1). The strains in sublineage a were all from food sources.

Sublineage b contained more strains and diverse sources. Moreover, *C. sakazakii* GZfs and all clinical source strains (*C. sakazakii* KMB-550, MOD1-1121-73, and CDC1121-73) in public databases belonged to this cluster. *C. sakazakii* MOD1-1121-73 and CDC1121-73 were isolated from bronchial washes; there was no other patient or disease information regarding those clinical strains.

The genome-wide substitution rate of *C. sakazakii* ST64 was estimated to be 2.3×10^6 substitutions/site/year (95% CI 1.0×10^7–5.3×10^6 substitutions/site/year). According to the maximum clade credibility (MCC) tree (Figure 2), the likely most recent common ancestor of CRISPR sublineage b was 47,500 (95% CI 11,600–300,700) years ago, earlier than for sublineage a, which was 10,900 (95% CI 1,300–11,600) years ago. *C. sakazakii* GZfs had a relatively close phylogenetic relationship with the food-source *C. sakazakii* strain ZV-3645-16 in Slovenia; environmental strains *C. sakazakii* Crono01, Crono02-YL, and Crono03-YL; and the food-source strain *C. sakazakii* cro3825W in China (Figures 1, 2). This finding indicates a close environment food-clinic relationship in dissemination.

We identified acquired drug resistance genes by using ResFinder 2.1. (https://cge.cbs.dtu.dk/services/ResFinder-2.1). All 3 strains in sublineage a acquired the antimicrobial resistance (AMR) genes *tet(A)/aadA2/dfrA12/qacE/sul1*, in accordance with their resistance to tetracycline and trimethoprim/sulfamethoxazole (Figure 1; Appendix Table). Five strains in sublineage b had AMR genes; 3 strains (*C. sakazakii* cro645A3-1, cro3040W, cro4114A1) and 1 strain (*C. sakazakii* cro4114B2) isolated from vegetables harbored the AMR genes *bla*$_{TEM-116}$ and *qnrA3*. Four strains were susceptible to all tested antimicrobial drugs (Appendix Table). One environmental strain, *C. sakazakii* FDA1024695-210-001, had *tet(B)/aph(3'')-Ib/aph(6)-Id* genes. A total of 92.1% (58/63) strains in sublineage b lacked AMR genes. All 4 clinical strains, including *C. sakazakii* GZfs, did not have AMR genes. In a previous study, *C. sakazakii* ST494 strain was sensitive to all antimicrobial drugs used for treatment; however, the patient died (*12*). Those results suggested that AMR might not be the major reason for the high case-fatality rate associated with this pathogenic infection. Both *C. sakazakii* ST494 and ST64 did not belong to the common pathogenic clonal complex. Enhanced surveillance and pathogenesis research of this organism are warranted.

The virulence genes in *C. sakazakii* remain unclear (*13*), and 2 T6SS and 1 prophage on the chromosome of GZfs might contribute to pathogenicity. The capsular profile of the GZfs was determined to be K1:CA1, as in our previous study (*1*), and plasmid pFS1 was closely related to the IncFIB-type virulence plasmid pESA3 identified in pathogenic *C. sakazakii* strains and pGW2 in *C. sakazakii* GZcsf-1, which causes
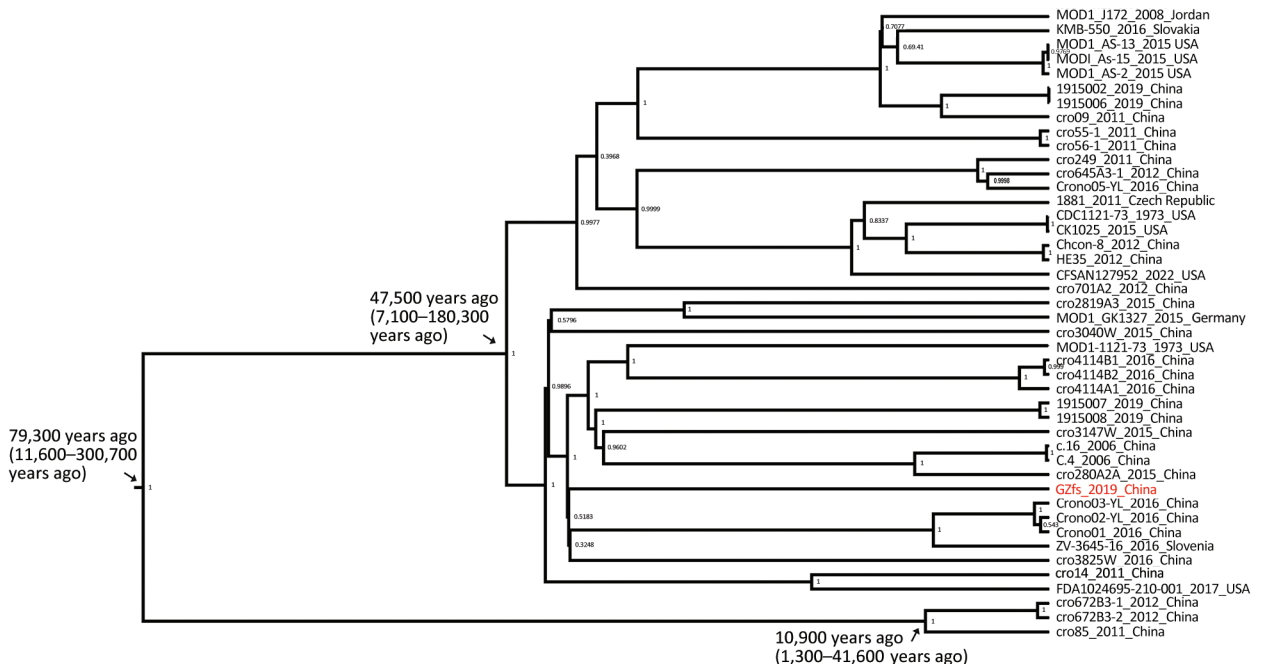
Figure 2. Timed phylogeny in maximum clade credibility tree of *Cronobacter sakazakii* ST64 strain from a fatal case of necrotizing enterocolitis in a 17-day-old male neonate, China, compared with reference strains. Red text indicates isolate from the neonate. Numbers along branches are bootstrap values. Posterior probabilities are shown in the nodes. ST, sequence type.

meningitis in neonates (*1,14*). More attention should be given to the study of virulence and pathogenesis.

Conclusions

We report 1 *C. sakazakii* ST64 strain, GZfs, causing fatal neonatal necrotizing enterocolitis in China that did not belong to the previously identified common pathogenic clonal complexes or STs (*3*). It belongs to the ancient, widespread, and antimicrobial drug–sensitive CRISPR cluster b of ST64. AMR might not be the major reason for the high case-fatality rate for this pathogen. Public health would benefit from identification of virulence genes and pathogenic mechanisms of *C. sakazakii*.

About the Author

Dr. Zeng is an associate professor at Guangdong University of Technology, Guangzhou, China. Her primary research interests are adaptive evolution, drug resistance, and phage treatment of major pathogenic bacteria.

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Fatal Necrotizing Enterocolitis in Neonate Caused by *Cronobacter sakazakii* Sequence Type 64 Strain of CRISPR Sublineage b

Appendix

Appendix Table. Antimicrobial drug susceptibility profiles

Antimicrobial group	Antibiotic	Antimicrobial susceptibility (KB)[#]								
		GZfs[*]	cro3147W[*]	cro701A2[*]	cro3825W[*]	cro249[*]	cro55-1[*]	cro56-1[*]	cro672B3-1[*]	cro672B3-2[*]
Penicillins	Ampicillin	S	S	S	S	S	S	S	S	S
	Ampicillin/sulbactam	S	S	S	S	S	S	S	S	S
	Amoxicillin/Clavulanic	S	S	S	S	S	S	S	S	S
Cephalosporins	Cefepime	S	S	S	S	S	S	S	S	S
	Ceftriaxone	S	S	S	S	S	S	S	S	S
	Cefazolin	S	S	S	S	S	S	S	S	S
	Cephalothin	R	R	R	S	S	S	S	S	S
Aminoglycosides	Tobramycin	S	S	S	S	S	S	S	S	S
	Gentamicin	S	S	S	S	S	S	S	S	S
	Amikacin	S	S	S	S	S	S	S	S	S
Quinolones	Ciprofloxacin	S	S	S	S	S	S	S	S	S

Antimicrobial group	Antibiotic	Antimicrobial susceptibility (KB)[#]								
		GZfs[*]	cro3147W[*]	cro701A2[*]	cro3825W[*]	cro249[*]	cro55-1[*]	cro56-1[*]	cro672B3-1[*]	cro672B3-2[*]
Carbapenems	Imipenem	S	S	S	S	S	S	S	S	S
Sulfonamides	Trimethoprim/sulfameth-oxazole	S	S	S	S	S	S	S	R	R
Monobactams	Aztreonam	S	S	S	S	S	S	S	S	S
Amphenicols	Chloramphenicol	S	S	S	S	S	S	S	S	S
Tetracyclines	Tetracycline	S	S	S	S	S	S	S	R	R

Antimicrobial group	Antibiotic	Antimicrobial susceptibility (KB)[#]							
		cro85[*]	cro2819A3	cro14	cro109	cro645A3-1	cro2802A1	cro3040W	cro4114A1
Penicillins	Ampicillin	S	S	S	S	S	S	S	S
	Ampicillin/sulbactam	S	S	S	S	S	S	S	S
	Amoxicillin/Clavulanic	S	S	S	S	S	S	S	S
Cephalosporins	Cefepime	S	S	S	S	S	S	S	S
	Ceftriaxone	S	S	S	S	S	S	S	S
	Cefazolin	S	S	S	S	S	S	S	S
	Cephalothin	R	R	S	S	S	R	S	S
Aminoglycosides	Tobramycin	S	S	S	S	S	S	S	S
	Gentamicin	S	S	S	S	S	S	S	S
	Amikacin	S	S	S	S	S	S	S	S
Quinolones	Ciprofloxacin	S	S	S	S	S	S	S	S
Carbapenems	Imipenem	S	S	S	S	S	S	S	S
Sulfonamides	Trimethoprim/sulfameth-oxazole	R	S	S	S	S	S	S	S
Monobactams	Aztreonam	S	S	S	S	S	S	S	S
Amphenicols	Chloramphenicol	S	S	S	S	S	S	S	S
Tetracyclines	Tetracycline	R	S	S	S	S	S	S	S

Antimicrobial group	Antibiotic	Antimicrobial susceptibility (KB)[#]	
		cro4114B1	cro4114B2
Penicillins	Ampicillin	S	S
	Ampicillin/sulbactam	S	S
	Amoxicillin/Clavulanic	S	S
Cephalosporins	Cefepime	S	S
	Ceftriaxone	S	S
	Cefazolin	S	S
	Cephalothin	S	S
Aminoglycosides	Tobramycin	S	S
	Gentamicin	S	S
	Amikacin	S	S
Quinolones	Ciprofloxacin	S	S
Carbapenems	Imipenem	S	S
Sulfonamides	Trimethoprim/sulfameth-oxazole	S	S
Monobactams	Aztreonam	S	S
Amphenicols	Chloramphenicol	S	S
Tetracyclines	Tetracycline	S	S

[#] The results are interpreted using the Enterobacteriaceae data from the Clinical and Laboratory Standards Institute (CLSI).

* These strains are newly sequenced, the genome sequence of other strains have been reported in our previous stud

Dataset Code of CRISPR types (CTs), order of CRISPR array spacers, and dictionary of CRISPR spacers in _C. sakazakii_ ST64

CT	CRISPR1-Serial number	CRISPR2-Serial number	CRISPR3-Serial number	CRISPR6-Serial number
82	58	34	2	
83	58	35	2	
84*	~~58~~	~~33~~	~~2~~	
85*	~~58~~	~~31~~	~~2~~	
86	59	31	2	
87	60	32	2	
88	61	30	2	
89	62	33	2	
137	58	111	2	
138	58	31	2	
141	97	111	2	
142	98	112	2	
179	57	31	2	
180*	58	139	~~2~~	
191	57	150	2	
192	58	150	2	
193	58	151	2	
194	58	153	2	
195	58	154	2	
196	97	31	2	
197	134	149	2	
198	135	150	2	
199	136	150	2	
200	137	31	2	
201	138	152	2	
202	139	153	2	
203	138	31	2	
204	59	158	2	
205	57	159	2	
206	60	31	2	
207	60	157	2	

Some CT codes were modified for CRISPR3 was omitted in the previous study.

* These CTs (n = 3) after the addition of CRISPR3 serial number were the same as the existing CTs, they were canceled in the new CTs code table.

Order of CRISPR array spacers

CRISPR1-Serial number	CRISPR1-spacers-order (key-order)
57	209,133,132,131,130,129,128,127,126,126,125,124,123,122,121,94,93,514,92
58	209,133,132,131,130,129,128,127,126,125,124,123,122,121,94,93,514,92
59	133,132,131,130,129,128,127,126,125,124,123,122,121,94,93,514,92
60	209,133,132,131,130,129,128,127,126,124,123,122,121,94,93,514,92
61	131,130,129,128,127,126,122,121,94,93,514,92
62	209,133,132,131,130,129,128,121,94,93,514,92
97	209,133,132,131,130,129,128,127,125,124,123,122,121,94,93,514,92
98	209,133,132,131,130,127,126,125,124,123,122,121,94,93,514,92
134	130,129,128,127,126,125,124,123,122,121,94,93,514,92
135	132,131,130,129,128,127,126,126,126,125,124,123,122,121,94,93,514,92
136	209,133,132,131,130,129,128,127,126,126,125,124,123,122,121,93,514,92
137	132,131,130,129,128,127,126,125,124,123,122,121,94,93,514,92
138	209,133,126,125,124,123,122,121,94,93,514,92
139	209,125,124,123,122,121,94,93,514,92

CRISPR2-Serial number	CRISPR2-spacers-order (key-order)
30	177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160,159,332,158,157,156,158,157,156,155,154,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
31	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160,159,332,158,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
32	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
33	180,179,178,177,176,175,174,173,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
34	180,179,178,177,176,175,174,173,172,171,179,169,168,167,166,165,164,163,162,161,160,159,332,158,157,156,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
35	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160,159,332,158,157,156,158,157,153,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
111	180,179,178,177,176,175,174,173,172,142,49,48,45,28
112	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160,159,332,158,157,156,154,153,152,151,150,149,148,147,146,45,28

CRISPR2-Serial number	CRISPR2-spacers-order (key-order)
149	180,179,178,177,176,175,174,173,172,171,170,169,168,167,165,164,163,162,161,157,156, 155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
150	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160, 159,332,158,157,156,155,154,153,152,151,150,149,147,146,145,144,143,142,49,48,45,28
151	180,179,178,177,176,175,174,173,172,171,170,169,168,162,161,160,159,157,156,155,154, 153,152,150,149,148,147,146,145,144,143,142,49,48,45,28
152	180,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160,159, 332,158,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
153	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160, 159,332,158,157,156,155,154,153,152,151,149,148,147,146,145,144,143,142,49,48,45,28
153	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,158,157, 156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
154	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,158,157, 156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
157	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160, 332,158,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
158	180,179,178,177,176,175,174,173,172,171,170,169,168,167,166,165,164,163,162,161,160, 332,158,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28
159	180,179,178,177,176,175,174,172,171,170,169,168,167,166,165,164,163,162,161,160,159, 332,158,157,156,155,154,153,152,151,150,149,148,147,146,145,144,143,142,49,48,45,28

CRISPR3-Serial number	CRISPR3-spacers-order (key-order)
2	164,1

Dictionary of CRISPR1 spacers

key	AnnotatedSpacer	sequence
92	sak1–92	CGGTGATGCACCTCGCCGCGATAGATGATCAA
93	sak1–93	CGACGTTTTTACCTACACCGTGCAAATACTGGC
94	sak1–94	ACGGCGACGCTTGTCAGCCCGCCCGAAGGCGT
121	sak1–121	TTGCCGAACGCCACCCAGATAATTTCCGTGCC
122	sak1–122	AAATCTGCATGTTCTGCGGCGAGCCAGCATCG
123	sak1–123	AACCCGGCACTGACAGCCGCCAATGAGTGTAT
124	sak1–124	GTTAATTGCCCGCAGTGCGGGCACCTTTTTGA
125	sak1–125	CCGAGCGCCTTTACATCCTTGCCAGTCGGCTC
126	sak1–126	CGGCGGCCGGGTGCCGTGACTTATTGGGCGTA

key	AnnotatedSpacer	sequence
127	sak1–127	GTACCGCCGCCAGTCTGAGTTGTATTTCTGCA
128	sak1–128	CAACGATTTATCCAGGACGAAGTACACAAAAA
129	sak1–129	GACTCGTTGCTTGTCATTCAGCGGGCATTCAC
130	sak1–130	CAGCATAACCGGATCTGTCGTCTGGCCGTTCA
131	sak1–131	AGAAAGATCAAAATTGCCGGCAACCAATTCTT
132	sak1–132	CGCGATATTATGATCATCACCGGACGGCGCCC
133	sak1–133	AAAGACGGATCCGACATGCGCACGCGAAAACA
209	sak1–209	ATATCATCCCGTCCTGCCTGTCCGTTCACCGT
514	sak1–514	CCGCCTGCGTCCTAACCGGCCTGTAGGCTTTT

Dictionary of CRISPR2 spacers

key	AnnotatedSpacer	sequence
28	sak2–28	ATCGCCAGTATTTTCAACGTGCCGGCGCACAT
45	sak2–45	TTTTCGAAATTGAGCATATTTAACCTATGATT
48	sak2–48	GCGCGACGTATCGCACCGTTGCGCAGGATACC
49	sak2–49	GCCATCGGACGCTTCTGCCAATCTTAACAGGA
142	sak2–142	GGTCATGACGGAAGAGGATGAAGAGGACGCCG
143	sak2–143	GTGTGAGACTTTTGCGCTGCCTAACTTGATCG
144	sak2–144	CCACAGCGCCGCCCTCGCCGGCGGCCGAGTAT
145	sak2–145	TGTACGGGGTAAGGCTGCACCGCATACGTGCG
146	sak2–146	ATTAATATTCATCCTGAACCAGTTAACACCCC
147	sak2–147	ATCGCCTTCGACAGGTCGCCAGGTTCGTCGTA
148	sak2–148	CAGGCGCGCGGTTACGCCTGGGCGGAGAAATA
149	sak2–149	TCGTCGAGGGTTATTTGCATGGCGGCTCGGGG
150	sak2–150	GAGGAACGCCGCCAATTTCAGGAATGTATTTT
151	sak2–151	GGTGTGAAGCTGTTAAGCGTTTTTGAAGATCT
152	sak2–152	TTCGACGATCTCCCCGTCGGCGTGAATGTTCG
153	sak2–153	CATCGGCGTCGCTTGTTCGAGCGCGTCCATCA
154	sak2–154	TCAGTTTCCCGGAGGCGAGCGCCCCTTCAAGC
155	sak2–155	GCTGCGCGACTCGCTGGCCCGTCTCGATACCA
156	sak2–156	ATGTGCCAGAAGTGAAAGCGGCTATCAAACGG
157	sak2–157	GCCGGAACCGGCGCGGAACTGAGGCAGGCATA
158	sak2–158	TGCTGTTATCAGCACTCGTCTGGAACTGGATA
159	sak2–159	CTTACCGCTGGCTGGCAGAGCAGCTCGGCATC
160	sak2–160	TCTGCCGCCCCGGTGCCCAGCGGCCGGACGTG
161	sak2–161	GCAAACTTTCAGTCACTGCAATCGCGTTTATC
162	sak2–162	CATGCGGCCATCGTCGTCAGTGCGCTGTATTC
163	sak2–163	CGCAATCCAAAAATACTGTATATTTGTACAGT

key	AnnotatedSpacer	sequence
164	sak2–164	CCGCTGGTGGGCGTGGGGTGGATATCCGGGCG
165	sak2–165	CGCCTGCGGGCTGTGTCATTACTGGCGTGAAA
166	sak2–166	TCAGGCGGTGTCAGTGGCCGCCTTCACAAGCA
167	sak2–167	CCCGCCGTTCTCGTATTTATTTTTAAATGCCG
168	sak2–168	GCTTCCTGCGTTTTACTGCCTGATAAATCCTG
169	sak2–169	CGTAACGAACTACTGCAATCCAGCGGTTATTG
170	sak2–170	GCCATGAGAACGCCAAAGGCATGTCCGTCACT
171	sak2–171	CGATGTGGGCGGCGGGCGCCACGCGCGTAACG
172	sak2–172	TACACCCGAGACGGGTTTAACAGCAGGTGGGA
173	sak2–173	AGGGTTGAGGCATTCGTCATTGAGGCGCTCCA
174	sak2–174	AATGACCAAAAAAACGCAGACTTTTTTCTTGC
175	sak2–175	AGTTGGTCGCCGAGCGACGCCGCCTGCCGGTC
176	sak2–176	AACTTTCCCGCGATTACAGCCAGGTGAGCTAC
177	sak2–177	CCCCTTTGTTTTACCGAGGCTAAATGCCCAGC
178	sak2–178	AACCGGATAGACAGTTAAATACCAACTATCTA
179	sak2–179	CAACTCGCACACGTCAGCGGAGGAACGCGCTC
180	sak2–180	GAAAAACAGGTGGGTTTTGCCTGTGAGTGTGA
332	sak2–332	GCTCATATTTTTTAAAATTTTTAATATCTAAC

Dictionary of CRISPR3 spacers

key	AnnotatedSpacer	sequence
1	sak3–1	AGACGCGCCGGAAGGGGAGTTGGTGCGCCAGCT
93	sak3–164	CATTGCCTCGATAACCTGCAAATCCCCCTCAC