

P030 Non-natural amino acid incorporation into protein by mitochondrial tRNAs having non-standard structure
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In the *in vitro* and *in vivo* translation system, non-natural amino acids can be introduced into proteins by using orthogonal tRNAs which encode the non-natural amino acids. An orthogonal tRNA must avoid accepting all 20 types of amino acids. In translation systems with canonical tRNAs, such as *Escherichia coli* system, tRNAs having non-standard structure should be orthogonal because such tRNAs probably is recognized by none of 20 types of aminoacyl-tRNA synthetases. Thus, we here applied mitochondrial tRNAs having non-standard secondary structure to introduce non-natural amino acid into protein in *E. coli in vitro* translation system. In this study, we prepared engineered mitochondrial tRNAs having a CCCG four-base anticodon corresponding to a CCCG four-base codon. Using these tRNAs, non-natural amino acid (nitrophenylalanine) could successfully incorporated at CGGG codon in engineered streptavidine mRNA.