Augustana College Augustana Digital Commons

Meiothermus ruber Genome Analysis Project

Biology

2019

Effects of Temperature on CRISPR/Cas System

Eddie Beckom Augustana College, Rock Island Illinois

Dr. Lori Scott Augustana College, Rock Island Illinois

Follow this and additional works at: https://digitalcommons.augustana.edu/biolmruber Part of the <u>Bioinformatics Commons</u>, <u>Biology Commons</u>, <u>Genomics Commons</u>, and the <u>Molecular Genetics Commons</u>

Augustana Digital Commons Citation

Beckom, Eddie and Scott, Dr. Lori. "Effects of Temperature on CRISPR/Cas System" (2019). *Meiothermus ruber Genome Analysis Project.* https://digitalcommons.augustana.edu/biolmruber/45

This Student Paper is brought to you for free and open access by the Biology at Augustana Digital Commons. It has been accepted for inclusion in Meiothermus ruber Genome Analysis Project by an authorized administrator of Augustana Digital Commons. For more information, please contact digitalcommons@augustana.edu.

Eddie Beckom BIO 375 Dr. Lori R. Scott Biology Department, Augustana College 639 38th Street, Rock Island, IL 61201

Temperature Effect on Complexity of CRISPR/Cas Systems

What is *Meiothermus ruber?*

Meiothermus ruber is a Gram-negative thermophilic rod-shaped eubacteria. The genus name derives from the Greek words 'meion' and 'thermos' meaning 'lesser' and 'hot' to indicate the thermophilic characteristics of Meiothermus ruber. (Nobre et al., 1996; Euzeby, 1997). It lives in thermal environments with an optimal temperature of 60°C. *Meiothermus ruber* belongs to the bacterial phylum Deinococcus-Thermus. The order Thermales, which is housed within the Thermus group and consists of 6 genera (Vulcanithermus, Oceanithermus, Thermus, Marinithermus, Meiothermus, Rhabdothermus), all containing genera with proteins that are thermostable. (Albuquerque and Costa, 2014). M. ruber is one of eight currently known species in the genus *Meiothermus* (Euzeby, 1997). As of 2017, five Meiothermus ruber genomes have been sequenced and uploaded into Genbank. Our study uses the Meiothermus ruber DSM 1279 genome (GenBank Name ASM2442v1) sequenced through a collaboration between the U.S. Joint Genome Institute and Leibniz-Institut DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), which is called the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project. The many projected benefits of the GEBA project are novel gene discoveries, the identification of novel biochemical processes, and a better understanding of the processes underlying the evolutionary diversification of microbes (e.g., lateral gene transfer and gene duplication) which aids in the comparative analysis of *M. ruber* with model organisms. The complete genome of Meiothermus ruber DSM 1279 consists of over 3 million base pairs, over 3000 protein-coding genes, 53 RNA genes and 6 CRISPR repeats; the genome has 63.4% GC content (Tindall et al., 2010). The phylogenetic relationship of Deinococcus-Thermus to phyla contain thermophiles is seen in Figure 1.



Figure 1. Phylogenetic tree showing the relationships between bacterial phyla based on W. Ludwig and H. P. Klenk's "Bergey's Manual of Systemic Bacteriology". Relationship between thermophiles based on the analysis of 16SrRNa genes with ARB parsimony tool. Of the phyla presented, Thermodesulfobacteria and Thermomicrobia are missing. (Lebedinsky *et al., 2007*)

Why study Meiothermus ruber?

Many physical and genome features make *M. ruber* an interesting organism to study. As a GEBA organism, little is known about the Deinococcus-Thermus phylum, with the exception of growing knowledge about members of the Thermus species. The notable characteristic is the thermophilic characteristics of *M. ruber* and how that impacts the biochemical processes that take place within the cells. Details about how thermophilic cells endure and adapt to temperature extremes in terms of the proteins and pathways that produce them are not well known. The study of CRISPR/Cas system in *M. ruber* is one of the many processes that have gone unstudied. As we learned from the GEBA project, most of what we know about biological processes in prokaryotes comes from studying a small percentage of the known microbes known as model organisms such as Escherichia coli. Expanding our knowledge to include poorly studied microbes from diverse branches of the tree of life is likely to identify diverse strategies to achieve many biological processes. To date, only 41 articles were pulled from the Pubmed search engine using the phrase Meiothermus ruber, while more than 366688 entries were pulled with E. coli. An understanding of the CRISPR/Cas system in Meiothermus ruber is essential in understanding the consensus between biosynthetic pathways between organisms and in what ways they may differ. Given the thermostability of proteins in thermophiles, studying the CRISPR/Cas system may expand on the knowledge of how defense mechanisms against viral infections take place in series of bacteria.

What are the parts of the CRISPR/Cas System?

CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR associated) adaptive immune systems are found in roughly 50% of bacteria and 90% of archaea (Makarova *et al.*, 2015). CRISPR/Cas systems are one of the many prokaryotic defense systems present in bacterial cells (Labrie et al., 2010) Similar to the immune systems of some eukaryotic organisms, such as humans, bacteria maintain a record of infections in regions of the CRISPR/Cas system sequence called CRISPR spacer-repeat arrays which allows for a quick response by the organism if a viral infection of that nature happens again. (Wright *et al.*, 2016). CRISPR/Cas systems were first identified in Escherichia coli (Jiang & Doudna, 2015) along with an understanding of their sequence parts: a set of universal cas genes, series of cas genes varying by CRISPR type, and a spacer-array sequence. This makes *Escherichia coli* a model organism for the study of CRISPR/Cas systems in comparison to Meiothermus ruber and other bacterial organisms. An analysis of the Escherichia coli CRISPR/Cas system using Ecocyc (Keseler et al., 2013), a database devoted to the study of E. coli, and KEGG (Kanehisa et al., 2019) showed that the CRISPR/Cas system in E. coli is a singular operon with no evidence of genes for other types as shown in Figure 2 and Figure 3. This finding means that all genes shown in the KEGG analysis are found to be apart of the functional CRISPR-associated gene cascade or the universal cas gene complex.



Figure 2. Ecocyc (Keseler *et al., 2013*) analysis of the CRISPR/Cas operon in *Escherichia coli*. Shows the coding of the CRISPR/Cas system starting with signature gene *cas3* on the right. The system is coded for moving downstream, or from right to left. The system is made up of the signature gene and cas cascade genes.

 CRISPR-Cas system Universal Cas proteins b2755 cas1; multifunctional nuclease Cas1 b2754 cas2; CRISPR-associated endoribonuclease Cas2 K09951 cas2 Type I CRISPR-Cas system Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-A factors
 Universal Cas proteins b2755 cas1; multifunctional nuclease Cas1 b2754 cas2; CRISPR-associated endoribonuclease Cas2 K09951 cas2 Type I CRISPR-Cas system Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-A factors
 b2755 cas1; multifunctional nuclease Cas1 b2754 cas2; CRISPR-associated endoribonuclease Cas2 Type I CRISPR-Cas system Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-B factors
 b2754 cas2; CRISPR-associated endoribonuclease Cas2 K09951 cas2 Type I CRISPR-Cas system Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-A factors
 Type I CRISPR-Cas system Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-B factors
 Type I signiture cas proteins b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors Subtype I-A factors
b2761 cas3; CRISPR-associated endonuclease/helicase Cas3 K07012 cas3 Subtype I-A factors
Subtype I-A factors
Subtype I-R factors
Subtype I-D factors
Subtype I-D factors
Subtype I-D factors
block and the I R CRISE support Canada suburit Cash M10102 and
b2760 cash; type 1-t CRISER System Cascade Suburit Cash R19125 cash
b2/59 casb; type 1-L CRISPR System Cascade Suburit Casb R19040 casb
b2/55 casc; type 1-L CRISPR system cascade subunit casc R19124 casc
b2/5/ casD; type 1-E CRISPR system cascade subunit casD R19125 casD
p2/56 casE; pre-CRISPE RNA endonuclease R19126 casE
Subtype 1-F factors
Subtype 1-0 factors
 Type II CRISPR-Cas system
Type II signiture cas proteins
Subtype II-A factors
Subtype II-B factors
 Type III CRISPR-Cas system
Type III signiture cas proteins
Subtype III-A factors
Subtype III-B factors
Subtype III-U factors

Figure 3. KEGG (Kanehisa *et al., 2019*) analysis of the CRISPR/Cas system in *Escherichia coli*. Evidence of one (1) functional operon made up of universal Cas proteins b2755 (Cas1) and b2754 (Cas2), Type 1 signature protein b2761 (Cas3), Subtype 1-E proteins b2760 (CasA), b2759 (CasB), b2758 (CasC), b2757 (CasD), b2756 (CasE).

The CRISPR operon structure begin with a signature protein, a necessary protein component for the production of an effective CRISPR/Cas operon, called *cas3*. *Cas3* downstream of following CRISPR associated cas genes: *casA*, *casB*, *casC*, *casD*, *casE* as seen in Figure 1. This sequence of downstream genes make up the cas cascade with *cas1* and *cas2* downstream from the final gene of the operon. *Cas1* and *cas2* are the universal Cas proteins present in all CRISPR/Cas operons. A classification of CRISPR/Cas operon types (Makarova *et al.*, 2011) compared to the KEGG and Ecocyc results demonstrates that *E. coli* has a Type I-E CRISPR/Cas operon as represented in Figure 3.



Nature Reviews | Microbiology

Figure 4. A classification of CRISPR/Cas type based on the presence of universal cas genes and CRISPR-associated genes that must be present in order to have a function CRISPR/Cas system. (Makarova *et al., 2011*)

How do bacteria adapt to temperature?

It is shown that *Escherichia coli* has a simple Type I-E CRISPR/Cas system as shown in Figure 3. E. coli is also a mesophile meaning that it grows an optimal temperature between 20-45°C. Bacteria in general grow at a wide range of temperatures: psychrophile below 20°C, mesophiles between 20-45°C, thermophiles 45-80°C, and hyperthermophiles above 80°C. (Goldstein 2007) The protein stability in these species of bacteria is referred to as the thermostability of the proteins. The interactions between amino acids during the folding is subject to the temperature of the bacterial environment and gives rise to functionally folded proteins or misfolded proteins based on the temperature effect. A bacterial protein in an organism outside of its temperature range will likely misfold due to the impact the temperature has on the interactions between the amino acids within the protein domains. An organismal adaptation to temperature-based impacts on interactions is for produced proteins to incorporate amino acids that are similar enough that the function of the protein remains but that the protein is more fitted for that specific temperature range.. Previous studies look at the interaction of thermophilic proteins and concludes that interactions in the protein domain to maintain thermostability are based on the hydrophobic effect of the environment and the enthalpic contributions amongst the protein domain (Berezovsky et al., 2005). The hydrophobic effect is the tendency for nonpolar molecules to interact in an aqueous environment. At room temperatures the hydrophobic effect is relatively low, but is directly related to the environmental temperatures. Proteins in various temperature environments interact in a continuously changing manner based on the effect. Another study looking at the thermostability of proteins in bacteria list the greater number of G+C content in a sequence as the region for stability in thermophilic bacteria proteins. (Lebedinsky et al., 2007) In comparing the G+C % content of thermophiles to mesophile, they approximately maintain 65 mol% to 55 mol%, respectively.

One group of thermophilic bacteria is the phylum Deinococcus-Thermus which consist of Meiothermus ruber, an organism known to have a CRISPR/Cas system. Lebedinsky et al shows the phylogenetic relationship between the Firmicutes and Deinococcus-Thermus which leads to the hypothesis that organisms within both phylum have a CRISPR/Cas system given *M. ruber* does. A study on the CRISPR/Cas system in Streptococcus thermophilus, an organism within the Firmicutes phylum, details the complexity of the S. thermophilus CRISPR/Cas system. (Horvath & Barrangou, 2010) S. thermophilus is known to have four individual operons with their own unique spacer-repeat CRISPR arrays. A previous study explored the classification and diversity of CRISPR/Cas systems based on the conclusion that the high energy cost of maintaining and expressing several CRISPR/Cas genes must offer major advantages on to the cell. (Garrett et al., 2011) The presence of multiple spacer-repeat arrays in a CRISPR/Cas systems likely confers the conclusion that parts of the system work independently of each other. This means that phase 1, comprised of cas1 and cas2, works independently of the function CRISPR/Cas operon, comprised of a universal cas gene and CRISPR-associated cascade. With the presence of CRISPR/Cas systems in 50% of bacteria and 90% of archaea (Makarova et al., 2015) and some organisms, such as Streptococcus thermophilus, having multiple CRISPR/Cas operons, types, and arrays leads to question on the complexity of CRISPR/Cas systems. The goal of this study will be to analyze the CRISPR/Cas system in a model organism *Escherichia coli* to other mesophiles,

thermophiles, and psychrophiles to assess the complexity of the CRISPR/Cas operons across the classifications to maintain thermostability and effective CRISPR/Cas systems.

Purpose

The purpose of this study will be to compare the CRISPR/Cas system in a model organism *Escherichia coli* to other mesophiles, thermophiles, and psychrophiles to assess the complexity of the CRISPR/Cas systems across these bacterial classifications. We are interested in studying an environmental condition such as temperature might influence the complexity of the CRISPR-Cas system.

Methods

Dr. Wegman-Geedey provided her expertise in identifying suitable prokaryotes for this study. A literature search was performed to identify other suitable organisms for this project. In addition to using *E. coli* K12 MG1655 and *Meiothermus ruber* DSM1279, the following organisms were chosen for this project: *Salmonella enterica subsp. enterica serovar, Yersinia pseudotuberculosis, Xanthomonas albilineans, Fusobacterium hwasookii, Hymenobacter nivis, Psychrobacter, Listeria weinhenstephansis, Thermus aquaticus sp. G, Streptococcus thermophilus, Thermodesulfobium narugense, and Geobacillus stearothermophilus.* These species were chosen because they represent a diversity of temperature growth optimum (See Table 1) and their genomes are sequenced and available through GenBank. *Xanthomonas albilineans* (Willerslev *et. al.,* 2004) and *Fusobacterium hwasookii* (Simon, 1977) are capable of growth across multiple temperature classifications. *X. albilineans* demonstrates a temperature growth range that classifies it as both mesophilic and psychrophilic, while *Fusobacterium hwasookii* can grow across the full spectrum.

The bioinformatics tool KEGG (Kanehisa *et al., 2019*) was used to predict the components and organization of the CRISPR-Cas system in each of the chosen species. The KEGG Brite Hierarchy ko02048 categorized genes into different CRISPR-Cas system types and subtypes. It also identified specific *cas* genes as "universal" (*cas1* and *cas2*; found in all CRISPR-Cas systems) and as "signature" genes (cas3 for the type IE; found in type-specific systems). Genes that are components of the different subtypes are also predicted. In this study, a CRISPR-Cas system type was identified by the presence of genes that encode both the "universal" and "signature" proteins. In addition, a functional/complete CRISPR-Cas operon was defined as possessing all the components of a specific subtype, based on KEGG predictions. In general, genes in GenBank that are positioned in sequential order are given sequential locus tags. However, recent renumbering of locus tags by GenBank doesn't follow this rule. Consequently, if the genes for a particular CRISPR-Cas subtype are present but do not appear to be sequentially positioned on the chromosome, then we confirmed their position using IMG/M chromosome map. Also, if an organism is not predicted to have a CRISPR-Cas system, then this was also confirmed using IMG/M.

Results

As described in the literature (see Figure 3), the mesophilic *E. coli* has a single CRISPR-Cas operon, Type IE. This is supported by a KEGG (Kanehisa *et al., 2019*) analysis and the study reviewed in the Jiang & Dounda article.

As seen in Figure 5, A KEGG analysis of *Meiothermus ruber* shows that the CRISPR/Cas system is more complex with three (3) function operons and a series of cas genes, CRISPR-associated genes, as described in Figure 5. Of the three functional operons, one is a Type 3-A operon, one a Type 3-B, and the other a Type 1-E operon similar to that of *E. coli*. Cas genes for *M. ruber* contain Cas4 and Cas6 genes present across subtypes. The presence of the multiple universal cas genes with respective cas genes is evidence for the presence of similar genes coding for the different functional CRISPR/Cas operons.

<pre>V CRISPR-Cas system V Universal Cas proteins Mrub_0224 CRISPR-associated protein Casl Mrub_1477 CRISPR-associated protein Casl Mrub_1476 CRISPR-associated protein Casl Mrub_1476 CRISPR-associated protein Casl Mrub_0225 CRISPR-associated protein Casl Mrub_0225 CRISPR-associated protein Casl Mrub_0225 CRISPR-associated protein Casl Mrub_0225 CRISPR-associated protein Casl Mrub_0220 CRISPR-associated protein Casl Mrub_0202 CRISPR-associated protein Casl Mrub_0202 CRISPR-associated protein Casl Mrub_1476 CRISPR-associated protein Casl Mrub_1475 CRISPR-associated</pre>	Meiothermus ruber	KO
<pre>v Universal Cas proteins Mrub 024 CH3PR-associated protein Cas1 K15942 cas1; Mrub_1477 CH3PR-associated protein Cas1 K15942 cas1; Mrub_0132 CH3PR-associated protein Cas1 K15942 cas1; Mrub_0126 CH3PR-associated protein Cas2 K09951 cas2; Mrub_0212 CH3PR-associated protein Cas4 K0964 cas4; Mrub_0212 CH3PR-associated protein Cas4 K07464 cas4; Mrub_0212 CH3PR-associated protein Cas4 K07464 cas4; Mrub_1478 CH3PR-associated protein Cas4 K07464 cas4; Mrub_1485 CH3PR-associated protein Cas4 K07664 cas4; Mrub_0180 CH3PR-associated protein Cas4 K07664 cas4; Mrub_0190 CH3PR-associated protein Cas4 K07664 cas4; Mrub_0019 CH3PR-associated protein Cas5 family K1913 cas50 Mrub_0019 CH3PR-associated protein Cas5 family K1912 cas57 Mrub_0019 CH3PR-associated protein Cas5 family K1912 cas57 Mrub_0019 CH3PR-associated protein Cas5 family K19126 cas57 Mrub_0019 CH3PR-associated protein K19138 cam37 Mrub_0019 CH3PR-associated protein K19138 cam37 Mrub_0019 CH3PR-associated protein K19138 cam37 Mrub_0019 CH3PR-associated Protein K19138 cam37 Mrub_0019 CH3PR-associated Protein K19138 cam37 Mrub_001</pre>	CRISPR-Cas system	
Mrub_0224 ChiPSR-associated protein Casl K15942 casl; Mrub_013 CRIPSR-associated protein Casl K15942 casl; Mrub_0205 CRIPSR-associated protein Casl K05981 cas2; Mrub_0205 CRIPSR-associated protein Casl K05981 cas2; Mrub_012 CRIPSR-associated protein Casl K05981 cas2; Mrub_012 CRIPSR-associated protein Casl K05981 cas2; Mrub_012 CRIPSR-associated protein Casl K05981 cas2; Mrub_010 CRIPSR-associated helicase Cas3 K07012 cas3; Mrub_020 CRIPSR-associated protein Cas4 K07464 cas4; Mrub_1475 CRIPSR-associated protein Cas4 K07464 cas4; Mrub_01202 CRIPSR-associated protein Cas4 K07464 cas4; Mrub_0120 CRIPSR-associated protein Cas4 K19919 cas5 Mrub_0120 CRIPSR-associated protein Cas4 K19919 cas5; Mrub_0120 CRIPSR-associated protein Cas4 K19912	Vniversal Cas proteins	
Meub_1477 CRISPR-secolated protein Casi RISS42 casi; Meub_1301 CRISPR-associated protein Casi RISS42 casi; Meub_0028 CRISPR-associated protein Casi ROSS1 cas2; Meub_0020 CRISPR-associated protein Casi ROSS1 cas2; Meub_0020 CRISPR-associated protein Casi ROSS1 cas2; Meub_0120 CRISPR-associated protein Casi ROSS1 cas2; Meub_1476 CRISPR-associated protein Casi ROT012 cas3; Muub_020 CRISPR-associated protein Casi ROT464 cas4; Muub_020 CRISPR-associated protein Casi ROT464 cas4; Muub_1476 CRISPR-associated protein Casi ROT464 cas4; Muub_1470 CRISPR-associated protein Casi ROT464 cas4; Muub_1470 CRISPR-associated protein Casi RUS112 casi; Muub_010 CRISPR-associated protein Casi RUS12 casi; Muub_010 CRISPR-associated protein Casi RUS12 casi; Muub_010 CRISPR-associated protein Casi RUS12 casi; Muub_010 CRISPR-associated protein RUS123 cas2;	Mrub 0224 CRISPR-associated protein Cas1	K15342 cas1;
Meub_2013 CRISPR-associated protein Cas1 RISS42 cas1; Meub_2023 CRISPR-associated protein Cas2 ROSS1 cas2; Meub_2013 CRISPR-associated protein Cas2 ROSS1 cas2; Meub_2013 CRISPR-associated protein Cas2 ROSS1 cas2; Meub_2012 CRISPR-associated protein Cas2 ROSS1 cas2; Type I signiture cas proteins ROT012 cas3; Muub_2020 CRISPR-associated protein Cas4 ROT046 cas4; Muub_1470 CRISPR-associated protein Cas4 ROT464 cas4; Muub_1470 CRISPR-associated protein RI9123 cas2;	Mrub 1477 CRISPR-associated protein Casl	K15342 cas1;
Meub_1476 CRISPR-associated protein Cas2 R0981 cas2; Mrub_0012 CRISPR-associated protein Cas2 R09851 cas2; Type I Signitume cas proteins R07012 cas5; Mrub_0020 CRISPR-associated protein Cas3 R07012 cas5; Mult_170 CRISPR-associated protein Cas3 R07012 cas5; Mult_171 CRISPR-associated protein Cas4 R07464 cas4; Mult_171 CRISPR-associated protein Cas4 R07464 cas4; Mult_1470 CRISPR-associated protein Cas4 R07464 cas4; Mult_01010 CRISPR-associated protein Cas4 R07464 cas4; Mult_01010 CRISPR-associated protein Cas5 Rult_0114 cas5; Mult_01010 CRISPR-associated protein Cas5 Rult_014 cas2; Mult_01010 CRISPR-associated protein R1913 cas2; Mult_01010 CRISPR-associated protein R1914 cas2;	Mrub 3013 CRISPR-associated protein Cas1	K15342 cas1;
Menkb 2012 CRISPR-associated protein Cas2 R09851 cas2; Type I CRISPR-Cas system R09951 cas2; Type I reginiture cas proteins R07012 cas3; Munub 2020 CRISPR-associated helicase Cas3 R07012 cas3; Munub 2020 CRISPR-associated protein Cas4 R07464 cas4; Munub 1475 CRISPR-associated protein Cas4 R07464 cas4; Munub 2020 CRISPR-associated protein Cas4 R07464 cas4; Munub 2012 CRISPR-associated protein R19123 cas5, Munub 2020 CRISPR-associated protein R19212 cas5, Munub 2012 CRISPR-associated protein R19212 cas5, <td>Mrub 1476 CRISPR-associated protein Cas2</td> <td>K09951 cas2;</td>	Mrub 1476 CRISPR-associated protein Cas2	K09951 cas2;
Meub_2012 CRISPR-casesociated protein Cas2 R09981 cas2; * Type I signiture cas proteins Meub_2020 CRISPR-associated helicase Cas3 R07012 cas3; * Subtype I-A factors R07464 cas4; Meub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mub_1475 CRISPR-associated protein Cas4 R07464 cas4; * Subtype I-C factors R19117 cad1; Muub_1475 CRISPR-associated protein R19117 cad1; Muub_1475 CRISPR-associated protein R19117 cad1; Muub_1475 CRISPR-associated protein Cas5 family R19119 cas56 Muub_1475 CRISPR-associated protein Cas5 family R19119 cas56 Muub_2022 CRISPR-associated protein R19123 cas1; Muub_2015 CRISPR-associated protein R19123 cas2; Muub_2015 CRISPR-associated protein R19123 cas2; Muub_2015 CRISPR-associated protein R19123 cas2; Muub_2016 CRISPR-associated protein R19123 cas2; Muub_2016 CRISPR-associated protein R19126 cas2; Muub_2015 CRISPR-associated protein R19126 cas2; Muub_2016 CRISPR-associated	Mrub 0225 CRISPR-associated protein Cas2	K09951 cas2:
<pre>Y Type I Signiture cas proteins Muu_3020 CRISPR-associated helicase Cas3 R07012 cas3; % Subtype I-A factors Muu_1478 CRISPR-associated protein Cas4 R07464 cas4; % Subtype I-B factors Muu_1478 CRISPR-associated protein Cas4 R07464 cas4; % Subtype I-C factors Muu_1478 CRISPR-associated protein Cas4 R07464 cas4; % Muu_1478 CRISPR-associated protein Cas4 R07464 cas4; % Muu_1478 CRISPR-associated protein Cas4 R07464 cas4; % Muu_1478 CRISPR-associated protein Cas5 family R19119 cas50 % Subtype I-D factors Muu_1478 CRISPR-associated protein Cas5 family R19119 cas50 % Subtype I-D factors Muu_1478 CRISPR-associated protein Cas5 family R19119 cas50 % Subtype I-D factors Muu_1478 CRISPR-associated protein Cas5 family R19119 cas50 % Subtype I-D factors Muu_1478 CRISPR-associated protein Cas5 family R19123 cas5; Muu_03019 CRISPR-associated protein Cas5 family R19123 cas5; Muu_3019 CRISPR-associated protein Cas5 family R19123 cas5; Muu_3019 CRISPR-associated protein Cas5 family R19123 cas5; Muu_3016 CRISPR-associated protein M1904 cas5; Muu_3016 CRISPR-associated protein R19123 cas5; Muu_3016 CRISPR-associated protein M1904 cas5; Muu_3016 CRISPR-associated protein M1904 cas5; Muu_3016 CRISPR-associated protein R19123 cas5; Muu_3016 CRISPR-associated protein M1904 cas5; Muu_3016 CRISPR-associated protein M1904 cas5; Muu_3016 CRISPR-associated protein Cas5 family R19123 cas5; Muu_3014 CRISPR-associated protein Cas5 family R19125 cas5; Muu_3014 CRISPR-associated protein Cas5 family R19125 cas5; Muu_3014 CRISPR-associated protein Cas5 family R19135 cas5; Muu_3014 CRISPR-associated protein Cas5 family R19126 cas5; Muu_3020 CRISPR-associated protein R19136 cas1; Muu_3020 CRISPR-associated protein R19136 cas1; Muu_3020 CRISPR-associated protein R1920 cas5; Muu_3020 CRISPR-associated RMP protein R1920 cas5; Muu_3020 CRISPR-associated RMP prote</pre>	Mrub 3012 CRISPR-associated protein Cas2	K09951 cas2:
<pre>* Type I signiture cas proteins Mcud 3020 CRISPR-associated helicase Cas3 Mcud 1475 CRISPR-associated protein Cas4 Mcud 1475 CRISPR-associated protein Mcud 1475 CRISPR-associated protein Cas4 Mcud 1475 CRISPR-associated protein Cas5 Mcud 1475 CRISPR-associated protein Cas5 Mcud 1475 CRISPR-associated protein Cas5 Mcud 1475 CRISPR-associated protein Cas6 Mcud 2020 CRISPR-associated protein Cas6 Mcud 3012 CRISPR-associated protein Mcud 3015 CRISPR-associated protein Mcud 3015 CRISPR-associated protein Mcud 3014 CRISPR-associated protein Mcud 3014 CRISPR-associated protein Mcud 3014 CRISPR-associated protein Mcud 3015 CRISPR-associated protein Mcud 3015 CRISPR-associated protein Mcud 1476 CRISPR-associated protein Mcud 3015 CRISPR-associated protein Mcud 2015 CRISPR-associated protein Mcud 2016 CRISPR-associated RAMP protein Mcud 2016 CRISPR-associated RAMP protein Mcud 1485 CRISPR-associated RAMP protein M</pre>	V Type I CRISPR-Cas system	
Mrub 3020 CRISPR-associated helicase Cas3 Mrub 3020 CRISPR-associated protein Cas4 Mrub 1478 CRISPR-associated protein Cas6 Mrub 1478 CRISPR-associated protein Cas6 Mrub 1478 CRISPR-associated protein Cas6 Mrub 1478 CRISPR-associated protein Mrub 3019 CRISPR-associated protein Mrub 3014 CRISPR-associated protein Mrub 3015 CRISPR-associated protein Mrub 3014 CRISPR-associated protein Mrub 3014 CRISPR-associated protein Mrub 3014 CRISPR-associated protein Mrub 2016 CRISPR-associated AMP protein Mrub 2016 CRISPR-associated AMP protein Mrub 2019 CRISPR-associated AMP protein Mrub 2019 CRISPR-associated AMP protein Mrub 1485 CRISPR-associated AMP protein Mrub 1485 CRISPR-associated Protein Mrub 1485 CRISPR-associated Protein Mrub 1485 CRISPR-associated Protein Mrub 1485 CRISPR-associated Protein Mr	V Type I signiture cas proteins	
<pre>Subtype I-A factors Mrub 1475 CRISPR-associated protein Cas4 Mrub 1475 CRISPR-associated protein Cas6 Subtype I-B factors Mrub 1476 CRISPR-associated protein Cas4 Subtype I-C factors Mrub 1487 CRISPR-associated protein Mrub 1487 CRISPR-associated protein Cas4 Mrub 1487 CRISPR-associated protein Cas6 Subtype I-D factors Mrub 0019 CRISPR-associated protein Mrub 0019 CRISPR-associated protein Mrub 0010 CRISPR-associated AMP protein Mrub 0010 CRISPR-associa</pre>	Mrub 3020 CRISPR-associated helicase Cas3	K07012 cas3:
Mrub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub_0222 CRISPR-associated protein Cas4 R19051 cas6; Mrub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1475 CRISPR-associated protein R19117 csd1; Mrub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1475 CRISPR-associated protein Cas5 R19119 cas50 * Subtype I-D factors R19123 cas4; Mrub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1475 CRISPR-associated protein Cas5 R19123 cas4; Mrub_0101 CRISPR-associated protein R19123 cas4; Mrub_0101 CRISPR-associated protein R19123 cas2; Mrub_0101 CRISPR-associated protein R19124 cas2; Mrub_0101 CRISPR-associated protein R19124 cas2; Mrub_011 CRISPR-associated protein R19124 cas2; Mrub_011 CRISPR-associated protein R19124 cas2; Mrub_011 CRISPR-associated protein R07464 cas4; Mrub_012 factors R07464 cas4; Type II CRISPR-associated protein Cas4 R0	Subtype I-A factors	
Mrub_0222 CRISPR-associated protein Cas6 R19091 cas6; Subtype I-5 factors R07464 cas4; Mrub_1475 CRISPR-associated protein R19117 csd1; Mrub_1486 CRISPR-associated protein R19117 csd1; Mrub_1486 CRISPR-associated protein R19118 csd2; Mrub_1486 CRISPR-associated protein R19118 csd2; Mrub_1486 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub_0182 CRISPR-associated protein Cas4 R07464 cas4; Mrub_0122 CRISPR-associated protein Cas4 R07464 cas4; Mrub_0122 CRISPR-associated protein Cas4 R19091 cas5; Mrub_0122 CRISPR-associated protein R19123 cash; Mrub_0101 CRISPR-associated protein R19123 cash; Mrub_0101 CRISPR-associated protein R19123 cash; Mrub_0101 CRISPR-associated protein R19124 cas2; Mrub_0101 CRISPR-associated protein R19126 casE; Subtype I-F factors R19126 casE; Subtype I-F factors R19126 casE; Mrub_011 CRISPR-associated protein R19126 casE; Subtype I-F factors R07464 cas4; Type II cRISPR-cas system R19126 casE; Mrub_0121 CRISPR-associated protein R07464 cas4; Mrub_0121 CRISPR-associated protein R07464 cas4; Mrub	Mrub 1478 CBISPB-associated protein Cas4	K07464 cas4:
<pre>Subtype I-B factors Mrub 1472 CRISPR-associated protein Cas4 Wrub 1472 CRISPR-associated protein Mrub 1487 CRISPR-associated protein Mrub 1486 CRISPR-associated protein Cas4 Mrub 1488 CRISPR-associated protein Cas5 family Subtype I-D factors Mrub 1478 CRISPR-associated protein Cas4 Mrub 2012 CRISPR-associated protein Mrub 2019 CRISPR-associated protein Mrub 2019 CRISPR-associated protein Mrub 2019 CRISPR-associated protein Mrub 2019 CRISPR-associated protein Mrub 2018 CRISPR-associated RAMP protein Mrub 2019 CRISPR-</pre>	Mrub 0222 CRISPR-associated protein Cas6	K19091 ca=6:
Mrub 1475 CRISPR-associated protein Cas4 K07464 cas4; Mrub 1487 CRISPR-associated protein K19117 csdl; Mrub 1486 CRISPR-associated protein Cas4 Mrub 1487 CRISPR-associated protein Cas5 family K19118 csd2; Mrub 1475 CRISPR-associated protein Cas5 family K19119 cas60 Subtype I-D factors K07464 cas4; Mrub 1478 CRISPR-associated protein Cas6 K19081 cas6; Mrub 2012 CRISPR-associated protein K19122 cash; Mrub 3019 CRISPR-associated protein K19123 cash; Mrub 3016 CRISPR-associated protein K19124 casc; Mrub 3016 CRISPR-associated protein K19126 casp; Mrub 3016 CRISPR-associated protein K19126 casp; Mrub 3016 CRISPR-associated protein K19126 casp; Mrub 3014 CRISPR-associated protein K19126 casp; Mrub 1476 CRISPR-associated protein K19126 casp; Subtype II-D factors K07464 cas4; K07464 cas4; Mrub 1476 CRISPR-associated protein K07464 cas4; Mrub 1477 CRISPR-associated protein K07464 cas4; <tr< td=""><td>T Subture I-B factors</td><td></td></tr<>	T Subture I-B factors	
<pre>Number 1-C factors Number 1</pre>	Mrub 1478 CBISPB-associated protein Cas4	K07464 ca=4-
Mrub_1487 CRISPR-associated protein R19117 csdl; Mrub_1486 CRISPR-associated protein Cas4 R07466 cas4; Mrub_1485 CRISPR-associated protein Cas5 family R19118 csdl; Mrub_1485 CRISPR-associated protein Cas5 Family Mrub_1485 CRISPR-associated protein Cas6 R07466 cas4; Mrub_0222 CRISPR-associated protein Cas6 R19123 cas5; Mrub_3018 CRISPR-associated protein R19123 cas5; Mrub_3016 CRISPR-associated protein R19124 cas6; Mrub_3016 CRISPR-associated protein R19126 cas5; Mrub_3016 CRISPR-associated protein R19126 cas2; Mrub_3014 CRISPR-associated protein R19126 cas2; Mrub_3014 CRISPR-cas system R19126 cas2; Subtype II-A factors R07466 cas4; Nurb_1476 CRISPR-cas system R07466 cas4; Type III crispr-Cas system R07466 cas4; Mrub_10215 CRISPR-associated protein R07016 csml; Mrub_0216 CRISPR-associated protein R19138 csm2; Mrub_0216 CRISPR-associated RMM protein R19138 csm2;<	V Subture I-C factors	
Mrub_1486 CRISPR-associated protein R19118 csd2; Mrub_1486 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub_0222 CRISPR-associated protein Cas6 R19091 cas6; Subtype I-E factors R19123 cas1; Mrub_3019 CRISPR-associated protein R19124 cas2; Mrub_3016 CRISPR-associated protein R19124 cas2; Mrub_3016 CRISPR-associated protein Cas5 family R19124 cas2; Mrub_3016 CRISPR-associated protein Cas5 family R19126 cas5; Mrub_3016 CRISPR-associated protein Cas5 family R19126 cas2; Mrub_3016 CRISPR-associated protein Cas5 family R19126 cas2; Mrub_3016 CRISPR-associated protein R19126 cas2; Subtype I-F factors R07464 cas4; Nrub_1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub_1478 CRISPR-associated protein Cas4 R07464 cas4; Nrub_1478 CRISPR-associated protein R07016 csm1; Mrub_0215 CRISPR-associated protein R07016 csm1; Mrub_0216 CRISPR-associated protein R09002 csm3; Mrub	Mrub 1487 CRISPB-associated protein	K19117 ced1:
Mrub 1475 CRISPR-associated protein Cas4 R07464 cas4; Mrub 1485 CRISPR-associated protein Cas5 family R19119 cas5d Subtype I-D factors Nrub 1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub 0222 CRISPR-associated protein Cas6 R19991 cas6; Subtype I-D factors R19123 cash; Mrub 2019 CRISPR-associated protein R19123 cash; Mrub 2016 CRISPR-associated protein R19124 casc; Mrub 2015 CRISPR-associated protein R19126 casE; Subtype I-U factors R19126 casE; Subtype I-U factors R07464 cas4; Type II CRISPR-Cas system R07464 cas4; Type III cRISPR-Cas system R07464 cas4; Mrub 0215 CRISPR-associated protein R07464 cas4; Mrub 0215 CRISPR-associated protein R07464 cas4; Type III signiture cas proteins R07464 cas4; Mrub 0215 CRISPR-associated protein R07464 cas4; </td <td>Mruh 1486 CRISPB-associated protein</td> <td>K19118 c=d2:</td>	Mruh 1486 CRISPB-associated protein	K19118 c=d2:
Mrub 1488 CRISER-associated protein Cas1 family R19119 cas5d * Subtype I-D factors R07464 cas4; Mrub 1478 CRISER-associated protein Cas4 R07464 cas4; Mrub 0222 CRISER-associated protein Cas5 R19123 cas3; Mrub 019 CRISER-associated protein R19123 cas2; Mrub 016 CRISER-associated protein R19123 cas2; Mrub 016 CRISER-associated protein R19123 cas2; Mrub 016 CRISER-associated protein R19124 cas2; Mrub 016 CRISER-associated protein R19125 cas2; Mrub 016 CRISER-associated protein R19126 cas2; Subtype I-F factors R07464 cas4; Ype II RISER-Cas system R07464 cas4; Type II CRISER-cassociated protein Cas4 R07016 csm1; * Type III CRISER-associated protein R19128 csm2; Mrub 0215 CRISER-associated protein R09002 csm2; Mrub 0216 CRISER-associated protein R19138 csm2; Mrub 0216 CRISER-associated RAMP protein R19138 csm2; Mrub 0216 CRISER-associated RAMP protein R19138 csm2; Mrub 0216 CRISER-associated RAMP protein R19140 csm5; Subtype III-5 factors	Mrub 1478 CBISDB-sepociated protein Cae4	K07464 ca=4.
Subtype I-D factors R07464 cas4; Mrub_1478 CRISFR-associated protein Cas6 R07464 cas4; Mrub_2022 CRISFR-associated protein Cas6 R19019 cas6; Subtype I-E factors R19123 cash; Mrub_3016 CRISFR-associated protein R19123 cash; Mrub_3016 CRISFR-associated protein R19123 cash; Mrub_3016 CRISFR-associated protein R19124 casC; Mrub_3016 CRISFR-associated protein R19126 casE; Mrub_3016 CRISFR-associated protein R19126 casE; Mrub_3014 CRISFR-associated protein R19126 casE; Subtype I-F factors R19126 casE; Subtype I-U factors R19126 casE; Subtype II-S factors R07464 cas4; Type II signiture cas proteins R07464 cas4; Mrub_0215 CRISFR-associated protein Cas4 R07464 cas4; Type III cRISFR-Cas system R07464 cas4; Type III signiture cas proteins R07016 csm1; Mrub_0215 CRISFR-associated protein R0902 csm3; Mrub_0216 CRISFR-associated protein R09002 csm3; Mrub_0217 CRISFR-associated RMP protein R19139 csm4; Mrub_0219 CRISFR-associated RMP protein R19140 csm5; Subtype III-5 factors	Mrub 1488 CDISDB-associated protein Cast family	K19119 ca=5d:
Mrub 1478 CRISPR-associated protein Cas4 R07464 cas4; Mrub 0222 CRISPR-associated protein Cas6 R19091 cas6; Subtype I-E factors R19123 cash; Mrub 3018 CRISPR-associated protein R19126 casb; Mrub 3014 CRISPR-associated protein Cas5 family R19126 casb; Mrub 3014 CRISPR-associated protein Cas5 family R19126 casb; Subtype I-F factors Subtype I-F factors Subtype II-S factors Subtype II-A factors Subtype II-A factors K07464 cas4; Type III signiture cas proteins Mrub 0215 CRISPR-associated protein Cas4 Mrub 0215 CRISPR-associated protein K07016 csml; Subtype III-A factors K07016 csml; Mrub 0216 CRISPR-associated protein K09002 csm3; Mrub 0216 CRISPR-associated protein K19138 csm3; Mrub 0217 CRISPR-associated RAMP protein K19140 csm3; Mrub 0218 CRISPR-associated RAMP protein K19140 csm3; Mrub 0219 CRISPR-associated RAMP protein K19140 csm5; Subtype III-5 factors K09012	T Subture I-D factors	Aller Calou,
Mrub 0222 CRISPR-associated protein Cas6 R1909 cas6; Subtype I-E factors R1909 CRISPR-associated protein R1904 cas5; Mrub 3019 CRISPR-associated protein R1904 cas5; Mrub 3016 CRISPR-associated protein Cas5 family R19124 cas0; Mrub 3016 CRISPR-associated protein Cas5 family R19126 cas5; Mrub 3016 CRISPR-associated protein Cas5 family R19126 cas5; Mrub 3016 CRISPR-associated protein Cas5 family R19126 cas5; Mrub 3014 CRISPR-associated protein Cas5 family R19126 cas5; Subtype I-F factors R1998 cas proteins Subtype II-A factors R1998 cas proteins Subtype II-A factors R1998 cas proteins R1998 cas proteins Mrub 1475 CRISPR-associated protein Cas4 R07464 cas4; Type III cRISPR-associated protein Cas4 R07464 cas4; Type III signiture cas proteins Mrub 0215 CRISPR-associated protein R298 R1998 cas9; Mrub 0216 CRISPR-associated protein R398 R1998 cas9; Mrub 0216 CRISPR-associated R309 protein R19138 cas2; Mrub 0219 CRISPR-associated R309 protein R19139 cas6; Mrub 0219 CRISPR-associated R309 protein R1998 cas9; Mrub 1495 CRISPR-associated R309 protein R1998 cas9; Mrub 1495 CRISPR-associated R309 protein R19976 cas9; Mrub 1495 CRISPR-associated Protein R10976 cas9; Mrub 1495 CRISPR-associated Protein R10977 cas9; Mrub 14	Mrub 1478 CDISDE-seconisted protein Car4	K07464 car4.
Nutrype I-E factors K1901 Call Mrub 3019 CRISPR-associated protein K19123 cash; Mrub 3018 CRISPR-associated protein K19124 casC; Mrub 3015 CRISPR-associated protein Cas5 family K19125 casD; Mrub 3014 CRISPR-associated protein Cas5 family K19126 casE; Subtype I-F factors K19126 casE; Subtype II-F factors K19126 casE; Subtype II-F factors K07464 cas4; Type II CRISPR-Cas system K07464 cas4; Type III CRISPR-Cas system K07016 ceml; Mrub 2015 CRISPR-associated protein K07016 ceml; Mrub 2016 CRISPR-associated protein K09002 cem2; Mrub 2018 CRISPR-associated RAMP protein K19138 cem2; Mrub 2019 CRISPR-associated RAMP protein K19130 cem3; Mrub 2019 CRISPR-associated RAMP protein K19140 cem5; Subtype III-5 factors K19140 cem5; Mrub 1485 CRI	Mrub 0222 CDISDD-resociated protein Cast	K19091 cash:
NubleyNuble	Rubture T-F factors	RIPOPI CRSC,
Mrub_3018 CRISPR-associated protein K19046 cas5; Mrub_3016 CRISPR-associated protein K19046 cas5; Mrub_3014 CRISPR-associated protein K19125 cas5; Mrub_3014 CRISPR-associated protein K19126 cas5; Subtype I-F factors K19126 cas5; Subtype I-U factors K19126 cas5; Subtype I-U factors K19126 cas5; Subtype II-S factors Subtype II-A factors Subtype II-S factors K07464 cas4; Type III CRISPR-Cas system K07016 css1; Type III CRISPR-Cas system K07016 css1; Subtype II-A factors K07016 css1; Mrub_0215 CRISPR-associated protein K07016 css1; Mrub_0216 CRISPR-associated protein K19138 css2; Mrub_0217 CRISPR-associated protein K19138 css2; Mrub_0219 CRISPR-associated RAMP protein K19138 css3; Mrub_0219 CRISPR-associated RAMP protein K19139 css3; Mrub_0219 CRISPR-associated RAMP protein K19140 css3; Subtype III-5 factors K19140 css3; Mrub_1485 CRISPR-associated RAMP protein K19140 css3; Subtype III-5 factors K19140 css3; Mrub_1485 CRISPR-associated RAMP protein K19076 cmr2;	Mayb 2019 CDTSDD accisted protein	F10122
Mrub_3016 CRI3PR-associated protein K19124 casC; Mrub_3015 CRI3PR-associated protein K19126 casD; Mrub_3014 CRI3PR-associated protein K19126 casD; Subtype I-F factors Subtype I-U factors Subtype II-U factors Y Type II signiture cas proteins Subtype II-A factors Subtype II-B factors Y Mrub_1475 CRI3PR-associated protein Cas4 K07464 cas4; Type III signiture cas proteins Y Mrub_1475 CRI3PR-associated protein Cas4 K07464 cas4; Type III signiture cas proteins Y Mrub_0215 CRI3PR-associated protein K07016 csml; Subtype III-A factors Y Mrub_0216 CRI3PR-associated protein K09002 csm3; Mrub_0216 CRI3PR-associated Protein K09002 csm3; Mrub_0216 CRI3PR-associated RAMP protein K19138 csm2; Mrub_0216 CRI3PR-associated RAMP protein K19139 csm4; Mrub_0216 CRI3PR-associated RAMP protein K19140 csm5; Subtype III-B factors Y Mrub_1485 CRI3PR-associated RAMP protein K19100 csm5; Subtype III-B factors Y Mrub_1485 CRI3PR-associated RAMP protein K07061 cmr1; <t< td=""><td>Mauk 2018 CDISDE-sessized protein</td><td>V10046 case.</td></t<>	Mauk 2018 CDISDE-sessized protein	V10046 case.
Mrub_3015 CRISPR-associated protein Cas5 family K19126 cas0; Mrub_3014 CRISPR-associated protein K19126 cas0; Subtype I-F factors Subtype I-U factors Subtype I-U factors Yope II cRISPR-Cas system Type II signiture cas proteins Subtype II-5 factors Subtype II-5 factors Nrub_1478 CRISPR-associated protein Cas4 K07464 cas4; Type III cRISPR-Cas system Type III signiture cas proteins K07016 cas1; Mrub_0215 CRISPR-associated protein K07016 cas1; Subtype III-A factors Mrub_0215 CRISPR-associated protein K07016 cas1; Subtype III-A factors Mrub_0216 CRISPR-associated protein K19138 cas2; Mrub_0216 cRISPR-associated Protein Mrub_0216 CRISPR-associated Protein K19138 cas2; Mrub_0216 CRISPR-associated RAMP protein K19139 cas3; Mrub_0219 CRISPR-associated RAMP protein K19140 cas5; Subtype III-5 factors K19140 cas5; Mrub_1485 CRISPR-associated RAMP protein K19140 cas5; Mrub_1483 CRISPR-associated Protein K07061 cmr1; Mrub_1485 CRISPR-associated Protein K07061 cmr1; Mrub_1483 CRISPR-associated Protein K09127 cmr3;	March 2016 CRISPR associated protein	Kigigi and
Mrub_3014 CRISPR-associated protein Case family K19126 casE; Mrub_3014 CRISPR-associated protein K19126 casE; Subtype I-F factors Type II CRISPR-Cas system Type II cRISPR-Cas system Subtype II-5 factors Mrub_1478 CRISPR-associated protein Cas4 K07464 cas4; Type III cRISPR-Cas system Type III cRISPR-cas system Type III cRISPR-cas system Mrub_0215 CRISPR-associated protein Cas4 K07016 cas1; Subtype III-A factors Mrub_0216 CRISPR-associated protein K19138 cas2; Mrub_0216 CRISPR-associated protein K19138 cas2; Mrub_0216 CRISPR-associated RAMP protein K19139 cas4; Mrub_0219 CRISPR-associated RAMP protein K19139 cas4; Mrub_0219 CRISPR-associated RAMP protein K19130 cas5; Subtype III-5 factors Mrub_1485 CRISPR-associated RAMP protein K19140 cas5; Mrub_1485 CRISPR-associated Protein K19076 cmr2; Mrub_1484 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K19076 cmr2;	March 2015 CDISDB-separational probain Care family	MIGIOS
Milb_0014 CRISPR associated protein R19120 Cas2, Subtype I-D factors Subtype I-D factors Type II cRISPR-Cas system Type II signiture cas proteins Subtype II-B factors Nrub 1478 CRISPR-associated protein Cas4 Mrub 1478 CRISPR-cas system R07464 cas4; Type III CRISPR-Cas system R07464 cas4; Type III CRISPR-cas system R07016 csm1; Mrub 0215 CRISPR-associated protein R07016 csm1; Subtype III-A factors R07016 csm2; Mrub 0216 CRISPR-associated protein R19138 csm2; Mrub 0217 CRISPR-associated RAMP protein R09002 csm3; Mrub 0218 CRISPR-associated RAMP protein R19139 csm4; Mrub 0219 CRISPR-associated RAMP protein R19140 csm5; Subtype III-B factors R07061 cm11; Mrub 1485 CRISPR-associated protein R07061 cm12; Mrub 1483 CRISPR-associated protein R19076 cm22; Mrub 1483 CRISPR-associated protein R19076 cm22; Mrub 1483 CRISPR-associated protein R09127 cm73;	March 2014 CDISDB-second sectors	Vioios cast,
Subtype I - U factors Subtype II - U factors Type II CRISPR-Cas system Type II signiture cas proteins Subtype II-A factors Mrub_1478 CRISPR-associated protein Cas4 Type III CRISPR-Cas system Type III signiture cas proteins Mrub 0215 CRISPR-associated protein Mrub 0215 CRISPR-associated protein Mrub 0216 CRISPR-associated protein Mrub 0217 CRISPR-associated Protein Mrub 0217 CRISPR-associated RAMP protein Mrub 0218 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 1485 CRISPR-associated RAMP protein Mrub 1485 CRISPR-associated Protein Mrub 1484 CRISPR-associated protein Mrub 1483 CRISPR-associated protein Mrub 1485 CRISPR-Associated protein Mrub	MIDD 3014 CRIDER ASSociated protein	AISI20 CASE,
<pre>Subsyst 1 o factors Type II CRISPR-Cas system Type II signiture cas proteins Subtype II-A factors Subtype II-5 factors Mrub_1478 CRISPR-associated protein Cas4 Type III CRISPR-Cas system Type III signiture cas proteins Mrub_0215 CRISPR-associated protein Mrub_0215 CRISPR-associated protein Mrub_0216 CRISPR-associated protein Mrub_0216 CRISPR-associated RAMP protein Mrub_0218 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_1485 CRISPR-associated RAMP protein Mrub_1484 CRISPR-associated protein Mrub_1483 CRISPR-associated protein Mru</pre>	Subtype I'l factors	
Type II ckilsk cas system Type II signiture cas proteins Subtype II-5 factors Mrub_1478 CRISPR-associated protein Cas4 Type III CRISPR-cas system Type III cRISPR-cas system Type III signiture cas proteins Mrub_0215 CRISPR-associated protein Mrub_0216 CRISPR-associated protein Mrub_0217 CRISPR-associated Protein Mrub_0218 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Subtype III-5 factors Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_1485 CRISPR-associated Protein Mrub_1484 CRISPR-associated protein Mrub_1483 CRISPR-associated protein	Time IT CDIRDECas suctors	
Subtype II-A factors Subtype II-B factors Mrub_1478 CRISPR-associated protein Cas4 Type III CRISPR-Cas system Type III signiture cas proteins Mrub_0215 CRISPR-associated protein Mrub_0216 CRISPR-associated protein Mrub_0217 CRISPR-associated Protein Mrub_0218 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_1485 CRISPR-associated RAMP protein Mrub_1484 CRISPR-associated protein Mrub_1483 CRISPR-associated protein Mrub_1484 CRISPR-associated protein Mrub_1484 CRISPR-associated protein Mrub_1484 CRISPR-associated protein Mrub_1484 CRISPR-associated protein Mrub_1484 CRISPR-associated protein Mrub_1485 CRISPR-as	Ture II chipth cas system	
Subtype II-5 factors Subtype II-5 factors Mrub_1478 CRISPR-associated protein Cas4 Type III CRISPR-Cas system Type III signiture cas proteins Mrub_0215 CRISPR-associated protein Mrub_0216 CRISPR-associated protein Mrub_0217 CRISPR-associated Protein Mrub_0218 CRISPR-associated RAMP protein Mrub_0219 CRISPR-associated RAMP protein Mrub_1485 CRISPR-associated RAMP protein Mrub_1484 CRISPR-associated protein Mrub_1483 CRISPR-associated protein Mrub_1485 CRIS	Rubture II-1 factors	
Mrub 1478 CRISPR-associated protein Cas4 K07464 cas4; Type III CRISPR-Cas system Type III signiture cas proteins Mrub 0215 CRISPR-associated protein K07016 csml; Subtype III-A factors K19138 csm2; Mrub 0216 CRISPR-associated protein K09002 csm3; Mrub 0217 CRISPR-associated RAMP protein K19138 csm2; Mrub 0218 CRISPR-associated RAMP protein K19139 csm4; Mrub 0219 CRISPR-associated RAMP protein K19140 csm5; Subtype III-B factors K19146 cRISPR-associated RAMP protein Mrub 1485 CRISPR-associated protein K190766 cmr2; Mrub 1484 CRISPR-associated protein K19076 cmr2; Mrub 1483 CRISPR-associated protein K19076 cmr2;	Public TT-E factors	
<pre>* Type III CRISPR-Cas system * Type III signiture cas proteins Mrub 0215 CRISPR-associated protein Mrub 0216 CRISPR-associated protein Mrub 0216 CRISPR-associated protein Mrub 0217 CRISPR-associated RAMP protein Mrub 0218 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 0219 CRISPR-associated RAMP protein Mrub 1485 CRISPR-associated RAMP protein Mrub 1485 CRISPR-associated protein Mrub 1484 CRISPR-associated protein Mrub 1483 CRISPR-associated protein K19076 cmr2; Mrub 1483 CRISPR-associated protein K07016 cmr3; </pre>	March 1478 CDISDE-sessisted protein CarA	V07464
<pre>Nype III CRIDER Cas system Type III cRIDER Cas system Type III signiture cas proteins Mrub 0215 CRISER-associated protein Mrub 0216 CRISER-associated protein Mrub 0217 CRISER-associated RAMP protein Mrub 0218 CRISER-associated RAMP protein Mrub 0219 CRISER-associated RAMP protein Mrub 0219 CRISER-associated RAMP protein Mrub 1485 CRISER-associated RAMP protein Mrub 1485 CRISER-associated RAMP protein Mrub 1485 CRISER-associated protein Mrub 1484 CRISER-associated protein Mrub 1483 CRISER-associated protein K19002 cmr2; Mrub 1483 CRISER-associated protein K0902 cmr3;</pre>	Time III CDISDD-Cas sustan	RUJTOT CAST,
Mrub 0215 CRISPR-associated protein K07016 csml; Subtype III-A factors Nrub 0216 CRISPR-associated protein K19138 csm2; Mrub 0217 CRISPR-associated RAMP protein K09002 csm3; Mrub 0218 CRISPR-associated RAMP protein K19139 csm4; Mrub 0219 CRISPR-associated RAMP protein K19139 csm4; Mrub 0219 CRISPR-associated RAMP protein K19140 csm5; Subtype III-B factors K19140 csm5; Mrub 1485 CRISPR-associated RAMP protein K07061 cmr1; Mrub 1485 CRISPR-associated protein K19076 cmr2; Mrub 1483 CRISPR-associated protein K19076 cmr2;	Tune III eigniture one proteine	
Mrub_0216 CRISPR-associated protein K19138 csm2; Mrub_0216 CRISPR-associated protein K19138 csm2; Mrub_0217 CRISPR-associated RAMP protein K19139 csm4; Mrub_0218 CRISPR-associated RAMP protein K19140 csm5; Mrub_0219 CRISPR-associated RAMP protein K19140 csm5; Subtype III-5 factors K07061 cmr1; Mrub_1485 CRISPR-associated RAMP protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K09127 cmr3;	Verype III Signiture cas proteins	K07016
Mrub_0216 CRISPR-associated protein K19138 csm2; Mrub_0217 CRISPR-associated RAMP protein K09002 csm3; Mrub_0218 CRISPR-associated RAMP protein K19139 csm4; Mrub_0219 CRISPR-associated RAMP protein K19140 csm5; * Subtype III-B factors K07061 cmr1; Mrub_1485 CRISPR-associated PAMP protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K19076 cmr2;	White TITE factors	RUJUIO CEMI,
Mrub_0217 CRISFR-associated protein RAMP protein R09002 csm3; Mrub_0218 CRISFR-associated RAMP protein R19139 csm4; Mrub_0219 CRISFR-associated RAMP protein R19140 csm5; * Subtype III-5 factors Mrub_1485 CRISFR-associated RAMP protein R07061 cmr1; Mrub_1484 CRISFR-associated protein R19076 cmr2; Mrub_1483 CRISFR-associated protein R09127 cmr3;	March 0216 CDTSDD-second sectors	¥101282-
Mrub_0218 CRISPR-associated RAMP protein K19139 csm4; Mrub_0218 CRISPR-associated RAMP protein K19140 csm5; Subtype III-B factors Mrub_1485 CRISPR-associated RAMP protein K07061 cmr1; Mrub_1484 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K09127 cmr3;	Mauk 0217 CDISDR-associated Division	Kipido China;
Mrub_0219 CRISPR-associated RAMP protein K19139 Csmt, Mrub_0219 CRISPR-associated RAMP protein K19140 csm5; Mrub_1485 CRISPR-associated RAMP protein K07061 cmr1; Mrub_1484 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K09127 cmr3;	March 0219 CRIDER associated DAMD protein	K09002 CBms,
Wilb_0219 CRISER-associated RAMP protein R19190 Casso, * Subtype III-5 factors Mrub 1485 CRISER-associated RAMP protein R07061 cmr1; Mrub 1484 CRISER-associated protein K19076 cmr2; Mrub_1483 CRISER-associated protein K09127 cmr3;	March 0210 CRISPR ASSociated RAMP protein	Migias Cont,
Mrub_1485 CRISPR-associated RAMP protein K190761 cmr1; Mrub_1484 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K09127 cmr3;	With Jais CRIDER ASSociated RAMP protein	REPIRO CBIND,
Mrub_1484 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K19076 cmr3;	Much 1485 CDTRDD-sectional DAWD support	¥07051
Mrub_1483 CRISPR-associated protein K19076 cmr2; Mrub_1483 CRISPR-associated protein K09127 cmr3;	March 1404 CDIODD-1405 AMAR protein	KU/UCI CMT1;
MIND_1403 CRIDERTASSOCIATED protein R09127 cmr3;	MILD 1909 CRIDEN-ASSOCIATED Protein	Kisu/o cmr2;
March 1400 CRTCRR and RIVE and A	March 1403 CRIDER associated protein	RUDIA/ CMTS;
White 1992 Chief associated Amer protein R05000 cmr4;	March 1401 CRISPR-associated KAMP protein	ROSOOD CMI4;
Mult 100 ChipfK-associated pictein River Chip	March 1400 CDTSDD-sesses and DIMD sesses	Kisiai cmro;
All 100 Chief associated Ant protein All 100 Chief All 100	TILD_190 CRIDER associated whit protein	ALFINE CMIC;

Figure 5. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Meiothermus ruber*. Evidence of four (4) functional operons. Operon 1: made up of universal Cas genes Mrub_0224 (*cas1*) and Mrub_0225 (*cas2*), Type 3 signature gene Mrub_0215 (*csm1*), Subtype 3-A genes Mrub_0216 (*csm2*), Mrub_0217 (*csm3*), Mrub_0218 (*csm4*), Mrub_0219 (*csm5*). Operon 2: universal cas genes Mrub_3013 (*cas1*) and Mrub_3012 (*cas2*), Type 1 signature gene Mrub_3020 (*cas3*), Subtype 1-E genes Mrub_3019 (*casA*), Mrub_3018 (*casB*), Mrub_3016 (*casC*), Mrub_3015 (*casD*), Mrub_3014 (*casE*). Operon 3: universal cas genes Mrub_0224 (*cas1*) and Mrub_0225 (*cas2*), Type 3 signature gene Mrub_0215 (*csm1*), Subtype 3-B genes Mrub_1485 (*cmr1*), Mrub_1484 (*cmr2*), Mrub_1483 (*cmr3*), Mrub_1482 (*cmr4*), Mrub_1481 (*cmr5*), Mrub_1480 (*cmr6*). Operon 4: universal cas genes Mrub_3013 (*cas1*) and Mrub_3012 (*cas2*), Type 1 signature gene Mrub_3020 (*cas3*), Subtype 1-E genes Mrub_3020 (*cas3*), Mrub_1487 (*cas4*), Mrub_1487 (*cas4*), Mrub_1488 (*cmr5*), Mrub_1480 (*cmr6*). Operon 4: universal cas genes Mrub_3013 (*cas1*) and Mrub_3012 (*cas2*), Type 1 signature gene Mrub_3020 (*cas3*), Subtype 1-E genes Mrub_3020 (*cas3*), Subtype genes cas genes Mrub_1478 (*cas4*),

Mrub_1487 (*csd1*), Mrub_1486 (*csd2*), Mrub_1488 (*cas5d*). Cas genes Mrub_1477 (*cas1*), Mrub_1476 (*cas2*), Mrub_0222 (*cas6*).

X. albilineans has a temperature growth range that classifies it as mesophilic and psychrophilic. KEGG analysis of the organism (Figure 6) shows that its CRISPR/Cas system contains a functional Type 1-C operon, functional Type 1-F operon, and cas gene Cas4 that's present across subtypes. The presence of a Type 1 operon is similar to *E. coli*, which is also mesophilic.

Xanthomonas albilineans	KO
CRISPR-Cas system	
Universal Cas proteins	
XALC 2891 probable crispr-associated protein cas1	K15342 cas1;
XALC 3048 probable crispr-associated protein cas1	K15342 cas1;
XALC 2892 probable crispr-associated protein cas2	K09951 cas2;
Type I CRISPR-Cas system	
Type I signiture cas proteins	
XALC 2885 probable crispr-associated helicase cas3 protein	K07012 cas3;
XALC 3049 probable crispr-associated helicase cas3 protein	K07012 cas3;
Subtype I-A factors	
XALC_2890 probable crispr-associated protein cas4	K07464 cas4;
Subtype I-B factors	
XALC_2890 probable crispr-associated protein cas4	K07464 cas4;
Subtype I-C factors	
XALC_2888 probable crispr-associated protein	K19117 csd1;
XALC_2889 probable crispr-associated protein	K19118 csd2;
XALC_2890 probable crispr-associated protein cas4	K07464 cas4;
XALC_2887 probable crispr-associated protein cas5d	K19119 cas5d;
Subtype I-D factors	
XALC_2890 probable crispr-associated protein cas4	K07464 cas4;
Subtype I-E factors	
Subtype I-F factors	
XALC_3050 probable crispr-associated protein	K19127 csy1;
XALC_3051 probable crispr-associated protein	K19128 csy2;
XALC_3052 probable crispr-associated protein	K19129 csy3;
XALC_3053 probable crispr-associated protein	K19130 csy4;
Subtype I-U factors	
🔻 Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
 Subtype II-B factors 	
XALC_2890 probable crispr-associated protein cas4	K07464 cas4;
 Type III CRISPR-Cas system 	
Type III signiture cas proteins	
Subtype III-A factors	
Subtype III-B factors	
Subtype III-U factors	

Figure 6. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Xanthomonas albilineans*. Evidence of two (2) functional operons. First made up universal Cas genes XALC_2891 (*cas1*) and XALC_2892 (*cas2*), Type 1 signature gene XALC_2885 (*cas3*), and Subtype 1-C genes XALC_2888 (*csd1*), XALC_2889 (*csd2*), XALC_2890 (*cas4*), XALC_2887 (*cas5d*). Second made up of universal Cas gene XALC_3048 (*cas1*), Type 1 signature gene XALC_3049 (*cas3*), and Subtype 1-F genes XALC_3050 (*csy1*), XALC_3051 (*csy2*), XALC_3052 (*csy3*), XALC_3053 (*csy4*).

KEGG analysis of *F. hwasookii* (Figure 7), classified in all three temperature classifications, shows evidence of a functional Type 1-A operon, functional Type 3-A operon, and cas genes *cas4* and *cas6* that are apparent across subtypes. Presence of a Type 1 operon is similar to *E. coli* and further evidence of relationship, while presence of a Type 3 operon and repeating *cas4* and *cas6* genes is similar to the thermophilic *M. ruber* (Figure 5).

Fusobacterium hwasookii	KO	
CRISPR-Cas system		
Universal Cas proteins		
RN87 08950 type I-B CRISPR-associated endonuclease Cas1	K15342 c	as1;
RN87 05765 CRISPR-associated protein Cas1	K15342 c	as1:
BN87 08945 CBISPR-associated protein Cas2	K09951 c	as2:
BN87 05770 CRISPR-associated protein Cas2	K09951 c	a = 2 :
V Type I CRISPR-Cas system		
V Type I signiture cas proteins		
BN87 08960 CBISPB-associated belicase Cas3	K07012 c	as3:
Subtype I-1 factors		
BNS7 08970 type I-B CRISPR-associated protein Cas7/Cst2/DevR	K19075 c	st2:
BNS7 08955 CRISPR-associated protein Cas4	K07464 c	a=4:
BNS7 08965 CBISPB-associated protein Cas5	K19090 c	asst:
RNS7 08980 CRISPB-associated endoribonuclease Cas6	K19091 c	as6:
Subture I_B factors		
BNR7 08955 CBISPB-associated protein Cas4	K07464 c	as4:
Subtype I-C factors	101101	
BNS7 08955 CBISPB-associated protein Cas4	K07464 c	as4.
Subture I_D factors		
BNST 08955 CBISDB_associated protein Cas4	K07464 c	ae4.
BNS7 08980 CRISPB-associated endoribonuclease Cas6	K19091 c	aak.
Subtupe I-F factors		
Subtype I - Factors		
Subtype I I factors		
Tune II CBISDE-Cas sustem		
Type II signiture cas proteins		
Subture II-1 factors		
Subtype II-B factors		
DNS7 08055 CDISDLassociated protein Cas4	K07464 c	·se4·
Tune III (DISDE-Cas system	10,101 0	
V Type III signiture cas proteins		
BN87 05730 type III-1 CBISPB-associated protein Cas10/Cam1	K07016 c	em1.
Subtra III-1 factors	10/010 0	in the second se
DN97 05735 tune III_1 CPISPD_associated protein Cam2	K19139 C	·sm2 ·
RNS7 05740 tune III-1 CRISPR-associated RIMP protein Cem3	K09002 c	em3.
BN87 05745 ture III-1 CBISDE associated BMP protein Cem4	K19130 A	sam4 .
RN87 05750 type III-A CRISPR-associated RAMP protein Com5	K19140 c	sem5.
Subture III-B factors	115140 G	, one
Subtype III-D factors		
equally are a resource		

Figure 7. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Fusobacterium hwasookii*. Evidence of 2 (two) functional operons. First made up of universal cas genes RN87_08950 (*cas1*) and RN87_08945 (*cas2*), Type 1 signature gene RN87_08960 (*cas3*), Subtype 1-A genes RN87_08970 (*cst2*), RN87_08955 (*cas4*), RN87_08965 (*cas5t*), RN87_08980 (*cas6*). Second made up of universal cas genes RN87_05765 (*cas1*) and RN87_05770 (*cas2*), Type 3 signature gene RN87_05745 (*csm1*), Subtype 3-A genes RN87_05735 (*csm2*), RN87_05740 (*csm3*), RN87_05745 (*csm4*), RN87_05750 (*csm5*).

The remaining psychrophilic organisms - *Hymenobacter nivis* (Figure 8), *Psychrobacter sp. G* (Figure 9,10), *Listeria weihenstephanensis* (Figure12)-- were determined to have either one or no functional CRISPR-Cas operons. The notable piece of evidence from the KEGG analysis is that all the psychrophiles had present a repeating cas gene, *cas4*, similar to *X. albilineans* and *F. hwasookii*. The repeating *cas4* gene among all psychrophilic provides some evidence for a relationship within the classification related to the cas gene. Specific to the analysis of *Psychrobacter sp. G*, argument of a functional operon can made based on locus tag cluster similarity that exist if the need for *cas2* is excluded. Looking at the KEGG analysis of *Psychrobacter immobilis DSM 7229*, an organism in the same genus as *Psychrobacter sp. G*, you can see the presence of both universal cas genes *cas1 and cas2*. Given the relationship between the two *Psychrobacter* organisms, it is possible that the KEGG analysis *P. sp. G* is simply missing the *cas2* gene and a functional system can actually be predicted. The same argument is however void for *H. nivis* as it lacks a majority of the genes for each CRISPR/Cas system subtype. *H. nivis* also couldn't be found in IMG/M database to confirm or refute the KEGG analysis.

Hymenobacter nivis	KO
CRISPR-Cas system	
Universal Cas proteins	
DDQ68 04835 subtype I-B CRISPR-associated endonuclease Cas1	K15342 cas1;
DDQ68 04830 cas2; CRISPR-associated endonuclease Cas2	K09951 cas2;
🔻 Type I CRISPR-Cas system	
Type I signiture cas proteins	
DDQ68_04855 cas3; CRISPR-associated helicase Cas3'	K07012 cas3;
DDQ68_04875 CRISPR-associated endonuclease Cas3''	K07012 cas3;
Subtype I-A factors	
DDQ68_04840 cas4; CRISPR-associated protein Cas4	K07464 cas4;
Subtype I-B factors	
DDQ68_04840 cas4; CRISPR-associated protein Cas4	K07464 cas4;
 Subtype I-C factors 	
DDQ68_04840 cas4; CRISPR-associated protein Cas4	K07464 cas4;
Subtype I-D factors	
DDQ68_04840 cas4; CRISPR-associated protein Cas4	K07464 cas4;
Subtype I-E factors	
Subtype I-F factors	
Subtype 1-0 factors	
Type 11 CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
Subtype 11-b lactors	1207464
DDD00_04040 Cas4; Ckisrk-associated protein Cas4	KU/464 Ca84;
Type III CHISTR-Cas System	
Subture III A factors	
Subture III-R factors	
Subture TIT-II factors	
ampabe iii o recora	

Figure 8. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system of *Hymenobacter nivis*. Evidence of zero (0) functional operons. Presence of universal Cas genes DDQ68_04835 (*cas1*) and DDQ68_04830 (*cas2*), Type 1 signature genes DDQ68_04855 (*cas3*) and DDQ68_04875 (*cas3*), cas gene DDQ68_04840 (*cas4*).

Psychrobacter sp. G	KO
CRISPR-Cas system	
Universal Cas proteins	
PSYCG 09140 CRISPR-associated protein Cas1	K15342 cas1;
▼ Type I CRISPR-Cas system	
Type I signiture cas proteins	
PSYCG_09145 hypothetical protein	K07012 cas3;
Subtype I-A factors	
Subtype I-B factors	
Subtype I-C factors	
Subtype I-D factors	
Subtype I-E factors	
Subtype I-F factors	
PSYCG_09150 hypothetical protein	K19127 csy1;
PSYCG_09155 hypothetical protein	K19128 csy2;
PSYCG_09160 hypothetical protein	K19129 csy3;
PSYCG_09165 hypothetical protein	K19130 csy4;
Subtype I-U factors	
🔻 Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
Subtype II-B factors	
🔻 Type III CRISPR-Cas system	
Type III signiture cas proteins	
Subtype III-A factors	
Subtype III-B factors	
Subtype III-U factors	

Figure 9. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Psychrobacter sp. G.* Evidence of zero (0) functional operons. Presence of universal cas gene PSYCG_09140 (*cas1*), Type 1 signature gene PSYCG_09145 (*cas3*), Subtype 1-F genes PSYCG_09150 (*csy1*), PSYCG_09155 (*csy2*), PSYCG_09160 (*csy3*), PSYCG_09165 (*csy4*).



Figure 10. IMG/M (Markowitz, 2012) analysis of *Psychrobacter sp. G* confirming the presence of *cas1*, *cas3*, *csy1*, *csy2*, *csy3*, *csy4* in an operon downstream from CRISPR spacer-repeat array. The operon lacks the presence of *cas2*.



Figure 11. IMG/M (Markowitz) analysis of *Psychrobacter immobilis DSM 7229*, an organism apart of the same genus as *Psychrobacter sp. G*. Analysis shows the presence of signature gene *cas3* and universal proteins *cas1* and *cas2*.

Listeria weihenstephanensis	KO
CRISPR-Cas system	
Universal Cas proteins	
UE46 03610 CRISPR-associated protein Cas1	K15342 cas1;
UE46 03615 CRISPR-associated protein Cas2	K09951 cas2;
🔻 Type I CRISPR-Cas system	
Type I signiture cas proteins	
UE46 03600 CRISPR-associated protein Cas3	K07012 cas3;
Subtype I-A factors	
UE46_03585 CRISPR-associated protein Cst1	K19088 cst1;
UE46_03590 CRISPR-associated protein	K19075 cst2;
UE46_03605 CRISPR-associated exonuclease Cas4	K07464 cas4;
UE46_03595 CRISPR-associated protein Cas5	K19090 cas5t;
UE46_13090 CRISPR-associated protein Cas6	K19091 cas6;
UE46_03580 hypothetical protein	K19091 cas6;
Subtype I-B factors	
UE46_03605 CRISPR-associated exonuclease Cas4	K07464 cas4;
Subtype I-C factors	
UE46_03605 CRISPR-associated exonuclease Cas4	K07464 cas4;
Subtype I-D factors	
UE46_03605 CRISPR-associated exonuclease Cas4	K07464 cas4;
UE46_13090 CRISPR-associated protein Cas6	K19091 cas6;
UE46_03580 hypothetical protein	K19091 cas6;
Subtype I-E factors	
Subtype I-F factors	
Subtype I-U factors	
🔻 Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
Subtype II-B factors	
UE46_03605 CRISPR-associated exonuclease Cas4	K07464 cas4;
Type III CRISPR-Cas system	
Type III signiture cas proteins	
Subtype III-A factors	
Subtype III-B factors	
Subtype III-U factors	

Figure 12. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Listeria weihenstephanensis*. Evidence of one (1) functional operon made up of universal cas genes UE46_03610 (*cas1*) and UE46_03615 (*cas2*), Type 1 signature genes UE46_03600 (*cas3*), Subtype 1-A genes UE46_03585 (*cst1*), UE46_03590 (*cst2*), UE46_03605 (*cas4*), UE46_03595 (*cas5t*), UE46_03580 (*cas6*).

KEGG analysis of the organisms within the thermophilic classification predicted the presence of complex CRISPR/Cas systems based on the presence of either multiple functional operons or repeating *cas4*, *cas6* genes similar to *M. ruber* with *Streptococcus thermophilus* being the exception the trend. Of the organisms following the trend -- *M. ruber* (Figure 5), *F. hwasookii* (Figure 8), *T. aquaticus* (Figure 13), *T. narugense* (Figure 14), *G. stearothermophilus* (Figure 15) -- have a seemingly functional Type 1 and Type 3 operon with *G. stearothermophilus* missing a type 3 signature gene. While *G. stearothermophilus* lacks a type 3 signature gene, the Subtype 3-B gene cluster contains locus tags similar to the universal cas genes and Subtype 1-B functional operon cluster. Given the similarity between locus tag clusters, evidence for a functional Type 3 operon in *G. stearothermophilus* is provided. Including all the thermophilic organisms, excluding *S. thermophilus*, the repetition of *cas4*, *cas6* genes is evidence of relationship based on these cas genes that exist between thermophilic CRISPR/Cas systems.

Repetition of *cas4* gene across thermophiles and psychrophiles provides evidence that the cas gene is involved in the complexity or maintenance of thermostability the CRISPR/Cas system.

Thermus aquaticus	KO
CRISPR-Cas system	
Iniversal Cas proteins	
T073 0109 CDISDD-associated protein Casi	K15342 cae1:
TOT3 0985 CDISDD-seccisted protein Casl	K15342 cas1:
TOTS 0108 CDISD-associated protein Cas2	K09951 cas2:
TOTS_ORDS CRIEDE associated protein Cas2	K09951 0002;
	RUDDOI CHEZ,
Type I CRISFR-Cas System	
Type I signifulle cas proteins	102010
TO'S DESS CRISPR-ASSOCIATED RELICASE CASS	KU/UIZ CHES,
* Subtype 1-A factors	
1073 0984 CRISPR-associated RecB family exonuclease Casea	KU7464 Cas4;
1073 1387 CRISPR repeat RNA endoribonuclease Case	K19091 Cas6;
10/3_0982 CRISPR repeat RNA endoribonuclease Case	K19091 Cas6;
 Subtype I-B factors 	2221212-21221-2122-2122
T073_0981 CRISPR-associated protein	K19114 csh1;
T073_0980 CRISPR-associated protein	K19115 csh2;
T073_0984 CRISPR-associated RecB family exonuclease Cas4a	K07464 cas4;
Subtype I-C factors	
T073_0984 CRISPR-associated RecB family exonuclease Cas4a	K07464 cas4;
Subtype I-D factors	
T073 0984 CRISPR-associated RecB family exonuclease Cas4a	K07464 cas4;
T073 1387 CRISPR repeat RNA endoribonuclease Cas6	K19091 cas6;
T073 0982 CRISPR repeat RNA endoribonuclease Cas6	K19091 cas6;
Subtype I-E factors	
Subtype I-F factors	
Subtype I-U factors	
V Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subture II-A factors	
Subture II-B factors	
TOTA 0844 CDISDD-seconisted Deck family evolutionse Cas4s	K07464 0004.
Time III CONSDECTS sustem	NO/101 C151/
Time III carber cas system	
TOTAL SIGNATURE Cas proteins	K07016 acm1.
School III & School Bedelik	KOVOIG CBRII,
· Subtype III-A Iscols	K10100
10/3 13/8 CRISPR-associated protein	KISI38 CSM2;
1073 1375 CRISPR-associated RAMP Cama	KU9UU2 CBM3;
10/3_1380 CRISPR-associated RAMP protein	K19139 CEM4;
T073_1381 CRISPR-associated protein	K19140 csm5;
 Subtype III-B factors 	000000000000000000000000000000000000000
T073_1912 CRISPR-associated RAMP Cmr1	K07061 cmr1;
T073_1914 CRISPR-associated RAMP Cmr2	K19076 cmr2;
T073_1913 CRISPR-associated RAMP Cmr3	K09127 cmr3;
T073_1911 CRISPR-associated RAMP Cmr4	K09000 cmr4;
T073_1910 CRISPR-associated RAMP Cmr5	K19141 cmr5;
T073_1909 CRISPR-associated RAMP Cmr6	K19142 cmr6;
Subturna TIT-II factors	

Figure 13. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Thermus aquaticus*. Evidence of three (3) functional operons. First made up of universal cas genes TO73_0109 (*cas1*) and TO73_0108 (*cas2*), Type 3 signature gene TO73_1377(*csm1*), Subtype 3-A genes TO73_1378 (*csm2*), TO79_1379 (*csm3*), TO73_1380 (*csm4*), TO73_81 (*csm5*). Second made up of universal cas genes TO73_0985 (*cas1*) and TO73_0978 (*cas2*), Type 1 signature gene TO73_0983 (*cas3*), Subtype 1-B genes TO73_0981 (*csh1*), TO73_0980 (*csh2*), TO73_0984 (*cas4*). Third made up of universal cas genes TO73_0109 (*cas1*) and TO73_0108 (*cas2*), Type 3 signature gene TO73_1377(*csm1*), Subtype 3-B genes TO73_1912 (*cmr1*), TO73_1914 (*cmr2*), TO73_1913 (*cmr3*), TO73_1911 (*cmr4*), TO73_1910 (*cmr5*), TO73_1909 (*cmr6*). Cas genes TO73_1387 (*cas6*), TO73_0982 (*cas6*).

Thermodesulfobium narugense	KO
CRISPR-Cas system	
Universal Cas proteins	
Thena 0820 CRISFR-associated protein Cas1	K15342 cas1;
Thena 1727 CRISPR-associated protein Casi	K15342 cas1:
Thena 1726 CRISPR-associated protein Cas2	K09951 cas2:
Thena 0821 CRISPR-associated protein Cas2	K09951 cas2:
V Type I CRISPR-Cas system	000000000000000000000000000000000000000
V Type I signiture cas proteins	
Thena 0818 CRISPR-associated HD domain protein	K07012 cas3:
Subtype I-A factors	
Thena 0819 CRISPR-associated protein Cas4	K07464 cas4:
Thena 0814 CRISPR-associated protein Cas6	K19091 cas6:
Subtype I-B factors	
Thena 0815 CRISPR-associated protein Cshl domain protein	K19114 csh1:
Thena 0816 CRISPR-associated protein	K19115 csh2;
Thena 0819 CRISPR-associated protein Cas4	K07464 cas4:
Thena 0817 CRISPR-associated protein Cas5	K19116 cas5h:
V Subtype I-C factors	
Thena 0819 CRISPR-associated protein Cas4	K07464 cas4:
Subtype I-D factors	
Thena 0819 CRISPR-associated protein Cas4	K07464 cas4;
Thena 0814 CRISPR-associated protein Cas6	K19091 cas6;
Subtype I-E factors	
Subtype I-F factors	
Subtype I-U factors	
Thena 0826 protein of unknown function DUF324	K19134 csx10;
Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
Subtype II-B factors	
Thena 0819 CRISPR-associated protein Cas4	K07464 cas4;
Type III CRISPR-Cas system	
Type III signiture cas proteins	
Thena 1733 CRISPR-associated protein	K07016 csm1;
Subtype III-A factors	
Thena 1731 CRISPR-associated RAMP protein	K09002 csm3;
Thena 1730 hypothetical protein	K19139 csm4;
Thena 1729 CRISPR-associated RAMP protein	K19140 csm5;
Subtype III-B factors	
Subtype III-U factors	

Figure 14. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Thermodesulfobium nargusense*. Evidence of two (2) functional operons. First made up of universal cas genes Thena_0820 (*cas1*) and Thena_0821 (*cas2*), Type 1 signature gene Thena_0818 (*cas3*), Subtype 1-B genes Thena_0815 (*csh1*), Thena_0816 (*csh2*), Thena_0819 (*cas4*), Thena_0817 (*cas5h*). Second made up of universal cas genes Thena_1727 (*cas1*) and Thena_1726 (*cas2*), Type 3 signature gene Thena_1733 (*csm1*), Subtype 3-A genes Thena_1731 (*csm3*), Thena_1730 (*csm4*), Thena_1729 (*csm5*).

Geobacillus stearothermophilus	KO
CRISPR-Cas system	
V Universal Cas proteins	
GT50 11265 CRISPR-associated protein Cas1	K15342 cas1;
GT50 11270 CRISPR-associated protein Cas2	K09951 cas2;
V Type I CRISPR-Cas system	
V Type I signiture cas proteins	
GT50 11255 CRISPR-associated protein Cas3	K07012 cas3;
V Subtype I-A factors	
GT50 11260 CRISPR-associated protein Cas4	K07464 cas4;
GT50 11275 CRISPR-associated protein Cas6	K19091 cas6;
V Subtype I-B factors	
GT50 11240 CRISPR-associated protein	K19114 csh1;
GT50 11245 CRISPR-associated protein Csh2	K19115 csh2;
GT50 11260 CRISPR-associated protein Cas4	K07464 cas4;
GT50 11250 CRISPR-associated protein Cas5	K19116 cas5h;
V Subtype I-C factors	
GT50 11260 CRISPR-associated protein Cas4	K07464 cas4;
V Subtype I-D factors	
GT50 11260 CRISPR-associated protein Cas4	K07464 cas4;
GT50 11275 CRISPR-associated protein Cas6	K19091 cas6;
Subtype I-E factors	
Subtype I-F factors	
Subtype I-U factors	
GT50_01510 hypothetical protein	K19134 csx10;
Type II CRISPR-Cas system	
Type II signiture cas proteins	
Subtype II-A factors	
Subtype II-B factors	
GT50_11260 CRISPR-associated protein Cas4	K07464 cas4;
Type III CRISPR-Cas system	
Type III signiture cas proteins	
Subtype III-A factors	
Subtype III-B factors	
GT50 11305 CRISPR-associated protein	K07061 cmr1;
GT50 11310 CRISPR-associated protein Crm2	K19076 cmr2;
GT50 11315 CRISPR-associated protein Cmr3	K09127 cmr3;
GT50 11320 CRISPR-associated protein Cmr4	K09000 cmr4;
Subtype III-U factors	

Figure 15. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Geobacillus stearothermophilus*. Evidence of one (1) functional operon made up of universal cas genes GT50_11265 (*cas1*) and GT50_11270 (*cas2*), Type 1 signature gene GT50_11255 (*cas3*), Subtype 1-B genes GT50_11240 (*csh1*), GT50_11245 (*csh2*), GT50_11260 (*cas4*), GT50_11250 (*cas5h*). Cas genes GT50_11275 (*cas6*), GT50_11305 (*cmr1*), GT50_11310 (*cmr2*), GT50_11315 (*cmr3*), GT50_11320 (*cmr4*).

KEGG analysis of *Streptococcus thermophilus* (Figure 16) in comparison to KEGG data of other thermophilic organisms studied demonstrates major differences and lack of correspondence to apparent trends. Analysis showed no evidence of a functional operon but presence of universal cas genes, signature genes for Type 2 and Type 3, and complete set of genes for Subtype 3-A. Analyzing the locus tag cluster similarities the type 3 signature gene and respective subtype genes have a corresponding universal cas gene, *cas2*. The type 2 signature gene corresponds to universal cas genes *cas1, cas2* based on locus tag clustering method. Presence of corresponding Type 3-A nonfunctional operon is seemingly evidence that expands upon relationship amongst CRISPR/Cas system in thermophilic bacteria based on presence of Type 3 operons.

Streptococcus thermophilus	KO
CRISPR-Cas system	
Universal Cas proteins	
AVT04 01625 type II CRISPR-associated endonuclease Cas1	K15342 cas1;
AVT04 00350 CRISPR-associated protein Cas2	K09951 cas2;
AVT04 01620 CRISPR-associated protein Cas2	K09951 cas2;
V Type I CRISPR-Cas system	
Type I signiture cas proteins	
Subtype I-A factors	
Subtype I-B factors	
Subtype I-C factors	
Subtype I-D factors	
Subtype I-E factors	
Subtype I-F factors	
Subtype I-U factors	
Type II CRISPR-Cas system	
Type II signiture cas proteins	
AVT04 01630 type II CRISPR RNA-guided endonuclease Cas9	K09952 csn1;
Subtype II-A factors	
Subtype II-B factors	
Type III CRISPR-Cas system	
Type III signiture cas proteins	
AVT04 00340 type III-A CRISPR-associated protein Cas10/Csml	K07016 csm1;
Subtype III-A factors	
AVT04 D0335 type III-A CRISPR-associated protein Csm2	K19138 csm2;
AVT04 00330 type III-A CRISPR-associated RAMP protein Csm3	K09002 csm3;
AVT04 00325 type III-A CRISPR-associated RAMP protein Csm4	K19139 csm4;
AVT04 00320 type III-A CRISPR-associated RAMP protein Csm5	K19140 csm5;
Subtype III-B factors	
Subture III-U factors	

Figure 16. KEGG (Kanehisa *et al., 2019*) analysis of CRISPR/Cas system in *Streptococcus thermophilus*. Evidence of (1) functional operons made up of universal cas genes AVT04_01625 (*cas1*), AVT04_01620 (*cas2*), Type 3 signature gene AVT04_00340 (*csm1*), Subtype 3-A genes AVT04_00335 (*csm2*), AVT04_00330 (*csm3*), AVT04_00325 (*csm4*), AVT04_00320 (*csm5*). Cas genes AVT04_00350 (*cas2*), Type 2 signature gene AVT04_01630 (*cas9*).

Table 1 summarizes the data from all the organisms based on temperature classification $(N_{meosphiles}=5, N_{Psychrophiles}=5, N_{Thermophiles}=6)$.

Organism Name	Temperature range#	Predicted Number of CRISPR/Cas Operons	Predicted Number of CRISPR arrays
Hymenobacter nivis	Psychro	0	na
Psychrobacter sp. G	Psychro	1	
Psychrobacter immobilis DSM 7229**		1	1
Listeria weihenstephanensis	Psychro	1	6
Escherichia coli	Meso	1	2
Salmonella enterica subsp. enterica serovar	Meso	1	2
Yersinia pseudotuberculosis	Meso	1	2
Meiothermus ruber DSM1279	Thermo	4	6
Thermus aquaticus Y51MC23	Thermo	3	7
Streptococcus thermophilus LMD-9	Thermo	1	3
Thermodesulfobium narugense	Thermo	2	2
Geobacillus stearothermophilus	Thermo	1	1
Xanthomonas albilineans*	Meso-Psychro	2	6
Fusobacterium hwasookii*	Meso-Psychro- Thermo	2	3

Table 1. A comparison of the number of putative CRISPR/Cas operons and CRISPR arrays in a select group of psychrophilic, mesophilic and thermophilic prokaryotes.

#Bacteria in general grow at a wide range of temperatures: psychrophile below 20°C, mesophiles between 20-45°C, thermophiles 45-80°C, and hyperthermophiles above 80°C. (Goldstein 2007)

*Organisms demonstrating a wider temperature range for characterized growth **Aditional organisms were added to the list based on the absence of related organisms not being found in IMG/M database. These organisms are related to the chosen organisms and thus their IMG/M (Markowitz, 2012) analysis is relevant to the the chosen organisms.

Discussion

The KEGG data, complementary IMG/M data, and temperature classifications reveal that several relationships may exist within and between classifications that alludes to the complexity in CRISPR/Cas systems that arise in psychrophiles and thermophiles compared to mesophiles such as model organism Escherichia coli. CRISPR/Cas systems in thermophilic bacteria demonstrate a trend of containing a components of a functional Type 3 operon. Mesophilic and and some psychrophilic bacteria, such as *Listeria weihenstephanensis*, have components that correspond to a functional Type 1 operon. Differing from mesophiles, the CRISPR/Cas systems in psychrophiles and thermophiles seem to be related by the presence of *cas4* which is presence across subtypes of Type 1 and Type 2. Thermophiles, in addition to *cas4*, demonstrate the repetition of cas6 which follows similar trends of presence in subtypes of Type 1 and Type 2. All studied organisms, except S. thermophilus and Hymenobacter nivis, have at least components of or a functional Type 1 operon. This data presents the possibility of evolutionary significance linked to Type 1 CRISPR/Cas systems as being shared across organisms The presence of Type 1 signature genes and cas genes also suggest the possibility of this relationship. Given the data collected however, the conclusion that complexity arises outside of thermophilic conditions can be made with a limited certainty until data is collected for a larger pool of organisms that fall within the the specified temperature classifications. In addition to conclusions based on the bioinformatic data, conclusions of functionality despite absence of specific genes can be made as the function of the operon may not be completely dependent on the presence of that specific gene (Richter et. al., 2012) and the biological principle that a previous study explored relating the classification and diversity of CRISPR/Cas systems to the conclusion that the high energy cost of maintaining and expressing several CRISPR/Cas genes must offer major advantages on to the cell. (Garrett et al., 2011). This means that the absence of a specific cas gene from prediction outputted by KEGG may be due to the lack identification of that gene somewhere else in the genome of an organisms. Analysis of *Psychrobacter sp. G* with KEGG (Figure 9) and IMG/M (Figure 10) suggest this when compared to the IMG/M analysis of *Psychrobacter immobilis* DSM 7229 (Figure 11) as cas2 is absent in the first but predicted to be present in the latter. A limitation of the methodology used, as mentioned earlier, is the renumbering of the locus tags for the genes analyzed by GenBank, a database KEGG and IMG/M pulls from. Due to this limitation, the method of predicting number of operons and functional systems based on locus tag similarities is solely applicable to analysis of the subtypes. In analyzing the entire functionality of the system, the method of identifying a complete set of universal cas genes, a type-specific signature gene, and a complete set of subtype genes without correspondence between the locus tags yielded adequate results that were reaffirmed by IMG/M. Given the results, this methodology was validated by the predicted number of CRISPR arrays which suggest that the phases of the CRISPR/Cas systems work independently of each other; cas1 and cas2 form a functional protein, cas cascade genes of a subtype form a functional protein.

Literature Cited

Willerslev, E., Hansen, A.J., Ronn, R., Brand, T.B., Barnes, I., Wiuf, C., Gilichinsky, D., Mitchell, D., Cooper, A., 2004. Long-term persistence of bacterial DNA. Curr. Biol., R9eR10.

Simon PC. The effect of temperature on growth and survival of Fusobacterium necrophorum isolated from bovine liver abscesses. *Can J Comp Med.* 1977;41(2):169-73.

Brady MF, Bhimji SS. Yersinia Pseudotuberculosis. [Updated 2018 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan-.https://www.ncbi.nlm.nih.gov/books/NBK430717/

Giannella RA. Salmonella. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 21.https://www.ncbi.nlm.nih.gov/books/NBK8435/

Makarova, Kira S and Eugene V Koonin. "Annotation and Classification of CRISPR-Cas Systems" *Methods in molecular biology (Clifton, N.J.)* vol. 1311 (2015): 47-75. Richter, Corinna et al. "Function and regulation of clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR associated (Cas) systems" *Viruses* vol. 4,10 2291-311. 19 Oct. 2012, doi:10.3390/v4102291

Jeanthon, C.; S. L'Haridon, V. Cueff, A. Banta, A. L. Reysenbach and D. Prieur. "Thermodesulfobacterium hydrogeniphilum sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent at Guaymas Basin, and emendation of the genus Thermodesulfobacterium." International Journal of Systematic and Evolutionary Microbiology, Vol 52, 765-772, Copyright © 2002 by Society for General Microbiology.

Tindall, B. J., Sikorski, J., Lucas, S., Goltsman, E., Copeland, A., Glavina Del Rio, T., ... Lapidus, A. (2010). Complete genome sequence of Meiothermus ruber type strain (21T). Standards in Genomic Sciences, 3(1), 26–36. <u>http://doi.org/10.4056/sigs.1032748</u>

Garrett, R. A., & Shah, S. A. (2011). CRISPR/Cas and Cmr modules, mobility and evolution of adaptive immune systems. *Research in Microbiology*, *162*(1), 27-38. doi:10.1016/j.resmic.2010.09.001

Euzéby JP. List of bacterial names with standing in nomenclature: A folder available on the Internet. Int J Syst Bacteriol 1997; 47:590-592. PubMed doi:10.1099/00207713-47-2-590

Nobre MF, Trüper HG, Da Costa MS. Transfer of Thermus ruber (Loginova et al. 1984), Thermus silvanus (Tenreiro et al. 1995), and Thermus chliarophilus (Tenreiro et al. 1995) to Meiothermus gen. nov. as Meiothermus ruber comb. nov., Meiothermus silvanus comb. nov., and Meiothermus chliarophilus comb. nov., respectively, and emendation of the genus Thermus. Int J Syst Bacteriol 1996; 46:604-606. doi:10.1099/00207713- 46-2-604

Makarova, K. S., Haft, D. H., Barrangou, R., Brouns, S. J., Charpentier, E., Horvath, P., ... Koonin, E. V. (2011, May 09). Evolution and classification of the CRISPR–Cas systems. Retrieved from <u>https://www.nature.com/articles/nrmicro2577</u>

Dupuis, M., Villion, M., Magadán, A. H., & Moineau, S. (2013, July 02). CRISPR-Cas and restriction–modification systems are compatible and increase phage resistance. Retrieved from <u>https://www.nature.com/articles/ncomms3087</u>

Terashima, M., Ohashi, K., Takasuka, T. E., Kojima, H., & Fukui, M. (2018, November 15). Antarctic heterotrophic bacterium Hymenobacter nivis P3T displays light-enhanced growth and expresses putative photoactive proteins. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1111/1758-2229.12702

Lebedinksy, A. V., Chernyh, N. A., & Bonch-Osmolovskaya, E. A. (2007). Phylogenetic Systematics of Microorganisms Inhabiting Thermal Environments [Abstract]. *BIOCHEMISTRY* (*Moscow*), 72(12), 1299-1311. Retrieved January 12, 2019.

Jiang, F., & Doudna, J. A. (2015). The Structural Biology of CRISPR-Cas Systems. *Curr Opin Struct Biol,30*, 100-111. Retrieved February 5, 2019.

Horvath, & Barrangou. (2010). CRISPR/Cas, the Immune System of Bacteria and Archaea. *SCIENCE*, *327*, 167-169. Retrieved February 5, 2019.

Wright, A., Nuñez, J., & Doudna, J. (2016). Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering. *Cell*, *164*(1-2), 29-44. doi:10.1016/j.cell.2015.12.035

Keseler, I.M., Mackie, A., Peralta-Gil, M., Santos-Zavaleta, A., Gama-Castro, S., Bonavides-Martinez, C., Fulcher, C., Huerta, A.M., Kothari, A., Krummenacker, M., Latendresse, M., Muniz-Rascado, L., Ong, Q., Paley, S., Schroder, I., Shearer, A., Subhraveti, P., Travers, M., Weerasinghe, D., Weiss, V., Collado-Vides, J., Gunsalus, R.P., Paulsen, I., and Karp, P.D. 2013. <u>EcoCyc: fusing model organism databases with systems biology</u> *Nucleic Acids Research* 41:D605-612.

Markowitz VM, Chen IA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, et al. 2012. IMG: The integrated microbial genomes database and comparative analysis system. Nucleic Acids Research 40(D1):D115-22. Available from: http://nar.oxfordjournals.org/content/40/D1/D115.full

Kanehisa, M., Sato, Y., Furumichi, M., Morishima, K., and Tanabe, M.; New approach for understanding genome variations in KEGG. Nucleic Acids Res. 47, D590-D595 (2019).