Supplementary information to:

Ultradeep characterisation of translational sequence determinants refutes rare-codon hypothesis and unveils quadruplet base pairing of initiator tRNA and transcript

Simon Höllerer[1] and Markus Jeschek[1,2]*

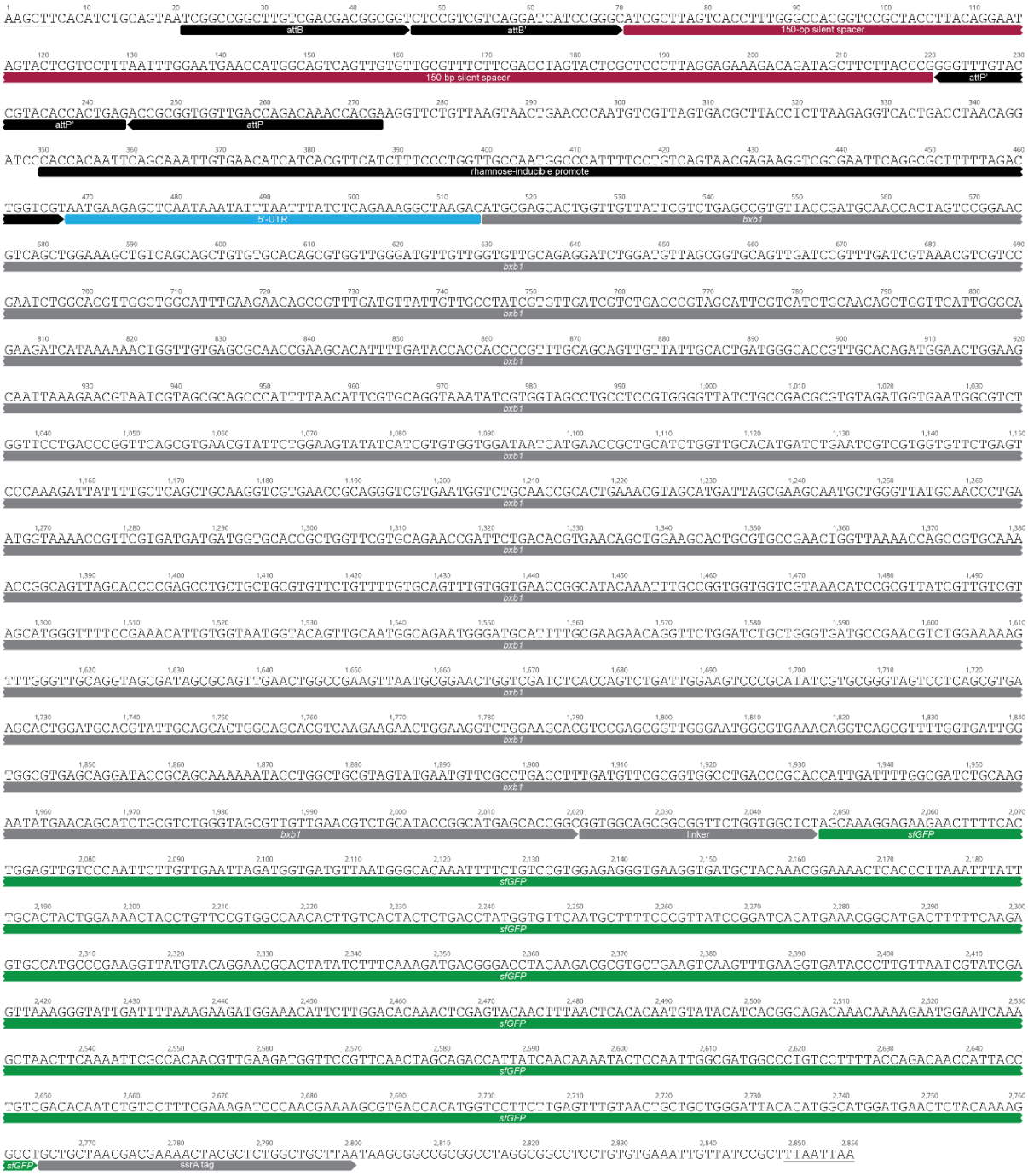
[1]Department of Biosystems Science and Engineering, Swiss Federal Institute of Technology – ETH Zurich, Basel, CH-4058, Switzerland

[2]Institute of Microbiology, Synthetic Microbiology Group, University of Regensburg, Regensburg, D-93053, Germany

*To whom correspondence should be addressed: E-mail: markus.jeschek@ur.de

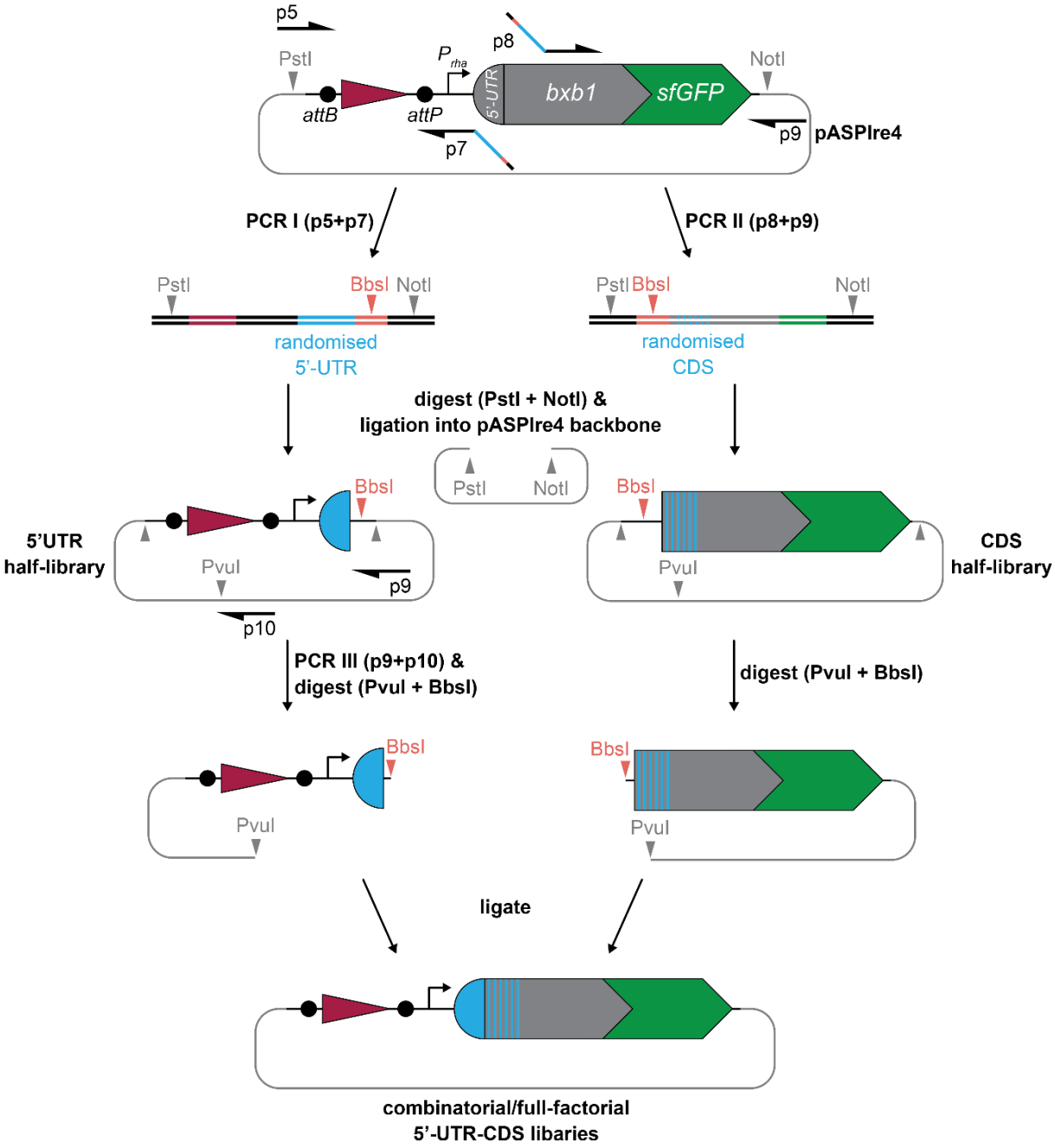
Supplementary figures

All DNA sequences follow the IUPAC nucleotide code; N: A/C/G/T; H: A/C/T; Y: C/T.



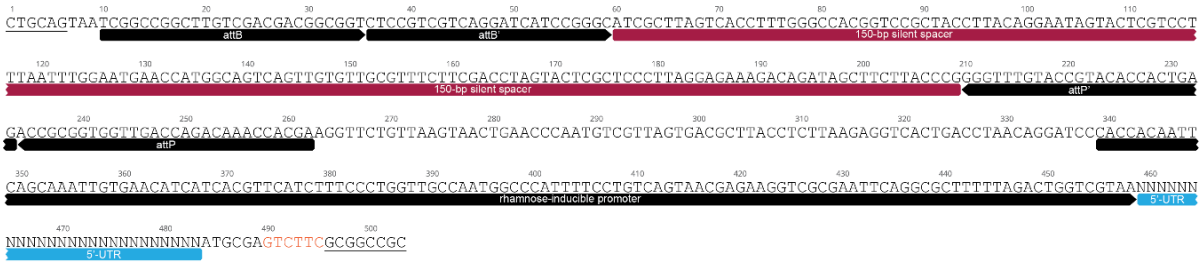
Supplementary Figure 1: Graphical representation of pASPIre4. The displayed sequence corresponds to the insert between HindIII and PacI restriction sites (underlined) in pSEVA291. ssrA: proteolytic degradation tag. The full sequence is available in text format in **Supplementary Note 2**.

Supplementary Figure 2: Graphical representation of pASPIre4$_{lib}$. The displayed sequence corresponds to the insert between HindIII and PacI restriction sites (underlined) in pASPIre4. ssrA: proteolytic degradation tag. The full sequence is available in text format in **Supplementary Note 3.**

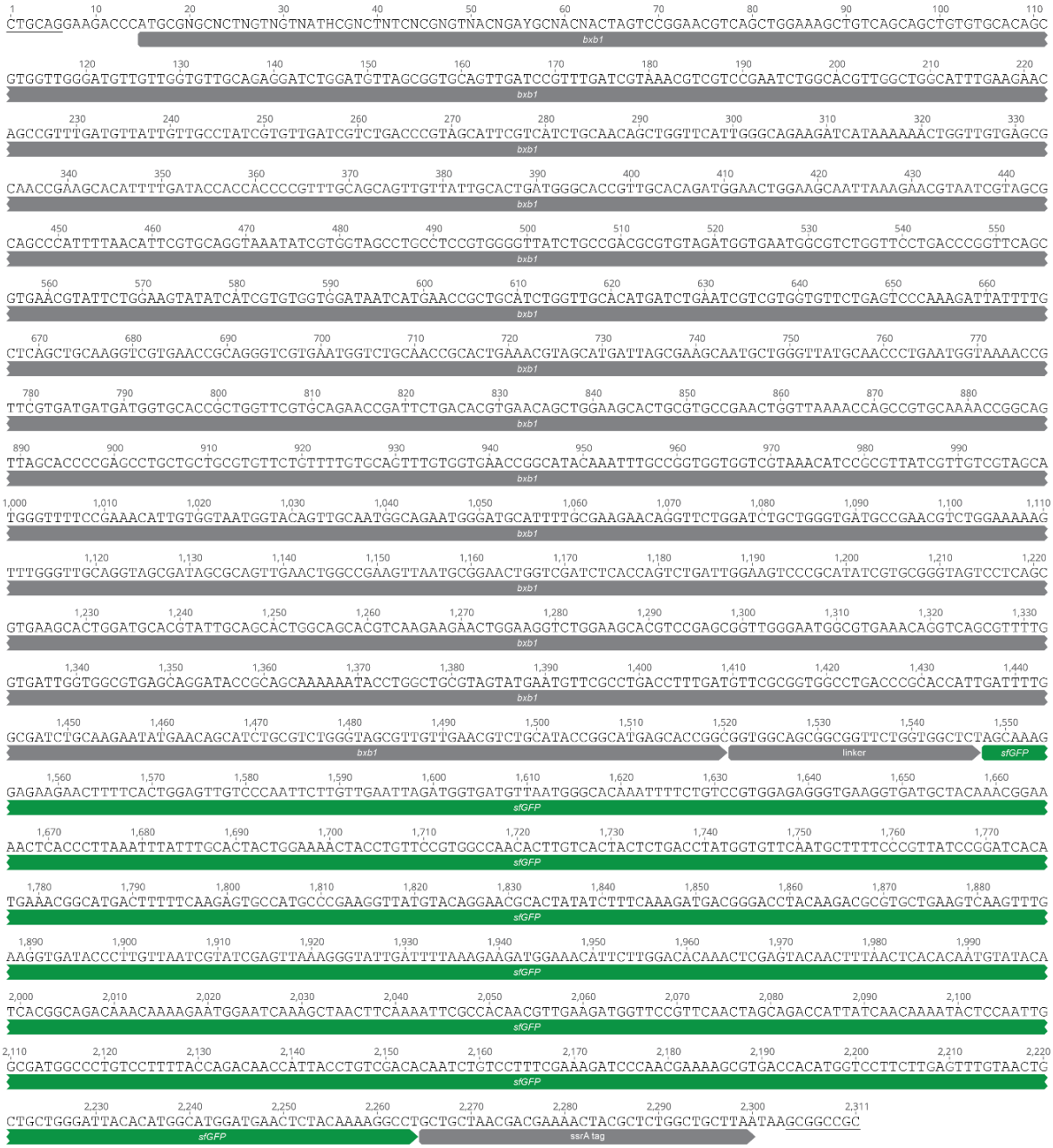


Supplementary Figure 3: Cloning scheme for the generation of combinatorial and full-factorial 5'-UTR-CDS libraries. Libraries were generated from 5'-UTR and CDS half-libraries as described in the **Methods** section. The 5'-UTR (left) half-library was generated by PCR with primers p5 and p7 with pASPIre4 as template. Primer p7 introduces degeneracy in the 5'-UTR and a BbsI site between the randomised 5'-UTR and the NotI site. The CDS half-library (right) was generated by PCR with primers p8 and p9 on pASPIre4 as template. Primer p8 introduces degeneracy in the CDS and a BbsI site between the CDS and the PstI site. The resulting PCR products were then sub-cloned into pASPIre4 to retrieve half libraries. In a second step, 5'-UTR and CDS half-libraries were combined to generate full libraries Lib_{comb1}, Lib_{comb2} and Lib_{fact}. To achieve this, plasmid DNA of the 5'-UTR half-library was PCR-amplified with primers p9 and p10, and the product was digested with BbsI and PvuI and ligated into the backbone prepared from the CDS half-library via digestion with PvuI and BbsI. Note that the

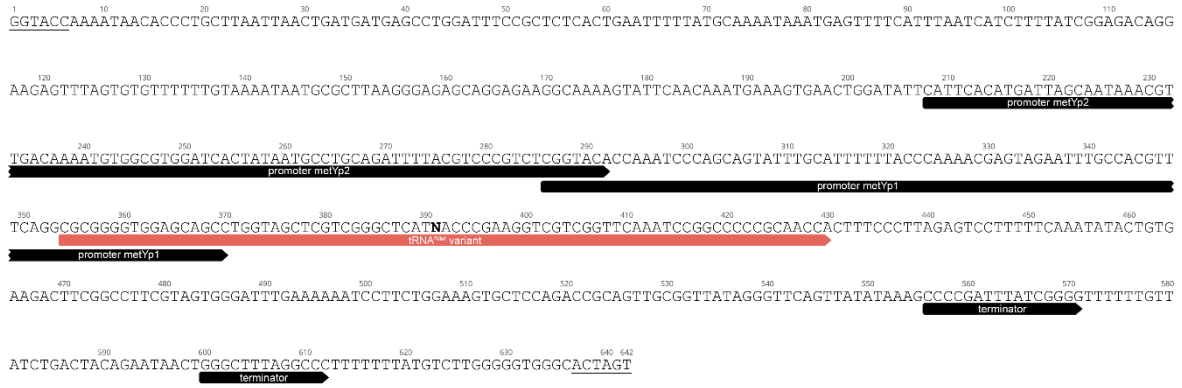
BbsI type IIS restriction site enables scarless joining of 5'-UTR and CDS half-libraries using ATGC (start codon ATG + first downstream base) as sticky ends for ligation.



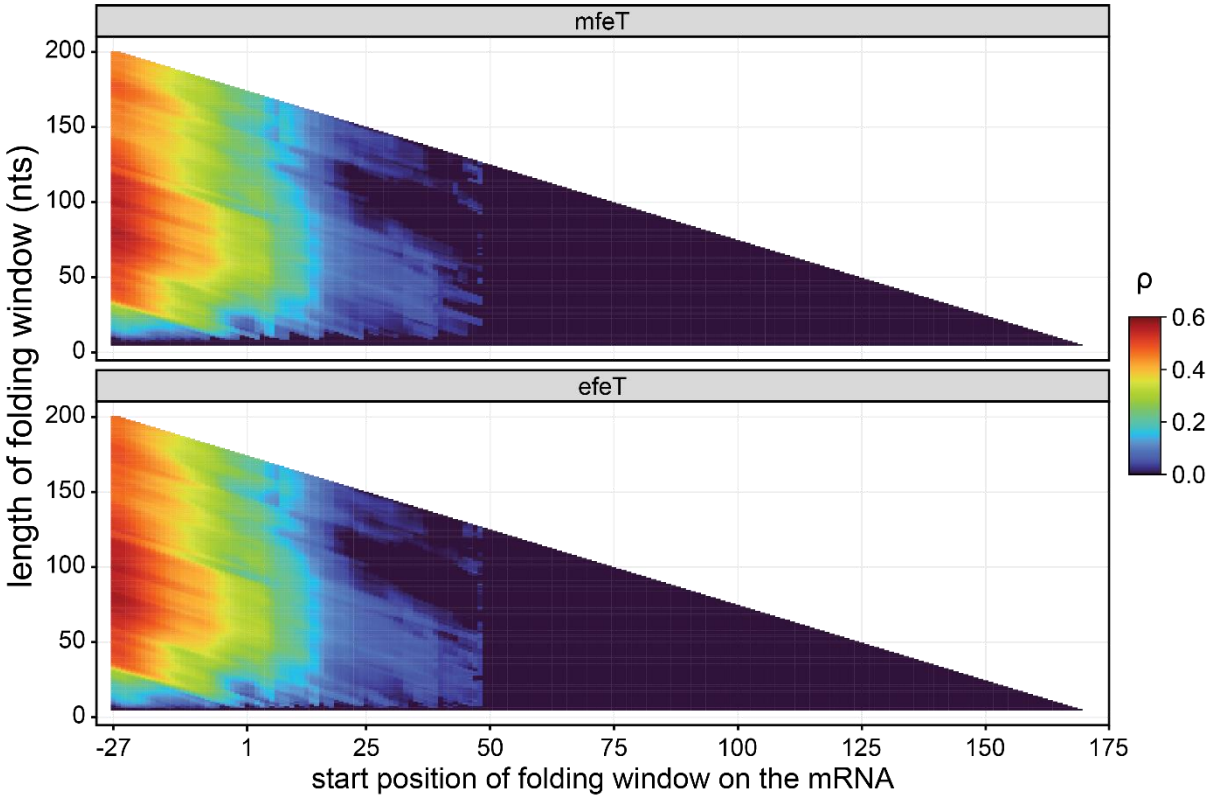
Supplementary Figure 4: Graphical representation of 5'-UTR half-libraries. The displayed sequence corresponds to the insert between PstI and NotI restriction sites (underlined) in pASPIre4. The introduced BbsI restriction site (orange) is used for scarless joining of 5'-UTR and CDS half-libraries. The full sequence is available in text format in **Supplementary Note 4**.



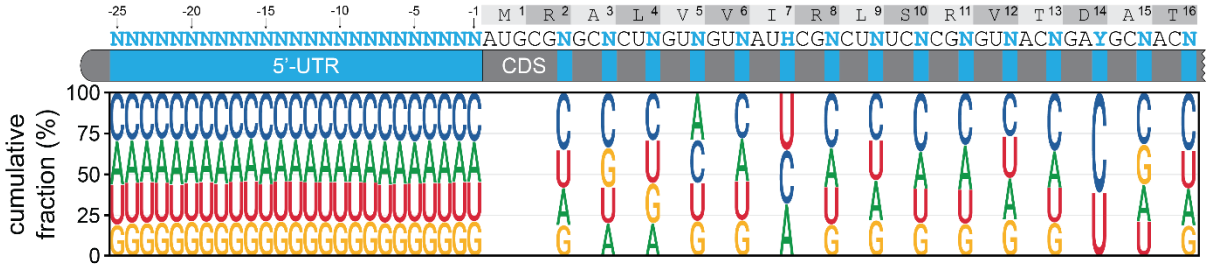
Supplementary Figure 5: Graphical representation of CDS half-libraries. The displayed sequence corresponds to the insert between PstI and NotI restriction sites (underlined) in pASPIre4. The introduced BbsI restriction site (orange) is used for scarless joining of 5'-UTR and CDS half-libraries. The full sequence in text format is available in **Supplementary Note 5.**



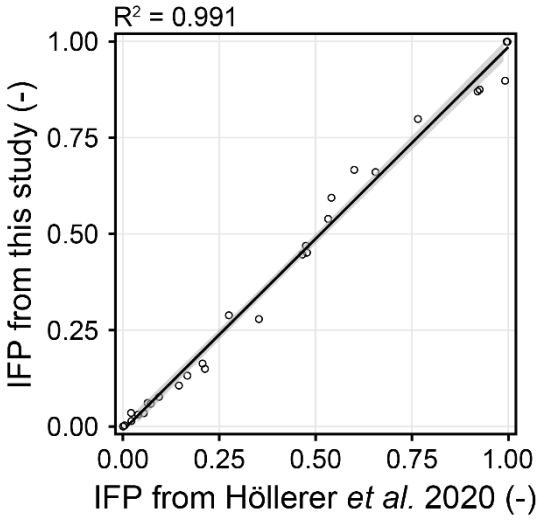
Supplementary Figure 6: Graphical representation of plasmids for the overexpression of tRNA^{fMet} variants. The displayed sequence corresponds to the native chromosomal locus of *metY* including regulatory sequences. The bold N nucleotide indicates the mutated position 37 (p37) in tRNA^{fMet}. The window between KpnI and SpeI restriction sites (underlined) in pSEVA361 is shown. The full sequence is available in text format in **Supplementary Note 6**.



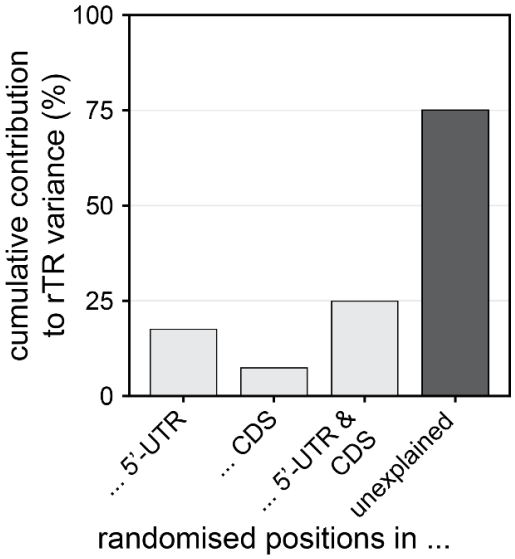
Supplementary Figure 7: Correlation between predicted folding energy and rTR for different mRNA windows. Folding energies (mfeT and efeT) of all possible mRNA sequence windows with lengths between 5 and 200 bases were predicted using *RNAfold* of the *Vienna RNA* package and the resulting values were correlated with rTR using Spearman's correlation (**Methods**).



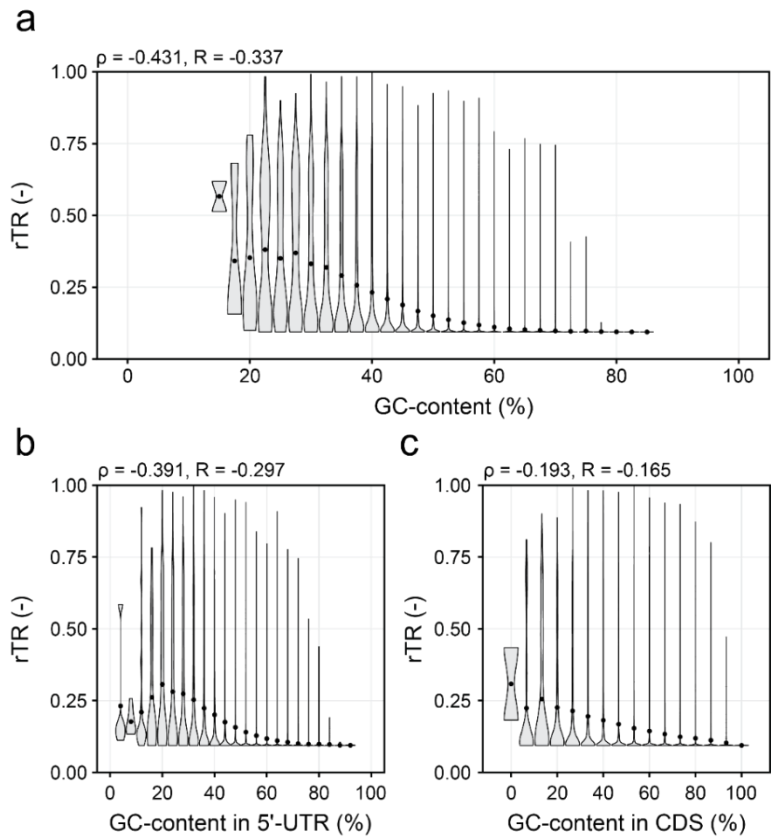
Supplementary Figure 8: Base distribution in Lib$_{random}$. The base distribution amongst all 198,174 variants of Lib$_{random}$ above high quality read count threshold is displayed for each randomised position. Bases are ordered from the most (top) to the least (bottom) frequent for each position. Note that in codons 7 (Ile) and 14 (Asp) only three (A, C, U) and two (C, U) bases were allowed, respectively, to avoid non-synonymous mutations.



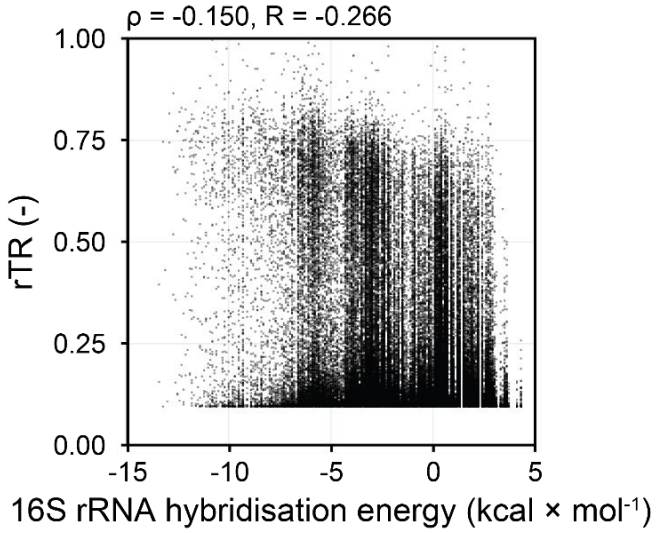
Supplementary Figure 9: Cross-study reproducibility of uASPIre. The IFP of 31 standard RBSs as measured in this study is plotted over the IFP of the same standard RBSs as determined in a previous study (1). The black line corresponds to a linear regression with 95% confidence interval (grey area).



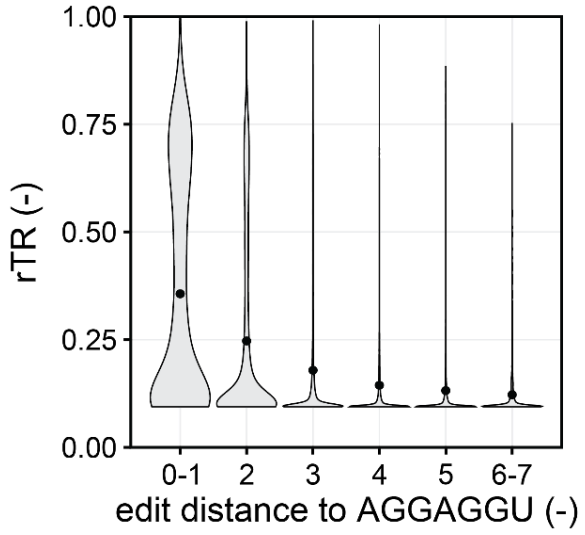
Supplementary Figure 10: Cumulative impact of randomised positions in 5'-UTR and CDS on the rTR. Bars are the cumulative contribution of all randomised positions in the mRNA part(s) indicated on the horizontal axis except for "unexplained" which corresponds to the residuals. The contribution of individual positions (**Fig. 2a**) was determined by ANOVA (**Methods**).



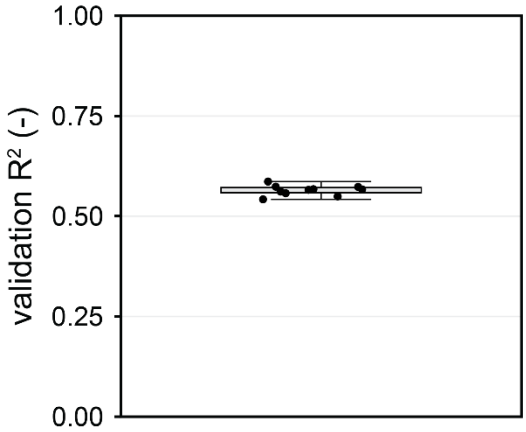
Supplementary Figure 11: Impact of GC-content on rTR. The correlation of rTR and GC-content in all randomised positions (**a**) as well as in the randomised positions in 5'-UTR (**b**) and CDS (**c**) individually was assessed. Variants were binned according to their GC-content in the respective mRNA part, and the rTR distribution of bins is displayed as violin with each bin's mean rTR (black circles).



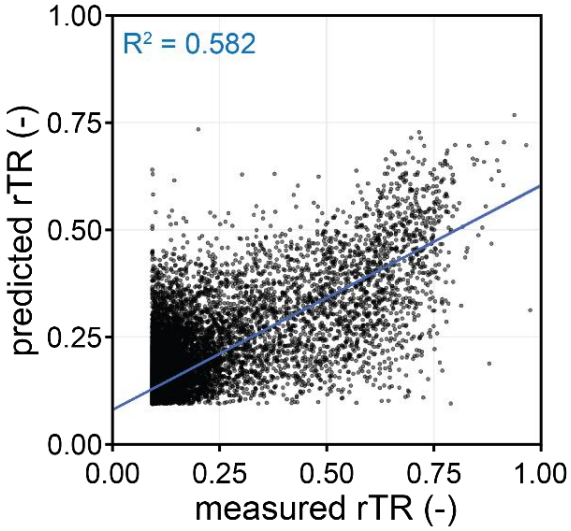
Supplementary Figure 12: Correlation between predicted 16S rRNA hybridisation energy and rTR. The hybridisation energy of the 3'-end of *E. coli*'s 16S rRNA (sequence: 5'-ACCUCCUUA-3') and the 5'-UTR between position -18 and -4 was predicted using *RNAduplex* of the *Vienna RNA* package and the resulting energy values were correlated with rTR.



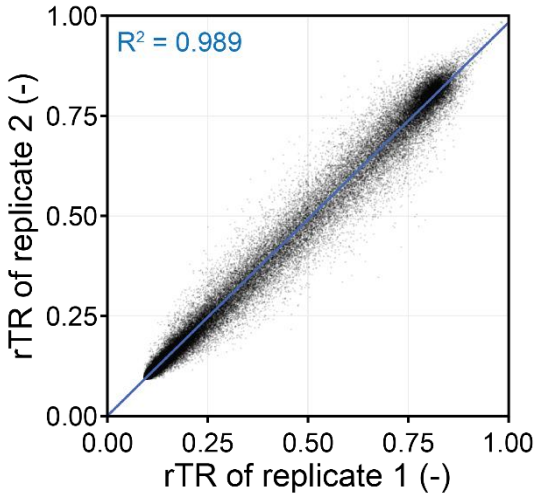
Supplementary Figure 13: Impact of similarity to the SD motif on rTR. The minimum edit distance between the canonical SD motif AGGAGGU and all 7-nt windows between 5'-UTR positions -18 and -4 was calculated for each variant (**Methods**). Violins are rTR distributions amongst variants grouped by minimum edit distance with group means (black circles).



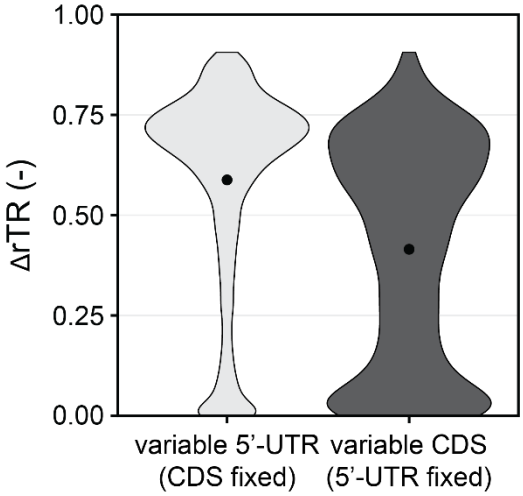
Supplementary Figure 14: Cross-validation of random forest model (Fig. 3e). Validation R^2 values are shown for 10 independent validation runs as circles. Box represents interquartile range with whiskers marking the 1.5-fold interquartile range.



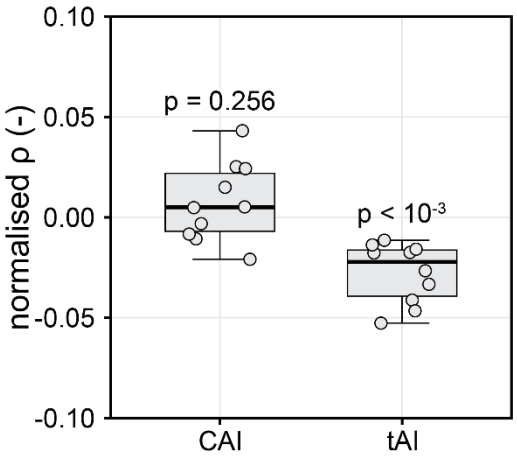
Supplementary Figure 15: Random forest performance. The rTR values predicted by the random forest model trained on Lib_{random} (compare **Fig. 3e**) are plotted over the experimentally measured rTRs for 19,816 randomly selected test set variants strictly held out during training. The blue line corresponds to a linear regression with corresponding Pearson's R^2.



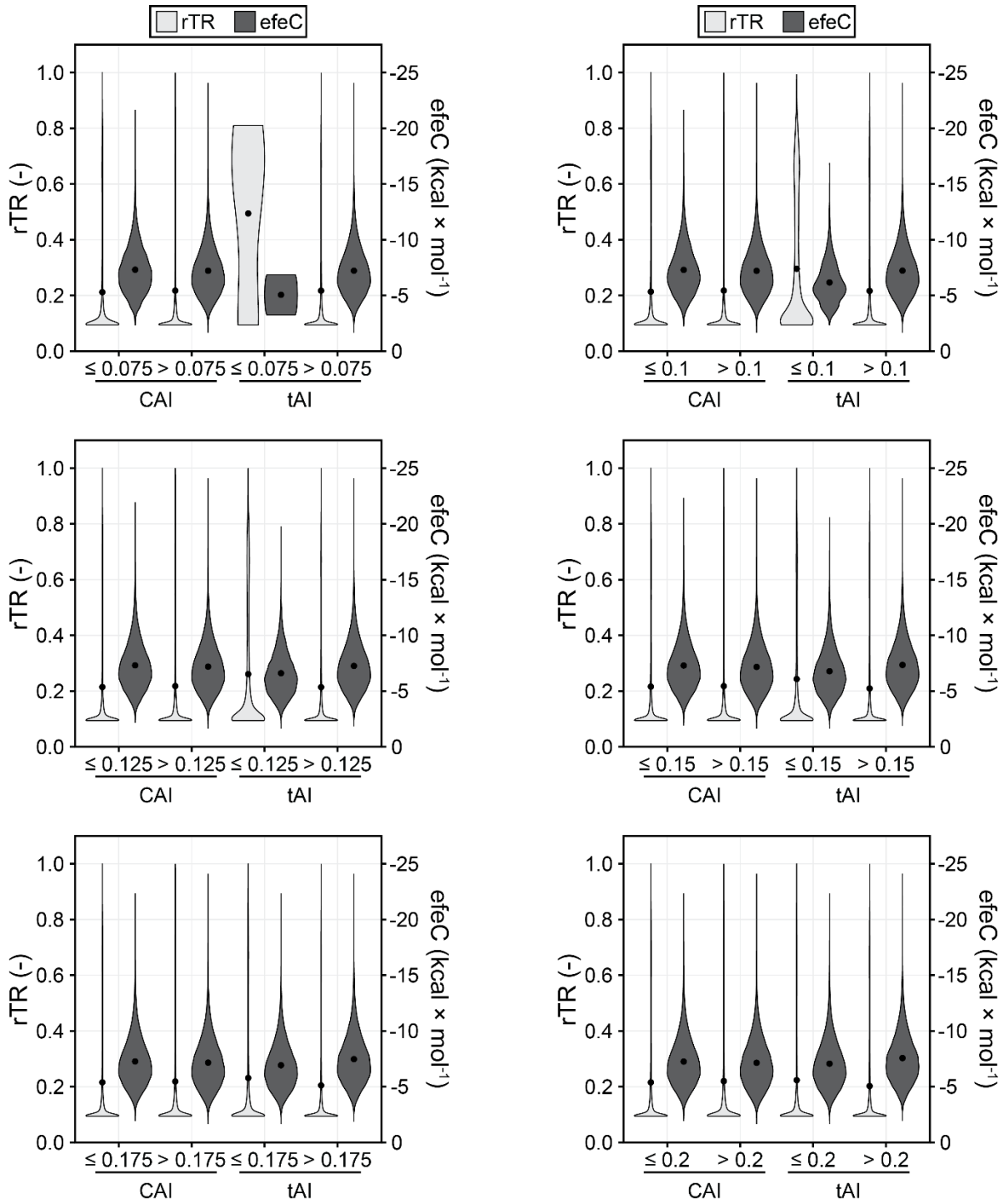
Supplementary Figure 16: Biological reproducibility within Lib_{fact}. rTR values of two independently cultured, biological replicates of Lib_{fact} are shown (112,296 variants). The blue line corresponds to a linear regression with corresponding Pearson's R^2.



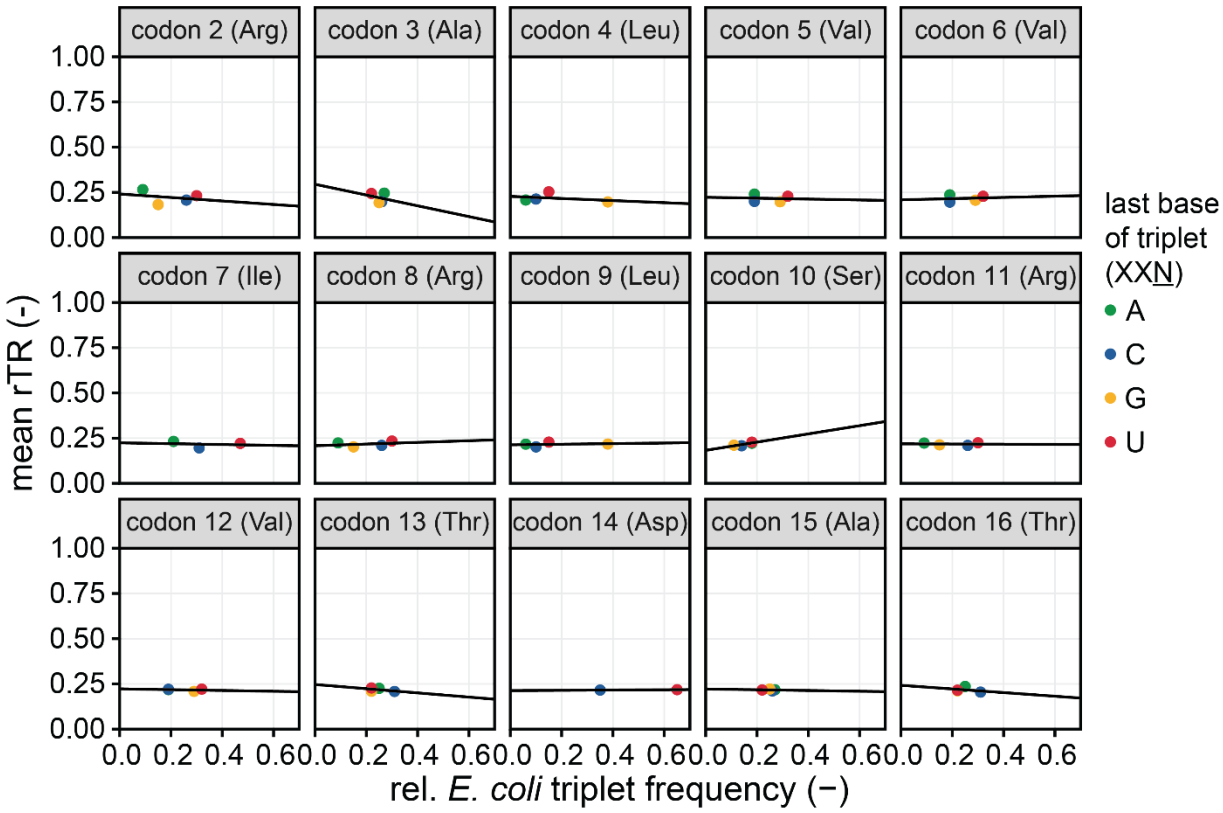
Supplementary Figure 17: Coverage of rTR range upon exchange of 5'-UTR (left) and CDS (right). For each CDS (or 5'-UTR) that appeared at least twice in all libraries, the absolute difference in rTR between the strongest and weakest 5'-UTR (or CDS) combined with this CDS (or 5'-UTR) is displayed (ΔrTR). Violins indicate the distribution of ΔrTR with black circles representing the mean value.



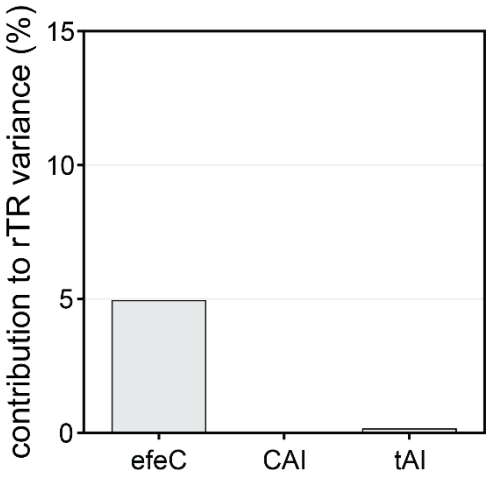
Supplementary Figure 18: Correlation between codon indices and the CDS-derived contribution to rTR variance. Pearson's correlation between CAI/tAI and the mean rTR of each CDS (over all 5'-UTRs combined with that CDS) was calculated for each of the ten batches of Lib_{fact} (grey circles) and normalised to the mean effect of the CDS on rTR (Fig. 4c). Boxes represents interquartile range with whiskers marking the 1.5-fold interquartile range and medians as solid black lines. P-values of one sample t-tests are indicated above each box.



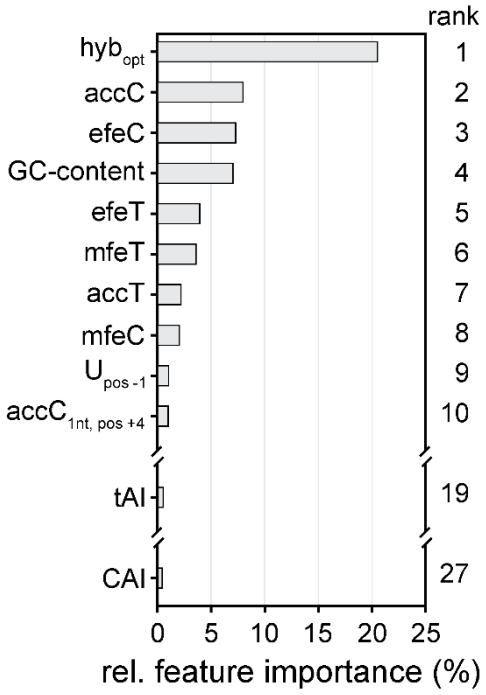
Supplementary Figure 19: Comparison of rTRs and predicted folding energies (efeC) of variants with low and high CAI/tAI. Different thresholds were tested for CAI and tAI. Black circles within violins are mean rTR/efeC values (compare **Fig. 4e**).



Supplementary Figure 20: Dependence between triplet frequency in *E. coli* and rTR. Mean rTRs of variants from all libraries with the same triplet in the randomised codons are plotted over the relative codon frequency of the respective triplet in *E. coli*. Triplet frequencies can be found in **Supplementary Table 6**. Black lines are best linear fits of data points. Note that no consistent up- or downward trend is observed across all codons as well as amongst triplets coding for the same amino acid but in different positions.



Supplementary Figure 21: Contribution of efeC, CAI and tAI to the rTR variance on weakly folding mRNAs. An ANOVA with efeC, CAI and tAI as only covariates was performed to assess their impact on the rTR amongst the 727,570 variants with low tendency for mRNA folding (i.e. efeC ≥ -7.5 kcal mol^{-1}, compare **Fig. 4f**).



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148 **Supplementary Figure 22: Feature importance of random forest model trained with codon usage**
149 **and folding metrics.** Relative importance of the ten most important features as well as tAI and CAI for
150 the model displayed in **Figure 5g** (second bar from left) is shown. Ranking of features is indicated to
151 the right. $AccC_{1nt,\ pos\ +4}$: AccC score for position +4 of the CDS.

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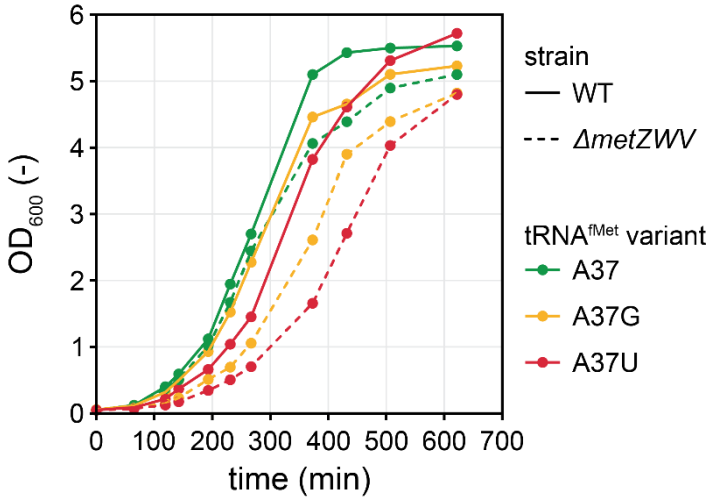

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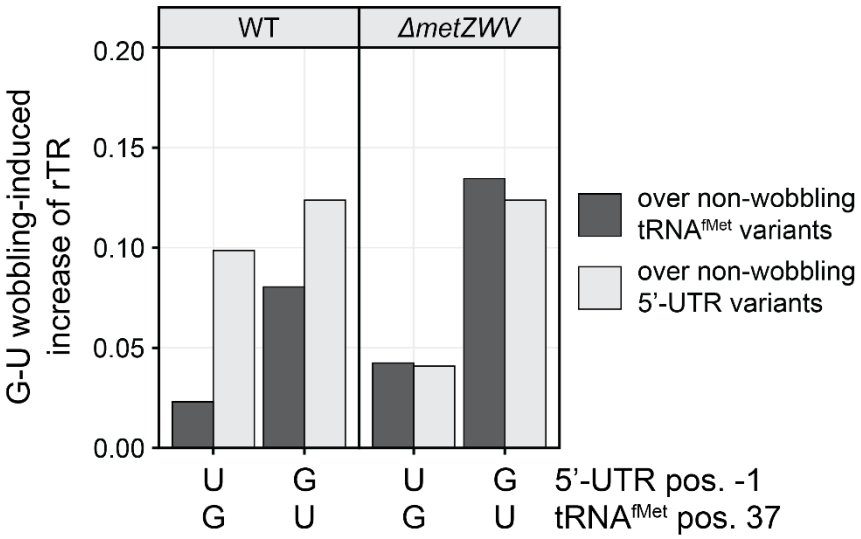
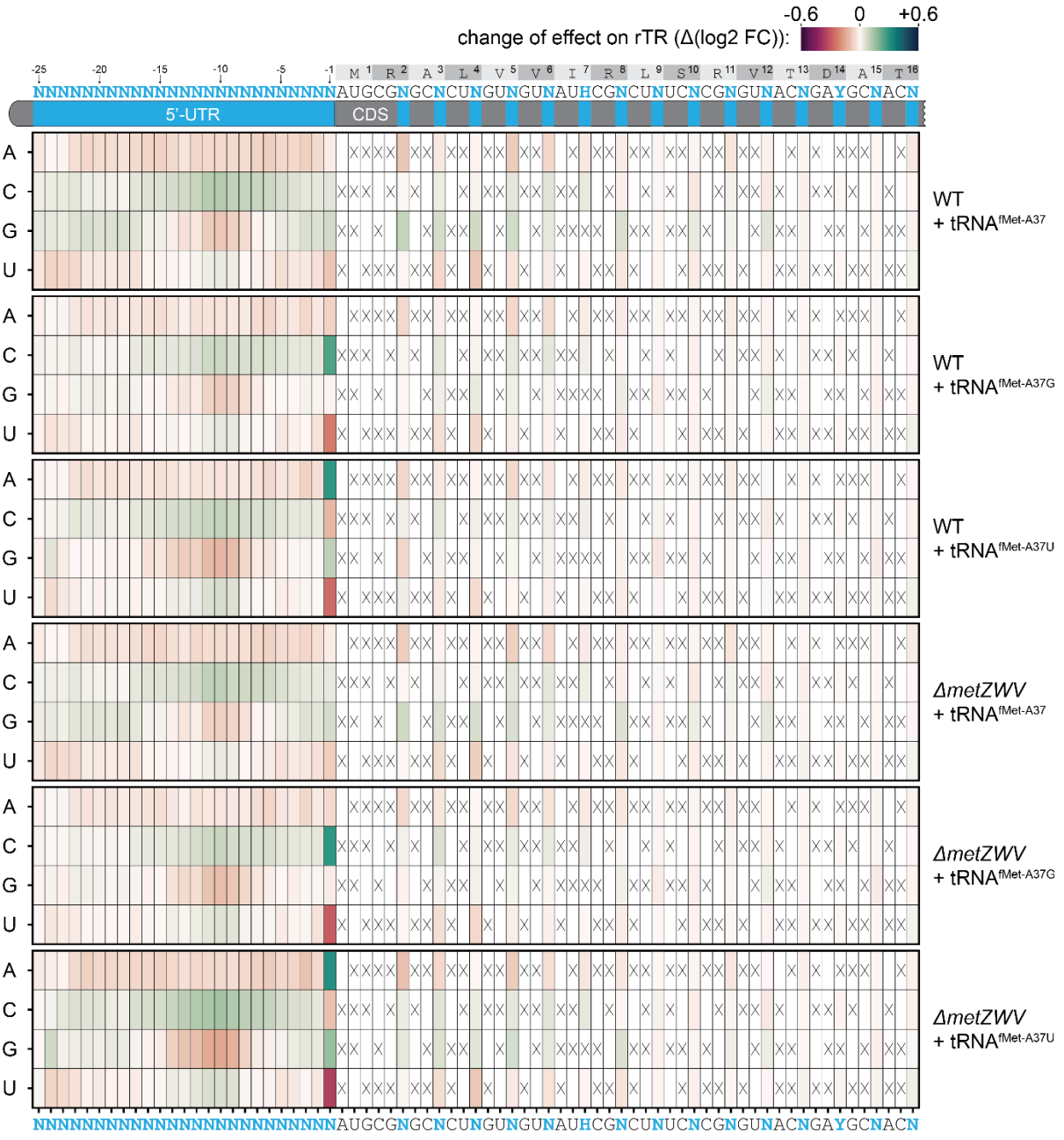
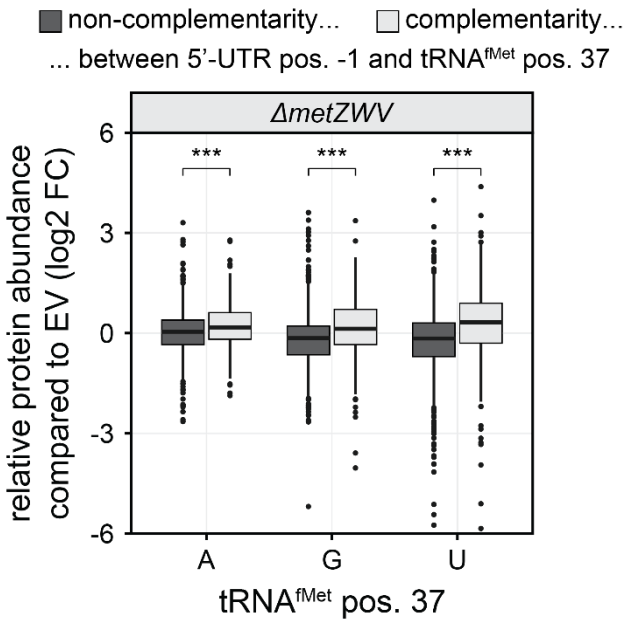
155 **Supplementary Figure 23: Growth of *E. coli* WT and *ΔmetZWV* expressing different $tRNA^{fMet}$**
156 **variants.** Approximately 50,000 variants of Lib_{random} were co-cultivated in shake flasks (**Methods**) for
157 each of the six combinations of *E. coli* strains (WT or *ΔmetZWV*) and overexpressed $tRNA^{fMet}$ variants
158 (A37, A37G or A37U).



Supplementary Figure 24: Effect of G-U wobbling on rTR. The mean increase of the rTR is shown for different combinations of G-U wobbling base pairs between 5'-UTR position -1 and $tRNA^{fMet}$ position 37 as indicated on the horizontal axis. The rTR increases of the respective wobbling pair over either all non-wobbling and non-complementary $tRNA^{fMet}$ variants (dark grey) or over all non-wobbling and non-complementary 5'-UTR variants (light grey) are displayed. In order to facilitate comparison between the different experimental groups (i.e. six combinations of strain and $tRNA^{fMet}$ variant), rTR values were normalised to the mean rTR of each group prior to determination of the G-U wobbling-induced increase of rTR.



Supplementary Figure 25: Base- and position-specific change of the rTR upon overexpression of tRNA^{fMet} variants. The change of the effect on rTR compared to the case without tRNA overexpression in the WT strain (**Fig. 2b**) is shown for different combinations of strains (WT vs. Δ*metZWV*) and overexpressed tRNA^{fMet} variants (A37, A37G or A37U). This change of effect was calculated as follows: First, log2-transformed fold changes (log2 FC) of the mean rTR of all variants with a given base at the respective position over the mean rTR of all variants with any other base permitted at that position were determined for variants of Lib$_{random}$. Afterwards, the log2 FCs obtained for the WT strain without tRNA^{fMet} overexpression (**Fig. 2b**) were subtracted (i.e. Δ(log2 FC)). Crossed boxes indicate non-permitted bases.



Supplementary Figure 26: Impact of overexpression of tRNA^{fMet} variants on endogenous protein expression. Label-free proteomics were performed on strain *ΔmetZWV* cells overexpressing tRNA^{fMet} variants with an A, G or U nucleotide at tRNA^{fMet} position 37 (see **Methods**). Relative protein abundances compared to an empty vector control (EV) are shown as log2 fold-changes (log2 FC) for the 1,098 endogenous proteins detectable in all strains and replicates. Proteins are grouped according to their complementarity or non-complementarity between 5'-UTR position -1 and tRNA^{fMet} position 37. *** denote p-values < 10^{-4} in a one-sided Welch two sample t-test.

Supplementary tables

Supplementary Table 1: Primers used in this study. Restriction sites (BbsI/NotI/PstI/SpeI) are underlined. The introduced BbsI site is marked in orange. Primer binding regions are highlighted in bold.

Name	Sequence (5'-3')	Description
p1	TTTGTTCAAAATCATGCCAAATCCGTGATCGGGGTAAAAAA**TGATCGGCAC GTAAGAGGTTCC**	Forward primer for the generation of the knockout cassette *ΔmetZWV::specR*
p2	GAGAAGGGGATGATAAAAAGGCGCTGAATGGCGCTTTTTTA**TTATTGGCTG GCACCAAGCAG**	Forward primer for the generation of the knockout cassette *ΔmetZWV::specR*
p3	**GCGGCAAGCATTGCCACAACCGTGC**	Forward genotyping primer for *ΔmetZWV*
p4	**CGTATTTTGCCGATGGGGCGACGCTGG**	Reverse genotyping primer for *ΔmetZWV*
p5	**TCTATCAACAGGAGTCCAAG**	Forward primer for the generation of all libraries
p6	TATATAACTAGTNGTNGCRTCNGTNACNCGNGANAGNCGDATNACNACNAG NGCNCGCATNNNNNNNNNNNNNNNNNNNNNNNNNN**TTACGACCAGTCTAAAA AGCGCC**	Reverse primer for the generation of Lib_{random}
p7	ATATATGCGGCCGCGAAGACTCGCATNNNNNNNNNNNNNNNNNNNNNNNNN **TTACGACCAGTCTAAAAAGCGCC**	Reverse primer for the generation of 5'-UTR half-library
p8	ATATATCTGCAGGAAGACCCATGCGNGCNCTNGTNGTNATHCGNCTNTCNC GNGTNACNGAYGCNACN**ACTAGTCCGGAACGTCAGCTGG**	Forward primer for the generation of CDS half-library
p9	**GCCTTTCGTTTTATTTGATGCC**	Reverse primer for the generation of CDS half-library and reverse primer for amplification of 5'-UTR half-library insert
p10	**GGTTATCCAGGCTAAAATCG**	Forward primer for the amplification of 5'-UTR half-library insert

Supplementary Table 2: Strains used in this study.

Name	Genotype	Description	Source/Reference
E. coli TOP10 *ΔrhaA*	*F^- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Str^R) endA1 $λ^-$ ΔrhaA*	Rhamnose utilisation-deficient derivative of *E. coli* TOP10.	Hoellerer *et al.* (1)
E. coli TOP10 *ΔrhaA ΔmetZWV*	*F^- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Str^R) endA1 $λ^-$ ΔrhaA ΔmetZWV::specR*	Derivative of *E. coli* TOP10 *ΔrhaA* with deleted *metZWV* locus.	This study (**Methods**)

Supplementary Table 3: Plasmids used in this study.

Name	Description	Source
pSEVA291	contains a pBR322 replicon, a kanamycin resistance cassette, and a multiple cloning site	Silva-Rocha *et al.* (2)
pSEVA361	Backbone for ptRNA^{fMet} plasmids; contains a p15A replicon, a chloramphenicol resistance cassette, and a multiple cloning site	Silva-Rocha *et al.* (2)
pASPIre3	Derivative of pSEVA291; contains a *bxb1-sfGFP* gene (translational fusion) under control of P_{rha} and an *attB-/attP-* flanked 150 bp stretch of silent DNA	Hoellerer *et al.* (1)
pASPIre4	Derivative of pASPIre3 with SpeI site in the CDS of *bxb1*. PCR template and backbone for all 5'-UTR and CDS libraries	This study
pASPIre4$_{lib}$	Derivative of pASPIre4 with randomised 5'-UTR and CDS	This study
ptRNA$^{fMet\text{-}A37}$	Derivative of pSEVA361 with expression cassette for tRNA^{fMet} with the native A at position 37	This study
ptRNA $^{fMet\text{-}A37C}$	Derivative of ptRNA$^{fMet\text{-}A37}$ with A37C substitution	This study
ptRNA $^{fMet\text{-}A37G}$	Derivative of ptRNA$^{fMet\text{-}A37}$ with A37G substitution	This study
ptRNA $^{fMet\text{-}A37U}$	Derivative of ptRNA$^{fMet\text{-}A37}$ with A37T/U substitution	This study

Supplementary Table 4: DNA adapters for NGS. Adapters contain NcoI- and SpeI-compatible overhangs (orange) for ligation. Binding regions for Illumina flow cell (blue) and sequencing primers (green) are highlighted. Sample-specific indices are shown in red. P: 5'-phosphorylation.

Name	Sequence
NcoI$_1$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTATCACGC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAATAGTGCGGTAC-P 3′
NcoI$_2$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGATGTC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGATAGCTACAGGTAC-P 3′
NcoI$_3$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCTTGTAC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGACTAGAACATGGTAC-P 3′
NcoI$_4$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGCCAATC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGCTACGGTTAGGTAC-P 3′
NcoI$_5$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATACAGTGC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAAGCTATGTCACGGTAC-P 3′
NcoI$_6$	5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGATACTTGAC 5′ 3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGATAGCTATGAACTGGTAC-P 3′
SpeI$_1$	5′ P-CTAGAAATAACGTAAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TTTATTGCATTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′
SpeI$_2$	5′ P-CTAGATTCTTGAAATAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TAAGAACTTTATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′
SpeI$_3$	5′ P-CTAGAGGCAGATCATCAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TCCGTCTAGTAGTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′
SpeI$_4$	5′ P-CTAGACTATGTTAATCGAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TGATACAATTAGCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′
SpeI$_5$	5′ P-CTAGAGTTGACGCATCGAAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TCAACTGCGTAGCTTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′
SpeI$_6$	5′ P-CTAGAATCTACGAATCGATAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG 5′ 3′ TTAGATGCTTAGCTATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGAGCATACGGCAGAAGACGAAC 3′

Supplementary Table 5: NGS runs and sample-specific adapter combinations from this study.

NGS run	SRA number	Library	Sample number	Time point (min)	Forward adapter	Reverse adapter
1	SAMN27867644	Lib_{random}	1	0	$NcoI_1$	$SpeI_1$
			2	95	$NcoI_2$	$SpeI_2$
			3	225	$NcoI_3$	$SpeI_3$
			4	290	$NcoI_4$	$SpeI_4$
			5	360	$NcoI_5$	$SpeI_5$
			6	480	$NcoI_6$	$SpeI_6$
		Lib_{comb1}	1	0	$NcoI_1$	$SpeI_2$
			2	95	$NcoI_2$	$SpeI_3$
			3	225	$NcoI_3$	$SpeI_4$
			4	290	$NcoI_4$	$SpeI_5$
			5	360	$NcoI_5$	$SpeI_6$
			6	480	$NcoI_6$	$SpeI_1$
		Lib_{comb2}	1	0	$NcoI_1$	$SpeI_3$
			2	95	$NcoI_2$	$SpeI_4$
			3	225	$NcoI_3$	$SpeI_5$
			4	290	$NcoI_4$	$SpeI_6$
			5	360	$NcoI_5$	$SpeI_1$
			6	480	$NcoI_6$	$SpeI_2$
		Standard RBSs	1	0	$NcoI_1$	$SpeI_4$
			2	95	$NcoI_2$	$SpeI_5$
			3	225	$NcoI_3$	$SpeI_6$
			4	290	$NcoI_4$	$SpeI_1$
			5	360	$NcoI_5$	$SpeI_2$
			6	480	$NcoI_6$	$SpeI_3$
2	SAMN27867645	Lib_{fact} (replicate 1)	1	0	$NcoI_1$	$SpeI_1$
			2	95	$NcoI_2$	$SpeI_2$
			3	225	$NcoI_3$	$SpeI_3$
			4	290	$NcoI_4$	$SpeI_4$
			5	360	$NcoI_5$	$SpeI_5$
			6	480	$NcoI_6$	$SpeI_6$
		Lib_{fact} (replicate 2)	1	0	$NcoI_1$	$SpeI_6$
			2	95	$NcoI_2$	$SpeI_5$
			3	225	$NcoI_3$	$SpeI_4$
			4	290	$NcoI_4$	$SpeI_3$
			5	360	$NcoI_5$	$SpeI_2$
			6	480	$NcoI_6$	$SpeI_1$
3	SAMN27867646	Lib_{random} in WT strain with $ptRNA^{fMet\text{-}A37}$	1	0	$NcoI_1$	$SpeI_1$
			2	95	$NcoI_2$	$SpeI_2$
			3	225	$NcoI_3$	$SpeI_3$
			4	290	$NcoI_4$	$SpeI_4$
			5	360	$NcoI_5$	$SpeI_5$
			6	480	$NcoI_6$	$SpeI_6$
		Lib_{random} in WT strain with $ptRNA^{fMet\text{-}A37G}$	1	0	$NcoI_1$	$SpeI_2$
			2	95	$NcoI_2$	$SpeI_3$
			3	225	$NcoI_3$	$SpeI_4$
			4	290	$NcoI_4$	$SpeI_5$
			5	360	$NcoI_5$	$SpeI_6$
			6	480	$NcoI_6$	$SpeI_1$
		Lib_{random} in WT strain with $ptRNA^{fMet\text{-}A37U}$	1	0	$NcoI_1$	$SpeI_3$
			2	95	$NcoI_2$	$SpeI_4$
			3	225	$NcoI_3$	$SpeI_5$
			4	290	$NcoI_4$	$SpeI_6$
			5	360	$NcoI_5$	$SpeI_1$
			6	480	$NcoI_6$	$SpeI_2$
			1	0	$NcoI_1$	$SpeI_4$

3	SAMN27867646	Lib_{random} in *ΔmetZWV* strain with $ptRNA^{fMet-A37}$	2	95	$NcoI_2$	$SpeI_5$
			3	225	$NcoI_3$	$SpeI_6$
			4	290	$NcoI_4$	$SpeI_1$
			5	360	$NcoI_5$	$SpeI_2$
			6	480	$NcoI_6$	$SpeI_3$
		Lib_{random} in *ΔmetZWV* strain with $ptRNA^{fMet-A37G}$	1	0	$NcoI_1$	$SpeI_5$
			2	95	$NcoI_2$	$SpeI_6$
			3	225	$NcoI_3$	$SpeI_1$
			4	290	$NcoI_4$	$SpeI_2$
			5	360	$NcoI_5$	$SpeI_3$
			6	480	$NcoI_6$	$SpeI_4$
		Lib_{random} in *ΔmetZWV* strain with $ptRNA^{fMet-A37U}$	1	0	$NcoI_1$	$SpeI_6$
			2	95	$NcoI_2$	$SpeI_1$
			3	225	$NcoI_3$	$SpeI_2$
			4	290	$NcoI_4$	$SpeI_3$
			5	360	$NcoI_5$	$SpeI_4$
			6	480	$NcoI_6$	$SpeI_5$

Supplementary Table 6: Codon weight and frequencies for calculation of CAI and tAI. w(CAI)/w(tAI): weight for the calculation of CAI (Sharp *et al.* (3))/tAI (dos Reis *et al.* (4)). *E. coli* fraction: fraction of each triplet amongst all triplets coding for the same amino acid in the *E. coli* genome (according to: https://www.kazusa.or.jp/codon/).

Triplet	Amino acid	w(CAI)	w(tAI)	Rel. codon frequency
GCA	Ala	0.586	0.375	0.270
GCC	Ala	0.122	0.250	0.260
GCG	Ala	0.424	0.120	0.250
GCU	Ala	1.000	0.110	0.220
AGA	Arg	0.004	0.125	0.130
AGG	Arg	0.002	0.165	0.070
CGA	Arg	0.004	0.000	0.090
CGC	Arg	0.356	0.360	0.260
CGG	Arg	0.004	0.125	0.150
CGU	Arg	1.000	0.500	0.300
AAC	Asn	1.000	0.500	0.410
AAU	Asn	0.051	0.220	0.590
GAC	Asp	1.000	0.375	0.350
GAU	Asp	0.434	0.165	0.650
UGC	Cys	1.000	0.125	0.480
UGU	Cys	0.500	0.055	0.520
CAA	Gln	0.124	0.250	0.350
CAG	Gln	1.000	0.330	0.650
GAA	Glu	1.000	0.500	0.640
GAG	Glu	0.259	0.160	0.360
GGA	Gly	0.010	0.125	0.190
GGC	Gly	0.724	0.500	0.290
GGG	Gly	0.019	0.165	0.180
GGU	Gly	1.000	0.220	0.340
CAC	His	1.000	0.125	0.370
CAU	His	0.291	0.055	0.630
AUA	Ile	0.003	0.163	0.210
AUC	Ile	1.000	0.375	0.310
AUU	Ile	0.185	0.165	0.470
CUA	Leu	0.007	0.125	0.060
CUC	Leu	0.037	0.125	0.100

CUG	Leu	1.000	0.540	0.380
CUU	Leu	0.042	0.055	0.150
UUA	Leu	0.020	0.125	0.180
UUG	Leu	0.020	0.165	0.130
AAA	Lys	1.000	0.750	0.710
AAG	Lys	0.253	0.240	0.290
AUG	Met	1.000	1.000	1.000
UUC	Phe	1.000	0.250	0.360
UUU	Phe	0.296	0.110	0.640
CCA	Pro	0.135	0.125	0.230
CCC	Pro	0.012	0.125	0.160
CCG	Pro	1.000	0.165	0.370
CCU	Pro	0.070	0.055	0.240
AGC	Ser	0.410	0.125	0.200
AGU	Ser	0.085	0.055	0.180
UCA	Ser	0.077	0.125	0.180
UCC	Ser	0.744	0.250	0.140
UCG	Ser	0.017	0.165	0.110
UCU	Ser	1.000	0.110	0.180
ACA	Thr	0.076	0.125	0.250
ACC	Thr	1.000	0.250	0.310
ACG	Thr	0.099	0.290	0.220
ACU	Thr	0.965	0.110	0.220
UGG	Trp	1.000	0.165	1.000
UAC	Tyr	1.000	0.375	0.350
UAU	Tyr	0.239	0.165	0.650
GUA	Val	0.495	0.625	0.190
GUC	Val	0.066	0.250	0.190
GUG	Val	0.221	0.200	0.290
GUU	Val	1.000	0.110	0.320

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Supplementary Table 7: Optimisation of hybridisation window. Correlation between rTR and the mean hybridisation energy of different sequence windows with different lengths. The mean of the hybridisation energy at position -11 and position -10 (rank 1) was denoted hyb_{opt}. The data was ranked by the sum of R and ρ.

Rank	Window length (nt)	Window start (5'-UTR pos.)	Pearson's R	Spearman's ρ
1	2	-11	-0.298	-0.170
2	3	-11	-0.298	-0.167
3	4	-12	-0.299	-0.166
4	3	-12	-0.294	-0.165
5	5	-12	-0.295	-0.161
6	5	-13	-0.292	-0.160
7	6	-13	-0.293	-0.159
8	2	-10	-0.288	-0.162
9	4	-11	-0.289	-0.159
10	7	-13	-0.287	-0.152
11	2	-12	-0.280	-0.159
12	4	-13	-0.281	-0.156
13	6	-12	-0.285	-0.152
14	7	-14	-0.283	-0.151
15	8	-14	-0.281	-0.148
16	6	-14	-0.278	-0.150
17	3	-10	-0.275	-0.151
18	5	-11	-0.274	-0.147
19	8	-13	-0.274	-0.143
20	9	-14	-0.272	-0.140

Supplementary Notes

Supplementary Note 1: Sequence of spectinomycin resistance gene (*specR*) including constitutive promoter used for knockout of *metZWV*. The start and stop codon of *specR* are underlined.

TGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATC
GAGATTTTCAGGAGCTAAGGAAGCTACATATGAGTGAAAAAGTGCCCGCCGAGATTTCGGTGCAACTATCACAAG
CACTCAACGTCATCGGGCGCCACTTGGAGTCGACGTTGCTGGCCGTGCATTTGTACGGCTCCGCACTGGATGGCG
GATTGAAACCGTACAGTGATATTGATTTGCTGGTGACTGTAGCTGCACCGCTCAATGATGCCGTGCGGCAAGCCC
TGCTCGTCGATCTCTTGGAGGTTTCAGCTTCCCCTGGCCAAAACAAGGCACTCCGCGCCTTGGAAGTGACCATCG
TCGTGCACAGTGACATCGTACCTTGGCGTTATCCGGCCAGGCGGGAACTGCAGTTCGGAGAGTGGCAGCGCAAAG
ACATCCTTGCGGGCATCTTCGAGCCCGCCACAACCGATTCTGACTTGGCGATTCTGCTAACAAAGGCAAAGCAAC
ATAGCGTCGTCTTGGCAGGTTCAGCAGCGAAGGATCTCTTCAGCTCAGTCCCAGAAAGCGATCTATTCAAGGCAC
TGGCCGATACTCTGAAGCTATGGAACTCGCCGCCAGATTGGGCGGGCGATGAGCGGAATGTAGTGCTTACTTTGT
CTCGTATCTGGTACACCGCAGCAACCGGCAAGATCGCGCCAAAGGATGTTGCTGCCACTTGGGCAATGGCACGCT
TGCCAGCTCAACATCAGCCCATCCTGTTGAATGCCAAGCGGGCTTATCTTGGGCAAGAAGAAGATTATTTGCCCG
CTCGTGCGGATCAGGTGGCGGCGCTCATTAAATTCGTGAAGTATGAAGCAGTTAAACTGCTTGGTGCCAGCCAATAA

Supplementary Note 2: Sequence of pASPIre4. The displayed sequence corresponds to the insert between HindIII and PacI restriction sites (underlined) in pSEVA291. A graphical representation of pASPIre4 is shown in **Supplementary Figure 1**.

AAGCTTCACATCTGCAGTAATCGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGC
TTAGTCACCTTTGGGCCACGGTCCGCTACCTTACAGGAATAGTACTCGTCCTTTAATTTGGAATGAACCATGGCA
GTCAGTTGTGTTGCGTTTCTTCGACCTAGTACTCGCTCCCTTAGGAGAAAGACAGATAGCTTCTTACCCGGGGTT
TGTACCGTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCACGAAGGTTCTGTTAAGTAACTGAACCCAAT
GTCGTTAGTGACGCTTACCTCTTAAGAGGTCACTGACCTAACAGGATCCCACCACAATTCAGCAAATTGTGAACA
TCATCACGTTCATCTTTCCCTGGTTGCCAATGGCCCATTTTCCTGTCAGTAACGAGAAGGTCGCGAATTCAGGCG
CTTTTTAGACTGGTCGTAATGAAGAGCTCAATAAATATTTAATTTATCTCAGAAAGGCTAAGACATGCGAGCACT
GGTTGTTATTCGTCTGAGCCGTGTTACCGATGCAACCACTAGTCCGGAACGTCAGCTGGAAAGCTGTCAGCAGCT
GTGTGCACAGCGTGGTTGGGATGTTGTTGGTGTTGCAGAGGATCTGGATGTTAGCGGTGCAGTTGATCCGTTTGA
TCGTAAACGTCGTCCGAATCTGGCACGTTGGCTGGCATTTGAAGAACAGCCGTTTGATGTTATTGTTGCCTATCG
TGTTGATCGTCTGACCCGTAGCATTCGTCATCTGCAACAGCTGGTTCATTGGGCAGAAGATCATAAAAAACTGGT
TGTGAGCGCAACCGAAGCACATTTTGATACCACCACCCCGTTTGCAGCAGTTGTTATTGCACTGATGGGCACCGT
TGCACAGATGGAACTGGAAGCAATTAAAGAACGTAATCGTAGCGCAGCCCATTTTAACATTCGTGCAGGTAAATA
TCGTGGTAGCCTGCCTCCGTGGGGTTATCTGCCGACGCGTGTAGATGGTGAATGGCGTCTGGTTCCTGACCCGGT
TCAGCGTGAACGTATTCTGGAAGTATATCATCGTGTGGTGGATAATCATGAACCGCTGCATCTGGTTGCACATGA
TCTGAATCGTCGTGGTGTTCTGAGTCCCAAAGATTATTTTGCTCAGCTGCAAGGTCGTGAACCGCAGGGTCGTGA
ATGGTCTGCAACCGCACTGAAACGTAGCATGATTAGCGAAGCAATGCTGGGTTATGCAACCCTGAATGGTAAAAC
CGTTCGTGATGATGATGGTGCACCGCTGGTTCGTGCAGAACCGATTCTGACACGTGAACAGCTGGAAGCACTGCG
TGCCGAACTGGTTAAAACCAGCCGTGCAAAACCGGCAGTTAGCACCCCGAGCCTGCTGCTGCGTGTTCTGTTTTG
TGCAGTTTGTGGTGAACCGGCATACAAATTTGCCGGTGGTGGTCGTAAACATCCGCGTTATCGTTGTCGTAGCAT
GGGTTTTCCGAAACATTGTGGTAATGGTACAGTTGCAATGGCAGAATGGGATGCATTTTGCGAAGAACAGGTTCT
GGATCTGCTGGGTGATGCCGAACGTCTGGAAAAAGTTTGGGTTGCAGGTAGCGATAGCGCAGTTGAACTGGCCGA
AGTTAATGCGGAACTGGTCGATCTCACCAGTCTGATTGGAAGTCCCGCATATCGTGCGGGTAGTCCTCAGCGTGA
AGCACTGGATGCACGTATTGCAGCACTGGCAGCACGTCAAGAAGAACTGGAAGGTCTGGAAGCACGTCCGAGCGG
TTGGGAATGGCGTGAAACAGGTCAGCGTTTTGGTGATTGGTGGCGTGAGCAGGATACCGCAGCAAAAAATACCTG
GCTGCGTAGTATGAATGTTCGCCTGACCTTTGATGTTCGCGGTGGCCTGACCCGCACCATTGATTTTGGCGATCT
GCAAGAATATGAACAGCATCTGCGTCTGGGTAGCGTTGTTGAACGTCTGCATACCGGCATGAGCACCGGCGGTGG
CAGCGGCGGTTCTGGTGGCTCTAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGA
TGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCT
TAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCA
ATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACA
GGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCT
TGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAA
CTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCA

CAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCT
TTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGT
CCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAACTCTACAAAAGGCCTGCTGCTAACGA
CGAAAACTACGCTCTGGCTGCTTAATAAGCGGCCGCGGCCTAGGCGGCCTCCTGTGTGAAATTGTTATCCGCTTT
AATTAA

Supplementary Note 3: Sequence of pASPIre4$_{lib}$. The displayed sequence corresponds to the insert between PstI and NotI restriction sites (underlined) in pASPIre4. A graphical representation of pASPIre4$_{lib}$ is shown in **Supplementary Figure 2**.

CTGCAGTAATCGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGCTTAGTCACCTT
TGGGCCACGGTCCGCTACCTTACAGGAATAGTACTCGTCCTTTAATTTGGAATGAACCATGGCAGTCAGTTGTGT
TGCGTTTCTTCGACCTAGTACTCGCTCCCTTAGGAGAAAGACAGATAGCTTCTTACCCGGGGTTTGTACCGTACA
CCACTGAGACCGCGGTGGTTGACCAGACAAACCACGAAGGTTCTGTTAAGTAACTGAACCCAATGTCGTTAGTGA
CGCTTACCTCTTAAGAGGTCACTGACCTAACAGGATCCCACCACAATTCAGCAAATTGTGAACATCATCACGTTC
ATCTTTCCCTGGTTGCCAATGGCCCATTTTCCTGTCAGTAACGAGAAGGTCGCGAATTCAGGCGCTTTTTAGACT
GGTCGTAANNNNNNNNNNNNNNNNNNNNNNNNNNATGCGNGCNCTNGTNGTNATHCGNCTNTCNCGNGTNACNGAY
GCNACNACTAGTCCGGAACGTCAGCTGGAAAGCTGTCAGCAGCTGTGTGCACAGCGTGGTTGGGATGTTGTTGGT
GTTGCAGAGGATCTGGATGTTAGCGGTGCAGTTGATCCGTTTGATCGTAAACGTCGTCCGAATCTGGCACGTTGG
CTGGCATTTGAAGAACAGCCGTTTGATGTTATTGTTGCCTATCGTGTTGATCGTCTGACCCGTAGCATTCGTCAT
CTGCAACAGCTGGTTCATTGGGCAGAAGATCATAAAAAACTGGTTGTGAGCGCAACCGAAGCACATTTTGATACC
ACCACCCCGTTTGCAGCAGTTGTTATTGCACTGATGGGCACCGTTGCACAGATGGAACTGGAAGCAATTAAAGAA
CGTAATCGTAGCGCAGCCCATTTTAACATTCGTGCAGGTAAATATCGTGGTAGCCTGCCTCCGTGGGGTTATCTG
CCGACGCGTGTAGATGGTGAATGGCGTCTGGTTCCTGACCCGGTTCAGCGTGAACGTATTCTGGAAGTATATCAT
CGTGTGGTGGATAATCATGAACCGCTGCATCTGGTTGCACATGATCTGAATCGTCGTGGTGTTCTGAGTCCCAAA
GATTATTTTGCTCAGCTGCAAGGTCGTGAACCGCAGGGTCGTGAATGGTCTGCAACCGCACTGAAACGTAGCATG
ATTAGCGAAGCAATGCTGGGTTATGCAACCCTGAATGGTAAAACCGTTCGTGATGATGATGGTGCACCGCTGGTT
CGTGCAGAACCGATTCTGACACGTGAACAGCTGGAAGCACTGCGTGCCGAACTGGTTAAAACCAGCCGTGCAAAA
CCGGCAGTTAGCACCCCGAGCCTGCTGCTGCGTGTTCTGTTTTGTGCAGTTTGTGGTGAACCGGCATACAAATTT
GCCGGTGGTGGTCGTAAACATCCGCGTTATCGTTGTCGTAGCATGGGTTTTCCGAAACATTGTGGTAATGGTACA
GTTGCAATGGCAGAATGGGATGCATTTTGCGAAGAACAGGTTCTGGATCTGCTGGGTGATGCCGAACGTCTGGAA
AAAGTTTGGGTTGCAGGTAGCGATAGCGCAGTTGAACTGGCCGAAGTTAATGCGGAACTGGTCGATCTCACCAGT
CTGATTGGAAGTCCCGCATATCGTGCGGGTAGTCCTCAGCGTGAAGCACTGGATGCACGTATTGCAGCACTGGCA
GCACGTCAAGAAGAACTGGAAGGTCTGGAAGCACGTCCGAGCGGTTGGGAATGGCGTGAAACAGGTCAGCGTTTT
GGTGATTGGTGGCGTGAGCAGGATACCGCAGCAAAAAATACCTGGCTGCGTAGTATGAATGTTCGCCTGACCTTT
GATGTTCGCGGTGGCCTGACCCGCACCATTGATTTTGGCGATCTGCAAGAATATGAACAGCATCTGCGTCTGGGT
AGCGTTGTTGAACGTCTGCATACCGGCATGAGCACCGGCGGTGGCAGCGGCGGTTCTGGTGGCTCTAGCAAAGGA
GAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTC
CGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCT
GTTCCGTGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAA
CGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGG
ACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGAT
TTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCA
GACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCA
GACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAA
TCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATT
ACACATGGCATGGATGAACTCTACAAAAGGCCTGCTGCTAACGACGAAAACTACGCTCTGGCTGCTTAATAAGCG
GCCGC

Supplementary Note 4: Sequence of 5'-UTR half-library. The displayed sequence corresponds to the insert between PstI and NotI restriction sites (underlined) in pASPIre4. The BbsI restriction site for scarless cloning of 5'-UTR-CDS combinations is marked in orange. A graphical representation is available in **Supplementary Figure 4**.

CTGCAGTAATCGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGCTTAGTCACCTT
TGGGCCACGGTCCGCTACCTTACAGGAATAGTACTCGTCCTTTAATTTGGAATGAACCATGGCAGTCAGTTGTGT
TGCGTTTCTTCGACCTAGTACTCGCTCCCTTAGGAGAAAGACAGATAGCTTCTTACCCGGGGTTTGTACCGTACA
CCACTGAGACCGCGGTGGTTGACCAGACAAACCACGAAGGTTCTGTTAAGTAACTGAACCCAATGTCGTTAGTGA

CGCTTACCTCTTAAGAGGTCACTGACCTAACAGGATCCCACCACAATTCAGCAAATTGTGAACATCATCACGTTC
ATCTTTCCCTGGTTGCCAATGGCCCATTTTCCTGTCAGTAACGAGAAGGTCGCGAATTCAGGCGCTTTTTAGACT
GGTCGTAANNNNNNNNNNNNNNNNNNNNNNNNNATGCGAGTCTTCGCGGCCGC

Supplementary Note 5: Sequence of CDS half-library. The displayed sequence corresponds to the insert between PstI and NotI restriction sites (underlined) in pASPIre4. The BbsI restriction site for scarless cloning of 5'-UTR-CDS combinations is marked in orange. A graphical representation is available in **Supplementary Figure 5**.

CTGCAGGAAGACCCATGCGNGCNCTNGTNGTNATHCGNCTNTCNCGNGTNACNGAYGCNACNACTAGTCCGGAAC
GTCAGCTGGAAAGCTGTCAGCAGCTGTGTGCACAGCGTGGTTGGGATGTTGTTGGTGTTGCAGAGGATCTGGATG
TTAGCGGTGCAGTTGATCCGTTTGATCGTAAACGTCGTCCGAATCTGGCACGTTGGCTGGCATTTGAAGAACAGC
CGTTTGATGTTATTGTTGCCTATCGTGTTGATCGTCTGACCCGTAGCATTCGTCATCTGCAACAGCTGGTTCATT
GGGCAGAAGATCATAAAAAACTGGTTGTGAGCGCAACCGAAGCACATTTTGATACCACCACCCCGTTTGCAGCAG
TTGTTATTGCACTGATGGGCACCGTTGCACAGATGGAACTGGAAGCAATTAAAGAACGTAATCGTAGCGCAGCCC
ATTTTAACATTCGTGCAGGTAAATATCGTGGTAGCCTGCCTCCGTGGGGTTATCTGCCGACGCGTGTAGATGGTG
AATGGCGTCTGGTTCCTGACCCGGTTCAGCGTGAACGTATTCTGGAAGTATATCATCGTGTGGTGGATAATCATG
AACCGCTGCATCTGGTTGCACATGATCTGAATCGTCGTGGTGTTCTGAGTCCCAAAGATTATTTTGCTCAGCTGC
AAGGTCGTGAACCGCAGGGTCGTGAATGGTCTGCAACCGCACTGAAACGTAGCATGATTAGCGAAGCAATGCTGG
GTTATGCAACCCTGAATGGTAAAACCGTTCGTGATGATGATGGTGCACCGCTGGTTCGTGCAGAACCGATTCTGA
CACGTGAACAGCTGGAAGCACTGCGTGCCGAACTGGTTAAAACCAGCCGTGCAAAACCGGCAGTTAGCACCCCGA
GCCTGCTGCTGCGTGTTCTGTTTTGTGCAGTTTGTGGTGAACCGGCATACAAATTTGCCGGTGGTGGTCGTAAAC
ATCCGCGTTATCGTTGTCGTAGCATGGGTTTTCCGAAACATTGTGGTAATGGTACAGTTGCAATGGCAGAATGGG
ATGCATTTTGCGAAGAACAGGTTCTGGATCTGCTGGGTGATGCCGAACGTCTGGAAAAAGTTTGGGTTGCAGGTA
GCGATAGCGCAGTTGAACTGGCCGAAGTTAATGCGGAACTGGTCGATCTCACCAGTCTGATTGGAAGTCCCGCAT
ATCGTGCGGGTAGTCCTCAGCGTGAAGCACTGGATGCACGTATTGCAGCACTGGCAGCACGTCAAGAAGAACTGG
AAGGTCTGGAAGCACGTCCGAGCGGTTGGGAATGGCGTGAAACAGGTCAGCGTTTTGGTGATTGGTGGCGTGAGC
AGGATACCGCAGCAAAAAATACCTGGCTGCGTAGTATGAATGTTCGCCTGACCTTTGATGTTCGCGGTGGCCTGA
CCCGCACCATTGATTTTGGCGATCTGCAAGAATATGAACAGCATCTGCGTCTGGGTAGCGTTGTTGAACGTCTGC
ATACCGGCATGAGCACCGGCGGTGGCAGCGGCGGTTCTGGTGGCTCTAGCAAAGGAGAAGAACTTTTCACTGGAG
TTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTG
ATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTG
TCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGA
GTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTG
AAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACA
TTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAA
TCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATA
CTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATC
CCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAAC
TCTACAAAAGGCCTGCTGCTAACGACGAAAACTACGCTCTGGCTGCTTAATAAGCGGCCGC

Supplementary Note 6: Sequence of ptRNA^{fMet} variants. The displayed sequence corresponds to the insert between KpnI and SpeI restriction sites (underlined) in pSEVA361. The mutated position 37 in tRNA^{fMet} is highlighted in bold. A graphical representation is available in **Supplementary Figure 6**.

GGTACCAAAATAACACCCTGCTTAATTAAAGCGGATAACAATTTCACACAGGAGGCCGCCTAGGCCGCGGCCGCG
CGAATTCGAGCTCGGTACCAAAATAACACCCTGCTTAATTAACTGATGATGAGCCTGGATTTCCGCTCTCACTGA
ATTTTTATGCAAAATAAATGAGTTTTCATTTAATCATCTTTTATCGGAGACAGGAAGAGTTTAGTGTGTTTTTTG
TAAAATAATGCGCTTAAGGGAGAGCAGGAGAAGGCAAAAGTATTCAACAAATGAAAGTGAACTGGATATTCATTC
ACATGATTAGCAATAAACGTTGACAAAATGTGGCGTGGATCACTATAATGCCTGCAGATTTTACGTCCCGTCTCG
GTACACCAAATCCCAGCAGTATTTGCATTTTTTACCCAAAACGAGTAGAATTTGCCACGTTTCAGGCGCGGGGTG
GAGCAGCCTGGTAGCTCGTCGGGCTCAT**N**ACCCGAAGGTCGTCGGTTCAAATCCGGCCCCCGCAACCACTTTCCC
TTAGAGTCCTTTTTTCAAATATACTGTGAAGACTTCGGCCTTCGTAGTGGGATTTGAAAAAATCCTTCTGGAAAGT
GCTCCAGACCGCAGTTGCGGTTATAGGGTTCAGTTATATAAAGCCCCGATTTATCGGGGTTTTTTGTTATCTGAC
TACAGAATAACTGGGCTTTAGGCCCTTTTTTTATGTCTTGGGGGTGGGCACTAGT

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