Ribosomal Synthesis of Unnatural Peptides

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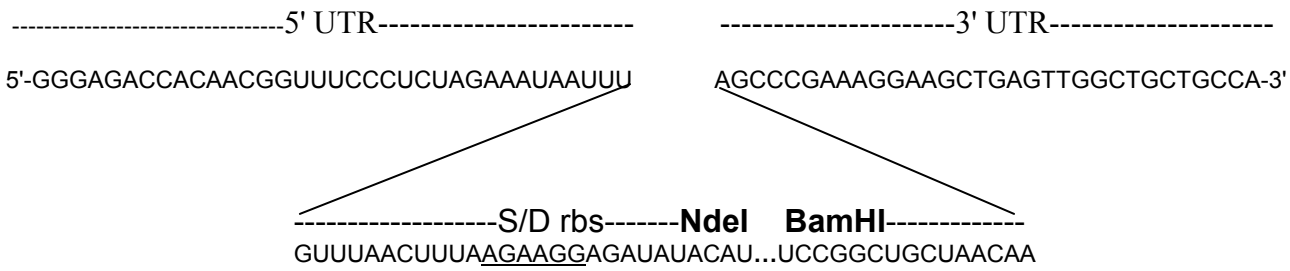
Supporting information

IF1 FWD	5-GCTACGACCATATGGCCAAAGAAGACAATATTGAAATGC-3
IF1 REV	5-CGATGGATCCTCAGCGACTACGGAAGACAATGCG-3
IF2 FWD	5-GCTACGACCATATGACAGATGTAACGATTAAAACGCTGGCC-3
IF2 REV	5-CGATGGATCCTTAAGCAATGGTACGTTGGATCTCG-3
IF3 FWD	5-GCTACGACCATATGAAAGGCGGAAAACGAGTTCAAACG-3
IF3 REV	5-CGATGGATCCTTACTGTTTCTTCTTAGGAGCGAGCACC-3
EF-Tu FWD	5-CTACTAGCTAGCGAAAAGTTTGAACGTACAAAACCGCACG-3
EF-Tu REV	5-TATCAAATGCGGCCGCGCTCAGAACTTTTGCTACAACGCC-3
EF-Ts FWD	5- CTACTAGCTAGCGCTGAAATTACCGCATCCCTGG-3
EF-Ts REV	5-CATAGCCTCGAGAGACTGCTTGGACATCGCAGC-3
EF-G FWD	5-CATATGGCTCGTACAACACCCATCGCAC-3
EF-G REV	5-CTCGAGTTTACCACGGGCTTCAATTACGGC-3
RF1 FWD	5-ATGTCCATGGCTAAGCCTTCTATCGTTGCCAAACTG-3
RF1 REV	5-TAATGCGGCCGCTTCCTGCTC GGACAACGCCGC-3
RF3 FWD	5-ATGTCCATGGCTCACCACCACCACCACCACACGTTGTCTCCTTAT TTGCAAGAGGTG-3
RF3 REV	5-TAATGCGGCCGCTTAATGCTCGCGGGTCTGGTGG-3
RRF FWD	5-ATGTCCATGGCTAGCGATATCAGAAAAGATGC-3
RRF REV	5-TATCAAATGCGGCCGCTCATCATCAGTGGTGGTGGTGGTG GTGGAACTGCATCAGTTCTGCTTC-3
MTF FWD	5-CTACTAGCTAGCGAATCACTACGTATTATTTTTGCGGG-3
MTF REV	5-TATCAAATGCGGCCGCGACCAGACGGTTGCCCGG-3
MetRS FWD	5-GCTAGCACTCAAGTCGCGAAGAAAATTCTGGTG-3
MetRS REV	5-CTCGAGTTTCACCTGATGACCCGGTTTAGC-3
GluRS FWD	5-GGGAATTCATATGAAAATCAAAACTCGCTTCGC-3
GluRS REV	5-CCGCTCGAGCTGCTGATTTTCAGCAT-3
AspRS FWD	5-GGGAATTCCATATGCGTACAGAATATTGTGGACAGT-3
AspRS REV	5-CCGCTCGAGGTTATTCTCCAGCCTTCTTCACAACC-3
ThrRS FWD	5-CTACTAGCTAGCATGCCTGTTATAACTCTTCCTGATG-3
ThrRS REV	5-CGCGGATCCTTATTCCTCCAATTGTTTAAGACTGCG-3
PheRS FWD	5-GGGAATTCCATATGTCACATCTCGCAGAACTGGT-3
PheRS REV	5-CCGCTCGAGTCAATCCCTCAATGATGCCTG-3
Mutagenic Primers	(bold-underline is site of mutation)
mutPheRS FWD	5-TACTCTGGTTTCG**G**CTTCGGGATGGGGATG-3
mutPheRS REV	5-CATCCCCATCCCGAAG**C**CGAAACCAGAGTA-3
mutLeuRS RWD	5-CTGGCTTTAAAGCGGTTCACC-3
mutLeuRS REV	5-GGATAACCGGTTTGATGTTCAGGCCGTATTTAGAGGCAAACTCGT G**G**CGCGCTGGTCGTGCCCCG-3

Table S1. PCR primers used to make expression vectors and PheRS and LeuRS mutants.

Factor	Location of His_6 tag	Vector	Restriction Sites
IF1	N	pET28a	NdeI / BamHI
IF2	N	pET28a	NdeI / BamHI
IF3	N	pET28a	NdeI / BamHI
EF-Tu	C	pET24a	NheI / NotI
EF-Ts	C	pET24a	NheI / XhoI
EF-G	C	pET24a	NdeI / XhoI
RF1	C	pET24d	NcoI / NotI
RF3	N (primers)	pET24d	NcoI / NotI
RRF	C (primers)	pET24d	NcoI / NotI
MTF	C	pET24a	NheI / NotI
MetRS	C	pET24a	NheI / XhoI
GluRS	C	pET24a	NdeI / XhoI
AspRS	C	pET24a	NdeI / XhoI
ThrRS	N	pET28a	NheI / BamHI
PheRS	N	pET28a	NdeI / XhoI
SerRS	C	pET24a	NheI / NotI
TyrRS	C	pET20b	NdeI / XhoI

Table S2. Expression vectors for the translation factors



NdeI **PsiI** **BamHI**

(1) AUG CUG GAA CCG CAG GAU UAU AAA GAC GAC GAU GAC AAA UAG UAG GGA
M L E P Q D Y K D D D D K stop stop
FLAG affinity tag

(2) AUG CAU UUU AGC UGG GAU UAU AAA GAC GAC GAU GAC AAA UAG UAG GGA
M H F S W D Y K D D D D K stop stop

(3) AUG AUC ACC AAC CCG GAU UAU AAA GAC GAC GAU GAC AAA UAG UAG GGA
M I T N P D Y K D D D D K stop stop

(4) AUG ACC AUU AAC CGU GAU UAU AAA GAC GAC GAU GAC AAA UAG UAG GGA
M T I N R D Y K D D D D K stop stop

(5) AUG GAU UAU AAA AUG CAU CAC CAU CAC CAU CAC UAG UAG GAU UAU AAA
M D Y K M H H H H H H stop stop
GAC GAC GAU GAC AAA UAG UAG GGA

(6) AUG CAC CAC CAU CAC CAU CAC AAA UUC UAC AUC GAC CCG AUG UGG CGU
M H H H H H H K F Y I D P M W R
CUG ACC CAU AAA CAC CGG CUA TAG UUA UAA AGA CGA CGA UGA CAA AUA
L T H K H R L stop
GUA GGG A

Figure S1. mRNAs in vitro transcribed from pET12b. The sequences of the 5' and 3' untranslated regions (UTR) of mRNAs transcribed from the pET12b T7 promoter are shown with the ribosome binding site (rbs) underlined. The first mRNA (1) was constructed by ligating complementary oligonucleotides that correspond to the entire open reading frame with the appropriate overhangs into pET12b opened with NdeI and BamHI. The codons for the FLAG tag were chosen to provide a unique PsiI site for the in-frame ligation of complementary oligonucleotides for mRNAs (2)-(4) with the with

the C-terminal FLAG tag. Longer complementary oligonucleotides with stop codons prior to the C-terminal FLAG tag were inserted into the NdeI and PsiI sites for mRNAs (5) and (6).