Supplementary Material for

Bipartite recognition of target RNAs activates DNA cleavage by the Type III-B CRISPR-Cas system

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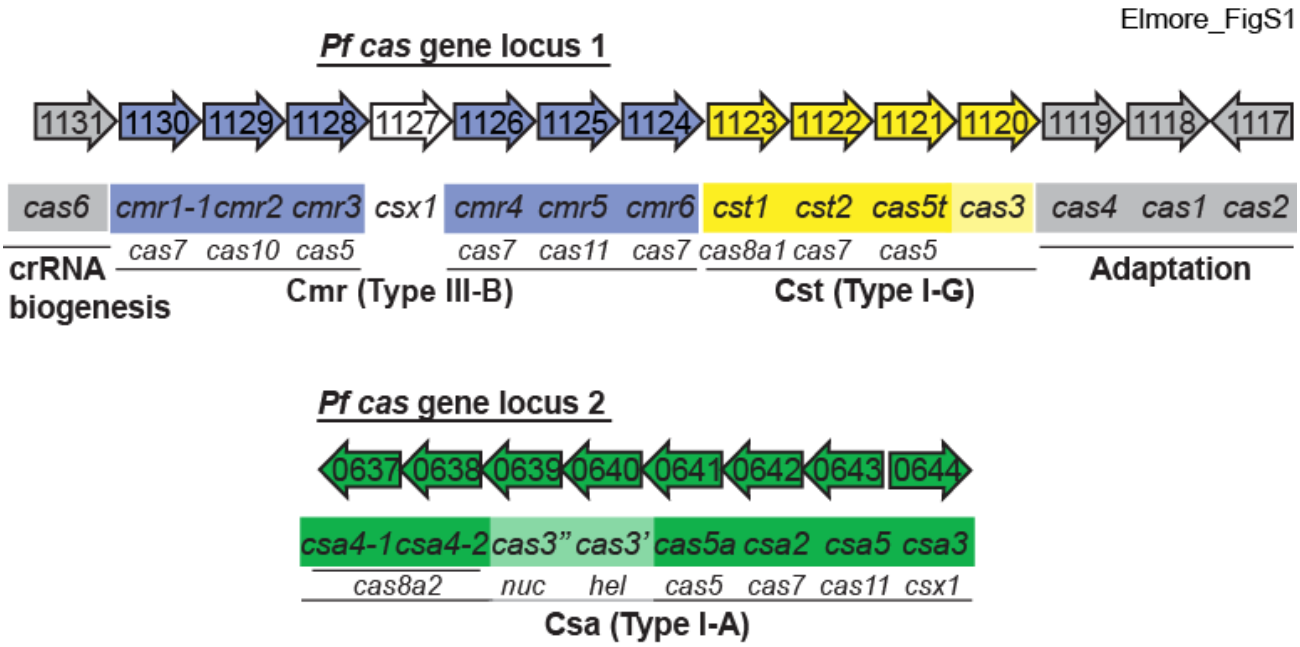
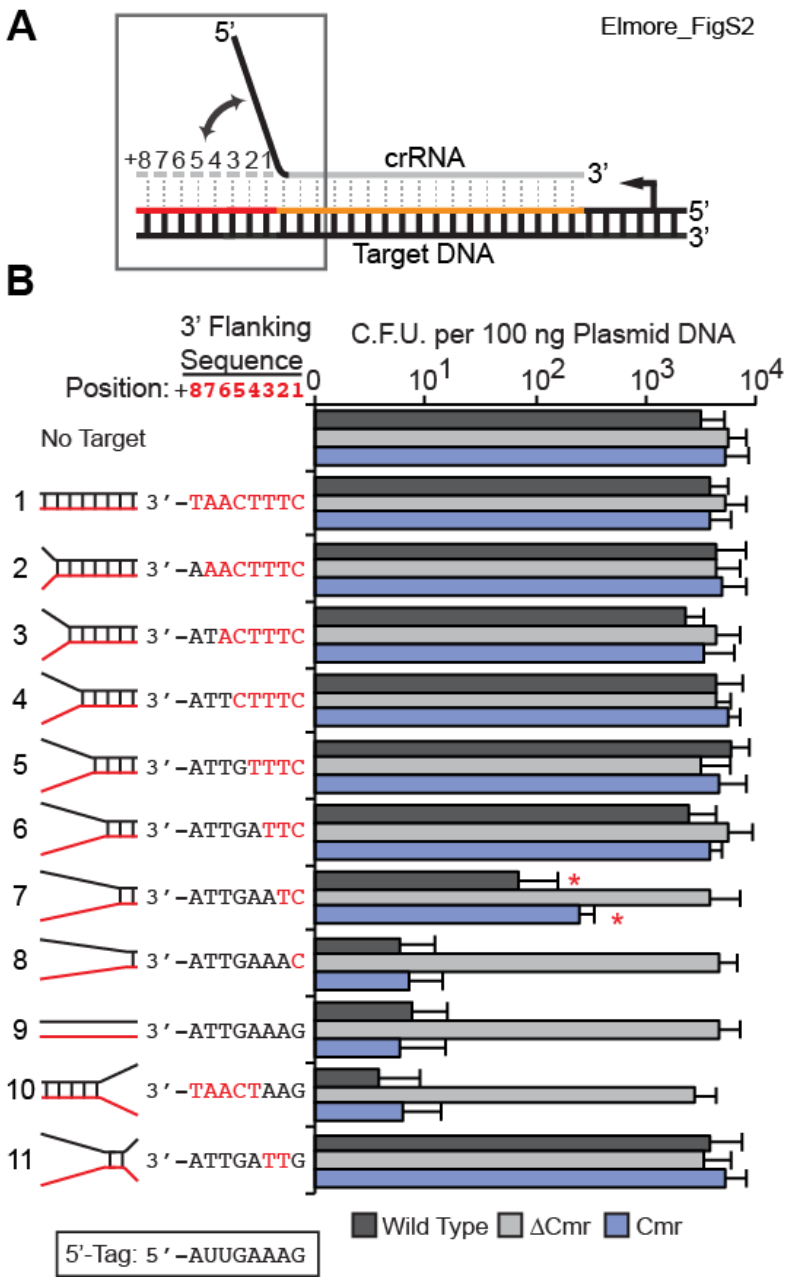
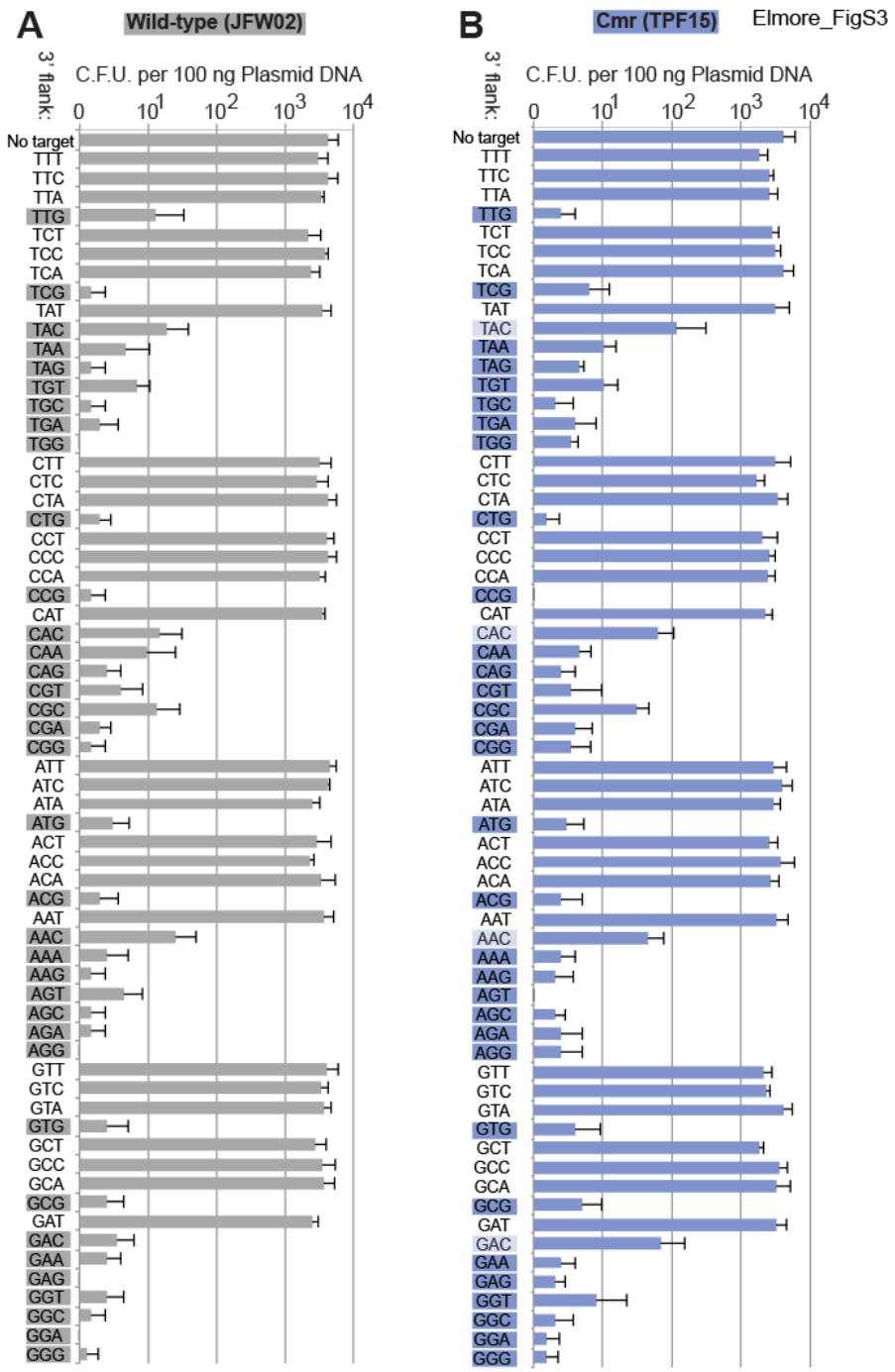


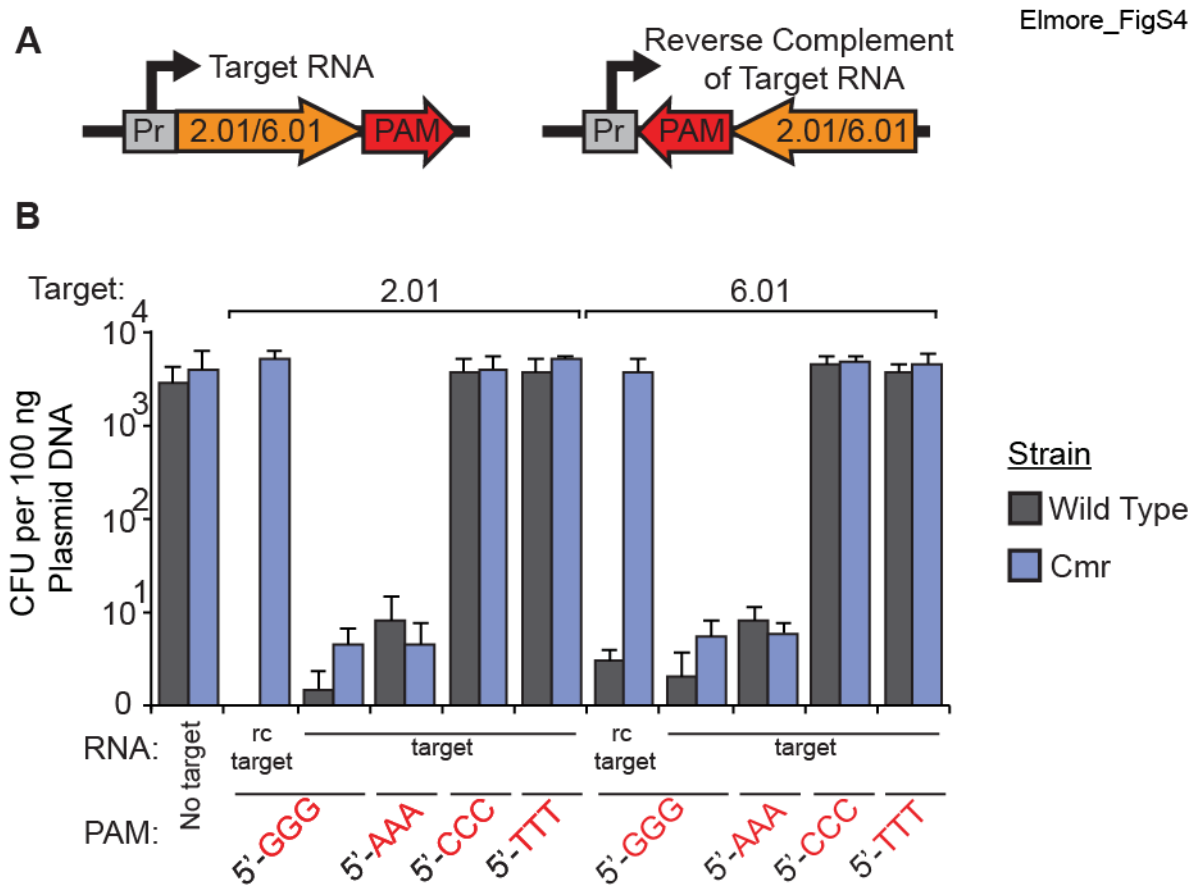
Supplemental Figure S1. *Pyrococcus furiosus cas* gene locus organization. The genome organization and annotations of the predicted cas genes were adapted from the NCBI database (http://www.ncbi.nlm.nih.gov). Type III-B Cmr (blue), Type I-G Cst (yellow), Type I-A Csa (green), and adaptation/biogenesis *cas* genes (gray) are indicated with cas gene superfamily designations indicated below relevant *csa*, *cst*, and *cmr* genes. The *csx1* gene (white) is often found in association with Type III-B systems and is encoded in between the *cmr* genes in *Pf*.



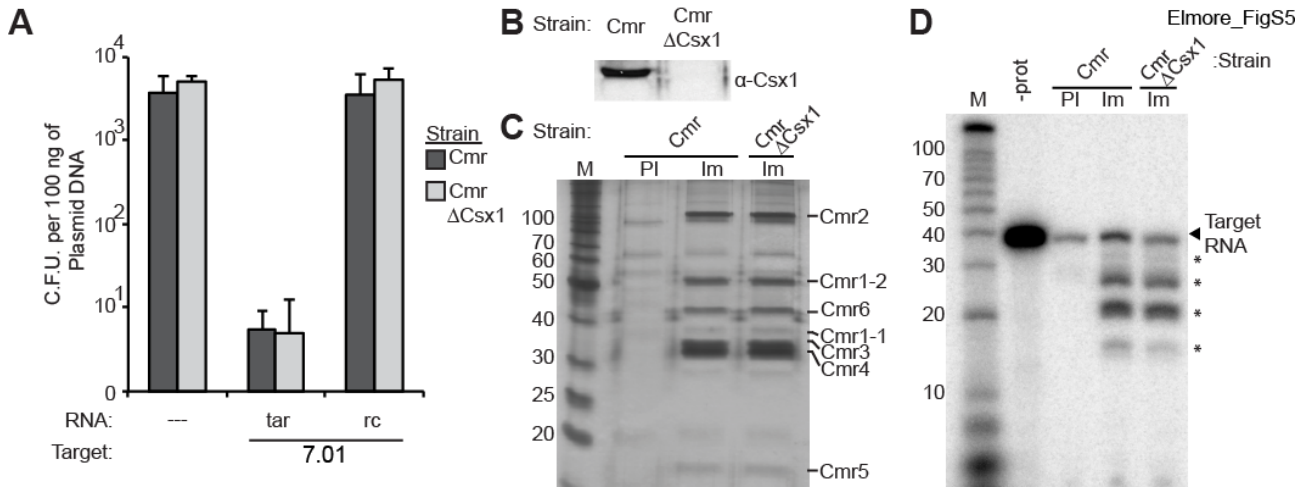
Supplemental Figure S2. 3' flanking positions +1/2/3 are important for self versus non-self discrimination by Cmr. (A) Location of tested flanking sequences (red) relative to the target sequence in the DNA (orange) with aligned crRNA (grey) with an indication of potential complementarity between crRNA 5' tag (black) and the flanking sequence. (B) Colonies produced by infection of 12 plasmids in wild-type (3 endogenous CRISPR-Cas systems, dark grey), ΔCmr (lacking Cmr system, medium grey), Cmr (Cmr only, blue). Colony numbers are plotted with the standard deviation in 9 replicates indicated by error bars. All plasmids, except a negative control (no target), produce a 7.01 target RNA, but vary in 3' flanking sequence. The 3' flanking sequence is mutated sequentially from fully complementary with the 5' tag (red) to fully non-complementary (black). Complementarity is indicated graphically with 5' tag (black) and target flanking region (red). Red asterisks on the chart indicate intermediate silencing phenotypes.



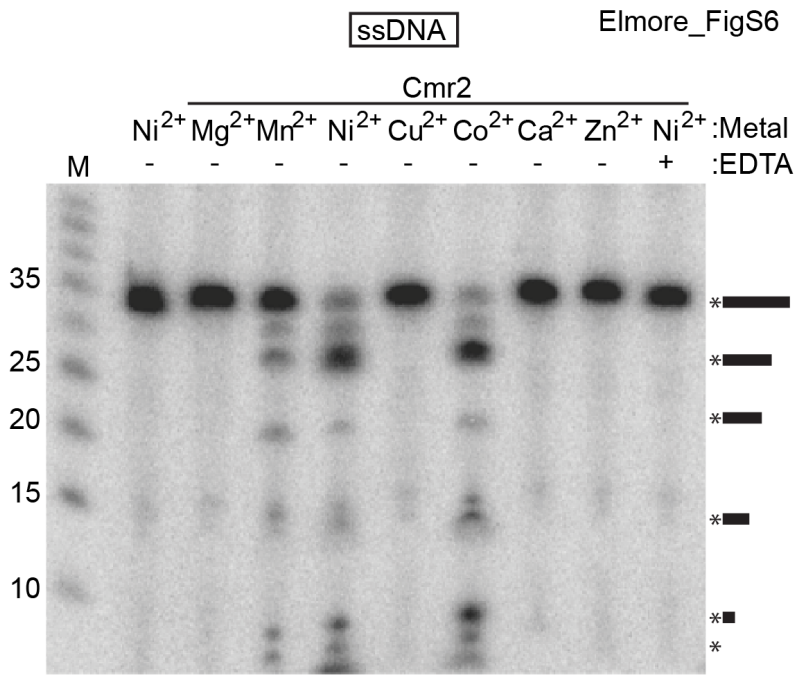
Supplemental Figure S3. The Cmr system in *Pfu* utilizes a protospacer adjacent motif (PAM) to distinguish invader from host. Colonies produced by infection with 65 plasmids in wild-type (A) and Cmr (B) strains. Colony numbers are the average of at least 3 replicates with the standard deviation indicated by error bars. All plasmids, except a negative control (no target), produce 7.01 target RNAs that differ by the 3 nucleotides immediately 3' of the 7.01 target sequence (see Fig. 2A), as indicated. Target-adjacent sequences that activated CRISPR-Cas targeting resulting in greater than 100-fold reduction in colony numbers relative to negative control plasmid are shaded dark blue or grey. Sequences that conferred 30-fold to 100-fold reduction in colony numbers are shaded light blue or grey.



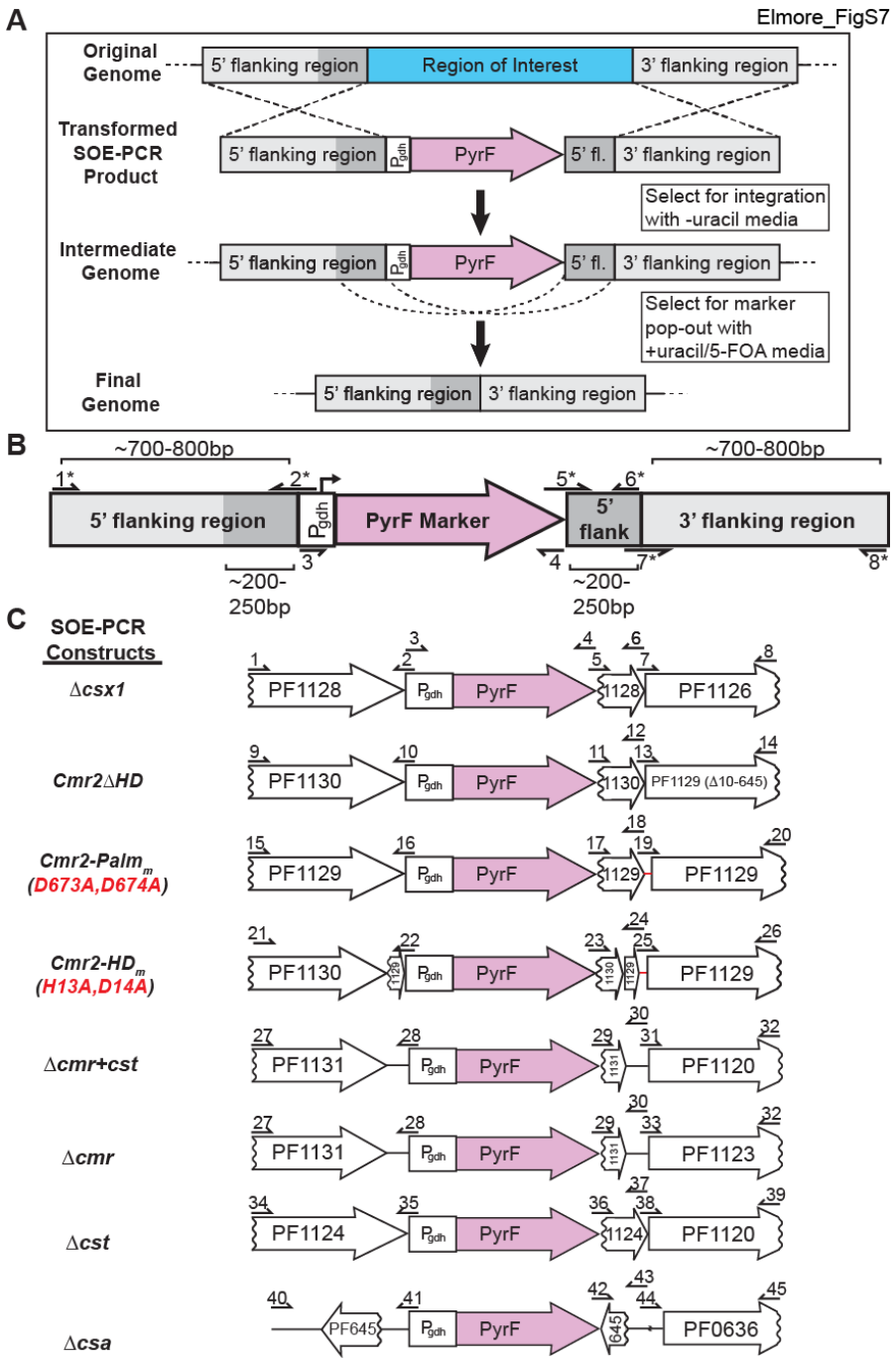
Supplemental Figure S4. Cmr silences additional CRISPR target sequences in a target transcription and PAM dependent manner. (A) Target sequence transcription configuration of the various plasmids. Orientation of the 2.01/6.01 crRNA target sequence relative to the promoter and target-adjacent PAM region is shown. Plasmids are designed for transcription of a target RNA complementary to the endogenous 2.01/6.01 crRNAs (target) or transcription of an RNA that is not complementary to the 2.01/6.01 crRNAs (rc target). (B) Colonies produced by infection with 11 plasmids in wild-type (grey) and Cmr (blue) strains. Plasmids with 2.01 or 6.01 target sequences are indicated above. The presence of target region transcript is indicated below graph as "no target", "target", and "rc of target" with the target-adjacent sequences indicated beneath. Colony numbers are the average of three replicates with error bars indicating the standard deviation.



Supplemental Figure S5. Csx1 is not required for plasmid interference by Cmr in *Pfu.* (A) Plasmid infection of Csx1 deletion strain. Cmr strains with (Cmr, dark grey) or without Csx1 (Cmr ΔCsx1, light grey) were infected with plasmids expressing no target RNA (---), crRNA 7.01 target RNA (tar), or the reverse complement RNA (rc). Colonies numbers are plotted with error bars indicating standard deviation in three replicates. Both target plasmids contain a GGG target-adjacent sequence. (B) Western blot analysis of Csx1 expression. S20 extract containing 50 µg protein from either Cmr and Cmr ΔCsx1 strains were probed with polyclonal antibodies against *Pfu* Csx1. (C) Cmr complex from Cmr and Cmr ΔCsx1 strains. Proteins immunoprecipitated with preimmune (PI, Cmr only) or immune (Im) antibodies again Cmr2 from Cmr strains with (Cmr) or without Csx1 (Cmr ΔCsx1) were analyzed by SDS-PAGE and silver staining. Cmr protein identities are indicated based on predicted molecular weights and mass spectrometry. (D) RNA cleavage activity of Cmr complexes with (Cmr) or without (Cmr ΔCsx1) Csx1. Complexes immunopurified from Cmr strains were incubated with 5' end-labeled crRNA 7.01 target RNA. Products were analyzed by denaturing PAGE. Decade Marker RNAs (M) were included for size estimations. Asterisks mark primary RNA cleavage products.



Supplemental Figure S6. Cmr2 ssDNA cleavage is divalent cation dependent. Wild-type Cmr2 protein was incubated with a 5' radiolabeled single stranded DNA and several different metal chlorides (indicated above) in the absence (-) or presence of EDTA (+). The resulting products were separated by denaturing PAGE, and visualized by phosphorimaging. A DNA size ladder (M) is used in the left-most lane for sized identification, and graphical representation of cleavage products is indicated on the right.



Supplemental Figure S7. Construction of *Pfu* strains. (A) Steps in *Pfu* strain construction by homologous recombination of transformed SOE-PCR (splicing by overlap extension polymerase chain reaction) constructs. (B) Generic SOE-PCR construct with approximate sizes and primer locations indicated. Initially, four distinct PCR products are generated by PCR using primer pairs 1*/2*, 3/4, 5*/6*, and 7*/8*. The final product displayed is generated by two additional rounds of SOE-PCR with two PCR products acting as templates in a PCR reaction with the outer primers of the two products. Primers 3 and 4 are used to amplify the Pgdh-*pyrF* selection marker in all constructs. Primers 1*-2* & 5*-8* are specific for a given construct (primer numbers indicated in panel C). To mediate splicing events, primers 2*, 5* and 7* also contain sequences that overlap with the adjacent PCR products. (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. For the Cmr2ΔHD strain, the deleted nucleotides are indicated next to the PF1129 (Cmr2) ORF. For amino acid substitution SOE-PCR products, a thin red line indicates the mutated sequences.

Supplemental Experimental Procedures

P.furiosus strain construction

Pfu strains were constructed using a variant of the previously described pop-in/pop-out marker replacement technique (Supplemental Figure 7)(Lipscomb et al. 2011; Farkas et al. 2012). The transformed PCR products were generated by splicing 4 PCR products together with Splicing by Overlap Extension PCR (SOE-PCR). A schema of the SOE-PCR products guiding each mutation is shown in Supplemental Figure 7C.

PCR primers used to generate SOE-PCR products are listed in Supplemental Table S2. Strains were constructed as follows. TPF06 (ΔCmr) was constructed by deletion of PF1130-PF1124 from wild-type CRISPR-Cas strain JFW02. TPF15 (Cmr; Δ*csa*Δ*cst*) and TPF20 (null; Δ*csa*Δ*cmr+cst*) were each constructed from JFW02 by stepwise deletion of PF0637-0644 (ΔCsa) and either PF1121-PF1123 (Δcst) for TPF15 or PF1121-1130 (ΔCmr+cst) for TPF20. TPF24 (ΔCsx1) was constructed by deletion of PF1127 from TPF15. Cmr2 mutant strains TPF25 (Cmr2ΔHD) and TPF27 (Cmr2-Palm$_m$) were constructed via mutation of Cmr2 in TPF15. Double Cmr2 mutant strains TPF35 (Cmr2ΔHD) and TPF37 (Cmr2-HD$_m$) were constructed via further mutation of Cmr2 in TPF27. PCR constructs used in each case are denoted in parentheses.

Protein expression and purification

Csx1 antigen (PF1127) was cloned from *Pf* gDNA into a modified pET24D vector with an N-terminal 6x His-tag. Protein expression and purification was performed as previously described for Cmr proteins (Hale et al. 2009; Hale et al. 2014).

Western Blot analysis

As previously described, antibodies against recombinant *Pf* Csx1 were generated (Carte et al. 2010). Western blot analysis was carried out as previously described (Hale et al. 2012) with the following modifications: S20 cell extracts containing 50 µg of protein were boiled in Laemmli buffer for 5 minutes, centrifuged briefly, and separated on 12.5% SDS-PAGE. *Pf* Csx1 antibody was used for primary antibody incubation at 0.5 µg/mL in TBST.

Cmr2 DNA cleavage assay

Metal utilization by Cmr2 for DNA cleavage was assayed using conditions described in the main text with the following modifications. Other metals are substituted for $NiCl_2$ at 2 mM where noted. 5'-radiolabeled DNA1 (Supplemental Table S2) was used as a substrate for ssDNA cleavage.

Supplemental Table S1

***E.coli* Strains**	**Revelevant Characteristics**	**Source or Reference**
Top10	F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 araD139 Δ(ara leu) 7697 galU galK rpsL (StrR) endA1 nupG	Invitrogen
BL21-CodonPlus(DE3)-RIPL	*E. coli* B F– *ompT hsdS*(r – m –) *dcm*+ Tetr *gal* λ(DE3) *endA* Hte [*argU proL Camr] [argU ileY leuW Strep/Specr]*	Novagen

***P.furiosus* Strains**	**Revelevant Characteristics**	**Source or Reference**
JFW02 (WT)	Δ*pyrF* Δ*trpAB*	(Farkas et al. 2011)
TPF06 (ΔCmr)	JFW02 Δ*cmr* (Δ*PF1124-PF1130*)	This study
TPF15 (Cmr)	JFW02 Δ*csa* (Δ*PF0637-0644*) Δ*cst* (Δ*PF1121-1123*)	This study
TPF20 (null)	JFW02 Δc*mr+cst* (Δ*PF1121-PF1130*) Δ*csa* (Δ*PF0637-0644*)	This study
TPF24 (ΔCsx1)	TPF15 Δ*csx1* (ΔPF1127)	This study
TPF25 (Cmr2ΔHD)	TPF15 c*mr2::cmr2*Δ*HD*	This study
TPF27 (Cmr2-DD-AA)	TPF15 c*mr2::cmr2-D673A,D674A*	This study
TPF35 (Cmr2::Cmr2ΔHD, DD-AA)	TPF27 c*mr2::cmr2*Δ*HD*	This study
TPF37 (Cmr2::Cmr2-HD-AA, DD-AA)	TPF27 c*mr2::cmr2-H13A,D14A*	This study

Plasmids	**Revelevant Characteristics**	**Source or Reference**
pJFW17	AprR general cloning vector with *E.coli* OriT, and *Pfu* Pgdh-pyrF cassette	(Farkas et al. 2011)
pJFW18	pJFW17 derivative; *Pfu* OriC for replication in *P.furiosus*	(Farkas et al. 2011)
pJE47	pJFW18 derivative; Tk-csg promoter/Tk-chiA terminator expression cassette	This study
pJE65	pJE47 derivative; 7.01 spacer, GGG flank, target strand transcribed	This study
pJE66	pJE47 derivative; 7.01 spacer, GGG flank, non-target strand transcribed	This study
pJE75	pJE47 derivative; 7.01 spacer, flank pos +1-8 are 5' tag comp., target strand trans.	This study
pJE76	pJE47 derivative; 7.01 spacer, flank pos +1-7 are 5' tag comp., target strand trans.	This study
pJE77	pJE47 derivative; 7.01 spacer, flank pos +1-6 are 5' tag comp., target strand trans.	This study
pJE78	pJE47 derivative; 7.01 spacer, flank pos +1-5 are 5' tag comp., target strand trans.	This study
pJE79	pJE47 derivative; 7.01 spacer, flank pos +1-4 are 5' tag comp., target strand trans.	This study
pJE80	pJE47 derivative; 7.01 spacer, flank pos +1-3 are 5' tag comp., target strand trans.	This study
pJE81	pJE47 derivative; 7.01 spacer, flank pos +1-2 are 5' tag comp., target strand trans.	This study
pJE82	pJE47 derivative; 7.01 spacer, flank pos +1 is 5' tag comp., target strand trans.	This study
pJE83	pJE47 derivative; 7.01 spacer, flank pos +1-8 are 5' tag reversed target strand trans.	This study

pJE84	pJE47 derivative; 7.01 spacer, flank pos +4-8 are 5' tag comp., target strand trans.	This study
pJE85	pJE47 derivative; 7.01 spacer, flank pos +2-3 are 5' tag comp., target strand trans.	This study
pJE186	pJE47 derivative; 7.01 spacer, TTT flank, target strand transcribed	This study
pJE187	pJE47 derivative; 7.01 spacer, TTC flank, target strand transcribed	This study
pJE188	pJE47 derivative; 7.01 spacer, TTA flank, target strand transcribed	This study
pJE189	pJE47 derivative; 7.01 spacer, TTG flank, target strand transcribed	This study
pJE190	pJE47 derivative; 7.01 spacer, TCT flank, target strand transcribed	This study
pJE191	pJE47 derivative; 7.01 spacer, TCC flank, target strand transcribed	This study
pJE192	pJE47 derivative; 7.01 spacer, TCA flank, target strand transcribed	This study
pJE193	pJE47 derivative; 7.01 spacer, TCG flank, target strand transcribed	This study
pJE194	pJE47 derivative; 7.01 spacer, TAT flank, target strand transcribed	This study
pJE195	pJE47 derivative; 7.01 spacer, TAC flank, target strand transcribed	This study
pJE196	pJE47 derivative; 7.01 spacer, TAA flank, target strand transcribed	This study
pJE197	pJE47 derivative; 7.01 spacer, TAG flank, target strand transcribed	This study
pJE198	pJE47 derivative; 7.01 spacer, TGT flank, target strand transcribed	This study
pJE199	pJE47 derivative; 7.01 spacer, TGC flank, target strand transcribed	This study
pJE200	pJE47 derivative; 7.01 spacer, TGA flank, target strand transcribed	This study
pJE201	pJE47 derivative; 7.01 spacer, TGG flank, target strand transcribed	This study
pJE202	pJE47 derivative; 7.01 spacer, CTT flank, target strand transcribed	This study
pJE203	pJE47 derivative; 7.01 spacer, CTC flank, target strand transcribed	This study
pJE204	pJE47 derivative; 7.01 spacer, CTA flank, target strand transcribed	This study
pJE205	pJE47 derivative; 7.01 spacer, CTG flank, target strand transcribed	This study
pJE206	pJE47 derivative; 7.01 spacer, CCT flank, target strand transcribed	This study
pJE207	pJE47 derivative; 7.01 spacer, CCC flank, target strand transcribed	This study
pJE208	pJE47 derivative; 7.01 spacer, CCA flank, target strand transcribed	This study
pJE209	pJE47 derivative; 7.01 spacer, CCG flank, target strand transcribed	This study
pJE210	pJE47 derivative; 7.01 spacer, CAT flank, target strand transcribed	This study
pJE211	pJE47 derivative; 7.01 spacer, CAC flank, target strand transcribed	This study
pJE212	pJE47 derivative; 7.01 spacer, CAA flank, target strand transcribed	This study
pJE213	pJE47 derivative; 7.01 spacer, CAG flank, target strand transcribed	This study
pJE214	pJE47 derivative; 7.01 spacer, CGT flank, target strand transcribed	This study
pJE215	pJE47 derivative; 7.01 spacer, CGC flank, target strand transcribed	This study
pJE216	pJE47 derivative; 7.01 spacer, CGA flank, target strand transcribed	This study
pJE217	pJE47 derivative; 7.01 spacer, CGG flank, target strand transcribed	This study
pJE218	pJE47 derivative; 7.01 spacer, ATT flank, target strand transcribed	This study
pJE219	pJE47 derivative; 7.01 spacer, ATC flank, target strand transcribed	This study
pJE220	pJE47 derivative; 7.01 spacer, ATA flank, target strand transcribed	This study
pJE221	pJE47 derivative; 7.01 spacer, ATG flank, target strand transcribed	This study
pJE222	pJE47 derivative; 7.01 spacer, ACT flank, target strand transcribed	This study
pJE223	pJE47 derivative; 7.01 spacer, ACC flank, target strand transcribed	This study
pJE224	pJE47 derivative; 7.01 spacer, ACA flank, target strand transcribed	This study
pJE225	pJE47 derivative; 7.01 spacer, ACG flank, target strand transcribed	This study
pJE226	pJE47 derivative; 7.01 spacer, AAT flank, target strand transcribed	This study
pJE227	pJE47 derivative; 7.01 spacer, AAC flank, target strand transcribed	This study

pJE228	pJE47 derivative; 7.01 spacer, AAA flank, target strand transcribed	This study
pJE229	pJE47 derivative; 7.01 spacer, AAG flank, target strand transcribed	This study
pJE230	pJE47 derivative; 7.01 spacer, AGT flank, target strand transcribed	This study
pJE231	pJE47 derivative; 7.01 spacer, AGC flank, target strand transcribed	This study
pJE232	pJE47 derivative; 7.01 spacer, AGA flank, target strand transcribed	This study
pJE233	pJE47 derivative; 7.01 spacer, AGG flank, target strand transcribed	This study
pJE234	pJE47 derivative; 7.01 spacer, GTT flank, target strand transcribed	This study
pJE235	pJE47 derivative; 7.01 spacer, GTC flank, target strand transcribed	This study
pJE236	pJE47 derivative; 7.01 spacer, GTA flank, target strand transcribed	This study
pJE237	pJE47 derivative; 7.01 spacer, GTG flank, target strand transcribed	This study
pJE238	pJE47 derivative; 7.01 spacer, GCT flank, target strand transcribed	This study
pJE239	pJE47 derivative; 7.01 spacer, GCC flank, target strand transcribed	This study
pJE240	pJE47 derivative; 7.01 spacer, GCA flank, target strand transcribed	This study
pJE241	pJE47 derivative; 7.01 spacer, GCG flank, target strand transcribed	This study
pJE242	pJE47 derivative; 7.01 spacer, GAT flank, target strand transcribed	This study
pJE243	pJE47 derivative; 7.01 spacer, GAC flank, target strand transcribed	This study
pJE244	pJE47 derivative; 7.01 spacer, GAA flank, target strand transcribed	This study
pJE245	pJE47 derivative; 7.01 spacer, GAG flank, target strand transcribed	This study
pJE246	pJE47 derivative; 7.01 spacer, GGT flank, target strand transcribed	This study
pJE247	pJE47 derivative; 7.01 spacer, GGC flank, target strand transcribed	This study
pJE248	pJE47 derivative; 7.01 spacer, GGA flank, target strand transcribed	This study
pJE249	pJE47 derivative; 7.01 spacer, GGG flank, target strand transcribed	This study
pJE271	pJE65 derivative; Pcsg deleted	This study
pJE272	pJE66 derivative; Pcsg deleted	This study
pJE275	pJE47 derivative; 6.01 spacer, GGG flank, target strand transcribed	This study
pJE276	pJE47 derivative; 6.01 spacer, GGG flank, non-target (guide) strand transcribed	This study
pJE294	pJE47 derivative; mutated non-target spacer, GGG flank either end	This study
pJE299	pJE47 derivative; 6.01 spacer, AAA flank, target strand transcribed	This study
pJE300	pJE47 derivative; 6.01 spacer, CCC flank, target strand transcribed	This study
pJE301	pJE47 derivative; 6.01 spacer, TTT flank, target strand transcribed	This study
pJE302	pJE47 derivative; 2.01 spacer, GGG flank, target strand transcribed	This study
pJE303	pJE47 derivative; 2.01 spacer, GGG flank, non-target (guide) strand transcribed	This study
pJE304	pJE47 derivative; 2.01 spacer, AAA flank, target strand transcribed	This study
pJE305	pJE47 derivative; 2.01 spacer, CCC flank, target strand transcribed	This study
pJE306	pJE47 derivative; 2.01 spacer, TTT flank, target strand transcribed	This study
pLC64-ChiA	*T.kodakaraensis* shuttle vector with Pcsg-ChiA expression cassette	(Elmore et al. 2013)

Table S2 - Oligos

Northern Probe Oligos

Northern Probe	Sequence (5'-3')
7.01 antisense	GCTCTCAGCCGCAAGGACCGCATAC
7.01 sense	GTATGCGGTCCTTGCGGCTGAGAGC
Pfu 5S rRNA antisense	CCCGGCTTCCCGCCCCCTCT

SOE-PCR Construct Primer Oligos

Primer	Sequence (5'-3')
Pgdh_PyrF_F [3]	GATTGAAAATGGAGTGAGCTGAG
Pdgh_PyrF_R [4]	TTATCTTGAGCTCCATTCTTTCACC
ΔCsx1_1 [1]	GGCAGAATTTACCCCCTTCC
ΔCsx1_2 [2]	CTCAGCTCACTCCATTTTCAATCTCATTCCCATATCCCTCCTAAAGC
ΔCsx1_5 [5]	GGTGAAAGAATGGAGCTCAAGATAATCCCACAATAGGGAAAGTTGG
ΔCsx1_6 [6]	TCATTCCCATATCCCTCCTAAAGC
ΔCsx1_7 [7]	GCTTTAGGAGGGATATGGGAATGACTGCAAATCTCGCTTATGAAG
ΔCsx1_8 [8]	CCTTTGCCCTGGGAGTTACA
Cmr2ΔHD_1 [9]	TGTTACACCGCTTAGTTCTCCA
Cmr2ΔHD_2 [10]	CTCAGCTCACTCCATTTTCAATCGTTAACCACTCCAACCACC
Cmr2ΔHD_5 [11]	GGTGAAAGAATGGAGCTCAAGATAATGGATTGCCTCGATTTAAGC
Cmr2ΔHD_6 [12]	GTTAACCACTCCAACCACC
Cmr2ΔHD_7 [13]	GGTGGTTGGAGTGGTTAACGTTAAGGATCCCACTTTGCTC
Cmr2ΔHD_8 [14]	GGCACTTCCATCCTTTGAGT
Cmr2-D673A,D674A_1 [15]	TGGATAGCCTGGGAGAGAGA
Cmr2-D673A,D674A_2 [16]	CTCAGCTCACTCCATTTTCAATCCCCTCCAGCGTATATTAGC
Cmr2-D673A,D674A_5 [17]	GGTGAAAGAATGGAGCTCAAGATAATTATGGATGGCGACGATATG
Cmr2-D673A,D674A_6 [18]	CCCTCCAGCGTATATTAGC
Cmr2-D673A,D674A_7 [19]	GCTAATATACGCTGGAGGGGCAGCAGTCCTAGCAATTTTGCCAGTC
Cmr2-D673A,D674A_8 [20]	AAATTCGGGTTCCTCCTCAC
Cmr2-H13A,D14A_1 [21]	ATCCTCCTGGGAGCAGATTT
Cmr2-H13A,D14A_2 [22]	CTCAGCTCACTCCATTTTCAATCAAGGTATACAAAAAGTTTCTCTTTGATG
Cmr2-H13A,D14A_5 [23]	GGTGAAAGAATGGAGCTCAAGATAAAGGAGAGCTTCTCCCCTTTG
Cmr2-H13A,D14A_6 [24]	AAGGTATACAAAAAGTTTCTCTTTGATG
Cmr2-H13A,D14A_7 [25]	CATCAAAGAGAAACTTTTTGTATACCTTGCAGCACCACCAGACAAGGCTCTAA
Cmr2-H13A,D14A_8 [26]	CCGAACTTGTCCACTATCACC
ΔCmr_1 [27]	TCCAATCCGAAGCTTGCAACATA

ΔCmr_2 [28]	CTCAGCTCACTCCATTTTCAATCGCTACCTCACCGAGCCAAATAAAGTG
ΔCmr_5 [29]	GGTGAAAGAATGGAGCTCAAGATAACTGGGCTTCGGAATGGTTAAGG
ΔCmr_6 [30]	GCTACCTCACCGAGCCAAATAAAGTG
ΔCmr_7 [31]	CACTTTATTTGGCTCGGTGAGGTAGCTTGCCGTTGGTGGCAGAGATAG
ΔCmr_8 [32]	GCCTTTGGTACCCTCTCCCAGA
ΔCmr+Cst_7 [33]	CACTTTATTTGGCTCGGTGAGGTAGCATGAAACCGTGCTTTGCAAAATTTCTTC
ΔCst_1 [34]	TCGTTGCCAATTGAAACTAAGGT
ΔCst_2 [35]	CTCAGCTCACTCCATTTTCAATCCTAAACATATTCAACAAGCCTCCCATAG
ΔCst_5 [36]	GGTGAAAGAATGGAGCTCAAGATAAATGTCCCACCTCCTGGGGACT
ΔCst_6 [37]	CTAAACATATTCAACAAGCCTCCCATAG
ΔCst_7 [38]	CTATGGGAGGCTTGTTGAATATGTTTAGATGAAACCGTGCTTTGCAAAATTTCTTC
ΔCst_8 [39]	GGGCCGCTTCAGTCTTTCCATA
ΔCsa_1 [40]	GGATTTTGTATTGCCTCACGGTTA
ΔCsa_2 [41]	CTCAGCTCACTCCATTTTCAATCGTTTTTCTGTATCGAATATTCCCCGAATG
ΔCsa_5 [42]	GGTGAAAGAATGGAGCTCAAGATAATCCCAGGTTCTGGTTTGACAAG
ΔCsa_6 [43]	GTTTTTCTGTATCGAATATTCCCCGAATG
ΔCsa_7 [44]	CATTCGGGGAATATTCGATACAGAAAAACAGCTTTATCTTTTCCCATAACCATTAGG
ΔCsa_8 [45]	TGGCTCCCTTAACTCGCTGGA

***numbers in brackets refer to Supplemental Figure 7C.**

PCR Screening Oligos

Primer	Sequence (5'-3')
ΔCsx1_seq_For	GTGTTGGAGTGGGTGAGGAG
ΔCsx1_seq_Rev	TCTGGAGATATTTGCCGTTAATC
ΔCsx1_seq_Int	TCCCACAATAGGGAAAGTTGG
Cmr2ΔHD_seq_For	GTTTTTGGGAGCACAAAGGA
Cmr2ΔHD_seq_Rev	GGTTCCTCATCAAGCCACAA
Cmr2ΔHD_seq_Int	TGGATTGCCTCGATTTAAGC
Cmr2-D673A,D674A_seq_For	GGGTCTCTCGGATGAAGATG
Cmr2-D673A,D674A_seq_Rev	TTCTGCCTTTCTCTGTTCCAA
Cmr2-D673A,D674A_seq_Int	TTATGGATGGCGACGATATG
Cmr2-D673A,D674A_scr_Mu	GACTGGCAAAATTGCTAGGACTGCTGC
Cmr2-D673A,D674A_scr_WT	GACTGGCAAAATTGCTAGGACATCATC
Cmr2-H13A,D14A_seq_For	GTTTTTGGGAGCACAAAGGA
Cmr2-H13A,D14A_seq_Rev	TTCAGCCTCCTTTCCTGAGA
Cmr2-H13A,D14A_seq_Int	AGGAGAGCTTCTCCCCTTTG

Cmr2-H13A,D14A_scr_Mu	TTAGAGCCTTGTCTGGTGGTGCTGC
Cmr2-H13A,D14A_scr_WT	TTAGAGCCTTGTCTGGTGGATCATG
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

Oligos for Target Plasmid Cloning

Oligos	**Sequence (5'-3')**
7.01_TT_GGG+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAAgggATCCGAGG
7.01_TT_GGG-	[phos]GATCCCTCGGATcccTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
7.01_GT_GGG+	[phos]TATGCTCGGATcccTTGTAGTATGCGGTCCTTGCGGCTG AGAGCACTTCAGAGGATCCG
7.01_GT_GGG-	[phos]GATCCGGATCCTCTGAAGTGCTCTCAGCCGCAAGGAC CGCATACTACAAgggATCCGAGCA
7.01_TT_tagc_1-8+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActttcaatAGG
7.01_TT_tagc_1-8-	[phos]GATCCCTattgaaagTTGTAGTATGCGGTCCTTGCGGCTGA GAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-7+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActttcaaAAGG
7.01_TT_tagc_1-7-	[phos]GATCCCTTttgaaagTTGTAGTATGCGGTCCTTGCGGCTGA GAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-6+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActttcaTAAGG
7.01_TT_tagc_1-6-	[phos]GATCCCTTAtgaaagTTGTAGTATGCGGTCCTTGCGGCTG AGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-5+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActttcTTAAGG
7.01_TT_tagc_1-5-	[phos]GATCCCTTAAgaaagTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-4+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActttGTTAAGG
7.01_TT_tagc_1-4-	[phos]GATCCCTTAACaaagTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-3+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAActtAGTTAAGG
7.01_TT_tagc_1-3-	[phos]GATCCCTTAACTaagTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-2+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC

	GCATACTACAActAAGTTAAGG
7.01_TT_tagc_1-2-	[phos]GATCCCTTAACTTagTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_1-1+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAAcAAAGTTAAGG
7.01_TT_tagc_1-1-	[phos]GATCCCTTAACTTTgTTGTAGTATGCGGTCCTTGCGGC TGAGAGCACTTCAGAGGATCCCA
7.01_TT_rtag+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAAGAAAGTTAAGG
7.01_TT_rtag-	[phos]GATCCCTTAACTTTCTTGTAGTATGCGGTCCTTGCGGC TGAGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_4-8+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAAGAAtcaatAGG
7.01_TT_tagc_4-8-	[phos]GATCCCTattgaTTCTTGTAGTATGCGGTCCTTGCGGCTG AGAGCACTTCAGAGGATCCCA
7.01_TT_tagc_2-3+	[phos]TATGGGATCCTCTGAAGTGCTCTCAGCCGCAAGGACC GCATACTACAAGttAGTTAAGG
7.01_TT_tagc_2-3-	[phos]GATCCCTTAACTaaCTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGATCCCA
pJE47_nontarg_+	[phos]TATGCTCGGATcccAGTCCTGTAGAGACTAATACCTTCA ATACGCAGCACCAGGATCCG
pJE47_nontarg_+	[phos]GATCCGGATCCTGGTGCTGCGTATTGAAGGTATTAGT CTCTACAGGACTgggATCCGAGCA
6.01_GT_GGG+	[phos]TATGCTCGGATCCCAGTGAAGAATTTGACGTACAAAT GTCCTTAGTGGAACAGGATCCG
6.01_GT_GGG-	[phos]GATCCGGATCCTGTTCCACTAAGGACATTTGTACGTC AAATTCTTCACTGGGATCCGAGCA
2.01_GT_GGG+	[phos]TATGCTCGGATCCCTGTTCATCGCACTTCTTCTTCTGA CTCTGCTCCACTTAGAGGATCCG
2.01_GT_GGG-	[phos]GATCCGGATCCTCTAAGTGGAGCAGAGTCAGAAGAA GAAGTGCGATGAACAGGGATCCGAGCA
701 NNN _F	GGTGTTGTCATATGGGTTCCTCTGAAGTGCTCTCAGCCGCA AGGACCGCATACTACAANNNTTCCGAGGGATCCCCCCTCT
701 NNN _R	AGAGGGGGGATCCCTCGGA
701_NNN_TCC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAtcctTCCGAGG
701_NNN_TCC-	[Phos]GATCCCTCGGAaggaTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_CCT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAccttTCCGAGG
701_NNN_CCT-	[Phos]GATCCCTCGGAaaggTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_CAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAcactTCCGAGG
701_NNN_CAC-	[Phos]GATCCCTCGGAagtgTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_ATC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAatctTCCGAGG
701_NNN_ATC-	[Phos]GATCCCTCGGAagatTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_ACC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAacctTCCGAGG
701_NNN_ACC-	[Phos]GATCCCTCGGAaggtTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_AAT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG

	CATACTACAAaattTCCGAGG
701_NNN_AAT-	[Phos]GATCCCTCGGAaattTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_TAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAtactTCCGAGG
701_NNN_TAC-	[Phos]GATCCCTCGGAagtaTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_CTT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAActttTCCGAGG
701_NNN_CTT-	[Phos]GATCCCTCGGAaaagTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_CCA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAccatTCCGAGG
701_NNN_CCA-	[Phos]GATCCCTCGGAatggTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_CAG+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAcagtTCCGAGG
701_NNN_CAG-	[Phos]GATCCCTCGGAactgTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_ACT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAacttTCCGAGG
701_NNN_ACT-	[Phos]GATCCCTCGGAaagtTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_ACA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAacatTCCGAGG
701_NNN_ACA-	[Phos]GATCCCTCGGAatgtTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_AAA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAaaatTCCGAGG
701_NNN_AAA-	[Phos]GATCCCTCGGAatttTTGTAGTATGCGGTCCTTGCGGCTG AGAGCACTTCAGAGGAaCCCA
701_NNN_AAG+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAaagtTCCGAGG
701_NNN_AAG-	[Phos]GATCCCTCGGAacttTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_AGT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAagttTCCGAGG
701_NNN_AGT-	[Phos]GATCCCTCGGAaactTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_GTT+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAgtttTCCGAGG
701_NNN_GTT-	[Phos]GATCCCTCGGAaaacTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_GAC+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAgactTCCGAGG
701_NNN_GAC-	[Phos]GATCCCTCGGAagtcTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
701_NNN_GAA+	[Phos]TATGGGtTCCTCTGAAGTGCTCTCAGCCGCAAGGACCG CATACTACAAgaatTCCGAGG
701_NNN_GAA-	[Phos]GATCCCTCGGAattcTTGTAGTATGCGGTCCTTGCGGCT GAGAGCACTTCAGAGGAaCCCA
601_GGG+	[Phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCA AATTCTTCACTgggATCCGAGG
601_GGG-	[Phos]GATCCCTCGGATcccAGTGAAGAATTTGACGTACAAAT GTCCTTAGTGGAACAGGATCCCA

601_AAA+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCA AATTCTTCACTaaaATCCGAGG
601_AAA-	[phos]GATCCCTCGGATtttAGTGAAGAATTTGACGTACAAATG TCCTTAGTGGAACAGGATCCCA
601_CCC+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCA AATTCTTCACTcccATCCGAGG
601_CCC-	[phos]GATCCCTCGGATgggAGTGAAGAATTTGACGTACAAAT GTCCTTAGTGGAACAGGATCCCA
601_TTT+	[phos]TATGGGATCCTGTTCCACTAAGGACATTTGTACGTCA AATTCTTCACTtttATCCGAGG
601_TTT-	[phos]GATCCCTCGGATaaaAGTGAAGAATTTGACGTACAAAT GTCCTTAGTGGAACAGGATCCCA
Pcsg_F	AACGAAGCGGCCGCTATCGGCAAAAGG
Term_R	AACGAAGATATCGAGGAAGCGGAGGTTCCAAG
Pcsg_R	GGATCCGATTCGTTCATATGACAACACCTCCTTGGGTTG
Term_F	GTTGTCATATGAACGAATCGGATCCCCCCTCTCTTCTCCTCT TTTG

Oligos for *in vitro* Cmr2/Cmr4 Mutations

Oligos	Sequence (5' - 3')
Cmr2_D673A,D674A_qc_F	GCTAATATACGCTGGAGGGGCAGCAGTCCTAGCAATTTTGC CAGTC
Cmr2_D673A,D674A_qc_R	GACTGGCAAAATTGCTAGGACTGCTGCCCCTCCAGCGTATA TTAGC
Cmr2_H13A,D14A_qc_F	CATCAAAGAGAAACTTTTTGTATACCTTGCAGCACCACCAG ACAAGGCTCTAA
Cmr2_H13A,D14A_qc_R	TTAGAGCCTTGTCTGGTGGTGCTGCAAGGTATACAAAAAGT TTCTCTTTGATG

Assay Oligos for Figures 3,5 & 6

Oligos	Sequence (5' - 3')
7.01_DNA_target [se] (2397)	GGCGACCGTATGCGCGTAGTGCCGTGCAGTCGCCGTACCCC TGAAGTGCTCTCAGCCGCAAGGACCGCATACTACAAGGGA GTTACTCGCGTGCACTCCGCCTTGGTGGAGCACTGA
7.01_DNA_target [as] (2398)	TCAGTGCTCCACCAAGGCGGAGTGCACGCGAGTAACTCCCT TGTAGTATGCGGTCCTTGCGGCTGAGAGCACTTCAGGGGTA CGGCGACTGCACGGCACTACGCGCATACGGTCGCC
7.01_DNA_bubble [as] (3124)	TCAGTGCTCCACCAAGGCGGAGTGCACGCGAGTAACTCCCA ACATCATACGCCAGGAACGCCGACTCTCGTGAAGTCGGGTA CGGCGACTGCACGGCACTACGCGCATACGGTCGCC
non-target_DNA_[se] (2765)	GGCGACCGTATGCGCGTAGTGCCGTGCAGTCGCCGTACCCA GTCCTGTAGAGACTAATACCTTCAATACGCAGCACCGGGAG TTACTCGCGTGCACTCCGCCTTGGTGGAGCACTGA
non-target_DNA_[as] (2766)	TCAGTGCTCCACCAAGGCGGAGTGCACGCGAGTAACTCCCG GTGCTGCGTATTGAAGGTATTAGTCTCTACAGGACTGGGTA CGGCGACTGCACGGCACTACGCGCATACGGTCGCC
45-mer 7.01 crRNA (RNA 1)	AUUGAAAGUUGUAGUAUGCGGUCCUUGCGGCUGAGAGCA CUUCAG
37-mer 7.01 target (RNA 2)	CUGAAGUGCUCUCAGCCGCAAGGACCGCAUACUACAA

Supplemental Figure 6 Assay Oligos

Oligos	Sequence (5' - 3')
DNA 1	TCGATGTAACGTATGCAAATGACAATTATTACTA

IVT Template PCR Primers

Oligos	Sequence (5' - 3')
117-mer F T7 (3110)	aagcaagaattcTAATACGACTCACTATAGGGAGAGGCGACCGTATGCG
117-mer R (3112)	TCAGTGCTCCACCAAG
117-mer F (3114)	GGCGACCGTATGCG
117-mer R T7 (3115)	aagcaaggatccTAATACGACTCACTATAGGGAGATCAGTGCTCCACCAAG
pJE47_IVT_T7_F (2798)	TAATACGACTCACTATAGGGAGACAACACTTAGTAGGGGCTA
pJE47_IVT_R (2801)	GCTTCCTTAGCTGTTTCTCCA

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