Supplementary file

Required elements in tRNA for methylation by the eukaryotic tRNA (guanine-N^2-) methyltransferase (Trm11-Trm112 complex)

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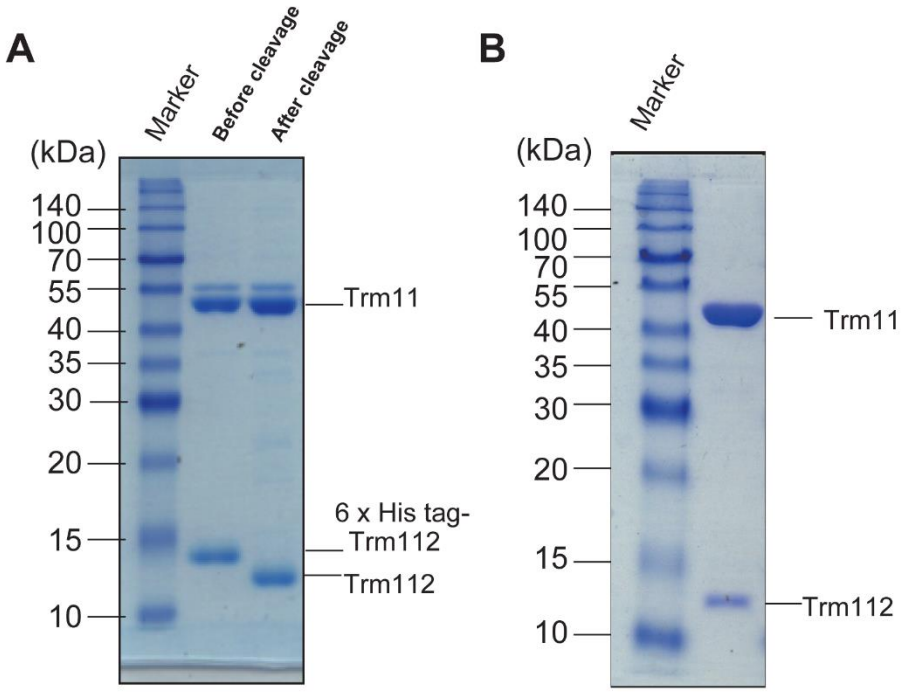
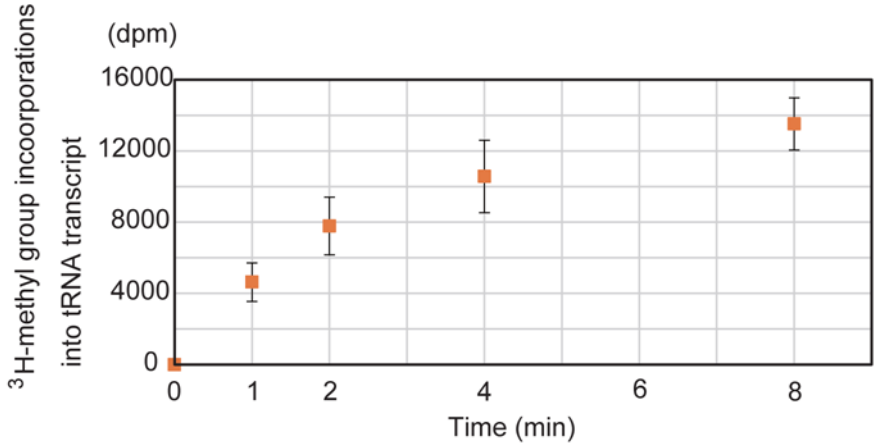
Co-expression of Trm11 and Trm112 in *Escherichia coli* cells and purification

The *trm112* with a His x 6 tag coding region fused at the end of gene was connected upstream of the *trm11* gene and these genes were inserted into the pET21a plasmid vector. The constructed vector, pET21a-*trm112*His x 6 tag-*trm11*, was introduced into *E. coli* BL21 (DE3) Rosetta 2 strain and recombinant proteins were expressed. The complex of Trm11-Trm112-His x 6 tag was purified by NiNTA super-flow column chromatography (Supplementary Figure 1A left lane), and then the His x 6 tag was cleaved by HRV3C protease. The cleaved His x 6 tag and HRV3C protease were removed from the sample by passing through the NiNTA super-flow column again (Supplementary Figure 1A right lane). The sample was further purified by Superdex-75 High Resolution gel-filtration column chromatography as shown in Supplementary Figure 1B.



Supplementary Figure S1. Purification of Trm11-Trm112 complex. A After NiNTA super-flow column chromatography (left lane), the His x 6 tag was cleaved by HRV3C protease (right lane). 6.8 μg of each of sample was analyzed by 15% SDS-polyacrylamide gel electrophoresis (PAGE). The gel was stained with Coomassie Brilliant Blue. **B** Purified Trm11-Trm112 (7.4 μg) was analyzed by 15% SDS-PAGE. The gel was stained with Coomassie Brilliant Blue.

Measurement of methyltransferase activity of Trm11- Trm112 complex



Supplementary Figure S2. Methyl-transfer activity measurement of purified Trm11-Trm112 complex. ^{3}H-methyl group incoorporation into $tRNA^{Phe}$ transcript was measured at 0, 1, 2, 4 and 8 min. The data is an average of three independent experiments.