

Comparative patterns of modified nucleotides in individual tRNA species from a mesophilic and two thermophilic archaea

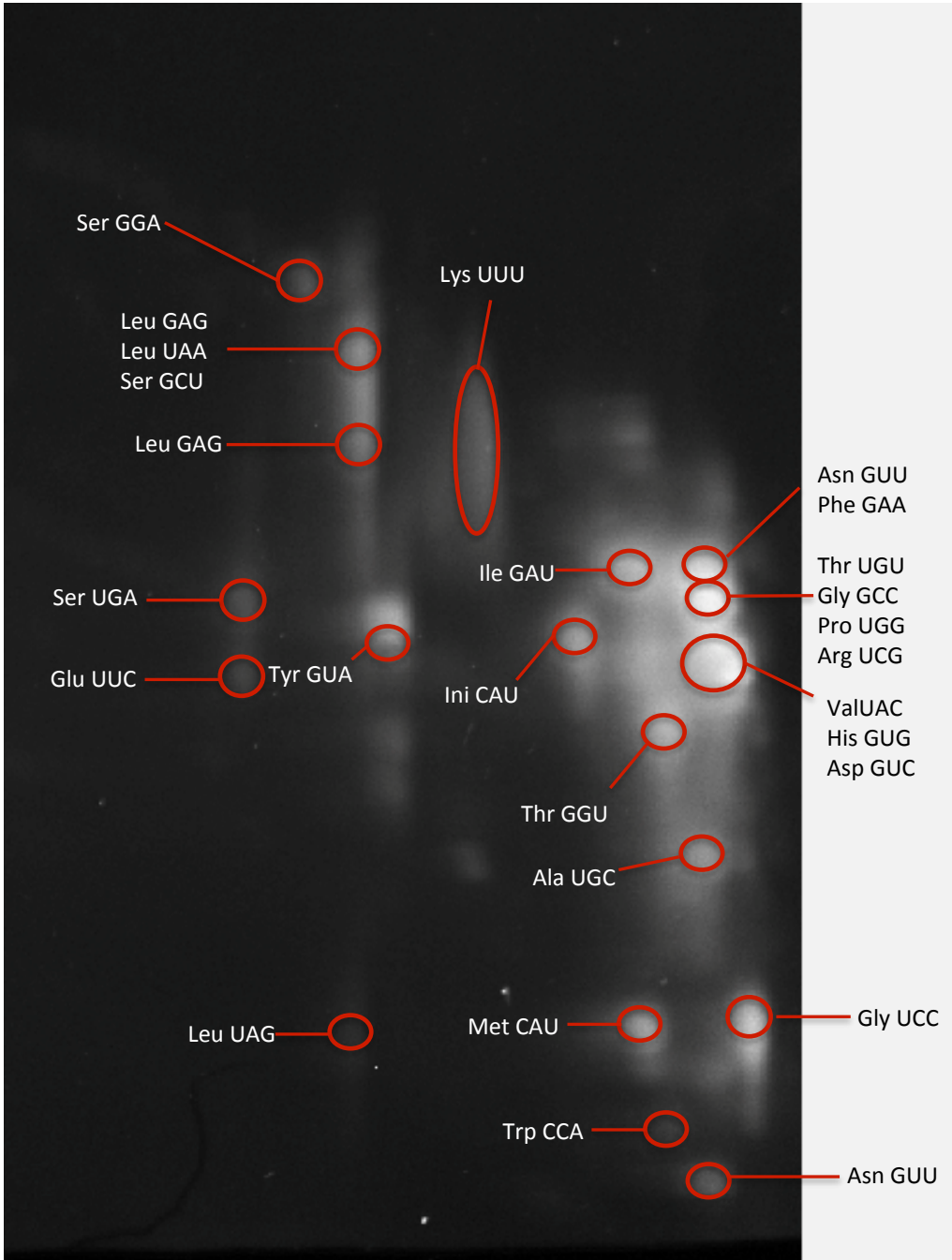
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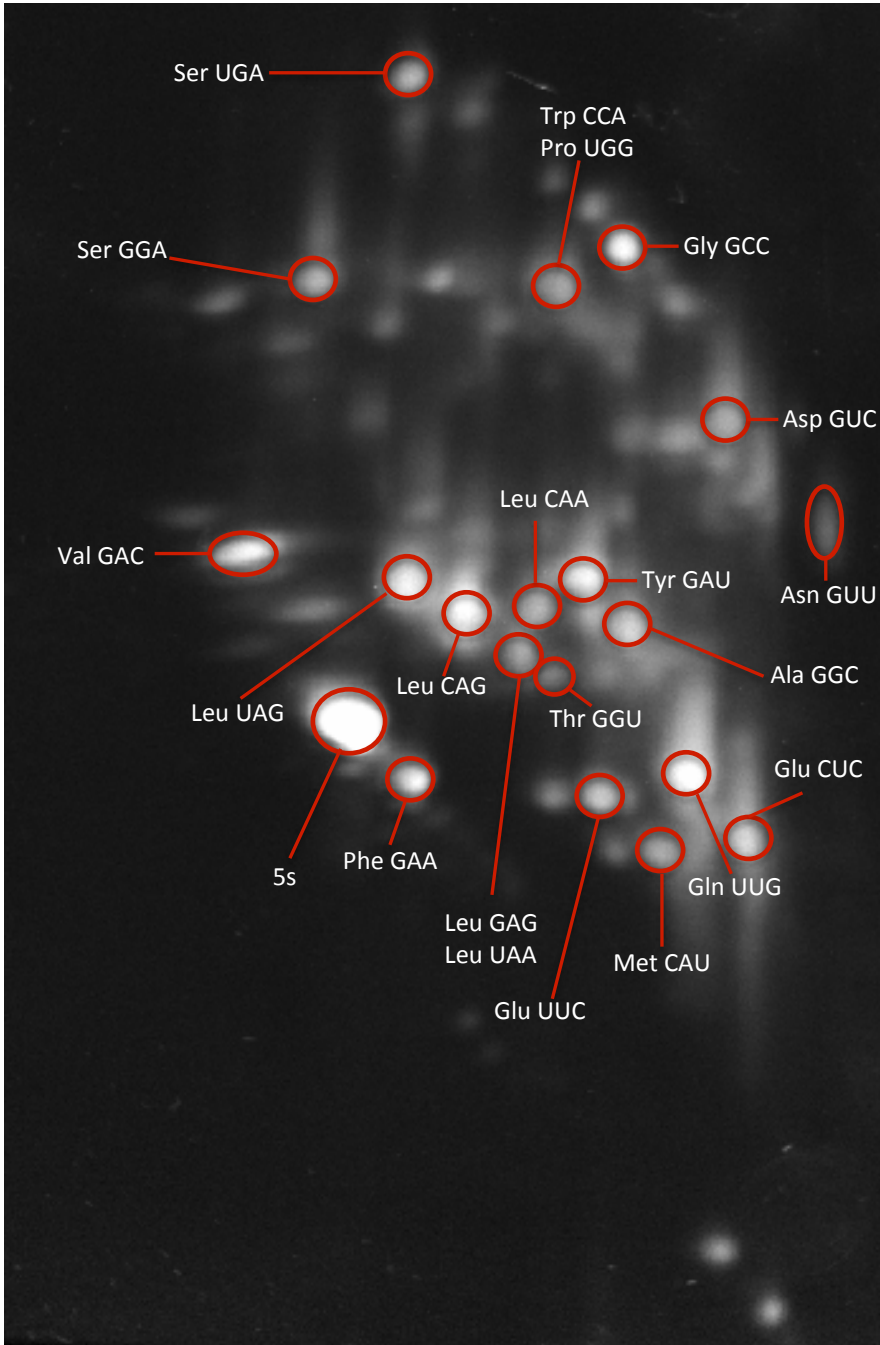
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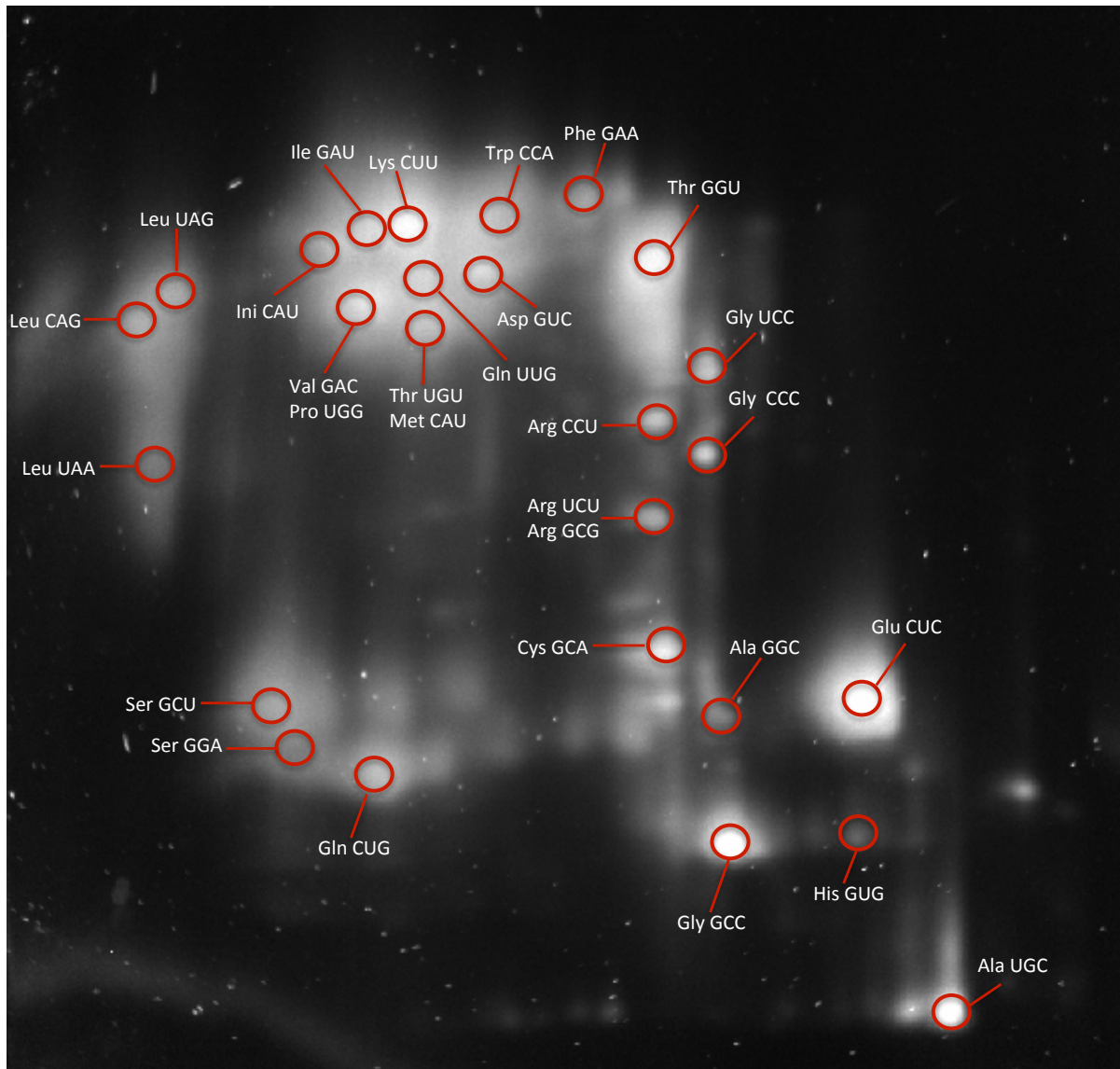
SUPPLEMENTAL MATERIAL



A



B



C

Figure S1

20% 2D polyacrylamide gel of total tRNAs. The spots containing tRNAs that were identified by MS/MS sequencing are circled in red. A) *M. maripaludis*; B) *S. acidocaldarius*; C) *P. furiosus*.

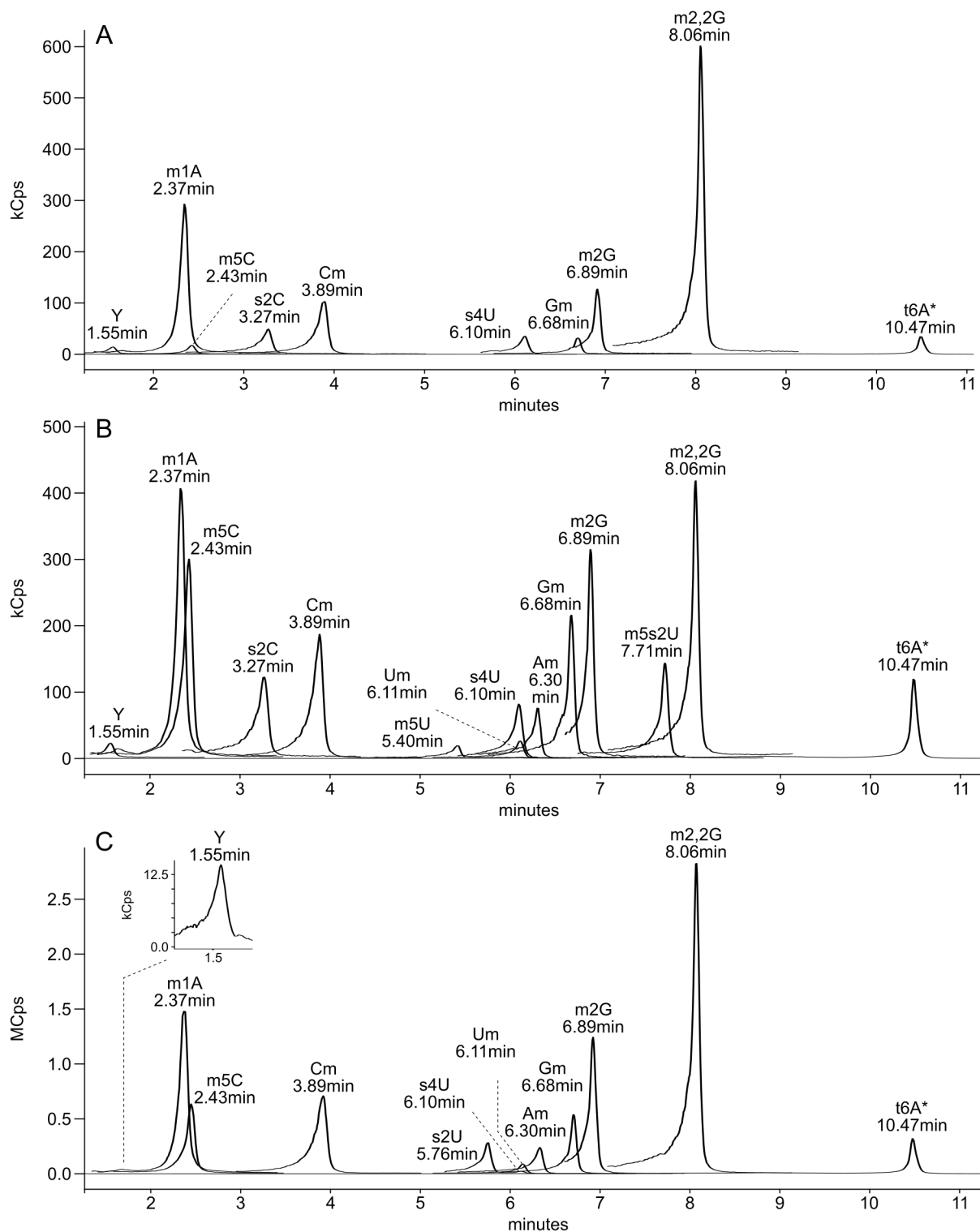


Figure S2

Extracted ions chromatograms for the following 15 nucleosides: m¹A, m²G, Am, Cm, Gm, Um, s²C, s²U, s⁴U, m⁵s²U, m⁵C, m⁵U, m²₂G, Y and t⁶A. (*) For t⁶A, the modifications are analyzed by following only the precursor m/z 413 and the product m/z 281. A) *M. maripaludis*; B) *P. furiosus*; C) *S. acidocaldarius*. Notice that the relative amount of pseudouridine (Y) in *S. acidocaldarius* is remarkably low compared to *M. maripaludis* and *P. furiosus*. Also, m⁵s²U is absent in the profile of *S. acidocaldarius* compared to *P. furiosus*.

a)

	m1A	m2G	Am	Cm	Gm	Um	s2C	s2U	s4U	m5s2U	m5C	m5U	m2,2G	Y
<i>M. maripaludis</i>	1,20E+06	4,97E+05	0,00E+00	2,60E+05	1,28E+05	0,00E+00	1,41E+05	0,00E+00	1,23E+05	0,00E+00	7,98E+04	0,00E+00	2,39E+06	3,26E+04
	1,21E+06	4,83E+05	0,00E+00	2,85E+05	1,27E+05	0,00E+00	1,39E+05	0,00E+00	1,38E+05	0,00E+00	8,44E+04	0,00E+00	2,53E+06	3,28E+04
	1,53E+06	5,65E+05	0,00E+00	4,63E+05	1,43E+05	0,00E+00	2,13E+05	0,00E+00	1,76E+05	0,00E+00	8,70E+04	0,00E+00	2,87E+06	3,21E+04
standard deviation	1,85E+05	4,37E+04	0,00E+00	1,10E+05	8,64E+03	0,00E+00	4,19E+04	0,00E+00	2,75E+04	0,00E+00	3,61E+03	0,00E+00	2,47E+05	3,83E+02
<i>P. furiosus</i>	2,07E+06	1,35E+06	3,89E+05	6,48E+05	9,33E+05	1,29E+05	5,00E+05	0,00E+00	3,58E+05	6,64E+05	1,69E+06	7,52E+04	1,91E+06	6,01E+04
	1,82E+06	1,23E+06	3,63E+05	5,76E+05	8,44E+05	1,18E+05	3,70E+05	0,00E+00	3,28E+05	5,76E+05	1,52E+06	6,92E+04	1,85E+06	5,66E+04
	2,06E+06	1,19E+06	3,54E+05	5,78E+05	8,23E+05	1,26E+05	4,17E+05	0,00E+00	3,21E+05	6,11E+05	1,62E+06	6,63E+04	1,89E+06	5,88E+04
standard deviation	1,44E+05	8,17E+04	1,81E+04	4,10E+04	5,85E+04	5,63E+03	6,54E+04	0,00E+00	1,95E+04	4,40E+04	8,84E+04	4,55E+03	3,12E+04	1,76E+03
<i>S. acidocaldarius</i>	5,65E+06	4,90E+06	1,05E+06	1,79E+06	1,79E+06	3,36E+05	0,00E+00	9,59E+05	3,18E+04	0,00E+00	2,77E+06	0,00E+00	1,12E+07	3,98E+04
	5,58E+06	4,86E+06	1,06E+06	1,97E+06	1,90E+06	3,70E+05	0,00E+00	1,01E+06	3,11E+04	0,00E+00	2,83E+06	0,00E+00	1,09E+07	3,88E+04
	6,89E+06	6,08E+06	1,07E+06	1,92E+06	1,77E+06	3,40E+05	0,00E+00	1,04E+06	3,23E+04	0,00E+00	2,99E+06	0,00E+00	1,12E+07	3,79E+04
standard deviation	7,39E+05	6,94E+05	9,61E+03	9,43E+04	7,23E+04	1,87E+04	0,00E+00	3,84E+04	5,97E+02	0,00E+00	1,15E+05	0,00E+00	1,57E+05	9,34E+02

b)

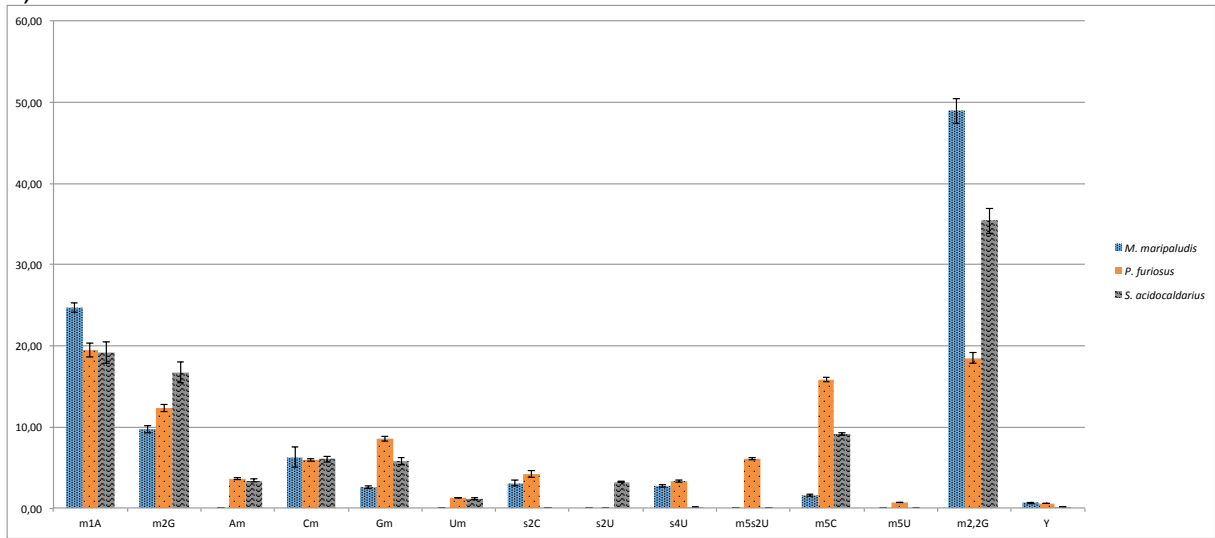
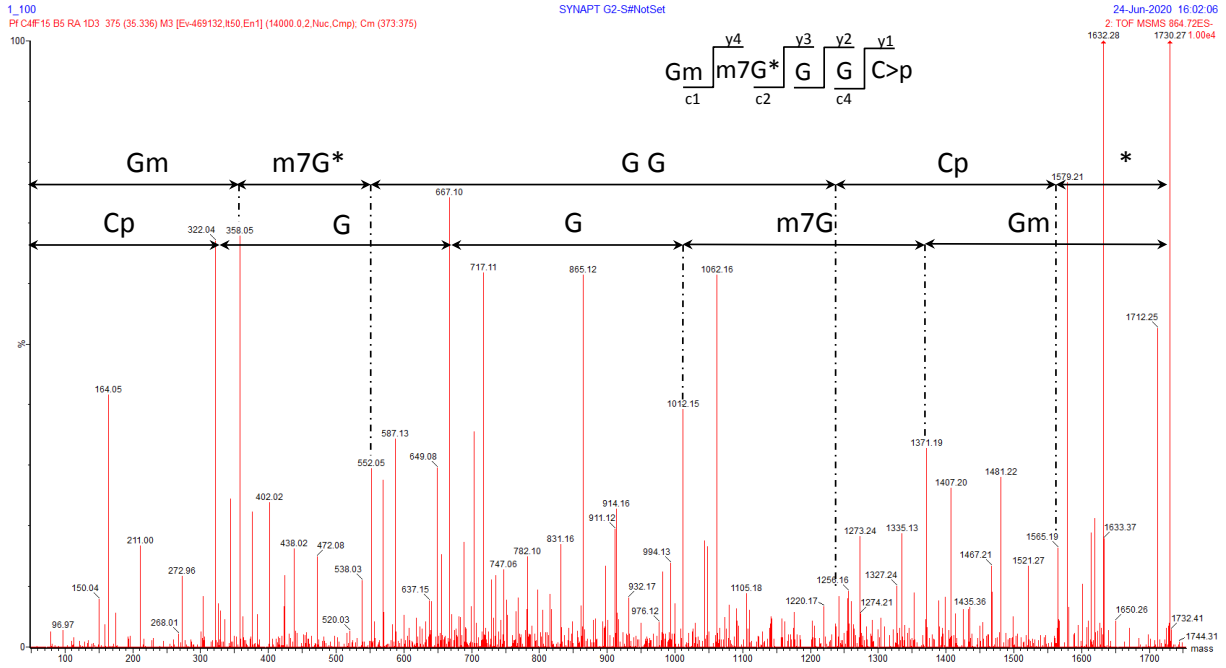


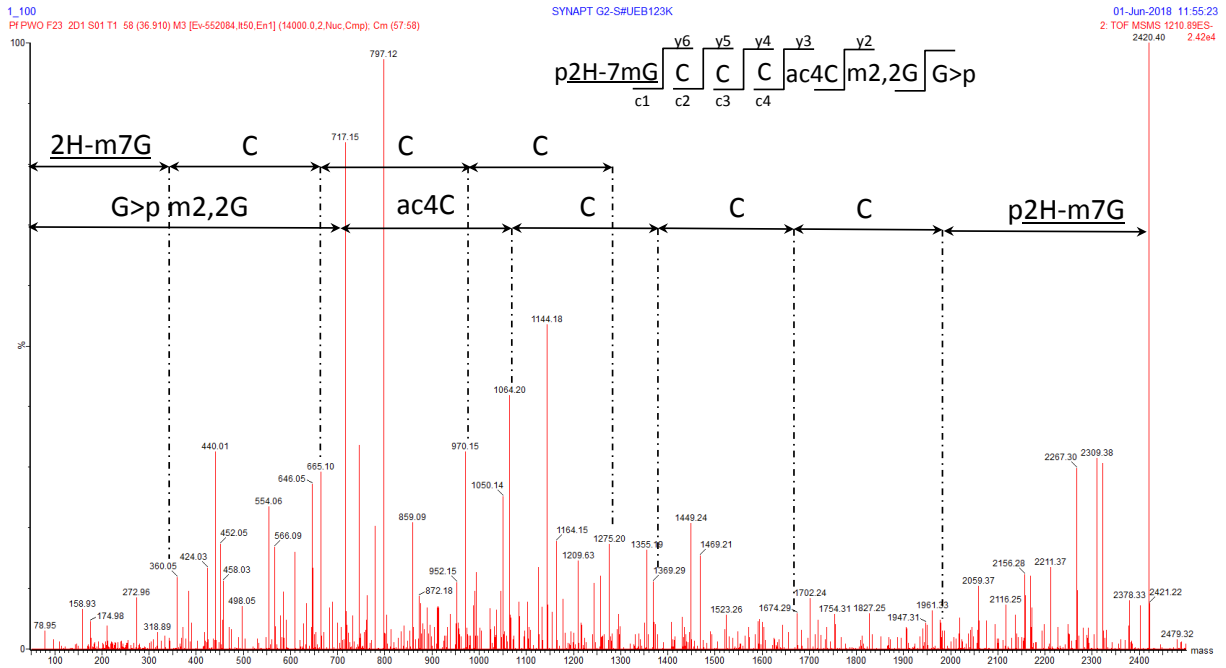
Figure S3

Relative quantification of nucleosides by MRM (multiple reaction monitoring). a) Table of area of nucleosides for each species. The analysis was performed with three technical replicates. b) Relative quantification histogram of nucleoside MRM analysis. Values are normalized by using the sum of area per nucleoside for each archaeon (light blue *M. maripaludis*, orange for *P. furiosus*, and grey for *S. acidocaldarius*).

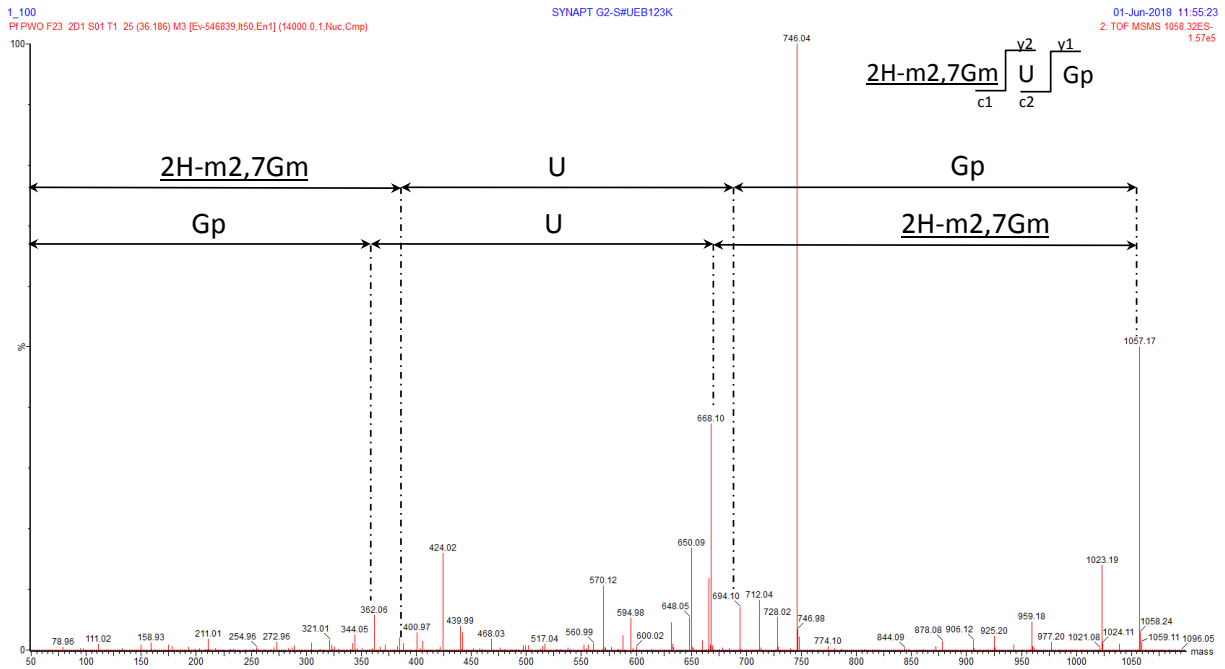
A



B



C



D

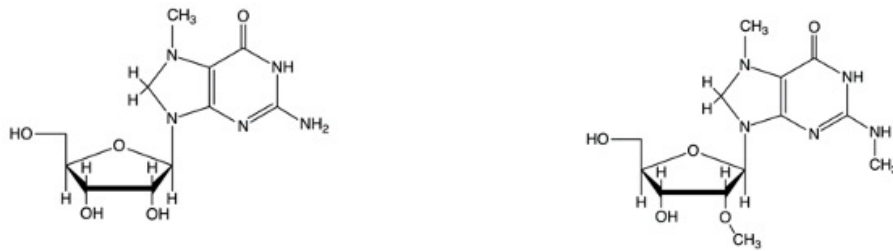


Figure S4

MS/MS sequencing spectra containing m⁷G. A) MS/MS spectrum [Gm][m⁷G]GGC>p of tRNAⁱⁿⁱ of *P. furiosus* after RNase A digestion (m/z 864.63, z = 2-). The spectrum shows a neutral loss of 165 Da, which is specific to m⁷G and corresponding to the loss of the positively charged modified base. The mass of m⁷G nucleotide is 359 Da. B) MS/MS spectrum p[2H-m⁷G]CCC[ac4C]G>p of tRNA^{Glu} of *P. furiosus* after RNase T1 digestion (m/z 1209.66, z = 2-). The mass of m⁷G nucleotide is 361 Da which could correspond to the reduced form of m⁷G (Wintermeyer and Zachau 1975). C) MS/MS spectrum [2H-m²,7Gm]UGp of tRNA^{Glu} of *P. furiosus* after RNase T1 digestion (m/z 1057.17, z = 1-). The mass of 2H-m²,7Gm nucleotide is

389 Da which could correspond to the reduced form of m²-Gm. 2H-m⁷G and 2H-m^{2,7}Gm are underlined because they cannot be unambiguously characterized by the techniques used and unknown modified guanosine nucleotides cannot be excluded. D) The chemical structures of the reduced forms of m⁷G derivatives.

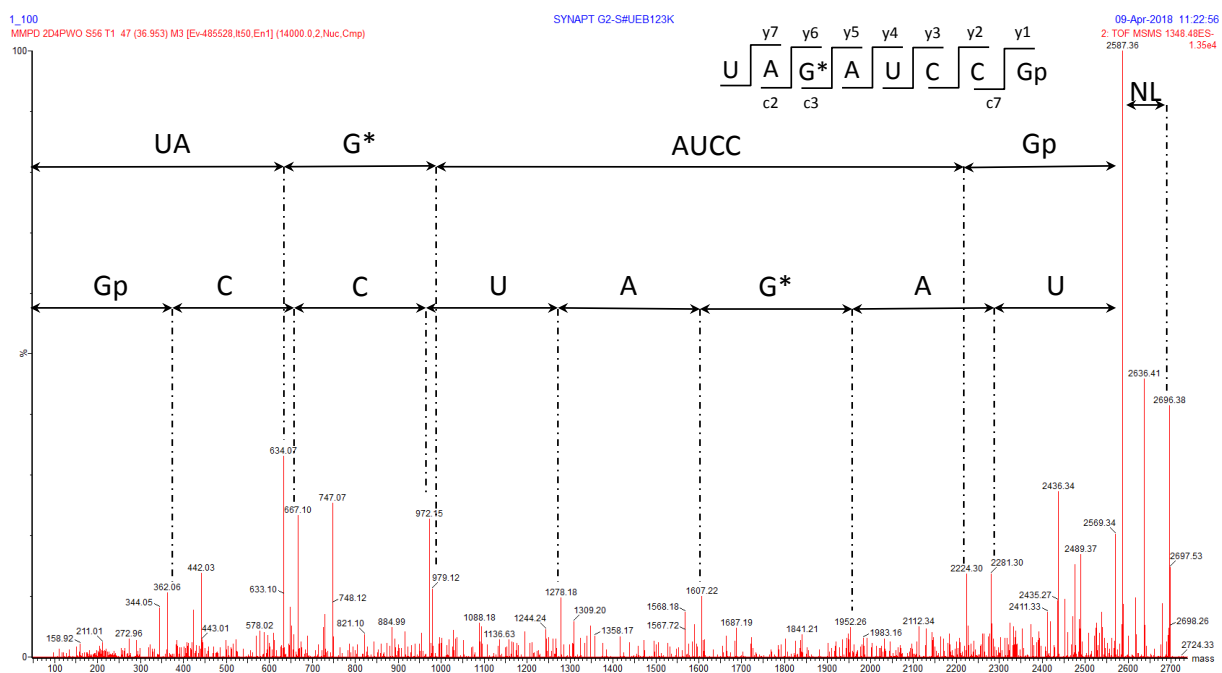


Figure S5

MS/MS sequencing spectrum UA[xG]AUCCGp of tRNA^{Tyr} of *M. maripaludis* after RNase T1 digestion (m/z 1347.71, z = 2-). The spectrum shows a neutral loss of 109 Da corresponding to the loss of an unknown modification from G37. The mass of xG nucleotide is 454 Da. * corresponds to the probable location of the neutral loss.

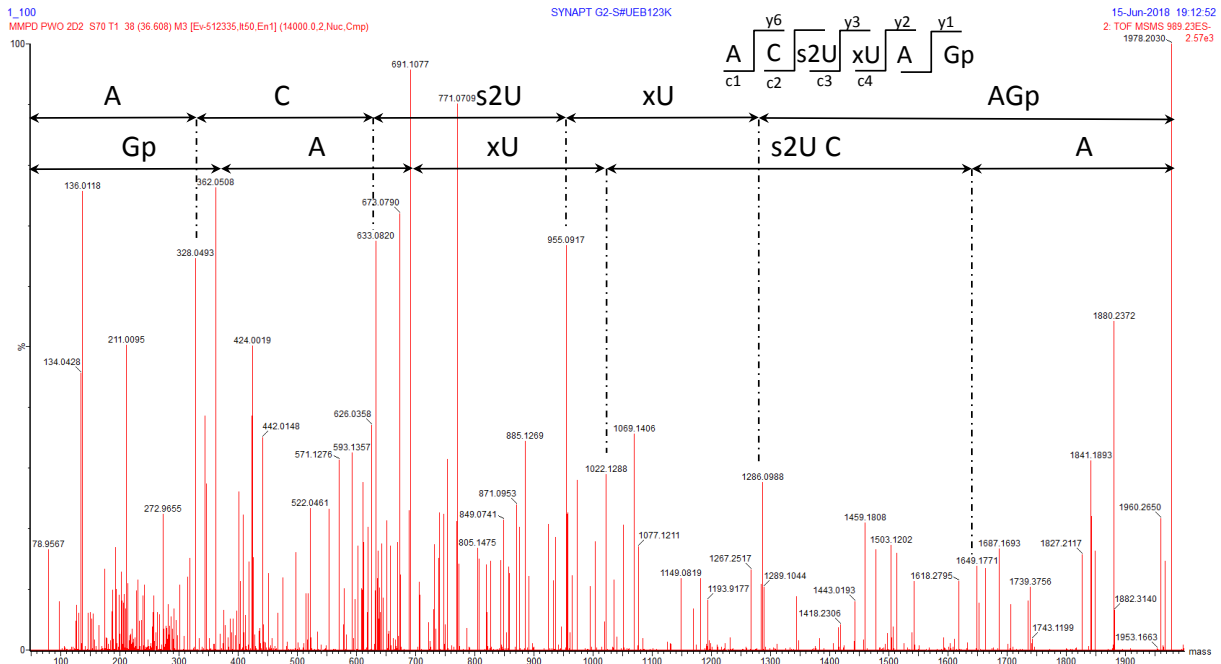
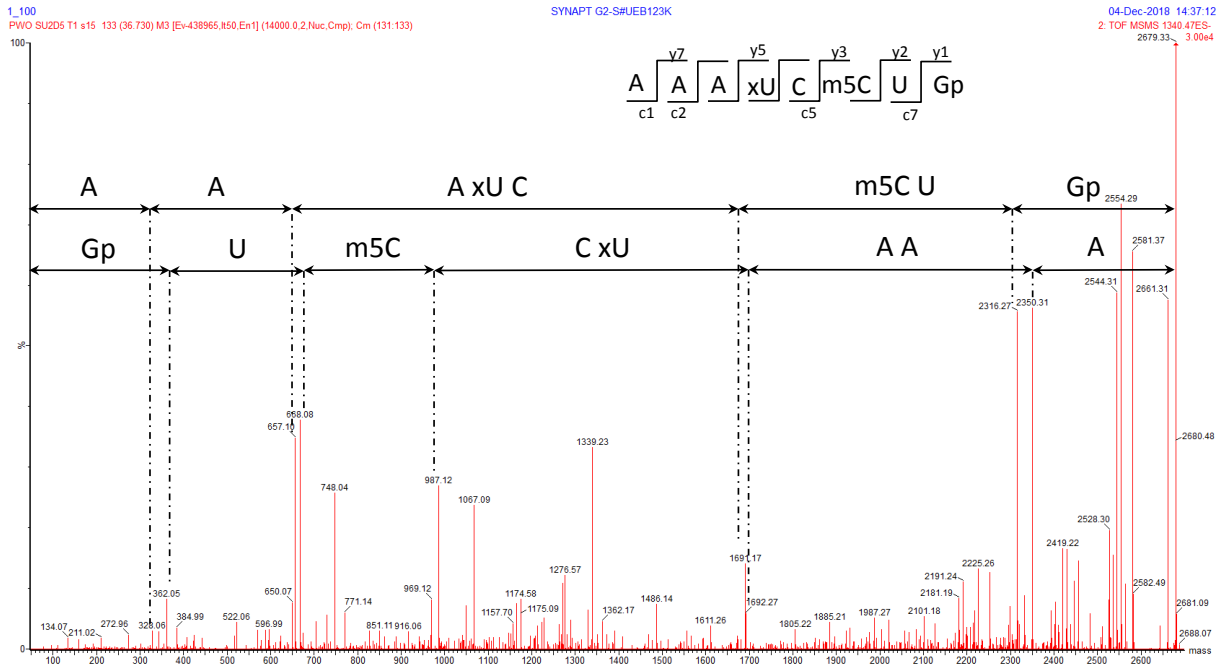


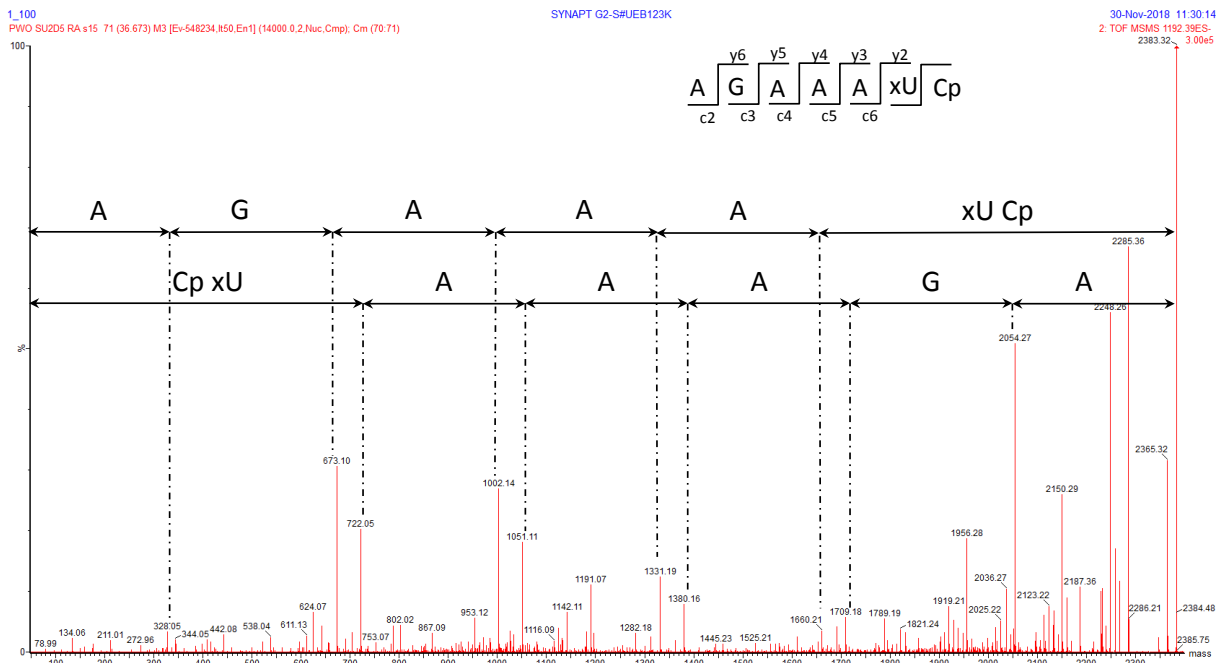
Figure S6

MS/MS sequencing spectrum AC[s²U][xU]AGp of tRNA^{Leu} of *M. maripaludis* after RNase T1 digestion (m/z 988.61, z = 2-). The mass of xU nucleotide is 331 Da.

A



B



C

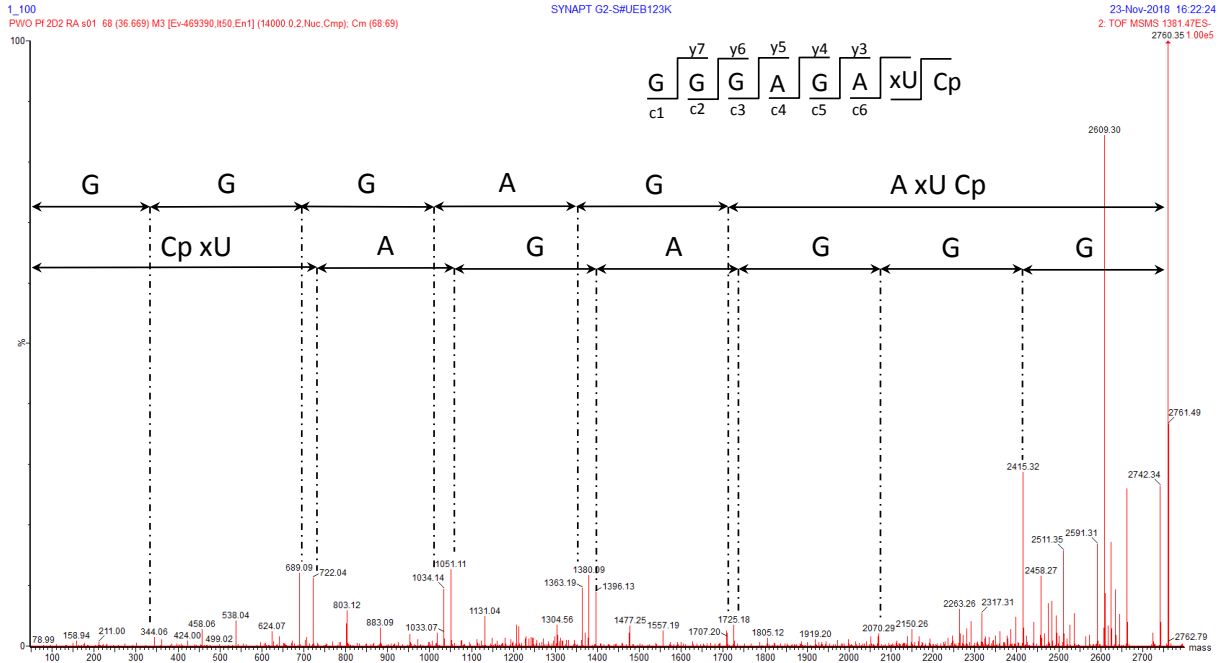


Figure S7

MS/MS sequencing spectra containing xU47 (nucleotide mass of 400 Da). A) MS/MS sequencing spectrum AAA[xU]C[m5C]UGp of tRNA^{Val} of *S. acidocaldarius* after RNase T1 digestion (m/z 1339.17, z = 2-). B) MS/MS sequencing spectrum AGAAA[xU]Cp of tRNA^{Val} of *S. acidocaldarius* after RNase A digestion (m/z 1191.14, z = 2-). C) MS/MS sequencing spectrum GGGAGA[xU]Cp of tRNA^{Met} of *P. furiosus* after RNase A digestion (m/z 1379.67, z = 2-).

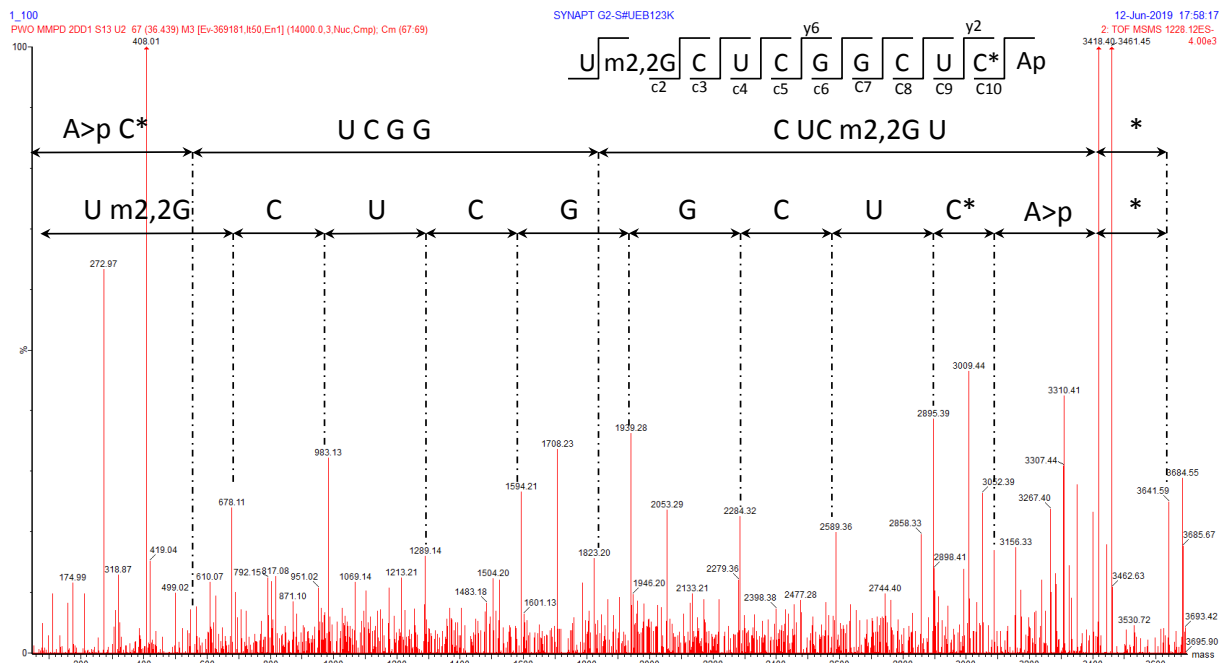
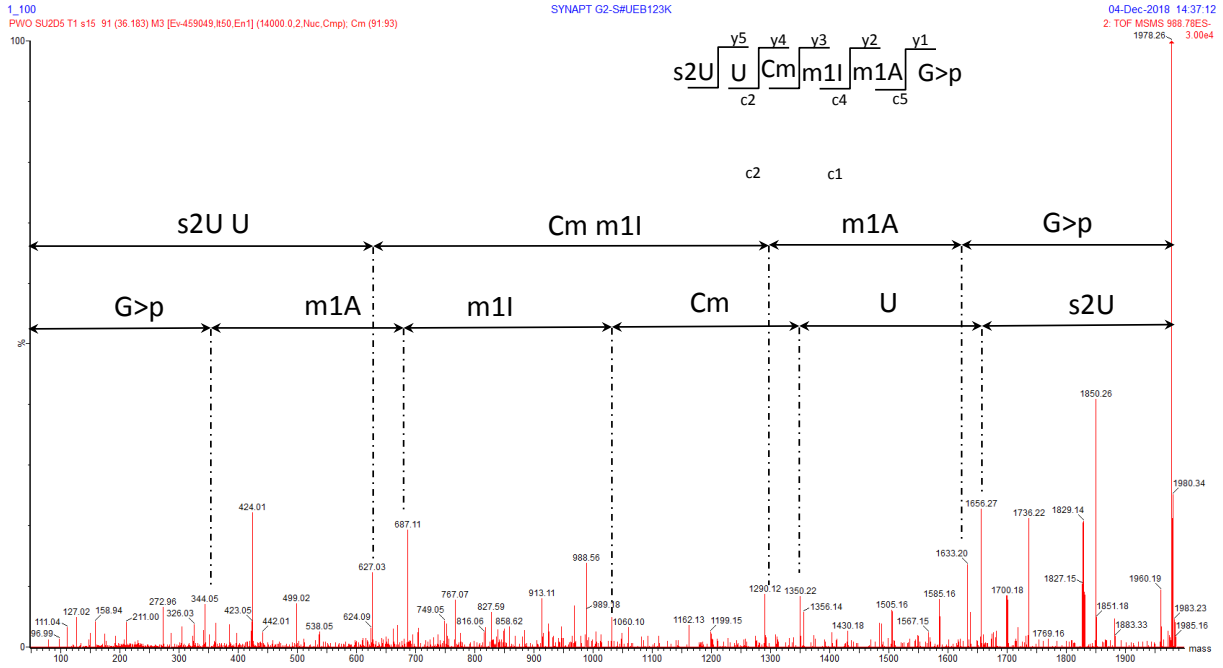


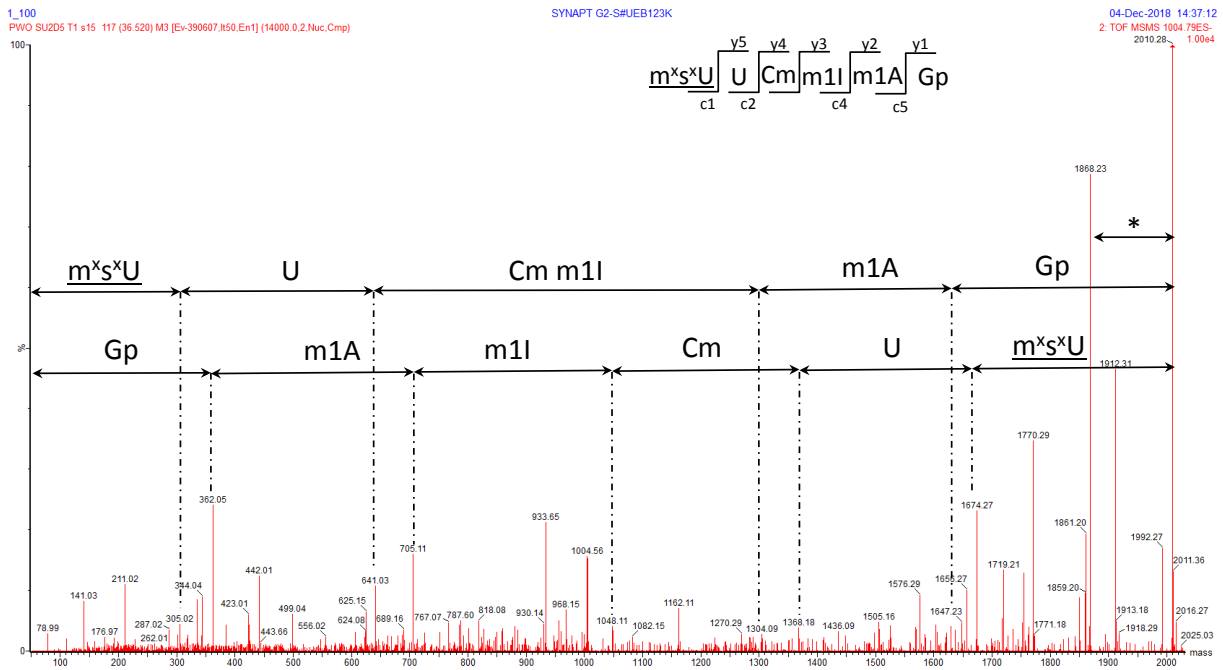
Figure S8

MS/MS sequencing spectrum U[m²Gm]CUCGGCU[C+>A>p of tRNA^{Ile} of *M. maripaludis* after RNase U2 digestion (m/z 1227.51, z = 3-). The spectrum shows a neutral loss of 223 Da, which corresponds to the loss of the modification from the cytosine base.

A



B



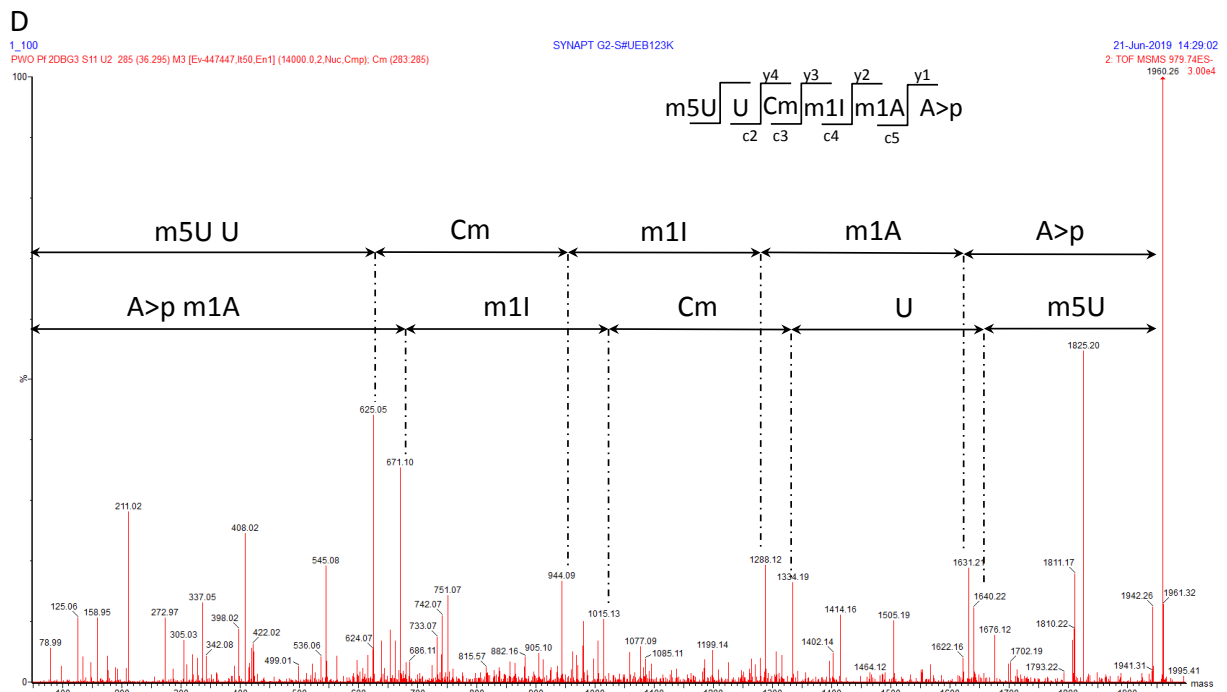
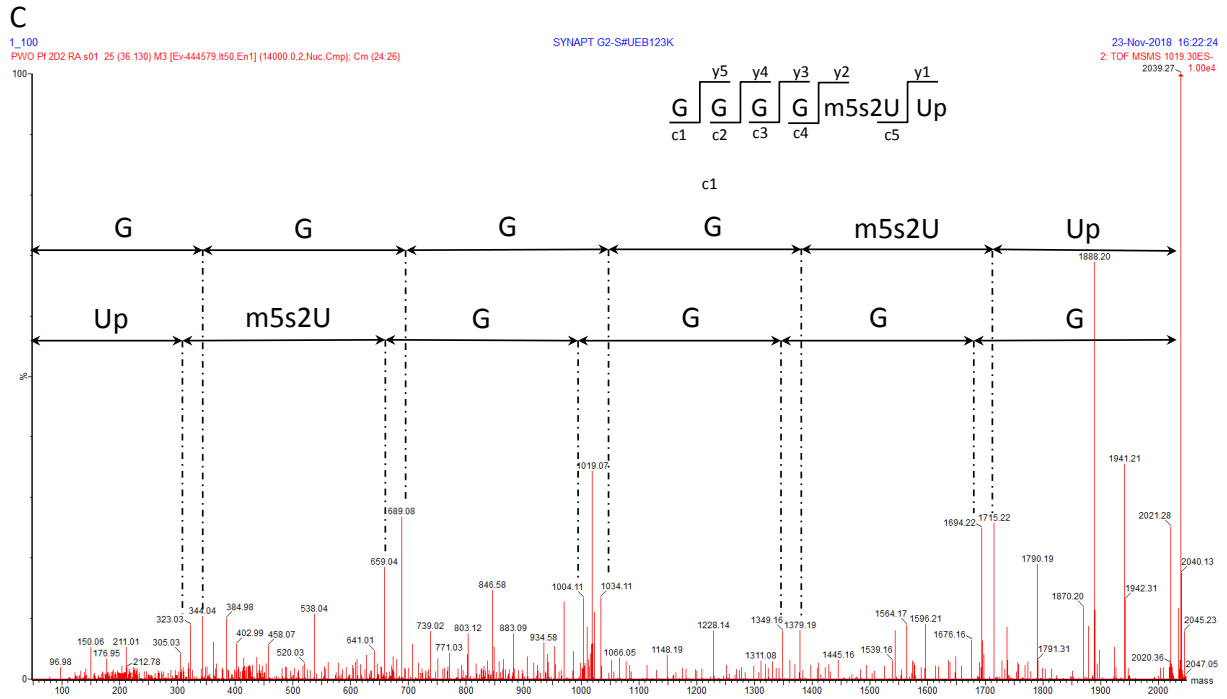


Figure S9

MS/MS sequencing spectra containing U54. A) MS/MS spectrum $[s^2U]U[Cm][m^1I][m^1A]G>p$ of tRNA^{Val} of *S. acidocaldarius* after RNase T1 digestion (m/z 988.62, $z = 2^-$). B) MS/MS spectrum $[s^x m^x U]U[Cm][m^1I][m^1A]Gp$ of tRNA^{Val} of *S. acidocaldarius* after RNase T1 digestion (m/z 1004.65, $z = 2^-$). The spectrum shows a neutral loss of 142 Da, which corresponds to the loss of a methyl-thio uracil (or pseudouracil) base. $[s^x m^x U]$ is underlined because the position

of methyl and thio groups are unambiguously localized. C) MS/MS spectrum GGGG[m⁵s²U]Up of tRNA^{Met} of *P. furiosus* after RNase A digestion (m/z 1019.12, z = 2-). D) MS/MS sequencing spectrum [m⁵U]U[Cm][m¹I][m¹A]A>p of tRNA^{Lys} of *P. furiosus* after RNase A digestion (m/z 979.63, z = 2-).

Methanococcus marisplacidis

AA AC	Acc-5'	D-5'	D-loop	D-3'	AC-5'	Ac-loop	AC-3'	V-region	E-5'	T-loop	E-3'	Acc-3'	
1	8	1	1	2	2	3	3	4	4	5	6	6	
1	0	4	4	2	2	2	3	4	4	4	6	6	
H_GHG	GCCGGGG	UA GGGG	AG U	GGCU A UCCU	G AGGGA	CU GUG	m1G A UCCU	CGA	C GGGG	UUC	G A	UUCCGG	UCGCGG
L_CAG	GCAGGG	UU UUGG	AG+ CCU	GGUUA AGGG	*G CAGGA	Cs2U XLAG	A A UCCU	UCCAGUAGGGU	C GAGG	UUC	A A	AUCCGG	CCCCGC
L_CAG	GCAGGG	UU UUGG	AG+ CCU	GGUUA AGGG	*G CAGGA	CU GUC	G G UCCG	UCCAGUAGGGU	C GAGG	UUC	G m1A	AUCCGG	CCCCGC
P_HSG	GGGCGG	UU GGGU	AG+ CCU	GGUC A UCCU	U UGGGA	UU UGG	m1G A UCCU	AAA	C CCGG	UUC	G m1A	AUCCGG	CAGGCC
A_HGC	GGGCGG	UA GGGU	AG+ UU	GG A GAGG	G CCGCC	CU UMG[C/*C]	A A GGGG	AGCU	C GGGG	m1YUcm	m1I m1A	AUCCGG	CUAGGU
G_GCC	GGGCGG	UU AUGG	AG+ ACU	GGU A UCAU	A GCGCC	CU GCC	A C GGGG	ACA	C CCGG	UUC	A A	AUCCGG	AGGCGG
L_HGC	GGGCGG	UU GGGU	AG+ CCU	GGU A UCAU	U UUGG	CU cmm5UCC	A A GGGG	UAA	C GGGG	m1YUcm	m1I m1A	AUCCGG	CAGCGG
R_HGC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CAGGA	CU GUC	A A UCCU	UCCAGUAGGGU	C GAGG	UUC	A A	AUCCGG	CAGCGG
R_HGC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CAGGA	CU GUC	A A UCCU	UCCAGUAGGGU	C GAGG	UUC	A A	AUCCGG	CAGCGG
V_UAC	GGACCU*AG	UU GGGU	AG+ UU	GGCU A UGAC	A UCGCC	CU UAC	A A GGGG	GGU	C GGGG	UUC	G A	AUCCGG	UAGGUAC
V_GAC	GAGUUCG	UU GGGU	AG+ UU	GGCU A UGAC	A CCGCC	CU GAC	A C GGGG	UGAU	C GGGG	UUC	G m1A	AUCCGG	CGGACU
F_GAA	GCCAGG	UA GGUU	AG CCU	GGG A GAGC	*G CUGGA	CmU GAA	img-14 A UCCG	UUGU	C GGGG	UUCm	m1I m1A	AUCCGG	CCUUGG
T_GAA	GGGCGG	UU GGGU	AG CCU	GGUUA AGGG	*G CAGGA	CU C+AU	A A GGGG	UCCAGUAGGGU	C GAGG	UUC	A A	AUCCGG	UCCCGC
T_GAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CAGGA	CU UGA	A A UCCG	UCCAGUAGGGU	C GAGG	m1YUcm	m1I m1A	AUCCGG	UCCCGC
S_GGA	GCAGGA	UA GGUU	AG+ CCU	GGG A AGGC	*G UACCG	CU GGA	A A UCCG	UAGGCUUUGCCUCC	C GGGG	UUC	A A	AUCCGG	UCCCGC
V_GUA	CCCGGA	UA GGUU	AG+ CCU	GGG A AGGC	*G UACCG	CU GGA	A A UCCG	UAGGCUUUGCCUCC	C GGGG	UUC	A A	AUCCGG	UCCCGC
M_CAA	GGGAGU	UU GGGU	AG+ Ua2CU	GGUUA UCAU	C GGGGA	CU CCA	A A UCCU	GGA	C UGGG	UUC	A A	AUCCGG	UACUCC
T_GAA	AGGCGG	UU GGUU	AG+ Cs2CU	GGUUA A GAGU	*G CUCGG	CU GAA	hn6A A CCGG	UUGU	C GGGG	UUCm	G m1A	AUCCGG	CUGCGG
T_GAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CAGGA	CU C+AU	A A GGGG	UCCAGUAGGGU	C GAGG	m1YUcm	m1I m1A	AUCCGG	UCCCGC
R_HHU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CAGGA	CU UGA	A A UCCG	UCCAGUAGGGU	C GAGG	UUC	A A	AUCCGG	UCCCGC
M1_CAU	AGGCGG	UA GGGU	AG+ CCA	GGUUA UCCG	G CCGCG	CU CAU	A A CCGG	AGAU	C AGAG	UUC	A A	AUCCGG	CUCUCG
M2_CAU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GGGG	m1YUcm	m1I A	AUCCGG	CCGCGG
N_CAU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GAGG	UUC	G A	AUCCGG	AGGAGG
R_HHU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GAGG	m1YUcm	G m1A	AUCCGG	UCCCGC
R_HHU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GAGG	UUC	A A	AUCCGG	UCCCGC
R_HHU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GAGG	UUC	A A	AUCCGG	UCCCGC
R_HHU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CUCGG	CU GAA	hn6A A CCGG	AGGU	C GAGG	UUC	A A	AUCCGG	UCCCGC
1	8	1	1	2	2	3	3	4	4	5	6	6	
.....	UR G..Y	AG		GG R ..Y	R ..G.	CU	A ..C..	4	C ..G..	UUC	R m1A	RYD..

Pyrococcus furiosus

AA AC	Acc-5'	D-5'	D-loop	D-3'	AC-5'	Ac-loop	AC-3'	V-region	E-5'	T-loop	E-3'	Acc-3'	
1	8	1	1	2	2	3	3	4	4	5	6	6	
1	0	4	4	2	2	2	3	4	4	4	6	6	
L_UAG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*Gm CGGGA	U cmm5UAG	G G UCCCG	UCCCGUAGGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
L_CAG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*Gm CGGGA	U CAG	G G UCCCG	UCCCGUAGGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
P_HSG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*Gm CGGGA	U CAG	G G UCCCG	UCCCGUAGGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
Q_UUG	AGCCCGG	UU GGGU	AG+ C	GmGCCAA GCAU	G CCGGA	CU cmm5UAG	A A UCCG	UCCAGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
Q_UUG	AGCCCGG	UU GGGU	AG+ C	GmGCCAA GCAU	G CCGGA	CU cmm5UAG	A A UCCG	UCCAGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
R_GCC	GGGGGG	UU GGGU	AG+ CCU	GGUUA AGGG	m2G CCGG	CU GCG	m1mG A CUCUG	AGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
A_HGC	GGG*Cs2GmUA	UU GGGU	AG+ CCU	GGUUA AGGG	Gm CCGCC	CU cmm5UCC	A A GGGG	AGGC	C GGGG	m5UUCm	m1I m1A	AUCCGG	CGGGG
A_HGC	GGGCGG	UA GGGU	AG+ CCU	GGUUA AGGG	G CCGCC	CU GGC	A A GGGG	AGGC	C GGGG	UUC	A A	AUCCGG	CGGGG
D_GUC	GGGCGG	UU GGGU	AG+ CCC	GGUUA AGGG	m2G CCGG	CU GGC	A A UCCG	CGA	C CCGG	UUC	A A	AUCCGG	CGGGG
E_UCC	m7GCC*+GGUG2_7GmUA	UU GGGU	AG+ CCC	GGUUA AGGG	m2G CCGG	CU CUC	m1G A UCCG	CGA	m5C CCGG	UUC	A A	AUCCGG	CGGGG
G_GCC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	G CCGCC	CU CCC	A A GGGG	CGA	m5C CCGG	UUC	A A	AUCCGG	CGGGG
V_UAC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	m2G CCGG	CU cmm5UCC	m1G A GCGG	CGA	C CCGG	UUC	A A	AUCCGG	CGGGG
G_GCC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	G CCGCC	CU GGC	A A GGGG	AGGC	C GGGG	UUC	A A	AUCCGG	CGGGG
V_UAC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	G CCGCC	CU UAC	A A GGGG	AGGU	C GGGG	UUC	G m1A	AUCCGG	CGGGG
V_GAC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGCC	CU GAC	A A GGGG	AGGU	C GGGG	UUC	A A	AUCCGG	CGGGG
C_GCA	GGG*Cs2GmUA	Um1AGGU	AG+ A	GGC C AGGC	*Gm GGGGA	CU GCA	G A UCCG	UUUA	C GGGG	m5s2UUCm	m1I m1A	AUCCGG	UCCCGG
F_GAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	A CCGGA	CU GAA	m1mG A A UCCG	UUGU	C GGGG	UUC	A A	AUCCGG	CGGGG
L_UAG	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*Gm CGGGA	CU UAA	G UCCG	UCCAGUAGGGU	C GGGG	UUC	G A	AUCCGG	CCCCCG
S_GGA	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	*Gm CGGGA	CU UGA	img-2/m1mG A CCGG	UCCCGUAGGGU	C GGGG	m5U/m5s2UUC	A A	AUCCGG	UCCCGG
S_GGA	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	G CCGG	CU GGA	m1mG A G/a2_2G UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	UCCCGG
M_CAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	C CCGG	CU CCA	m1mG A C CCGG	GGA	m5C GGGG	UUCm	m1I m1A	AUCCGG	CCCCCG
I_GAU	GGGCGG	UU GGGU	AG+ Cs2CU	GGUUA AGGG	Gm CCGCC	CU GAA	hn6A A CCGG	AGGU	C GGGG	UUC	G A	AG CCGG	CGGGG
K_CAU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*Gm CCGGA	CU CAU	ms2t6A A UCCG	AGGU	C GGGG	m5UUCm	m1I m1A	AUCCGG	CGGGG
M1_CAU	AGGCGG	UA GGGU	AG+ CUA	GGUUA AGGG	*Gm CCGGA	CU CAU	A A CCGG	AGGU	C CCGG	m5s2UUCm	m1I m1A	AUCCGG	CGGGG
M2_CAU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	G C CCGG	CU CAU	hn6A A CCGG	AGGU	C CCGG	m5s2UUCm	G m1A	AG CCGG	CGGGG
R_HHU	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	*Gm CCGG	CU CCU	t6A A CCGG	AGGU	C GGGG	UUC	A A	AUCCGG	CGGGG
R_HHU	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	G CCGG	CU cmm5UUC	A A CCGG	AGGU	C GGGG	UUC	G m1A	AUCCGG	CGGGG
S_GGU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*Gm CCGG	CU GCU	t6A A CCGG	AGGU	C GGGG	UUC	A A	AUCCGG	CGGGG
T_GGU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*Gm GGGGA	CU cmm5UUC	hn6A A UCCG	AGGU	C GGGG	UUC	A A	AUCCGG	CGGGG
T_GGU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*Gm CCGG	CU GGU	t6A/hn6A A CCGG	AGGU	C CCGG	UUCm	m1I m1A	AG CCGG	CGGGG
1	8	1	1	2	2	3	3	4	4	5	6	6	
.....	UR G..Y	AG		GG R ..Y	R ..G.	CU	R R ..C..	4	C ..G..	UUC	R m1A	AY C..

Sulfolobus acidocaldarius

AA AC	Acc-5'	D-5'	D-loop	D-3'	AC-5'	Ac-loop	AC-3'	V-region	E-5'	T-loop	E-3'	Acc-3'	
1	8	1	1	2	2	3	3	4	4	5	6	6	
1	0	4	4	2	2	2	3	4	4	4	6	6	
L_GAG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*G CCGGA	CU GAG	m1G C UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
L_CAG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*G CCGGA	CU GAG	m1G C UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
L_CAG	GGGGGG	UU GGGG	AG+ CCU	GGUCAA AGGC	*G CCGGA	CU GAG	m1G C UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
E_UUG	GGGGGG	UU GGGU	AG+ CCU	GGUCAA AGGC	*G CCGGA	CU GAG	m1G C UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
Q_UUG	AGCCCGG	UU GGGU	AG+ C	GmGCCAA GCAU	G CCGGA	CU GAG	m1G C UCCG	UCCCGUAGGGU	C GGGG	UUC	A A	AUCCGG	CCCCCG
A_GGC	GGGCGG	UA GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
A_UUC	GGGCGG	UA GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
L_GCC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
V_GAG	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
D_GUC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
E_UUC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
E_UUC	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
F_GAA	GGGCGG	UA GGGU	AG+ CCC	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
L_UAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
L_CAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
S_GGA	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
S_GGA	GGGCGG	UU GGGU	AG+ CCA	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
V_GUA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
M_CAA	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C CCGG	UUC	A A	AUCCGG	CGGGG
N_GUU	GGGCGG	UU GGGU	AG+ CCU	GGUUA AGGG	*G CCGG	CU GGC	A U CCGG	AGGU	C C				

Figure S10

Structural alignments of the purified and analyzed tRNAs from each species. The secondary structural elements of the tRNA cloverleaf are indicated above and below each alignment with key positions numbered following the standard nomenclature of yeast tRNA-Phe. On the last line, highly conserved residues are indicated. For clarity, the base-paired stems are colored (yellow for the AA-stem; green for the D-stem; cyan for the AC-stem; purple for the TΨC-stem throughout the alignments). For the long-arm tRNAs, tRNA-Ser and tRNA-Leu, the possible pairings of the additional helix are in italics. The highly conserved residues are in bold and those quasi-conserved not bolded in the consensus lines below each alignment. Square brackets are used when there is a potential ambiguity in nomenclature. The amino acids are arranged alphabetically with at the top (in blue) those coded by CG-rich codons (1st group: C1-quadrant corresponding to G36-containing tRNAs – Figure 2); 2nd group: G1-quadrant corresponding to C36-containing tRNAs) and below (in red) those coded by UA-rich codons (1st group: U1-quadrant corresponding to A36-containing tRNAs; 2nd group: A1-quadrant corresponding to U36-containing tRNAs - Figure 2). The code for modified nucleosides follows the one in MODOMICS (Boccaletto et al. 2018). Thus, the modification is always indicated in front of the letter with the atom number of the base modified (m5C, s2U, m1G or m5s2U etc.). The exception is 2'O-methylation on the ribose indicated after the base without atom number (Gm, Cm, ...). Because, we cannot always assign the methylation position, we underline such residues (for example, Gm means the nucleotide could be either m1G, m2G... or Gm). Brackets are used when there is a mixture of modifications, e.g. [A/Am] means some of those As are methylated on the O2' and [A/Am] means that the methylation position of the A is unknown. For shortness, we introduced here: ac6A = *A; ac4C = *C; m22G = *G. An "x" before a nucleotide, e.g. xG, or after a modification symbol, e.g. m^x, means that the modification has not been formally identified (see text). Pseudouridines are not represented, except when the available evidence points formally to their presence, as for position 54 (the code MODOMICS is "Y").

Figure S11

Drawings of tertiary pairs discussed in the text. A: the *trans* Watson-Crick/Watson-Crick G15/C48 (from PDB 1EHZ). B: the *trans* Watson-Crick/Hoogsteen T54/m1A58 (from PDB 1EHZ). C: the C32/A38N contact (from PDB 1EHZ). D: the G26/A44 base pair (from PDB 1EHZ). Bottom right: the *trans* Watson-Crick/Hoogsteen A54/m1A58 (from PDB 1YFG) seen from the top (above) and along the m22G26 (below). Drawings made using Pymol (PyMOL(TM) 1.7.7.6 – Incentive Product Copyright © Schrodinger LLC.

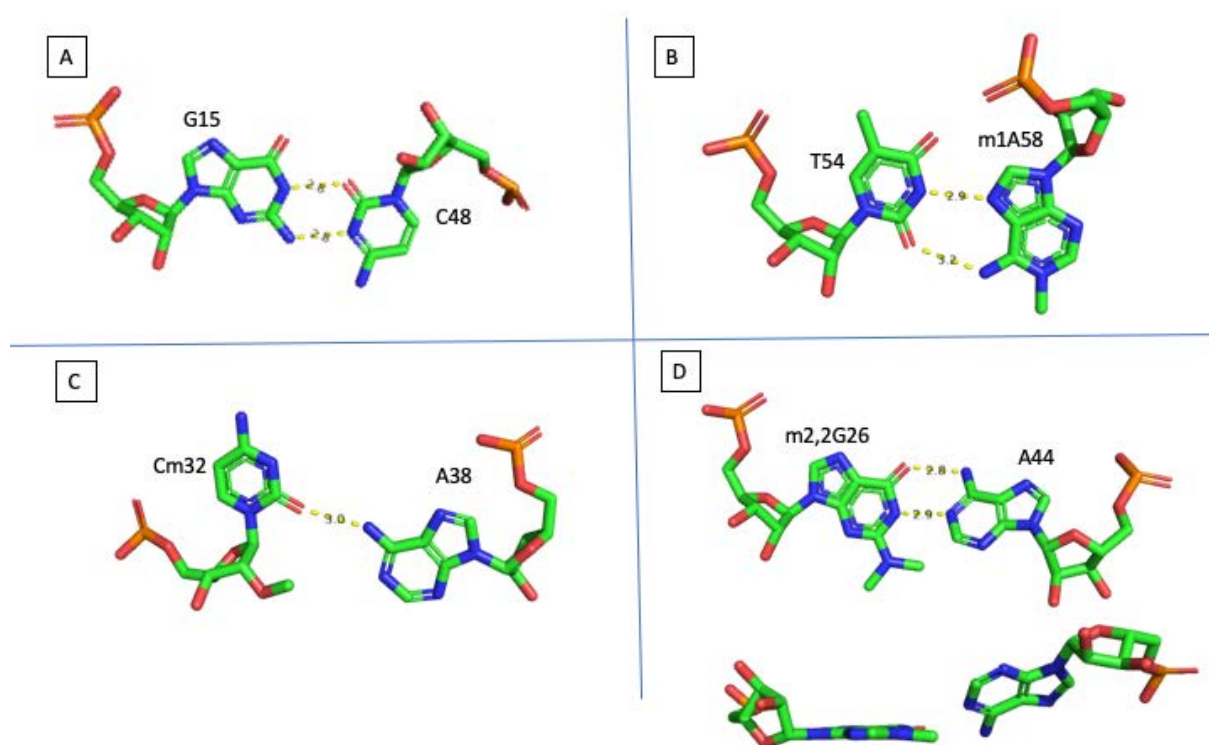


Figure S12

Contacts between the P-site tRNA and the large rRNA in bacterial ribosome (A and B) (from Watson et al. 2020). The contacts occur in the minor groove of base pairs 29-41 and 30-40 in the anticodon stem. Notice how the hydroxyl groups of 40 and 41 are forming H-bonds locking in those residues. Further A1338 forms a *trans* Hoogsteen/Watson-Crick pair (a “sheared” base pair) with G944. C: The environment around residues 59 and 60 (from PDB 2DU3). D: The H-bond between O2'(A37) and N1(A1913) (from Watson et al. 2020). Drawings made using Pymol (PyMOL(TM) 1.7.7.6 – Incentive Product Copyright © Schrodinger LLC.

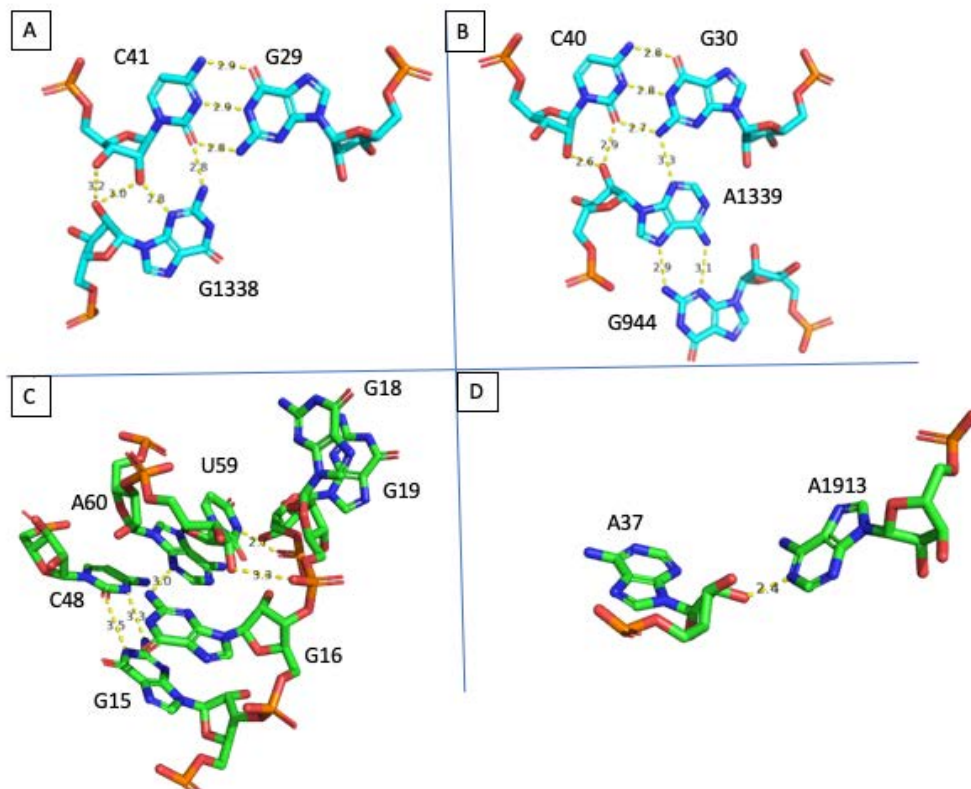


Table S1

Oligonucleotide fragments found in RNase digests that contain a methylation. In the case of RNase T1, m2G and m22G could be cleaved (depending tRNA as well as on experimental conditions) but m7G, m1G and Gm was never cleaved. In the case of RNase A, m5C and m1Y are cleaved and Cm, Um, m5U and m5s2U are not cleaved.

m/z	z	Molecular mass (Da)	Sequence	tRNA	Modification	T1 cleavage
<i>M. maripaludis</i>						
719,13	2	1439,3	A[Gm]AGp + NA + K	Ala UGC	[Gm] 22	no
1070,47	3	3213,4	CCCU[mnm5U]UC[m1G]AG>p	Glu UUC	[m1G] 37	no
1135,7	2	2272,4	GmAUCCUGp	Pro UGG	[m1G] 37	no
1440,64	2	2882,3	GmAUCCUGp	His GUG	[m1G] 37	no
1154,21	2	2309,4	AAC[m2,2G]CUGp	Phe GAA	[m2,2G] 26	no
667,11	2	1335,2	AAC[m2,2G]>p	Tyr GUA	[m2,2G] 26	no
824,62	2	1650,2	C[m2,2G]CCGp	Ser UGA	[m2,2G] 26	no
827,64	2	1656,3	C[m2,2G]ACG>p	Asn GUU	[m2,2G] 26	no
968,62	2	1938,2	C[m2,2G]CCUG>p	Lys UUU	[m2,2G] 26	no
968,67	2	1938,3	C[m2,2G]CCUG>p	Thr GGU	[m2,2G] 26	no
977,61	2	1956,2	C[m2,2G]CCUGp	Lys UUU	[m2,2G] 26	no
978,17	2	1957,3	U[m2,2G]CUCGp	Ile	[m2,2G] 26	no
980,68	2	1962,4	C[m2,2G]UACG>p	Ser GGA	[m2,2G] 26	no
1001,62	2	2004,2	AU[m2,2G]CAGp	Leu UAG	[m2,2G] 26	no
<i>P. furiosus</i>						
856,14	2	1713,3	[m2,2G]AU[Am]Gp	Cys GCA	[m2,2G] 6	no
684,1	2	1369,2	C[ac4C][m2,2G]Gp	Ala UGC	[m2,2G] 6	no
679,13	2	1359,3	C[m2,2Gm]CGp	Leu CAG	[m2,2Gm] 26	no
822,61	2	1646,2	C[m2,2Gm]CCG>p	Ala UGC	[m2,2G] 26	no
824,64	2	1650,3	C[m2,2G]CCGp	Leu CAG	[m2,2G] 26	no
857,65	2	1716,3	AC[ac4C][m2,2G]Gp	Arg CCU	[m2,2G] 26	no
834,64	2	1670,3	C[m2,2Gm]CCGp	Ala UGC	[m2,2G] 26	no
729,13	2	1459,3	A[mimG]A[m2,2G]p	Ser GGA	[m2,2G] 39	yes
843,64	2	1688,3	A[m5C][m5C][Gm]Gp	Gln CUG	[Gm] 50	no
1039,15	1	1039,1	[m2,7Gm]UG>p	Glu CUC	[m2,7Gm] 10	no
1209,66	2	2420,3	p[m7G]CCC[ac4C][m2,2G]>p	Glu CUC	[m7G] 10	no
<i>S. acidocaldarius</i>						
1291,5	2	3876,5	UC[m2G]UCUAG+C[Cm]UG>p	Val GAC	[m2G] 10	no
1134,17	2	2269,3	CCCAU[Am][m2G]p	Trp CCA	[m2G] 10	yes
1100,48	2	3303,4	UAm[m22G]UCUAG+CG>p	Asp GUC	[m22G] 10	no
1328,69	2	2658,4	UCUAG+C[Gm]Gp	Gln UUG	[Gm] 18	no
1432,89	2	4300,7	UA[m22G]UAUAG+CCC[Gm]Gp	Glu UUC	[Gm] 19	no
1154,16	2	2309,3	GmAUCmCAGp	Gln UUG	[Gm] 23	no
670,6	2	1342,2	[m2,2G][Cm]UG>p	Ser UGA	[m2,2G] 26	no
672,6	2	1346,2	U[m2,2G]CGp	Ala GGC	[m2,2G] 26	no
816,61	2	1634,2	C[m2,2G]UUGp	Thr GGU	[m2,2G] 26	no
1142,13	2	2285,3	AC[m2,2G]CCUGp	Gly GCC	[m2,2G] 26	no
1154,17	2	2309,3	AU[m2,2G]CCAGp	Pro UGG	[m2,2G] 26	no
984,15	2	1969,3	Cm[m2,2G]CCCGp	Phe TRUE	[m2,2G] 26	no
987,64	2	1976,2	ACm[m2,2G]CUG>p	Val GAC	[m2,2G] 26	no
832,13	2	1665,3	U[m2,2Gm]CCGp	Trp CCA	[m2,2Gm] 26	no
984,13	2	1969,3	C[m2,2Gm]CCCGp	Tyr GUA	[m2,2Gm] 26	no
820,61	2	1642,2	AC[m1G]CG>p	Val GAC	[m1G] 37	no
1123,12	2	2247,2	[m1G]CCCCUGp	Gln UUG	[m1G] 37	no
961,63	2	1924,3	[m1G]CCCCUG>p	Pro UGG	[m1G] 37	no
961,63	2	1924,3	[m1G]CUCCG>p	Leu GAG	[m1G] 37	no
975,64	2	1952,3	CCmU[m2,2G]CG>p	Leu UAA	[m2,2G] 47	no
1162,78	3	3490,3	UUAUCCCU[m2,2G]CG>p	Ser UGA	[m2,2G] 47	no

m/z	z	Molecular mass (Da)	sequence	tRNA	Modification	RNase A cleavage
<i>M. maripaludis</i>						
821,10	2	1643,2	GGG[Cm]U>p	Phe GAA	[Cm]32	no
1030,64	2	2062,3	GGGGG[m1Y]p	Arg UCU	[m1Y]54	yes
1030,61	2	2062,2	GGGGG[m1Y]p	Ser UGA	[m1Y]54	yes
819,63	2	1640,3	[Cm][m1I][m1A]AU>p	Ser UGA	[Cm]56	no
820,12	2	1641,2	[Cm]G[m1A]AU>p	Ile GAU	[Cm]56	no
<i>P. furiosus</i>						
691,13	1	691,1	[m2,2G][m5C]>p	Met CAU	[m5C] 27	yes
1030,16	2	2061,3	[Cm]AU[m6t6A]AC>p	Met CAU	[Cm]32	no
1014,16	2	2029,3	GGAGA[m5C]p	Gly GCC	[m5C]48	yes
846,60	2	1694,2	GGG[m5U]Up	Cys GCA	[m5U]54	no
1019,12	2	2039,2	GGGG[m5U]Up	Met CAU	[m5U]54	no
828,63	2	1658,3	[Cm][m1I][m1A]Up	Gly GCC	[Cm]56	no
1000,66	2	2002,3	[Cm][m1I][m1A]AGCp	Thr GGU	[Cm]56	no
1001,16	2	2003,3	[Cm]G[m1A]AGCp	Met CAU	[Cm]56	no
854,64	2	1710,3	A[mimG]A[Cm]Cp	Trp CCA	[Cm]39	no
1016,18	2	2033,3	[Cm][Gm]GGAC[m5C]p	Trp CCA	[Cm]41	no
1016,18	2	2033,3	[Cm][Gm]GGAC[m5C]p	Trp CCA	[m5C]48	yes
1014,13	2	2029,3	GGAGA[m5C]p	Gly GCC	[m5C]48	yes
897,46	2	2694,4	AGGAG[Um][Gm]Cp	Ini	[Um] 21	no
1011,12	2	2023,2	GAGG[m5s2U]Up	Ini	[m5s2U]54	no
<i>S. acidocaldarius</i>						
819,62	2	1640,2	[Cm]O[Am]AU>p	Met CAU	[Cm]56	no
827,09	2	1655,2	[Um]GG[s2U]Up	Met CAU	[Um] 51	no
828,62	2	1658,2	[Cm]O[Am]AUp	Gly GCC	[Cm]56	no
830,12	2	1661,2	GGG[Cm]Up	Leu GAG	[Cm]32	no
836,62	2	1674,2	[Cm][m1I][Am]GUp	Tyr GUA	[Cm]32	no
836,63	2	1674,3	[Cm]AG[m1G]Cp	Leu CAG	[Cm]34	no
844,62	2	1690,2	G[Cm]GG[m5C]p	Asp GUC	[Cm]69	no
844,62	2	1690,2	G[Cm]GG[m5C]p	Asp GUC	[m5C]72	yes
854,64	2	1710,3	A[mimG]A[Cm]Cp	Trp CCA	[Cm]39	no
999,11	2	1999,2	[Cm]GGGs2UUp	Gly GCC	[Cm] 50	no
999,62	2	2000,2	[Um]GGGs2UUp	Val GAC	[Um] 50	no
1006,13	2	2013,3	[Cm]GmGGs2UUp	Asp GUC	[Cm]50	no
1009,65	2	2020,3	A[Gm]GG[Cm]Up	Met	[Cm]32	no
1066,12	2	2133,2	GG[Cm]G[xG]Up	Gly GCC	[Cm]50	no
1131,83	3	3397,5	GGGAGAG[Cm][m2,2G]Cp	Phe GAA	[Cm]25	no
1172,13	2	2345,3	G[Um]GGGs2UUp	Leu GAG	[Um] 50	no
1172,13	2	2345,3	G[Um]GGG[s2U]Up	Leu CAG	[Um] 50	no
1172,14	2	2345,3	G[Um]GGGs2UUp	Ser UGA	[Um] 50	no
1200,68	2	2402,4	AAGGG[m2,2G][m5C]p	Ser UGA	[m5C]27	yes
1247,18	3	3743,5	AGGA[Um][m2,2G]GGGGCp	Asp GUC	[Um] 25	no

Table S2

Types and positions of chemical modifications of nucleotides in naturally occurring tRNAs from selected Archaea and their corresponding modification enzymes.

The compilation includes data from tRNAs purified from: two Methanococcales, i.e. the mesophile *Methanococcus maripaludis* (this work), and the hyperthermophile *Methanocaldococcus jannaschii* (Yu et al. 2019); three closely related hyperthermophiles from the Pyrococcale genus, i.e. *Pyrococcus furiosus* (this work), *Pyrococcus abyssi* and *Pyrococcus horikoshii* together with data of the unique tRNA-Trp from *Thermococcus kodakarensis* analyzed so far. They all belong to the Euryarchaeal subgroup of Archaea. The last group of tRNAs analyzed belongs to the Sulfolobales phylum of the Crenarchaeota subgroup: *Sulfolobus acidocaldarius* (this work), *Sulfolobus solfataricus* and *Sulfolobus tokadai*. When known, are also listed the conventional acronyms of corresponding enzymes, for which the specificity has been experimentally validated, the group of ortholog genes they belong to (COG) and eventually the precise genomic orf (when known) obtained from MODOMICS (<https://iimcb.genesilico.pl/modomics/>) or PubMed/Medline. The associated reference list corresponds to the selection of only one, often among many other valuable ones, that best describes the tRNA(s) or the modification enzyme(s). Some modifications are catalyzed by stand-alone enzymes while others, especially in hyperthermophilic archaea, result from more complexed enzymatic machinery guided by sRNA (aFlpA C/D box sRNP in the case of 2'-O-methylations and Cbf5 H/ACA sRNP in the case of isomerisation of U into pseudouridine). In bold are the new modifications, observed in the present study, and for which the corresponding enzymes are not yet known. When underlined [X- methylated], it means that the nucleotide X is monomethylated but we do not know yet whether it is on the base or on the 2'-O-ribose. xU, and xG correspond to yet undetermined chemical modifications of the base that do not appear to correspond to any modification identified so far. Together with all the modified nucleotides reported in this work, are also listed those not indicated in Table 1 and Figure 3 but that have been experimentally identified in independent works, the archaeal species, the gene coding for the corresponding enzyme with references are clearly mentioned. Numbering positions of nucleotides in tRNA are the conventional ones used in the tRNA data bank (<http://trnadb.bioinf.uni-leipzig.de>).

MODOMICS of archaeal tRNA			Methanococcales			
Pos	MODS	ENZYME (COG)	Orf	Archaea genus	References	Pmid
6	m2G	TrmN/Trm14 (COG0116)	MJ0438	<i>M. jannaschii</i>	Menezes et al, 2011	.21693558
7	ac6A (see text)	unknown		<i>M. maripaludis</i>	this work	
8	s4U	Thil (COG0301)	Mmp1354/Mj0931	<i>M. maripal/jann</i>	Liu et al, 2012	.22904325
10	m2G/m2,2G	Trm-G10 (COG1041)	Mmp0149/Mj0710	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
15	G+	TgtA + (arcS) (COG1549)	Mj0436 +(Mj1022)	<i>M. Jannaschii</i>	Bai et al, 2000	.10862614
15	G+	TgtA + (arcS) (COG1549)	Mmp0610+(Mj1595)	<i>M. maripaludis</i>	Phillips et al, 2012	.22032275
17	s2C	unknown		<i>M. maripaludis</i>	this work	
22	Gm	unknown		<i>M. maripaludis</i>	this work	
26	m2G/m2,2G	Trm1 (COG1867)	Mmp0228/Mj0946	<i>M. maripal/jann</i>	Graham/Kramer, 2007	.17673084
32	s2C	<i>TtcA-like</i> (COG0037)	<i>Mmp1356/Mj1157</i>	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
32	Cm/Um (+34?)	<i>FlpA +snoRNP</i> (COG1889)	<i>Mmp0597/Mj0697</i>	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
33	s2U	unknown		<i>M. maripaludis</i>	this work	
34	C+	TiaS (COG1571)	Mmp0616/Mj1095	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
34	mcm5(s2)U	<i>Elp3-like</i> (COG1243)	<i>Mmp1577/Mj1136</i>	<i>M. maripal/jann</i>	Huang et al, 2008	.18755837
34	s2(mnm5)U	Ncs6/Ctu1-like+(unknown)	Mmp1356/Mj1157	<i>M. maripal/jann</i>	Liu et al, 2014	.24530533
34	se2(mnm5)U	<i>ybbB-like /selU+(unknown)</i>	<i>Mmp0899/Mj0053</i>	<i>M. maripal/jann</i>	Su et al, 2012	.22293502
34	s2(cnm5)U	Ncs6 + (unknown)		<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
34	Cm (+32?)	<i>FlpA +snoRNP</i> (COG1889)	<i>Mmp0597/Mj0697</i>	<i>M. maripal/jann</i>	Singh et al, 2004	.15347671
34	xU(cnU) see text	unknown		<i>M. maripaludis</i>	this work	
37	t6A	Tsa2+TsaB/D/E	Mj0062 + Mj1130	<i>M. Jannaschii</i>	Wan et al, 2016	.27302132
37		(t6A complex)	Mj0594 + Mj0186	<i>M. Jannaschii</i>		
37	t6A	Tsa2+TsaB/D/E	Mmp0186 + 0415	<i>M. maripaludis</i>	Thiaville et al, 2014	.25629598
37		(t6A complex)	Mmp0247 + 0897	<i>M. maripaludis</i>		
37	ms2t6A	MtaB (+ t6A complex)	Mmp0412/Mj0867	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
37	ms2hn6A	(MtaB) + hn6A complex	<i>t6A-related enzyme</i>	<i>M. maripal/jann</i>	Reddy et al, 1992	.1280806
37	m1G	Trm5b (COG2520)	Mmp0323/Mj0883	<i>M. maripal/jann</i>	Go-Ito et al, 2008	.18384044
37	imG-14	(Trm5b)+Taw1 (COC0731)	Mmp1440/Mj0257	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
37	imG	(Trm5b+Taw1)+Taw3	Mmp0310/Mj1510	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
37	yW	(Trm5+Taw1)+Trm2(+Taw3)	Mmp0560/Mj1557	<i>M. maripal/jann</i>	Umitsu et al, 2009	.19717466
37	mimG	Trm5b+Taw1+Trm5a+Taw3		<i>M. maripal/jann</i>	deCrecy-Lagard et al,2010	.20382657
37	xG (see text)	<i>wyosine metabolism</i>		<i>M. maripaludis</i>	this work	
37	f6A (see text)	unknown (or TrmM-like)		<i>M. maripaludis</i>	(Golovina et al, 2016)	.26707202
39	s2U	unknown		<i>M. maripaludis</i>	this work	
48	m5C (+49)	Trm4 (COG0144)	Mmp1126/Mj0026	<i>M. maripal/jann</i>	Kuratani et al, 2010	.20600111
54	Psi (+55)	Pus10 (COG1258)	Mmp0924/Mj0041	<i>M. maripal/jann</i>	Gurha & Gupta, 2008	.18952823
54	m1Psi	(Pus10) >>TrmY(COG1901)	Mmp0094/Mj1640	<i>M. maripal/jann</i>	Chatterjee et al, 2012	.22274953
54	s4(m1Psi)	TtuA + (TtuB) (COG0037)	Mj1478	<i>M. jannaschii</i>	Shigi, 2014	.24765101
55	Psi	<i>probably present</i> (Pus10)		<i>M. maripal/jann</i>	Fitzek et al, 2018	.29349599
56	Cm	Trm56 (COG1303)	Mmp1425/Mj1385	<i>M. maripal/jann</i>	Yu et al, 2019	.30745370
57	(m1A) >> m1I	(TrmI) >> + unknown		<i>H. volcanii</i>	Grosjean et al, 1996	.7501451
58	m1A	TrmI (COG2519)	Mmp1375/Mj0134	<i>M. maripal/jann</i>	Graham & Kramer, 2007	.17673084
67	m2G	unknown		<i>M. jannaschii</i>	Yu et al, 2019	.30745370

MODOMICS of archaeal tRNA				Pyrococcales		
Pos	MODS	ENZYME (COG)	Orf	Archaea genus	References	Pmid
1	2H-m7G (see text)	unknown		<i>P. furiosus</i>	this work	
5	ac4C	unknown		<i>P. furiosus</i>	this work	
6	m2G/m22G	TrmN/Trm14 (COG0116)	PF1002	<i>P. furiosus</i>	Fislage et al, 2012	.22362751
	Cm-6 / Um-8	unknown/unknown		<i>T. kodak/furios</i>	Hirata et al, 2019	.31405913
8	s4U-8(+9)	Thil (COG0301)	PF1288/TK0368	<i>P. furios/kodak</i>	Cavuzic & Liu, 2017	.28287455
9	m1A/m1G	Trm10 (COG2419)	PF0678/TK1257	<i>P. furios/kodak</i>	Krihnamohan et al, 2019	.30704107
10	m2G/m2,2G	Trm-G10 (COG1041)	PF0400/TK0980	<i>P. furios/kodak</i>	Armengaud et al, 2004	.15210688
10	2H-m27Gm (text)	unknown		<i>P. furiosus</i>	this work	
15	G+	TgtA + (arcS) (COG1549)	PF0071/TK2156	<i>P. furios/horik</i>	Ishitani et al, 2002	.12054814
17	s2C	unknown		<i>T. furios/kodak</i>	Hirata et al, 2019	.31405913
18	Gm	<i>FlpA +sRNP (or TrmH-like?)</i>	PF0059	<i>P. furiosus</i>	this work	
	Um-21; Gm-22	unknown or <i>FlpA +sRNP</i>		<i>T. kodak/furios</i>	Hirata et al, 2019	.31405913
26	m2G/m2,2G(m)	Trm1(+unknown)(COG1867)	PF1871/TK0970	<i>P. furios/kodaka</i>	Constantinesco et al,1998	.9685492
32	s2C	<i>TtcA-like</i> (COG0037)	PF0273/TK1821	<i>P. furios/kodaka</i>	Cavuzic & Liu, 2017	.28287455
32	m5C(m)	unknown (<i>+FlpA-snoRNA</i>)		<i>T. kodakarensis</i>	Hirata et al, 2019	.31405913
34	C+	TiaS (COG1571)	PF1855/TK0553	<i>P. furios/kodak</i>	Ikeuchi et al, 2010	.20139989
34	s2(mnm5)U	Ncs6/Ctu1-like (+unknown)	PF0273/TK1821	<i>P. furios/kodak</i>	Cavuzic and Liu, 2018	.28287455
34	(s2)mnm5U	Elp3-like (COG1243)				
34	Cm/Um(+39)	FlpA + Nop5 + snoRNA	PF0059/TK0183	<i>P. furios/kodak</i>	Clouet-d'Orval et al, 2001	.11713301
34	cnm5U	unknown		<i>Euryarchaeota</i>	see Mandal et al, 2014	.24344322
34	ac4C(m)	<i>TmcA-like(+FlpA)</i> (COG1444)	PF0504/TK0754	<i>P. furios/kodak</i>	(Bortolin et al, 2003)	.14602911
37	t6A	Tsa2 (+TsaB/D/E)	PF0306/TK0948	<i>P. furios/kodak</i>	Perrochia et al, 2012	.23258706
37	ms2(hn6A)	<i>MtaB-like (+unknown)</i>	PF1912/TK2064	<i>P. furios/kodak</i>	Reddy et al, 1992	.1280806
37	m1G	Trm5b (COG2520)	PF1415/TK0497	<i>P. furios/kodak</i>	Wu et al, 2017	.28911863
37	imG2	(Trm5a+Taw1) + Taw2	PF0089/TK2223	<i>P. furios/kodak</i>	Urbonavicius et al, 2014	.24837075
37	mimG	(Trm5b+Taw1+ 22) +Taw3	PF2034/TK0175	<i>P. furios/kodak</i>	Perche-Letuvée et al,2012	.23043105
37	yW-86	(Trm5b+Taw1) + Taw2	PF0500/TK0071	<i>P. furios/kodak</i>	Umitsu et al, 2009	.19717466
38	Am	<i>probably FlpA + snoRNP</i>		<i>P. furiosus</i>	Singh al 2004	.15347671
39	m22G	unknown		<i>P. furiosus</i>	this work	
39	Cm (+34)	FlpA-Nop5+snoRNP	PF0059/TK0183	<i>P. furios/kodak</i>	Bortolin et al, 2003	.14602911
	Cm-41/42,Gm-42	<i>probably all FlpA + snoRNP</i>		<i>T. kodak/furios</i>	Hirata et al, 2019	.31405913
42	ac4C	unknown		<i>P. furiosus</i>	this work	
44	Um	unknown		<i>P. furiosus</i>	this work	
47	xU (see text)	unknown		<i>P. furiosus</i>	this work	
48	m5C +49 (+27)	Trm4/ unknow-27 (COG0144)	PF1553/TK0360	<i>P.furios/kodak</i>	Auxilien et al, 2017	.17470432
50	Gm	unknown		<i>P. furiosus</i>	this work	
54	s2(m5U)	TtuA (+TrmU54) (COG2265)	PF1172/TK1116	<i>P. furios/kodak</i>	Rose et al, 2020	.32459340
55	Psi (+54)	Pus10 (COG1258)	PF1139/TK0903	<i>P. furios/kodak</i>	Gurha & Gupta, 2008	.18952823
55	Psi	Cbf5 (+Gar1; no gRNA)	PF1785/TK1509	<i>P. furios/kodak</i>	Müller et al, 2007	.17704128
56	Cm	Trm56 (<i>or FlpA +snoRNP</i>)	PF0461/TK0060	<i>P.furios/kodak</i>	Renalier et al, 2005	.15987815
57	m1A (+58)	TrmI (COG2519)	PF1896/TK1328	<i>P. furios/kodak</i>	Roovers et al, 2004	.14739239
57	(m1A) >> m1I	(TrmI) >> + unknown		<i>P. furiosus</i>	Grosjean et al, 1999	.9973619
63	ac4C (+64)	unknown		<i>P. furiosus</i>	this work	
	m2G-67, Gm-68	unknown-67/68		<i>T. kodak/P.furios</i>	Hirata et al, 2019	.31405913
72	m5C	NSun6 (COG0144)	PF0169/TK2122	<i>P. furios/kodak</i>	Li et al, 2019	.30541086

MODOMICS of archaeal tRNA				Sulfolobales		
Pos	MODS	ENZYME (COG)	Orf	Archaea genus	References	Pmid
3	ac4C	unknown		<i>S. acidocaldarius</i>	this work	
9	m1A	Trm10 (COG2419)	Saci_1677	<i>S. acidocaldarius</i>	Kempnaers et al, 2010	.20525789
10	m2G/m2,2G	Trm-G10 (COG1041)	Saci_1283	<i>S. acidocaldarius</i>	Wagner et al, 2004	.17150579
15	G+	TgtA+ArcS-like (COG0343)	Saci0659+Saci0657	<i>S. acidocaldarius</i>	Phillips et al, 2012	.22032275
17	<u>C/A</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
18	Gm (+34)	FlpA+ Nop5 + snoRNP	Saci_1346	<i>S. acidocaldarius</i>	Omer et al, 2006	.16861619
20	<u>A</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
23	Gm	probably FlpA+Nop5+snoRN	Saci_1346	<i>S. acidocaldarius</i>	this work	
25	Cm/Um	probably FlpA+Nop5+snoRN	Saci_1346	<i>S. acidocaldarius</i>	this work	
26	m2G/m2,2G(m)	Trm1(+unknown)(COG1867)	Saci_1343	<i>S. acidocaldarius</i>	≈ ubiquitous in Archaea	
27	m5C	unknown		<i>S. acidocaldarius</i>	this work	
29	Gm	unknown		<i>S. acidocaldarius</i>	this work	
32	Cm	TrmJ (COG0565)	Saci_0621	<i>S. acidocaldarius</i>	Somme et al, 2014	.24951554
34	C+	TiaS (COG1571)	Saci_0732	<i>S. acidocaldarius</i>	Mandal et al, 2010	.20133752
34	Um/Cm (+18)	FlpA-Nop5 + snoRNP	Saci_1346	<i>S. acidocaldarius</i>	Ziesche et al, 2004	.15522081
34	s2U(m)	<i>Ncs6/Ctu1-like</i> (COG0037)	<i>Saci_1570</i>	<i>S. acidocaldarius</i>	Liu et al, 2016	.27791189
34	mchm5Um	<i>Elp3-like</i> (COG1243)	<i>Saci_1044</i>	<i>S. acidocaldarius</i>	this work	
37	t6A	TsaC+ (Tsc complex)	Saci_1642+Saci0851	<i>S. tokadai</i>	Kuratani et al, 2011	.21538543
37	m1G	Trm5c (COG2520)	Saci_0559	<i>S. acidocaldarius</i>	Urbonavicius et al, 2016	.27852927
37	imG-14	(Trm5c) +Taw1 (COG0731)	Scai_1331	<i>S. acidocaldarius</i>	Urbonavicius et al, 2016	.27852927
37	imG2	(Trm5c +Taw1) +Trm5a	Scai_0030	<i>S. acidocaldarius</i>	deCrecy-Lagard et al,2010	.20382657
37	mimG	(Trm5c +Taw2) +Taw3	Saci_1603	<i>S. acidocaldarius</i>	McCloskey et al, 1987	.27932585
39	Cm	probably FlpA+Nop5+snoRN	<i>Saci_1346</i>	<i>S. acidocaldarius</i>	see in <i>P. furiosus</i>	
40	m5C	probably Trm4 (like in HVO)		<i>S. acidocaldarius</i>	Grosjean et al, 2008	.18844986
41	ac4C (+40)	unknown		<i>S. acidocaldarius</i>	this work	
47	<u>A</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
47	m2,2G	unknown		<i>S. acidocaldarius</i>	this work	
47	xU (see text)	unknown		<i>S. acidocaldarius</i>	this work	
48	m5C (+49)	Trm4 (COG0144)	Saci_0717	<i>S. acidocaldarius</i>	almost in all Archaea	
50	Um/Cm (+18)	FlpA-Nop5+snoRNP	Saci_1346	<i>S. acidocaldarius</i>	Tang et al, 2005	.15659164
51	Gm	FlpA-Nop5+snoRNP		<i>S. acidocaldarius</i>	Zago et al, 2005	.15752202
54	Um?? > see text	unknown		<i>S. acidocaldarius</i>	Kuchino et al, 1982	.6178978
54	s2U/s4Psi?	TtuA+TtuB see text	Saci_0378+Saci1570	<i>S. acidocaldarius</i>	Rose et al, 2020	.32459340
54	<u>mxsU/Psi</u> >text	unknown		<i>S. acidocaldarius</i>	this work	
55	Psi	possibly Cbf5	Scai_0811	<i>S. acidocaldarius</i>	Majumber et a, 2016	.27539785
56	Cm	Trm56 (COG1303)	Saci_1040	<i>S. acidocaldarius</i>	Renalier et al, 2005	.15987815
57	(m1A) >> m1I	(TrmI) + unknown		<i>S. acidocaldarius</i>	Yamaizumi et al, 1982	.7183961
58	m1A	TrmI (COG2519)	Saci_0844	<i>S. acidocaldarius</i>	Guelorget et al, 2011	.22168821
62	<u>C</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
64	<u>U</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
69	<u>C</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	
72	m5C	NSun6 (COG0144)	Saci_1487	<i>S. acidocaldarius</i>	Wagner et al, 2004	.17150579
72	<u>U</u> methylated	unknown		<i>S. acidocaldarius</i>	this work	

