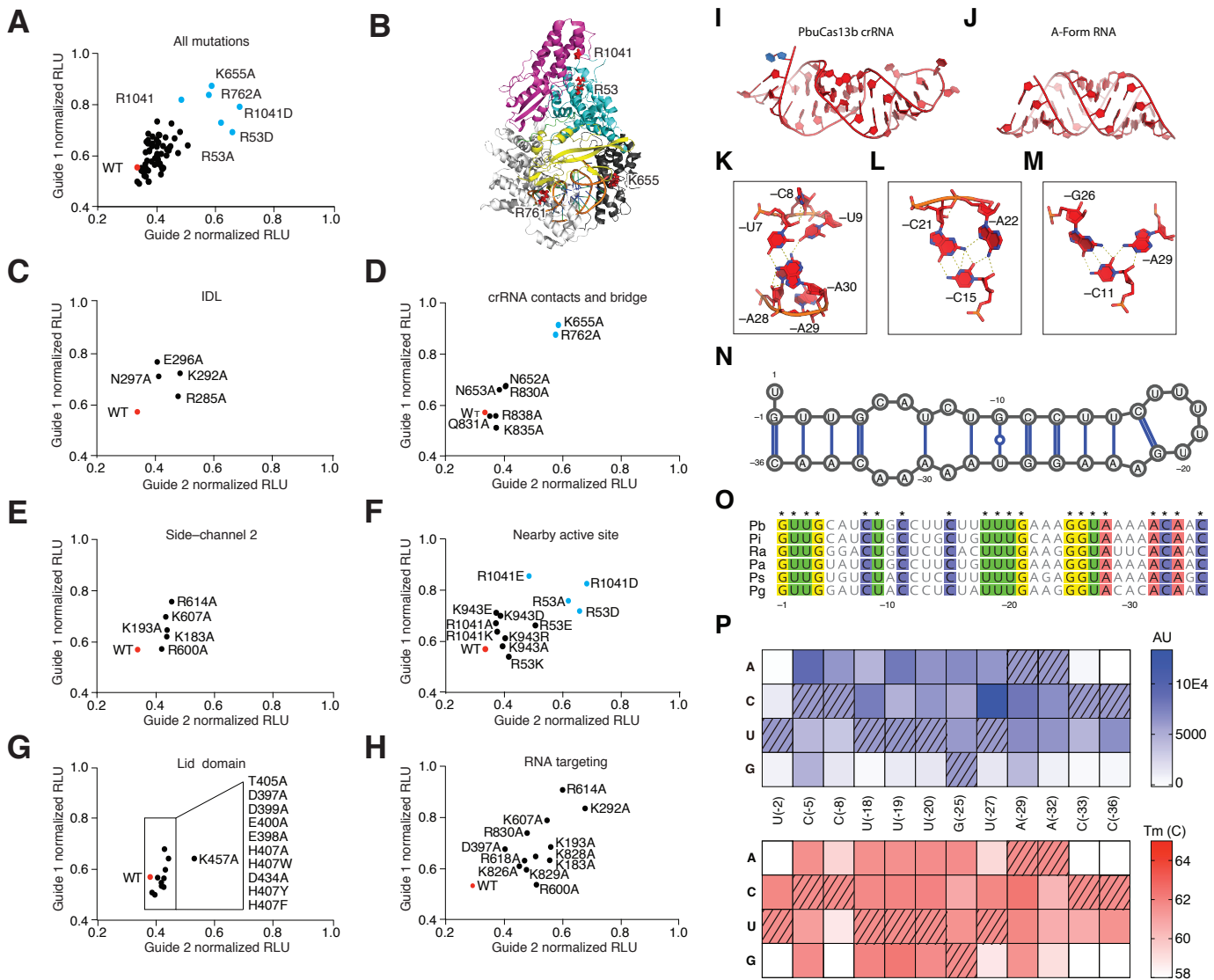
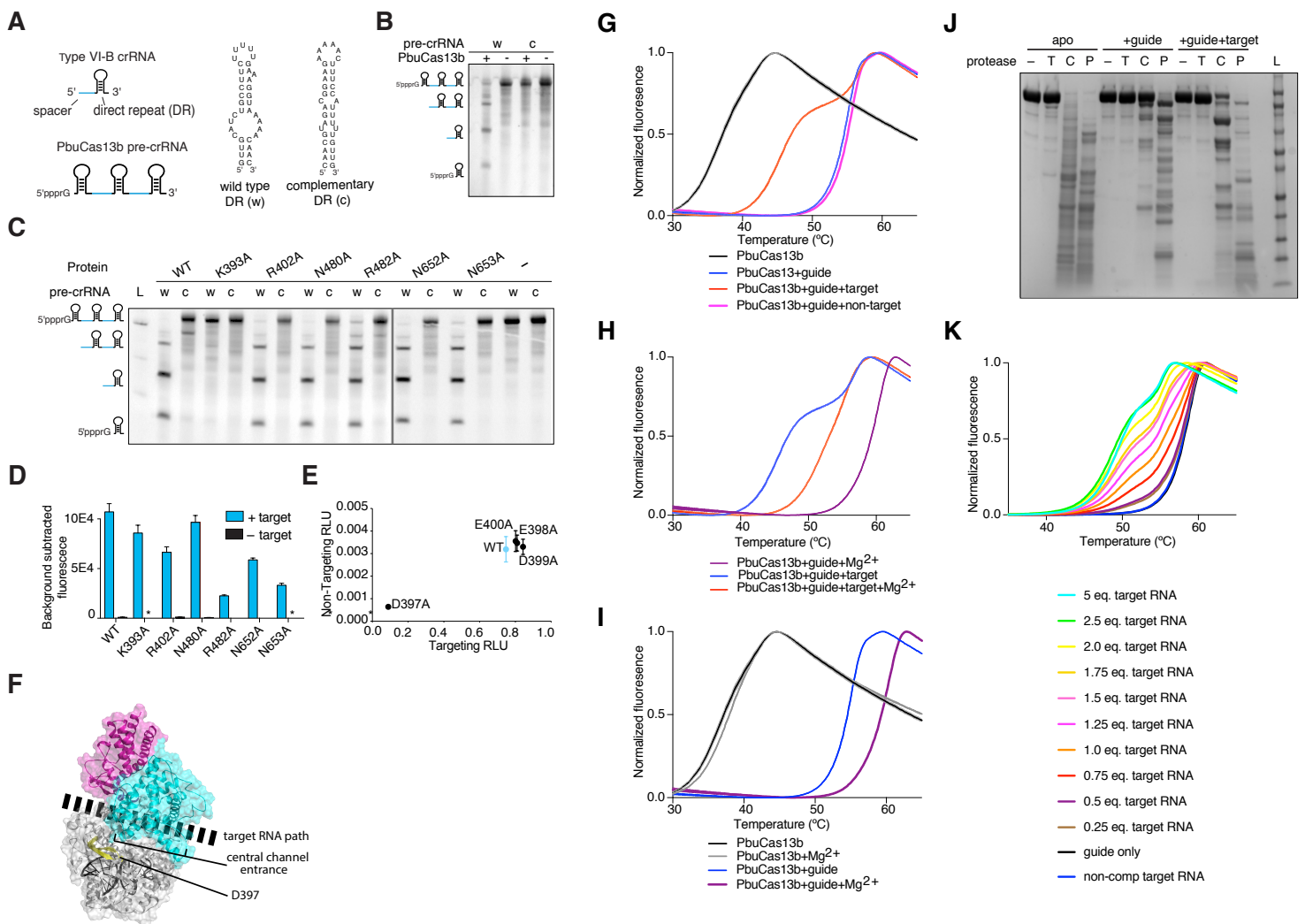


**Supplemental Figure 1, Related to Figure 1 (A - D)** Surface electrostatics of PbuCas13b. (A) Surface electrostatics of PbuCas13b with the active site and side channels labeled and crRNA shown in red. (B) Surface electrostatics of PbuCas13b rotated 180° from panel A with the second side channel labeled. (C) Surface electrostatics of PbuCas13b with the Lid domain removed, showing highly positively charged inner channel. (D) Surface electrostatics of the putative crRNA processing active site involving K393. (E) Grouped topology map of PbuCas13b secondary structure.

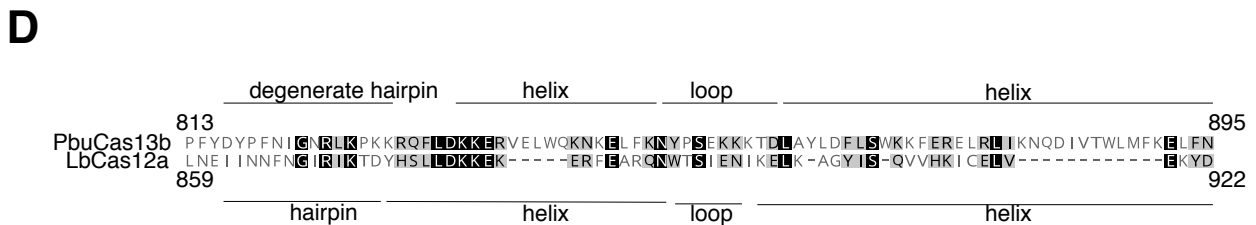
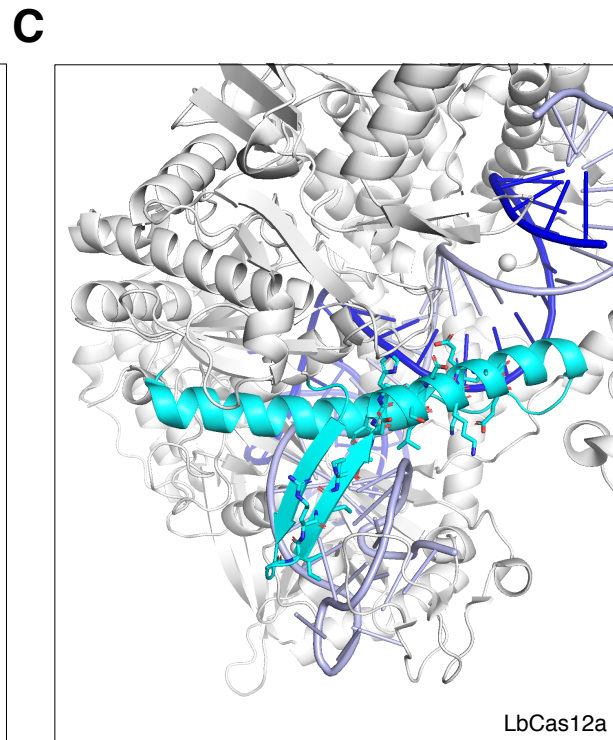
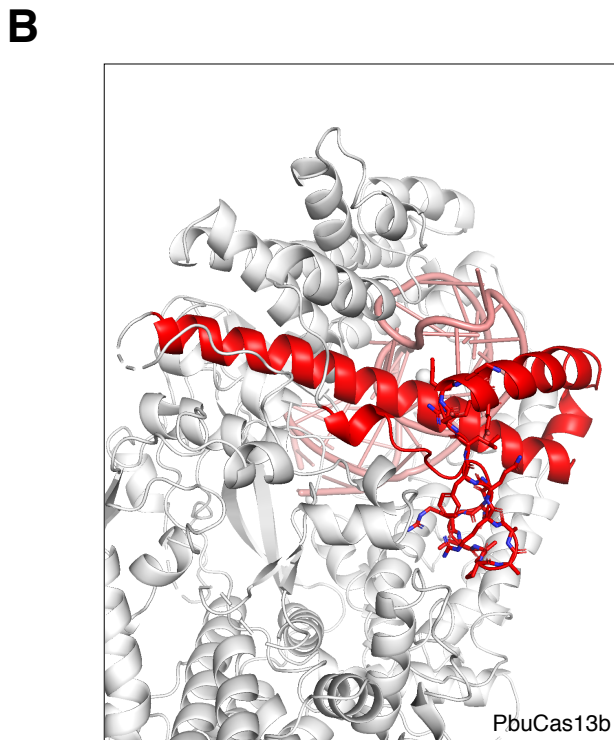
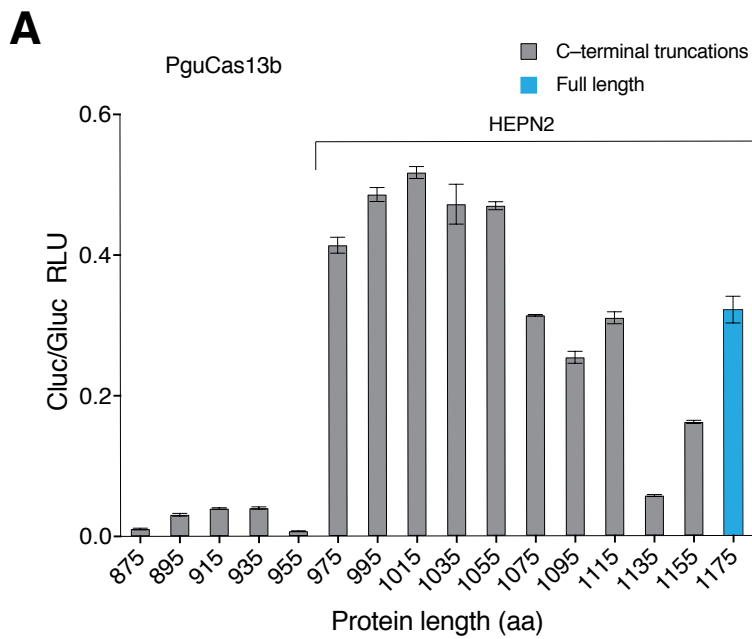


**Supplementary Figure 2, Related to Figure 3, 4 (A - H)** Mutagenesis Cas13b residues impacts RNA interference. (A) Effect of all PbuCas13b site-directed mutations on RNA (luciferase) interference in mammalian cells. Strongest interference knockdowns are colored in blue. (B) PbuCas13b with mutations that substantially affected knockdown labeled and colored in red. (C) Mutations made in the inter-domain linker (IDL). (D) Mutations made to crRNA contacts and the bridge helix. (E) Mutations made around side-channel 2. (F) Mutations made nearby the HEPN active site. (G) Mutations made in the Lid domain. (H) Mutations predicted to mediate RNA targeting. (I - P) Direct repeat RNA structure and sequence. (I) The structure of crRNA within the crystallized PbuCas13b complex. (J) Ideal A-form RNA for comparison to crRNA structure. (K) crRNA multiplete one. (L) crRNA multiplete two. (M) crRNA multiplete three. (N) Diagram of direct repeat base pairing and secondary structure. (O) Alignment of PbuCas13b direct repeat sequences with conserved residues highlighted according to base identity and denoted with an asterisk. (P) Changes in the crRNA impact PbuCas13b nuclease activity and complex stability. crRNA base identity swapping experiments data shown as a heatmap. Upper panel shows fluorescent collateral RNA-cleavage assays; lower panel shows thermal stability. The hashed fill denotes wild-type base identities

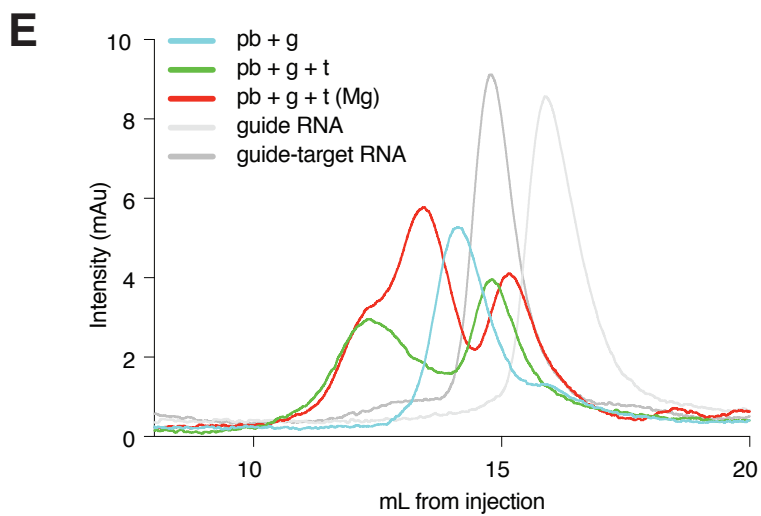
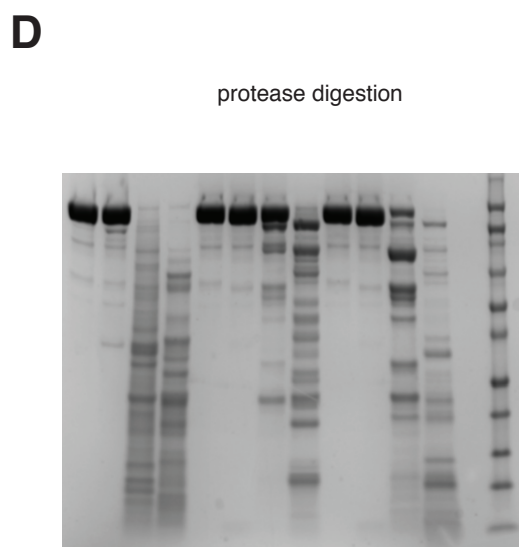
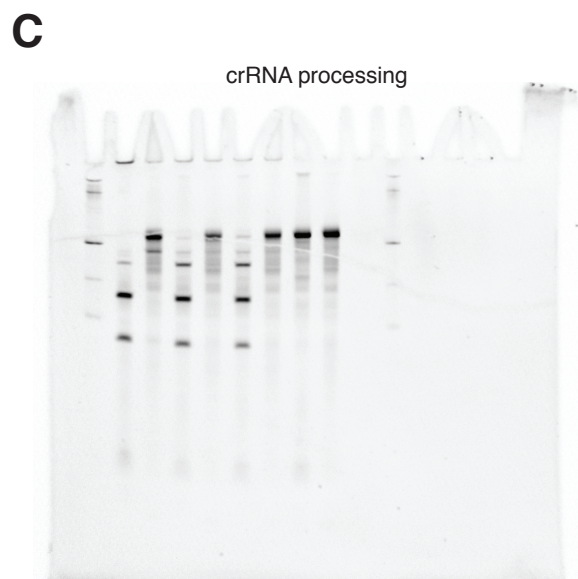
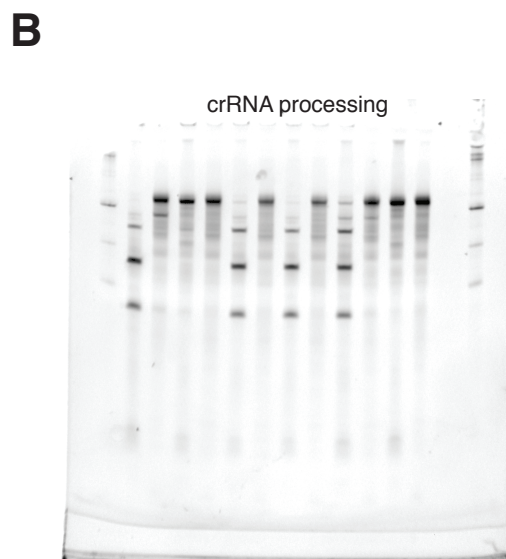
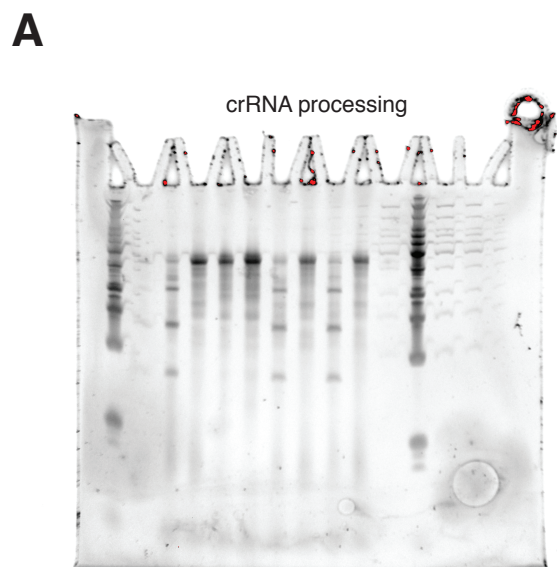


Supplementary Figure 3, Related to Figure 3 - 5. (A - F) Identification of RNA processing center and RNA escorting hairpin. (A) Schematics of crRNA (top left); pre-crRNA array used for the crRNA processing assays (bottom left); and primary and secondary structure of direct repeat (DR) for wildtype (w) and sequence complement control (c). (B) Gel showing processing of wild-type pre-crRNA array and lack of processing of complementary pre-crRNA array. Cartoons of the expected cleavage products are shown to the left of the gel. (C) Gel showing processing of pre-crRNA array with wild-type direct repeat (w) by PbuCas13b variants (see also Figure 2B). Pre-crRNA with complementary direct-repeat (c) is not processed. Cartoons of the expected cleavage products are shown to the left of the gel. (D) SHERLOCK assay measuring collateral activity of crRNA processing mutants. Asterisk denotes nearly undetectable levels of activity. (E) REPAIR activity with PbuCas13b mutants of the Lid domain hairpin (wild-type PbuCas13b is shown in blue). (F) Model showing the path that target RNA (black) is predicted to take through the protein. The Lid domain hairpin bearing D397 is colored yellow and labeled, as is the central channel entrance, which is the predicted site of domain opening. (G - I) Melting curves of PbuCas13b with substrate RNA. (G) The effect of RNA substrate on PbuCas13b thermal stability. (H) The effect of PbuCas13b RNA cleavage on thermal stability. (I) The effect of magnesium on PbuCas13b thermal stability. (J) Limited proteolysis of PbuCas13b. T, Trypsin; C, Chymotrypsin; P, Pepsin; L, ladder. (K) Titration of target RNA (as measured in molar ratio of RNA to protein) into PbuCas13b-crRNA complexes in a thermal denaturation assay.





Supplementary Figure 5, related to Figures 3 - 6. (A) RNA editing with smaller Cas13b variants. REPAIR assay of PguCas13b C-terminal truncations. (B - D) Comparison of PbuCas13b structure to LbCas12a. (B) PbuCas13b with bridge-helix highlighted in red. RNA is colored in pink. (C) LbCas12a (PDBID: 5XUS, previously known as Cpf1) with bridge-helix highlighted in cyan. RNA is colored in light purple, DNA dark purple. (D) Manual sequence alignment of bridge helix from PbuCas13b and LbCas12a.



Supplementary Figure 6, related to Figure 3 and Star Methods. (A - D) Raw uncropped gels and (E) gel filtration of PbuCas13b complexes.

**Supplementary Table 1, related to Star Methods.** Mutations tested for RNA interference. List of mutations and averaged normalized fluorescent values from 3 biological replicates.

<b>Mutation</b>	<b>Guide 1 normalized RLU</b>	<b>Guide 2 normalized RLU</b>
R53A	0.747858	0.618255
R53K	0.533437	0.415443
R53D	0.708809	0.656473
R53E	0.653859	0.505983
Y164A	0.560344	0.423418
Y164F	0.555361	0.419603
Y164W	0.578905	0.411809
K183A	0.611807	0.434156
K193A	0.637075	0.435537
R285A	0.621679	0.473138
K292A	0.709821	0.47966
E296A	0.753062	0.402674
N297A	0.697938	0.407599
T405A	0.668786	0.366621
H407A	0.541401	0.358297
H407Y	0.503637	0.335036
H407W	0.528546	0.359063
H407F	0.495551	0.341844
K457A	0.632984	0.441894
H500A	0.549885	0.34935
K570A	0.575468	0.362485
K590A	0.587262	0.383572
R600A	0.565624	0.417064
K607A	0.687397	0.430726
R614A	0.744806	0.450827
N634A	0.661617	0.386325
R638A	0.696471	0.410163
Q646A	0.675146	0.372062
N647A	0.677884	0.400227
N652A	0.665943	0.406755
N653A	0.650794	0.384887
K655A	0.900461	0.58631
S658A	0.625349	0.363679
K741A	0.648908	0.401644
K744A	0.651516	0.42232
N756A	0.650638	0.447333
S757A	0.618393	0.402225
R762A	0.862666	0.577076
R791A	0.644193	0.444169
K826A	0.600621	0.395086
K828A	0.619022	0.416127
K829A	0.593882	0.404272
K846A	0.576794	0.407463
K857A	0.595231	0.40528

R877A	0.683229	0.461362
K943A	0.573508	0.394379
K943R	0.60418	0.403167
K943D	0.69041	0.386955
K943E	0.702192	0.372508
R1041A	0.662393	0.371243
R1041K	0.629986	0.376016
R1041D	0.813623	0.680736
R1041E	0.842593	0.484389
D397A	0.63295	0.376658
E398A	0.557761	0.365275
D399A	0.590279	0.368724
E400A	0.560351	0.349016
D434A	0.524497	0.364659
R618A	0.611197	0.401871
R830A	0.663284	0.405163
Q831A	0.548391	0.351777
K835A	0.503864	0.373504
K836A	0.503571	0.374439
R838A	0.549749	0.372399
WT	0.563282	0.335038



Supplemental Data S1: Alignments of Cas13b proteins annotated with crystal structure secondary structure and colored by conservation. Pgu, *Porphyromonas gulae*; Fbr, *Flavobacterium branchiophilum*; Cca, *Capnocytophaga canimorsus*; Bzo, *Bergeyella zoohelcum*; Ran, *Riemerella anatipestifer*; Psa, *Prevotella saccharolytica*; Pgi, *Porphyromonas gingivalis*; Pin, *Prevotella intermedia*; Pau, *Prevotella aurantiaca*.

*PbuCas13b* α1

1 10 20 30 40 50

*PbuCas13b* MQKQDKLFPVDRKKNAIFAFPKYITIMENKEKPEPIY **YE**...LTD**KHF**...**WAAFLNLA**RH  
*PguCas13b* .....MTEQSERPYNGTY **YT**...LED**KHF**...**WAAFLNLA**RH  
*FbrCas13b* .....MENLNKILD...KEN**EIC**...**TSKIFN**TKGI  
*Alistipes\_ZOR0009\_Cas13b* .....MSNEIGAFREHQ **FAY**APGNE**QEE**EA**T**F**ATYF**N**LALS**  
*CcaCas13b* .....MKNIQRLGKGN**E**SP**FKED**K**FY**...**FGGFLN**LANN  
*BzoCas13b* .....MENK**T**SLGNN**IY**NP**FKPQD****KSY**...**FAGYF**N**AAME**  
*Prevotella\_P5-125* .....MNIPAL**VE**...**NQK****Y**...**FGTYS**V**MAML**  
*RanCas13b* .....MEK**PL**LN**PV**Y**T**...**LKH****KF**...**WGAFLN**L**ARH**  
*Prevotella\_MA2016\_Cas13b* .....MSKE**CK**Q**RQE**K**RR**L**Q**KA**N****F**S**IS**LT**G**K**HV**...**F**GA**YF**N**MART**  
*PsaCas13b* .....MED**KF**...**WAAFLN**L**ARH**  
*PgiCas13b* .....MTEQ**NE**K**P**YNG**TY** **YT**...LED**KHF**...**WAAFLN**L**ARH**  
*PinCas13b-1* .....MED**D**K**T**KE**ST**N**M**...**LDN****KHF**...**WAAFLN**L**ARH**  
*PauCas13b* .....MED**D**K**T**T**G**SI**S** **YE**...**LKD****KHF**...**WAAFLN**L**ARH**  
*PinCas13b-2* .....MED**D**K**T**T**D**SI**R** **YE**...**LKD****KHF**...**WAAFLN**L**ARH**  
*PinCas13b-3* .....MED**D**K**T**T**D**SI**R** **YE**...**LKD****KHF**...**WAAFLN**L**ARH**

*PbuCas13b* η1 α2

60 70 80

*PbuCas13b* **NV**Y**T****T****I****NH****I****NRR****L**E**IAE**..L**KD****D**G**Y**.....**MMGI**K**GS**  
*PguCas13b* **NA**Y**I****T****L****T****H****I****D**R**L**A**Y**S**K**A**D**I**T**N**D****Q**D.....**VLSF**K**AL**  
*FbrCas13b* **AA**P**I****T**E**K**A**L****D**N**I**K**S**Q**K**N**D**L**N**K**E**A**R**L**H**Y**F**S**I**G**H**S**F**K.....**Q**.....**ID**F**K**.**KV**  
*Alistipes\_ZOR0009\_Cas13b* **NV**E**G**M**M**.....**ET**F**G**E**KI**  
*CcaCas13b* **N**I**E**D**F**F**K**E**I****I**T**R**F**G**I**V**..I**T**D**E**N**K**K**P**K.....**E**T**F**G**E**K**I**  
*BzoCas13b* **N**T**D**S**V**F**R**E**L****G**K**R**L**G**K**E**..**Y**T**S****E**N**F**.....**E**T**F**G**E**K**I**  
*Prevotella\_P5-125* **NA**Q**T**V**L****D**H**I****Q**K**V**A**D**I**E**G..**E**Q**N**E**N**E**N**L**W**.....**F**.....**H**P**V**M**S**H**L**  
*RanCas13b* **NA**F**I****T****I****C**H**I****N**E**Q**L**G**L**T**..**P**S**N****D****K**I.....**V**D**V**V**C**E**T**  
*Prevotella\_MA2016\_Cas13b* **N**F**V**K**T****I****N**Y**I****L**P**I**A**G**V**R**G..**N**Y**S****E**N**Q**I**N**K**M**L**H**A**L**F**L**I**Q**A**G**R**N**E.....**L**T**T**E**Q**K**Q**  
*PsaCas13b* **N**V**Y**L**T****V****N**H**I****N**K**L****D**L**E**K..**L**Y**D**E**G**K**H**K**E**I**F**E**R**E**D**I**F**N**I**S**D**D**V**M**N**D**A**N**S**G**K**R**K**L**D**I**K**K**I**  
*PgiCas13b* **NA**Y**I****T****L**A**H****I****D**R**L**A**Y**S**K**A**D**I**T**N**D**E**D**.....**I**L**F**F**K**G**Q**  
*PinCas13b-1* **N**V**Y**I**T****V****N**H**I****N**K**V**L**E**L**K**N..**K**K**D****I**I**D**N**D**Q**D**.....**I**L**A**I**K****T****H**  
*PauCas13b* **N**V**Y**I**T****I****N**H**I****N**K**L**E**I**R**E**..**I**D**N****D**E**K**.....**V**L**D**I**K**T**L**  
*PinCas13b-2* **N**V**Y**I**T****V****N**H**I****N**K**I**L**E**E**D**E..**I**N**R****D**G**Y**.....**E**N**T**L**E**N**S**  
*PinCas13b-3* **N**V**Y**I**T****V****N**H**I****N**K**I**L**E**E**G**E..**I**N**R****D**G**Y**.....**E**T**L**T**K**N**T**

*PbuCas13b* α3 α4

90 100 110 120

*PbuCas13b* **W**N**E**Q...**A**K**K****L**D**K**V**R**L**R**D**L**I**M****K**H**F**P**F**L**E**A**A**.**Y**.....**E**M**T**N**S**K**S**P**N**N**K**E  
*PguCas13b* **W**K**N**F...**D**N**D****L**E**R**K**S****R**L**R**S**L**I**L**K**H**F**S**F**L**E**G**A**A**.**Y**G**K**K**L**F**E**S**K**S**S**N**K**S**K**N**K**E...**L**T**K**K  
*FbrCas13b* **F**D**Y**V**L**I**E**L**K**D**E**K**P**L**K**F**I****T**L**Q**K**D**E**F**T**K**E**F**S**I**K.....**L**Q**L**I**N**S**I**R**N**N**H**Y  
*Alistipes\_ZOR0009\_Cas13b* **F**G**E**V...**E**S**N**P**D**K.....**I**E**K**S**L**D**T**L**P**P**A**I.....**L**R**Q**I**A**S**F**I**W**L  
*CcaCas13b* **L**N**E**I**F**K**K**D**I**S**I**V**D**Y**E**K**W**V**N**I**F**A**D**Y**F**P**F**T**K**Y**L**S**L**Y**L**E**M**Q**F**K**N**R**V**I**C**F**R**D**V**M**K**E**L**L**T**V**E**A  
*BzoCas13b* **F**D**A**I**F**K**E**N**I**S**L**V**E**Y**E**R**Y**V**K**L**L**S**D**Y**F**P**M**A**R**L**L**D..**K**K**E**V**P**I**K**E**R**K**E**N**F**K**K**N**F**K**G**I**I**A**V**R**D**  
*Prevotella\_P5-125* **Y**N**A**K**N**G**Y**D**K**Q**P**E**K**T**M**F**I**E**R**L**O**S**Y**F**P**F**L**K**I**M**A**.....**E**N**Q**R**E**Y**S**N**G**Y  
*RanCas13b* **W**N**I**L**N**D**H**D**L**L**K**S**Q**L**T**E**L**I**L**K**H**F**P**F**L**T**A**M**C**.**Y**.....**H**P**P**K**K**E**G**K**K**G**H**Q**E**Q**Q**E**K**E  
*Prevotella\_MA2016\_Cas13b* **W**E**K**K**L**..**R**L**N**P**E**Q**T**K**F**Q**L**L**F**K**H**F**P**V**L**G**P**M**M**.**A**.....**D**V**A**D**H**K**A**Y**L**N**K**K**S**T**V**L**Q**E  
*PsaCas13b* **W**D**D**L...**D**T**D**L**T**R**K**Y**Q**L**R**E**L**I**L**K**H**F**P**F**I**Q**P**A**I**.**I**G**A**Q**T**K**E**R**T**I**D**K**D**R**S**T**S**N**D**S**L**K**T**  
*PgiCas13b* **W**K**N**L...**D**N**D****L**E**R**K**A****R**L**R**S**L**I**L**K**H**F**S**F**L**E**G**A**A**.**Y**G**K**K**L**F**E**S**Q**S**S**N**K**S**K**K**E**...**L**S**K**K  
*PinCas13b-1* **W**E**K**V...**N**G**D****L**N**K**T**E**R**L**R**E**L**M**T**K**H**F**P**F**L**E**T**A**I.**Y**.....**T**K**N**K**E**...**D**K**E**E  
*PauCas13b* **W**Q**K**G...**N**K**D**L**N**Q**K**A**R**L**R**E**L**M**T**K**H**F**P**F**L**E**T**A**I**.**Y**.....**T**K**N**K**E**...**D**K**E**E  
*PinCas13b-2* **W**N**E**I...**K**D**I**N**K**K**D****R**L**S**K**L**I**I**K**H**F**P**F**L**E**A**T**T**.**Y**.....**R**Q**N**P**T**...**D**T**T**K  
*PinCas13b-3* **W**N**E**I...**K**D**I**N**K**K**D****R**L**S**K**L**I**I**K**H**F**P**F**L**E**A**T**T**.**Y**.....**R**L**N**P**T**...**D**T**T**K

*PbuCas13b* α5 α6

130 140 150 160 170

*PbuCas13b* **Q**R**E**K**E**Q**S**E**A**L**S**L**N**N**L**K**N**V**L**F**I**F**L**E**K**L.....**Q**V**L**R**N**Y**Y**S**H**...**Y**K**Y**S**E**S**P**K**P**I**F**...**E**T  
*PguCas13b* **E**K**E**L**Q**A**N**A**L**S**L**D**N**L**K**S**L**F**D**F**L**Q**K**L.....**K**D**F**R**N**Y**Y**S**H**...**Y**R**H**S**G**S**S**E**L**P**L**F...**D**G  
*FbrCas13b* **V**H**F**N**D**I**N**L**N**K**I**D**S**N**V**F**H**L**K**E**S**F**L**.....**A**I**E**K**Y**Y**K**V...**N**K**K**P**D**A...**E**K  
*Alistipes\_ZOR0009\_Cas13b* **S**K**E**D**H**P**D**K**A**Y**S**T**E**E**V**K**V**I**V**D**L**V**R**R**L**.....**C**F**Y**R**N**Y**Y**S**H**...**C**F**Y**L**D**T**Q**Y**F**...**L**  
*CcaCas13b* **L**R**N**F**Y**T**H**D**H**E**P**I**K**I**E**D**R**V**F**Y**F**L**D**K**V**L**L**D**V**S**L**T**V**K**N**K**Y**L**K**...**T**D**K**T**K**E**F**L**N**Q**H**I**G**E**L**K  
*BzoCas13b* **L**R**N**F**Y**T**H**K**E**H**G**E**V**E**I**T**D**E**I**F**G**V**D**E**M**L**K**S**T**V**L**T**V**K**K**K**V**K...**T**D**K**T**K**E**L**L**K**S**I**B**K**Q**L**D  
*Prevotella\_P5-125* **K**Q**N**R**V**E**V**N**S**N**D**I**F**E**V**L**K**R**A**F**G**V**L**K**M**Y...**R**D**L**T**N**H**Y**K**T**...**Y**E**E**K**L**N**D**G**C**E**F**L**T**S**T**Q  
*RanCas13b* **S**E**A**Q**S**Q**A**E**A**L**N**P**S**K**L**I**E**A**L**E**I**L**V**N**L**.....**H**S**L**R**N**Y**Y**S**H**...**Y**K**H**...**K**K**P**D**A**...**E**K  
*Prevotella\_MA2016\_Cas13b* **D**E**T**F**A**M**L**K**G**V**S**L**A**D**C**L**D**I**T**C**L**M**A**D**T**L.....**T**E**C**R**N**F**Y**T**H**K**D**P**Y**N**K**P**S**Q**L**A**D**Q**Y**L...**H**Q  
*PsaCas13b* **T**E**G**E**D**I**N**D**L**L**S**L**S**N**V**K**S**M**F**F**R**L**L**Q**I**L.....**E**Q**L**R**N**Y**Y**S**H**...**V**R**H**S**K**S**L**T**M**P**N**F...**D**E  
*PgiCas13b* **E**K**E**L**Q**A**N**A**L**S**L**D**N**L**K**S**L**F**D**F**L**Q**K**L.....**K**D**F**R**N**Y**Y**S**H**...**Y**R**H**P**E**S**S**E**L**P**L**F...**D**G  
*PinCas13b-1* **V**K**Q**E**K**Q**A**E**A**Q**S**F**D**S**L**K**H**K**L**F**L**F**L**E**K**L.....**Q**E**A**R**N**Y**Y**S**H**...**Y**K**Y**S**E**S**T**K**E**P**M**L...**E**K  
*PauCas13b* **V**K**Q**E**K**Q**A**E**A**Q**S**L**E**S**L**K**D**C**L**F**L**F**L**D**K**L.....**Q**E**A**R**N**Y**Y**S**H**...**Y**K**Y**S**E**F**S**K**E**P**F**...**E**E  
*PinCas13b-2* **Q**K**E**E**K**Q**A**E**A**Q**S**L**E**S**L**K**K**S**F**F**V**F**I**Y**K**L.....**R**D**L**R**N**H**Y**S**H**...**Y**K**H**S**K**S**L**E**R**P**K**F...**E**E  
*PinCas13b-3* **Q**K**E**E**K**Q**A**E**A**Q**S**L**E**S**L**R**K**S**F**F**V**F**I**Y**K**L.....**R**D**L**R**N**H**Y**S**H**...**Y**K**H**S**K**S**L**E**R**P**K**F...**E**E

*PbuCas13b* α7 η2

180 190 200 210

*PbuCas13b* **S**L**L**K**N**Y**K**Y**F**D**A**N**V**R**L**V**K**R**D**Y**M**..**H**H**E**.**N**T**D**M**Q**R**D**..**F**T**H**L**N**R**K**.....**K**.....  
*PguCas13b* **N**M**L**Q**R**L**Y**N**V**F**D**V**S**V**Q**R**V**K**I**D**H**E..**H**N**D**.**E**V**D**P**H**Y**H**..**F**N**H**L**V**R**K**.....**G**.....  
*FbrCas13b* **E**I**V**L**F**L**K**E**L**F.....**I**K**D**E**N**T**A**L**L**N**Y**.**F**T**N**L**S**K**D**E**A**I**E**Y**I**L.....  
*Alistipes\_ZOR0009\_Cas13b* .....**Y**S**D**E**L**V**D**T**A**I**G**E**K**L**P**..**Y**N**F**..**H**H**F**I**T**N**R**L**F**R**Y**.....  
*CcaCas13b* **E**L**C**K**Q**R**K**D**Y**L**V**G**K**R**I**D**E**.....**S**E.**I**N**G**I**Y**N**N**A**F**K**D**F**I**C**K**R**E**Q**D**D**K**E**N**H**N**S**V**E**K**I  
*BzoCas13b* **I**L**C**Q**K**K**L**E**Y**L**R**D**T**A**R**K**I**E**E**K**R**R**N**Q**R**E**R**G**E**K**L**V**A**P..**F**K**Y**S**D**K**R**D**L**I**A**A**I**Y**N**.....  
*Prevotella\_P5-125* **P**L**S**G**M**I**N**N**Y****Y**T**V**A**L**R**N**M**N**E**R**Y**G**..**Y**K**T**E**D**L**A**F**I**Q**D**K**R**F**K**F**V**K**D**A...**Y**G.....  
*RanCas13b* **D**I**F**K**H**L**Y**K**A**F**D**A**S**L**R**M**V**K**E**D**Y**K..**A**H**F**.**T**V**N**L**T**R**D**..**F**A**H**L**N**R**K**.....**G**.....  
*Prevotella\_MA2016\_Cas13b* **E**M**I**A**K**K**L**D**K**V**V**V**A**S**R**R**I**L**D**R**E**G**L**S**V**N..**E**V**E**F**L**T**G**..**I**D**H**L**H**Q**E**V**L**K**D**E**F**G**N**A.....  
*PsaCas13b* **D**L**L**N**W**M**R**Y**I**F**I**D**S**V**N**K**V**K**E**D**Y**S..**S**N**S**.**V**I**D**P**N**T**S**..**F**S**H**L**I**Y**K**.....  
*PgiCas13b* **N**M**L**Q**R**L**Y**N**V**F**D**V**S**V**Q**R**V**K**I**D**H**E..**H**N**D**.**K**V**D**P**H**R**H**..**F**N**H**L**V**R**K**.....**G**.....  
*PinCas13b-1* **E**L**L**K**M**Y**N**I**F**D**D**N**I**Q**L**V**I**K**D**Y**Q**..**H**N**K**.**D**I**N**P**D**E**D**..**F**K**H**L**D**R**T**.....**E**.....  
*PauCas13b* **G**L**L**E**K**M**Y**N**I**F**G**N**N**I**Q**L**V**I**N**D**Y**Q..**H**N**K**.**D**I**N**P**D**E**D**..**F**K**H**L**D**R**K**.....**G**.....  
*PinCas13b-2* **D**L**Q**N**K**M**Y**N**I**F**D**V**S**I**Q**F**V**K**E**D**Y**K..**H**N**T**.**D**I**N**P**K**D..**F**K**H**L**D**R**K**.....**R**.....  
*PinCas13b-3* **G**L**L**E**K**M**Y**N**I**F**N**A**S**I**R**L**V**K**E**D**Y**Q..**Y**N**K**.**D**I**N**P**D**E**D**..**F**K**H**L**D**R**T**.....**E**.....

*PbuCas13b* TT 220 230

*PbuCas13b* .....QVGRTKNIIDSPN**F**.HYH**F**A.DKEG.....  
*PguCas13b* .....K.KDRYGHNDNPS**F**.KHH**F**V.DGEG.....  
*FbrCas13b* .....TFTTENKIWNIN**N**EHNL**L**NEIKGK.....  
*Alistipes\_ZOR0009\_Cas13b* .....S**L**PE**I**TL**F**R**W**NEGERK.....  
*Cca* LCNKEPQNKQKSSATVWELCSKSSSK**Y**TEKS**F**PNREND.....KHCLE  
*BzoCas13b* .....DAFDVYIDKKKDSLKESKAK**Y**NTKS**D**PQOEEG.....DLK  
*Prevotella\_P5-125* .....KKKSQVNTGFLL**S**LQDY**N**GDTQKKL.....  
*RanCas13b* .....K.....NKQDN**P**D**F**NR**Y**R**F**.....EKDG.....  
*Prevotella\_MA2016\_Cas13b* .....KVKGDKVMK**T**FVE**Y**DD**F**Y**F**KISGKRLVNGY**T**V**T**T**K**DD**K**PVN**V**NT**M**L  
*PsaCas13b* .....DEQ**G**K**I**K**P**CR**Y**P**F**T.SKD**G**.....  
*PgiCas13b* .....K.KDKYGNND**N**P**F**.KHH**F**V.DREG.....  
*PinCas13b-1* .....E.....E**F**.N**Y**Y**F**T**N**KK**G**.....  
*PauCas13b* .....Q**F**.K**Y**S**F**A.DNE**G**.....  
*PinCa13b-2* .....K.....G**K****F**.H**Y**S**F**A.DNE**G**.....  
*PinCas13b-3* .....E.....E**F**.N**Y**Y**F**T**K**DNE**G**.....

*PbuCas13b* α8 α9 α10 α11

240 250 260 270 280  
*PbuCas13b* .N**M**T**I**A**G**LL**F**F**V**S**I****F**L**D**K**R**D**A**I**W**M**Q**K**K**L**R**G**F**K**D**G.....RNLRE**M**T**N**E  
*PguCas13b* .M**V**T**E**A**G**LL**F**F**V**S**I****F**L**E**K**R**D**A**I**W**M**Q**K**K**I**R**G**F**K**G**G.....TETY**Q**O**M**T**N**E  
*FbrCas13b* .Y**L**T**F**E**A**M**L**F**L**I**T**I**F**L**Y**K**N**E**A**N**H**L**L**P**K**L**Y**D**F**K**N**N.....K**S**K**Q**E  
*Alistipes\_ZOR0009\_Cas13b* Y**E**I**L**R**D****G**L**I**F**F**C**C**L**F**L**K**R**G**Q**A**E**R**L**N**E**L**R**F**K**R**T.....D**E**E**G**I**K**R**T**  
*Cca* V**F**I**S**Q**K****G**I**V**F**L**L**S**F**L**N**K**G**E**I**Y**A**L**T**S**N**I**K**G****F**K**A**K**I**.....T**K**E**E**P**V**T**Y**D**K**N**S**I**R**Y**M**A**T**H**R**  
*BzoCas13b* I**P**I**S**K**N****G**V**V**F**L**L**S****F**L**T**K**Q**E**I**H**A**F**K**S**K**I**A**G**F**K**A**T**V**I**D**E**A**T**V**S**E**A**T**V**S**H**G**K**N**S**I**C**F**M**A**T**H**E  
*Prevotella\_P5-125* .H**L**S**G**V**G**I**A**L**L**I**C**L**F**L**D**K**Q**I**N**I**F**L**S**R**L**P**T****F**S**S**Y.....N**A**Q**S**E**R**R**I**I**R**  
*RanCas13b* .**F**F**T**E**S****G**L**L**F**F**T**N**L**F**L**D**K**R**D**A**Y**W**M**L**K**V**S**F**K**A**S.....H**K**O**R**E**K**M**T**E  
*Prevotella\_MA2016\_Cas13b* P**A**L**S**D**F****G**L**L**Y**F**C**V**L**F**L**S**K**F**Y**A**K**L**F**I**D**E**V**R**L**F**E**Y**S.....P**F**D**D**K**E**N**M**I**S**E  
*PsaCas13b* .**S**I**N**A**F****G**L**L**F**F**V**S**I**F**L**E**K**Q**D**S**I**W**M**Q**K**K**I**P**G**F**K**K**A.....S**E**N**Y**M**K**M**T**E  
*PgiCas13b* .**T**V**T**E**A****G**L**L**F**F**V**S**I**F**L**E**K**R**D**A**I**W**M**Q**K**K**I**R**G**F**K**G**G.....T**E**A**Y**Q**O****M**T**N**E  
*PinCas13b-1* .**N**I**T**A**S****G**L**L**F**F**V**S**I**F**L**E**K**K**D**A**I**W**M**Q**K**K**L**R**G**F**K**D**N.....R**E**S**K**K**M**T**H**E  
*PauCas13b* .**N**I**T**E**S****G**L**L**F**F**V**S**I**F**L**E**K**K**D**A**I**W**M**Q**K**L**N**G**F**K**D**N**.....L**E**N**K**K**M**T**H**E  
*PinCa13b-2* .**N**I**T**E**S****G**L**L**F**F**V**S**I**F**L**E**K**K**D**A**I**W**V**Q**K**L**E**G****F**K**C**S.....N**K**S**Y**Q**K**M**T**H  
*PinCas13b-3* .**N**I**T**E**S****G**L**L**F**F**V**S**I**F**L**E**K**K**D**A**I**W**M**Q**K**L**R**G****F**K**D**N.....R**E**N**K**K**K****M**T**N**E

*PbuCas13b* α12 α13

290 300 310 320  
*PbuCas13b* V**F**C.....R**S**R**I**S**L**P**K**L**K**L**E**.....N**V**Q**T**K**D**W**M**L**D**M**L**N**E**L**V**R**C**K**S**L**Y**E**R**L**R**E  
*PguCas13b* V**F**C.....R**S**R**I**S**L**P**K**L**K**L**E**.....S**L**R**M**D**D**W**M**L**L**D**M**L**N**E**L**V**R**C**K**P**L**Y**D**R**L**R**E**  
*FbrCas13b* L**F**T.....F**F**S**K**K**F**T**S**Q**D**I**D**.....A**E**E**G**H**L**I**K**F**R**D**M**I**Q**Y**L**N**H**Y**E**T**A**W**N**N**D**L**K**L  
*Alistipes\_ZOR0009\_Cas13b* I**F**T.....K**Y**C**T**R**E**S**H****G****H**I**G**.....I**E**E**Q**D**F**L**I**F**Q**D**I**G**D**L**N**V**R**V**K**V**C**D**G**V**V**D**L**  
*Cca* M**F**S**F**L**A**Y**K**G**L**K**R**K**I**R**T**S**E**I.....N**Y**N.....E**D**G**Q**A**S**S**T**Y**E**K**E**T**L**M**L**Q**M**D**E**L**N**V**R**C**K**S**L**Y**R**L**T**P  
*BzoCas13b* I**P**S**H**L**A**Y**K**K**L**K**R**K**V**R**T**A**E**I.....N**Y**G**E**A**E**N**A**E**Q**L**S**V**Y**A**K**E**T****L**M**M**Q**M**L**D**E**L**S**K**V**P**D**V**Y**Q**N**L**S**E**  
*Prevotella\_P5-125* S**F**G.....I**N**S**I**K**L**P**K**D**R**I**H**.....S**E**K**S**N**K**S**V**A**M**D**M**L**N**E**V**K**R**C**P**D**L**F**T**L**S**A  
*RanCas13b* V**F**C.....R**S**R**I**L**L**P**K**L**R**L**E**.....S**R**Y**D**H**N**Q**M**L**L**D**M**L**S**E**L**S**R**C**P**D**L**Y**E**K**L**H  
*Prevotella\_MA2016\_Cas13b* M**L**S.....I**Y**R**I**R**T**P**R**L**K**H**I**D.....S**H**D**S**K**A**T**L**A**M**D**I**F**G**E**L**R**C**P**M**E**L**Y**N**L**D**K  
*PsaCas13b* V**F**C.....R**N**H**I**L**L**P**K**I**R**L**E**.....T**V**Y**D**K**D**W**M**L**L**D**M**L**N**E**V**R**C**P**L**S**L**Y**R**L**T**P  
*PgiCas13b* V**F**C.....R**S**R**I**S**L**P**K**L**K**L**E**.....S**L**R**T**D**D**W**M**L**L**D**M**L**N**E**L**V**R**C**K**S**L**Y**D**R**L**R**E**  
*PinCas13b-1* V**F**C.....R**S**R**M**L**L**P**K**L**R**L**E**.....S**T**Q**T**Q**D**W**I**L**L**D**M**L**N**E**L**I**R**C**P**K**S**L**Y**E**R**L**Q**G  
*PauCas13b* V**F**C.....R**S**R**I**L**M**P**K**L**R**L**E**.....S**T**Q**T**Q**D**W**I**L**L**D**M**L**N**E**L**I**R**C**P**K**S**L**Y**E**R**L**Q**G  
*PinCa13b-2* V**F**C.....R**S**R**M**L**L**P**K**L**R**L**E**.....S**T**Q**T**Q**D**W**I**L**L**D**M**L**N**E**L**I**R**C**P**K**S**L**Y**E**R**L**Q**G  
*PinCas13b-3* V**F**C.....R**S**R**M**L**L**P**K**L**R**L**Q**.....S**T**Q**T**Q**D**W**I**L**L**D**M**L**N**E**L**I**R**C**P**K**S**L**Y**E**R**L**R**E

*PbuCas13b* α14 η3 β1 β2 α15

330 340 350 360 370  
*PbuCas13b* K**D**R**E**S**F**K**V**F**F**D**I**F**S**D**Y**N**A**E.....E**E**F**F**K**N**T**L**V**R**H**Q**D**R**F**P**Y**F**V**L**R**F**D**L**N**E**I**F**E**O**L**R**  
*PguCas13b* D**D**R**A**C**F**R**V**F**V**D**I**L**P**D**E**D**D**T**D**.....G**G**G**E**D**F**F**K**N**T****L**V**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**F**D**L**K**K**V**F**T****S**L**R**  
*FbrCas13b* E**S**E**N**K**N**K**I**M**T**T**K**L**I**D**S**I.....I**E**F**E**L**N**S**N**Y**P**S**F**A.....T**D**I**Q**  
*Alistipes\_ZOR0009\_Cas13b* S**K**E**N**E**R**Y**I**K**N**R**E**T**S**N**E**S**D**E**N**.....K**A**R**Y**R**L**I**R**E**K**D**K**F**P**Y**L**M**R**Y**I**V**D**F**G**V**L**P**C**I**T**  
*Cca* D**V**Q**K**T**F**I**E**D**W**N**E**Y**L**K**N**N**G**D**V**G**T**M**E**E**E**Q**V**I**H**P**V**I**R**K**R**Y**E**D**K**F**N**Y**F**A**I**R**F**L**D**E**F**A**Q**F**T****L**R  
*BzoCas13b* D**V**Q**K**T**F**I**E**D**W**N**E**Y**L**K**N**N**G**D**V**G**T**M**E**E**E**Q**V**I**H**P**V**I**R**K**R**Y**E**D**K**F**N**Y**F**A**I**R**F**L**D**E**F**A**Q**F**T****L**R  
*Prevotella\_P5-125* E**K**Q**S**R**F**R**I**.....I**S**D**H**N**E**V.....M**K**R**S**S**D**R**F**V**P**L**L**Q**Y**D**Y**G**K**L**D**P**H**I**R**  
*RanCas13b* E**N**K**K**H**F**O**V**E**A**D**G**F**L**E**I**E**E**.....Q**N**F**F**K**D**T**L**I**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**L**D**B**K**L**S**F**S**I**R**  
*Prevotella\_MA2016\_Cas13b* N**A**G**Q**P**F**F**H**D**E**V**K**H**P**N**S**H**T**P**D**.....V**S**K**R**L**R**Y**D**R**F**P**T**A**L**R**Y**I**D**E**T**E**L**F**K**R**I**R  
*PsaCas13b* A**A**Q**N**K**F**V**P**E**K**S**S**D**N**A**N**R**Q**E.....D**D**N**F**F**S**R**I****L**V**R**H**Q**N**R**F**P**Y**F**V**L**R**F**F**D**L**N**E**I**T**L**R  
*PgiCas13b* E**D**R**A**R**F**R**V**F**V**D**I**L**S**D**E**D**D**T**D**.....G**T**E**E**D**F**F**K**N**T****L**V**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**F**D**L**K**K**V**F**T****S**L**R**  
*PinCas13b-1* E**Y**R**K**K**F**N**V**F**D**S**A**D**E**D**Y**D**A**E.....Q**E**F**F**K**N**T**L**V**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**F**D**Y**N**E**I**F**T**N**L**R  
*PauCas13b* D**D**R**E**K**F**K**V**F**D**P**A**D**E**D**Y**D**A**E.....Q**E**F**F**K**N**T**L**I**R**H**Q**D**R**F**P**Y**F**V**L**R**Y**F**D**Y**N**E**I**F**K**N**L**R  
*PinCa13b-2* V**N**R**K**K**F**Y**V**S**F**D**P**A**D**E**D**Y**D**A**E**.....Q**E**F**F**K**N**T**L**V**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**F**D**Y**N**E**V**F**A**N**L**R  
*PinCas13b-3* E**D**R**E**K**F**R**V**F**I**E**I**A**D**E**D**Y**D**A**E**.....Q**E**F**F**K**N**T**L**V**R**H**Q**D**R**F**P**Y**F**A**L**R**Y**F**D**Y**N**E**I**F**T**N**L**R

*PbuCas13b* β3 β4 η4 η5 α16

380 390 400 410 420  
*PbuCas13b* F**R**I**D**L**G**T**Y**H**F**S**I**Y**N**K**R**.....G**D**E**D**E**V**R**H**L**T**H**L**Y**G**F**A**R**I**O**D**F**A**P**Q**.....N**O**P**E**E**W**R**K**L..  
*PguCas13b* F**R**I**D**L**G**T**Y**H**F**A**I**Y**K**K**M**.....I**G**E**Q**P**E**D**R**H**L**T**R**N**L**Y**G**F**G**R**I**O**D**F**A**E**E**.....H**R**P**E**E**W**K**R**L..  
*FbrCas13b* F**R**I**D**L**G**T**Y**H**F**A**S**N**K**K**R**.....N**O**T**S**F**S**N**K**S**Y**N**E**I**R**H**N**P**H**I**K**Q.....Y**R**D**E**I**A**S**A**L**T**P  
*Alistipes\_ZOR0009\_Cas13b* F**R**I**D**L**G**T**Y**H**F**S**I**Y**N**K**R**.....E**G**R**G**Q**F**H**Y**Q**D**A**A**V**A**Q**E**E**R**C**Y**N**F**V.....Y**R**D**E**I**A**S**A**L**T**P  
*Cca* F**R**I**D**L**G**N**Y**L**C**D**K**R**T**K**Q**.....I**C**D**T**T**E**R**E**V**K**K**I**T**V**F**G**R**L**S**E**L**E**N**K**A**I**F**L**N**E**R**E**.....  
*BzoCas13b* F**R**I**D**L**G**N**Y**L**H**D**S**R**F**K**Q**.....N**L**I**S**D**R**R**I**K**E**K**I**T**V**F**G**R**L**S**E**L**E**H**K**.....K**A**L**F**..  
*Prevotella\_P5-125* F**R**I**D**L**G**T**Y**H**F**L**L**K**A**D**K**T**C**I**D**.....G**Q**T**R**V**R**V**I**E**Q**P**L**N**G**F**G**R**L**E**E**.**A**E**T**.....M**R**K**Q**E**N**G**T**F**G**N  
*RanCas13b* F**R**I**D**L**G**T**Y**H**F**C**I**Y**D**K**K**.....I**D**G**E**Q**E**K**R**H**L**T**R**L**L**S**F**G**R**L**Q**D**F**T**E**I.....N**R**P**Q**E**W**K**A**L..  
*Prevotella\_MA2016\_Cas13b* F**R**I**D**L**G**S**F**R**Y**K**F**Y**D**K**E**N**C**I**D**.....G**D**G**R**V**R**V**R**R**I**O**K**E**I**N**G**Y**G**R**M**Q**E**V**A**D**K**.....R**M**D**K**W**G**D**L**..  
*PsaCas13b* F**R**I**D**L**G**C**Y**H**F**A**I**C**K**Q**Q**.....I**G**D**K**K**E**V**H**H**L**I**R**T**L**Y**G**F**S**R**L**O**N**F**T**Q**N**.....T**R**P**E**W**N**T**L**..  
*PgiCas13b* F**R**I**D**L**G**T**Y**H**F**A**I**Y**K**K**N**.....I**G**E**Q**P**E**D**R**H**L**T**R**N**L**Y**G**F**G**R**I**O**D**F**A**E**E**.....H**R**P**E**E**W**K**R**L..  
*PinCas13b-1* F**R**I**D**L**G**T**Y**H**F**S**I**Y**K**K**L**.....I**G**Q**K**E**D**R**H**L**T**H**K**L**Y**G**F**E**R**I**O**E**F**A**K**Q.....N**R**F**T**D**E**W**K**A**I**..  
*PauCas13b* F**R**I**D**L**G**T**Y**H**F**S**I**Y**K**K**L**.....I**G**Q**K**E**D**R**H**L**T**H**K**L**Y**G**F**E**R**I**O**E**F**A**K**Q.....N**R**F**T**D**E**W**K**A**I**..  
*PinCa13b-2* F**R**I**D**L**G**T**Y**H**F**S**I**Y**K**K**L**.....I**G**Q**K**E**D**R**H**L**T**H**K**L**Y**G**F**E**R**I**O**E**F**D**K**Q.....N**R**F**T**D**E**W**K**A**I**..  
*PinCas13b-3* F**R**I**D**L**G**T**Y**H**F**S**I**Y**K**Q**Q**.....I**G**Q**Y**K**E**S**H**H**L**T**H**K**L**Y**G**E**R**I**O**E**F**T**K**Q.....N**R**F**T**D**E**W**R**K**F**..

*PbuCas13b*

430 440 β5 β6 \*450

```

PbuCas13b      ....VK.....DLDF...ETSQE.....FYISKTAAPHYHLE.....
PguCas13b      ....VR.....DLDYF...ETGDK.....PYISQTSAPHYHLE.....
FbrCas13b      ISFNVKED.....KFKIFVKKHVLE.....EYFPNSIGYEFKLE.....
Alistipes_ZOR0009_Cas13b
Cca            ....VR.....NGNVYY.....SYMPQAQNVVRI.....
BzoCas13b      ....IK.....GWEVF.....PNPSYDFPKENISVNYKDFP
Prevotella_P5-125
RanCas13b      ....IR.....NTET...NEDREHYWEIFPNPNYDFPKENISVNDKDFP
Prevotella_MA2016_Cas13b
PsaCas13b      ....TK.....DLDYK...ETSNO.....FFISKITTPHYHIT.....
PgiCas13b      ....IQKREERSVKLEHEELYINLDFLEDTADST...PVTDRRPAYNH.....
PinCas13b-1    ....VK.....DLDYF...ETGDK.....FYITQITAPHYHLE.....
PauCas13b      ....VR.....DFDTY...ETSEE.....PYISETTAPHYHLE.....
PinCas13b-2    ....VK.....DLDYF...ETSNK.....RYISETTAPHYHLE.....
PinCas13b-3    ....VK.....DSDTFKKKEEKEEEK...PYISETTAPHYHLE.....
                ....VK.....TFNSF...ETSKE.....FYIPEITAPHYHLE.....

```

*PbuCas13b*

TT β7 460 470 β8 TT β9 \*480

```

PbuCas13b      ....NEKIGIRFCSA...HNNLF...FSLQTDKTCNGRSKFNLG..
PguCas13b      ....KGGKIGLRFMPE...GQHLW...FSPVEVGTTRGRSKYAQD..
FbrCas13b      ....YDNDFTEKEKEDFLGLKLYSNPK...TNKLI...ERIDNHKLVKSHGRNQDR..
Alistipes_ZOR0009_Cas13b
Cca            ....ELQGTISVEEL...RNMVY...ASINGKDVNKSVQYLYH..
BzoCas13b      ....IVGSILDREKQPVSNKIGIRVKIAD.ELQREID...KAIKCKKLRNPKNRKANQDE
Prevotella_P5-125
RanCas13b      ....IAGSILDREKQPVAGKIGIRVKLLNQYVSEVD...KAVKAHQKQKAKSKPSI..
PsaCas13b      ....NNKVMFINDKED.SAPLL...RYIVEDD...RYVVVK..
PgiCas13b      ....DNKIGFRLGT...SKEYLY...PSLEIKDGANRIAKYPYN..
PinCas13b-1    ....ANRIGLYWEDSQNPQYKVFDENGMYTPELVVTEDEKKAFIGMP..
PauCas13b      ....NEKIGIKIFDGDTAVDTDIWSVSTEKQLNKPDKYTLT..
PinCas13b-2    ....KGGKIGLRFVPE...GQHLW...FSPVEGATRGRSKYAQD..
PinCas13b-3    ....NQKIGIRFRND...NDEIW...PSLKTNGENNEKRKYKLD..
                ....NQKIGIRFRNG...NKEIW...ESLKTNDENNEKSKYKLD..
                ....NKKIGIAFK...NHNH...ESTQTELTNNKRRKYVNLG..
                ....NQKIGIRFRND...NDKIW...ESLKTNSEKNKSKYKLD..

```

*PbuCas13b*

η6 β10 η7 α17

490 500 510

```

PbuCas13b      ....TQFTAFAFLSVHELLEPMMFYLLTKDYSR.....
PguCas13b      ....KRLTAEAFLSVHELLEPMMFYLLLREKYSE.....
FbrCas13b      ....FMDFSMRFLEANNYFGKDAFFKCYKFYDTQEQDEFLO
Alistipes_ZOR0009_Cas13b
Cca            ....LHLLYEKILTL...LHLLYEKILTL...LHLLYEKILTL...
BzoCas13b      ....QNIIEEIVPINESNPKEAIVFGGQPTAYLSMNDIHSVLYEFLINKISG...
Prevotella_P5-125
RanCas13b      ....T.IPSCRMSTLEIPAMAFHMLF...
PsaCas13b      ....SGFVAHAFISVHELLEPMMFYLLT...
PgiCas13b      ....APRCALSVDLPAMLFYELREQQ...
PinCas13b-1    ....PGFKADVFLSVHELLEPMMFYLLLCEGMLK...
PauCas13b      ....KRLTAEAFLSVHELLEPMMFYLLLREKYSE...
PinCas13b-2    ....KQYQAEAFLSVHELLEPMMFYLLLKKKEFPNND...
PinCas13b-3    ....KQYQAEAFLSVHELLEPMMFYLLLKKKEFPNND...
                ....TSIKAEAFLSVHELLEPMMFYLLLKTENTKNDNKVGG
                ....KSFQAEAFLSVHELLEPMMFYLLLKTENTDNDNEIET

```

*PbuCas13b*

α18 α19

520 530 540 550 560

```

PbuCas13b      ....KESADKVEGTRKEITSNIT...YAIYDAFANNEINSI...ADLTRRLQN
PguCas13b      ....EVSAAERVOGRIRKRVIEDV...YAVYDAFARDEINTR...DELDACLAD
FbrCas13b      ....SNENNDDVKFHKGVTTYIKYEEHLKN...YSYWDGPFVEENNSMS...VKISIGSEE
Alistipes_ZOR0009_Cas13b
Cca            ....ISGQTIKEGRV...DVEDY...RPLLDKLLLRASNG...ELRRLR
BzoCas13b      ....EALETKIVEKIE.TQIKQIIGKDATTKILKPYTNANSINREKLLRDLEQE
Prevotella_P5-125
RanCas13b      ....KELRKEIGKELEKKIVGKIQ.AQIQIIDKDTNAKILKPYQDGNSTAIKPKLIKDLKQE
PsaCas13b      ....GSKKTEKLI.VDVHNNRY...KRLFQAMQKEEVTA...ENIASF...
PgiCas13b      ....GKSEDLK.EVVRHI...QRIYKDFEERINTI...DLEKANQG
PinCas13b-1    ....DNEFPFAEQVYI.EYEDDY...RKFVKAVAEGLKPKFKRPF.EFRDFLKK
PauCas13b      ....TDAGNAVEKVLIDTRNAI...FNLYDAFVQEKINTI...DLENYLQD
PinCas13b-2    ....EVSAAEKVOGRIRKRVIEDV...YAVYDAFARDEINTR...DELDACLAD
PinCas13b-3    ....KKNASIVVEGFKREIRDI...YKLYDAFANGEINNI...DLEKYCED
                ....EINASIVVEGFKREIRDI...FKLYDAFANGEINNI...DLEKYCAD
                ....KKETK...KQGHKHEAIIIE.SKIKDI...YALYDAFANGEINSE...DELKEYLKG
                ....KKKENKNDKQEKHKLEEIIE.NKITEI...YALYDTEFANGEIKSI...DELEEYCKG

```

*PbuCas13b*

η8 α20 α21

570 580 590 600 \*

```

PbuCas13b      T.NTILQGHLPKQMISTIT...KGRQKDMGKEAER...KIGEMIDDTQRRDLDL*
PguCas13b      K.GIRRGHLPRQMIAIL...SQEHKDMEEKIRK...KLQEMMADTDRHLDMLDR
FbrCas13b      KILKIQRNLMIIYFLENALYN...ENVENQGYKLVNNS...YRELLKADVEEIASLDL
Alistipes_ZOR0009_Cas13b
Cca            Q.QILKTLLEEQ...QREK...RLKAILLRHEQLL...
BzoCas13b      Q.NILQKLEKQTVR...EKEYNDFIAYQDK...NREINKVRDRNHKQYLKD
Prevotella_P5-125
RanCas13b      R.GIAESDLPQRILDLTSG...NAHGKDVDAFIRL...TVDDMLDTERRIKRFD
PsaCas13b      R.LPLGAFPKQMLGILL...QNKQPDLSKAKI...KIEKLIATFKLLSHRLNT
PgiCas13b      EYPKLRMADIPKKLQLFCSHGCLYNNKPEPVYERLDRLTLQHLBEERELHQNRRLEHYQK
PinCas13b-1    K.PILIGHLPKQMIDLL...KGHORMLKAVEQ...KKAMLIKDTERRLKLKLDK
PauCas13b      K.GIRRGHLPRQMIAIL...SQEHKDMEEKVRK...KLQEMMADTDRHLDMLDR
PinCas13b-2    K.GIPKRHLPKQMVAIL...YDEHKDMAEAKR...KQKEMVVKDTKKLLATLEK
PinCas13b-3    K.GIPKRHLPKQMVAIL...YDEHKDMVKEAKR...KQKEMVVKDTKKLLATLEK
                K.DIKIVHLPKQMIAIL...KNEHKDMAEAKR...KQKEMVVKDTKKLLATLEK
                K.DIEIGHLPKQMIAIL...KDEHKVMATEAER...KQEEMLVDVQKSLDNLN

```



*PbuCas13b* η15     β12  
 2...22     2...2  
 830     830

*PbuCas13b* ..... IGNRLKP...KKRQFLDK...K  
*PguCas13b* ..... VGNLSLKP...KKGRFLSK...E  
*FbrCas13b* ..... WIETNKP...TNNENRYTL...E  
*Alistipes\_ZOR0009\_Cas13b* ..... LGK...REMSFAAFIRQDK  
*Cca* .....  
*BzoCas13b* .....  
*Prevotella\_P5-125* ..... MDMLKGEYDRKGSLOHCFTSVE  
*RanCas13b* ..... L...E...AKAPL...LKR...E  
*Prevotella\_MA2016\_Cas13b* KKDTFFPFEMIPFLTSDEIQERLSAKLLDGDGNPVPEVGEKGPATDSQGNITWKRRRIYS  
*PsaCas13b* ..... VGNVSKP...TEANYLSK...G  
*PgiCas13b* ..... VGNLSLKP...KKGRFLSK...E  
*PinCas13b-1* ..... VGNHKKP...DEKDFLPS...E  
*PauCas13b* ..... VGNHKKP...KEKDFLHR...E  
*PinCas13b-2* ..... VGDVSKP...KEEDFLHR...E  
*PinCas13b-3* ..... VGNINIKP...DEKNFLNC...E

*PbuCas13b* α32  
 2...22...22...22  
 840     850

*PbuCas13b* ERV<sup>E</sup>LWQKNKEL...<sup>F</sup>KNY...  
*PguCas13b* ERA<sup>E</sup>EWERGKER...<sup>F</sup>RDLEAWSYSAARRIEDAFAGIYASPGNKKKIEQLLRDL<sup>S</sup>LW  
*FbrCas13b* NYKN<sup>L</sup>LILQKQVFINVYH<sup>F</sup>SKY...  
*Alistipes\_ZOR0009\_Cas13b* KAQ<sup>K</sup>LWLRFNYT...<sup>V</sup>ENI...  
*Cca* KVE<sup>E</sup>...<sup>K</sup>EK...<sup>F</sup>KRN...  
*BzoCas13b* . . . V<sup>E</sup>LEKKQADR...  
*Prevotella\_P5-125* EREG<sup>L</sup>LWKERASR...<sup>T</sup>ERY...  
*RanCas13b* EHY<sup>E</sup>YWQONKP...  
*Prevotella\_MA2016\_Cas13b* EVD<sup>D</sup>YAEKLTDRDMKIS<sup>F</sup>KGE...KNDVYTLMM...K...W  
*PsaCas13b* KRE<sup>E</sup>LFKSYQNK...<sup>F</sup>KNN...  
*PgiCas13b* KRA<sup>E</sup>EWESGKER...<sup>F</sup>FR...  
*PinCas13b-1* ERK<sup>K</sup>LWGDKKYK...<sup>F</sup>KGY...  
*PauCas13b* ERI<sup>E</sup>LWDK<sup>K</sup>KKDK...<sup>F</sup>KGY...  
*PinCas13b-2* ERI<sup>E</sup>LWDK<sup>K</sup>KKDK...<sup>F</sup>KGY...  
*PinCas13b-3* ERR<sup>E</sup>LLR<sup>K</sup>KKKDE...<sup>F</sup>...

*PbuCas13b* α33     α34  
 2...22...22...22...22...22...22...22  
 860     870     880

*PbuCas13b* .....PSEK...KKTDLAYLD...FLSWKKF<sup>E</sup>RE<sup>LR</sup>...L<sup>I</sup>KNQD<sup>I</sup>V  
*PguCas13b* EAFESKLVKVRADRI<sup>N</sup>LAKLKEILEAQEH<sup>P</sup>YHD...FKSWQKF<sup>E</sup>RE<sup>LR</sup>...L<sup>V</sup>KNQD<sup>I</sup>I  
*FbrCas13b* .....LIDKNL<sup>L</sup>NSEN...NV<sup>I</sup>O...YKSLENV<sup>E</sup>YL<sup>I</sup>SD<sup>F</sup>YFQSK<sup>L</sup>S  
*Alistipes\_ZOR0009\_Cas13b* .....KTLQKRAA<sup>A</sup>ADA...CKKLYNL<sup>V</sup>G...<sup>E</sup>VE...R<sup>V</sup>HTND<sup>K</sup>V  
*Cca* .....KQ<sup>I</sup>NKQK...KNDVYTL<sup>M</sup>M...<sup>V</sup>EY<sup>I</sup>I<sup>O</sup>...K<sup>I</sup>FGDK<sup>F</sup>V  
*BzoCas13b* .....KRKT<sup>K</sup>IYQ<sup>Q</sup>K...KNDVYTL<sup>M</sup>M...<sup>A</sup>KH<sup>I</sup>IF<sup>K</sup>...S<sup>V</sup>FKQD<sup>S</sup>I  
*Prevotella\_P5-125* RKQASNKIRSNRQ<sup>M</sup>RNAS...SEEI<sup>E</sup>TI<sup>L</sup>DKRLSNSRNEYQ<sup>K</sup>SK<sup>V</sup>IR...R<sup>Y</sup>RVQD<sup>A</sup>L  
*RanCas13b* .....QPTESQ<sup>R</sup>LEL<sup>H</sup>T...SDRWK<sup>D</sup>Y<sup>L</sup>L...YKR<sup>W</sup>QHL<sup>E</sup>KK<sup>L</sup>R...L<sup>R</sup>NRQD<sup>V</sup>M  
*Prevotella\_MA2016\_Cas13b* EKLP<sup>R</sup>WQDK<sup>I</sup>IK<sup>R</sup>RD<sup>E</sup>T...RRQ<sup>M</sup>RDE<sup>L</sup>LQ<sup>R</sup>MP<sup>R</sup>Y<sup>I</sup>RD<sup>I</sup>KD<sup>N</sup>ERT<sup>L</sup>R...R<sup>Y</sup>KTQD<sup>M</sup>V  
*PsaCas13b* .....T<sup>P</sup>AEK...TKEYREY<sup>K</sup>N...FSLWKK<sup>F</sup>RE<sup>LR</sup>...L<sup>I</sup>KNQD<sup>I</sup>V  
*PgiCas13b* .....LAKL<sup>K</sup>KEI<sup>L</sup>EAK<sup>E</sup>...HPY<sup>H</sup>D...FKSWQKF<sup>E</sup>RE<sup>LR</sup>...L<sup>V</sup>KNQD<sup>I</sup>I  
*PinCas13b-1* .....KAKV<sup>K</sup>SK<sup>L</sup>TD<sup>K</sup>E...KEEY<sup>R</sup>SY<sup>L</sup>E...FQSWNK<sup>F</sup>RE<sup>LR</sup>...L<sup>V</sup>NRQD<sup>I</sup>V  
*PauCas13b* .....KEK<sup>I</sup>KSK<sup>L</sup>TE<sup>K</sup>D...KEE<sup>F</sup>RSY<sup>L</sup>E...FQSWNK<sup>F</sup>RE<sup>LR</sup>...L<sup>V</sup>NRQD<sup>I</sup>V  
*PinCas13b-2* .....KAKK<sup>K</sup>FK<sup>E</sup>MT<sup>D</sup>KE...KEE<sup>H</sup>RSY<sup>L</sup>E...FQSWNK<sup>F</sup>RE<sup>LR</sup>...L<sup>V</sup>NRQD<sup>I</sup>V  
*PinCas13b-3* .....KK<sup>M</sup>TD<sup>K</sup>E...KEE<sup>N</sup>PSY<sup>L</sup>E...FKSWNK<sup>F</sup>RE<sup>LR</sup>...L<sup>V</sup>NRQD<sup>I</sup>V

*PbuCas13b* η16     α35  
 2...22...22...22...22...22...22...22  
 890     900     910     920

*PbuCas13b* ..TW<sup>L</sup>MF<sup>K</sup>E<sup>L</sup>FN<sup>M</sup>AT<sup>V</sup>EG<sup>L</sup>KI...GE<sup>T</sup>H<sup>L</sup>RD<sup>I</sup>DT<sup>N</sup>TAN...EE  
*PguCas13b* ..TW<sup>M</sup>MR<sup>D</sup>LM<sup>E</sup>ENK<sup>V</sup>EGL<sup>D</sup>T...GT<sup>L</sup>Y<sup>L</sup>KD<sup>I</sup>IR<sup>P</sup>NVQ<sup>E</sup>...QG  
*FbrCas13b* IDQ<sup>Y</sup>KT<sup>C</sup>CG<sup>L</sup>FN<sup>K</sup>LK<sup>S</sup>NK<sup>L</sup>...ED<sup>C</sup>L<sup>Y</sup>E<sup>I</sup>AY<sup>N</sup>IDK...KN  
*Alistipes\_ZOR0009\_Cas13b* ..LV<sup>L</sup>V<sup>Q</sup>RY<sup>R</sup>ER<sup>L</sup>LV<sup>G</sup>SK...CA<sup>V</sup>T<sup>L</sup>DN<sup>P</sup>ERQ<sup>Q</sup>KL...AD  
*Cca* EEN<sup>F</sup>L<sup>V</sup>LK<sup>G</sup>I<sup>F</sup>Q<sup>S</sup>KA...ER<sup>Q</sup>Q<sup>N</sup>NT<sup>H</sup>AAT<sup>Q</sup>E...RN  
*BzoCas13b* ..D<sup>Q</sup>FS<sup>L</sup>ED<sup>L</sup>Y<sup>Q</sup>SRE...ER<sup>L</sup>GN<sup>Q</sup>ER<sup>A</sup>RQ<sup>T</sup>GE...RN  
*Prevotella\_P5-125* ..L<sup>F</sup>LL<sup>A</sup>KK<sup>T</sup>L<sup>T</sup>EL<sup>A</sup>DFD<sup>G</sup>...ER<sup>F</sup>K<sup>L</sup>E<sup>K</sup>IMP<sup>D</sup>AEK...DA  
*RanCas13b* ..L<sup>W</sup>ML<sup>T</sup>EL<sup>L</sup>TK<sup>N</sup>H<sup>F</sup>KL<sup>N</sup>LN<sup>Y</sup>...H<sup>Q</sup>L<sup>K</sup>EN<sup>L</sup>AV<sup>N</sup>QEA...DA  
*Prevotella\_MA2016\_Cas13b* ..L<sup>F</sup>LL<sup>A</sup>E<sup>K</sup>M<sup>F</sup>T<sup>N</sup>I<sup>I</sup>SE<sup>Q</sup>SSE<sup>F</sup>NW...K<sup>Q</sup>M<sup>R</sup>L<sup>S</sup>K<sup>V</sup>CNEA...AG  
*PsaCas13b* ..I<sup>W</sup>LM<sup>C</sup>KN<sup>L</sup>F<sup>D</sup>E<sup>K</sup>I<sup>K</sup>PK<sup>K</sup>D<sup>I</sup>LE<sup>P</sup>RIA<sup>V</sup>SY<sup>I</sup>K<sup>L</sup>DS<sup>L</sup>Q<sup>T</sup>NT<sup>S</sup>T...AG  
*PgiCas13b* ..TW<sup>M</sup>MR<sup>D</sup>LM<sup>E</sup>ENK<sup>V</sup>EGL<sup>D</sup>T...GT<sup>L</sup>Y<sup>L</sup>KD<sup>I</sup>IR<sup>T</sup>DVQ<sup>E</sup>...QG  
*PinCas13b-1* ..TW<sup>L</sup>L<sup>C</sup>TEL<sup>L</sup>ID<sup>K</sup>LK<sup>V</sup>EGL<sup>N</sup>VEE...L<sup>K</sup>KL<sup>R</sup>L<sup>K</sup>D<sup>I</sup>DT<sup>D</sup>TAK...QE  
*PauCas13b* ..TW<sup>L</sup>L<sup>C</sup>TEL<sup>L</sup>ID<sup>K</sup>LK<sup>V</sup>EGL<sup>N</sup>VEE...L<sup>K</sup>KL<sup>R</sup>L<sup>N</sup>I<sup>D</sup>DT<sup>D</sup>TAK...KE  
*PinCas13b-2* ..TW<sup>L</sup>L<sup>C</sup>TEL<sup>L</sup>ID<sup>K</sup>LK<sup>V</sup>EGL<sup>N</sup>VEE...L<sup>K</sup>KL<sup>R</sup>L<sup>K</sup>D<sup>I</sup>DT<sup>D</sup>TAK...KE  
*PinCas13b-3* ..TW<sup>L</sup>L<sup>C</sup>ME<sup>L</sup>FN<sup>K</sup>KK<sup>I</sup>KL<sup>N</sup>VE...E<sup>K</sup>I<sup>Y</sup>L<sup>K</sup>N<sup>I</sup>NT<sup>N</sup>T<sup>T</sup>KK<sup>E</sup>KN<sup>T</sup>EE<sup>R</sup>KN<sup>G</sup>E<sup>E</sup>KN<sup>I</sup>KE

*PbuCas13b* η17     β13     TT     β14     η18     α36  
 2...22     930     940     950     960     970

*PbuCas13b* SNN<sup>I</sup><sup>L</sup>N<sup>R</sup>I<sup>M</sup>P<sup>M</sup>K<sup>T</sup>P<sup>V</sup>KTYE...TDN<sup>K</sup>GN<sup>L</sup>K<sup>E</sup>R<sup>R</sup>.PLAT<sup>F</sup>Y<sup>I</sup>EE<sup>T</sup>ET<sup>K</sup>V<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KAL<sup>V</sup>  
*PguCas13b* SLN<sup>V</sup><sup>L</sup>N<sup>R</sup>V<sup>K</sup>P<sup>M</sup>R<sup>L</sup>P<sup>V</sup>VVYR...ADSR<sup>G</sup>H<sup>V</sup>H<sup>K</sup>EAP<sup>L</sup>AT<sup>V</sup>Y<sup>I</sup>EE<sup>R</sup>DT<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KSF<sup>V</sup>  
*FbrCas13b* VHK<sup>I</sup>D<sup>I</sup>Q<sup>I</sup>K<sup>L</sup>IT<sup>S</sup>K<sup>I</sup>L<sup>T</sup>IND...ANT<sup>P</sup>Y<sup>K</sup>I<sup>S</sup>V<sup>F</sup>PN<sup>K</sup>ER<sup>S</sup>Y<sup>F</sup>MT<sup>A</sup>IK  
*Alistipes\_ZOR0009\_Cas13b* VYE<sup>V</sup><sup>Q</sup>NA<sup>W</sup>LS<sup>I</sup>RF...DD<sup>L</sup>D<sup>F</sup>IL<sup>T</sup>H<sup>V</sup>N<sup>L</sup>SN<sup>L</sup>R<sup>K</sup>AY<sup>N</sup>L<sup>I</sup>  
*Cca* LNG<sup>T</sup><sup>L</sup>NQ...P<sup>K</sup>D<sup>I</sup>K<sup>I</sup>Q<sup>G</sup>K...IT<sup>V</sup>K<sup>V</sup>G<sup>L</sup>R<sup>D</sup>I<sup>G</sup>N<sup>F</sup>RY<sup>K</sup>  
*BzoCas13b* TNY<sup>I</sup><sup>W</sup>NK...T<sup>V</sup>DL<sup>K</sup>L<sup>C</sup>D<sup>G</sup>K...IT<sup>V</sup>EN<sup>V</sup>K<sup>L</sup>R<sup>N</sup>V<sup>F</sup>RY<sup>K</sup>  
*Prevotella\_P5-125* ..G<sup>T</sup><sup>L</sup>SE<sup>I</sup>MP<sup>M</sup>S<sup>F</sup>TF<sup>E</sup>KGG...K<sup>K</sup>YT<sup>I</sup>SE<sup>G</sup>M<sup>K</sup>L<sup>N</sup>Y<sup>G</sup>DF<sup>V</sup>LA  
*RanCas13b* KLN<sup>P</sup><sup>L</sup>NQ<sup>T</sup>L<sup>P</sup>M<sup>V</sup>L<sup>P</sup>V<sup>K</sup>Y<sup>P</sup>...ATA<sup>F</sup>GE<sup>V</sup>Q<sup>Y</sup>H<sup>K</sup>T<sup>P</sup>IR<sup>T</sup>V<sup>Y</sup>IRE<sup>E</sup>HT<sup>K</sup>AL<sup>R</sup>K<sup>N</sup>G<sup>F</sup>KAL<sup>V</sup>  
*Prevotella\_MA2016\_Cas13b* ..F<sup>L</sup>R<sup>Q</sup>T<sup>L</sup>TF<sup>R</sup>V<sup>P</sup>V<sup>T</sup>VE...T<sup>T</sup>I<sup>Y</sup>V<sup>Q</sup>E<sup>N</sup>MS<sup>L</sup>R<sup>N</sup>Y<sup>G</sup>DF<sup>V</sup>LA  
*PsaCas13b* SLN<sup>A</sup><sup>L</sup>AK<sup>V</sup>V<sup>P</sup>MT<sup>L</sup>AI<sup>H</sup>IDS<sup>P</sup>K<sup>P</sup>.K<sup>G</sup>KAG<sup>N</sup>NE<sup>K</sup>EN<sup>K</sup>.KE<sup>F</sup>T<sup>V</sup>Y<sup>I</sup>KE<sup>E</sup>GT<sup>K</sup>L<sup>L</sup>K<sup>W</sup>G<sup>N</sup>F<sup>K</sup>T<sup>L</sup>  
*PgiCas13b* SLN<sup>V</sup><sup>L</sup>N<sup>R</sup>V<sup>K</sup>P<sup>M</sup>R<sup>L</sup>P<sup>V</sup>VVYR...ADSR<sup>G</sup>H<sup>V</sup>H<sup>K</sup>EAP<sup>L</sup>AT<sup>V</sup>Y<sup>I</sup>EE<sup>R</sup>DT<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KSF<sup>V</sup>  
*PinCas13b-1* KNN<sup>I</sup><sup>L</sup>N<sup>R</sup>V<sup>M</sup>PM<sup>D</sup>EL<sup>P</sup>V<sup>T</sup>VE...ID<sup>D</sup>SH<sup>N</sup>IV<sup>K</sup>D<sup>R</sup>.PL<sup>H</sup>T<sup>V</sup>Y<sup>I</sup>EE<sup>T</sup>ET<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KAL<sup>V</sup>  
*PauCas13b* KNN<sup>I</sup><sup>L</sup>N<sup>R</sup>V<sup>M</sup>PM<sup>D</sup>EL<sup>P</sup>V<sup>T</sup>VE...ID<sup>D</sup>SH<sup>K</sup>IV<sup>K</sup>D<sup>K</sup>.PL<sup>H</sup>T<sup>V</sup>Y<sup>I</sup>KE<sup>A</sup>ET<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KAL<sup>V</sup>  
*PinCas13b-2* KNN<sup>I</sup><sup>L</sup>N<sup>R</sup>V<sup>M</sup>PM<sup>D</sup>EL<sup>P</sup>V<sup>T</sup>VE...V<sup>N</sup>K<sup>G</sup>Y<sup>I</sup>IK<sup>N</sup>K<sup>K</sup>.PL<sup>H</sup>T<sup>V</sup>Y<sup>I</sup>KE<sup>A</sup>ET<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KAL<sup>V</sup>  
*PinCas13b-3* KNN<sup>I</sup><sup>L</sup>N<sup>R</sup>I<sup>M</sup>PM<sup>R</sup>L<sup>P</sup>L<sup>K</sup>V<sup>Y</sup>GRE<sup>N</sup>FS<sup>K</sup>N<sup>K</sup>K<sup>K</sup>IR<sup>R</sup>N<sup>N</sup>.TF<sup>F</sup>T<sup>V</sup>Y<sup>I</sup>EE<sup>K</sup>GT<sup>K</sup>L<sup>L</sup>KQ<sup>G</sup>N<sup>F</sup>KAL<sup>V</sup>

