Supporting information

A selective and sensitive detection system for 4-thiouridine modification in RNA

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MATERIALS AND METHODS

Nucleoside analysis of Linear-s^{4}U and -U RNAs.

Modified nucleosides in tRNA fractions were analyzed by the method reported previously (Tomikawa et al. 2010). Briefly, 0.30 A260units of RNA was digested with 0.5 units Nuclease P1 (Fuji Film-Wako, Tokyo, Japan), 20 μg RNase A (Invitrogen) and 0.125 units bacterial alkaline phosphatase (Takara, Kyoto, Japan) at 37ºC over-night. Nucleosides were analyzed on a Hitachi L-2000 HPLC system (Hitachi, Tokyo, Japan) equipped with a Nucleosil 7C18 column (Chemco Plus, Osaka, Japan).

Detection of s^{4}U in Linear-s^{4}U, Linear-U, Stem-s^{4}U, Stem-U RNAs and *Thermus thermophilus* tRNA mixtures.

The experimental procedures are described in the Materials and Methods section in the main text. In the case of experiment in SFig. 5, Linear-s^{4}U RNA (2.5, 5, and 10 pmol) and 250 pmol *T. thermophilus ΔthiI* tRNA mixture was mixed and then reacted with MTSEA biotin-XX. Negative control was prepared by addition of water instead of *T. thermophilus ΔthiI* tRNA mixture.

REFERENCES IN SUPPORTING INFORMATION

Tomikawa C, Yokogawa T, Kanai T, Hori H. 2010. N^7-Methylguanine at position 46 (m^7G46) in tRNA from *Thermus thermophilus* is required for cell viability at high temperatures through a tRNA modification network. *Nucleic Acids Res*. **38**: 942-957.

FIGURE LEGENDS

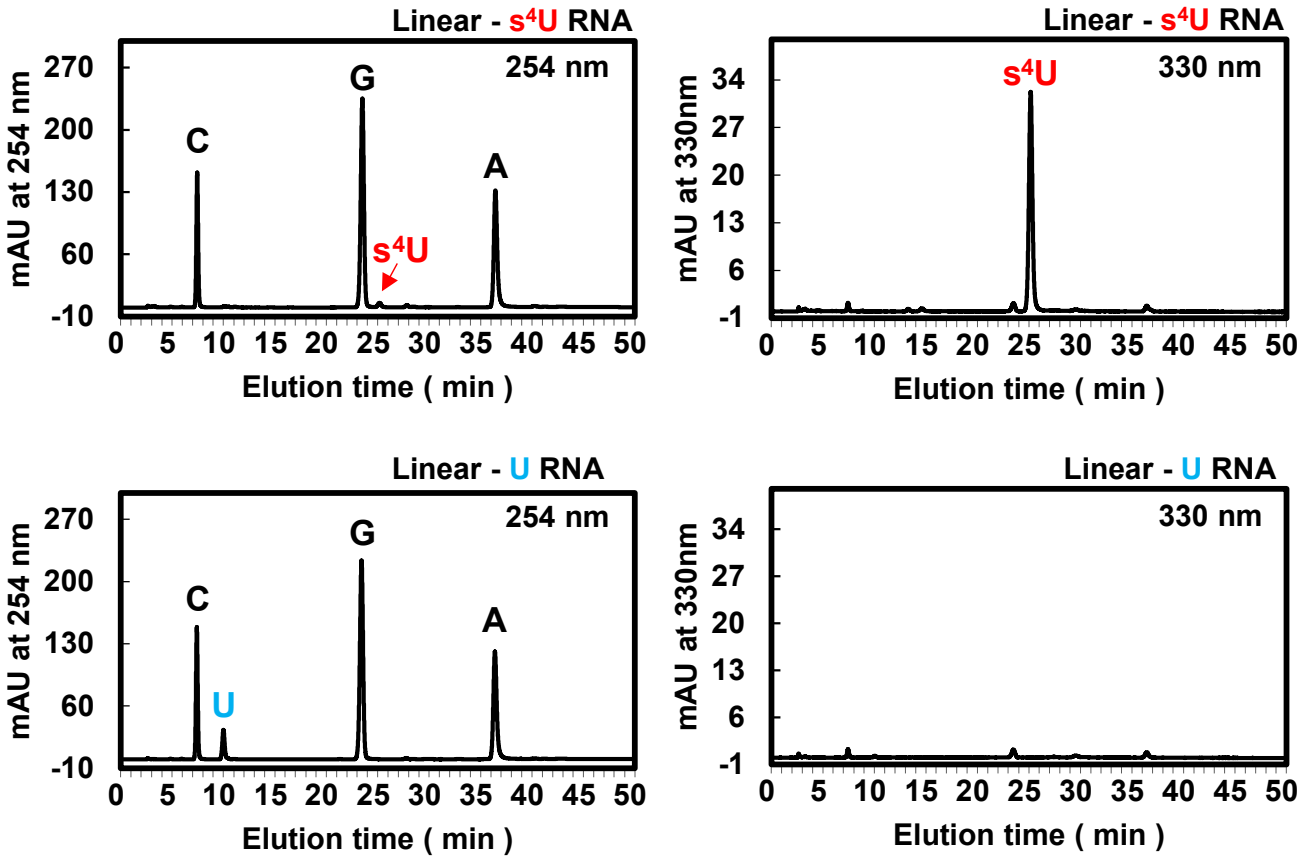
Supplementary Figure 1. Nucleosides in Linear-s^{4}U and -U RNAs were analyzed. To detect s4U clearly, absorbances at 254 nm (left panels) and 330 nm (right panels) were monitored. In the Linear-s^{4}U RNA, U was not detected. In contrast, U was detected in Linear-U instead of s^{4}U.

Supplementary Figure 2. (A) Chemiluminescence derived from 2.5 pmol Linear-U RNA (upper) and 2.5 pmol Linear-s^{4}U RNA are compared. Because only 2.5 pmol RNAs were used, spots of RNAs on the filter were not visible by methylene blue staining. (B) The intensities of chemiluminescence (exposure time 13 s) were measured with Lumino Graph II EM (code WSE-6270, Atto, Tokyo, Japan).

Supplementary Figure 3. (A) Chemiluminescence derived from 10 pmol annealed Linear-U RNA (upper) and 2.5 pmol annealed Linear-s^{4}U RNA are compared. (B) The intensities of chemiluminescence (exposure time 13 s) were measured with Lumino Graph II EM (code WSE-6270, Atto, Tokyo, Japan).

Supplementary Figure 4. (A) Chemiluminescence derived from 300 pmol *Thermus thermophilus* tRNA mixtures from the wild-type (upper) and *ΔthiI* (lower) strains are compared. (B) The intensities of chemiluminescence (exposure time 13 s) were measured with Lumino Graph II EM (code WSE-6270, Atto, Tokyo, Japan).

Supplementary Figure 5. Chemiluminescence derived from Linear-s4U RNA (2.5, 5, and 10 pmol) was monitored in the absence (upper) and presence (lower) of 250 pmol *T. thermophilus ΔthiI* tRNA mixture.


Linear - s4U RNA
254 nm
mAU at 254 nm
270
200
130
60
-10
C
G
A
s4U
0 5 10 15 20 25 30 35 40 45 50
Elution time (min)
Linear - s4U RNA
330 nm
mAU at 330nm
34
27
20
13
6
-1
s4U
0 5 10 15 20 25 30 35 40 45 50
Elution time (min)
Linear - U RNA
254 nm
mAU at 254 nm
270
200
130
60
-10
C
G
A
U
0 5 10 15 20 25 30 35 40 45 50
Elution time (min)
Linear - U RNA
330 nm
mAU at 330nm
34
27
20
13
6
-1
0 5 10 15 20 25 30 35 40 45 50
Elution time (min)

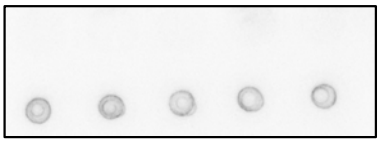
SFig.1

A

Linear- U RNA

Linear- s^{4}U RNA

Methylene blue staining

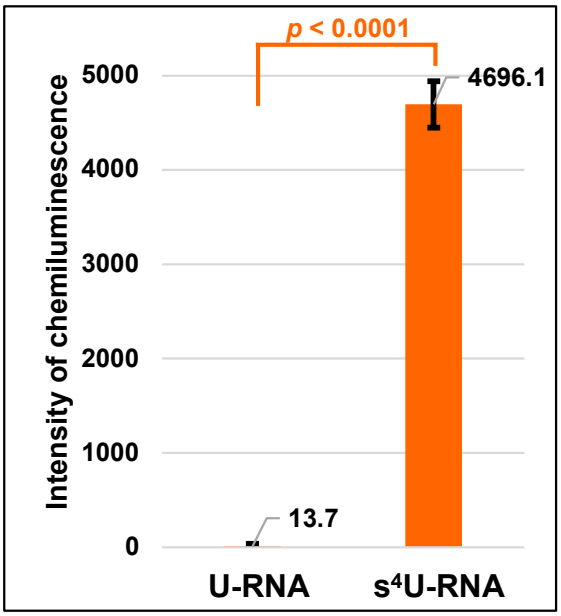


Linear- U RNA

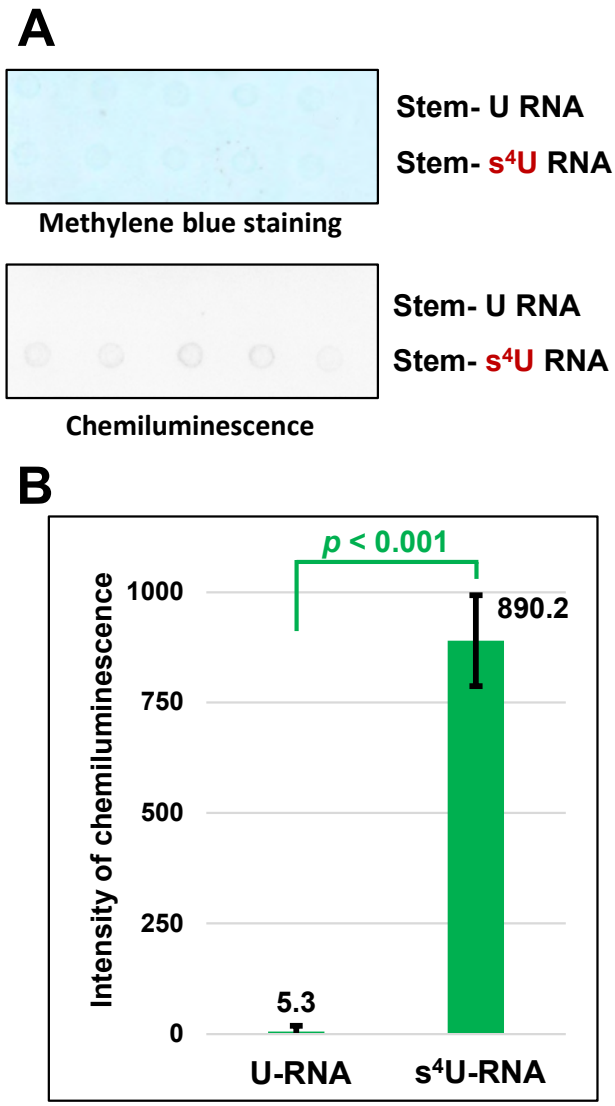
Linear- s^{4}U RNA

Chemiluminescence

B

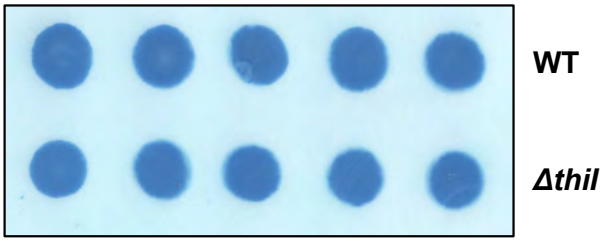


SFig.2

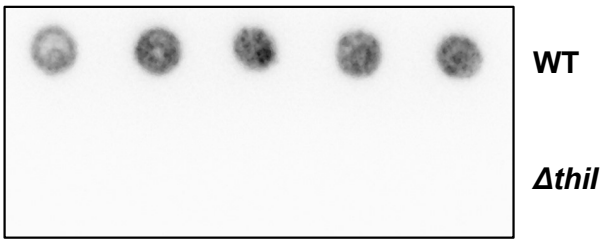

A
Stem- U RNA
Stem- s4U RNA
Methylene blue staining
Stem- U RNA
Stem- s4U RNA
Chemiluminescence
B
p < 0.001
Intensity of chemiluminescence
1000
750
500
250
0
890.2
5.3
U-RNA
s4U-RNA

SFig.3

A

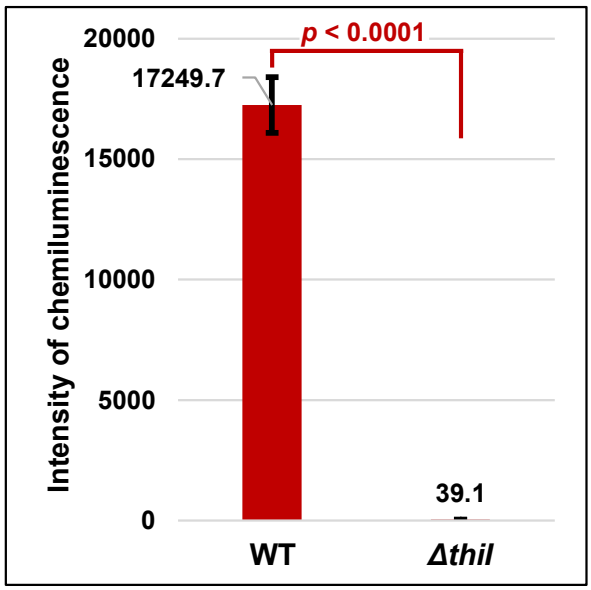


Methylene blue staining

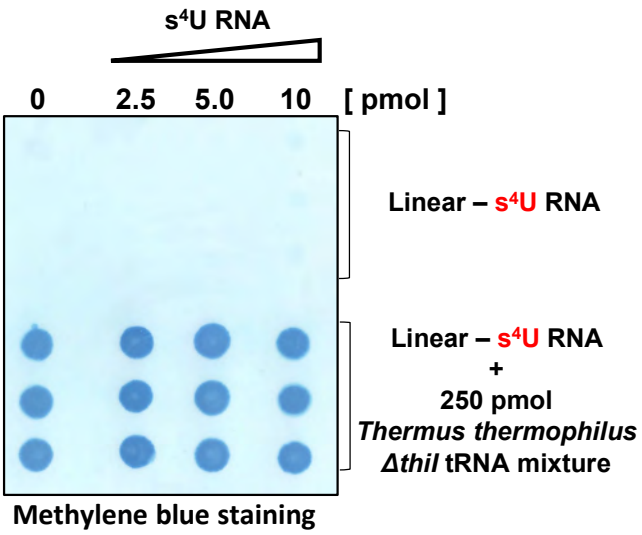


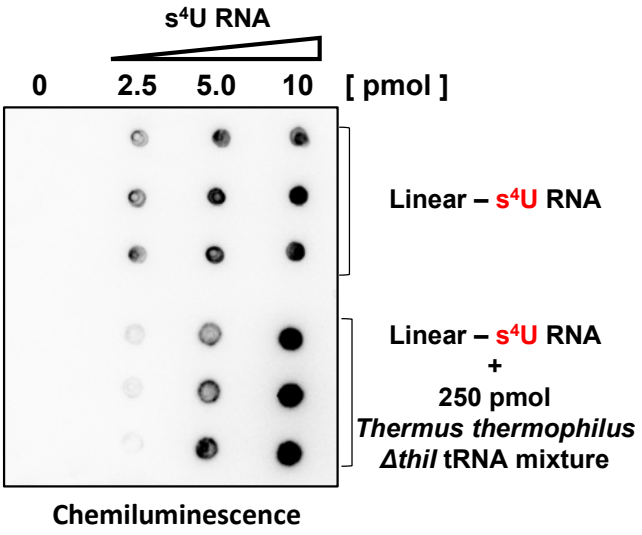
Chemiluminescence

B



SFig.4


s4U RNA
0
2.5
5.0
10
[pmol]
Linear – s4U RNA
Linear – s4U RNA
+
250 pmol
Thermus thermophilus
Δthil tRNA mixture
Methylene blue staining


s4U RNA
0
2.5
5.0
10
[pmol]
Linear – s4U RNA
Linear – s4U RNA
+
250 pmol
Thermus thermophilus
Δthil tRNA mixture
Chemiluminescence

SFig.5