Supplemental Material

The presence of the ACA box in the archaeal H/ACA guide RNA allows atypical pseudouridylation

Mrinmoyee Majumder, Shaoni Mukhopadhyay, Parinati Kharel and Ramesh Gupta

METHODS

Construction of cloning vectors

Plasmid pKS$^+$Metpro (Supplemental Fig. S5A) is a vector that contains an insert (sequence in Supplemental Fig. S6) within the multiple cloning site of pBlueScript KS$^+$ (Stratagene). The insert contains the promoter and terminator regions of the *H. volcanii* intron-containing elongator tRNA-Met gene, but not the structural gene. The two regions are separated by a *Sma*I site. A *Hin*dIII fragment of about 1.5 Kb in pHVH205-2 (Datta et al. 1989) contains the elongator tRNA-Met gene. This fragment of pHVH205-2 was subcloned into the *Hin*dIII site of pBlueScript KS$^+$, such that the promoter of the gene was towards the *Kpn*I site of the multiple cloning site of the vector. Stepwise partial deletions at the *Taq*I sites downstream of the terminator reduced the insert size to about 250 bases that contained the gene and its promoter and terminator regions. Digestion by *Sma*I and religation of this 250-base-containing clone removed about 140 bases and produced pKS$^+$Metpro. The 140-base fragment that was removed contained the main tRNA and its intron. This deletion was possible because there are two *Sma*I sites (CCCGGG) within the tRNA gene, one in each strand of the acceptor arm of the tRNA. In the tRNA transcript the two CCCGGG sequences pair in the acceptor stem.

Plasmid pDS2 is a derivative of the *E. coli-H. volcanii* shuttle vector pWL102 (Lam and Doolittle 1989) that contains the multiple cloning site of pBlueScript KS$^+$. It has ampicillin as the selectable marker for *E. coli* and mevinolin for *H. volcanii*. Its construction is shown schematically in Supplemental Fig. S5B. pWL102 was digested with *Asp*718 (a *Kpn*I isoschizomer) and *Bam*HI, filled with Klenow enzyme and religated to produce pWL102BA. This removed certain restriction sites from the plasmid. Both

pWL102BA and pBlueScript KS^+ plasmids were completely digested by *Afl*III and partially digested by *Ssp*I. The 8.2 Kb *Afl*III- *Ssp*I fragment of pWL102BA and 1.2 Kb *Afl*III- *Ssp*I fragment of pBlueScript KS^+ were ligated together to produce pDS1. Plasmid pDS2 is an improved version of pDS1 in which adjacent *Hin*dIII, *Cla*I and *Eco*RI sites near the Amp^r locus were eliminated, by partial digestion with individual enzyme, filling in and religation. Thus, only one site (within the multiple cloning site) for each of these enzymes was retained.

Gel-shift assays

About 0.1 μM of [α-^{32}P]NTP-labeled guide RNA (WT or mutant) was mock-treated or mixed with 0.1 μM recombinant *M. jannaschii* Cbf5 protein in 20 μL reactions (20 mM Na-HEPES, pH 7.0, 150 mM NaCl, 0.75 mM DTT, 1.5 mM EDTA, 10% glycerol and 1μg yeast tRNA) and incubated at 68^{o}C for 30 min. The samples were resolved by 4% native PAGE at 4^{o}C in 0.5X TBE buffer for 6-8 h at 125 V. Resolved RNPs were visualized by phosphorimaging after drying the gels.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Time course analyses of SL1 stem-loop RNP-mediated Ψ formation. Different sRNP:substrate (S) RNA ratios (as indicated on the side) were used in reactions similar to those shown in Fig. 3B and 3D to measure Ψ formation. The sRNP was 0.1 μM in each reaction (i.e., 0.1 μM of each of the four proteins and the guide RNA) and the substrate RNA was 0.01 μM for the single turnover conditions and 0.2, 0.5 and 1.0 μM for different multiple turnover conditions. Total mole Ψ/mole RNA was determined at different time points. Mean values of two independent reactions are plotted here using GraphPad Prism software. The inset amplifies the reaction curve for 0, 1, 2 and 5 min of the same plot.

Supplemental Figure S2. In vivo pseudouridylations by 1940 guide and 1942 guide were not consistent. The Δ*sR-h45* strain of *H. volcanii* was independently transformed with 1940 guide and 1942 guide (see schematics of the Ψ pockets in Figs. 4D and 6B). Total RNA of the transformants was treated as in Figs.

2C and 2E to determine Ψ formation at positions 1940 and 1942. Guides are indicated above the panels. Results of the two independent sets of the experiments are not similar (intensities of the bands at positions 1940 and 1942 are not similar in the two sets).

Supplemental Figure S3. The ACA box is required for binding of Cbf5 to RNA. Radiolabeled SL1 and SL2 RNAs and their variants (as in Fig. 6B) were incubated with recombinant *M. jannaschii* Cbf5, resolved by native PAGE and visualized by phosphorimaging. RNAs are designated above the panels and migration positions of free RNA (C0) and Cbf5-bound RNA (C1) are indicated on the sides.

Supplemental Figure S4. Time course analyses of Ψ formation using different mutations of SL1 guide RNA (schematics in Figs. 6B and 7A) under single turnover conditions (sRNP:substrate RNA - 10:1). Reaction conditions and analyses were done as in Supplemental Fig. S1.

Supplemental Figure S5. The Δ*sR-h45* strain of *H. volcanii* was independently transformed with various mutants of pHSL1 and pHSL2 (schematics of the mutants in Fig. 7). Total RNA of the transformants were treated as in Figs. 2B-D to determine the modification status of U's at position 2605 in (A) and (B) and at positions 1940 and 1942 in (C) and (D). Mutants are indicated above each panel. (*A*) and (*C*) are primer extensions for U-specific reactions and (*B*) and (*D*) are for CMCT reactions. Asterisks next to the bands indicate unmodified U2605 in (*A*), Ψ2605 in (*B*), unmodified U1940 and U1942 in (*C*), and Ψ1940 and Ψ1942 in (*D*). Some WT reaction figures are the same as in Fig. 2.

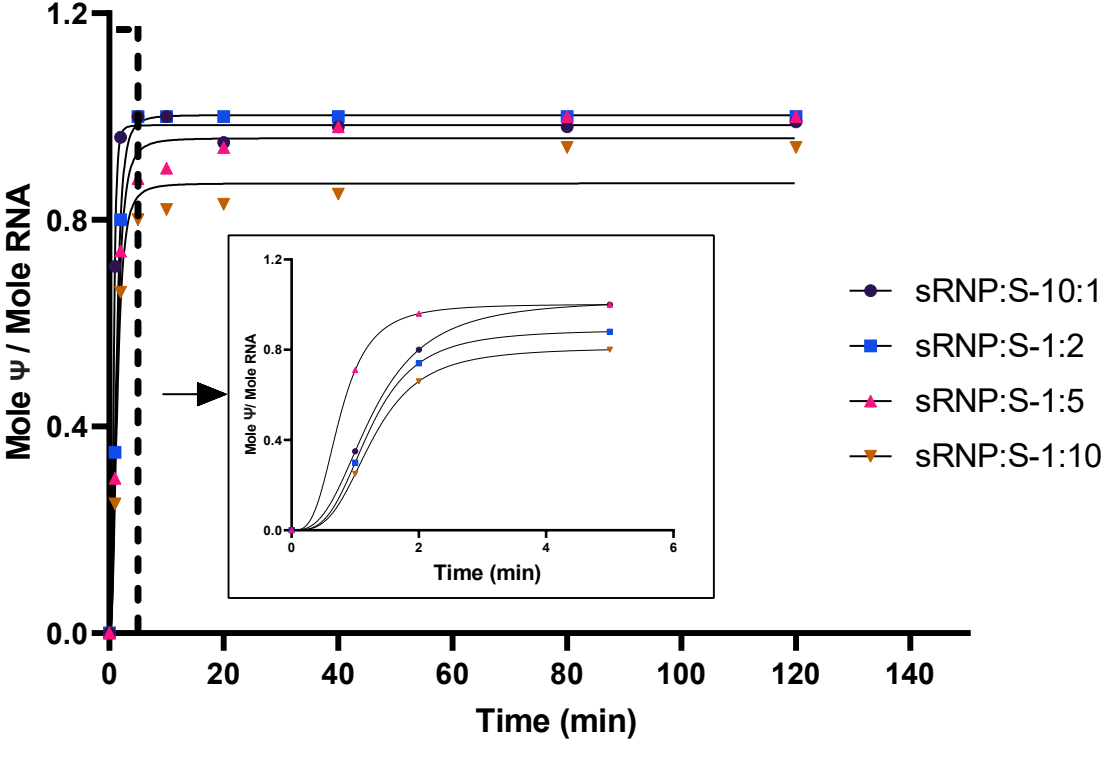
Supplemental Figure S6. Schematic presentation of cloning vectors. (A) pKS$^+$Metpro. Here an insert containing promoter (P) and terminator (T) of *H. volcanii* elongator tRNA-Met gene (Datta et al. 1989) that are connected by a *Sma*I site (sequence in Supplemental Fig. S6), is cloned in the multiple cloning site of pBlueScript KS$^+$. (B) Steps in conversion of pWL102 (Lam and Doolittle 1989) to pDS2 as described in the Methods section of Supplemental Material.

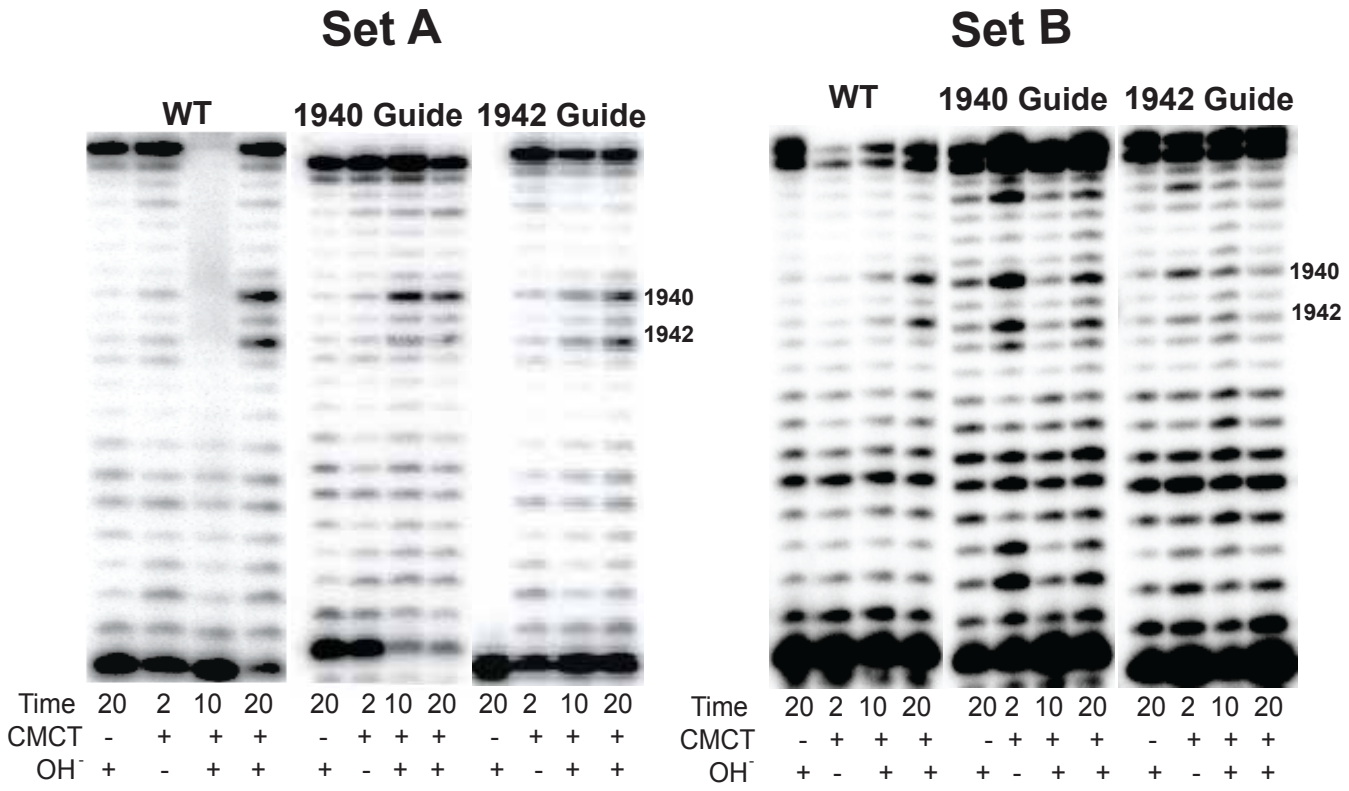
Supplemental Figure S7. Sequence of *Kpn*I-*Sac*I fragment of the pKS^{+}Metpro. The promoter region (marked by single underline) and terminator region (marked by double underline) are indicated by P and T within square boxes, respectively. Various restriction sites are indicated above the sequence. The *Sma*I sequence connecting the two regions is included in a box. The arrow indicates the transcription start site.

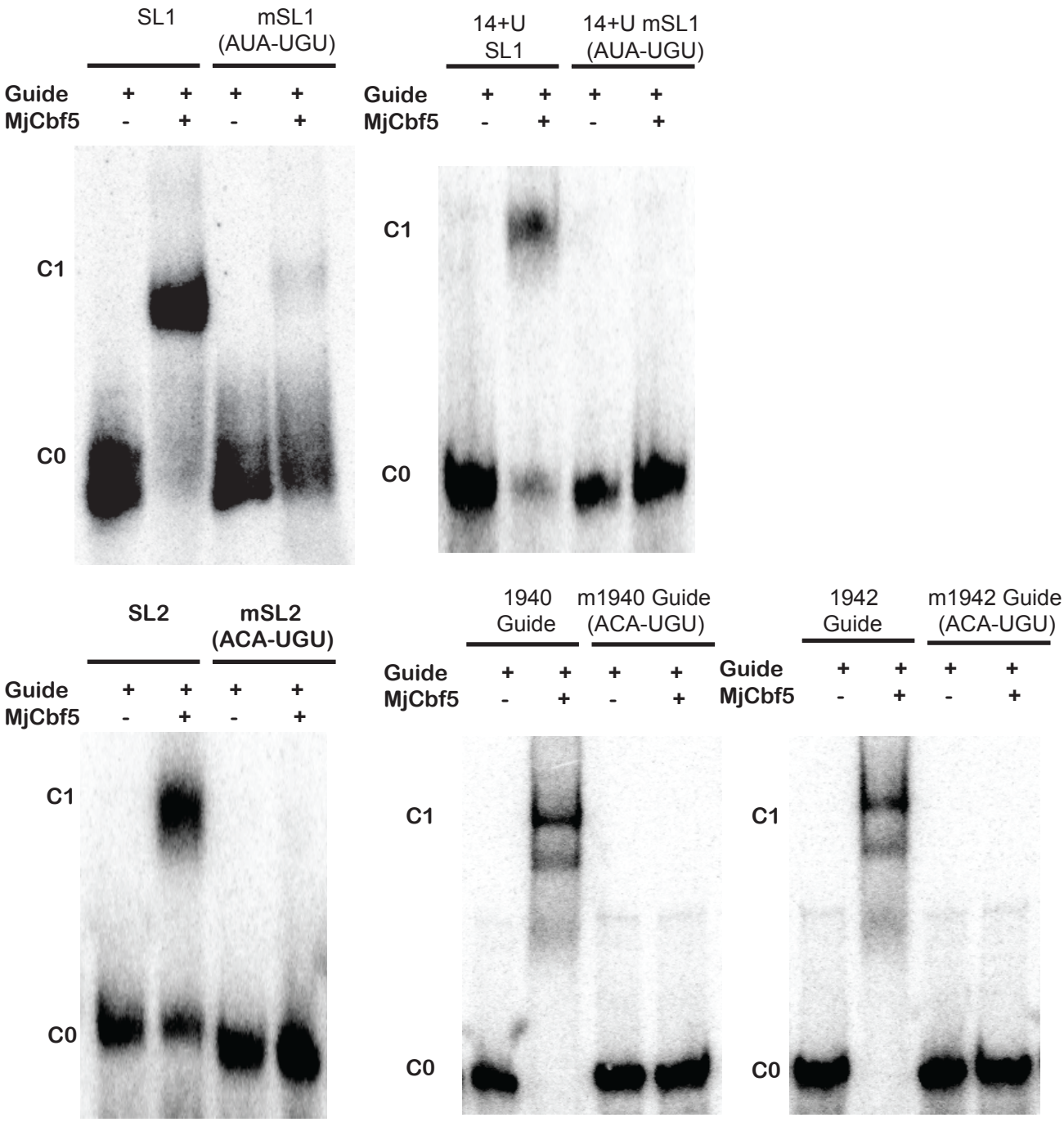
Supplemental Figure S8. Northern blot analyses to show in vivo expression of mutant RNAs that did not produce Ψ at the appropriate positions. The RNAs isolated from these strains were separated by 6% PAGE. Mutants of SL1 (A) and SL2 (B) are indicated above each lane. ^{32}P-labeled probes used for hybridization are listed on the side of panels. 5S rRNA was used as a loading control.

Supplemental Figure S9. Coomassie blue-stained 15% SDS-PAGE gels of different recombinant *M. jannaschii* proteins purified by Ni-NTA chromatography alongside a protein ladder. Calculated sizes of the Cbf5, L7Ae, Nop10 and Gar1 proteins are approximately 41, 12, 7 and 7 kDa.

Figure S1


Mole ψ / Mole RNA
1.2
0.8
0.4
0.0
0
20
40
60
80
100
120
140
Time (min)
sRNP:S-10:1
sRNP:S-1:2
sRNP:S-1:5
sRNP:S-1:10
Mole Ψ/ Mole RNA
1.2
0.8
0.4
0.0
0
2
4
6
Time (min)


Set A
WT
1940 Guide
1942 Guide
1940
1942
Time 20 2 10 20 20 2 10 20 20 2 10 20
CMCT - + + + - + + + - + + +
OH- + - + + + - + + + - + +
Set B
WT
1940 Guide
1942 Guide
1940
1942
Time 20 2 10 20 20 2 10 20 20 2 10 20
CMCT - + + + - + + + - + + +
OH- + - + + + - + + + - + +


SL1
mSL1 (AUA-UGU)
Guide + + + +
MjCbf5 - + - +
C1
C0
14+U SL1
14+U mSL1 (AUA-UGU)
Guide + + + +
MjCbf5 - + - +
C1
C0
SL2
mSL2 (ACA-UGU)
Guide + + + +
MjCbf5 - + - +
C1
C0
1940 Guide
m1940 Guide (ACA-UGU)
Guide + + + +
MjCbf5 - + - +
C1
C0
1942 Guide
m1942 Guide (ACA-UGU)
Guide + + + +
MjCbf5 - + - +
C1
C0

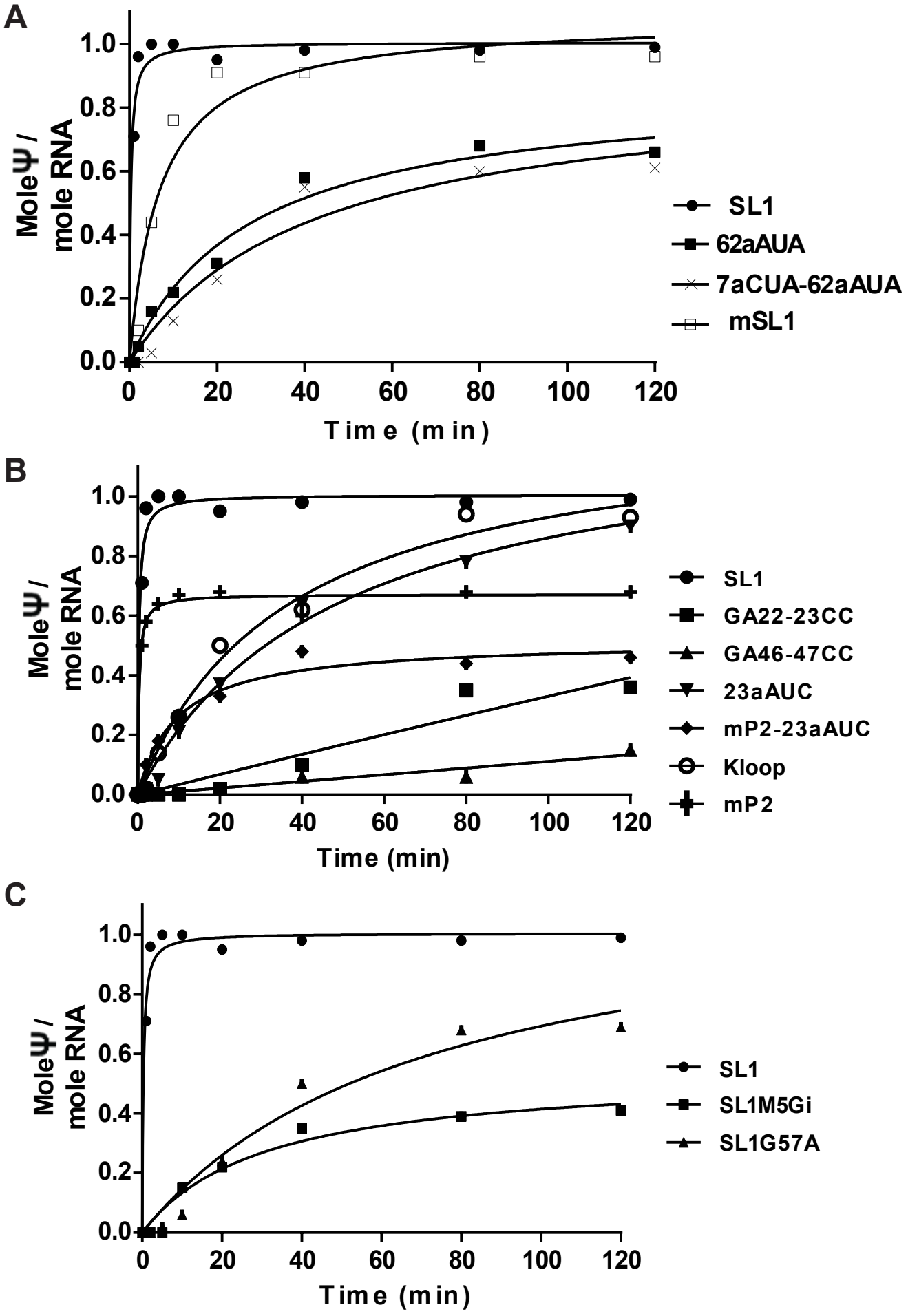
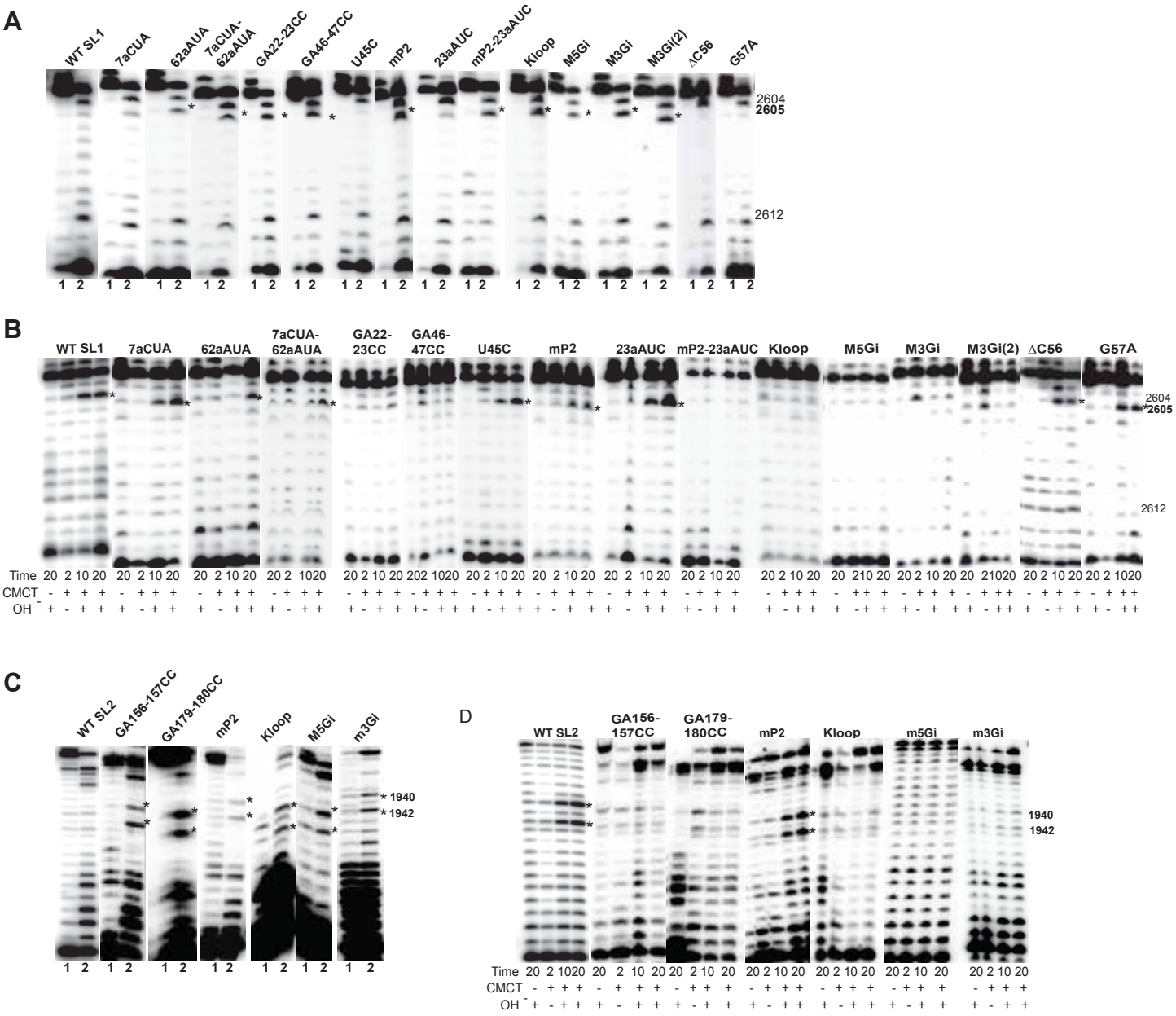

A
Mole Ψ / mole RNA
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (min)
SL1
62aAUA
7aCUA-62aAUA
mSL1
B
Mole Ψ / mole RNA
Time (min)
SL1
GA22-23CC
GA46-47CC
23aAUC
mP2-23aAUC
Kloop
mP2
C
Mole Ψ / mole RNA
Time (min)
SL1
SL1M5Gi
SL1G57A

Figure S5


A
WT SL1
7aCUA
62aAUA
7aCUA-62aAUA
GA22-23CC
GA46-47CC
U45C
mP2
23aAUC
mP2-23aAUC
Kloop
M5Gi
M3Gi
M3Gi(2)
ΔC56
G57A
2604
2605
2612
1 2
B
WT SL1
7aCUA
62aAUA
7aCUA-62aAUA
GA22-23CC
GA46-47CC
U45C
mP2
23aAUC
mP2-23aAUC
Kloop
M5Gi
M3Gi
M3Gi(2)
ΔC56
G57A
2604
2605
2612
Time
CMCT
OH
C
WT SL2
GA156-157CC
GA179-180CC
mP2
Kloop
M5Gi
m3Gi
1940
1942
1 2
D
WT SL2
GA156-157CC
GA179-180CC
mP2
Kloop
m5Gi
m3Gi
1940
1942
Time
CMCT
OH

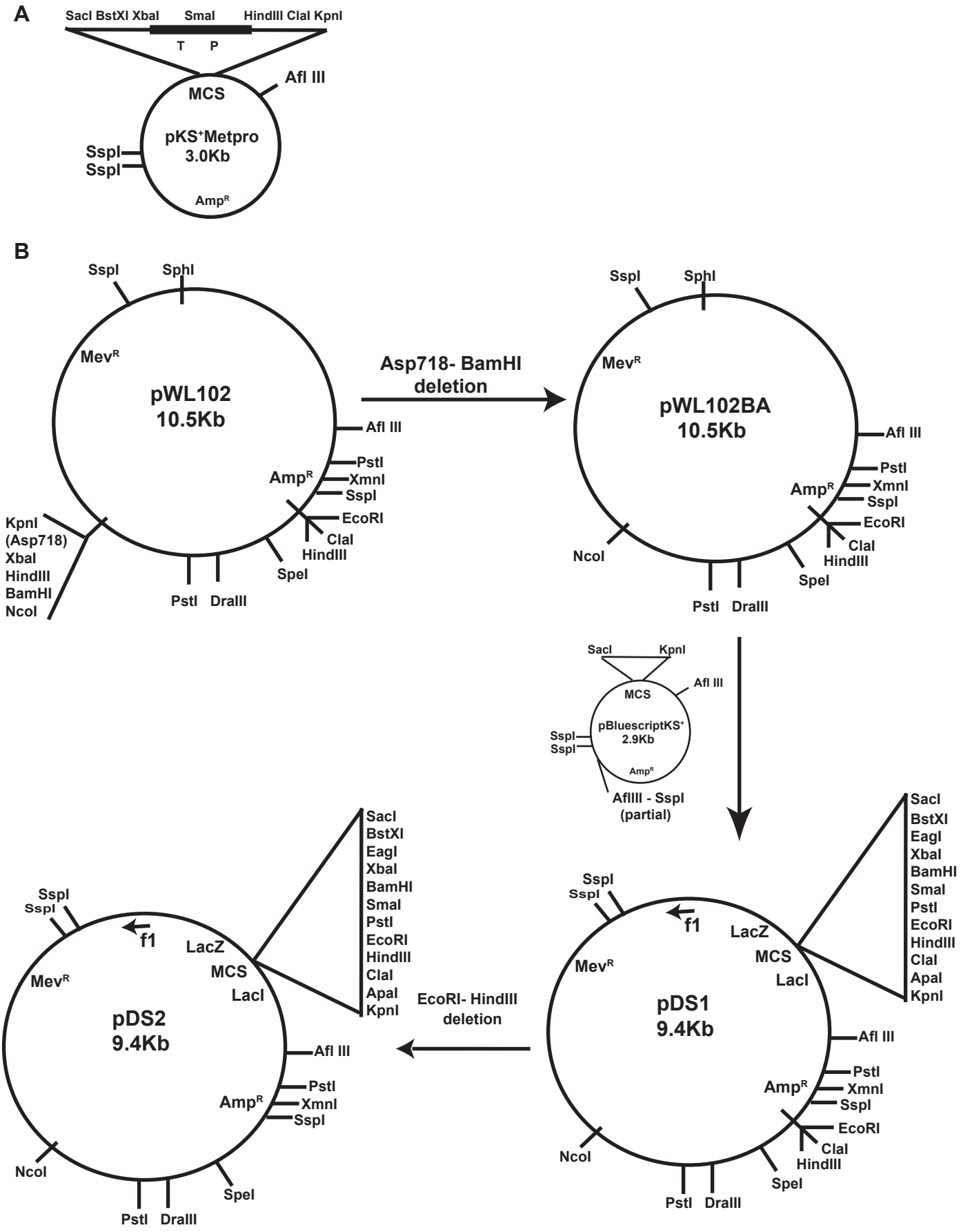
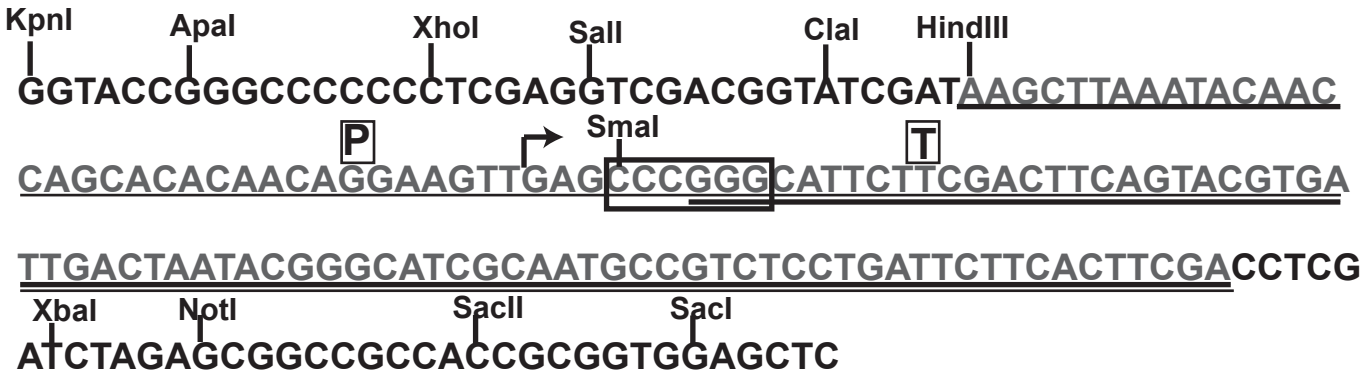

A
SacI BstXI XbaI
SmaI
HindIII ClaI KpnI
T
P
MCS
AfI III
pKS+Metpro
3.0Kb
SspI
SspI
Amp^R
B
SspI
SphI
Mev^R
pWL102
10.5Kb
AfI III
PstI
XmnI
SspI
EcoRI
ClaI
HindIII
SpeI
PstI
DraIII
KpnI
(Asp718)
XbaI
HindIII
BamHI
NcoI
Asp718- BamHI
deletion
pWL102BA
10.5Kb
SacI
KpnI
pBluescriptKS+
2.9Kb
AflIII - SspI
(partial)
EcoRI- HindIII
deletion
pDS1
9.4Kb
pDS2
9.4Kb
f1
LacZ
MCS
LacI
SacI
BstXI
EagI
XbaI
BamHI
SmaI
PstI
EcoRI
HindIII
ClaI
ApaI
KpnI
NcoI

Figure S7


KpnI
ApaI
XhoI
SalI
ClaI
HindIII
GGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTAAATACAAC
P
SmaI
T
CAGCACACAACAGGAAGTTGAGCCCGGGCATTCTTCGACTTCAGTACGTGA
TTGACTAATACGGGCATCGCAATGCCGTCTCCTGATTCTTCACTTCGACCTCG
XbaI
NotI
SacII
SacI
ATCTAGAGCGGCCGCCACCGCGGTGGAGCTC

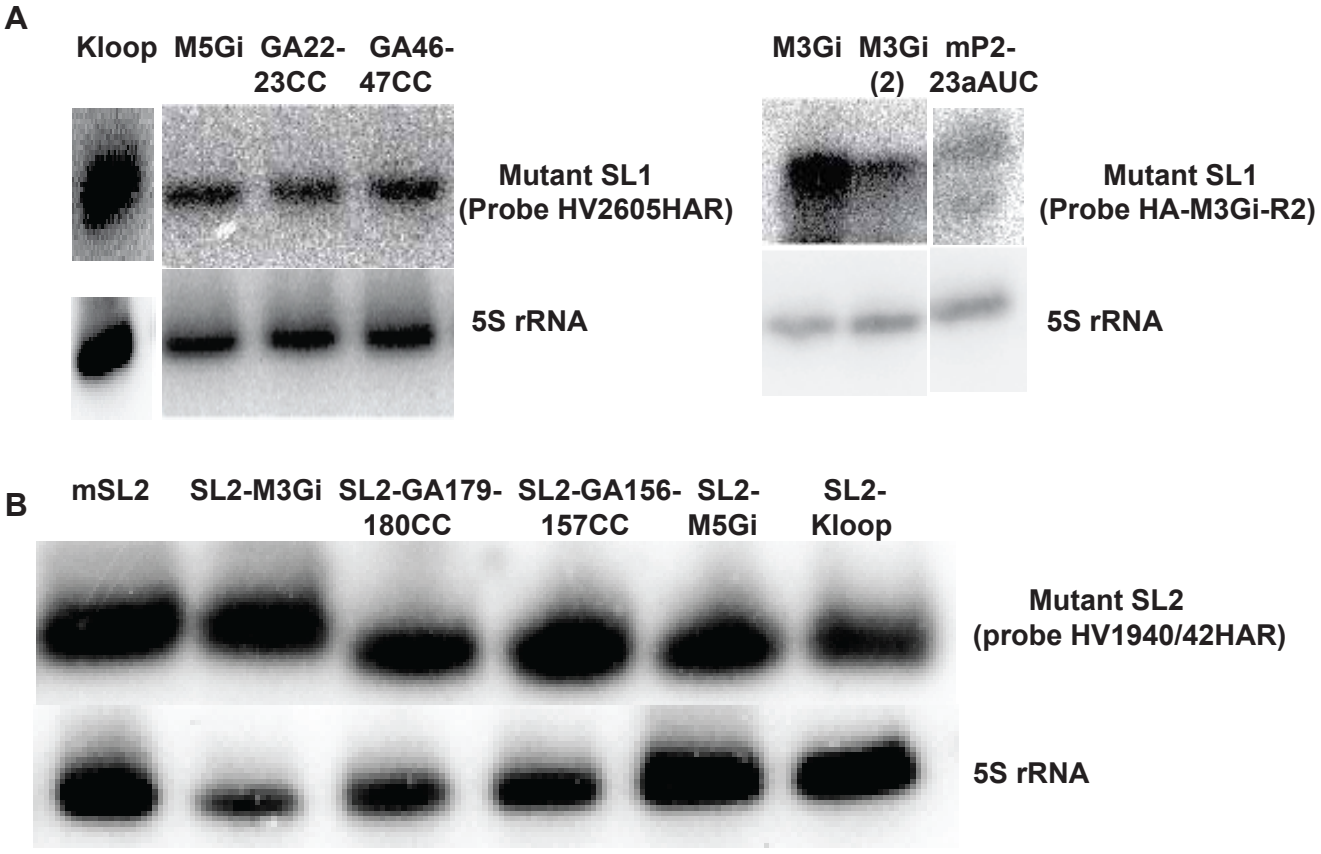
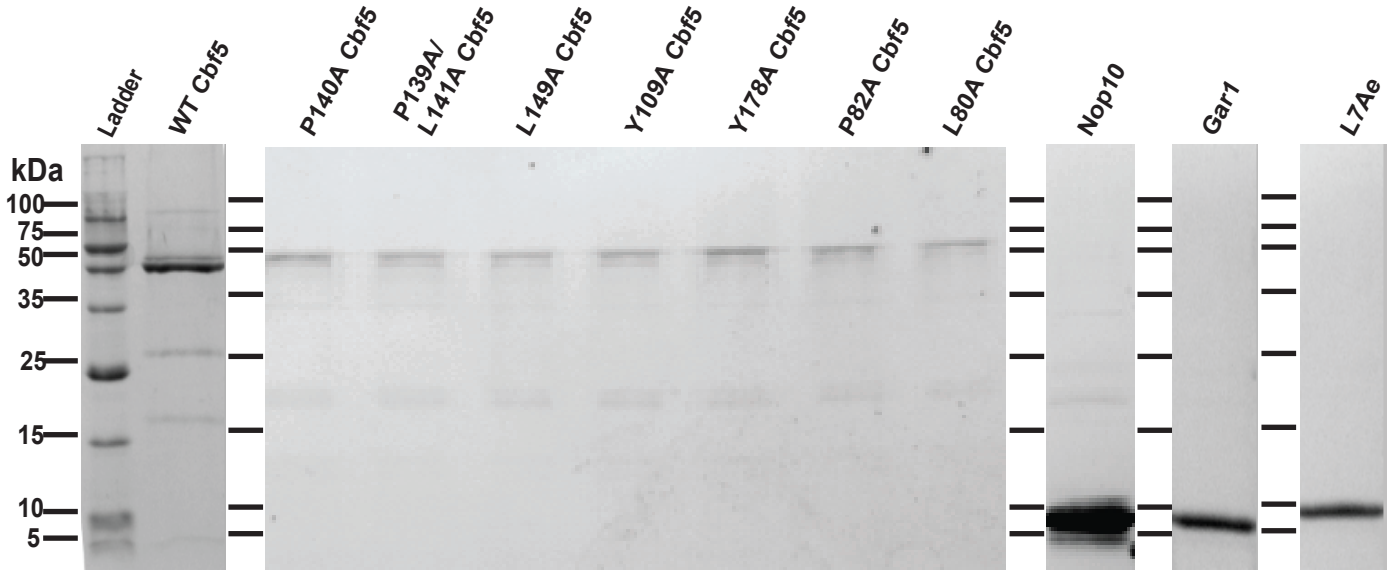

A
Kloop
M5Gi
GA22-23CC
GA46-47CC
Mutant SL1
(Probe HV2605HAR)
5S rRNA
M3Gi
M3Gi (2)
mP2-23aAUC
Mutant SL1
(Probe HA-M3Gi-R2)
5S rRNA
B
mSL2
SL2-M3Gi
SL2-GA179-180CC
SL2-GA156-157CC
SL2-M5Gi
SL2-Kloop
Mutant SL2
(probe HV1940/42HAR)
5S rRNA

Figure S9


Ladder
WT Cbf5
P140A Cbf5
P139A/ L141A Cbf5
L149A Cbf5
Y109A Cbf5
Y178A Cbf5
P82A Cbf5
L80A Cbf5
Nop10
Gar1
L7Ae
kDa
100
75
50
35
25
15
10
5

Supplemental Table S1: List of oligonucleotides used in this work and their functions

Oligonucleotide	Sequence	Purpose
Primers used to detect and clone sR-h45 gene and its shorter versions, and for northern analyses		
HV1940/42HAR	GTG TCG CCC AGA ACA CTA ACG GCC G	Probe 1 of Fig.1A. Also used as reverse primer for PCR to produce most SL2 guide RNA.
HV2605HAR	CTA TTG CGC ACC CGG GTT CGC CG	Probe 2 of Fig.1A. Also used as reverse primer for PCR to produce most SL1 guide RNA.
HVHAMID-F	GTC CAG CGT CAC CGA CTG TCT GAG	Forward primer starting ~200b upstream of sR-h45 gene, primer 1 of Fig. 1B.
HVHA-ER	GGG CTG CAG GAA TTC TGA GCA GCC TCG GCA AGG TGT TCC	Reverse primer starting ~1000b downstream of sR-h45 gene, has 14 extra bases at the 5' end, primer 2 of Fig. 1B.
2605HA-F	CGC GAA TTC GGG TGC GTA CCT CAA GTC CC	Forward primer at the 5' end of sR-h45 gene, has 13 extra bases at the 5' end, primer 3 of Fig. 1B.
1940/42HA-R	GCG CAA GCT TGT GTC GCC CAG AAC ACT AAC GGC CG.	Reverse primer at the 3' end of sR-h45 gene, has 9 extra bases at the 5' end, primer 4 of Fig. 1B.
HVHA-F2	CCG GCA GCT GGG TGC GTA CCT CAA GTC CC	Forward primer to amplify sR-h45 DNA, has *Pvu*II site, used to clone sR-h45 in pKS$^+$Metpro.
HVHA-R	GCC CTT TAA AGG TGT CGC CCA GAA CAC TAA CGG	Reverse primer to amplify sR-h45 DNA, has *Dra*I site, used to clone sR-h45 in pKS$^+$Metpro.
HV-dL-F	GGC GAA CCC GGG TGC GCA ATA GCT ACC GCC CGG CAC GAG GGT TTC CCG	Forward primer to delete linker between SL1 and SL2 (position 77-130) in pHsR-h45 to produce pΔLinker.
HV-dL-R	CGG GAA ACC CTC GTG CCG GGC GGT AGC TAT TGC GCA CCC GGG TTC GCC	Reverse primer to delete linker between SL1 and SL2 (position 77-130) in pHsR-h45 to produce pΔLinker.
HA-dLST2-F	CGG CGA ACC CGG GTG CGC ACA CCT TTG GGC ATT CTT CGA CTT C	Forward primer to delete linker and SL2 of sR-h45 to produce pHSL1.
HA-dLST2-R	GAA GTC GAA GAA TGC CCA AAG GTG TGC GCA CCC GGG TTC GCC G	Reverse primer to delete linker and SL2 of sR-h45 to produce pHSL1.

HA-dLST2-F2	GGC GAA CCC GGG TGC GCA ATA GCT ACC TTT GGG CAT TCT TCG ACT TC	Forward primer to delete linker and SL2 of sR-h45 to produce pHSL2.
HA-dLST2-R2	GAA GTC GAA GAA TGC CCA AAG GTA GCT ATT GCG CAC CCG GGT TCG CC	Reverse primer to delete linker and SL2 of sR-h45 to produce pHSL2.
5S rRNA	CCT CAC CGC TCT GGC CGC C	Complementary to *H. volcanii* 5S sequence, near its 3'-end.

Primers to detect in vivo production of Ψ		
HVLSUR1	GCA AGG TAC TAC GCT ACC	Primer to determine Ψ1940 and Ψ1942 production.
HVLSUR2	GCA GCC GAC CTG TCT C	Primer to determine Ψ2605 production.

Primers to mutate plasmids		
HA-dA71-F	CGA ACC CGG GTG CGC ATA GCT ACC TTT GGG CAT TCT TCG	Forward primer to delete A71 in pHSL1.
HA-dA71-R	CGA AGA ATG CCC AAA GGT AGC TAT GCG CAC CCG GGT TCG	Reverse primer to delete A71 in pHSL1.
HA-62aATA-F	GAC AGC ACG GCG AAC CCA TAG GGT GCG CAA TAG CTA CC	Forward primer to insert ATA after residue 62 in pHsR-h45.
HA-62aATA-R	GGT AGC TAT TGC GCA CCC TAT GGG TTC GCC GTG CTG TC	Reverse primer to insert ATA after residue 62 in pHsR-h45.
HA-7aCTA-F2	GGA AGT TGA GCG TAC CCT ATC AAG TCC CCG GCC G	Forward primer to change U8 of SL1 in pHSL1 to CUA.
HA-7aCTA-R2	CGG CCG GGG ACT TGA TAG GGT ACG CTC AAC TTC C	Reverse primer to change U8 of SL1 in pHSL1 to CUA.
HA-ATA72TGT-F2	CGA ACC CGG GTG CGC ATG TGC TAC CTT TGG GCA TTC TTC G	Forward primer to create ATA72TGT in pHSL1.
HA-ATA72TGT-R2	CGA AGA ATG CCC AAA GGT AGC ACA TGC GCA CCC GGG TTC G	Reverse primer to create ATA72TGT in pHSL1.
HA-ACA205TGT-F	GCC GTT AGT GTT CTG GGC GTG TCC TTT GGG CAT TCT TCG ACT TC	Forward primer to create ACA205TGT mutation in pHSL2
HA-ACA205TGT-R	GAA GTC GAA GAA TGC CCA AAG GAC ACG CCC AGA ACA CTA ACG GC	Reverse primer to create ACA205TGT mutation in pHSL2

HA-GA22CC-F	GTA CCT CAA GTC CCC GGC CCC GTG TTC CCG CTT CGG GAG C	Forward primer to create GA22CC mutation in pHsR-h45.
HA-GA22CC-R	GCT CCC GAA GCG GGA ACA CGG GGC CGG GGA CTT GAG GTA C	Reverse primer to create GA22CC mutation in pHsR-h45.
HA-GA46CC-F	CGC TTC GGG AGC GAT CCC AGC ACG GCG AAC C	Forward primer to create GA46CC mutation in pHsR-h45.
HA-GA46CC-R	GGT TCG CCG TGC TGG GAT CGC TCC CGA AGC G	Reverse primer to create GA46CC mutation in pHsR-h45.
HA-GA156CC-F	GCA CGA GGG TTT CCC GGT CCC CGC GGC ACG CCG CCT CGG G	Forward primer to create GA156CC mutation in pHsR-h45.
HA-GA156CC-R	CCC GAG GCG GCG TGC CGC GGG GAC CGG GAA ACC CTC GTG C	Reverse primer to create GA156CC mutation in pHsR-h45.
HA-GA179CC-F	GCA CGC CGC CTC GGG ATC CGA CCG GCC GTT AGT GTT CTG G	Forward primer to create GA179CC mutation in pHsR-h45.
HA-GA179CC-R	CCA GAA CAC TAA CGG CCG GTC GGA TCC CGA GGC GGC GTG C	Reverse primer to create GA179CC mutation in pHsR-h45.
HA-mP2-F	CCT CAA GTC CCC GTG CTG GAG TGT TCC GCT TCG G	Forward primer inserting residues to make perfect pairs in lower part of P2 stem of SL1 in pHsR-h45.
HA-mP2-R	CCG AAG CGG AAC ACT CCA GCA CGG GGA CTT GAG G	Reverse primer inserting residues to make perfect pairs in lower part of P2 stem of SL1 in pHsR-h45.
HA-mP2-F2	GAG GGT TTC CCG GTC GGG ACG CGG CAC GCC GCC TCG GGA TGA CCG ACC GGC CGT TAG TG	Forward primer making eight perfect pairs in lower part of P2 stem of SL2 in pHsR-h45.
HA-mP2-R2	CAC TAA CGG CCG GTC GGT CAT CCC GAG GCG GCG TGC CGC GTC CCG ACC GGG AAA CCC TC	Reverse primer making eight perfect pairs in lower part of P2 stem of SL2 in pHsR-h45
HA-23aATC-F2	CCT CAA GTC CCC GTG CTG GAA TCG TGT TCC CGC TTC GGG AGC	Forward primer to insert ATC between residues 23 & 24 and residues to make perfect pairs in lower part of P2 stem of SL1 in pHsR-h45
HA-23aATC-R2	GCT CCC GAA GCG GGA ACA CGA TTC CAG CAC GGG GAC TTG AGG	Reverse primer to insert ATC between residues 23 & 24 and residues to make perfect pairs in

		lower part of P2 stem in SL1 in pHsR-h45.
HA-KL-F	CGT ACC TCA AGT CCC CGG CCG ATC GAT GAC AGC ACG GCG AAC CC	Forward primer to create K-loop in upper part of P2 stem of SL1 in pHsR-h45.
HA-KL-R	GGG TTC GCC GTG CTG TCA TCG ATC GGC CGG GGA CTT GAG GTA CG	Reverse primer to create K-loop in upper part of P2 stem of SL1 in pHsR-h45.
HA-KL-F2	GGC ACG AGG GTT TCC CGG TCG ATG GAT GAG ACC GGC CGT TAG TG	Forward primer to create K-loop in P2 stem of SL2 in pHsR-h45.
HA-KL-R3	CAC TAA CGG CCG GTC TCA TCC ATC GAC CGG GAA ACC CTC GTG CC	Reverse primer to create K-loop in P2 stem of SL2 in pHsR-h45
HA-M5Gi-F	CGA AAG ACG GAC CGC CCG GCA CCT CCC ATT CCC GGT CGA CGC GGC ACG C	Forward primer to change 5' guide GAGGGT sequence of SL2 to CTCCCA in pHsR-h45.
HA-M5Gi-R	GCG TGC CGC GTC GAC CGG GAA TGG GAG GTG CCG GGC GGT CCG TCT TTC G	Reverse primer to change 5' guide GAGGGT sequence of SL2 to CTCCCA in pHsR-h45.
HA-M5Gi-F2	GAA GTT GAG CGT ACC TCA ACA GCC CGG CCG AGT GTT CC	Forward primer to change 5' guide GTC sequence of SL1 to CAG in pHsR-h45.
HA-M5Gi-R2	GGA ACA CTC GGC CGG GCT GTT GAG GTA CGC TCA ACT TC	Reverse primer to change 5' guide GTC sequence of SL1 to CAG in pHsR-h45.
HA-M3Gi-F	CCT CGG GAT GAG ACC GGC CCA ATG TGT TCT GGG CGA CAC C	Forward primer to change 3' guide GTTA sequence of SL2 in pHsR-h45 to CAAT
HA-M3Gi-R	GGT GTC GCC CAG AAC ACA TTG GGC CGG TCT CAT CCC GAG G	Reverse primer to change 3' guide GTTA sequence of SL2 in pHsR-h45 to CAAT.
HA-M3Gi-F2	GGA GCG ATG ACA GCA CGG CGT TGG AGG GTG CGC AAT AGC TAC C	Forward primer to change 3' guide AACCC sequence of SL1 in pHsR-h45 to TTGGA.
HA-M3Gi-R2	GGT AGC TAT TGC GCA CCC TCC AAC GCC GTG CTG TCA TCG CTC C	Reverse primer to change 3' guide AACCC sequence of SL1 in pHsR-h45 to TTGGA. Also used as probe in Northern Blots.
HA-M3Gi-F3	GGA GCG ATG ACA GCA CGG CGA ACG AGG GTG CGC AAT AGC TAC C	Forward primer to change 3' guide AACCC sequence of SL1 in pHsR-h45 to AACGA.
HA-M3Gi-R3	GGT AGC TAT TGC GCA CCC TCG TTC GCC GTG CTG TCA TCG CTC C	Reverse primer to change 3' guide AACCC sequence of SL1 in pHsR-h45 to AACGA.

HA-G57A-F	CGA TGA CAG CAC GGC AAA CCC GGG TGC GCA ATA GC	Forward primer to create G57A mutation in pHsR-h45.
HA-G57A-R	GCT ATT GCG CAC CCG GGT TTG CCG TGC TGT CAT CG	Reverse primer to create G57A mutation in pHsR-h45
HA-dC56-F	GCG ATG ACA GCA CGG GAA CCC GGG TGC GCA ATA GC	Forward primer to delete C56 of SL1 in pHsR-h45.
HA-dC56-R	GCT ATT GCG CAC CCG GGT TCC CGT GCT GTC ATC GC	Reverse primer to delete C56 of SL1 in pHsR-h45.

Primers for PCR to prepare guide RNAs		
T7HV2605HAF2	TAA TAC GAC TCA CTA TAG CGT ACC TCA AGT CCC CGG	RNA polymerase T7 promoter (17 nt) followed by 19 nt of SL1 of sR-h45 to produce Ψ2605. Also used as forward primer for PCR to produce most SL1 guide RNA.
T7HV2605mHAF	TAA TAC GAC TCA CTA TAG GCG TAC CTC AAC AGC CCG G	RNA polymerase T7 promoter (17 nt) followed by a G and 19 nt of 5' guide mutant of SL1 of sR-h45.
HV2605HAR2	CTG TGC GCA CCC GGG TTC GCC G	Reverse primer to produce SL1 changing AAUA to ACA.
HV2605HAR3	CTA TGC GCA CCC GGG TTC GCC G	Reverse primer to produce SL1 changing AAUA to AUA.
HV2605HAR4	CTT TTG CGC ACC CGG GTT CGC CG	Reverse primer to produce SL1 changing AAUA to AAAA.
HV2605HAR5	CAC ATG CGC ACC CGG GTT CGC CG	Reverse primer to produce SL1 changing AAUA to AUGU.
HV2605HAR6	CTG TTG CGC ACC CTA TGG GTT CGC	Reverse primer to produce SL1 with AUA inserted after residue 62.
HV2605HAR7	CTG TTG CGC ACC CTC CAA CGC CG	Reverse primer to produce SL1 with 3 'guide sequence changed from AACCC to TTGGA.
T7HV2605HAF3	TAA TAC GAC TCA CTA TAG CGT ACC TCA AGT CCC CG	RNA polymerase T7 promoter (17 nt) followed by 18 nt of SL1 of sR-h45 to produce Ψ2605.
T7HV2605HAF4	TAA TAC GAC TCA CTA TAG CGT ACC CTA TCA AGT CC	RNA polymerase T7 promoter (17 nt) followed by 18 nt of SL1 (inserting CUA in P1 stem)) to produce Ψ2605.
HV2605HAR9	CTA TTG CGC ACC CGG GTT TGC CGT GC	Reverse primer to produce SL2 with G57 changed to A57.
HV2605HAR10	GCG CAC CCG GGT TCG CCG TGC	Reverse primer to produce SL2 with deleted ACA region.
T7HV1940/42HAF	TAA TAC GAC TCA CTA TAG GAC CGC CCG GCA CGA GG	RNA polymerase T7 promoter (17 nt) followed by 18 nt of SL2 of sR-h45 to produce Ψ1940/Ψ1942. Also used as forward primer for PCR to produce most SL2 guide RNA.

HV1940/42HAR3	AGG ACA CGC CCA GAA CAC TAA CGG CCG GTC	Reverse primer to produce SL2 with ACA changed to TGT,
HV1940/42HAR4	GTG TCG CCC AGA ACT AAC GGC CG	Reverse primer to produce SL2 with deletion of GU (193-194).
HV1940/42HAR5	GTG TCG CCC AGA ACT AAC TAC CG	Reverse primer to produce SL2 with deletion of GU (193-194) and changing CC (187-188) to UA.
HV1940/1942HAR6	GAC ACG CCC AGA ACA CTA AC	Reverse primer for Ψ1940 of artificial perfect 1940 guide with box ACA changed to UGU.
HV1940/1942HAR7	GAC ACG CCC AGA ACTA AC	Reverse primer for Ψ1942 of artificial perfect 1942 guide with box ACA changed to UGU.
HA-dTTC147-F	CCG CCC GGC ACG AGG GTC CGG TCG ACG CGG CAC G	Forward primer to delete TTC (147-149) of SL2 cloned in pKS$^+$Metpro.
HA-dTTC147-R	CGT GCC GCG TCG ACC GGA CCC TCG TGC CGG GCG G	Reverse primer to delete TTC (147-149) of SL2 cloned in pKS$^+$Metpro.
HA-TTC147CA-F	CCG CCC GGC ACG AGG GTC ACC GGT CGA CGC GGC ACG	Forward primer to change TTC (147-149) of SL2 cloned in pKS$^+$Metpro to CA.
HA-TTC147CA-R	CGT GCC GCG TCG ACC GGT GAC CCT CGT GCC GGG CGG	Reverse primer to change TTC (147-149) of SL2 cloned in pKS$^+$Metpro to CA.
HA-dCC187-F	CTC GGG ATG AGA CCG GGT TAG TGT TCT GGG CGA CAC	Forward primer to delete CC (187-188) of SL2 cloned in pKS$^+$Metpro.
HA-dCC187-R	GTG TCG CCC AGA ACA CTA ACC CGG TCT CAT CCC GAG	Reverse primer to delete CC (187-188) of SL2 cloned in pKS$^+$Metpro.
HA-CC187TA-F	CTC GGG ATG AGA CCG GTA GTT AGT GTT CTG GGC GAC AC	Forward primer to change CC (187-188) of SL2 cloned in pKS$^+$Metpro to TA.
HA-CC187TA-R	GTG TCG CCC AGA ACA TCA ACT ACC GGT CTC ATC CCG AG	Reverse primer to change CC (187-188) of SL2 cloned in pKS$^+$Metpro to TA.
HA-d187CC-194aCC-F	GAT GAG ACC GGG TTA GTC CGT TCT GGG CGA CAC	Forward primer to delete CC (187-188) and insert CC after position 194 of SL2 cloned in pKS$^+$Metpro.
HA-d187CC-a194CC-R	GTG TCG CCC AGA ACG GAC TAA CCC GGT CTC ATC	Reverse primer to delete CC (187-188) and insert CC after position 194 of SL2 cloned in pKS$^+$Metpro.
HA-G181T-F	CGC CGC CTC GGG ATG ATA CCG GCC GTT AGT G	Forward primer to create G181T mutation in SL2 cloned in pKS$^+$Metpro.
HA-G181T-R	CAC TAA CGG CCG GTA TCA TCC CGA GGC GGC G	Reverse primer to create G181T mutation in SL2 cloned in pKS$^+$Metpro.

HA-CC187TAdGT193-F	CGG GAT GAG ACC GGT AGT TAG TTC TGG GCG ACA C	Forward primer to change CC (187-188) to TA and delete GT (193-194) of SL2 cloned in pKS$^+$Metpro to CA.
HA-CC187TAdGT193-R	GTG TCG CCC AGA ACT AAC TAC CGG TCT CAT CCC G	Reverse primer to change CC (187-188) to TA and delete GT (193-194) of SL2 cloned in pKS$^+$Metpro to CA.
HA-dGT193-F	GAG ACC GGC CGT TAG TTC TGG GCG ACA C	Forward primer to delete GT (193-194) of SL2 cloned in pKS$^+$Metpro.
HA-dGT193-R	GTG TCG CCC AGA ACT AAC GGC CGG TCT C	Reverse primer to delete GT (193-194) of SL2 cloned in pKS$^+$Metpro.

Primers for PCR to prepare target RNAs		
T7P	TAA TAC GAC TCA CTA TA	Upper strand of the T7 RNA polymerase promoter (-17 to -1).
HV2605TGT-R3	GCC GAC CTG TCT CAC GAC GGT CTA AAC CCA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce 40-mer target RNA (positions 2590-2629) to produce Ψ2605.
HV2605TGT-R4	GCC GAC CTG TCT CAC GAC GGT CTA TTG GAA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce a mutant 40-mer target RNA (positions 2590-2629) to produce Ψ2605.
HV2605TGT-R5	GCC GAC CTG TCT CAC GAC GGT CTG AAC CCA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce a mutant 40-mer target RNA (positions 2590-2629) changing U2605 to C2605.
HV2605TGT-R6	GCC GAC CTG TCT CAC GAC GGT CTT AAC CCA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce a mutant 40-mer target RNA (positions 2590-2629 changing U2605 to A2605.
HV2605TGT-R7	GCC GAC CTG TCT CAC GAC GGT CAG AAC CCA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce a mutant 40-mer target RNA (positions 2590-2629) changing UA2605-6 to CU2605-6.
HV2605TGT-R8	GCC GAC CTG TCT CAC GAC GGT CAA AAC CCA GCT CAC GAC CTA TAG TGA GTC GTA TTA	Lower strand to produce a mutant 40-mer target RNA (positions 2590-2629) changing A2605 to U2605.
HV1940/42TGT-R	TAC CTT AAG AGG GTC ATA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to produce 29-mer target RNA (positions 1929-1957

		with GGG1932-35CCC change) to produce Ψ1940 and Ψ1942
HV1940/42TGT-R2	TAC CTT AAG AGG GTC GTA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to produce 29-mer target RNA with T1942C change to produce Ψ1940 and block Ψ1942.
HV1940/42TGT-R4	TAC CTT AAG AGG GTC ATG GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to produce 29-mer target RNA with T1940C change to produce Ψ1942 and block Ψ1940.
HV1940/42TGT-R5	TAC CTT AAG AGG GTC AAA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to produce 29-mer target RNA) to produce Ψ1940 and Ψ1942 with A1941 changed to U.
HV1940/42TGT-R6	TAC CTT AAG AGG GTC TAA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to make 29-mer target RNA for Ψ1940/Ψ1942 with AU (1941-42) changed to UA.
HV1940/42TGT-R7	TAC CTT AAG AGG GTC AAG TTA CGG GCG CTA TAG TGA GTC GTA TTA	Lower strand to produce 28-mer target RNA for Ψ1940/Ψ1942 with deletion of A1941.
HV1940/42TGT-R8	TAC CTT AAG AGG GTC ACA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to produce 29-mer target RNA for Ψ1940/Ψ1942 with A1941 changed to G.
HV1940/42TGT-R9	TAC CTT AAG AGG GTT AGA GTT ACG GGC GCT ATA GTG AGT CGT ATT A	Lower strand to make 29-mer target RNA for Ψ1940/Ψ1942 with A1941C & G1943A change.

Primers to generate *M. jannaschii* Cbf5 mutants for in vitro assays		
MJCBF-L80A-F	GGA CAT GGT GGG ACA GCA GAC CCA AAG GTT ACT GG	Forward primer to change Leu80 (TTA) of *Mj* Cbf5 gene in pET28a to Ala (GCA).
MJCBF-L80A-R	CCA GTA ACC TTT GGG TCT GCT GTC CCA CCA TGT CC	Reverse primer to change Leu80 (TTA) of *Mj* Cbf5 gene in pET28a to Ala (GCA).
MJCBF-P140A-F	GGA AGG ATT TAT CAG AGA CCT GCA TTA AAA GCA GCT GTT AAA AG	Forward primer to change Pro140 (CGA) of *Mj* Cbf5 gene in pET28a to Ala (GCA).
MJCBF-P140A-R	CTT TTA ACA GCT GCT TTT AAT GCA GGT CTC TGA TAA ATC CTT CC	Reverse primer to change Pro140 (CGA) of *Mj* Cbf5 gene in pET28a to Ala (GCA).
MJCBF-L149A-F	CAT TAA AAG CAG CTG TTA AAA GAA GAG CGA GAA TTA GGA AGA TTC	Forward primer to change Leu149 (TTG) of *Mj* Cbf5 gene in pET28a to Ala (GCG).
MJCBF-L149A-R	GAA TCT TCC TAA TTC TCG CTC TTC TTT TAA CAG CTG CTT TTA ATG	Reverse primer to change Leu149 (TTG) of *Mj* Cbf5 gene in pET28a to Ala (GCG).

MJCBF-P139L141AA-F	GGA AGG ATT TAT CAG AGA GCT CCA GCA AAA GCA GCT GTT AAA AGA AG	Forward primer to change Pro139 (CCT) and L141 (TTA) of *Mj* Cbf5 gene in pET28a to Ala (GCT and GCA, respectively).
MJCBFP139L141AA-R	CTT CTT TTA ACA GCT GCT TTT GCT GGA GCT CTC TGA TAA ATC CTT CC	Reverse primer to change Pro139 (CCT) and L141 (TTA) of *Mj* Cbf5 gene to Ala (GCT and GCA, respectively).
MJCBF-Y178A-F	GGT TAA ATG TCA ATC TGG GAC TGC TAT AAG GAA ATT GTG TG	Forward primer to change Tyr178 (TAT) of *Mj* Cbf5 gene to Ala (GCT).
MJCBF-Y178A-R	CAC ACA ATT TCC TTA TAG CAG TCC CAG ATT GAC ATT TAA CC	Reverse primer to change Tyr178 (TAT) of *Mj* Cbf5 gene to Ala (GCT).
MJCBF-Y109A-F	GCA CAT TCC ACC TAA GGA GGC TGT TTG TTT GAT GCA TC	Forward primer to change Tyr109 (TAT) of *Mj* Cbf5 gene to Ala (GCT).
MJCBF-Y109A-R	GAT GCA TCA AAC AAA CAG CCT CCT TAG GTG GAA TGT GC	Reverse primer to change Tyr109 (TAT) of *Mj* Cbf5 gene to Ala (GCT).
MJCBF-P82A-F	GGT GGG ACA TTA GAC GCA AAG GTT ACT GGT GTT TTG C	Forward primer to change Pro82 (CCA) of *Mj* Cbf5 gene to Ala (GCA).
MJCBF-P82A-R	GCA AAA CAC CAG TAA CCT TTG CGT CTA ATG TCC CAC C	Reverse primer to change Pro82 (CCA) of *Mj* Cbf5 gene to Ala (GCA).