

**The influence of phylogeny on posttranscriptional modification of rRNA
in
thermophilic prokaryotes: The complete
modification map of 16S rRNA of *Thermus thermophilus****

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Supporting Information

Table S1. Assignments for monomer mass fragment ions used for detection of modified residues in RNase digest oligonucleotides

Nucleoside	<i>m/z</i>	assignment ^a
m ⁷ G, m ² G	164	mGua
	358	mG>p
	376	mGp
m ₂ ² G	178	m ₂ Gua
	372	m ₂ G>p
Ψ	225	Ψ – H ₂ O
	207	Ψ – 2H ₂ O
	189	207 – H ₂ O
	164	207 – HNCO
m ₂ ⁶ A	162	m ₂ Ade
	356	m ₂ ⁶ A>p
	374	m ₂ ⁶ Ap
m ⁵ C	124	mCyt
	336	mCp
m ⁴ Cm	124	mCyt
	332	mCm>p
	350	mCmp

Cm	398	pCm>p
m ³ U	125	mUra
	319	mU>p
	337	mUp

^a All are singly charged negative ions

Fig. S1

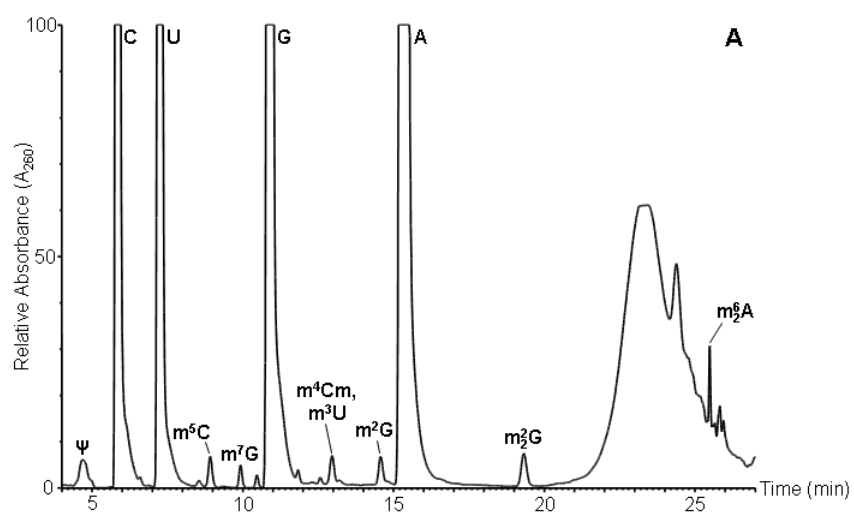


FIGURE S1. Census of modified nucleosides in *Thermus thermophilus* 16S rRNA by LC/MS analysis of a total nucleoside digest.

Fig. S2

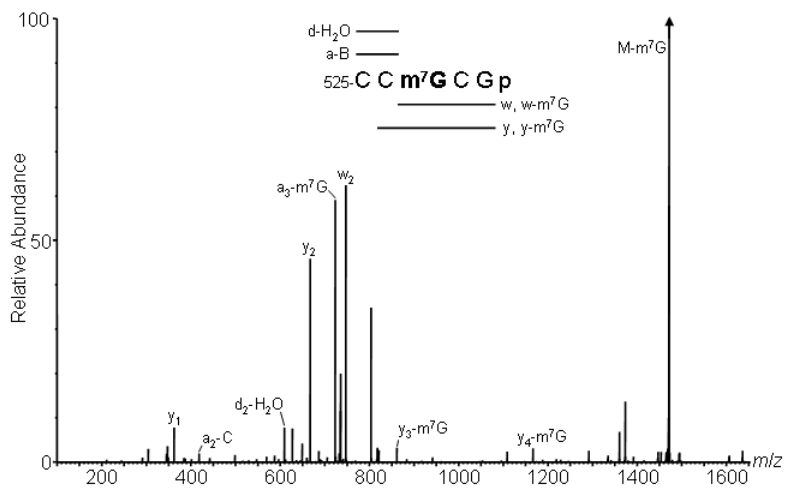


FIGURE S2. Mass spectrum from CID of 525-CCm⁷GCGp.

Fig. S3

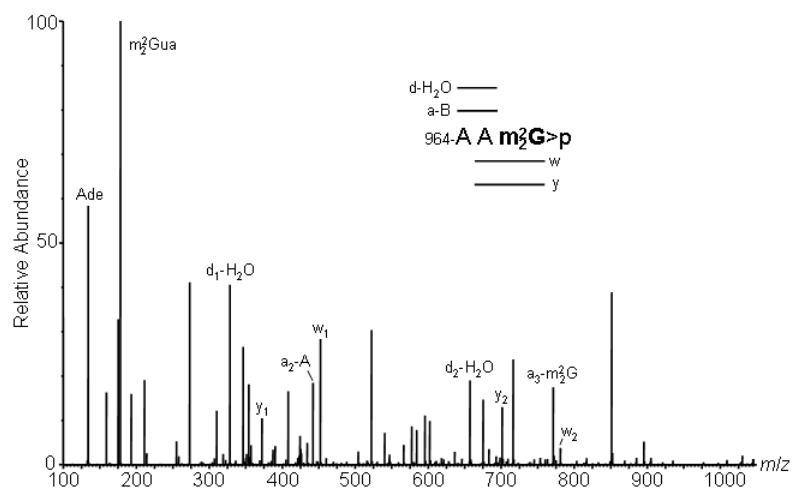


FIGURE S3. Mass spectrum from CID of 964-AAm₂G>p, with sequence ions annotated, for placement of m₂G-966.

Fig. S4

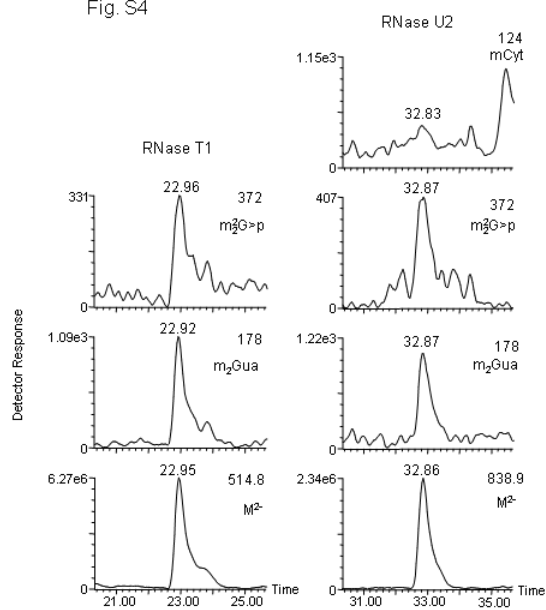


FIGURE S4. Chromatographic time alignments between characteristic low-mass ions and molecular ions in RNase T1 and U2 digests used for placement of adjacent modifications m_2^2G -966 and m^5C -967.

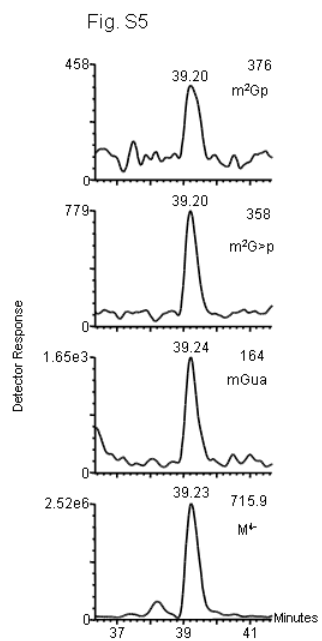


FIGURE S5. Chromatographic time alignments between methylguanine fragment ions and the molecular ion from U2 product M_r 2866, identifying the 1205–1213 oligonucleotide as containing m^2G .

Fig. S6

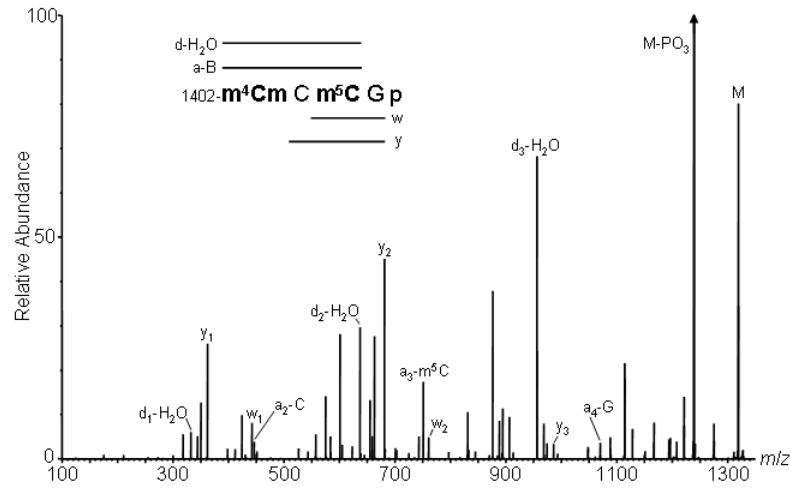


FIGURE S6. Mass spectrum from CID of RNase T1 oligonucleotide M_r 1320 (1402- m^4CmCm^5CGp), showing sequence ions used for distribution of three methyls in residues 1402 and 1404.

Fig. S7

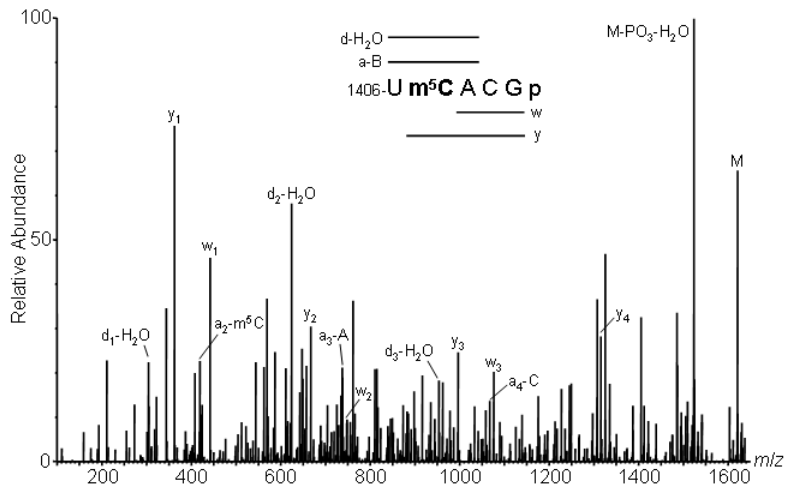


FIGURE S7. Mass spectrum from CID of RNase T1 oligonucleotide M_r 1622 (1406-Um⁵CACGp), showing position of m⁵C-1407 within the heavily methylated segment shown in Figure 6.

Fig. S8

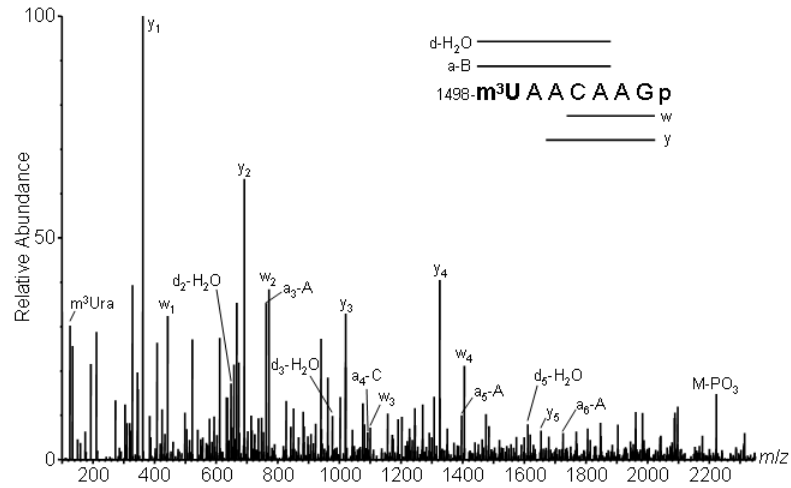


FIGURE S8. Mass spectrum from CID of RNase T1 oligonucleotide M_r 2304 (1498- $m^3\text{UAACAAGp}$), showing sequence ions consistent with placement of base-methylated uridine at the 5' terminus of the oligonucleotide.

Fig. S9

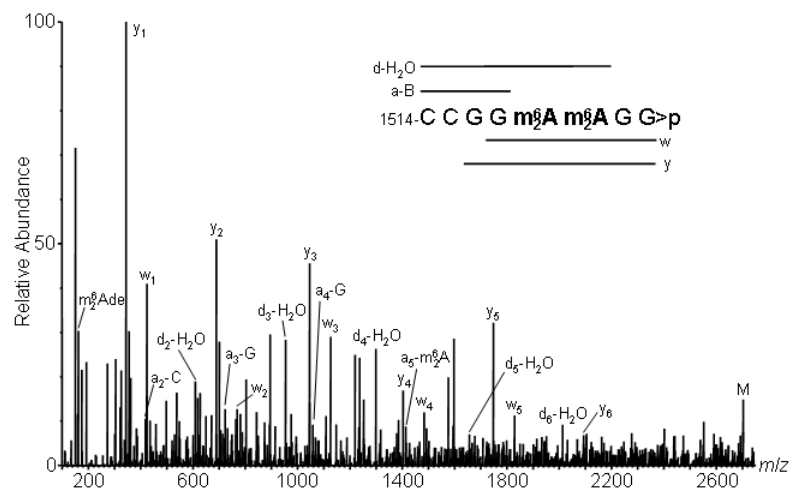


FIGURE S9. Mass spectrum following CID of RNase U2 oligonucleotide M_r 2704, confirming placement of the highly conserved tandem m_2^6A residues in the loop of helix 45.

Fig. S10

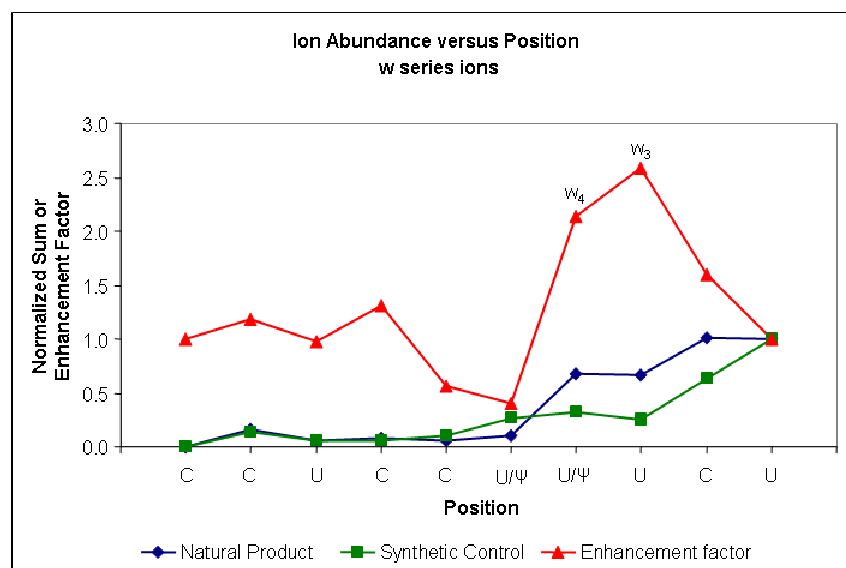


FIGURE S10. Normalized sum, encompassing all observed charge states, of the w series of sequence ions from the mass spectrum of RNase U2 oligonucleotide M_r 2993, compared with unmodified control oligonucleotide. Enhancement factors (ref 30) for ions w_3 and w_4 result from pseudouridylation of residues six and seven.