Supplemental Information

Contents

Supplemental Table 1. Average times for codon-specific decoding cycles in seconds as predicted by the computational model.

	2nd Base								
1st Base	A		C		G		U		3rd Base
A	Lys	0.052	Thr	0.390	Arg	0.0316	Ile	0.595	A
	Asn	0.121	Thr	0.117	Ser	0.327	Ile	0.087	C
	Lys	0.071	Thr	0.267	Arg	0.529	Met	0.172	G
	Asn	0.135	Thr	0.113	Ser	0.326	Ile	0.086	U
C	Gln	0.105	Pro	0.233	Arg	0.145	Leu	0.451	A
	His	0.251	Pro	0.918	Arg	0.103	Leu	1.411	C
	Gln	1.233	Pro	0.056	Arg	0.440	Leu	0.330	G
	His	0.184	Pro	0.927	Arg	0.101	Leu	0.496	U
G	Glu	0.069	Ala	0.495	Gly	0.253	Val	1.022	A
	Asp	0.151	Ala	0.139	Gly	0.070	Val	0.129	C
	Glu	0.569	Ala	0.056	Gly	0.323	Val	0.464	G
	Asp	0.157	Ala	0.140	Gly	0.068	Val	0.131	U
U	Stop		Ser	0.829	Stop		Leu	0.187	A
	Tyr	0.216	Ser	0.118	Cys	0.166	Phe	0.175	C
	Stop		Ser	1.372	Trp	0.067	Leu	0.119	G
	Tyr	0.226	Ser	0.121	Cys	0.166	Phe	0.174	U

Supplemental Table 2. Recombinant sequences used to generate data in figure 1.

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>minHIS3

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>staHIS3

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>minRLuc

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>staRLuc

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>mCherryv3

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>mCherryv4

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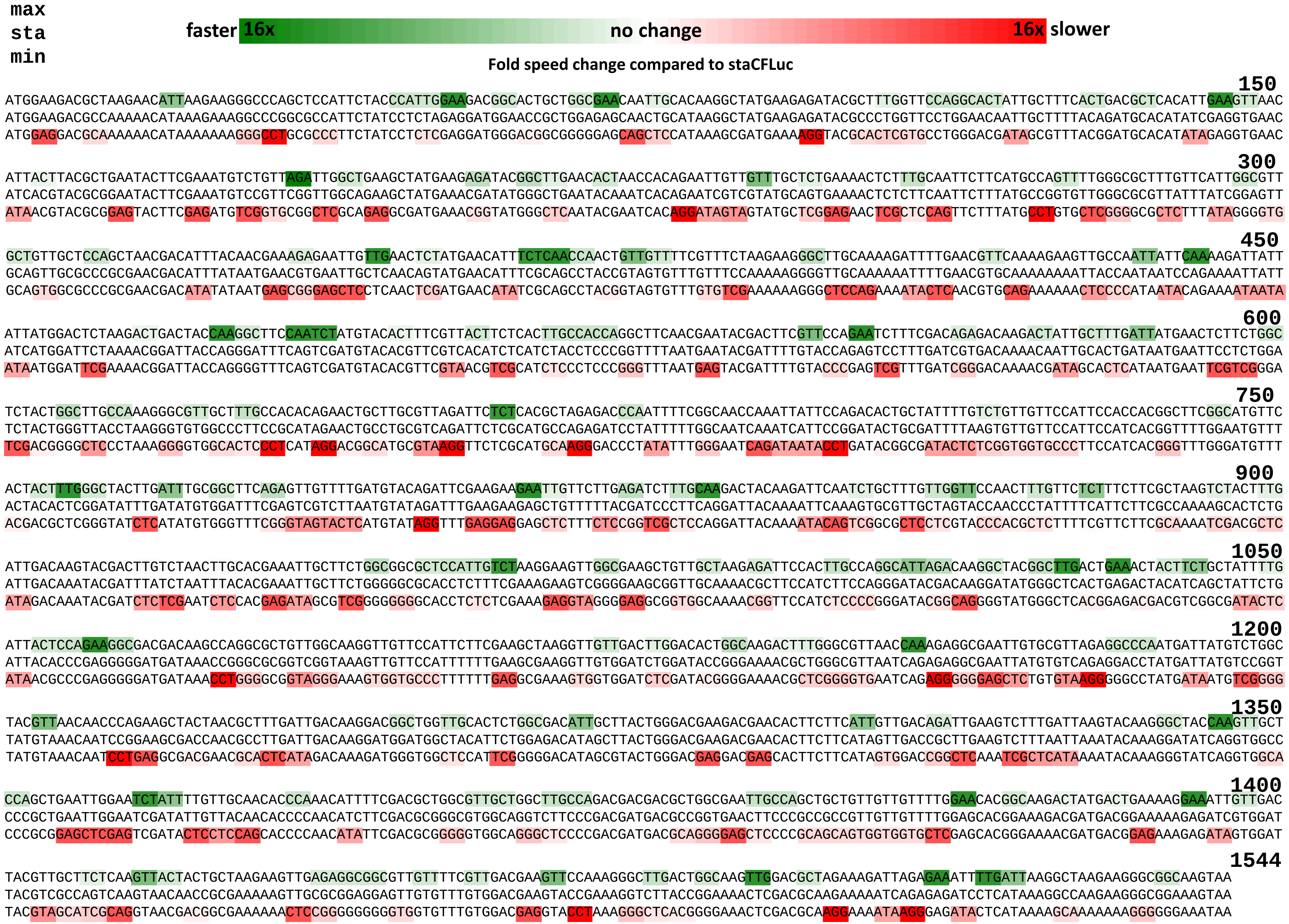
Firefly Luciferase (CFLuc) codon variants: codon substitutions and resulting decoding speed changes



Fold speed change compared to staCFLuc

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max
sta
min

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                                                                                                                                                 1544
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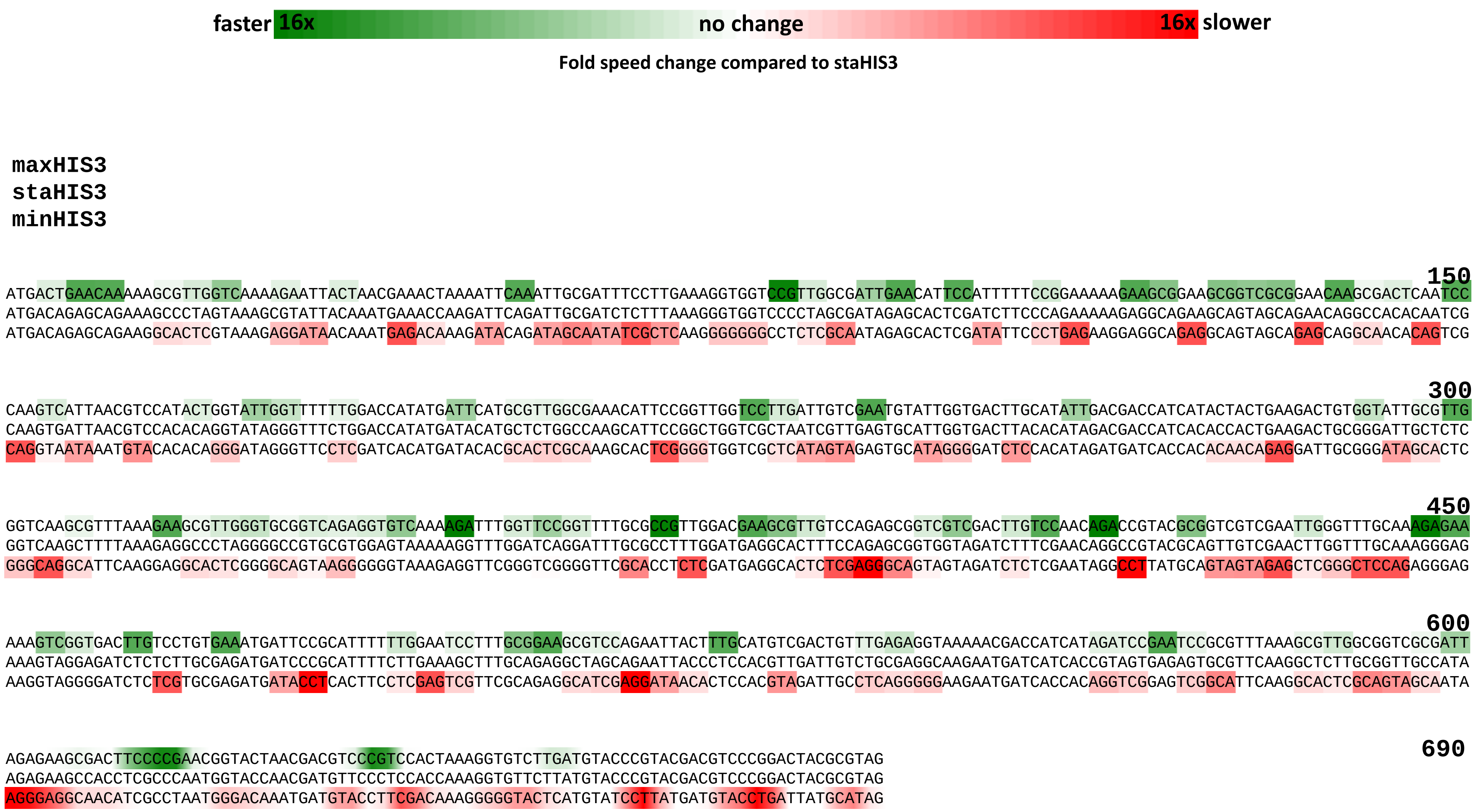
HIS3 codon variants: codon substitutions and resulting decoding speed changes



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maxHIS3
staHIS3
minHIS3

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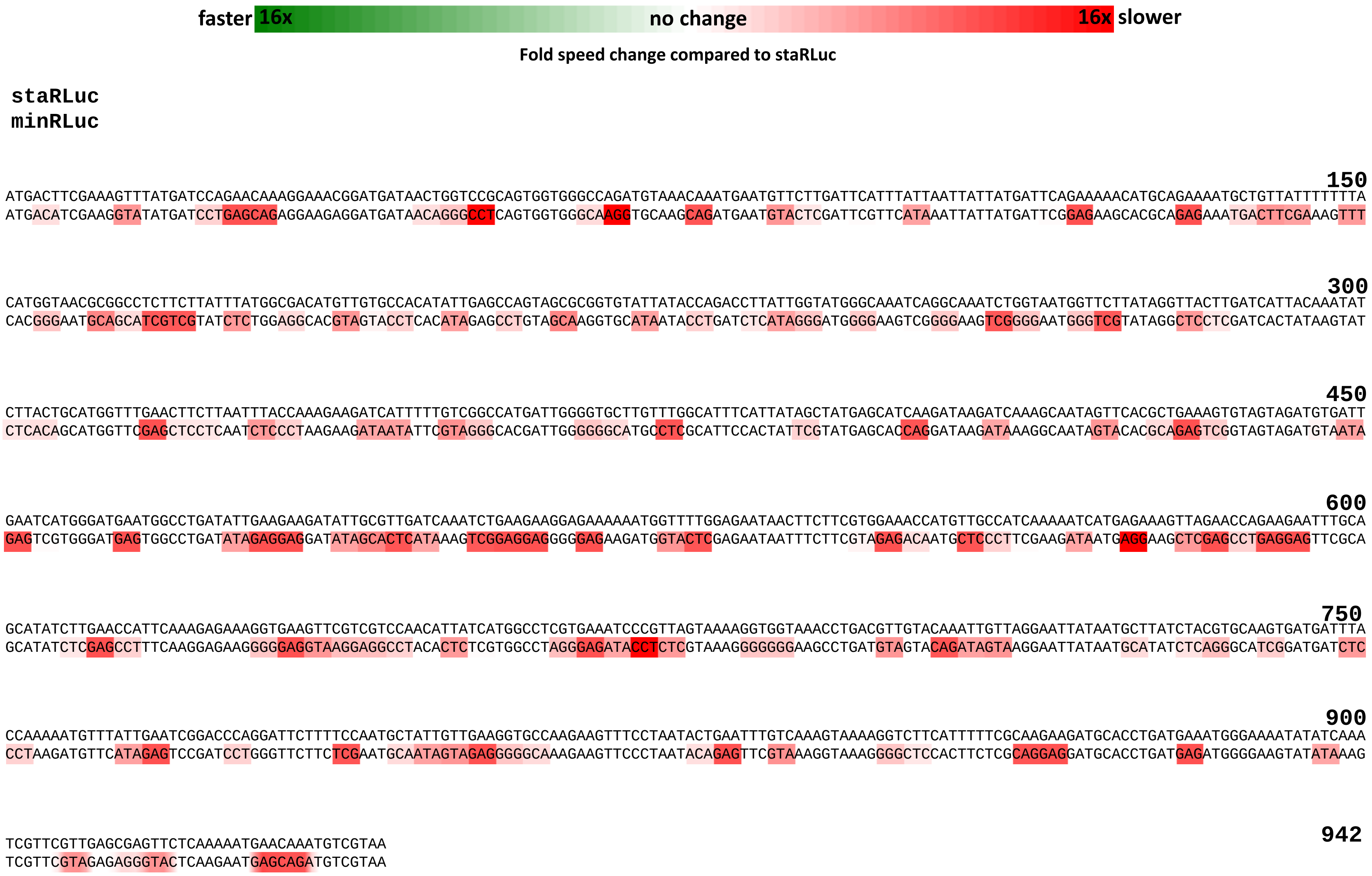
RLuc codon variants: codon substitutions and resulting decoding speed changes



staRLuc
minRLuc

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                                                                                                                                                   450
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                                                                                                                                                   600
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                                                                                                                                                   750
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                                                                                                                                                   900
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                                                                                                                                                   942
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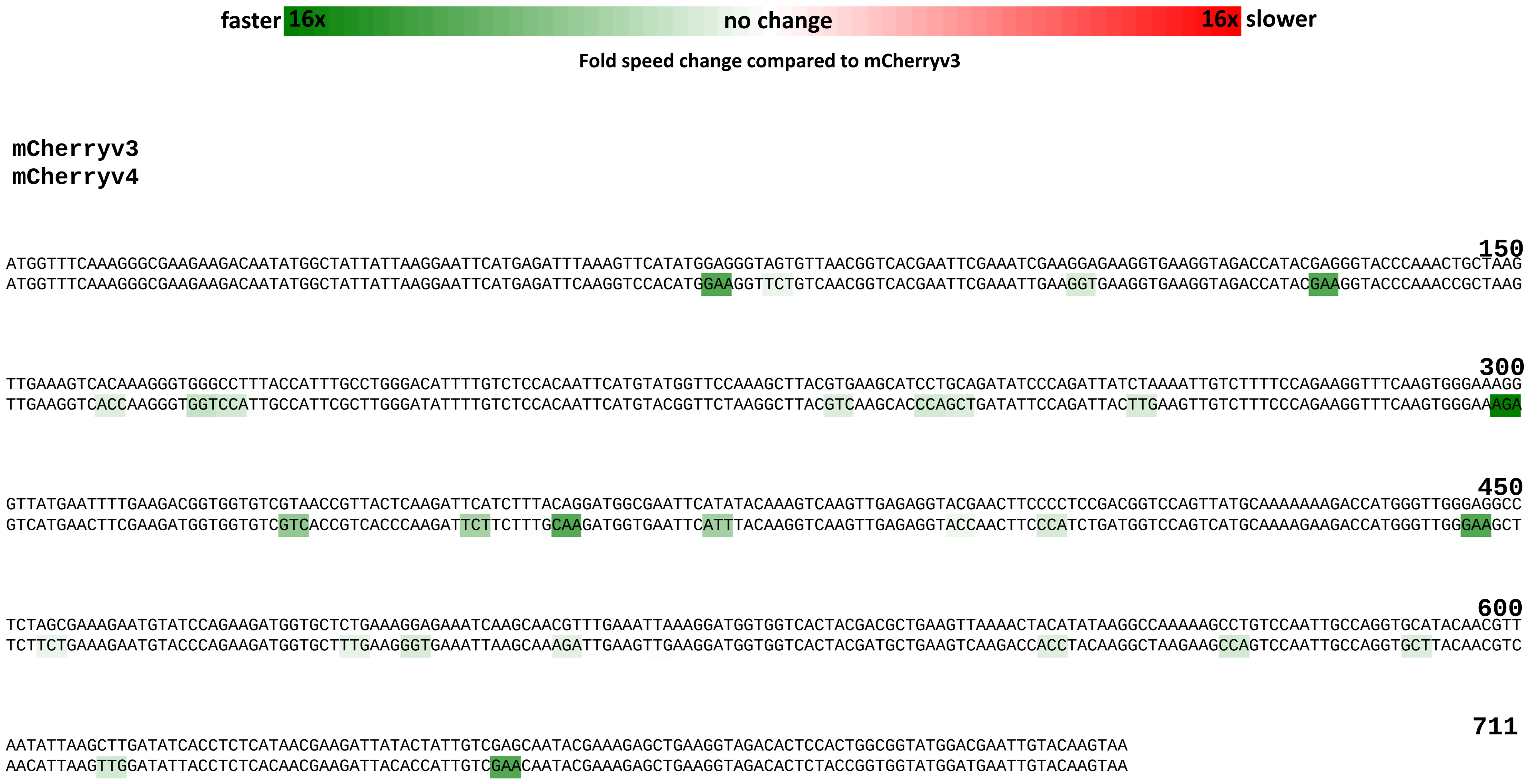
```

mCherry codon variants: codon substitutions and resulting decoding speed changes



```
mCherryv3
mCherryv4

                                                                                                                                                      150
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                                                                                                                                                      300
TTGAAAGTCACAAAGGGTGGGCCTTTACCATTTGCCTGGGACATTTTGTCTCCACAATTCATGTATGGTTCCAAAGCTTACGTGAAGCATCCTGCAGATATCCCAGATTATCTAAAATTGTCTTTTCCAGAAGGTTTCAAGTGGGAAAGG
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                                                                                                                                                      450
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                                                                                                                                                      600
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                                                                                                                                                      711
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AACATTAAGTTGGATATTACCTCTCACAACGAAGATTACACCATTGTCGAACAATACGAAAGAGCTGAAGGTAGACACTCTACCGGTGGTATGGATGAATTGTACAAGTAA
```

Supplemental Table 3. Plasmids used in this study.

“Addgene Ref.” numbers can be used to locate plasmid with sequence information and maps at the Addgene plasmid repository (www.addgene.org).

	Plasmid	Alt Name	Description	Ref.	Addgene Ref.
Basic CFLuc codon variants	pTH644	CENBEVY-U	Centromeric *URA3* marker plasmid containing bidirectional expression cassette, for simultaneous expression of genes from *TDH3* and *ADH1* promoters.	(Chu et al., 2011)	29695
	pTH645	CEN_R	CENBEVY-U with Renilla luciferase (RLuc) expressed from the *ADH1* promoter.	(Chu et al., 2011)	29694
	pTH726	CEN_R/minCFLuc	pTH645 with a slow codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter.	this study	38210
	pTH727	CEN_R/staCFLuc	pTH645 with normal codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter.	this study	38211
	pTH728	CEN_R/maxCFLuc	pTH645 with fast codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter.	this study	38212
	pTH786	CEN_R/GAA$_{10}$maxCFLuc	pTH728 including a run of 10 GAA codons following the maxCFLuc start codon	this study	45556
	pTH787	CEN_R/GAG$_{10}$maxCFLuc	pTH728 including a run of 10 GAG codons following the maxCFLuc start codon	this study	45557
Mixed CFLuc codon variants	pTH747	CEN_R/min4maxCFLuc	As pTH728, but first 4 codons replaced by slow codons	this study	38213
	pTH748	CEN_R/min8maxCFLuc	As pTH728, but first 8 codons replaced by slow codons	this study	38214
	pTH749	CEN_R/min12maxCFLuc	As pTH728, but first 12 codons replaced by slow codons	this study	38215
	pTH750	CEN_R/min16maxCFLuc	As pTH728, but first 16 codons replaced by slow codons	this study	38216
	pTH751	CEN_R/min53maxCFLuc	As pTH728, but first 53 codons replaced by slow codons	this study	38217

	pTH752	CEN_R/ min103maxCFLuc	As pTH728, but first 103 codons replaced by slow codons	this study	38218
	pTH753	CEN_R/ min346maxCFLuc	As pTH728, but first 346 codons replaced by slow codons	this study	38219
	pTH754	CEN_R/ max346minCFLuc	As pTH728, but last 201 codons replaced by slow codons	this study	40597
Basic CFLuc codon variants with slow initiation region	pTH738	CENBEVY-slow	Variant pTH644 with a uORF-containing leader in the bidirectional expression cassette, which slows initiation rates for mRNAs expressed from the *TDH3* promoter.	this study	38220
	pTH741	CENslow_R	Variant pTH645 with a uORF-containing leader in the bidirectional expression cassette, which slows initiation rates for mRNAs expressed from the *TDH3* promoter. Expresses Renilla luciferase from the *ADH1* promoter.	this study	38221
	pTH742	CENslow-R/ minCFLuc	pTH741 with a slow codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter via a uORF-containing 5-UTR.	this study	38222
	pTH743	CENslow-R/ staCFLuc	pTH741 with normal codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter via a uORF-containing 5-UTR.	this study	38223
	pTH744	CENslow-R/ maxCFLuc	pTH741 with fast codon variant of cytoplasmic FLuc expressed from the *TDH3* promoter via a uORF-containing 5-UTR.	this study	38224
Codon variants of other proteins	pTH729	CEN_minRLuc	pTH644 with slow codon variant of RLuc expressed from the *TDH3* promoter.	this study	38225
	pTH730	CEN_staRLuc	pTH644 with normal codon variant of RLuc expressed from the *TDH3* promoter.	this study	38226
	pTH735	CEN_minHIS3	pTH644 with slow codon variant of HA-tagged yeast HIS3 expressed from the *TDH3* promoter.	this study	38227
	pTH736	CEN_staHIS3	pTH644 with normal codon variant of HA-tagged yeast HIS3 expressed from the *TDH3* promoter.	this study	38228
	pTH737	CEN_maxHIS3	pTH644 with fast codon variant of HA-tagged yeast HIS3 expressed from the *TDH3* promoter.	this study	38229
	pTH760	CEN_mCherry_v3	Centromeric plasmid expressing v3 variant of mCherry fluorescent protein.	this study	40598
	pTH761	CEN_mCherry_v4	Centromeric plasmid expressing v4 variant of mCherry fluorescent protein.	this study	40599

tRNA plasmid	pTH490	tQSRTL	Centromeric plasmid, contains genes for the five essential tRNAs encoded by a single chromosomal gene in *S. cerevisiae.*	(Chu et al., 2011)	29699

qPCR data and mRNA analyses

1. qPCR primer sequences.

Primers were designed using the primer design tool provided by Genscript, Piscataway, NJ (https://www.genscript.com/ssl-bin/app/primer), with a target product size range of 80-150 nucleotides and a target primer Tm of 58-60 °C. qADH1 primers were designed manually.

Primer Name	Purpose	Sequence
qMaxFLuc_f	Target nucleotides 1263-1351 of the MaxCFLuc ORF	CGACATTGCTTACTGGGACG
qMaxFLuc_r		GAGCAACTTGGTAGCCCTTG
qMaxFLuc5_f	Target nucleotides 600-734 of the MaxCFLuc ORF	CTCTACTGGCTTGCCAAAGG
qMaxFLuc5_r		TGGTGGAATGGAACAACAGA
qMinFLuc5_f	Target nucleotides 32-137 of the MinCFLuc ORF	CTGCGCCCTTCTATCCTCTC
qMinFLuc5_r		TGTGCATCCGTAAACGCTAT
qREN_f	Target nucleotides 758-880 of the RLuc ORF	TGTTTATTGAATCGGACCCA
qREN_r		CATCAGGTGCATCTTCTTGC
qADH1_f	Target a 70 nt long sequence in the *ADH1*-derived 3'-UTR present in the recombinant protein expression constructs.	TGCAAGCTTTGGACTTCTTC
qADH1_r		CAAGGTAGACAAGCCGACAA
qHIS3_f	Target a 88 nt-long sequence in the *HIS3* mRNA starting at nucleotide 658 of the ORF and extending 82 nucleotides into the *HIS3* 3'-UTR	ATGTAGTGACACCGATTATTTA
qHIS3_r		TACATACTTACTGACATTCATAG
qLEU3_f	Target a 134-nt long sequence in the *LEU3* mRNA, comprising nucleotides 2125-2259 of the ORF	CAGCAACTAAGGACAAGG
qLEU3_r		GGTCGTTAATGAGCTTCC

2. qPCR protocol.

2 oD units of yeast cells transformed with the recombinant protein expressing plasmids and grown in SC medium lacking uracil to an oD_{600} of 0.5-0.9 were harvested and frozen at –20 °C. For data in figure 7, 2 oD units of cells grown in YPD to an oD_{600} of 0.7-0.8 were used. RNA was prepared from cells using an RNA easy kit (QIAgen, UK) including the optional DNAse digest step.

Apparent primer efficiencies were determined by preparing 2-fold serial dilutions of RNA samples containing target mRNA and analysing the resulting data as described in Pfaffl *et al.* (2001).

Primer specificity was verified by separating final PCR products on a 2% agarose gel where all primers yielded a single product of the expected size.

3. Primers used for different constructs.

qPCR data displayed in figure 2 were generated using primers qADH1 directed against the 3'-UTRs of the codon variant mRNAs, and primers qLEU3 which are directed against the internal standard mRNA.

qPCR data in figure 3 were generated using primers qADH1 directed against the 3'-UTRs of the different CFLuc mRNAs, and primers qREN for the internal standard.

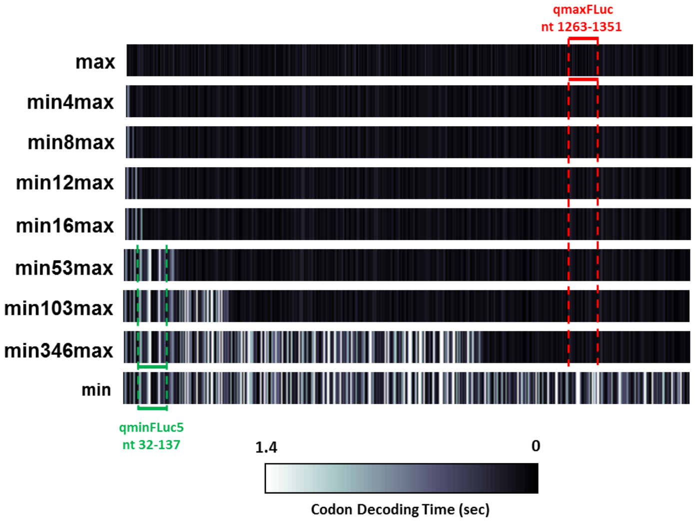
qPCR data in figure 4 were generated as described below.

qPCR data for the maxCFLuc/max346minCFLuc comparison in figure 5 were generated using primers qmaxFLuc5, which are directed against the codon optimised part of the sequence shared between maxCFLuc and max346minCFLuc, and using primers qREN for the internal standard. The data for minCFLuc in this figure are from the maxCFLuc/minCFLuc comparison in figure 4.

qPCR data in figure 6 were generated using primers qmaxFLuc, and using primers qREN for the internal standard.

qPCR data in figure 7 were generated using primers qHIS3 which are directed against the invariant 3'-UTR of the *HIS3* codon variant mRNAs, and using primers qADH1 for the internal standard

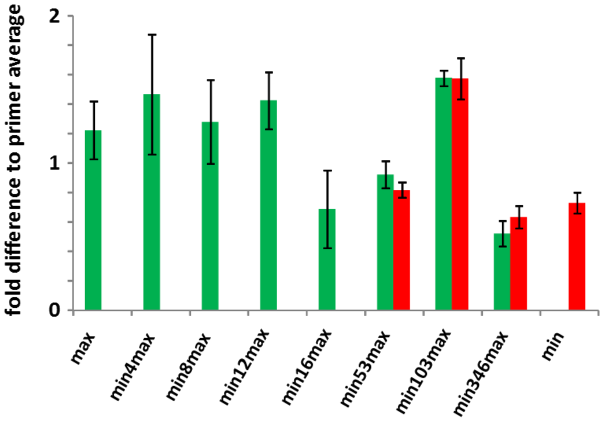
To generate data for the mixed codon variants in figure 4, we employed a strategy using two pairs of primers (qmaxFLuc and qminFLuc5) as follows, with qREN as internal standard for all reactions.

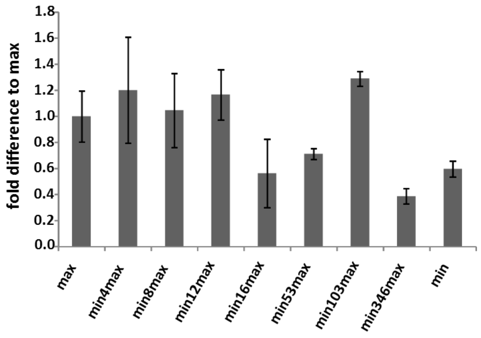


Primers qmaxFLuc anneal to the codon optimised sequence common to maxCFLuc and all mixed codon variants in this figure. Primers qminFLuc5 anneal to the codon-disoptimised sequence common to minCFLuc and min346/103/53CFLuc. qPCR reactions were run for each template with all primer pairs targeting that template.

RNA samples were prepared at least in triplicate from independently cultured cells expressing the different CFLuc constructs. qPCR reactions were prepared combining each RNA sample with each primer pair targeting the expressed CFLuc construct in that sample. qREN primers were used to generate an internal standard.

The fold difference to the average signal for the primer pair used was calculated, based on the ΔCt to the average Ct and the experimentally determined efficiency for that primer. All minFLuc5 values were adjusted so that the average value for min53max, min103max and min346max was the same as the average maxFLuc value





Data were normalised to the max value and where multiple primers were used for the same sample values were averaged to generate data used in figure 4.

4. References

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29:** e45.