

**Table S3 The top 100 PAMs ranked by fold-change.**

PAM preference of Cas $\pi$ -1					
50 mM NaCl		150 mM NaCl		300 mM NaCl	
PAM	Fold change	PAM	Fold change	PAM	Fold change
TCCCC	10.42	TGCCC	13.28	TGCCC	27.76
GTCCC	9.99	TCCCC	13.17	TACCC	26.72
TTCCT	9.74	GTCCC	13.01	GACCC	26.44
TGCCC	9.71	TACCC	12.56	TGCCT	26.39
GACCT	9.68	GACCG	12.37	GACCT	26.02
TACCG	9.65	AACCT	12.08	TACCT	25.43
TACCC	9.64	AACCC	12.03	TTCCC	25.23
TTCCC	9.59	TTCCC	11.99	AACCT	25.22
GACCC	9.58	GGCCT	11.98	GGCCT	24.95
GGCCT	9.56	TGCCT	11.98	TCCCC	24.90
AGCCC	9.50	GACCC	11.96	TTCCT	24.64
GGCCC	9.48	GGCCC	11.93	AACCC	24.58
TGCCT	9.44	AGCCC	11.88	TCCCT	24.26
AACCC	9.39	TACCA	11.83	GTCCT	24.15
TTCCG	9.35	ACCCC	11.76	GGCCC	23.99
GTCCT	9.33	CTCCC	11.71	ATCCC	23.99
TACCT	9.29	ATCCT	11.63	AGCCC	23.85
ACCCT	9.27	TACCG	11.62	ATCCT	23.67
TCCCT	9.27	TTCCT	11.59	GTCCC	23.58
AACCT	9.26	CCCCC	11.57	TACCG	23.26
AACCA	9.20	TCCCT	11.56	AGCCT	23.25
GACCA	9.18	TGCCA	11.55	CTCCC	22.62
CTCCC	9.17	GTCCG	11.53	CACCC	22.27
TGCCG	9.10	ATCCC	11.52	CGCCT	21.78
GACCG	9.09	GCCCC	11.51	CCCCC	21.40
GTCCG	9.09	TACCT	11.47	GCCCT	21.35
GCCCC	9.06	TTCCG	11.40	GACCG	21.25
AGCCA	9.04	ACCCT	11.35	CGCCC	20.82
ATCCT	9.04	GTCCT	11.34	ACCCC	20.80
TACCA	9.04	TGCCG	11.33	CACCT	20.61
ATCCC	8.98	GACCT	11.30	ACCCT	20.48
ACCCC	8.95	AGCCT	11.12	AACCG	20.03
CCCCC	8.93	AACCA	10.93	TGCCG	19.88
TTCCA	8.92	ATCCG	10.88	CTCCT	19.62
GCCCG	8.89	GACCA	10.88	GCCCC	19.41
AGCCG	8.87	CACCC	10.85	CCCCT	19.26
TCCCG	8.72	GGCCG	10.84	TACCA	17.08
CACCG	8.69	AGCCA	10.75	AGCCG	16.37

TGCCA	8.66	AGCCG	10.75	ATCCG	16.32
AACCG	8.65	CACCG	10.71	TTCCG	15.98
GGCCA	8.64	AACCG	10.68	CACCG	15.79
ATCCG	8.63	CCCCT	10.50	TGCCA	15.68
GGCCG	8.62	CACCT	10.35	CGCCG	15.53
AGCCT	8.62	TTCCA	10.33	GGCCG	15.26
GTCCA	8.56	CGCCG	10.29	GTCCG	15.19
ATCCA	8.56	CACCA	10.28	AACCA	14.96
CACCC	8.55	CGCCC	10.21	GACCA	14.70
TCCCA	8.39	TCCCG	10.16	CACCA	13.61
CGCCC	8.31	ATCCA	10.15	AGCCA	13.33
CGCCT	8.30	GCCCT	10.07	TTCCA	13.08
CACCT	8.19	CGCCT	10.04	TCCCG	12.92
ACCCG	8.18	CTCCT	9.93	CGCCA	12.90
CACCA	8.16	GTCCA	9.91	GGCCA	12.73
CTCCG	8.11	CGCCA	9.90	CTCCG	12.71
CTCCT	8.09	ACCCG	9.87	GTCCA	12.67
ACCCA	8.05	CTCCG	9.87	ATCCA	12.54
CCCCT	7.94	TCCCA	9.83	ACCCG	12.29
GCCCA	7.93	GGCCA	9.83	TCCCA	12.29
CGCCA	7.93	GCCCG	9.66	GCCCG	11.36
CGCCG	7.91	GCCCA	9.52	ACCCA	11.17
CTCCA	7.84	CCCCG	9.29	GCCCA	11.06
CCCCG	7.79	CCCCA	9.01	CCCCG	11.03
CCCCA	7.47	ACCCA	8.89	CTCCA	10.60
GCCCT	7.30	CTCCA	8.87	CCCCA	10.42
ACCTA	5.68	GCCTA	6.81	TACTC	2.81
GCCTA	5.65	ACCTA	6.64	GTACT	2.77
TACTC	5.61	TACTC	5.77	TAACT	2.64
TCCTA	5.54	TAACT	5.73	GAACT	2.50
GTACT	4.94	GTACT	5.47	GCACT	2.40
TCACT	4.82	GATCT	5.41	GATCT	2.32
TAACT	4.81	TTACT	5.31	TTACT	2.06
GAACT	4.71	GAACT	5.24	TCACT	2.01
TTACT	4.69	TCCTA	5.12	ATACT	1.99
GATCT	4.61	GCACT	5.04	AAACT	1.83
ACCGA	4.59	ATACT	4.99	GTACC	1.82
GCCGA	4.48	ACACT	4.79	ACACT	1.80
ATACT	4.38	GTACC	4.67	AACTC	1.75
GACTC	4.31	TACTT	4.65	GCCTA	1.75
AACTC	4.20	TCACT	4.64	GACTC	1.74
GTACC	4.19	TGTCT	4.63	ACCTA	1.70
ACACT	4.17	ACCGA	4.58	GATCC	1.69

CACTC	4.16	TGACT	4.56	TACTT	1.59
GCACT	4.13	AAACT	4.54	GAACC	1.57
AAACT	4.12	GATCC	4.49	TAACC	1.57
TCCGA	4.08	GGACT	4.41	CACTC	1.51
TGACT	4.06	GAACC	4.35	TGACT	1.50
GATCC	4.03	GCCGA	4.33	TGTCT	1.40
TGTCT	3.99	AACTC	4.28	TGCTC	1.37
GGACT	3.94	GACTC	4.24	GCACC	1.31
CCCTA	3.92	TGCTC	4.09	GGACT	1.31
GAACC	3.90	TATCT	4.07	CAACT	1.30
TACTT	3.90	CACTC	4.07	GCTCT	1.28
TGCTC	3.89	TAACC	4.01	ATACC	1.26
TATCT	3.80	TCTCT	3.97	GTTCT	1.25
AGACT	3.77	GTTCT	3.88	GACTT	1.24
CAACT	3.76	GACTT	3.88	AAACC	1.19
TCACC	3.76	CAACT	3.83	TTACC	1.19
TGACC	3.75	ATACC	3.81	TCTCT	1.18
GCACC	3.74	AACTT	3.74	TATCT	1.15
GACTT	3.71	GCACC	3.73	AACTT	1.15

PAM preference of Cas $\pi$ -2					
50 mM NaCl		150 mM NaCl		300 mM NaCl	
PAM	Fold change	PAM	Fold change	PAM	Fold change
TACCC	32.14	TGCCC	30.19	TGCCC	28.17
GTCCC	31.80	GTCCC	29.61	TACCC	27.20
GACCC	31.73	TACCC	29.21	TTCCC	27.09
AGCCC	30.35	AACCC	27.09	TCCCC	26.79
TGCCC	30.27	GACCC	27.02	GACCC	26.39
GGCCC	30.15	GGCCC	26.79	GGCCC	25.94
AACCC	29.18	AGCCC	26.45	GTCCC	25.16
TTCCC	28.05	TTCCC	26.43	AGCCC	24.84
ATCCC	27.76	TCCCC	26.17	ATCCC	24.80
CACCC	26.95	CTCCC	26.05	AACCC	24.18
TCCCC	26.93	ATCCC	25.76	CCCCC	24.16
CTCCC	26.85	CCCCC	25.28	GGCCT	23.99
CGCCC	26.29	CACCC	24.54	TGCCT	23.96
CCCCC	25.99	AACCT	24.06	TCCCT	23.46
GACCT	25.84	TACCT	23.97	GCCCT	23.42
TACCT	25.13	TGCCT	23.71	GACCT	23.42
TGCCT	24.67	GGCCT	23.47	CTCCC	23.25
GGCCT	24.40	ATCCT	23.22	TTCCT	23.13
AACCT	23.81	ACCCC	23.18	ACCCC	23.02

GTCCT	23.67	GACCT	23.00	TACCT	22.96
GCCCC	23.43	GTCCCT	22.70	GTCCCT	22.59
ACCCC	23.28	CGCCC	22.47	CACCC	22.55
TTCCT	23.09	AGCCT	22.38	AACCT	22.46
ATCCT	22.39	GCCCC	22.26	GCCCC	22.17
AGCCT	21.85	TTCCT	22.15	AGCCT	21.99
CACCT	21.50	ACCCT	20.80	ATCCT	21.84
CGCCT	21.26	CACCT	20.66	CGCCC	21.56
ACCCT	20.11	CCCCT	20.41	ACCCT	21.28
CTCCT	20.06	TCCCT	20.33	CGCCT	20.37
TCCCT	19.78	CTCCT	19.86	CCCCT	20.33
CCCCT	19.51	CGCCT	19.59	CTCCT	19.33
GCCCT	18.63	GCCCT	18.85	CACCT	18.96
GACCA	12.69	TACCA	15.07	TACCA	15.86
TACCA	12.59	GACCA	13.02	GACCA	13.87
AGCCA	11.05	AGCCA	12.51	AGCCA	13.47
AACCA	10.35	AACCA	12.32	TGCCA	13.09
CACCA	9.97	TGCCA	11.92	AACCA	12.75
CGCCA	9.77	CGCCA	11.58	CGCCA	12.59
TACTC	9.59	CACCA	11.54	CACCA	11.98
GGCCA	9.58	TACTC	10.97	TACTC	11.52
TACCG	9.57	TACCG	10.82	GGCCA	11.30
TGCCA	9.29	GGCCA	10.75	TACCG	10.74
GACCG	7.77	GACCG	9.88	GTCCA	9.36
GTCCA	7.39	ATCCA	8.81	ATCCA	9.36
GACTC	7.29	GTCCA	8.67	GACCG	7.97
ATCCA	7.27	AGCCG	8.25	CACTC	7.85
AGCCG	6.93	GACTC	8.06	GACTC	7.79
CACTC	6.83	TGCTC	8.04	TGCTC	7.78
CTCCA	6.55	CACTC	7.80	TTCCA	7.74
CCCCA	6.26	TGCCG	7.77	CTCCA	7.53
TGCTC	6.23	CCCCA	7.50	AGCCG	7.38
GGCCG	6.14	CGCTC	7.49	TGCCG	7.35
TGCCG	6.14	CTCCA	7.46	CGCTC	7.32
CGCTC	6.13	CACCG	7.44	CCCCA	7.25
CACCG	6.00	GGCCG	7.41	AACCG	6.98
TTCCA	5.92	AACCG	7.38	AACTC	6.74
AACCG	5.86	TTCCA	7.16	GGCCG	6.51
ACCCA	5.55	GTCCG	6.82	TCCCA	6.15
GTCCG	5.53	CGCCG	6.82	CGCCG	6.10
AACTC	5.50	AACTC	6.79	AGCTC	6.08
CGCCG	5.45	ATCCG	6.58	ACCCA	6.06
GCCCA	5.44	AGCTC	6.40	ATCCG	5.96

AGCTC	5.23	GCCCA	6.37	CACCG	5.93
ATCCG	5.23	ACCCA	6.23	GCCCA	5.86
TCCCA	5.06	TCCCA	6.11	GTCTC	5.81
GGCTC	4.80	GGCTC	5.75	ATCTC	5.68
GTCTC	4.59	GTCTC	5.66	GTCCG	5.55
ATCTC	4.09	TTCCG	5.05	GGCTC	5.37
TTCCG	4.04	CTCCG	4.99	GTACC	4.56
CTCCG	4.03	ATCTC	4.81	TTCCG	4.50
CTCTC	3.69	CTCTC	4.59	TTCTC	4.42
GTACC	3.60	TTCTC	4.23	CTCTC	4.35
TTCTC	3.56	TACTT	4.08	CTCCG	4.19
CCCCG	3.43	GTACC	4.02	TGACC	4.19
TGACC	3.29	CCCCG	3.79	TGTCC	3.62
TCCTC	2.94	TGACC	3.66	TCCTC	3.56
CCCTC	2.94	TCCTC	3.58	TACTT	3.49
TGTCC	2.81	TGTCC	3.44	CCCCG	3.47
TCCCG	2.68	ACCCG	3.39	TCCCG	3.19
TACTT	2.65	CCCTC	3.28	CCCTC	3.14
ACCCG	2.63	TGCTT	3.17	ACCCG	3.14
TTACC	2.57	TCCCG	3.10	TTACC	3.02
GCCCG	2.49	GTACTION	3.05	GTACTION	2.97
GTACTION	2.47	GCCTC	2.92	TGCTT	2.89
GCCTC	2.36	ACCTC	2.82	GCCTC	2.87
TGCTT	2.35	CGCTT	2.73	CGACC	2.85
CGTCC	2.31	GACTT	2.72	ACCTC	2.79
ACCTC	2.26	AGCTT	2.71	GTTCC	2.72
GTTCC	2.25	GCCCG	2.68	CGTCC	2.51
ATACC	2.18	CGTCC	2.63	ATACC	2.47
GACTT	2.12	TTACC	2.63	GTCTT	2.40
AGCTT	2.05	GTTCC	2.62	CGCTT	2.39
CGACC	2.01	GTCTT	2.62	GCACC	2.39
CGCTT	1.97	CGACC	2.52	GCCCG	2.37
TCACC	1.91	CACTT	2.42	TTACT	2.29
GCACC	1.85	GGCTT	2.42	TCACC	2.26
GCTCC	1.84	TTACT	2.32	GGACC	2.26
TTACT	1.84	AACTT	2.22	GACTT	2.22
GTCTT	1.81	ATACC	2.21	GGTCC	2.19
TCTCC	1.81	ATCTT	2.20	AGCTT	2.17