CRISPR-Cas systems in *Fusobacterium*: An untapped genetic frontier

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ABSTRACT

Fusobacterium nucleatum has recently received significant attention for its strong connection with the acceleration and gravity of multiple cancers (e.g., colorectal, pancreatic, esophageal). However, our understanding of the molecular mechanisms that drive infection by this ‘oncomicrobe’ have been hindered by a lack of universal genetic tools. Herein we report a global bioinformatic identification and characterization of multiple *Fusobacterium* CRISPR-Cas adaptive immune systems including Cas13c, and detailed report of the proteins, spacer/repeat loci, *trans*-activating CRISPR RNA (tracrRNA), and CRISPR RNA (cRNA) from a Type II-A CRISPR-Cas9 system. Since most *Fusobacterium* are currently genetically intractable, this CRISPR-Cas bioinformatic roadmap could be used to build new genome editing and transcriptional tuning tools to characterize an increasingly important genus of human opportunistic-pathogens connected to the onset, progression, and severity of cancer.

KEYWORDS: *Fusobacterium nucleatum*, CRISPR, Cas9, Cas13, bacterial genetics

25

26 INTRODUCTION

27 Fusobacteria are anaerobic Gram-negative bacteria, with the most well studied human and
 28 livestock opportunistic pathogens of this genus being *F. nucleatum* and *F. necrophorum*. Due to
 29 robust restriction modification systems that hinder genome manipulation, *Fusobacterium* genetics
 30 have classically been considered nearly impossible, resulting in few bacterial strains that can be
 31 genetically modified for detailed mechanistic studies. Despite recent advances in *F. nucleatum*
 32 genetic tools, mainly in the strain *F. nucleatum* subsp. *nucleatum* ATCC 23726, all other species
 33 outside of *F. nucleatum* have nearly fallen into the category of genetically intractable ¹⁻³, with the
 34 exception being a recent study highlighting a conjugation method for genetic manipulation of *F.*
 35 *necrophorum* ⁴.

36 Recent advances in our understanding of Clustered regularly interspaced short palindromic
 37 repeats-CRISPR-associated proteins (CRISPR-Cas) systems have been exploited by molecular
 38 biologists to allow for efficient and robust genome editing in both prokaryotic and eukaryotic
 39 systems ⁵⁻⁷. CRISPR and Cas proteins comprise the basis of adaptive immunity in bacteria and
 40 archaea ⁸. CRISPR-Cas systems identify and cleave non-host DNA and RNA (viruses and
 41 plasmids), and deposit a ~30 bp segment into its host genome at defined CRISPR loci (host DNA
 42 repeats and foreign protospacer, or ‘spacer’ DNA) to provide organism- and sequence-specific
 43 defenses ⁹. During repeat infections, CRISPR-Cas complexes recognize these specific sequences
 44 and rapidly clear the invading DNA through active nucleotide cleavage ¹⁰. CRISPR systems have
 45 been characterized in 87% of archaea and 47% of bacteria and have been proposed to proliferate
 46 through horizontal gene transfer across bacteria as a basis of adaptive immunity ⁹.

CRISPR arrays are divided into three main categories and 16 distinct subcategories based on the *cas* genes encoded within bacteria and archaea ¹¹. However, the majority of genome editing studies have used the Cas9 protein from *S. pyogenes* ¹¹. While the use of *S. pyogenes* Cas9 in prokaryotic and eukaryotic systems has been achieved, our goal was to characterize *Fusobacterium* CRISPR-Cas proteins to determine if these proteins will be more efficient for future gene modulation studies in an AT rich genus of bacteria. The discovery and characterization of native Class 2 effectors in *Fusobacterium* will potentially enhance the application and development of CRISPR systems for genome engineering. Herein, we report a comprehensive bioinformatic characterization of multiple *Fusobacterium* CRISPR-Cas adaptive immune systems, focusing on Cas9 and the newly described Cas13c present in *F. necrophorum* subsp. *funduliforme* 1_1_36S. This data, coupled with a recent study characterizing an active CRISPR system in the strain *F. nucleatum* subspecies *nucleatum* ATCC 25586 ¹², provides hope that the development of new CRISPR-based genetic tools will enhance our understanding of fusobacterial physiology and pathogenesis.

61

62 MATERIALS AND METHODS

63 Use of genomic information for bioinformatic analysis and identification of CRISPR-Cas 64 systems in eight *Fusobacterium* strains.

65 *Fusobacterium* genomes (*F. nucleatum* subsp. *nucleatum* ATCC 23726 (GCA_003019785.1), *F.*
66 *nucleatum* subsp. *nucleatum* ATCC 25586 (GCA_003019295.1), *F. necrophorum* subsp.
67 *funduliforme* 1_1_36S (GCA_003019715.1), *F. varium* 27725 (GCA_003019655.1), *F. ulcerans*
68 49185 (GCA_003019675.1), *F. mortiferum* 9817 (GCA_003019315.1), *F. gonidiaformans* 25563
69 (GCA_003019695.1), and *F. periodonticum* 2_1_31 (GCA_003019755.1) were used to extract all

70 sequences to analyze using the CRISPROne web server ¹³ and CRISPRCasFinder ¹⁴.
 71 CRISPROne and CRISPRCasFinder were used to predict all CRISPR-Cas associated elements as
 72 well as the repeat arrays.

73

74 **Bioinformatic analysis of CRISPR-Cas Class 2 systems and identification of effector domain**

75 The genome sequence from *F. necrophorum* subsp. *funduliforme* 1_1_36S (GCA_003019715.1)
 76 was used to predict the open reading frame for the CRISPR-Cas systems. An open reading frame
 77 of 1337 amino acids for Cas9 and 1121 amino acids for Cas13c was identified using the web
 78 server pHMMER ¹⁵ and stand-alone HMMER software package, respectively.

79

80 **Phylogenetic Tree construction**

81 Full CRISPR-Cas phylogenetic analysis was created using the micropan ¹⁶ plugin in Rstudio by
 82 using only CRISPR associated proteins to build the tree. The gene maps in Figure 1B were
 83 created using the gggenes ¹⁷ plugin in Rstudio. Additional phylogenetic trees for Cas9 and Cas13c
 84 were created in Geneious 9.18 utilizing the multiple sequence alignment from SMARTBLAST and
 85 adapted in Affinity Designer.

86

87 **Structure Prediction**

88 The structure prediction of Cas9 from *Fusobacterium necrophorum* subsp. *necrophorum* 1_1_36S
 89 was performed in the web-based suite Phyre2 ¹⁸. CRISPR RNA structure prediction was done
 90 using NUPACK ¹⁹.

91

92 **RESULTS**

Comparative Analysis of *Fusobacterium* CRISPR-Cas systems

A total of eight *Fusobacterium* genome sequences were utilized for bioinformatic comparative analysis and the composition, occurrence, and diversity of CRISPR-Cas systems. Presently, there are two classes and six types of CRISPR-Cas systems based in the organization of their effector modules. A majority of the identified CRISPR-Cas systems in *Fusobacterium* belong to Class 1 (77% of CRISPR-Cas systems in the eight genomes) and interestingly, only *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. ulcerans* 49185 encode for Class 2 systems (23% of CRISPR-Cas systems in the eight genomes). The overall analysis of CRISPR-Cas systems is shown in **Table S1**.

To depict the correlation among *Fusobacterium* species and strains, a CRISPR-Cas system-based phylogenetic tree was constructed. As shown in **Figure 1A**, the eight *Fusobacterium* strains are divided into five distinct clades according to their predicted CRISPR-Cas systems. Interestingly, *F. nucleatum* ATCC 23726 (Lineage 1) clades with *F. gonidiaformans* 25563 (Lineage 3) and *F. periodonticum* 2_1_31 (Lineage 1) clades with *F. necrophorum* subsp. *funduliforme* 1_1_36S (Lineage 3).

The rapid evolution of CRISPR systems explains the diversity of systems from highly similar strains. For instance, *F. nucleatum* ATCC 23726 and *F. nucleatum* ATCC 25586 are the closest phylogenetically when using whole genome phylogenetic analysis, yet as seen in **Figure 1A**, they are far more divergent in their CRISPR loci. Additionally, the analysis revealed that *F. mortiferum* 9817 CRISPR-Cas systems have less conservation when compared to the other *Fusobacterium* strains.

Figure 1. CRISPR-Cas systems in *Fusobacterium*. A. CRISPR-Cas phylogenetic tree of eight *Fusobacterium* strains. B. A total of eight *Fusobacterium* strains were utilized for bioinformatic analysis and identification of CRISPR-Cas systems. A majority of the identified CRISPR-Cas systems in *Fusobacterium* belong to Class 1 and only *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. ulcerans* 49185 encode for Class 2 systems.

Estimation of spacer numbers and sequences among *Fusobacterium* species exhibited substantial diversity with 0% (0/348) of the spacers shared by eight strains. As shown in **Table S1**, the quantity of predicted spacers varies among the strains, ranging from 4 to 83, demonstrating the biological heterogeneity of *Fusobacterium* strains that encode an adaptive defense mechanism.

Type I-B presented a higher number of spacers when compared to the other CRISPR-Cas system types.

28

29 **Classification of CRISPR-Cas systems in *Fusobacterium***

30 To determine the classification and assortment of CRISPR-Cas systems across the eight
 31 *Fusobacterium* strains, we utilized the CRISPRCasFinder and CRISPROne web server that
 32 enabled the discovery of direct repeat consensus sequences within a genome as well as the
 33 characterization of related spacers and *cas* genes^{13,14}. This analysis detected a total of 13
 34 CRISPR-Cas systems belonging to both Class 1 (Type I, Type III and Type IV,) and Class 2 (Type
 35 II, Type VI): six Type I (54.54%), one Type II (9.09%), two Type III (18.18%), and two Type VI
 36 (18.18%) while no Type IV or V was identified. Therefore, we conclude that Type I is the
 37 predominant CRISPR-Cas system among the studied *Fusobacterium* strains. Detailed illustrations
 38 of the CRISPR-Cas systems identified among the strains analyzed in this study are summarized in
 39 **Figure 1B**. *F. nucleatum* ATCC 23726, *F. nucleatum* ATCC 25586, *F. gonidiaformans* 25563 and
 40 *F. periodonticum* 2_1_31 Type I-B is a Class 1 CRISPR-Cas loci that includes full gene sets to
 41 achieve adaptive immunity: *cas1*, *cas2* and *cas4* for spacer integration or adaptation, *cas6* for
 42 crRNA-processing and *cas3*, *cas5*, *cas7*, and *cas8* for interference. Both *F. ulcerans* 49185 and *F.*
 43 *varium* 27725 include a nearly complete Type I loci, but they do not encode for the *cas8* gene.
 44 Additionally, most CRISPRs of Type I-B CRISPR-Cas systems discovered in this analysis were
 45 encoded downstream of the *cas2* gene and/or upstream of the *cas6* gene. Worth noting, *F.*
 46 *nucleatum* ATCC 23726 and *F. gonidiaformans* 25563 presented an inverted loci composition of
 47 the Type I-B CRISPR-Cas systems identified (**Figure 1B**).

48 Furthermore, in *F. periodonticum* 2_1_31, Type I and Type III systems appear to combine
 49 as they often coexist²⁰. Type III systems have been divided into four major subtypes (III-A to D)
 50 ^{21,22}. A complete operon of *cas* genes associated with adaptation, crRNA-processing and

66 The Type II CRISPR-Cas systems function through the assembly of an effector module consisting
67 of a single Cas9 protein, a crRNA and a tracrRNA. Cas9 is 1337 amino acids in size. A previous
68 genome assembly of *F. necrophorum* subsp. *funduliforme* 1_1_36S had this protein as 329 amino
69 acids, proving that complete and accurate genome sequences found in FusoPortal were key for
70 identifying the proper open reading frames of these proteins ²⁴. The predicted structure of Cas9
71 **(Figure 2B)** resembles the characteristic bi-lobed architecture from previously characterized Cas9
72 proteins, with the RuvC and HNH domains for nucleic acid cleavage **(Figure 2A)**.

73 In addition to Cas9, recognition and targeting of DNA is dependent on the crRNA and
74 tracrRNA complex ²⁵. For this reason, we characterized the tracrRNA element of the Type II
75 CRISPR-Cas system in *F. necrophorum* subsp. *funduliforme* 1_1_36S. The first nucleotides
76 predicted in the nexus include adenine residues that are known to be highly conserved among
77 tracrRNAs of Type II systems as shown in **Figure 3A** ²⁵ and the bulge interacts through a specific
78 side-chain with the Rec1 domain or lobe through specific side-chain ²⁶. Both bulge and nexus in
79 tracrRNA are required for DNA cleavage, and the spacer sequence detailed in **Figure 3B** will
80 determine the location of Cas9 endonucleolytic cleavage ²⁵. Finally, the stems interact with Cas9
81 mainly through sequence-independent interactions with the phosphate backbone ²⁵.

82 The phylogenetic analysis of Cas9 from *F. necrophorum* subsp. *funduliforme* 1_1_36S
83 revealed a conservation within the *Fusobacterium* genus **(Figure 2B)**. While the PAM sequence for
84 *F. necrophorum* subsp. *funduliforme* 1_1_36S has not been experimentally characterized, the
85 predicted PAM sequence for this Cas9 is NGG ²⁷; the same as used for the commonly used *S.*
86 *pyogenes* Cas9. By contrast, the predicted PAM sequence for the Type I-B CRISPR-Cas systems
87 from *F. nucleatum* ATCC 23726 is YNW ²⁷. Overall, a total of 3,427 spacers have been identified in
88

Figure 3. Characterization of CRISPR-Cas elements from *F. necrophorum* subsp. *funduliforme* 1_1_36S. **A.** Predicting the upstream *Trans*-activating CRISPR RNA (tracrRNA) of Cas9, coupled with the repeat region complementary to the tracrRNA. **B.** Prediction of the secondary structure of Cas9 tracrRNA. **C.** Overview of CRISPR repeats (36 bp) and spacers (30 bp) for the Cas9 locus of *F. necrophorum* subsp. *funduliforme* 1_1_36S.

Fusobacteria with only 629 matching viral, intergenic or ORFs²⁸. Utilizing viruSITE²⁹, the integrated database for viral genomics, most of the 13 spacers of the CRISPR array from *Fusobacterium* subsp. *funduliforme* 1_1_36S Cas9 were identified as a potentially part of *Synechococcus*, *Bacillus*, *Enterobacteria*, or *Aeromonas* phage genomes.

Identification of the *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. ulcerans* 49185 Type IV CRISPR-Cas systems

Figure 4. Analysis of Cas13c from a Type VI CRISPR-Cas system in *F. necrophorum* subsp. *funduliforme* 1_1_36S. A. SMART-BLAST phylogenetic analysis of Cas13c. B. domain organization of the subsets of Cas13 enzymes.

Finally, Type VI CRISPR-Cas systems are divided into three types due to their low sequence similarity: VI-A, VI-B and VI-C. However, they all share the catalytic motif of the HEPN domain³⁰. The R-X4-H (Figure S1) motifs which are characteristic of HEPN-binding domains in both Cas13c that potentially mediate RNA cleavage is highlighted in Figure 4³¹. *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. ulcerans* 49185 shared a 40.51% and 40.64% of identity when

!12 compared to the previously identified Cas13c from *F. perfoetens* ³². The Cas13c from *F. ulcerans*
!13 is composed of 1117 amino acids while the Cas13c from *F. necrophorum* is 1137 amino acids,
!14 which is consistent with the average size for Cas13c of approximately 1120 amino acids ³³.

!15 A phylogenetic analysis of bioinformatically predicted Cas13c uncovered the close
!16 phylogenetic relationship between Type VI effectors in *Fusobacterium* (**Figure 4A**). Finally, when
!17 compared to other Type VI effectors Cas13c are the least studied and no accessory proteins have
!18 been identified like the other recently discovered Type VI systems.

!19

!20 **DISCUSSION**

!21 As CRISPR loci consist of host repeats and segments of foreign DNA extracted from invading
!22 organisms and mobile genetic elements, these repetitive stretches of DNA have proven difficult to
!23 resolve using short read DNA technologies that are commonly used to sequence bacterial
!24 genomes. The emergence of long-read DNA technologies from Oxford Nanopore and Pacific
!25 Biosciences has opened the door for the completion of fragmented genomes; many of which are
!26 likely incomplete due to the inability to resolve repeat regions. Previous attempts to determine the
!27 conformation, quantity, and diversity of CRISPR-Cas systems in *Fusobacterium* were exceptionally
!28 challenging because the complete genome sequencing information was incomplete, restricted, and
!29 incorrect. Complete and accurate genome sequences as the ones provided by FusoPortal are
!30 crucial for the analysis of these systems as well as the recombinant production of Cas proteins, or
!31 incorporation into a plasmid or chromosome; potentially leading to the expansion of *Fusobacterium*
!32 genetic toolkit ^{24,34}.

!33 A large percentage of bacteria harbor adaptive defense systems known as CRISPR-Cas systems,
!34 the analysis of CRISPR-Cas systems in *Fusobacterium* uncovered Class 1 and Class 2 systems.

Class 2 systems are less common within prokaryotes when compared to Class 1 systems, at most 10% of CRISPR–Cas systems within sequenced genomes belong to Class 2 systems³⁵. For that reason, most of the identified CRISPR-Cas systems in *Fusobacterium* belong to Class 1 and interestingly, only *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. varium* 49185 encode for Class 2 systems which is similar to the CRISPR-Cas systems identified in *Leptotrichia*, which belongs to *Fusobacterium* genus, where they are unevenly distributed³⁶.

Type I CRISPR-Cas systems were found to be the most abundant in the eight *Fusobacterium* strains, which often coexist with Type III CRISPR-Cas systems as shown in *F. periodonticum* 2_1_31. It is suspected that Type I systems complement Type III systems functionally³⁷.

Nonetheless, whereas Type I systems especially degrade dsDNA, Type III systems target ssDNA, dsDNA, and ssRNA³⁷. CRISPR mediates adaptive immunity by ensuing three steps. Particularly, most of *Fusobacterium* presented a complete operon of Type I-B CRISPR-Cas systems which is required to achieve adaptive immunity. First, the adaption step by Cas1, Cas2 and Cas4 requires the acquisition and inclusion of short segments of exogenous nucleotides into CRISPR loci; then crRNA-processing by Cas6 where the crRNA expression and maturation take place, and the last step is interference by Cas3, Cas5, Cas7 and Cas8 which recognize the crRNA and target nucleic acid³⁸. Generally, the Type I-B CRISPR-Cas system utilizes a cascade to target foreign DNA by base pairing CRISPR RNA (crRNA) to protospacers³⁹. In the case of *F. ulcerans* 49185 and *F. varium* 27725 which lack the signature *cas8* gene, the interference step could be defective as it has been demonstrated that Cas8 plays a key role in targeting cascade to invader DNA³⁹. Most Type I-B CRISPR-Cas systems in the analyzed eight strains were encoded downstream of the *cas2* gene and/or upstream of the *cas6* gene which is in accordance with a CRISPR-Cas system present in the anaerobic rod *Clostridium thermocellum*⁴⁰.

Type III-A CRISPR-Cas adaptive immune systems are composed of a protein-RNA complex

²¹. The complete loci in *F. periodonticum* 2_1_31 is constituted of five proteins and additional proteins that contain the Csm targeting complex Cas10/Csm1, Csm2–Csm5 and the Csm6 ribonuclease while *F. mortiferum* 9817 lacks Csm1 and Csm6 which could result in a non-functional CRISPR-Cas system. The role of these accessory genes may be related to modulation, complementation, or extension of these systems ^{22,37,41}.

To determine how CRISPR-Cas systems will function the Type, Class and PAM recognition of the endonucleases needs to be characterized. For the Type II-A CRISPR-Cas system in *F. necrophorum* subsp. *necrophorum* 1_1_36S the PAM sequence was previously predicted as NGG and we further characterized its tracrRNA and spacer composition ²⁷. This system could be utilized for genome modification in *Fusobacterium* strains (**Figure 5**), however distinct strains of an organism encode as shown in this study, different elements to their CRISPR systems and consequently, may have unique requirements for genetic editing.

The most conserved trait in Type VI CRISPR-Cas is the HEPN domain ³⁰. Interestingly enough, Type III systems possess RNase activity through the HEPN domain contained in the Csm6 and Csx1, although their role in this specific system remains to be studied ⁴². The first report of a Type VI system bioinformatically identified in *Fusobacterium* belonged to *F. perfoetens* ³², a strain that was found in children with diarrhea, nursing infants and in feces of a piglet. Additionally, CRISPR-Cas V1-C systems were only found in *Clostridium* and *Fusobacterium* ³⁰.

The subtypes in the VI systems are based in the HEPN domains which are highly variable, the most widely studied being Type VI-A (**Figure 4**) ¹¹. The HEPN domains are found frequently in various defense systems ³⁰, in Cas13d proteins, this domain has been found to orchestrate CRISPR RNA maturation and target cleavage ³¹ which could suggest a potential role for the two

HEPN domains identified in Cas13c from *F. necrophorum* subsp. *funduliforme* 1_1_36S and *F. ulcerans* 49185.

Figure 5. The use of native CRISPR-Cas systems as genetic tools could lead to the development of powerful tools. These tools could improve genome editing of *Fusobacterium* by creating deletion systems, characterization of essential genes through the CRISPRi, or the overexpression proteins using CRISPRa.

Additionally, CRISPR-Cas spacers represent a successful defense mechanism from past intruders, and the conserved spacers reflect the evolutionary phylogeny or conservation of species and strains ³⁶. In the studied *Fusobacterium* strains, the spacers were not similar within them and the number variability suggests the biological heterogeneity of *Fusobacterium* species that encode a CRISPR-Cas system as a defense mechanism and consequently, for modulation of immunity ³⁶. Higher number of spacers was observed in Type I-B which coincides with the fact that Type I-B, II-C and III-like CRISPR-Cas systems overall contain an increased number of spacers when compared to Type III-A, III-D, and VI-A.

In conclusion, the entirety of the *Fusobacterium* strains studied have endogenous systems that could be exploited as genetic tools for genomic engineering. The development of a CRISPR-

Cas gene editing tool in *Fusobacterium* requires the description and detailed examination of the Type/Class of CRISPR array and identifying the PAM recognition sequence. CRISPR-Cas systems are a good candidate for genetic engineering since they depend on the endogenous systems that provide existing components and it is necessary to expand the genetic toolbox as *Fusobacterium* only has a few genetic systems described ^{1,2}.

The utilization of bioinformatics has contributed to the continued discovery of novel CRISPR-Cas systems, such as Type V and Type VI CRISPR-Cas systems. As this bioinformatic study validates the presence of diverse CRISPR-Cas systems in *Fusobacterium*, we hypothesize that this technology could be used to more efficiently introduce gene interruptions, point mutations, and transcriptional control, through CRISPR interference (CRISPRi) and activation (CRISPRa) **(Figure 5)** ⁴³. Furthermore, the CRISPR-Cas technologies and applications that have been developed are rapidly evolving and could enable countless novel applications ³², which is of substantial impact as most strains of *Fusobacterium* do not contain native plasmids and the need to create broad host plasmids that allow for pan-genetics through CRISPR technologies is a priority for the field.

Declaration of competing interests

The authors declare no competing interests.

Acknowledgments

The authors would like to acknowledge Dr. Kira Marakova (NCBI) for the insightful information regarding the Cas13c and HEPN domain composition. This work was funded by the USDA

i20 National Institute of Food and Agriculture and the Virginia Tech College of Agriculture and Life
i21 Sciences. Select figures were created through Biorender.com.

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59 SUPPLEMENTAL FIGURES

N-terminal HEPN domain

WP_101184979.1	SKQRRDGGKITQAKYVVTTID----KYLDRNEK---EKRFKSTERELPNDTLLMRYKQISG	143
WP_042678931.1	IKQDRTK--VTV--SITDNKDHFDSDNLGDKIRLS-RSLTQYTNRIILLDADVMKNYREIVC	176
WP_027128616.1	K--NDRT--IVT----KIKDCLETQKKEKFERENTRRLISETERKLLSEETQKTYSKIAC	157
WP_035906563.1	K--NPRV--VEA----EIEDLMNSTILKDGRRSA--RREKSMTERKLEEKVAENYSLAN	163
WP_062624740.1	K--NPRV--VEA----EIEDLMNSTILKDGRRSA--RREKSMTERKLEEKVAENYSLAN	163
WP_106894941.1	K--NPRV--VEA----EIEDLMNSTILKDGRRSA--RREKSMTERKLEEKVAENYSLAN	163
1_1_36S_FusoPortal_Gene_1180	K--NPRV--VEA----EIEDLMNSTILKDGRRSA--RREKSMTERKLEEKVAENYSLAN	163
WP_047396607.1	E--KKDT--VVA----KVTDLKAPDKIEGERNHRLKLSSTERKLLSKTLCKNYSEISK	157
BBA50510.1	KCKTGSM--ITA----AIEDLLEDDRVREGIRNP--RRKASKTERKLITESICHNYAQITQ	166
49185_FusoPortal_Gene_424	KCKTGSM--ITA----AIEDLLEDDIAREGIRNP--RRKASKTERKLIAESICHNYAQIAQ	168
	. * : . . * : *	
WP_101184979.1	FDT-----LTSKDIYKIKRYIDFKNEMLFYFQFIEEFFSPLLPGKT-----NF-	186
WP_042678931.1	SDSEKVDETINIDSQEIYKINRFLSYRSNMIIYYQMINNFFLLHYDGEEDKGGNDSINLIN	236
WP_027128616.1	CPSED-----IDSVKIYKIKRFLAYRSNMLFFSLINDIFVKGVVK--DN-----GEEVG	205
WP_035906563.1	CPMEE-----VDSIKIYKIKRFLTYRSNMLLYFASINSFLCEGIGK--KD-----NETE	210
WP_062624740.1	CPMEE-----VDSIKIYKIKRFLTYRSNMLLYFASINSFLCEGIGK--KD-----NETE	210
WP_106894941.1	CPMEE-----VDSIKIYKIKRFLTYRSNMLLYFASINSFLCEGIGK--KD-----NETE	210
1_1_36S_FusoPortal_Gene_1180	CPMEE-----VDSIKIYKIKRFLTYRSNMLLYFASINSFLCEGIGK--KD-----NETE	210
WP_047396607.1	TPIEE-----IDSIKIYKIKRFLNYRSNLIYFALINDFLCAGVKE-----DDIN	202
BBA50510.1	CPVEE-----IDAVKIYKVRFLSYRSNMLFFALINDFLCKNLKN--EK-----GEKIN	214
49185_FusoPortal_Gene_424	CPVEE-----IDAVKIYKVRFLSYRSNMLFFALINDFLCKNLKN--KK-----GEKIN	216
	: : . * : : : : : : * : : :	
WP_101184979.1	--YSLNIE-----QNKDKVVKYIVYRLNDDFKN-----	212
WP_042678931.1	EIWKYENKKNDEKE-----KIIERSYKS-----IEKSINQYILN-----	270
WP_027128616.1	EIWRIDS---KEIDEKRTYDLLVENFKKRMSQEFINYKQSIENKIEKNTNKIKEIQKL	262
WP_035906563.1	EIWLKDNNDVRK-----EKVKENFNKLIQSTENYNSSLKNQIEEKEKLLRKESK--	260
WP_062624740.1	EIWLKDNNDVRK-----EKVKENFNKLIQSTENYNSSLKNQIEEKEKLLRKESK--	260
WP_106894941.1	EIWLKDNNDVRK-----EKVKENFNKLIQSTENYNSSLKNQIEEKEKLLRKESK--	260
1_1_36S_FusoPortal_Gene_1180	EIWLKDNNDVRK-----EKVKENFNKLIQSTENYNSSLKNQIEEKEKLLRKESK--	260
WP_047396607.1	EWVLIQDKEHTAFLENR---IEKITDYIDPKLSKDIENKKNQFEKRIKKYKTSLEELKTET	260
BBA50510.1	EIWEMENKGNKKIDFDENYNILVAQIKEYFTKEIENYNNRIDNIIDKKELLYSEKKEE	274
49185_FusoPortal_Gene_424	EIWMENKGNKKIDFDENYNILVAQIKEYFTKEIENYNNRIDNIIDKKELLYSEKKEE	276
	: . :	
	R-X4-H	
WP_101184979.1	-----QSLNQFIKKTDTIKYDFLKIQKILSD	244
WP_042678931.1	-----HNTVESGDKKKIDISEERIKEDLKKTFILFSR	310
WP_027128616.1	---KKEKYKKEINRLKKQLIELNRENDLLEKDKIELSDEEIREDEIEKILKIYSDI	319
WP_035906563.1	---KGAFYRT-----IIKKLQERIKELSEKSLTEDCEKIIKLYSEI	304
WP_062624740.1	---KGAFYRT-----IIKKLQERIKELSEKSLTEDCEKIIKLYSEI	304
WP_106894941.1	---KGAFYRT-----IIKKLQERIKELSEKSLTEDCEKIIKLYSEI	304
1_1_36S_FusoPortal_Gene_1180	---KGAFYRT-----IIKKLQERIKELSEKSLTEDCEKIIKLYSEI	304
WP_047396607.1	LEKNKTFYID---SIKTK---ITNLENKITELSLYNSKESLKEDLIKIIISIFTNI	314
BBA50510.1	SEKNK-----K---LEELNKLSEKQLKILTDEEIKADVIKIIKIFSDI	319
49185_FusoPortal_Gene_424	SEKNK-----K---LEELNKLSEKQLKILTDEEIKADVIKIIKIFSDI	321
	. . : * * : : * :	
WP_101184979.1	RFDFDFIQKFDDDEL-----KNRFDISTISLIKTMLQEKEEKKYQEKNNYIEDS	294
WP_042678931.1	RNYKPFYENLYSGKNFIYNKDKSKSRRFSELDDLNFKELSK-IKLVKNRAVSNYLDKK	369
WP_027128616.1	RNYQYFENLFENKKIS---KEKNEDVNLTLLDLNLFRLPL-VRQLKLENKTYLEKE	375
WP_035906563.1	RYDYQYFENLFENKEN-----SELTKNLNLDIFKSLPL-VRKMKLNKNVNYLEDN	353
WP_062624740.1	RYDYQYFENLFENKEN-----DDLMDLNLNLDIFKSLPL-IRKMKLNKNVNYLEDG	353
WP_106894941.1	RYDYQYFENLFENKEN-----SELTKNLNLDIFKSLPL-VRKMKLNKNVNYLEDN	353
1_1_36S_FusoPortal_Gene_1180	RYDYQYFENLFENKEN-----SELTKNLNLDIFKSLPL-VRKMKLNKNVNYLEDN	353
WP_047396607.1	RYDYKSFENLFENIEN-----EELKNLLDLNLFKSI RM-SDEFKTKNRTNYLGT	363
BBA50510.1	RYEYKYFENLFENKKN-----EELAE LLNLFKNLTL-LRQMKIENKNTNYLEGD	368
49185_FusoPortal_Gene_424	RYEYKYFENLFENKKN-----EELAE LLNLFKNLTL-LRQMKIENKNTNYLEGR	370
	* : : . : : : . . : : : : *	
WP_101184979.1	DTLTLFDEKESNFSKIHNFYIKISQKKPAPFNKLINSFLSKDGVPNELKSYLAT-----	348
WP_042678931.1	TTIHVLNKN-INAIKLLDIYRDICETKNGFNFINNMMTISGEEDKEYKEMVTKHFNENM	428
WP_027128616.1	DKITVLGVS-DSAIKYYSYNFLCEQKNGFNFINSFNSDGEENKSFKEKINLSLEKEI	434
WP_035906563.1	DTLFLVLQKT-KKAKTLYQIYDALCEQKNGFNKFINDFVSDGEENTVFKQIINEKFQSEI	412
WP_062624740.1	DTLFLVLQKT-KKAKTLYQIYDALCEQKNGFNKFINDFVSDGEENTVFKQIINEKFQSEM	412
WP_106894941.1	DTLFLVLQKT-KKAKTLYQIYDALCEQKNGFNKFINDFVSDGEENTVFKQIINEKFQSEM	412
1_1_36S_FusoPortal_Gene_1180	DTLFLVLQKT-KKAKTLYQIYDALCEQKNGFNKFINDFVSDGEENTVFKQIINEKFQSEM	412
WP_047396607.1	ESFTIVKKH-QNLKLYTYNNLCKDKNGFNFINSFVTDGIENTDFKNLILHFKEEM	422
BBA50510.1	EKFNILGKD-VRAKNALGHYDLLVEQKNGFNFINSFVQDGTENLEFKKFIDENFIKAQ	427
49185_FusoPortal_Gene_424	EEFNIIGKN-IKAKEVLGHYNLAEQKNGFNFINSFVQDGTENLEFKKLIDEHFVNK	429
	: : . * : : * . * : : : . * : * . :	

C-terminal HEPN domain

WP_101184979.1	KLKEYFESTFIEKRFPHFCVKFFNKKTKKEETKQKNIFNLIENTLEELVKESPLLQIIT	522
WP_042678931.1	YLSKSLDRRD-----RFL-----KL--IKD-----YKFRDTEIDFCSDRNNLVKLYI	626
WP_027128616.1	YLEVKSEKKY-----I-T-----KI--L-----NSIEDIHNTWLKNQENNLKPKFY	611
WP_035906563.1	YLTFLKKEEKE-----KFNLE-----KL--QET-----IQKTGEENWLLPQNNLKFY	607
WP_062624740.1	YLTSLFKKEEKE-----KFNLE-----KM--QKI-----IQKTEEDWLLPETKNNLKFY	607
WP_106894941.1	YLTFLKKEEKE-----KFNLE-----KM--QKI-----IQKTEEDWLLPETKNNLKFY	607
l_1_36S_FusoPortal_Gene_1180	YLTFLKKEEKE-----KFNLE-----KM--QKI-----IQKTEEDWLLPETKNNLKFY	607
WP_047396607.1	YLYNTAPKNK-----FNFA-----KL--EELQKIQTSEMADYLVNSPENNLFKFI	626
BBA50510.1	YLYNYCMEKEEDGK-NYKFHFK-----EI--S-----EIKDTHNEEWLENTSENNLFKFI	643
49185_FusoPortal_Gene_424	YLYKYYKDK-EAQK-NYEFPE-----EI--F-----ENKDTHNEEWLENTSENNLFKFI	612
	* : : : *	
WP_101184979.1	LLYLFIPKELQGEFVGFIKLYHHHTKNITNDTKE-----DEKSIEDTQNSFSL	570
WP_042678931.1	LMYILLPVEIRGDFLGFVKKNYYDLKHVDIFDKRNNNDK-----DTEFFH	670
WP_027128616.1	LYTYLLPPEFRGDFLGFVKKHYYDIKNVDFDENNDRLT-----PEQLEKMNDSFFN	664
WP_035906563.1	LYTYLLPYELKGDFLGFVKKHYYDIKNVDFMDENQSS-----KIESKEDDFYH	656
WP_062624740.1	LYTYLLPYELKGDFLGFVKKHYYDIKNVDFIDENQNNIQ-----VSQTEKQEDYFYH	660
WP_106894941.1	LYTYLLPYELKGDFLGFVKKHYYDIKNVDFMDENQNNIQ-----VSQTEKQEDYFYH	660
l_1_36S_FusoPortal_Gene_1180	LYTYLLPYELKGDFLGFVKKHYYDIKNVDFMDENQNNIQ-----VSQTEKQEDYFYH	660
WP_047396607.1	LYTYMLPVEFKGDFLGFVKHYYINKNVDFMDESLLDENEVDNKNLNEIKENLSDSFFN	686
BBA50510.1	LYTYLLPMFEKGDFLGVVKHYYDIKNVDFDTESEKELS-----QEIDKMGDSFFH	696
49185_FusoPortal_Gene_424	LYTYLLPMFEKGDFLGVVKHYYDIKNVDFDTESEKELS-----QVQLDKMGDSFFH	665
	* : : : * : : : * : : : * : : : *	
WP_101184979.1	KLKILAKNLRGLQLFNYSLSHNTLYNT-----K-EHFF--YEKGNRWQSVYKSL	617
WP_042678931.1	DLRLFENKVRLEVTSYSLSDGFLGKKSREKFGKELEKFIYK-----NVSIA--	717
WP_027128616.1	KIRLFENKSKKYDILKESILTSERIGKYFSLNTGAKYFEY-----	705
WP_035906563.1	KIRLFENKTKKYEVIVKYSIVPDKKLQYFKDLGIDTK-----Y	694
WP_062624740.1	KIRLFENKTKKYEVIVKYSIVPNEKLQYFEDLGIDIKYLTGSVESGEKWLGENGIDIKY	720
WP_106894941.1	KIRLFENKTKKYEVIVKYSIVPNEKLQYFEDLGIDIKYLTGSVESGEKWLGENGIDIKY	720
l_1_36S_FusoPortal_Gene_1180	KIRLFENKTKKYEVIVKYSIVPNEKLQYFEDLGIDIKYLTGSVESGEKWLGENGIDIKY	720
WP_047396607.1	KIRLFENKIKKYEVIVKYSVSTQENMKYFKQLNLDIPYLDY-----	727
BBA50510.1	KIRLFENKTKRYEIKYISILTSDEIKKYFELLEKVPYLEY-----	737
49185_FusoPortal_Gene_424	KIRLFENKTKRYEIKYISILTSDEIKRYFRLLLEDVPPYFEY-----	707
	. : : : * : : : *	
WP_101184979.1	-----ISHNQDEFDIHLVPIVVIKYIYNLKNLIGDFEYIALLTYADKNS	660
WP_042678931.1	-----LPTNIDIEKFNKSLVPMKNYQIFKLLNDIEISALFLIAKKEG	762
WP_027128616.1	-----GGEENRGIFNKNIIIPIFKYQIVLKLNDVLEAMLLTLESDE	749
WP_035906563.1	LLLDQKSEVSGEKNKKVSLKNNGMFNKTILLFVFKYYQIAFKLFNDIELYSLFFLRKSG	754
WP_062624740.1	LTVEQKSEVSEEEKNNKVKSLKNNGMFNKTILLFVFKYYQIAFKLFNDIELYSLFFLRKSG	780
WP_106894941.1	LTVEQKSEVSEEEKNNKVKSLKNNGMFNKTILLFVFKYYQIAFKLFNDIELYSLFFLRKSG	780
l_1_36S_FusoPortal_Gene_1180	LTVEQKSEVSEEEKNNKVKSLKNNGMFNKTILLFVFKYYQIAFKLFNDIELYSLFFLRKSG	780
WP_047396607.1	-----KSTDEIGIFNKNMILPIFKYYQNVFKLCNDIEIHALLALANKKQ	771
BBA50510.1	-----KGIDEIGIFNKNIIIPFKYYQIFRFLYNDLEIHGLFNVSPDIN	781
49185_FusoPortal_Gene_424	-----KGTDEIGIFNKNIIITFKYYQIFRFLYNDLEIHGLFNISDDL	751
	: : : * : : : * : : : *	
WP_101184979.1	ITE-----KLSDITRRDDLKFRGYNFTLLFKTFM-----	691
WP_042678931.1	NEGSITPKKVIDKVRKEDMNGNINF-----SQVMK-----	792
WP_027128616.1	KDINKIKELV--TLKEKVPKKIDYKYYKFSVLLDCFNRRIINLGK--	793
WP_035906563.1	KPFEVFLKE--LKDGMIGKQLNF-----GQLLYVVVEVLVK--NKDLSEILS----	797
WP_062624740.1	KPLEIFRKE--LESKMKDGYLNF-----GQLLYVVVEVLVK--NKDLDKILS----	823
WP_106894941.1	KPFEVFLKE--LKDGMIGKQLNF-----GQLLYVVVEVLVK--NKDLDKILS----	823
l_1_36S_FusoPortal_Gene_1180	KPFEVFLKE--LKDGMIGKQLNF-----GQLLYVVVEVLVK--NKDLDKILS----	823
WP_047396607.1	QNL-----EY-----AIYCCSKKNSL--NNNELLK-----	794
BBA50510.1	KILSDLK-----SYGNENINF-----REFLYVIKQNNNSSTEEYQKIWEKLESK	826
49185_FusoPortal_Gene_424	KILRDLK-----SYGNKNINF-----REFLYVIKQNNNSSTEEYRKIWENLEAK	796
	. : :	
WP_101184979.1	-----INTNYEQNKSTQYIKOTRNDIAHNIENMLKAFENNEIFA-----	732
WP_042678931.1	-----MALNEKVNCOIRNSIAHINMKQLYIEPLNIYINNQN-----	829
WP_027128616.1	---KDFLA-----SEEVKAVKTFNLAYIRNKICHNLNYSKFIDDLITIDTNKST---	840
WP_035906563.1	-----ERI-----DYRKDMCFSAEIAIDIRNFLSHLNYSKFLDNFMKINTNK-----	839
WP_062624740.1	-----KKI-----DYRKDKSFSPEIAYIRNFLSHLNYSKFLDNFMKINTNK-----	865
WP_106894941.1	-----KKI-----DYRKDKSFSPEIAYIRNFLSHLNYSKFLDNFMKINTNK-----	865
l_1_36S_FusoPortal_Gene_1180	-----KKI-----DYRKDKSFSPEIAYIRNFLSHLNYSKFLDNFMKINTNK-----	865
WP_047396607.1	-----TFNRKTYQNLSTIRNKIAHLNYSKELFSDLFNN-----	826
BBA50510.1	YLKEPLLTPKEKEINKKTEKELKLDGISTIRNKISHLEYEKIEGVLTAVNGENKSTS	886
49185_FusoPortal_Gene_424	YLRHLHLLTPKEEIKTKTKEELEKLEISNIRNGICHNLNYSKIEIEELKTEISEKNK--	853
	** : . * : : : *	
WP_101184979.1	-----QREEIVNYLQKE--HKMQEILHYNPINDFTMKTVQYLKSLNHSQKE---	777
WP_042678931.1	-----KKTISEQMEEIIDICITK--GLTGKELNKNIIINDYMKKEKLVFNKLKRNNLVS	883
WP_027128616.1	TDSEGKLLINDIRIKLKFIRENNQKMNISIDYNYINDYMKKEKFIQGRQKAKTIIDS	900
WP_035906563.1	DENKEVLIPSIIQKMIQFIEECNLQSQIDFDNFVNDYFMRKEKMFQIQLKQIFPDINS	899
WP_062624740.1	DENKEVLIPSIIQKMIQFIEECNLQSQIDFDNFVNDYFMRKEKMFQIQLKQIFPDINS	925
WP_106894941.1	DENKEVLIPSIIQKMIQFIEECNLQSQIDFDNFVNDYFMRKEKMFQIQLKQIFPDINS	925
l_1_36S_FusoPortal_Gene_1180	DENKEVLIPSIIQKMIQFIEECNLQSQIDFDNFVNDYFMRKEKMFQIQLKQIFPDINS	925
WP_047396607.1	-----ELDLNTKVRCLIEFSQNNK--FDQIDLGMNFINDYMKKTRFIFNQRRRLDLNVPS	820
BBA50510.1	ETNADKVFLENEKIKKIINFIEKENE-LDKIELGFNFINDFFMKKEQFMFGQIKQVKEGN--	943
49185_FusoPortal_Gene_424	-----EATLNEKIRKVINFIKENE-LDKVELGFNFINDFFMKKEQFMFGQIKQVKEGN--	905
	: . : : : : * : : : * : : : *	
WP_101184979.1	--SKIAD-----IHKKESLVPNDYLYIKLV-----IELLKQ----	808
WP_042678931.1	IDA-----QQNMKEKSIILNKYDLNKNYDNL-----NIKEI---ILKVNLDNNKQ	925
WP_027128616.1	GKKA-----NKRNKAEELLMYRVKKN-----INLIYELSKKLNELTKSELFL	944
WP_035906563.1	TEKQ-----KMNEKEEILRNRYHLTDKKNQEIQDEHEAQSQLYEKILSLQKIFYSSD--	950
WP_062624740.1	TEKQ-----KMNEKEEILRNRYHLTDKKNQEIQDEHEAQSQLYEKILSLQKIFYSSD--	976
WP_106894941.1	TEKQ-----KKSEKEEILRKRYHLINKKNEQIKDEHEAQSQLYEKILSLQKIFYSCD--	976
l_1_36S_FusoPortal_Gene_1180	TEKQ-----KKSEKEEILRKRYHLINKKNEQIKDEHEAQSQLYEKILSLQKIFYSCD--	976
WP_047396607.1	KEKIDGKRKQONDNNELLKKYGLSRTN-----IKDIFNKAWY-----	919
BBA50510.1	-SDSITTERKKEENNRKLKITYGLNYYN-----LSKIYEFNTLRREIVNSPLFL	992
49185_FusoPortal_Gene_424	-SDSITTERERKKNKKLKYELNCDN-----LSEFYETSNLRERANSSSLL	954
	. . : : *	

R-X4-H

Figure S1. Multiple sequence alignment of Cas13c proteins. N-terminal and C-terminal HEPN domains are highlighted as well as the R-X4-H motif.

SUPPLEMENTAL TABLES

Table S1. CRISPR-Cas system information of the eight analyzed *Fusobacterium* strains

Species	Strain	CRISPR-Cas system Type	Subtype	Number of spacers	Conservation
<i>F. nucleatum</i>	25586	I	B1	15	0%
<i>F. nucleatum</i>	23726	I	B1	23	0%
<i>F. periodonticum</i>	2_1_31	I, III	B1, A2	L1:42, L2:13, L3:13, L4:28	L1:0%, L2:0%, L3:0%, L4:0%
<i>F. varium</i>	27725	I	B1	L1: 13, L2:13, L3:24, L4:18	L1: 9.75%, L2:0%, L3:0%, L4:2.04%
<i>F. ulcerans</i>	49185	I, VI	B1, C	L1:17, L2:23, L3:7	L1:0%, L2:0%, L3:0%
<i>F. mortiferum</i>	9817	III	A1	L1:4, L2:6	L1:0%, L2:0%
<i>F. gonidiaformans</i>	25563	I	B1	83	0%
<i>F. necrophorum</i>	1_1_36S	III, VI	A1, C	L1:13, L2:9	L1:0%, L2:0%

Table S2. CRISPR-Cas cluster elements in *F. nucleatum* ATCC 25586.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas2	1711	AVQ15518.1	92
I	Cas1	1712	AVQ15519.1	330
I	Cas4	1713	AVQ15520.1	164
I	Cas3	1714	AVQ15521.1	812
I	Cas5	1715	AVQ15522.1	366
I	Cas7	1716	AVQ15523.1	300
I	Cas8a	1717	AVQ15524.1	517
I	Cas6	1718	AVQ15525.1	250

Table S3. CRISPR-Cas cluster elements in *F. nucleatum* ATCC 23726.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas6	1183	AVQ23192.1	250
I	Cas8a	1184	AVQ23193.1	518
I	Cas7	1185	AVQ23194.1	300
I	Cas5	1186	AVQ23195.1	366
I	Cas3	1187	AVQ23196.1	812
I	Cas4	1188	AVQ23197.1	164
I	Cas2	1190	AVQ23199.1	92
I	Cas1	1189	AVQ23198.1	330

Table S4. CRISPR-Cas cluster elements in *F. ulcerans* 49185.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas2	2775	AVQ29172.1	94
I	Cas1	2776	AVQ29173.1	333
I	Cas4	2777	AVQ29174.1	160
I	Cas3	2778	AVQ29175.1	861
I	Cas5	2779	AVQ29176.1	251
I	Cas7	2780	AVQ29177.1	314
I	Cas6	2782	AVQ29179.1	213
VI	Cas13c	424	AVQ26923.1	1117

Table S5. CRISPR-Cas cluster elements in *F. mortiferum* 9817.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
III	Cas6	2336	AVQ19756.1	242
III	Csm5	2337	AVQ19757.1	383
III	Csm4	2338	AVQ19758.1	314
III	Csm3	2339	AVQ19759.1	231
III	Csm2	2340	AVQ19760.1	155
III	Cas10	2341	AVQ19761.1	797
III	Cas2v7	2344	AVQ19764.1	94
III	Cas2III8	2345	AVQ19765.1	96
III	Cas1	2346	AVQ19766.1	327

Table S6. CRISPR-Cas cluster elements in *F. gonidiaformans* 25563.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas6	753	AVQ16743.1	250
I	Cas8a	754	AVQ16744.1	471
I	Cas7	755	AVQ16745.1	293
I	Cas5	756	AVQ16746.1	369
I	Cas3	757	AVQ16747.1	733
I	Cas4	758	AVQ16748.1	164
I	Cas1	759	AVQ16749.1	330
I	Cas2	760	AVQ16750.1	92

Table S7. CRISPR-Cas cluster elements in *F. periodonticum* 2_1_31

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas2	316	AVQ24518.1	92
I	Cas1	317	AVQ24519.1	330
I	Cas4	318	AVQ24520.1	164
I	Cas3	319	AVQ24521.1	803
I	Cas5	320	AVQ24522.1	359
I	Cas7	321	AVQ24523.1	294
I	Cas8a	322	AVQ24524.1	514
I	Cas6	323	AVQ24525.1	250
III	Cas2	1051	AVQ25182.1	109
III	Cas1	1052	AVQ25183.1	335
III	Csm6	1053	AVQ25184.1	463
III	Cas6	1054	AVQ25185.1	240
III	Csm5	1055	AVQ25186.1	389
III	Csm4	1056	AVQ25187.1	334

III	Csm3	1057	AVQ25188.1	235
III	Csm2	1058	AVQ25189.1	122
III	Cas10	1059	AVQ25190.1	846

Table S8. CRISPR-Cas cluster elements in *F. necrophorum* subsp. *funduliforme* 1_1_36S.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
II	Cas9	859	AVQ20950.1	1337
II	Cas1	860	AVQ20951.1	290
II	Cas2	861	AVQ20952.1	106
II	Csn2	862	AVQ20953.1	226
VI	Cas13c	1180	AVQ21256.1	1137

Table S9. CRISPR-Cas cluster elements in *F. varium* 27725.

Type	Cas proteins	Gene_FusoPortal_ID	GenBank_ID	Size (AA)
I	Cas1	2395	AVQ31955.1	333
I	Cas2	2396	AVQ31956.1	94
I	Cas3	2393	AVQ31953.1	858
I	Cas4	2394	AVQ31954.1	160
I	Cas5	2392	AVQ31952.1	250
I	Cas6	2389	AVQ31949.1	231
I	Cas7	2391	AVQ31951.1	314

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