Supplementary information

$tRNA^{Val}$ allows four-way decoding with unmodified uridine at the wobble position in *Lactobacillus casei*

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Supplementary method

Nucleosides analysis of $tRNA^{Val}$ by HPLC

Isolated $tRNA^{Val}$ from *L. casei* was further purified by gel extraction. Nucleoside analysis of the purified $tRNA^{Val}$ was performed according to our previous literature [1]. 4 µg of purified $tRNA^{Val}$ was digested with 0.012 units of snake venom phosphodiesterase (Sigma), 20 µg of RNaseA (Roche), and 0.25 units of bacterial alkaline phosphatase BAP C75 (Takara) in 20 µl of 50 mM Tris-HCl (pH8.0) at 37°C overnight. The sample was purified busing a PVDF centrifugal filter (Ultra free-MC-HV 0.45 µm, Millipore). Nucleosides were analyzed on an HPLC (Hitachi L-2000 system) equipped with a reverse phase C18 column (NUCLEOSIL 100 C18; 25 cm x 4.6 mm, 7 µm; GL Sciences, Inc). The solvent system consisted of buffer A (50 mM sodium phosphate, pH5.1) and buffer B (buffer A containing 70% methanol). The nucleosides (20 µl) were chromatographed using a flow rate of 1 ml/min with a multistep linear gradient as follows: 3% buffer B from 0-50min, 3-98% buffer B from 50-65 min, and 98-100% buffer B from 65-75 min. Standard pm^6A was purchased from Sigma. The pm^6A nucleotide was dephosphorylated with bacterial alkaline phosphatase before use. The elution points of 7-methylguanosine (m^7G), 5-methyluridine (m^5U), and pseudouridine (Ψ) were determined by enzymatic formations using the tRNA modification enzymes, TrmB, TrmA, and TruB, respectively.

Reference in Supplementary method

1. Tomikawa C, Yokogawa T., Kanai T., & Hori H. (2010) N7-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.