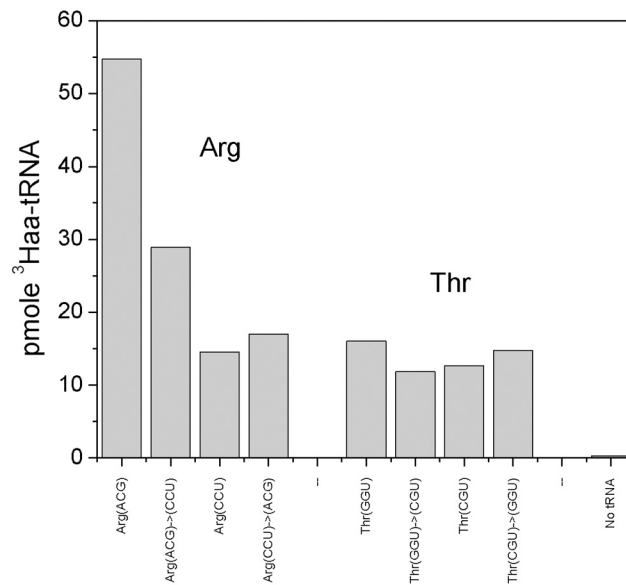
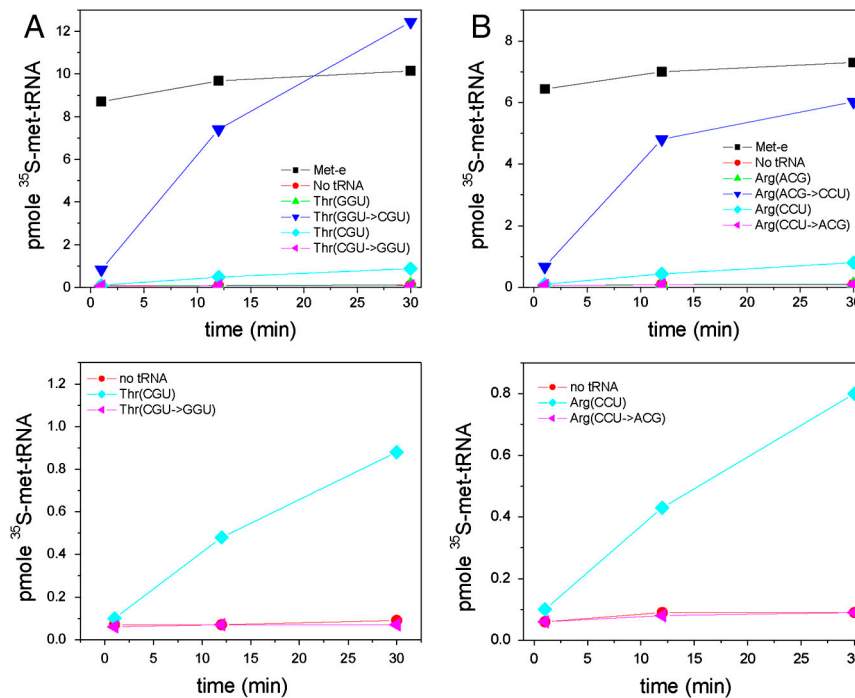


# Supporting Information

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**Fig. S1.** Plateau aminoacylation of tRNA<sup>Arg</sup> and tRNA<sup>Thr</sup> transcripts using *Escherichia coli* aaRS mixture. Each aminoacylation reaction includes 3 μM of total transcript based on A<sub>260</sub> absorbance. Fifty micromolar <sup>3</sup>H-arg or <sup>3</sup>H-thr was used for arg-tRNA and thr-tRNA transcripts, respectively. A main reason for observing the fraction aminoacylated below the amount of tRNA transcript used is due to heterogeneous 3' end addition in T7 RNA polymerase transcription.



**Fig. S2.** Misacylation of tRNA transcripts using N547EcMRS. (A) tRNA<sup>Thr</sup>. (B) tRNA<sup>Arg</sup>. Charging levels of all transcripts are shown in the top graph, whereas the y axis of the bottom graph is 1/10th of the y axis of the top graph.