Supplementary Information

Supplementary Figure Legends

Figure S1. Sequences of human cyto tRNA^{HisGUG} genes

Eleven cyto tRNA^{HisGUG} genes reported in the Genomic tRNA Database are shown. Upper case black letters designate the regions of mature tRNA^{HisGUG}. The nine genes from the top encode the identical "major" isodecoder on which this study focused. Sequences targeted by primers and the TaqMan probe in our TaqMan qRT-PCR analysis are indicated.

Figure S2. Primer extension using dideoxynucleotides

Synthetic tRNA^{HisGUG} harboring G_{-1} with *E. coli* tRNA carrier (left) and 70–90 nt mature tRNA fractions of BT-474 cells (right) were subjected to the primer extension assay using the indicated combinations of deoxy- and didecynucleotides. In didexynucleotide-containing reaction, 100 μM of either ddCTP or ddGTP was mixed with 10 μM dCTP, 50 μM dGTP, 50 μM dATP, or 50 μM dCTP, 10 μM dGTP, 50 μM dATP, respectively. The presence of ddCTP or ddGTP yielded 22- and 25-nt bands (shown with white squares) or 23- and 24-nt bands (shown with black squares), respectively, indicating that the reverse transcription was correctly run on "GCCG" sequences of tRNA^{HisGUG} in both synthetic tRNA and cellular tRNA samples.

Figure S3. Quantification of the bands yielded by the primer extension assay for 5′-SHOT-RNA^{HisGUG}

Three independent primer extension assays for an equal mixture of synthetic mature tRNA^{HisGUG} starting from G_1 and G_{-1}, or for a 30–50 nt small RNA fraction of BT-474 cells

were performed (one of the results are shown in **Fig. 2B**) and the intensities of the resultant 25- and 26-nt bands were quantified. The relative band intensities with SD bars are shown.

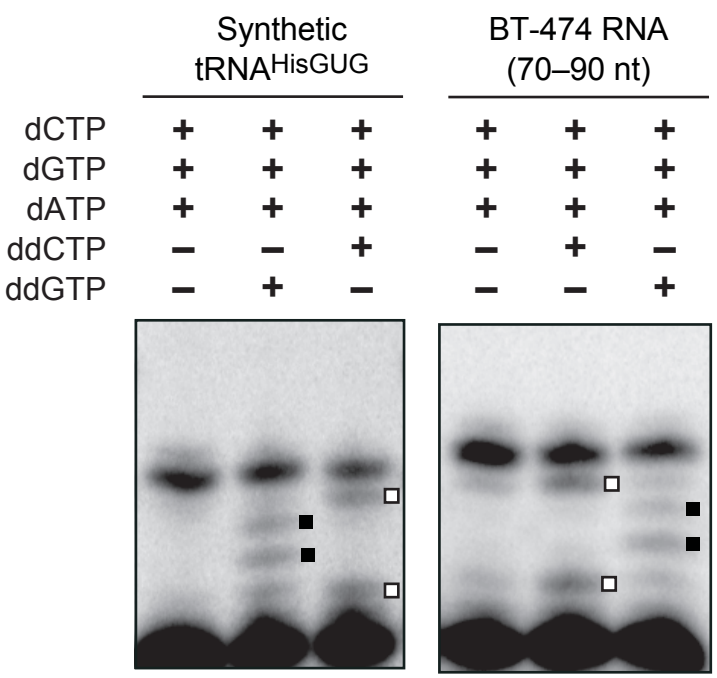
Figure S4. Schematics and sequences of the AS-disruptor, adapter, TaqMan probe, and primers for the TaqMan qRT-PCR method

The 5′-adaptor comprised DNA except for the last three 3′-terminal nucleotides, which were designed as RNA.

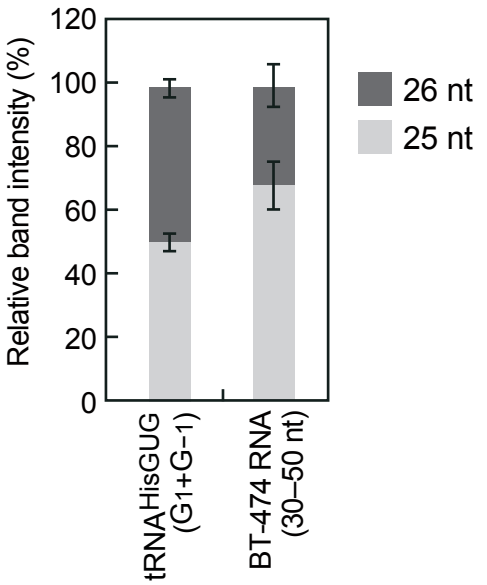
Figure S5. Proportional correlation of synthetic tRNA input with Ct values obtained using the TaqMan qRT-PCR method

Synthetic $tRNA^{HisGUG}$ containing either 5′-terminal G_{-1}, U_{-1}, C_{-1}, A_{-1}, and G_{1} was mixed with the *E. coli* tRNA fraction and used as a template. Each data set represents the average of three independent experiments with bars showing the SD.

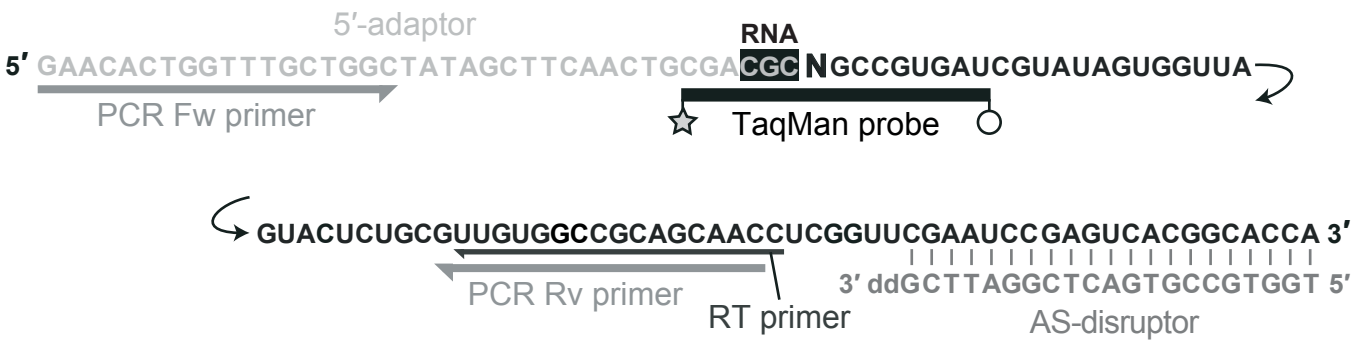
Shigematsu and Kirino, Supplementary Figure S1



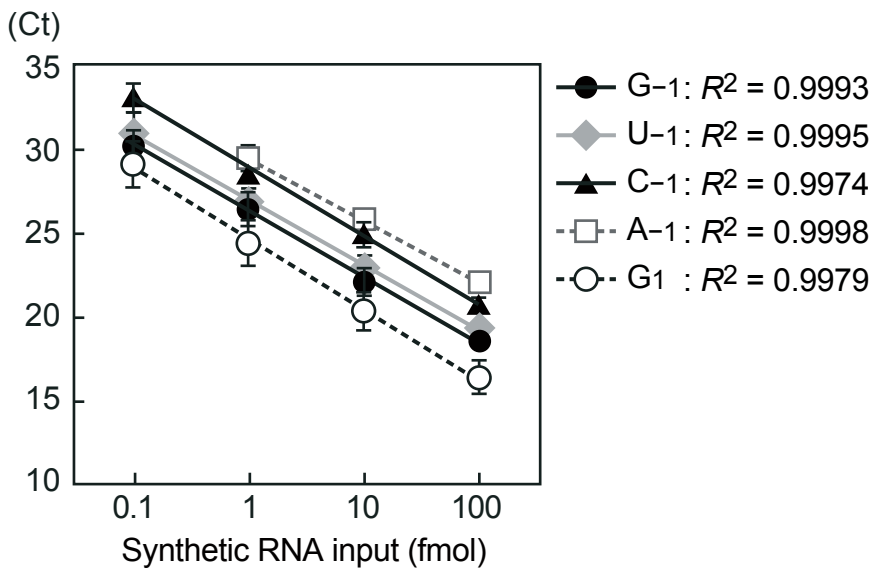
Shigematsu and Kirino, Supplementary Figure S2



Shigematsu and Kirino, Supplementary Figure S3



Shigematsu and Kirino, Supplementary Figure S4



Shigematsu and Kirino, Supplementary Figure S5

Supplementary Tables

Table S1. Validation of the specificity of our TaqMan qRT-PCR method

Template		TaqMan probe				
5'-end	Amount	G_{-1}	U_{-1}	C_{-1}	A_{-1}	G_1
G_{-1}	1 fmol	9.76 ± 0.02	N. D.	N. D.	N. D.	N. D.
	0.1 amol	26.90 ± 0.30	N. D.	N. D.	N. D.	N. D.
U_{-1}	1 fmol	N. D.	11.83 ± 0.15	N. D.	N. D.	N. D.
	0.1 amol	N. D.	25.02 ± 0.04	N. D.	N. D.	N. D.
C_{-1}	1 fmol	N. D.	N. D.	10.00 ± 0.06	N. D.	N. D.
	0.1 amol	N. D.	N. D.	23.93 ± 0.12	N. D.	N. D.
A_{-1}	1 fmol	N. D.	N. D.	N. D.	12.00 ± 0.09	N. D.
	0.1 amol	N. D.	N. D.	N. D.	25.66 ± 0.10	N. D.
G_1	1 fmol	N. D.	N. D.	N. D.	N. D.	10.44 ± 0.10
	0.1 amol	N. D.	N. D.	N. D.	N. D.	23.10 ± 0.03

A TaqMan qPCR method targeting 5′-terminal G_{-1}, U_{-1}, C_{-1}, A_{-1}, or G_1 was applied to plasmid DNA templates containing sequences of the ligation product of the 5′-adaptor and respective 5′-terminal variant of $tRNA^{HisGUG}$. Amplification signals were produced only when the TaqMan probe matched with its targeted template.

Table S2. Mature cyto $tRNA^{HisGUG}$ sequences present in the HEK293T tRNA sequence library

File name	Sample name		Total	Mapped to $tRNA^{HisGUG}$	Full length mature $tRNA^{HisGUG}$
SRR1836125	tRNA treated 1	Read	19182690	346179	5073
		%	100.00	1.80	0.03
		%	–	100.00	1.47
SRR1836125	tRNA treated 2	Read	8664537	157491	1932
		%	100.00	1.82	0.02
		%	–	100.00	1.23

SRR files were obtained from the Sequence Read Archive (SRA) as indicated in (Zheng et al. 2015). Raw reads of the two indicated samples (HEK293T tRNA library obtained after AlkB treatment) were mapped to human cyto $tRNA^{HisGUG}$ using Bowtie (two allowed mismatches). Among the mapped reads, mature tRNA sequences containing 3′-terminal CCA sequences with lengths of either 76 or 75 nt were extracted.

Table S3. 5′-terminal nucleotides of mature cyto tRNA^{HisGUG} in the HEK293T tRNA sequence library

File name	Sample name		Full length tRNA^{HisGUG}	Nucleotide of 5'-end				
				G_{-1}	U_{-1}	C_{-1}	A_{-1}	G_1
SRR1836125	tRNA treated 1	Read	5073	2381	909	112	132	1539
		%	100.00	46.93	17.92	2.21	2.60	30.34
SRR1836125	tRNA treated 2	Read	1932	927	331	47	63	564
		%	100.00	47.98	17.13	2.43	3.26	29.19

Table S4. Sequences of oligo DNA/RNA used in this study

Name	Sequence (5' to 3')
AS-disruptor	TGGTGCCGTGACTCGGATTCddG
5'-adaptor	GAACACTGCGTTTGCTGGCTATAGCTTCAACTGCGArCrGrC
RT primer	GGTTGCTGCGGCCACAA
PCR forward primer	GAACACTGCGTTTGCTGGC
PCR reverse primer	GTTGCTGCGGCCACAAC
TaqMan probe for G_{-1}	CGACGCGGCCGTGAT
TaqMan probe for U_{-1}	CGACGCTGCCGTGAT
TaqMan probe for C_{-1}	CGACGCCGCCGTGAT
TaqMan probe for A_{-1}	CGACGCAGCCGTGAT
TaqMan probe for G_1	CGACGCGCCGTGAT

TaqMan probes contain 6-carboxyfluorescein (FAM) and ZEN/Iowa Black as the fluorophore and quencher, respectively. The 5′-adaptor comprised DNA except for the last three 3′-terminal nucleotides, which were designed as RNA.