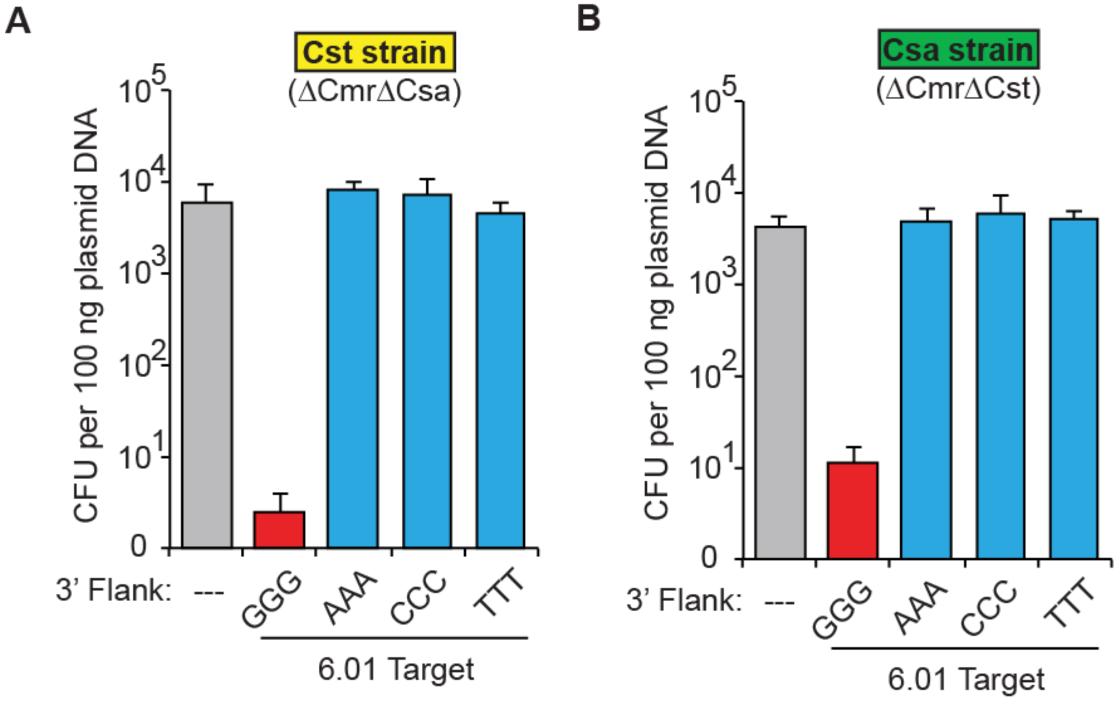
Supplementary Material for

DNA Targeting by the Type I-G and Type I-A CRISPR-Cas Systems of *Pyrococcus furiosus*

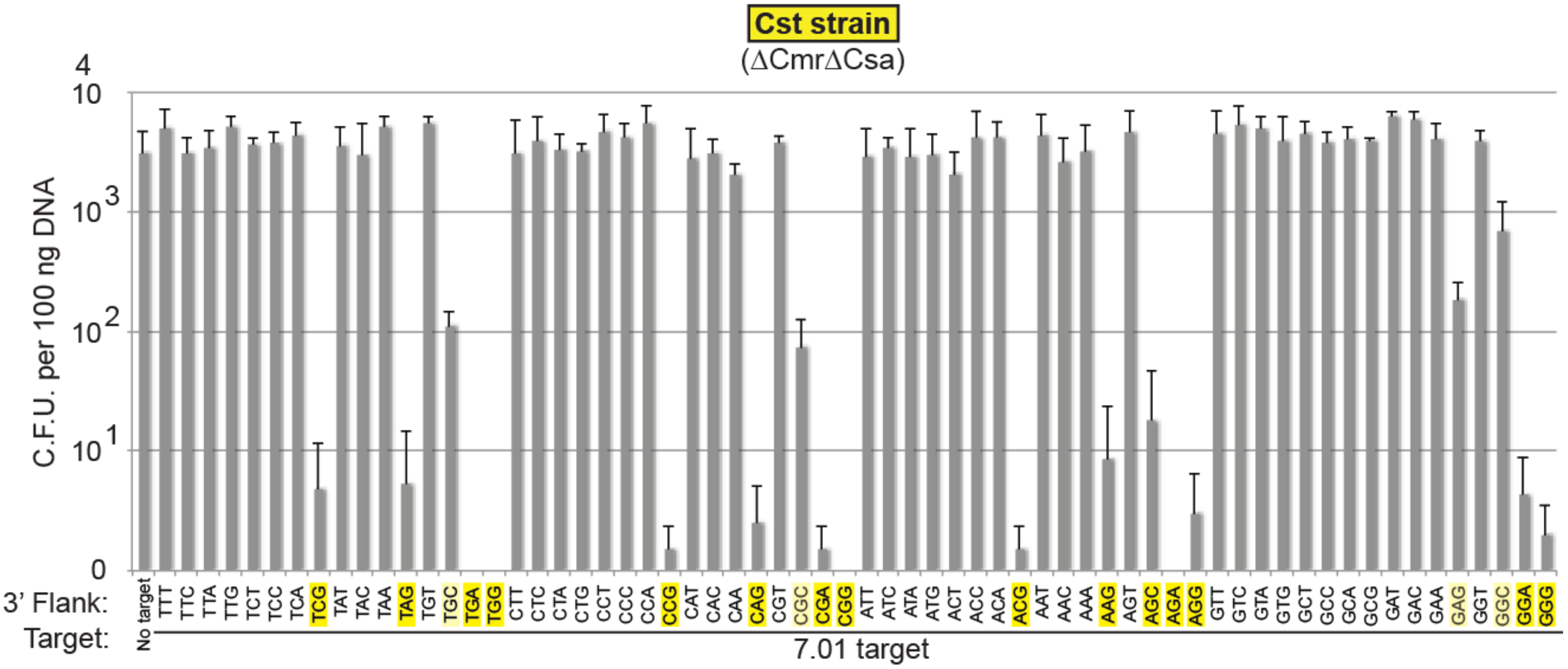
Joshua Elmore, Trace Deighan, Jan Westpheling, Rebecca M. Terns,
Michael P. Terns

Supplemental Figure S1



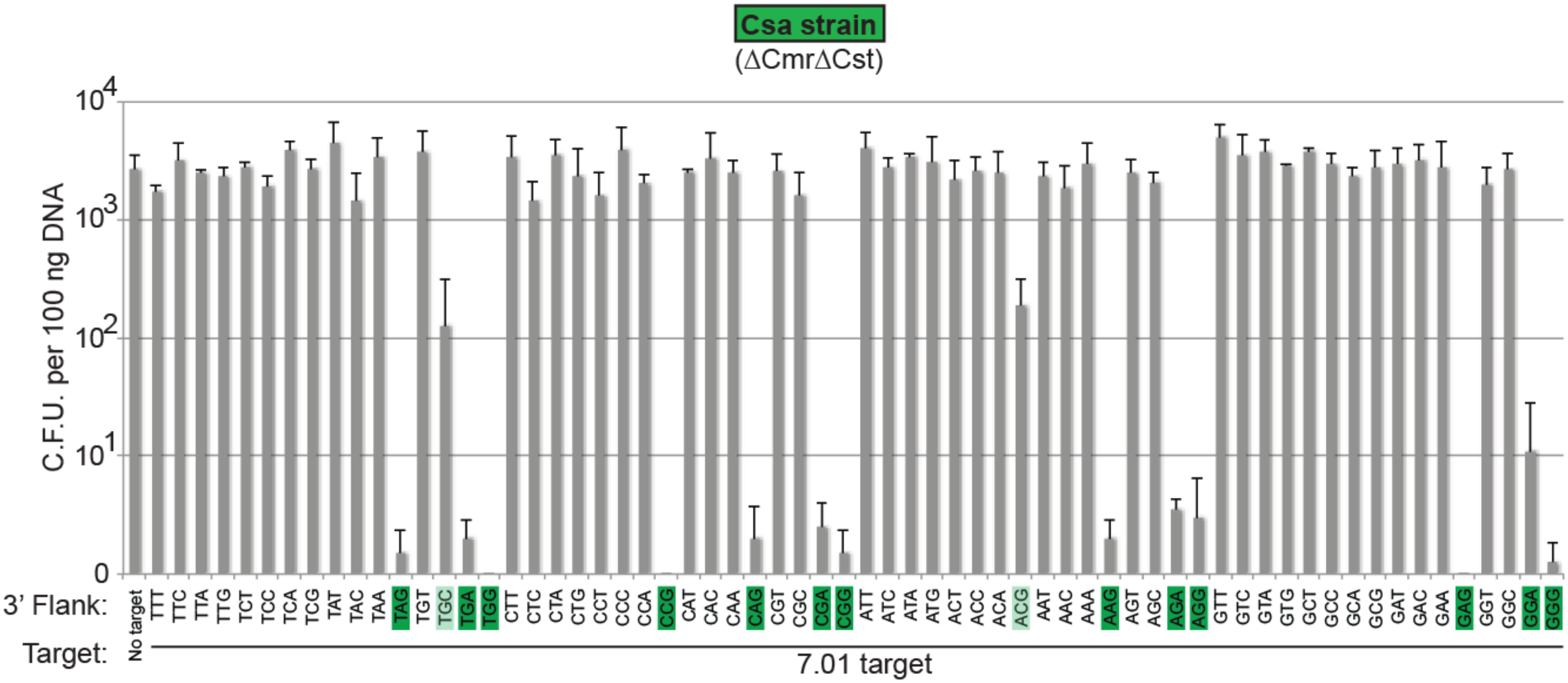
Supplemental Figure S1. *P. furiosus* Cst and Csa CRISPR-Cas systems silence plasmid DNA in a PAM-dependent manner. Plots of plasmid challenge assay results in *Pfu* strains harboring the Cst (**A**) or Csa (**B**) CRISPR-Cas systems. Colony forming units are plotted on the Y-axis, with the standard deviation in 3 replicates indicated by error bars. The CRISPR target and the sequence immediately downstream (3' flank) are indicated on the X-axis. Additionally, bar color indicates plasmids with no target (gray), with a CRISPR target and a predicted PAM (red) or predicted non-PAM (blue). The name and genotype of the assayed strain is indicated above each panel.

Supplemental Figure S2



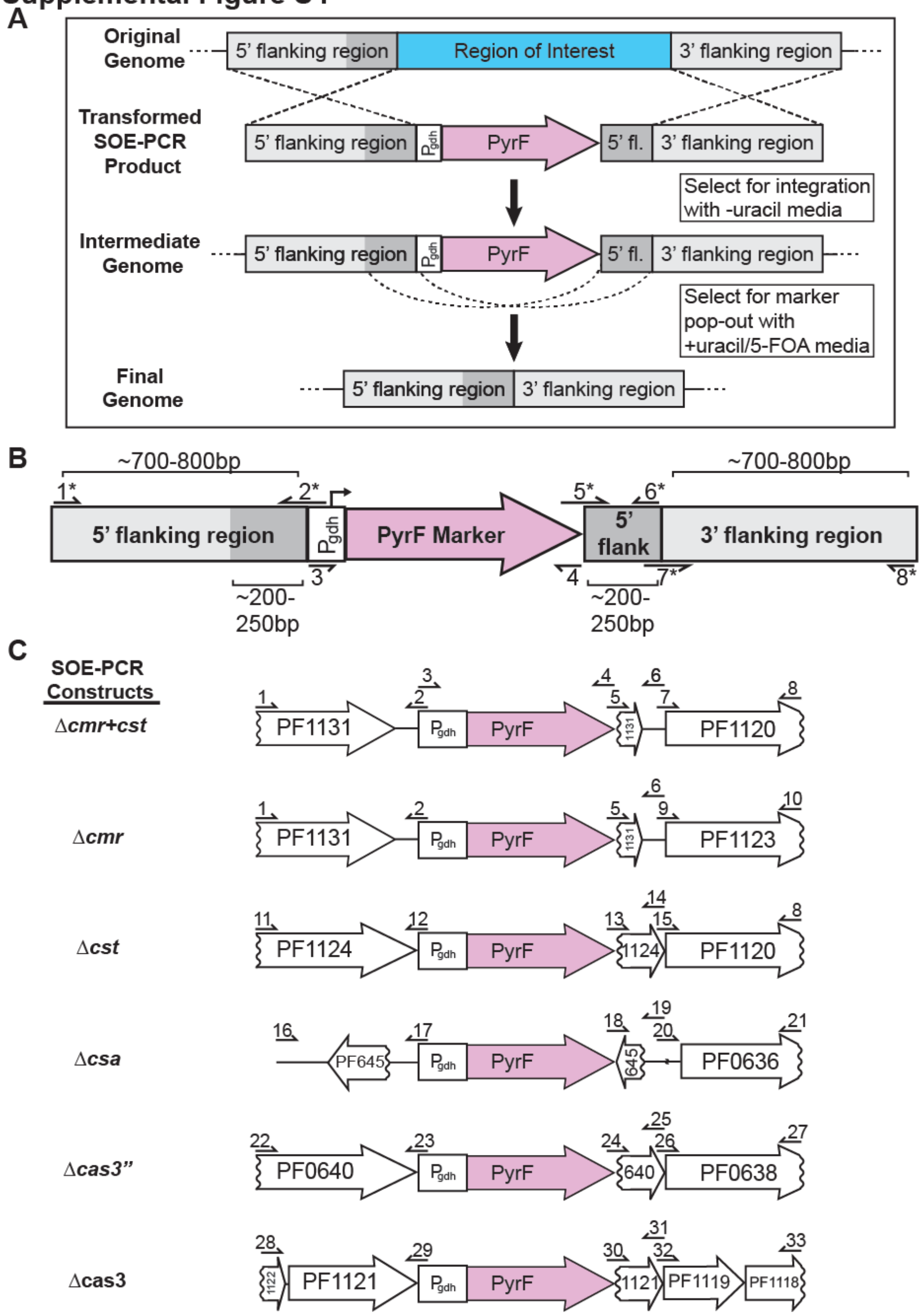
Supplemental Figure S2. ***P. furiosus*** **Cst plasmid interference PAM requirements.** Plot of plasmid challenge assay results in *Pfu* strain harboring the Cst CRISPR-Cas system. Colony forming units are plotted on the Y-axis, with the standard deviation in at least 3 replicates indicated by error bars. The CRISPR target and the sequence immediately downstream (3' flank) are indicated on the X-axis. Yellow boxes indicate plasmids with flanking sequences that are utilized as PAMs (dark) or weak PAMs (light) by the Cst system.

Supplemental Figure S3



Supplemental Figure S3. ***P. furiosus*** **Csa plasmid interference PAM requirements.** Plot of plasmid challenge assay results in *Pfu* strain harboring the Csa CRISPR-Cas system. Colony forming units are plotted on the Y-axis, with the standard deviation in at least 3 replicates indicated by error bars. The CRISPR target and the sequence immediately downstream (3' flank) are indicated on the X-axis. Green boxes indicate plasmids with flanking sequence that are utilized as PAMs (dark) or weak PAMs (light) by the Csa system.

Supplemental Figure S4



Supplemental Figure S4. Construction of *Pf* strains. (A) Graphic representation of the steps involved in *Pf* strain construction by homologous recombination of transformed SOE-PCR (splicing by overlap extension polymerase chain reaction) constructs. **(B)** Detailed graphic representation of a generic SOE-PCR construct containing the PyrF expression cassette, with approximate sizes and primer locations indicated. **(C)** Graphic representation of the individual SOE-PCR constructs used for strain construction in this study. Annotated *Pf* ORF numbers are indicated. Primer numbers refer to oligos in Table S2.

Table S1 - Strains and Plasmids

***E.coli* Strains**	**Relevant Characteristics**	**Source or Reference**
Top10	F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *galU galK rpsL* (StrR) *endA1 nupG*	Life Technologies

***P. furiosus* Strains**	**Relevant Characteristics**	**Source or Reference**
JFW02 (WT)	Δ*pyrF* Δ*trpAB*	(1)
TPF01 (ΔCas3")	JFW02 Δ*cas3"*	This study
TPF02 (ΔCas3)	JFW02 Δ*cas3*	This study
TPF04 (ΔCsa)	JFW02 Δ*csa* (Δ*PF0637-0644*)	This study
TPF06 (ΔCmr)	JFW02 Δ*cmr* (Δ*PF1124-PF1130*)	This study
TPF07 (Csa)	JFW02 Δ*cmr+cst* (Δ*PF1121-PF1130*)	This study
TPF10 (ΔCas3, ΔCas3")	TPF01 Δ*cas3*	This study
TPF15 (Cmr)	TPF04 Δ*cst* (Δ*PF1121-1123*)	This study
TPF17 (Cst)	TPF06 Δ*csa* (Δ*PF0637-0644*)	This study
TPF20 (null)	TPF07 Δ*csa* (Δ*PF0637-0644*)	This study
TPF29 (Cst ΔCas3)	TPF17 Δ*cas3 (*Δ*PF1120)*	This study
TPF30 (Csa ΔCas3")	TPF07 Δcas3" (Δ*PF0639*)	This study

Plasmids	**Relevant Characteristics**	**Source or Reference**
pJFW17	AprR general cloning vector with *E.coli* OriT, and *Pfu* Pgdh-pyrF cassette	(2)
pJFW18	pJFW17 derivative; *Pfu* OriC for replication in *P.furiosus*	(2)
pJE18	pJFW18 derivative; 7.01 spacer, GGG flank	This study
pJE19	pJFW18 derivative; 7.01 spacer, 5' tag complement flank	This study
pJE20	pJFW18 derivative; 1.01 spacer, GGG flank	This study
pJE21	pJFW18 derivative; 1.01 spacer, 5' tag complement flank	This study
pJE22	pJFW18 derivative; 7.01 spacer, GGG flank, reverse orientation	This study
pJE31	pJFW18 derivative; 7.01 spacer, GCA flank	This study
pJE33	pJFW18 derivative; 1.01 spacer, GCA flank	This study
pJE47	pJFW18 derivative; Tk-csg promoter/Tk-chiA terminator expression cassette	This study
pJE186	pJE47 derivative; 7.01 spacer, TTT flank	This study
pJE187	pJE47 derivative; 7.01 spacer, TTC flank	This study
pJE188	pJE47 derivative; 7.01 spacer, TTA flank	This study
pJE189	pJE47 derivative; 7.01 spacer, TTG flank	This study
pJE190	pJE47 derivative; 7.01 spacer, TCT flank	This study
pJE191	pJE47 derivative; 7.01 spacer, TCC flank	This study
pJE192	pJE47 derivative; 7.01 spacer, TCA flank	This study
pJE193	pJE47 derivative; 7.01 spacer, TCG flank	This study
pJE194	pJE47 derivative; 7.01 spacer, TAT flank	This study
pJE195	pJE47 derivative; 7.01 spacer, TAC flank	This study
pJE196	pJE47 derivative; 7.01 spacer, TAA flank	This study
pJE197	pJE47 derivative; 7.01 spacer, TAG flank	This study
pJE198	pJE47 derivative; 7.01 spacer, TGT flank	This study
pJE199	pJE47 derivative; 7.01 spacer, TGC flank	This study
pJE200	pJE47 derivative; 7.01 spacer, TGA flank	This study
pJE201	pJE47 derivative; 7.01 spacer, TGG flank	This study
pJE202	pJE47 derivative; 7.01 spacer, CTT flank	This study
pJE203	pJE47 derivative; 7.01 spacer, CTC flank	This study

Plasmids	**Relevant Characteristics**	**Source or Reference**
pJE204	pJE47 derivative; 7.01 spacer, CTA flank	This study
pJE205	pJE47 derivative; 7.01 spacer, CTG flank	This study
pJE206	pJE47 derivative; 7.01 spacer, CCT flank	This study
pJE207	pJE47 derivative; 7.01 spacer, CCC flank	This study
pJE208	pJE47 derivative; 7.01 spacer, CCA flank	This study
pJE209	pJE47 derivative; 7.01 spacer, CCG flank	This study
pJE210	pJE47 derivative; 7.01 spacer, CAT flank	This study
pJE211	pJE47 derivative; 7.01 spacer, CAC flank	This study
pJE212	pJE47 derivative; 7.01 spacer, CAA flank	This study
pJE213	pJE47 derivative; 7.01 spacer, CAG flank	This study
pJE214	pJE47 derivative; 7.01 spacer, CGT flank	This study
pJE215	pJE47 derivative; 7.01 spacer, CGC flank	This study
pJE216	pJE47 derivative; 7.01 spacer, CGA flank	This study
pJE217	pJE47 derivative; 7.01 spacer, CGG flank	This study
pJE218	pJE47 derivative; 7.01 spacer, ATT flank	This study
pJE219	pJE47 derivative; 7.01 spacer, ATC flank	This study
pJE220	pJE47 derivative; 7.01 spacer, ATA flank	This study
pJE221	pJE47 derivative; 7.01 spacer, ATG flank	This study
pJE222	pJE47 derivative; 7.01 spacer, ACT flank	This study
pJE223	pJE47 derivative; 7.01 spacer, ACC flank	This study
pJE224	pJE47 derivative; 7.01 spacer, ACA flank	This study
pJE225	pJE47 derivative; 7.01 spacer, ACG flank	This study
pJE226	pJE47 derivative; 7.01 spacer, AAT flank	This study
pJE227	pJE47 derivative; 7.01 spacer, AAC flank	This study
pJE228	pJE47 derivative; 7.01 spacer, AAA flank	This study
pJE229	pJE47 derivative; 7.01 spacer, AAG flank	This study
pJE230	pJE47 derivative; 7.01 spacer, AGT flank	This study
pJE231	pJE47 derivative; 7.01 spacer, AGC flank	This study
pJE232	pJE47 derivative; 7.01 spacer, AGA flank	This study
pJE233	pJE47 derivative; 7.01 spacer, AGG flank	This study
pJE234	pJE47 derivative; 7.01 spacer, GTT flank	This study
pJE235	pJE47 derivative; 7.01 spacer, GTC flank	This study
pJE236	pJE47 derivative; 7.01 spacer, GTA flank	This study
pJE237	pJE47 derivative; 7.01 spacer, GTG flank	This study
pJE238	pJE47 derivative; 7.01 spacer, GCT flank	This study
pJE239	pJE47 derivative; 7.01 spacer, GCC flank	This study
pJE240	pJE47 derivative; 7.01 spacer, GCA flank	This study
pJE241	pJE47 derivative; 7.01 spacer, GCG flank	This study
pJE242	pJE47 derivative; 7.01 spacer, GAT flank	This study
pJE243	pJE47 derivative; 7.01 spacer, GAC flank	This study
pJE244	pJE47 derivative; 7.01 spacer, GAA flank	This study
pJE245	pJE47 derivative; 7.01 spacer, GAG flank	This study
pJE246	pJE47 derivative; 7.01 spacer, GGT flank	This study
pJE247	pJE47 derivative; 7.01 spacer, GGC flank	This study
pJE248	pJE47 derivative; 7.01 spacer, GGA flank	This study
pJE249	pJE47 derivative; 7.01 spacer, GGG flank	This study
pJE252	pJE47 derivative; Pfu Cas3 (PF1120) expression vector	This study
pJE253	pJE47 derivative; Pfu Cas3" (PF0639) expression vector	This study
pJE257	pJE253 derivative; 7.01 spacer, GGG flank	This study
pJE258	pJE252 derivative; 7.01 spacer, GGG flank	This study

Plasmids	**Relevant Characteristics**	**Source or Reference**
pJE269	pJE47 derivative; Pfu Cas3'+3" (PF0639/PF0640) expression vector	This study
pJE270	pJE269 derivative; 7.01 spacer, GGG flank	This study
pJE275	pJE47 derivative; 6.01 spacer, GGG flank	This study
pJE299	pJE47 derivative; 6.01 spacer, AAA flank	This study
pJE300	pJE47 derivative; 6.01 spacer, CCC flank	This study
pJE301	pJE47 derivative; 6.01 spacer, TTT flank	This study
pLC64-ChiA	*T.kodakaraensis* shuttle vector with Pcsg-ChiA expression cassette	(3)

Table S2

Northern Probe Oligos

Northern Probe	Sequence (5'-3')
Repeat antisense (835)	CTTTCAATTCTTTTGTAGTCTTATTGGAAC
6.01 antisense (898)	CTAAGGACATTTGTACGTCAAATTCTTCAC
7.01 antisense (883)	GCTCTCAGCCGCAAGGACCGCATAC
Pfu 5S rRNA antisense (1008)	CCCGGCTTCCCGCCCCCTCT

SOE-PCR Construct Primer Oligos

Primer	Sequence (5'-3')
Pgdh_PyrF_F (3)	GATTGAAAATGGAGTGAGCTGAG
Pdgh_PyrF_R (4)	TTATCTTGAGCTCCATTCTTTCACC
ΔCmr_1 (1)	TCCAATCCGAAGCTTGCAACATA
ΔCmr_2 (2)	CTCAGCTCACTCCATTTTCAATCGCTACCTCACCGAGCCAAATAAAGTG
ΔCmr_5 (5)	GGTGAAAGAATGGAGCTCAAGATAACTGGGCTTCGGAATGGTTAAGG
ΔCmr_6 (6)	GCTACCTCACCGAGCCAAATAAAGTG
ΔCmr_7 (9)	CACTTTATTTGGCTCGGTGAGGTAGCTTGCCGTTGGTGGCAGAGATAG
ΔCmr_8 (10)	GCCTTTGGTACCCTCTCCCAGA
ΔCmr+Cst_7 (7)	CACTTTATTTGGCTCGGTGAGGTAGCATGAAACCGTGCTTTGCAAAATTTCTTC
ΔCst_1 (11)	TCGTTGCCAATTGAAACTAAGGT
ΔCst_2 (12)	CTCAGCTCACTCCATTTTCAATCCTAAACATATTCAACAAGCCTCCCATAG
ΔCst_5 (13)	GGTGAAAGAATGGAGCTCAAGATAAATGTCCCACCTCCTGGGGACT
ΔCst_6 (14)	CTAAACATATTCAACAAGCCTCCCATAG
ΔCst_7 (15)	CTATGGGAGGCTTGTTGAATATGTTTAGATGAAACCGTGCTTTGCAAAATTTCTTC
ΔCst_8 (8)	GGGCCGCTTCAGTCTTTCCATA
ΔCsa_1 (16)	GGATTTTGTATTGCCTCACGGTTA
ΔCsa_2 (17)	CTCAGCTCACTCCATTTTCAATCGTTTTTCTGTATCGAATATTCCCCGAATG
ΔCsa_5 (18)	GGTGAAAGAATGGAGCTCAAGATAATCCCAGGTTCTGGTTTGACAAG
ΔCsa_6 (19)	GTTTTTCTGTATCGAATATTCCCCGAATG
ΔCsa_7 (20)	CATTCGGGGAATATTCGATACAGAAAAACAGCTTTATCTTTTCCCATAACCATTAGG
ΔCsa_8 (21)	TGGCTCCCTTAACTCGCTGGA
ΔPF0639_1 (22)	ACGTTGGTGACATTGACACTGC
ΔPF0639_2 (23)	CTCAGCTCACTCCATTTTCAATCTCATACTCTCACCCCCCAGTTTTCG
ΔPF0639_5 (24)	GGTGAAAGAATGGAGCTCAAGATAATGGGTATGGAAAGTTCGGAAATCA
ΔPF0639_6 (25)	TCATACTCTCACCCCCCAGTTTTCG
ΔPF0639_7 (26)	CGAAAACTGGGGGGTGAGAGTATGAGGGGGAAGTTAAATGAAGCTTG
ΔPF0639_8 (27)	GAGCCCAGGCACTTCTTCAG
ΔPF1120_1 (28)	GAGCTAGGACTGGACATAGAGGCA
ΔPF1120_2 (29)	CTCAGCTCACTCCATTTTCAATCTCATGGCCTCCACCTATGCAGATAAAC
ΔPF1120_5 (30)	GGTGAAAGAATGGAGCTCAAGATAAGCTCCAATAGGAGGGACAGTTG
ΔPF1120_6 (31)	TCATGGCCTCCACCTATGCAGATAAAC

ΔPF1120_7 (32)	GTTTATCTGCATAGGTGGAGGCCATGAATGGAAGAGTACGCCTTAGAGGAGC
ΔPF1120_8 (33)	TGGCTCCTCCAACCACAAAG

***numbers in parentheses refer to Figure S4, panel C labels**

PCR Screening Oligos

Primer	Sequence (5'-3')
ΔCmr+Cst_seq_For	TTGGAGATAGGTTCACGTGGT
ΔCmr+Cst_seq_Rev	AAATCCCTGATGAGCTGTGG
ΔCmr+Cst_seq_Int	CTGGGCTTCGGAATGGTTAAGG
ΔCmr_seq_For	TTGGAGATAGGTTCACGTGGT
ΔCmr_seq_Rev	GCGTGAGCCACAAATCTAGTC
ΔCsa_seq_For	CGAGATTGAAACAGGAGCTG
ΔCsa_seq_Rev	TTGGGAGGAGCTGTAATTGG
ΔCsa_seq_Int	TCCCAGGTTCTGGTTTGACAAG
ΔCst_seq_For	CCTGGGGGAGAGACAGAACT
ΔCst_seq_Rev	AAATCCCTGATGAGCTGTGG
ΔCst_seq_Int	ATGTCCCACCTCCTGGGGACT
ΔPF0639_seq_For	ACCGGGAGAAGAAGGAGAAG
ΔPF0639_seq_Rev	GCACGTGCTTTCAAGTATCC
ΔPF0639_seq_Int	TGGGTATGGAAAGTTCGGAAATCA
ΔPF1120_seq_For	CCTGGGGATAATTGTCAACG
ΔPF1120_seq_Rev	CCTGCCTAATTCTTCCCTCA
ΔPF1120_seq_Int	GCTCCAATAGGAGGGACAGTTG

Oligos for Target Cloning

Oligos	Sequence (5'-3')
Pf_7.01_GGG+	[Phos]GGCCGCCTCGGATCCCTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGATCCGC
Pf_7.01_GGG-	[Phos]GGCCGCGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAGGGATCCGAGGC
Pf_7.01_tagcomp+	[Phos]GGCCGCCTATTGAAAGTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGATCCGC
Pf_7.01_tagcomp-	[Phos]GGCCGCGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAACTTTCAATAGGC
Pf_1.01_GGG+	[Phos]GGCCGCCTCGGATCCCTTATATGATTCATTTCTTGTCTTAAGCAATCTGATCTGACCAGAGCTGAGGATCCGC
Pf_1.01_GGG-	[Phos]GGCCGCGGATCCTCAGCTCTGGTCAGATCAGATTGCTTAAGACAAGAAATGAATCATATAAGGGATCCGAGGC
Pf_1.01_tagcomp+	[Phos]GGCCGCCTATTGAAAGTTATATGATTCATTTCTTGTCTTAAGCAATCTGATCTGACCAGAGCTGAGGATCCGC
Pf_1.01_tagcomp-	[Phos]GGCCGCGGATCCTCAGCTCTGGTCAGATCAGATTGCTTAAGACAAGAAATGAATCATATAACTTTCAATAGGC
Pf_1.01_GCA+	[Phos]GGCCGCCTCGGATTGCTTATATGATTCATTTCTTGTCTTAAGCAATCTGATCTGACCAGAGCTGAGGATCCGC
Pf_1.01_GCA-	[Phos]GGCCGCGGATCCTCAGCTCTGGTCAGATCAGATTGCTTAAGACAAGAAATGAATCATATAAGCAATCCGAGGC

Pf_7.01_GCA+	[Phos]GGCCGCCTCGGATTGCTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGATCCGC
Pf_7.01_GCA-	[Phos]GGCCGCGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAGCAATCCGAGGC
7.01 TIM Sat Mut _F	GGTGTTGTCATATGGGTTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAANNNTTCCGAGGGATCCCCCCTCT
7.01 TIM Sat Mut _R	AGAGGGGGGATCCCTCGGA
701_NNN_TCC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAtcctTCCGAGG
701_NNN_TCC-	[Phos]GATCCCTCGGAaggaTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_CCT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAccttTCCGAGG
701_NNN_CCT-	[Phos]GATCCCTCGGAaaggTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_CAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAcactTCCGAGG
701_NNN_CAC-	[Phos]GATCCCTCGGAagtgTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_ATC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAatctTCCGAGG
701_NNN_ATC-	[Phos]GATCCCTCGGAagatTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_ACC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAacctTCCGAGG
701_NNN_ACC-	[Phos]GATCCCTCGGAaggtTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_AAT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAaattTCCGAGG
701_NNN_AAT-	[Phos]GATCCCTCGGAaattTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_TAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAtactTCCGAGG
701_NNN_TAC-	[Phos]GATCCCTCGGAagtaTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_CTT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAActttTCCGAGG
701_NNN_CTT-	[Phos]GATCCCTCGGAaaagTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_CCA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAccatTCCGAGG
701_NNN_CCA-	[Phos]GATCCCTCGGAatggTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_CAG+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAcagtTCCGAGG
701_NNN_CAG-	[Phos]GATCCCTCGGAactgTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_ACT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAacttTCCGAGG
701_NNN_ACT-	[Phos]GATCCCTCGGAaagtTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_ACA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAacatTCCGAGG

701_NNN_ACA-	[Phos]GATCCCTCGGAatgtTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_AAA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAaaatTCCGAGG
701_NNN_AAA-	[Phos]GATCCCTCGGAatttTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_AAG+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAaagtTCCGAGG
701_NNN_AAG-	[Phos]GATCCCTCGGAacttTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_AGT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAagttTCCGAGG
701_NNN_AGT-	[Phos]GATCCCTCGGAaactTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_GTT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAgtttTCCGAGG
701_NNN_GTT-	[Phos]GATCCCTCGGAaaacTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_GAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAgactTCCGAGG
701_NNN_GAC-	[Phos]GATCCCTCGGAagtcTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
701_NNN_GAA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAgaatTCCGAGG
701_NNN_GAA-	[Phos]GATCCCTCGGAattcTTGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGAGGAaCCCA
601_GGG+	[Phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCAAATTCTTCACTgggATCCGAGG
601_GGG-	[Phos]GATCCCTCGGATcccAGTGAAGAATTTGACGTACAAATGTCCTTAGTGGAACAGGATCCCA
601_AAA+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCAAATTCTTCACTaaaATCCGAGG
601_AAA-	[phos]GATCCCTCGGATtttAGTGAAGAATTTGACGTACAAATGTCCTTAGTGGAACAGGATCCCA
601_CCC+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCAAATTCTTCACTcccATCCGAGG
601_CCC-	[phos]GATCCCTCGGATgggAGTGAAGAATTTGACGTACAAATGTCCTTAGTGGAACAGGATCCCA
601_TTT+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCAAATTCTTCACTtttATCCGAGG
601_TTT-	[phos]GATCCCTCGGATaaaAGTGAAGAATTTGACGTACAAATGTCCTTAGTGGAACAGGATCCCA

Other Oligos

Oligos	Sequence (5' - 3')
Pcsg_F	AACGAAGCGGCCGCTATCGGCAAAAGG
ChiA_Term_R	AACGAAGATATCGAGGAAGCGGAGGTTCCAAG
Pcsg_NdeI_Bam_R	GGATCCGATTCGTTCATATGACAACACCTCCTTGGGTTG

Term_NdeI_Bam_F	GTTGTCATATGAACGAATCGGATCCCCCCTCTCTTCTCCTCTTTTG
PF1120_pJE47_F	ccccaacaacccaaggaggtgttgtcatATGAAACCGTGCTTTGCAAAATTTC
PF1120_pJE47_R	ggacaaaagaggagaagagaggggggatccTTAGAGAATATCCCCCTCAC
PF0639_pJE47_F	ccccaacaacccaaggaggtgttgtcatATGAGTTGTAAGGCATTCCAA
PF0639_pJE47_R	ggacaaaagaggagaagagaggggggatccTCACTTCATCATTTCAACTCTAAG
PF0640_pJE47_F	ccccaacaacccaaggaggtgttgtcatATGGATACCGAAAAACTCTTC

References

1. Farkas, J., Stirrett, K., Lipscomb, G.L., Nixon, W., Scott, R.A., Adams, M.W. and Westpheling, J. (2012) Recombinogenic properties of Pyrococcus furiosus strain COM1 enable rapid selection of targeted mutants. *Applied and environmental microbiology*, **78**, 4669-4676.
2. Farkas, J., Chung, D., DeBarry, M., Adams, M.W. and Westpheling, J. (2011) Defining components of the chromosomal origin of replication of the hyperthermophilic archaeon Pyrococcus furiosus needed for construction of a stable replicating shuttle vector. *Applied and environmental microbiology*, **77**, 6343-6349.
3. Elmore, J.R., Yokooji, Y., Sato, T., Olson, S., Glover, C.V., 3rd, Graveley, B.R., Atomi, H., Terns, R.M. and Terns, M.P. (2013) Programmable plasmid interference by the CRISPR-Cas system in Thermococcus kodakarensis. *RNA biology*, **10**, 828-840.