

Supplementary Materials for

# Efficient Incorporation of Unnatural Amino Acids into Proteins With a Robust Cell-Free System

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Figure S1: Nucleotide and amino acid sequences of *pPaFRS*

Figure S2: Nucleotide and amino acid sequences of original *sfGFP*

Figure S3: Nucleotide and amino acid sequences of mutational *sfGFP2pPaF*

Figure S4: Nucleotide sequence of *o-tDNA*

Table S1: Primers used in this article

**Supporting Figure S1. Nucleotide and amino acid sequences of pPaFRS.**

**Nucleotide sequence of pPaFRS.**

ATGCATCACCATCACCATCATGGCGGAGCCATGGACGAGTTCGAAATGATTAACGCA  
ACACCAGCGAAATTATCTCTGAAGAAGAGCTGCGCGAGGTGCTGAAGAAAGACGAG  
AAGAGCGCGGCCATTGGCTTTGAGCCGTCCGGTAAAATTCACCTGGGTCACTACCTGC  
AAATCAAGAAGATGATTGATCTGCAAAACGCTGGTTTTGACATCATTATCCTGCTGGC  
GGACCTGCACGCCTACCTGAATCAAAGGGGCGAGCTGGATGAGATTGCAAGATCGG  
CGACTACAATAAGAAAGTCTTCGAAGCCATGGGTTTTGAAGGCTAAATACGTCTACGGT  
AGCCCTTTTCAGCTGGATAAGGATTACACGTTGAATGTGTACCGTCTGGCGCTGAAAA  
CCACGCTGAAACGCGCCCGTCTGTTCCATGGAGCTGATTGCGCGCGAGGATGAGAATC  
CAAAAGTTGCTGAGGTTATTTACCCTATTATGCAAGTTAATGCCATTCACTACGCGGGT  
GTTGATGTTGCCGTCGGTGGTATGGAGCAACGCAAATTCACATGCTGGCACGTGAAC  
TGCTGCCGAAAAGGTTGTCTGTATTCATAATCCGGTCTGACCGGCCTGGATGGCGA  
GGTAAAATGAGCAGCAGCAAGGGTAACTTTATTGCAGTTGACGATAGCCCGGAAGA  
AATCCGTGCGAAGATCAAGAAAGCGTACTGCCCGGCAGGCGTGGTTGAGGGTAACCC  
GATCATGGAAATCGCCAAGTATTTCTGGAATACCCACTGACGATTAAGCGCCCGGAG  
AAATTTGGCGGCGACCTGACCGTCAACAGCTACGAGGAGCTGGAAAGCTTGTTTAAG  
AACAAAGAACTGCATCCGATGCGCCTGAAAAACGCCGTGGCGGAAGAGCTGATTAA  
GATTCTGGAACCAATTCGCAAACGTCTGTAA

**Amino acid sequence of pPaFRS.**

MHHHHHHGGAMDEFEMIKRNTSEIIEEELREVLKKDEKSAAIGFEPGKIH LGHYLQIKK  
MIDLQNAGFDIII LLADLHAYLNQKGELDEIRKIGDYNKKVFEAMGLKAKYVYGS PFQLDK  
DYTLNVYRLALKTT LKRARRSMELIAREDENPKVAEVIYPIMQVNAIHYAGVDVAVGGME  
QRKIHMLARELLPKKV VCIHNPVLTGLDGEGKMSSSKGNFIAVDDSP EEIRAKIKKAYCPAG  
VVEGNPIMEIAKYFLEYPLTIKRPEKFGGDLTVNSYEELES LFKNKELHPMRLKN AVAEELIKI  
LEPIRKRL\*

**Figure S2: Nucleotide and amino acid sequences of original sfGFP.** Initiator AUG codon was indicated in green; and pPaF-encoding stop UAG codon was in bold and red. In the amino acid sequence, initiator (M) residues were green and in bold and pPaF residue was are highlighted and shown in red and bold. All the nucleotide and amino acid sequences of 6 × His tag were shown in purple.

**Nucleotide sequence of original sfGFP**

ATGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCTCCCTATTCTGGTGGAAGTGGATG  
GTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTA  
ATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTACCTTGGCC  
GACTCTGGTAAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGGACCATA  
TGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCA  
CGATTTCTTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAG  
GCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCA  
ATATCCTGGGCCATAAGCTGGAATACAATTTAACAGCCACAATGTTTACATCACCGCC  
GATAAACAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGAT  
GGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCCT  
GTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGA  
ACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGC  
ATGGTATGGATGAACTGTACAAA**CATCACCATCACCATCATTAA**

**Amino acid sequence of original sfGFP**

**MRKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLLKFICTTGKLPVPWPTLV**  
TTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVN  
RIELKGIDFKEDGNILGHKLEYNFNFSHNVYITADKQKNGIKANFKIRHNVEDGSQLADHY  
QQNTPIGDPVLLPDNHVLSLSTQSVLSKDPNEKRDHMLLEFVTAAGITHGMDELYK**HHH**  
**HHH\***

**Figure S3: Nucleotide and amino acid sequences of mutational sfGFP2pPaF.** Initiator AUG codon was indicated in green; and pPaF-encoding stop UAG codon was in bold and red. In the amino acid sequence, initiator (M) residues were green and in bold and pPaF residue was are highlighted and shown in red and bold. All the nucleotide and amino acid sequences of 6 x His tag were shown in purple.

**Nucleotide sequence of mutational sfGFP2pPaF**

**ATGTAG**AAAGGCGAAGAGCTGTTCACCTGGTGTCTCCCTATTCTGGTGGAAGTGGATG  
GTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTA  
ATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTACCTTGGCC  
GACTCTGGTAAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGGACCATA  
TGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCA  
CGATTTCCTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAG  
GCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCA  
ATATCCTGGGCCATAAGCTGGAATACAATTTAACAGCCACAATGTTTACATCACCGCC  
GATAAACAAAAAATGGCATTAAAGCGAATTTAAAATTCGCCACAACGTGGAGGAT  
GGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCCT  
GTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGA  
ACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGC  
ATGGTATGGATGAACTGTACAAA**CATCACCATCACCATCATTAA**

**Amino acid sequence of mutational sfGFP2pPaF**

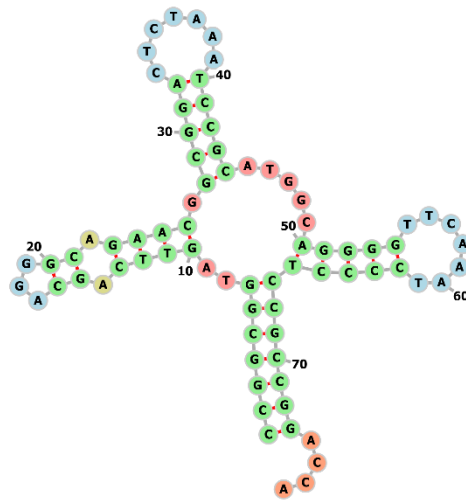
**MpPaF**KGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPT  
LVTTLTLYGVQCFARYPDHMKQHDFKSAPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV  
NRIELKGIDFKEDGNILGHKLEYNFNHNVYITADKQKNGIKANFKIRHNVEDGSVQLAD  
HYQONTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMLLEFVTAAGITHGMDELYKH  
**HHHHH\***

**Figure S4: Nucleotide sequence of o-tDNA and structure of o-tRNA.** The o-tDNA Sequence was indicated in red and bold. T7 promoter sequence was highlighted with yellow.

**Nucleotide sequence of o-tDNA**

GCTTTTAGATCT **TAATACGACTCACTATAGG** GAGACCGGCTGATGAGTCCGTGAGGAC  
GAAACGGTACCCGGTACCCGTCCCGGCGGTAGTTCAGCAGGGCAGAACGGCGGACT  
**CTAAATCCGCATGGCAGGGGTTCAAATCCCCTCCGCCGGACCA**

**Structure of o-tRNA.** Simulate on line via ViennaRNA Web Services



**Table S1.** Primers in this article.

<b>Primers</b>	<b>Sequence</b>
P1f	TTTCAGCTGGATAAGGATTACACGTTGAATGTGTACCGTCTGG
P1r	ACCACCGACGGCAACATCAACACCCGCGTAGTGAATGGCATTAAAC
P2f	AGAAAGACGAGAAGAGCGCGGCCATTGGCTTTGAGCCGTC
P2r	TAATCCTTATCCAGCTGAAAAGGGCTACCGTAGACGTATTTAGCC
P3f	TTGATGTTGCCGTCGGTGGTATGGAGCAACGCAAATTCACATG
P3r	CGCGCTCTTCTCGTCTTTCTTCAGCACCTCGCGCAG
P4f	TGGAGCCACCCGCAGTTCGAAAAGTAAGTCGACAAGCTTGCGGCCGC
P4r	TTACTTTTCGAACTGCGGGTGGCTCCATTTGTACAGTTCATCCATAACCATG CGTGATGCCC
P5f	CATATGTAGAAAGGCGAAGAGCTGTTC
P5r	GCCTTTCTACATATGTATATCTCCTTCTTAAAGTTAAAC
P6f	CCCTCCGCCGGACCAAAGCTTGCGGCCGCACTC
P6r	TTAAGATCTAAAAGCGTCGACGGAGCTCGAATTCGGATCC
P7f	GCTTTTAGATCTTAATACGACTCACTATAGGGAGACCG
P7r	TGGTCCGGCGGAGGGGAT