

Fig. S1 FpCas9(CN6), FpCas9(CN38), FpCas9(CN46) and *Flavobacterium columnare* Cas9 (FcCas9) protein alignment.

	1	10	20	30	40	50
FpCas9(CN6)	MKKILGLD	IGTNSIGWAF	INEAEN	IEVSSIVNT	GVRIVPLTTDEESD	FKKGN
FpCas9(CN38)	MKKILGLD	IGTNSIGWAF	INEAEN	IEVSSIVNT	GVRIVPLTTDEESD	FKKGN
FpCas9(CN46)	MKKILGLD	IGTNSIGWAF	TNNDF	DNKLGNI	EGLGSRIIPMSQD	VLGKFD
FcCas9	MKFNLGLD	IGTNSIGWAF	VNE	EDY	ENKKGKI	AAGSRIIPMTQD
	60	70	80	90	100	110
FpCas9(CN6)	DRTLKRGARR	GLQRFKQR	REALLLEIF	NRIRFI	ATDFVYAETGAATT	FSYQLRAKSA
FpCas9(CN38)	DRTLKRGARR	GLQRFKQR	REALLLEIF	NRIRFI	ATDFVYAETGAATT	FSYQLRAKSA
FpCas9(CN46)	ERTKYRGTR	LVQRFLL	RRELRHRL	ILNLD	FLPKHYAETID	FEKKGGQFKPEKEEK
FcCas9	RTLKYRGTR	LVQRFLL	RRELRHRL	ILNLD	FLPKHYAETID	FEKKGGQFKPEKEEK
	120	130	140	150	160	170
FpCas9(CN6)	VTKELVKKVL	LMLNKKRG	YKSSRKAK	TAEEDGAI	DGMKI	AKEIFENNLT
FpCas9(CN38)	VTKELVKKVL	LMLNKKRG	YKSSRKAK	TAEEDGAI	DGMKI	AKEIFENNLT
FpCas9(CN46)	YKNESGKNQ	LFLES	HQEMLA	EFQLIN	PDL	KQVPYDWTI
FcCas9	NQN	LFLES	HQEMLA	EFQLIN	PDL	KQVPYDWTI
	180	190	200	210	220	230
FpCas9(CN6)	GGKFIPDFYH	SDLQKELEKIVRF	QNFHSE	QINDKL	LADITQGKS	RTQTSQHFS
FpCas9(CN38)	GGKFIPDFYH	SDLQKELEKIVRF	QNFHSE	QINDKL	LADITQGKS	RTQTSQHFS
FpCas9(CN46)	EELAWILLNF	NQKRGYYQLRGE	EDNKNKLEEF	HTLT	VVDVIERE	KGKSGIWN
FcCas9	EELAWILLNF	NQKRGYYQLRGE	EDNKNKLEEF	HTLT	VVDVIERE	KGKSGIWN
	240	250	260	270	280	290
FpCas9(CN6)	AENKGTRE	ETKLQH	YQWRNE	ALANEL	DMKILAFI	IITEINN
FpCas9(CN38)	AENKGTRE	ETKLQH	YQWRNE	ALANEL	DMKILAFI	IITEINN
FpCas9(CN46)	ENGWIYK	RESKIAL	FDWIG	KQKNFI	VTTTEL	NDDGTVKLN
FcCas9	ENGWIYK	RESKIAL	FDWIG	KQKNFI	VTTTEL	NDDGTVKLN
	300	310	320	330	340	
FpCas9(CN6)	RSKELYFN	QETVGQY	QYKQLQEN	VHAKLKNQ	VFYRQD	YLD
FpCas9(CN38)	RSKELYFN	QETVGQY	QYKQLQEN	VHAKLKNQ	VFYRQD	YLD
FpCas9(CN46)	IRTEQIN	QSEKSV	GTFTI	YDAL	LNPNQKIK	GELLRT
FcCas9	IRTEQIN	QSEKSV	GTFTI	YDAL	LNPNQKIK	GELLRT
	350	360	370	380		
FpCas9(CN6)	INSSK	LYKACLEELYPK	NEIY	KSA	LKNKD	IAYLFIND
FpCas9(CN38)	INSSK	LYKACLEELYPK	NEIY	KSA	LKNKD	IAYLFIND
FpCas9(CN46)	INSSK	LYKACLEELYPK	NEIY	KSA	LKNKD	IAYLFIND
FcCas9	INSSK	LYKACLEELYPK	NEIY	KSA	LKNKD	IAYLFIND
	390	400	410	420		
FpCas9(CN6)	G	HAIPKSSP	IFQEFRI	WONLNN	VVVKNET	TKVEFELSE
FpCas9(CN38)	G	HAIPKSSP	IFQEFRI	WONLNN	VVVKNET	TKVEFELSE
FpCas9(CN46)	VPIKDENGIL	VKDDNGKQVVKPL	KCI	AKSNPI	IFQEFRL	IQFVKNLKI
FcCas9	VPIKDENGIL	VKDDNGKQVVKPL	KCI	AKSNPI	IFQEFRL	IQFVKNLKI
	430	440	450	460	470	480
FpCas9(CN6)	ELKQLIA	EELOFKES	MTDVQML	TFCE	LKKGHT	TVNFKKIE
FpCas9(CN38)	ELKQLIA	EELOFKES	MTDVQML	TFCE	LKKGHT	TVNFKKIE
FpCas9(CN46)	DFIKTEED	LVELFN	LNDRKE	IDQIS	LKHL	LRP
FcCas9	DFIKTEED	LVELFN	LNDRKE	IDQIS	LKHL	LRP
	490	500	510	520	530	540
FpCas9(CN6)	YEIDFSKMK	PLEIKET	VAP	IFETL	NI	NTVIL
FpCas9(CN38)	YEIDFSKMK	PLEIKET	VAP	IFETL	NI	NTVIL
FpCas9(CN46)	YEMDFS	KMKPLEIKET	VAP	IFETL	NI	NTVIL
FcCas9	YEMDFS	KMKPLEIKET	VAP	IFETL	NI	NTVIL

550 560 570 580 590
FpCas9 (CN6)DKLKETLKTKEFKENHLPFILNINLQADYGSLSAKAIKKLLPHLMDGHIYDK..
FpCas9 (CN38)DKLKETLKTKEFKENHLPFILNINLQADYGSLSAKAIKKLLPHLMDGHIYDK..
FpCas9 (CN46) IEITKAITTFACKHNLPDEFITNFSKLPYKKDYGSFSEAKAIKKLLPHLMDGHIYDK..
FcCas9 KQYEKALAKFAKLNHLDTKSFVNTFRKRIPPFPKNFYAIYSLAKAIKKLLPHLMDGHIYDK..

600
FpCas9 (CN6)A CLMVG.....YNHSSSL
FpCas9 (CN38)A CLMAG.....YNHSSSL
FpCas9 (CN46) IDIKTLARIDKIQTGEFDENMRTRVREKAHLSKTTDFQGLPLWLASYIYDRHSEASDV
FcCas9 IDPITKQKIQCIIIGENDTISTLVREKTNHFSNENDFKNLPWLALGYIYVNSHNESSSEI

610 620 630 640 650 660
FpCas9 (CN6) TTAQNNDRVLKDTLELLKKNSLRNPVVEKILNQMINVINATMKDPAMGKP...DEIRVEL
FpCas9 (CN38) TTAQNNDRVLKDTLELLKKNSLRNPVVEKILNQMINVINATMKDPAMGKP...DEIRVEL
FpCas9 (CN46) SKWKTTPADIQNFLKYDFKQHSRLRNPVVEQVITETLRVVKDITQHYGDKENFFTEIHIIEI
FcCas9 EKWKSPQDIDTFLK,KFKQHALLRNPVVEQVITETLRVVKDITQHYGDKENFFTEIHIIEI

670 680 690 700
FpCas9 (CN6) ARELKANNEQRRKATSDINTATTEHERIRKILHSEFN.....
FpCas9 (CN38) ARELKANNEQRRKATSDINAATTEHERIRKILHSEFN.....
FpCas9 (CN46) GREMKNDKATREMTKKISENENTNLRIKAILTELKNDGLNDIRPSPSQDDILKIYEFG
FcCas9 GKDLKNPVDKRRKLSSINTKNESSTNTRIKAILTELFTQGVENVKPNSPKHQEIILKIFEFG

710 720 730 740
FpCas9 (CN6)IPRVTRNDIIRYKLWLETGGISLYTGNPIKASDLFSKNYDI
FpCas9 (CN38)IPRVTRNDIIRYKLWLETGGISLYTGNPIKASDLFSKNYDI
FpCas9 (CN46) VYSNENKKEKLEEDIKIRKNAKPTPSEIIKYKLWLEQGYISPYTGKILPSELFTTKYQI
FcCas9 ALNSG..IEIEEDIKISNTDQPTKKQLELYKLWLEHKYKSPYTGQSTPLSKLFTPNYEI

750 760 770 780 790 800
FpCas9 (CN6) EHIIPKSRLFDDSF SNKTLCEERQINIDKSNKTAYSFLQEKLSENDFAAYENRVKDLFKTG
FpCas9 (CN38) EHIIPKSRLFDDSF SNKTLCEERQINIDKSNKTAYSFLQEKLSENDFAAYENRVKDLFKTG
FpCas9 (CN46) EHIIPKSRLFDDSF SNKTLCEERQINIDKSNKTAYSFLQEKLSENDFAAYENRVKDLFKTG
FcCas9 EHIIPKSRLFDDSF SNKTLCEERQINIDKSNKTAYSFLQEKLSENDFAAYENRVKDLFKTG

810 820 830 840
FpCas9 (CN6) KIKSYTKYKKLLMADN.....EIPLEGFIDRQLRETQYTAKKAKEILLEVSR.....
FpCas9 (CN38) KIKSYTKYKKLLMADN.....EIPLEGFIDRQLRETQYTAKKAKEILLEVSR.....
FpCas9 (CN46) YNQHIATAYSKNKSQKRLLESEIPEKFIERQLNDTKYISKIVKNLSRIVREEEQE..
FcCas9 YWNFVTTXYAKNKAARENLLLEIPEQMVVKQLNDTRVYSKYVMQLLSKILVRKEDGTDNG

850 860 870 880 890 900
FpCas9 (CN6)NVTATIGSVTDKLRQDWELVDVMKELNWEKYDKLGLTHIEEGKNGERLSKIKD..
FpCas9 (CN38)NVTATIGSVTDKLRQDWELVDVMKELNWEKYDKLGLTHIEEGKNGERLSKIKD..
FpCas9 (CN46) VTSKHIVSLNLSITSRMKQDWGLNDVWNNIVTPRFRERLNLALTNNSDFGQFEKLDAYGNK
FcCas9 INSTNLSISGKMTALKNDWDINDVWNNLLYRFRERLNLITKTNLFITYSEKQKQR...

910 920 930 940
FpCas9 (CN6)WTKRNDHRRHAMDATVAF TKPAYIQYLNNDNAKTQGENK..
FpCas9 (CN38)WTKRNDHRRHAMDATVAF TKPAYIQYLNNDNAKTQGENK..
FpCas9 (CN46) GKQIFQTTVPDAIAKGFTTKRIDHRRHAMDALVIA CVSRTHINYLNNNDNARDTDDKA..
FcCas9MPIVPDQYAKGFHKKRLDHRHAMDALVIA CVSRTHINYMNNQNIQGEKLLKNAI

950 960 970 980
FpCas9 (CN6)ANSIIGIENKHLRYDKNNKLRFISPMKNFR EEAQKQLESILISYK
FpCas9 (CN38)ANSIIGIENKHLRYDKNNKLRFISPMKNFR EEAQKQLESILISYK
FpCas9 (CN46)VKHELNRNKL CFKTKPDSN.GNYKWEFYKPKDFTTAEADKLNITTVSFK
FcCas9 EKENYREILQKSRLDIRAQ LCEKKYNDNDPTKYNWVFIKEWADFENTKDI LESIISFK

	990	1000	1010	1020	1030	1040
FpCas9 (CN6)	AKNKVVTKNKNTTKKSGGTNQKIQITPRGR	LHKETVY	GK	LQ	QYATKEEKVN	ASFTEDYTQ
FpCas9 (CN38)	AKNKVVTKNKNTTKKSGGTNQKIQITPRGR	LHKETVY	GK	LQ	QYATKEEKVN	ASFTEDYTQ
FpCas9 (CN46)	QNTTRVINKTIVNKYTNKDEKGNLNI	GTNGL	VBK	KIIT	QTKGDNWAIRKP	MHAETVSGKVF
FcCas9	QNLRIINKTIVNYYEKYN.ENG.	...	...	...	...	...

	1050	1060	1070	1080	1090
FpCas9 (CN6)	KVAKKEYREALLKRLLEN	DNDPKK	AFTGKN	ALN	KTPIYISL
FpCas9 (CN38)	KVAKKEYREALLKRLLEN	DNDPKK	AFTGKN	ALN	KTPIYISL
FpCas9 (CN46)	LKRIRKQSPITIANAIEQIE	FIVDK	EVKKQL	ASKIKQYPNNMVGLKKHLKAFP	VMDGKA
FcCas9	LSYKTPAKGKIL	...	...	...	...

	1100	1110	1120	1130	1140
FpCas9 (CN6)	VPEKVKTVWLETDYTIRK	DITPD	DKIDKVID	VGIRILONRL	NEFNGD.....PKKA
FpCas9 (CN38)	VPEKVKTVWLETDYTIRK	DITPD	DKIDKVID	VGIRILONRL	NEFNGD.....PKKA
FpCas9 (CN46)	DKVQVYETIEATATRKTL	DITFD	EKKIEKI	TDGTIQKILNHL	KQEMYQNAIHENGEKIP
FcCas9		...	...	...	...

	1150	1160	1170	1180	1190	1200
FpCas9 (CN6)	FVNLGESPIWLNKEKGI	AIKRV	TISGV	SNQAQLHTKKDHL	LGNEILD	KNGNPIPVDF
FpCas9 (CN38)	FVNLGESPIWLNKEKGI	AIKRV	TISGV	SNQAQLHTKKDHL	LGNEILD	KNGNPIPVDF
FpCas9 (CN46)	AHEVAFSENGLDELNKNL	TTLN	NGKKHQP	IKKVRVFE	EGGKFR	LGQTQNKADKYVEAAKG
FcCas9	..ELAFSEEGLEELNATIT	KYNN	GFHHKP	IYKVR	IYEEGV	KFPKLGNNKKSKYVEAEKG

	1210	1220	1230	1240	1250	1260
FpCas9 (CN6)	NNHHVAIYRDEKGNLQEE	VVSFYDAV	IRRNLCISVIN	KNHEK	GWEFLFSMKQ	NEFFIFPS
FpCas9 (CN38)	NNHHVAIYRDEKGNLQEE	VVSFYDAV	IRRNLCISVIN	KNHEK	GWEFLFSMKQ	NEFFIFPS
FpCas9 (CN46)	TNLFFAIYQDEKGRN	YKTI	IPFNEVIERQKQ	CLSAAQET	DENGNRL	DFTLSPNDLVYLP
FcCas9	TNLFFAIYQDENNR	YFET	IPFNDVLE	TLKKCMS	SAVPEKNQ	EEENTLVEALSPQDLVFVPT

	1270	1280	1290	1300	1310
FpCas9 (CN6)	DGFNPHEIDTLNPN	NNYHLISPN	IFRVQKISTKN	Y.....MFNHHLE	TKAVDGEMLKS
FpCas9 (CN38)	DDFNPHEIDTLNPN	NNYHLISPN	MFRVQSLSV	VQYGNSTIRDFKFRHHL	ETTVED.....
FpCas9 (CN46)	EDDENLTTINFDKLS	KEQTER	IYKFV	SCTSGEGHFVSNNYSKEII	SNENGSNNKNERML
FcCas9	LDIEIENPDIVDFKN	LTQDQ	TNR	IYKMS	SGKQCFFIKAEVATSIVN

	1320	1330	1340	1350
FpCas9 (CN6)	KKELSKTSYHF	IQTP	TNLN	.GIKVRINHLGKTVHLGEY
FpCas9 (CN38)	KKELTNITYKQIKSL	IPLN	.EIKVRINHLGKTVHVGEY	
FpCas9 (CN46)	ELNNSTIYDEKEKP	VMIK	SVCKLEVDR	LGKTRKIIIR.
FcCas9	EFSTSNKME	NSIEG	.IQITKSNCW	KLKLDRLGKTRVIRK.