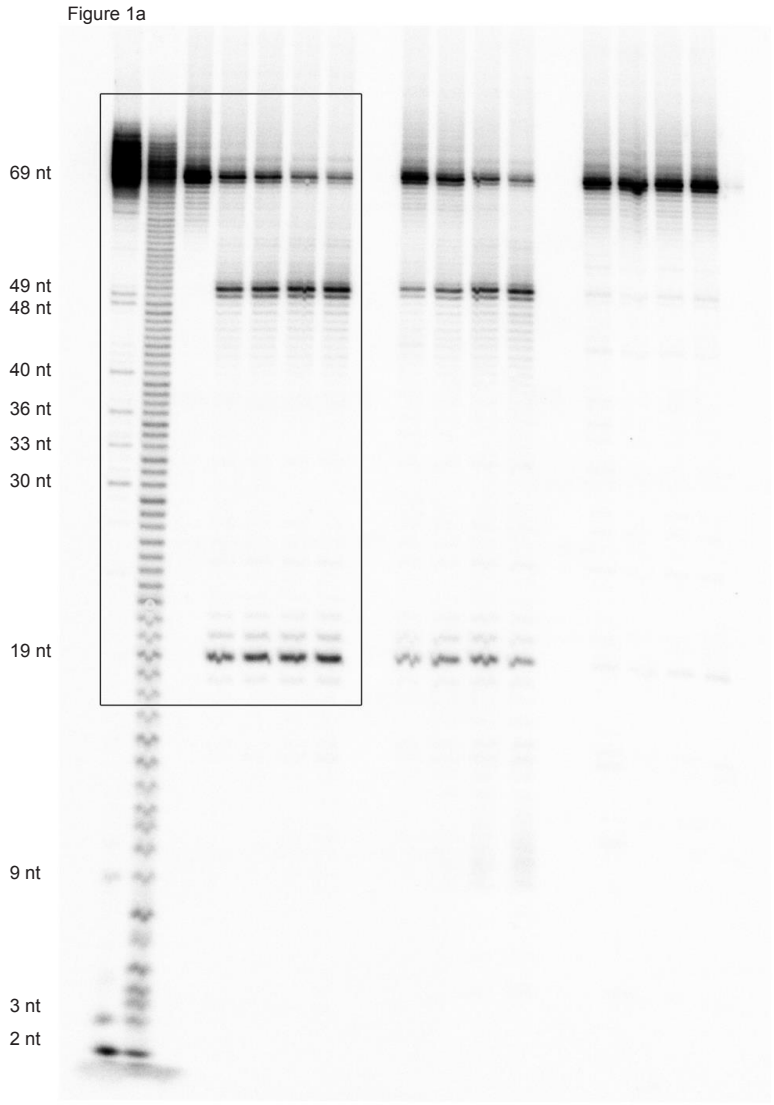
doi:10.1038/nature17945

The supplementary figures show the raw data of all gels shown in the main and Extended Data figures (Figure S1) and the multiple sequence alignment of Cpf1 orthologues (Figure S2). The table describes the list of strains (Table S1a), plasmids (Table S1b) and oligonucleotides (Table S1c) used in the study.

Supplementary Figures

Supplementary Figure 1: uncropped gel images



Continuation Supplementary Figure 1: uncropped gel images

Figure 2a

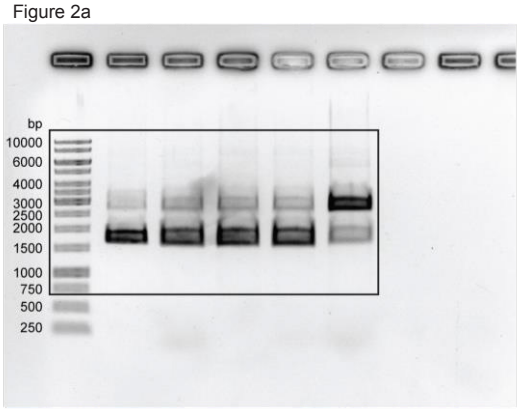


Figure 2b (left panel)

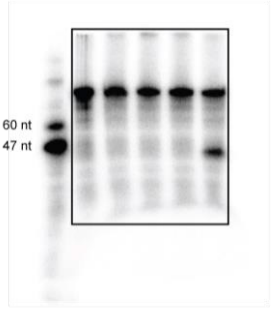


Figure 2b (right panel)

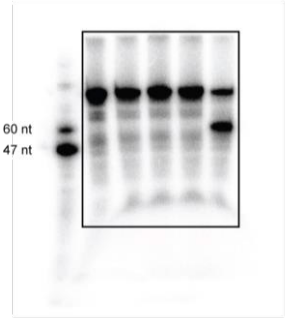


Figure 2d (upper panel)

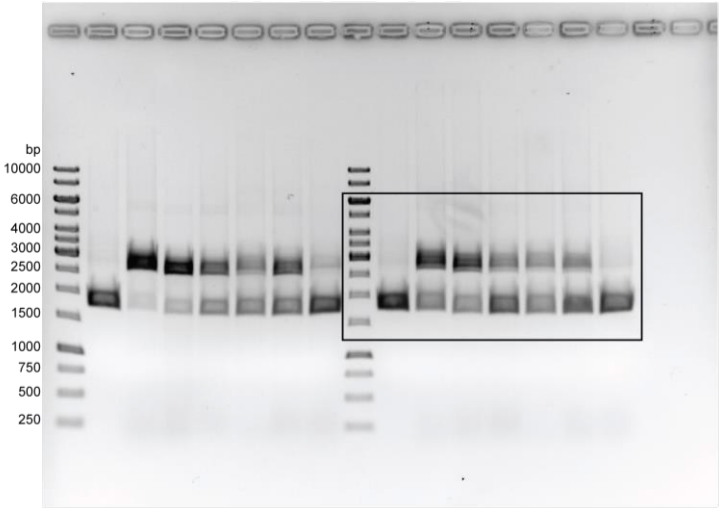


Figure 2d (lower panel)



Figure 2e (upper panel)

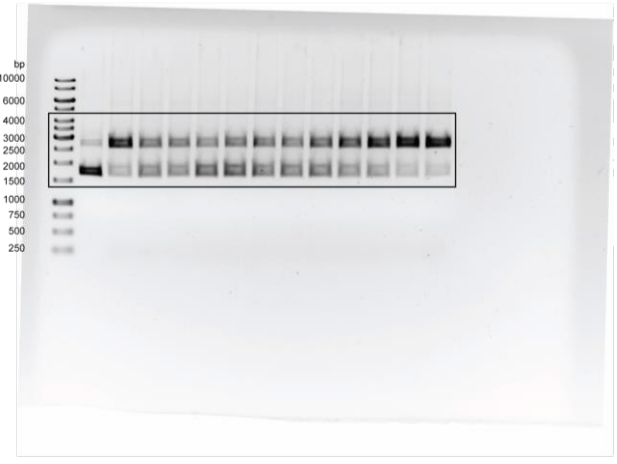


Figure 2e (lower panel)



Continuation Supplementary Figure 1: uncropped gel images

Figure 3a (upper panel)

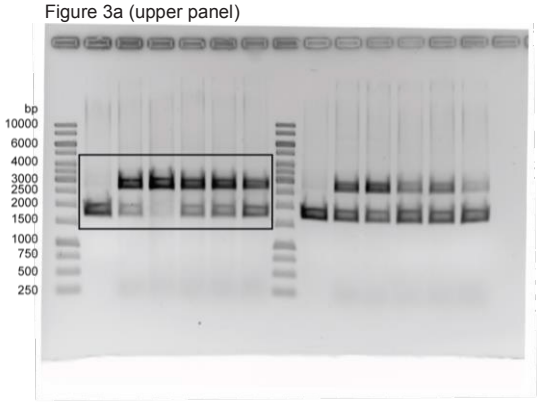


Figure 3a (middle panel)

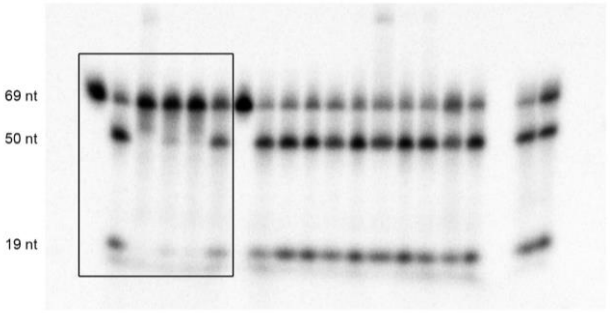


Figure 3a (lower panel)

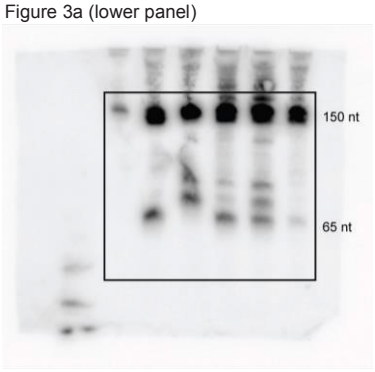


Figure 3b (upper panel)

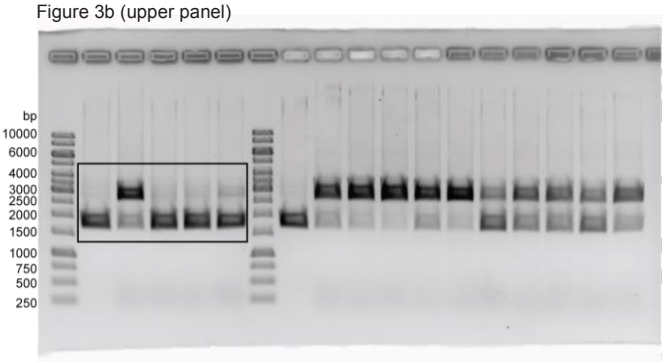
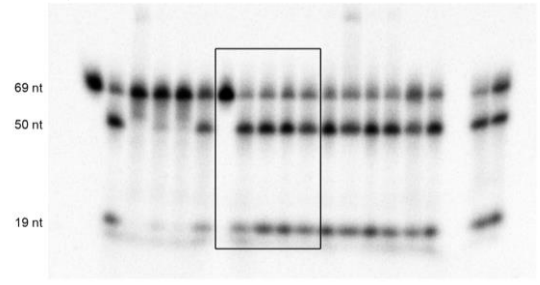


Figure 3b (lower panel)



Continuation Supplementary Figure 1: uncropped gel images

Figure 3c (first panel)

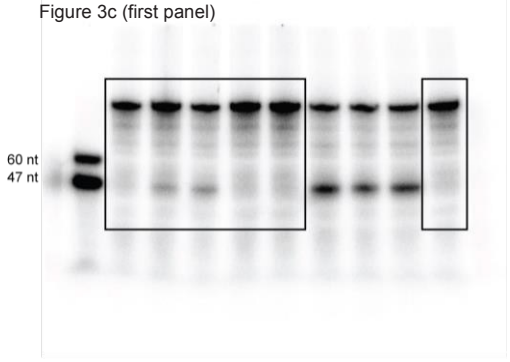


Figure 3c (second panel)

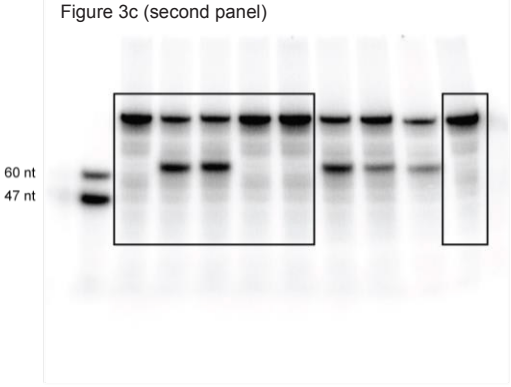


Figure 3c (third panel)

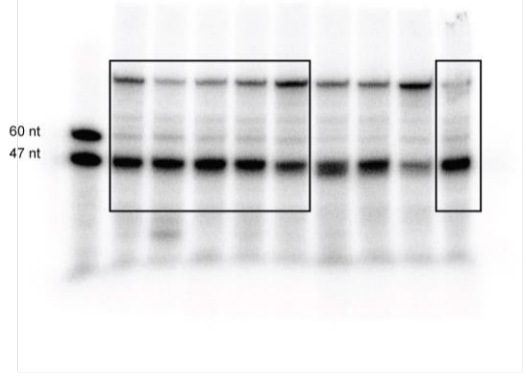
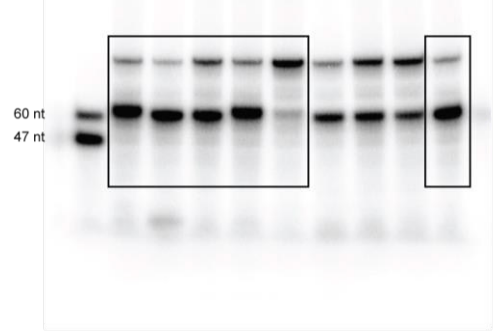
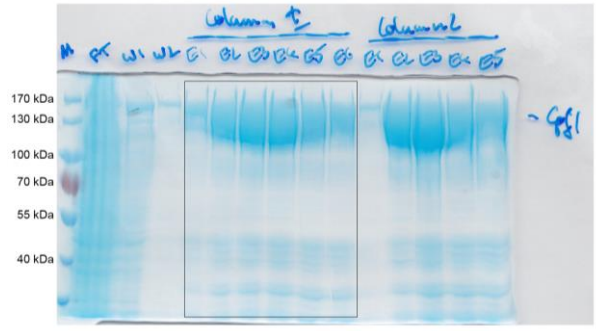


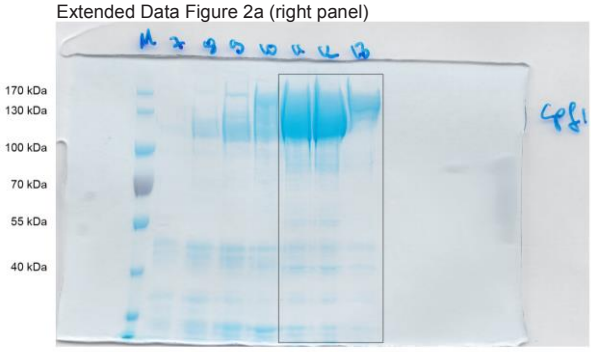
Figure 3c (fourth panel)



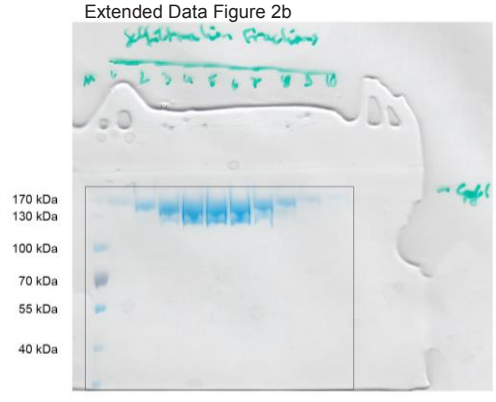
Extended Data Figure 2a (left panel)



Extended Data Figure 2a (right panel)

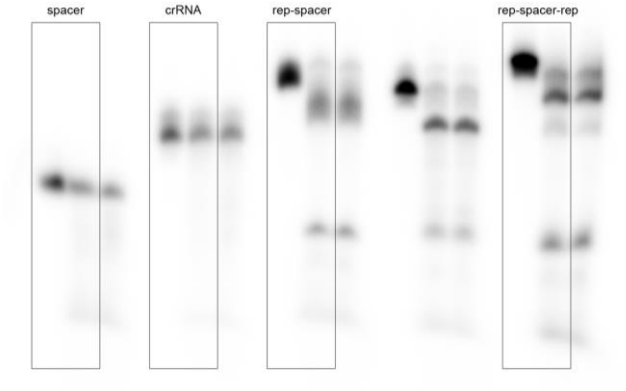


Extended Data Figure 2b

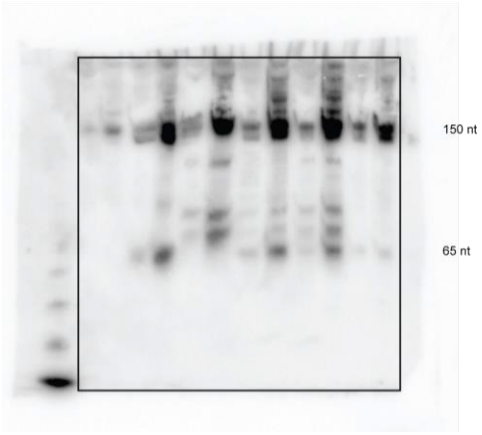


Continuation Supplementary Figure 1: uncropped gel images

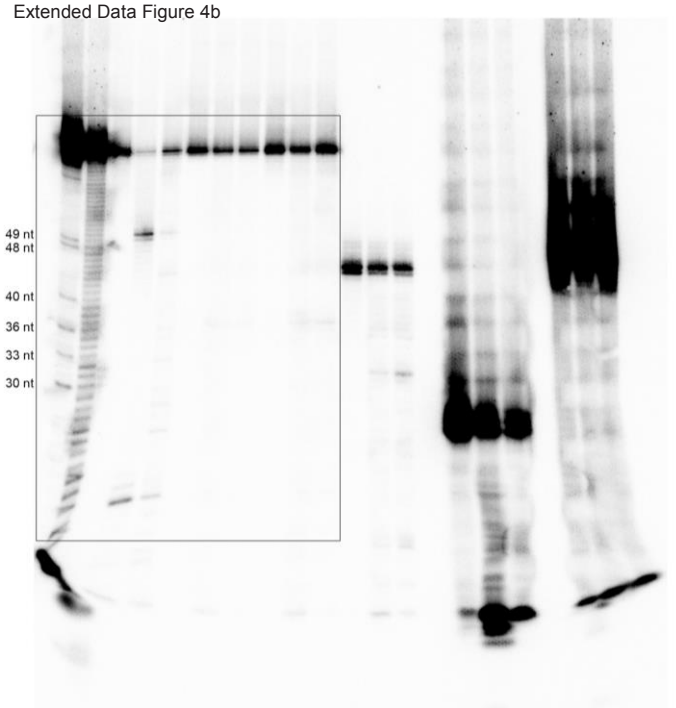
Extended Data Figure 3a



Extended Data Figure 3b

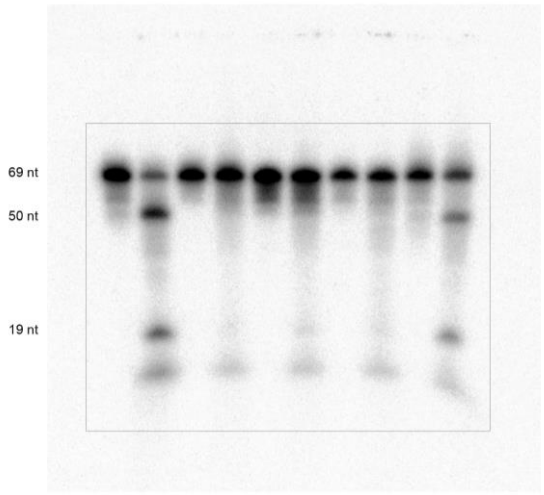


Extended Data Figure 4b

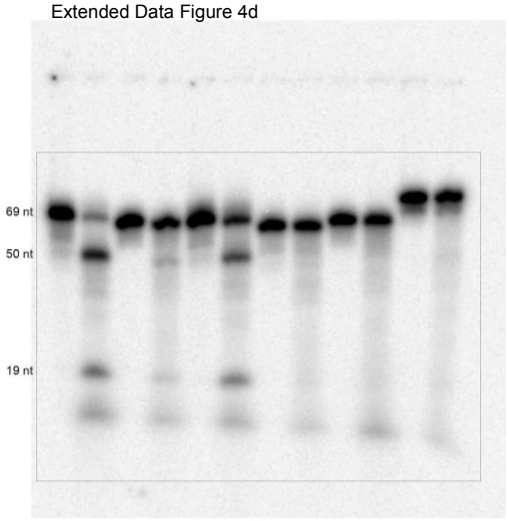


Continuation Supplementary Figure 1: uncropped gel images

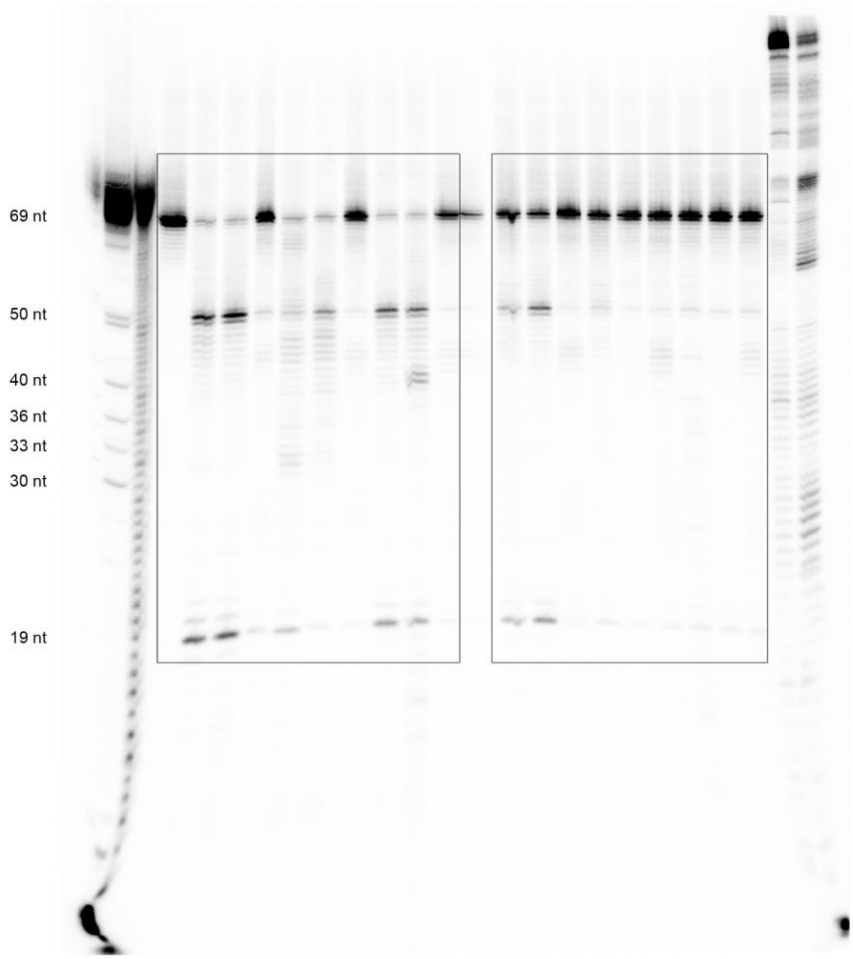
Extended Data Figure 4c



Extended Data Figure 4d

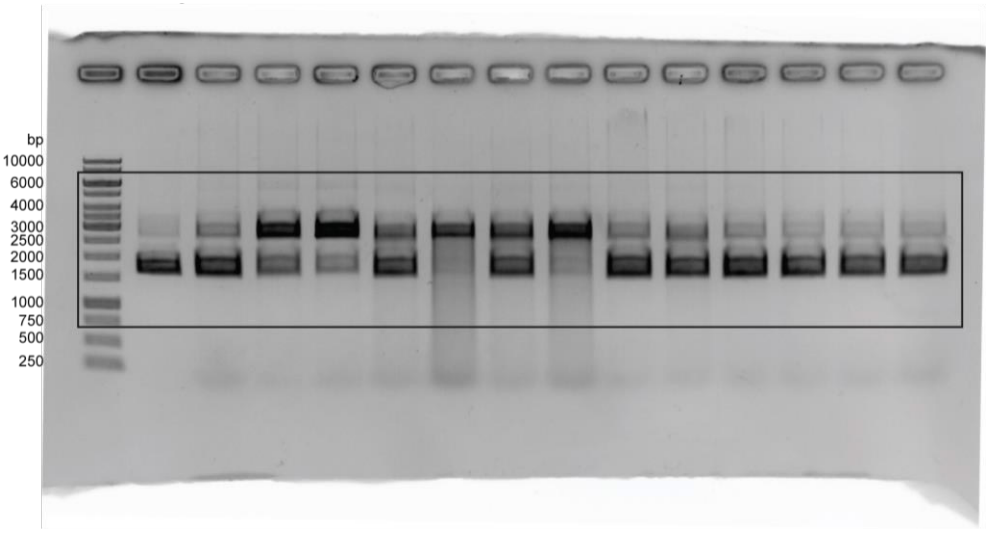


Extended Data Figure 5a



Continuation Supplementary Figure 1: uncropped gel images

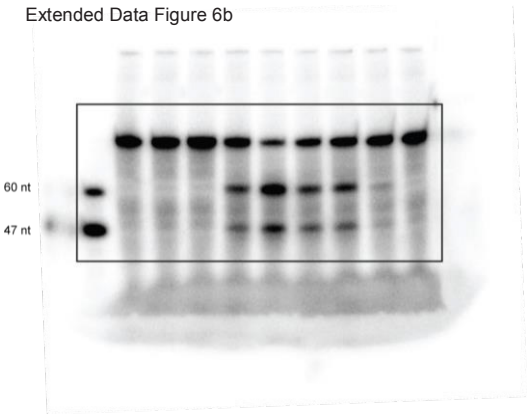
Extended Data Figure 5b



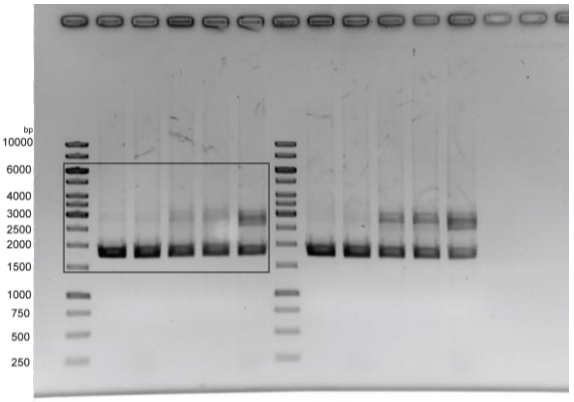
Extended Data Figure 6a



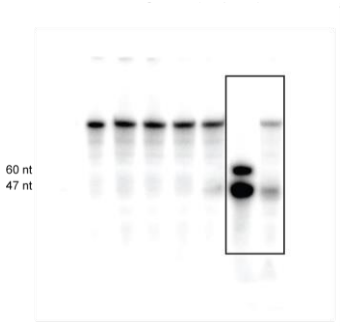
Extended Data Figure 6b



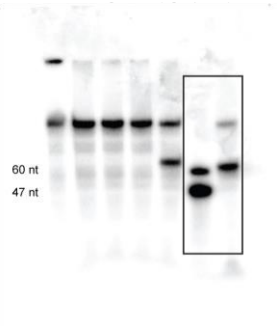
Extended Data Figure 7a



Extended Data Figure 7b (left panel)

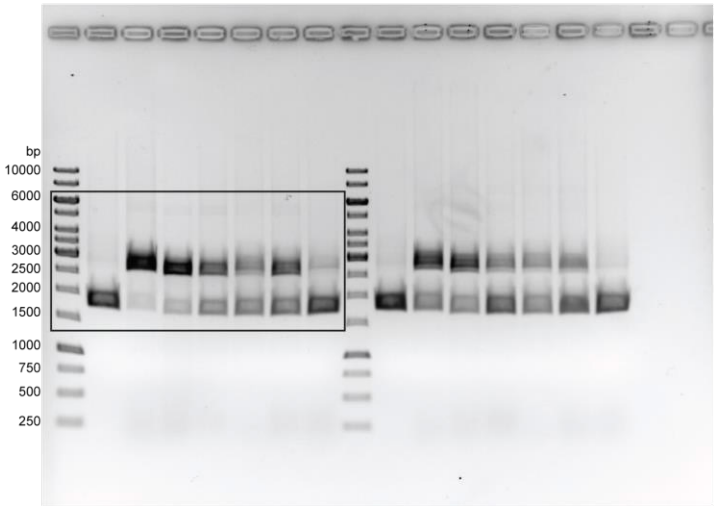


Extended Data Figure 7b (right panel)

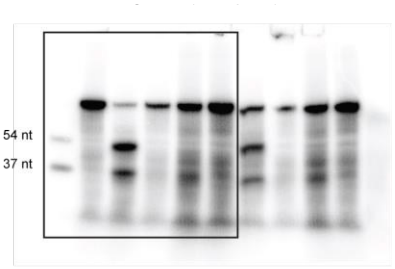


Continuation Supplementary Figure 1: uncropped gel images

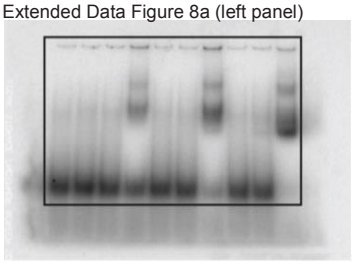
Extended Data Figure 7d (upper panel)



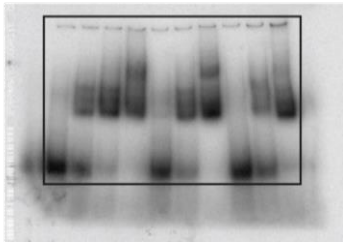
Extended Data Figure 7d (lower panel)



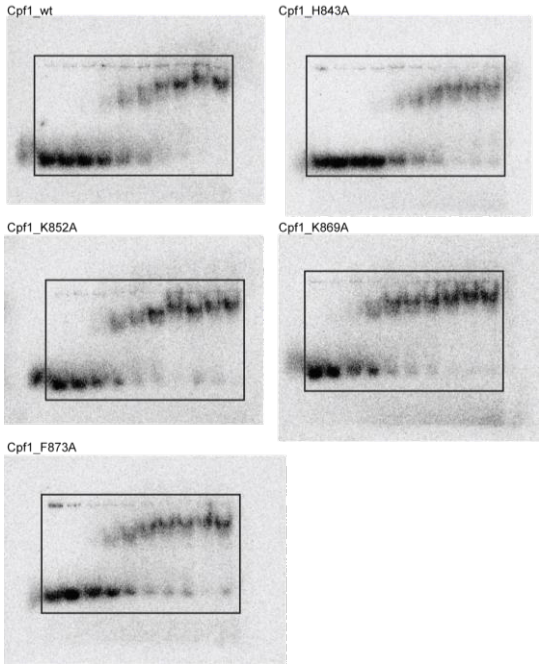
Extended Data Figure 8a (left panel)



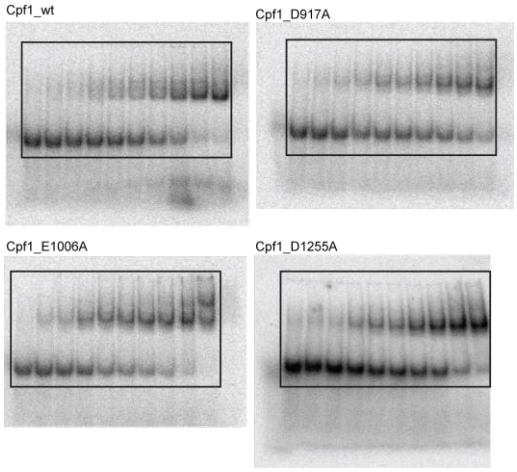
Extended Data Figure 8a (right panel)



Extended Data Figure 8b

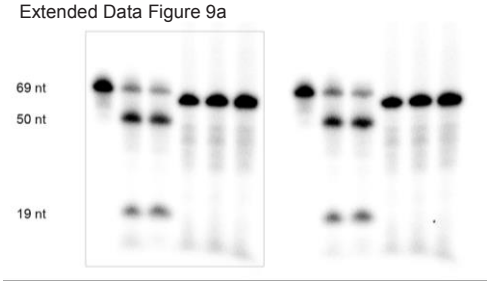


Extended Data Figure 8c

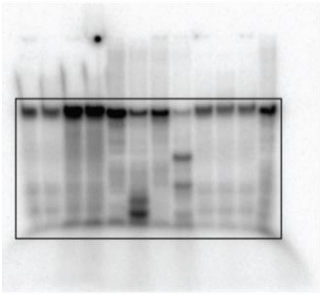


Continuation Supplementary Figure 1: uncropped gel images

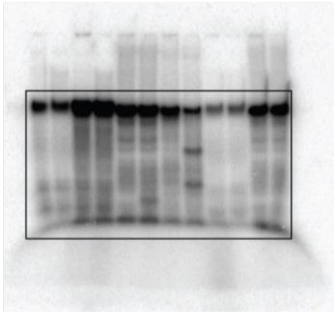
Extended Data Figure 9a

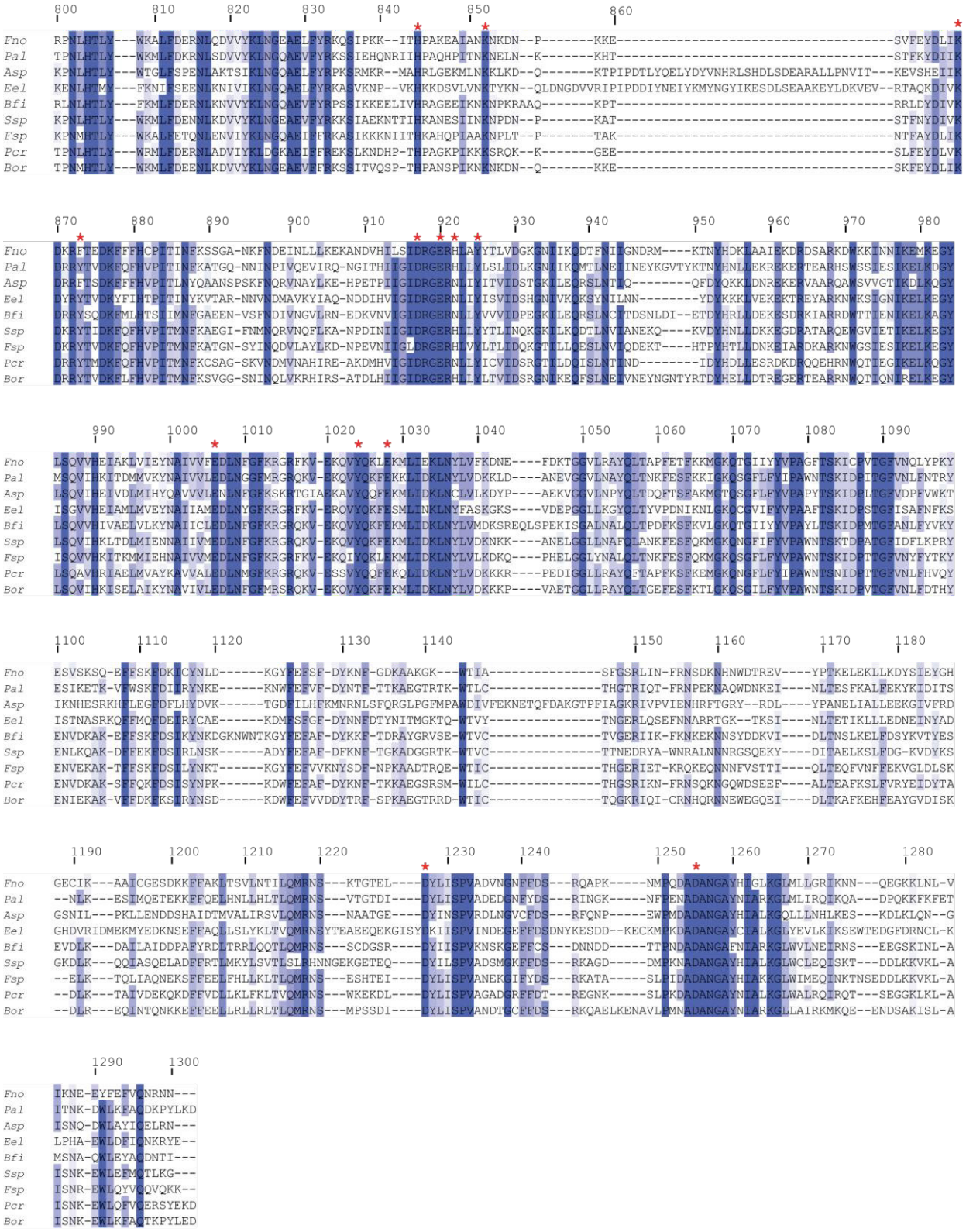


Extended Data Figure 9b (upper panel)



Extended Data Figure 9b (lower panel)





Supplementary Figure 2. Alignment of Cpf1 orthologues. Multiple sequence alignment of Cpf1 amino acid sequences of *F. novicida* U112 (Fno) (gi: 118496615), *Prevotella albensis* M384 (Pal) (gi: 640557447), *Acidaminococcus sp.* BV3L6 (Asp) (gi: 545612232), *Eubacterium eligens* CAG:72 (Eel) (gi: 547479789), *Butyrivibrio fibrisolvens* (Bfi) (gi: 652963004), *Smithella sp. SCADC* (Ssp) (gi:

739526085), *Flavobacterium* sp. 316 (Fsp) (gi: 800943167), *Porphyromonas crevioricanis* (Pcr) (gi: 739008549) and *Bacteroidetes* oral taxon 274 (Bor) (gi: 496509559) done with MUSCLE [1]. Only the C-terminal region corresponding to amino acid residues 800 - 1300 of *F. novicida* Cpf1 is visualised by JalView [2]. Conserved residues are highlighted in blue. Residues involved in RNA processing (H843, K852, K869, F873) and DNA targeting (D917, E920, H922, Y925, E1006, Y1024, E1028, D1227, D1255) are indicated by red asterisks.

Table S1a. Strains used in the study.

Strain	Relevant characteristics	Source
Francisella novicida		
EC1041	U112 (WT)	Anders Sjöstedt [3]
Escherichia coli		
RDN204	TOP10; Host for cloning	Invitrogen
RDN226	DH5α; Host for cloning	New England Biolabs
EC2212	NiCo21 (DE3); Expression strain	New England Biolabs

Table S1b. Plasmids used in the study.

Plasmids	Relevant characteristics	Source
Plasmids for *in vitro* protospacer study		
pUC19		New England Biolabs
pEC1664	pUC19ΩCpf1(psp5)	This study
pEC1688	pUC19Ωpsp5	This study
pEC1693	pUC19Ωpsp5_PAM_A2C	This study
pEC1703	pUC19Ωpsp5_A1C	This study
pEC1704	pUC19Ωpsp5_G2T	This study
pEC1705	pUC19Ωpsp5_A3C	This study
pEC1706	pUC19Ωpsp5_T4G	This study
pEC1707	pUC19Ωpsp5_A5C	This study
pEC1708	pUC19Ωpsp5_G6T	This study
pEC1709	pUC19Ωpsp5_A7C	This study
pEC1710	pUC19Ωpsp5_A8C	This study
pEC1711	pUC19Ωpsp5_T9G	This study
pEC1712	pUC19Ωpsp5_T10G	This study
pEC1713	pUC19Ωpsp5_A11C	This study
pEC1714	pUC19Ωpsp5_C12A	This study
pEC1715	pUC19Ωpsp5_C13A	This study
pEC1716	pUC19Ωpsp5_T14G	This study
pEC1717	pUC19Ωpsp5_T15G	This study
pEC1718	pUC19Ωpsp5_T16G	This study
pEC1719	pUC19Ωpsp5_T17G	This study
pEC1720	pUC19Ωpsp5_A18C	This study
pEC1721	pUC19Ωpsp5_A19C	This study
pEC1722	pUC19Ωpsp5_T20G	This study
pEC1723	pUC19Ωpsp5_C21A	This study
pEC1724	pUC19Ωpsp5_T22G	This study
pEC1725	pUC19Ωpsp5_mut1	This study
pEC1726	pUC19Ωpsp5_mut2	This study
pEC1731	pUC19Ωpsp5_PAM_G3T	This study
pEC1734	pUC19Ωpsp5_PAM_A2C,GG3,7TT	This study
pEC1735	pUC19Ωpsp5_PAM_A2G	This study
Plasmids for Cpf1 overexpression		
pEC621	pEC225 + *Not*I, *Sac*I, *Sal*I	[4]
pEC1611	pEC621Ω*cpf1*	This study
pEC1776	pEC621Ω*cpf1*(H843A)	This study
pEC1777	pEC621Ω*cpf1*(K852A)	This study
pEC1778	pEC621Ω*cpf1*(K869A)	This study
pEC1779	pEC621Ω*cpf1*(F873A)	This study
pEC1782	pEC621Ω*cpf1*(D917A)	This study
pEC1783	pEC621Ω*cpf1*(E920A)	This study
pEC1784	pEC621Ω*cpf1*(H922A)	This study
pEC1785	pEC621Ω*cpf1*(Y925A)	This study
pEC1788	pEC621Ω*cpf1*(E1006A)	This study
pEC1790	pEC621Ω*cpf1*(Y1024A)	This study

Plasmids	Relevant characteristics	Source
pEC1791	pEC621Ω*cpf1*(E1028A)	This study
pEC1796	pEC621Ω*cpf1*(D1227A)	This study
pEC1799	pEC621Ω*cpf1*(D1255A)	This study
Plasmids for Northern blot analysis of pre-crRNA processing		
pACYC184		New England Biolabs
pEC1690	pACYCΩsgRNA2	This study
pEC575	pCDF-1b	Novagen
pEC1701	pCDFΩ*cpf1*	This study

Table S1c. Oligonucleotides used in the study.

Purpose	Primer code	Sequence 5'-3'	F/R	Usage
Oligonucleotides for *in vitro* protospacer studies				
pEC1664	OLEC6213	AGCTGTAGCAAATATTAATCATATAGAAGAAAGCTCAGATCTCA ACAAGATAGAATTACCTTTTAATCTTAAATTATTATATCCAGAAA CTATTGATGGTAATTTACTTATC	F	Cloning
	OLEC6214	AGCTGATAAGTAAATTACCATCAATAGTTTCTGGATATAATAATT TAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTT CTATATGATTAATATTTGCTAC	R	Cloning
pEC1688	OLEC6283	AGCTGAGATAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6301	AGCTGAGATTAAAAGGTAATTCTATCTC	R	Cloning
pEC1693	OLEC6432	GACGGCCAGTGCAGTCGAGCTCGG	F	Mutage-nesis of pEC1688
	OLEC6433	CCTTTTAATCTCCGCTTGCATGCCTG	R	
pEC1703	OLEC6331	AGCTGCGATAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6332	AGCTGAGATTAAAAGGTAATTCTATCGC	R	Cloning
pEC1704	OLEC6333	AGCTGATATAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6334	AGCTGAGATTAAAAGGTAATTCTATATC	R	Cloning
pEC1705	OLEC6335	AGCTGAGCTAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6336	AGCTGAGATTAAAAGGTAATTCTAGCTC	R	Cloning
pEC1706	OLEC6337	AGCTGAGAGAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6338	AGCTGAGATTAAAAGGTAATTCTCTCTC	R	Cloning
pEC1707	OLEC6339	AGCTGAGATCGAATTACCTTTTAATCTC	F	Cloning
	OLEC6340	AGCTGAGATTAAAAGGTAATTCGATCTC	R	Cloning
pEC1708	OLEC6341	AGCTGAGATATAATTACCTTTTAATCTC	F	Cloning
	OLEC6342	AGCTGAGATTAAAAGGTAATTATATCTC	R	Cloning
pEC1709	OLEC6343	AGCTGAGATAGCATTACCTTTTAATCTC	F	Cloning
	OLEC6344	AGCTGAGATTAAAAGGTAATGCTATCTC	R	Cloning
pEC1710	OLEC6345	AGCTGAGATAGACTTACCTTTTAATCTC	F	Cloning
	OLEC6346	AGCTGAGATTAAAAGGTAAGTCTATCTC	R	Cloning
pEC1711	OLEC6347	AGCTGAGATAGAAGTACCTTTTAATCTC	F	Cloning
	OLEC6348	AGCTGAGATTAAAAGGTACTTCTATCTC	R	Cloning
pEC1712	OLEC6349	AGCTGAGATAGAATGACCTTTTAATCTC	F	Cloning
	OLEC6350	AGCTGAGATTAAAAGGTCATTCTATCTC	R	Cloning
pEC1713	OLEC6351	AGCTGAGATAGAATTCCCTTTTAATCTC	F	Cloning
	OLEC6352	AGCTGAGATTAAAAGGGAATTCTATCTC	R	Cloning
pEC1714	OLEC6353	AGCTGAGATAGAATTAACTTTTAATCTC	F	Cloning
	OLEC6354	AGCTGAGATTAAAAGTTAATTCTATCTC	R	Cloning
pEC1715	OLEC6355	AGCTGAGATAGAATTACATTTTAATCTC	F	Cloning
	OLEC6356	AGCTGAGATTAAAATGTAATTCTATCTC	R	Cloning
pEC1716	OLEC6357	AGCTGAGATAGAATTACCGTTTAATCTC	F	Cloning
	OLEC6358	AGCTGAGATTAAACGGTAATTCTATCTC	R	Cloning
pEC1717	OLEC6359	AGCTGAGATAGAATTACCTGTTAATCTC	F	Cloning
	OLEC6360	AGCTGAGATTAACAGGTAATTCTATCTC	R	Cloning
pEC1718	OLEC6361	AGCTGAGATAGAATTACCTTGTAATCTC	F	Cloning
	OLEC6362	AGCTGAGATTACAAGGTAATTCTATCTC	R	Cloning
pEC1719	OLEC6363	AGCTGAGATAGAATTACCTTTGAATCTC	F	Cloning
	OLEC6364	AGCTGAGATTCAAAGGTAATTCTATCTC	R	Cloning
pEC1720	OLEC6365	AGCTGAGATAGAATTACCTTTTCATCTC	F	Cloning
	OLEC6366	AGCTGAGATGAAAAGGTAATTCTATCTC	R	Cloning
pEC1721	OLEC6367	AGCTGAGATAGAATTACCTTTTACTCTC	F	Cloning

Purpose	Primer code	Sequence 5'-3'	F/R	Usage
	OLEC6368	AGCTGAGAGTAAAAGGTAATTCTATCTC	R	Cloning
pEC1722	OLEC6369	AGCTGAGATAGAATTACCTTTTAAGCTC	F	Cloning
	OLEC6370	AGCTGAGCTTAAAAGGTAATTCTATCTC	R	Cloning
pEC1723	OLEC6371	AGCTGAGATAGAATTACCTTTTAATATC	F	Cloning
	OLEC6372	AGCTGATATTAAAAGGTAATTCTATCTC	R	Cloning
pEC1724	OLEC6373	AGCTGAGATAGAATTACCTTTTAATCGC	F	Cloning
	OLEC6374	AGCTGCGATTAAAAGGTAATTCTATCTC	R	Cloning
pEC1725	OLEC6375	AGCTGCTCGAGAATTACCTTTTAATCTC	F	Cloning
	OLEC6376	AGCTGAGATTAAAAGGTAATTCTCGAGC	R	Cloning
pEC1726	OLEC6377	AGCTGAGATAGAATTACCTTTTACGAGC	F	Cloning
	OLEC6378	AGCTGCTCGTAAAAGGTAATTCTATCTC	R	Cloning
pEC1731	OLEC6432	GACGGCCAGTGCAGTCGAGCTCGG	F	Mutagenesis of pEC1688
	OLEC6499	CCTTTTAATCTCATCTTGCATGCCTG	R	
pEC1734	OLEC6432	GACGGCCAGTGCAGTCGAGCTCGG	F	
	OLEC6502	CCTTTTAATCTCCTCTTTCATGCCTG	R	
pEC1735	OLEC6432	GACGGCCAGTGCAGTCGAGCTCGG	F	
	OLEC6515	CCTTTTAATCTCGGCTTGCATGCCTG	R	
Substrates for PAM determination				
targ_wt	OLEC6503	AGCTGTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTATATCCAGAAACTATTGATGGTAC	F	
ntarg_wt	OLEC6504	AGCTGTACCATCAATAGTTTCTGGATATAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTAC	R	
targPAM_A2,3,7T	OLEC6507	AGCTGTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTTTATTTTTATATCCAGAAACTATTGATGGTAC	F	
ntargPAM_T2,3,7A	OLEC6508	AGCTGTACCATCAATAGTTTCTGGATATAAAAATAAAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTAC	R	
targPAM_A2,3T	OLEC6509	AGCTGTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTTTATTATTATATCCAGAAACTATTGATGGTAC	F	
ntargPAM_T2,3A	OLEC6530	AGCTGTACCATCAATAGTTTCTGGATATAATAATAAAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTAC	R	
targPAM_A2,3G	OLEC6531	AGCTGTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTGGATTATTATATCCAGAAACTATTGATGGTAC	F	
ntargPAM_T2,3C	OLEC6532	AGCTGTACCATCAATAGTTTCTGGATATAATAATCCAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTAC	R	
Substrate for radioactive cleavage assays and electrophoretic mobility shift assays				
Psp5	OLEC6213	AGCTGTAGCAAATATTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTATATCCAGAAACTATTGATGGTAATTTACTTATC	F	
	OLEC6214	AGCTGATAAGTAAATTACCATCAATAGTTTCTGGATATAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTAATATTTGCTAC	R	
D_cr5_rsp	OLEC6804	GTCTAAGAACTTTAAATAATTTCTACTGTTGTAGATAGATTAAAAGGTAATTCTATCTTGTTGAG	F	
targRNA	OLEC6805	GTACCATCAATAGTTTCTGGATATAATAATTTAAGATTAAAAGGTAATTCTATCTTGTTGAGATCTGAGCTTTCTTCTATATGATTACCCTATAGTGAGTCGTATTA	F	
nontargRNA	OLEC6806	GTAATCATATAGAAGAAAGCTCAGATCTCAACAAGATAGAATTACCTTTTAATCTTAAATTATTATATCCAGAAACTATTGATGGTACCCTATAGTGAGTCGTATTA	R	
***In vitro* transcription of crRNA**				
T7 promoter	OLEC4211	TAATACGACTCACTATA	F	IVT
T7-crRNA4_rep_sp_proc	OLEC6201	AAAAATGACCTTCATAAATCGCTAATCTACAACAGTAGAACCTATAGTGAGTCGTATTA	R	IVT

Purpose	Primer code	Sequence 5'-3'	F/R	Usage
T7-crRNA5_sp	OLEC6202	AGATAGAATTACCTTTTAATCTACCTATAGTGAGTCGTATTA	R	IVT
T7-crRNA5_rep_sp_proc	OLEC6203	AGATAGAATTACCTTTTAATCTATCTACAACAGTAGAACCTATAG TGAGTCGTATTA	R	IVT
T7-crRNA5_rep_sp_full	OLEC6204	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAGTAGAA ATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-crRNA5_sp_rep_full	OLEC6205	ATCTACAACAGTAGAAATTATTTAAAGTTCTTAGACCTCAACAAG ATAGAATTACCTTTTAATCTCCTATAGTGAGTCGTATTA	R	IVT
T7-crRNA5_rep_sp_rep	OLEC6206	ATCTACAACAGTAGAAATTATTTAAAGTTCTTAGACCTCAACAAG ATAGAATTACCTTTTAATCTATCTACAACAGTAGAAATTATTTAA AGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-crRNA5 no stem	OLEC6318	AGATAGAATTACCTTTTAATCTATGATGAACAGTAGAACCTATAG TGAGTCGTATTA	R	IVT
T7-crRNA5 seq mut	OLEC6440	AGATAGAATTACCTTTTAATCTATGATGTGTTCATCAACCTATAG TGAGTCGTATTA	R	IVT
T7-crRNA5 mut rep	OLEC6441	CTCAACAAGATAGAATTACCTTTTAATCTATGATGAACAGTAGA AATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-crRNA5 struct mut	OLEC6422	CTCAACAAGATAGAATTACCTTTTAATCTATGATGAACAGTAGA AATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_s_loop	OLEC6760	CTCAACAAGATAGAATTACCTTTTAATCTATCTACACAGTAGAAA TTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_l_loop	OLEC6761	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAAGTAGA AATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_s_stem	OLEC6762	CTCAACAAGATAGAATTACCTTTTAATCTATctcAACAgagAAATTA TTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_l_stem	OLEC6763	CTCAACAAGATAGAATTACCTTTTAATCTATctacgAACAcgtagAAA TTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_xl_stem	OLEC6764	CTCAACAAGATAGAATTACCTTTTAATCTATcgtgtacgAACAcgtacacg AAATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_rrs_1	OLEC6765	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAGTAGAA ATaATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_rrs_2	OLEC6766	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAGTAGAA AaTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_rrs_3	OLEC6767	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAGTAGAA tTTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-rep_rrs_4	OLEC6768	CTCAACAAGATAGAATTACCTTTTAATCTATCTACAACAGTAGAt ATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA	R	IVT
T7-cr5_rsp_2	OLEC6773	AGATAGAATTACCTTTTAATCTATCTACAACAGTAGAAATTCCTA TAGTGAGTCGTATTA	R	IVT
Cpf1 plasmids				
pEC1611	OLEC6138	ATGCAGGTCGACATGTCAATTTATCAAGAATTTG	F	Cloning
	OLEC6139	AGCTAGCGGCCGCTTAGTTATTCCTATTCTGCACG	R	Cloning
pEC1701	OLEC6287	ATGCAGGGTACCATGTCAATTTATCAAGAATTTG	F	Cloning
	OLEC6288	AGCTACGGCCGTTAGTTATTCCTATTCTGCACG	R	Cloning
Cpf1 mutagenesis				
pEC1776	OLEC6409	TCGTAAACAATCAATACCTAAAAAAATCACTGCCCCAGCTAAAG AGGCA	F	
	OLEC6410	TGCCTCTTTAGCTGGGGCAGTGATTTTTTTAGGTATTGATTGTTTA CGA	R	

Purpose	Primer code	Sequence 5'-3'	F/R	Usage
pEC1777	OLEC6561	CTCTTTTTTAGGATTATCTTTGTTTGCATTAGCTATTGCCTCTTTAGCTGGGT	F	Mutagenesis of pEC1611
	OLEC6562	ACCCAGCTAAAGAGGCAATAGCTAATGCAAACAAAGATAATCCTAAAAAAGAG	R	
pEC1778	OLEC6563	GAAAAACTTATCTTCAGTAAAGCGTTTATCTGCGATTAAATCATATTCAAAAACACTCTCTTTT	F	
	OLEC6564	AAAAGAGAGTGTTTTTGAATATGATTTAATCGCAGATAAACGCTTTACTGAAGATAAGTTTTTC	R	
pEC1779	OLEC6565	GGACAGTGAAAGAAAAACTTATCTTCAGTAGCGCGTTTATCTTTGATTAAATCATATTCAAA	F	
	OLEC6566	TTTGAATATGATTTAATCAAAGATAAACGCGCTACTGAAGATAAGTTTTTCTTTCACTGTCC	R	
pEC1782	OLEC6444	AAGCTAAATGTCTTTCACCTCTAGCTATACTTAATATATGAACATCATTTGCT	F	
	OLEC6445	AGCAAATGATGTTCATATATTAAGTATAGCTAGAGGTGAAAGACATTTAGCTT	R	
pEC1783	OLEC6476	TCATATATTAAGTATAGATAGAGGTGCAAGACATTTAGCTTACTATACTTTGG	F	
	OLEC6477	CCAAAGTATAGTAAGCTAAATGTCTTGCACCTCTATCTATACTTAATATATGA	R	
pEC1784	OLEC6411	CCATCTACCAAAGTATAGTAAGCTAAAGCTCTTTCACCTCTATCTATACTTAATAT	F	
	OLEC6412	ATATTAAGTATAGATAGAGGTGAAAGAGCTTTAGCTTACTATACTTTGGTAGATGG	R	
pEC1785	OLEC6464	CCTTTACCATCTACCAAAGTATAGGCAGCTAAATGTCTTTCACCTCTATC	F	
	OLEC6465	GATAGAGGTGAAAGACATTTAGCTGCCTATACTTTGGTAGATGGTAAAGG	R	
pEC1788	OLEC6446	CTCTTTTAAATCCAAAATTTAAATCCGCAAAAACCACAATAGCATTATACTCTATAACT	F	
	OLEC6447	AGTTATAGAGTATAATGCTATTGTGGTTTTTGCGGATTTAAATTTTGGATTTAAAAGAG	R	
pEC1790	OLEC6472	AATTAGCATTTTTTCTAACTTTTGAGCGACCTGCTTCTCTACCTTGAAACG	F	
	OLEC6473	CGTTTCAAGGTAGAGAAGCAGGTCGCTCAAAAGTTAGAAAAAATGCTAATT	R	
pEC1791	OLEC6448	GTTTAGTTTCTCAATTAGCATTTTTGCTAACTTTTGATAGACCTGCTTCTC	F	
	OLEC6449	GAGAAGCAGGTCTATCAAAAGTTAGCAAAAATGCTAATTGAGAAACTAAAC	R	
pEC1796	OLEC6419	CTGCTACTGGTGAAATTAGATAAGCTAACTCAGTACCTGTTTTTGAG	F	
	OLEC6420	CTCAAAAACAGGTACTGAGTTAGCTTATCTAATTTCACCAGTAGCAG	R	
pEC1799	OLEC6450	GATAAGCACCATTGGCAGCAGCATCTTGAGGCATA	F	
	OLEC6451	TATGCCTCAAGATGCTGCTGCCAATGGTGCTTATC	R	
Probes for Northern blot analysis of pre-crRNA processing				
spacer	OLEC6528	ATCAAGCCCTTCATGCGCTTCAAGGTGCA	R	
Size markers for radioactive RNA cleavage assays				
37 nt	OLEC5951	AGTTTAGGTACCTTATTTTCTCCACTCTAAACTTGAT		
47 nt	OLEC6260	ATATTCAACATATTGACCGGCCTGCAGAGTAAGGATGTTGGGTCTAC		
54 nt	OLEC6441	CTCAACAAGATAGAATTACCTTTTAATCTATGATGAACAGTAGAAATTATTTAAAGTTCTTAGACCCTATAGTGAGTCGTATTA		
60 nt	OLEC6489	ATGGGCCATCATCATCATCATCATCATCATCACACTACAGTAAAAAAAACAGAGCG		
Plasmid sequencing analysis				
proto-spacer	pUCM13-52	FROM SEQLAB	F	SEQ
	pUCM13-rev-157	FROM SEQLAB	R	SEQ
Plasmids containing Cpf1 or a	T7 prom	FROM SEQLAB	F	SEQ
	T7 term	FROM SEQLAB	R	SEQ
	OLEC6482	GGTGGTAAATTTG	F	SEQ

Purpose	Primer code	Sequence 5'-3'	F/R	Usage
variant thereof	OLEC6483	GTCAGTCAGAAG	F	SEQ
	OLEC6498	GGTTTATAAGCTAAATGGTGAGGC	F	SEQ
pEC1690	OLEC6319	GTCGCGAACGCCAGCAAG	R	SEQ
DNA insert for crRNA cloning				
pEC1690	IDT gBlock	ATGCAGAAGCTTTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCG TCTAAGAACTTTAAATAATTTCTACTGTTGTAGATTGCACCTTGAAGCG CATGAAGGGCTTGATGTCTAAGAACTTTAAATAATTTGTCTGTATATTA TTGATTTCTAAATTAGAATTTTCGGCCGATGCAG		Cloning

Supplementary References

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2 Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M. & Barton, G. J. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189-1191(2009).

3 Chylinski, K., Le Rhun, A. & Charpentier, E. The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems. *RNA Biol.* **10**, 726-737 (2013).

4 Fonfara, I. *et al.* Phylogeny of Cas9 determines functional exchangeability of dual-RNA and Cas9 among orthologous type II CRISPR-Cas systems. *Nucleic Acids Res.* **42**, 2577-2590 (2014).