

**Table S1.** Amplification primers used for the generation of FAM-labeled dsDNA PAM-templates.

Name	Structure (5'-3')
Uni-Spacer-F	caagaaggagatgtctggggatgtcagggatgcatttgggccat
FAM-Uni-R	tttcatctgtccagcaccttcatggattttaaagtgttcggcaaagaagaga
Spacer-PAM-R_1	tttcggcaaagaagagaggcttgttcttgacacttgaacaatggccacaaatgcatc
Spacer-PAM-R_2	tttcggcaaagaagagaggcttgttcttgacacttgaacaatggccacaaatgcatc
Spacer-PAM-R_3	tttcggcaaagaagagaggcttgttcttgacacttgaacaatggccacaaatgcatc
Spacer-PAM-R_4	tttcggcaaagaagagaggcttgttcttgacacttagaacaatggccacaaatgcatc
Spacer-PAM-R_5	tttcggcaaagaagagaggcttgttcttgacactttagaacaatggccacaaatgcatc
Spacer-PAM-R_6	tttcggcaaagaagagaggcttgttcttgacacttggatcaatggccacaaatgcatc
Spacer-PAM-R_7	tttcggcaaagaagagaggcttgttcttgacacttcatcaatggccacaaatgcatc
Spacer-PAM-R_8	tttcggcaaagaagagaggcttgttcttgacacttagatcaatggccacaaatgcatc
Spacer-PAM-R_9	tttcggcaaagaagagaggcttgttcttgacactttagcaatggccacaaatgcatc
Spacer-PAM-R_10	tttcggcaaagaagagaggcttgttcttgacacttggagcaatggccacaaatgcatc
Spacer-PAM-R_11	tttcggcaaagaagagaggcttgttcttgacacttggagcaatggccacaaatgcatc
Spacer-PAM-R_12	tttcggcaaagaagagaggcttgttcttgacacttagagcaatggccacaaatgcatc
Spacer-PAM-R_13	tttcggcaaagaagagaggcttgttcttgacactttagcaatggccacaaatgcatc
Spacer-PAM-R_14	tttcggcaaagaagagaggcttgttcttgacactttagcaatggccacaaatgcatc
Spacer-PAM-R_15	tttcggcaaagaagagaggcttgttcttgacactttagcaatggccacaaatgcatc
Spacer-PAM-R_16	tttcggcaaagaagagaggcttgttcttgacacttagaccaatggccacaaatgcatc
Spacer-PAM-R_18	tttcggcaaagaagagaggcttgttcttgacacgtcgaccaatggccacaaatgcatc
Spacer-PAM-R_19	tttcggcaaagaagagaggcttgttcttgacacctcgaccaatggccacaaatgcatc
Spacer-PAM-R_20	tttcggcaaagaagagaggcttgttcttgacacatcgaccaatggccacaaatgcatc
Spacer-PAM-R_21	tttcggcaaagaagagaggcttgttcttgacacttcgacctatggccacaaatgcatc
Spacer-PAM-R_22	tttcggcaaagaagagaggcttgttcttgacacgtcgacctatggccacaaatgcatc
Spacer-PAM-R_23	tttcggcaaagaagagaggcttgttcttgacacctcgacctatggccacaaatgcatc
Spacer-PAM-R_24	tttcggcaaagaagagaggcttgttcttgacacatcgacctatggccacaaatgcatc
Spacer-PAM-R_25	tttcggcaaagaagagaggcttgttcttgacacttcgaccgatggccacaaatgcatc
Spacer-PAM-R_26	tttcggcaaagaagagaggcttgttcttgacacgtcgaccgatggccacaaatgcatc
Spacer-PAM-R_27	tttcggcaaagaagagaggcttgttcttgacacctcgaccgatggccacaaatgcatc
Spacer-PAM-R_28	tttcggcaaagaagagaggcttgttcttgacacatcgaccgatggccacaaatgcatc
Spacer-PAM-R_29	tttcggcaaagaagagaggcttgttcttgacacttcgacctatggccacaaatgcatc
Spacer-PAM-R_30	tttcggcaaagaagagaggcttgttcttgacacgtcgacctatggccacaaatgcatc
Spacer-PAM-R_31	tttcggcaaagaagagaggcttgttcttgacacctcgacctatggccacaaatgcatc
Spacer-PAM-R_32	tttcggcaaagaagagaggcttgttcttgacacatcgacctatggccacaaatgcatc
Spacer-PAM-R_33	tttcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc
Spacer-PAM-R_34	tttcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc
Spacer-PAM-R_35	tttcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc
Spacer-PAM-R_36	tttcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc
Spacer-PAM-R_37	tttcggcaaagaagagaggcttgttcttgacacttcggcttatggccacaaatgcatc
Spacer-PAM-R_38	tttcggcaaagaagagaggcttgttcttgacacttcggcttatggccacaaatgcatc
Spacer-PAM-R_39	tttcggcaaagaagagaggcttgttcttgacacttcggcttatggccacaaatgcatc
Spacer-PAM-R_40	tttcggcaaagaagagaggcttgttcttgacacttcggcttatggccacaaatgcatc
Spacer-PAM-R_41	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_42	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_43	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_44	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_45	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_46	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_48	tttcggcaaagaagagaggcttgttcttgacacttcgcttatggccacaaatgcatc
Spacer-PAM-R_33	tttcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc

**Table S2.** Sequences of CRISPR array elements derived from the *Anoxybacillus flavithermus* genome.

Name	Structure (5'-3')
CRISPR repeat	gtaatagttcccctgaggttattgctgtgttatgat
CRISPR spacer 1	aaaatgacacacttatttgataaaaagaaa
CRISPR spacer 2	ccgaaagtttagactcactataaatcctc
CRISPR spacer 3	aaaaaggagatcatgaaaaagcagtagct
CRISPR spacer 4	cgtgggtcaattcggtaaatggaagtttt
CRISPR spacer 5	tgctacacttttgaccgaaacggagcgga
CRISPR spacer 6	agaccagtccaactagaaattaattttaga
CRISPR spacer 7	tgaagaaaaagaaaactgtagagaacaac
CRISPR spacer 8	tcatcagcaaaaatgtatagcgagaacggc
CRISPR spacer 9	tttctgtctagcagaacaactatttctgaa
CRISPR spacer 10	tttgctactatgatagaaagaacattagtg
CRISPR spacer 11	tttctgtctagcagaacaactatttccgaa
CRISPR spacer 12	tttctgtctagcagaacaactatttcggcg
CRISPR spacer 13	cagaaagggttggaaggtgattcgatgaag
CRISPR spacer 14	ccaaattcatgttttaagaacccgctgata
CRISPR spacer 15	atcatttaacgtcttactgatccgcgtatt
CRISPR spacer 16	tggtggtgatatgcctaataacgaattaaa

**Table S3.** Structure of dsDNA templates used as a library to determine AnCas9 PAM specificity. The PAM motif is highlighted in green.

Name	Structure (5'-3')
Spacer-PAM_1	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGTTCAA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_2	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGTTCCA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_3	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGTTCGA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_4	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGTTCTA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_5	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGATCAA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_6	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGATCCA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_7	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGATCGA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_8	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGATCTA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_9	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGCTCAA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_10	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGCTCCA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_11	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGCTCGA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_12	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGCTCTA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_13	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCAA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_14	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCCA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_15	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCGA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_16	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCTA</b> AGTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_18	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCGAC</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA

Spacer-PAM_19	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCGAGGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_20	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>TGGTCGATGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_21	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGTCTGAAGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_22	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGTCTGACGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_23	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGTCTGAGGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_24	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGTCTGATGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_25	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>CGGTCTGAAGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_26	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>CGGTCTGACGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_27	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>CGGTCTGAGGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_28	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>CGGTCTGATGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_29	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>GGGTCTGAAGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_30	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>GGGTCTGACGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_31	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>GGGTCTGAGGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM_32	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>GGGTCTGATGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_33	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AAGACGAAGT</b> GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGC TGGCACAGATGAGAA
Spacer-PAM-R_34	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ACGACGAAGT</b> GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGC TGGCACAGATGAGAA
Spacer-PAM-R_35	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGACGAAGT</b> GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGC TGGCACAGATGAGAA
Spacer-PAM-R_36	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ATGACGAAGTG</b> TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA

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Spacer-PAM-R_37	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AAGCCGAA</b> GT GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT TGGCACAGATGAGAA
Spacer-PAM-R_38	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ACGCCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_39	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGCCGAA</b> GT GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT TGGCACAGATGAGAA
Spacer-PAM-R_40	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ATGCCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_41	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AAGGCCAA</b> GT GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT TGGCACAGATGAGAA
Spacer-PAM-R_42	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ACGGCGAA</b> GT GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT TGGCACAGATGAGAA
Spacer-PAM-R_43	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AGGGCGAA</b> GT GTCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT TGGCACAGATGAGAA
Spacer-PAM-R_44	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ATGGCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_45	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>AAGTCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_46	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ACGTCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA
Spacer-PAM-R_48	CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCAT <b>ATGTCGAA</b> GTG TCAAGAACAAGCCTCTCTTCTTTGCCGACAACTTTACAAATCCATGAAGGGTGCT GGCACAGATGAGAA

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