Supplemental Information

Contents

1. Codon decoding times as estimated by the computational decoding model.
2. Recombinant sequences used to generate data in figure 1.
3. Alignments of codon variants and resulting speed changes.
4. Plasmids used in this study.
5. qPCR assays
   a. qPCR primer sequences
   b. qPCR protocol
   c. Primer strategy for comparison between codon variant transcripts
**Supplemental Table 1.** Average times for codon-specific decoding cycles in seconds as predicted by the computational model.

<table>
<thead>
<tr>
<th>1st Base</th>
<th>2nd Base</th>
<th>3rd Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>0.052</td>
<td>Thr 0.390</td>
</tr>
<tr>
<td>Asn</td>
<td>0.121</td>
<td>Thr 0.117</td>
</tr>
<tr>
<td>Lys</td>
<td>0.071</td>
<td>Thr 0.267</td>
</tr>
<tr>
<td>Asn</td>
<td>0.135</td>
<td>Thr 0.113</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>0.105</td>
<td>Pro 0.233</td>
</tr>
<tr>
<td>His</td>
<td>0.251</td>
<td>Pro 0.918</td>
</tr>
<tr>
<td>Gln</td>
<td>1.233</td>
<td>Pro 0.056</td>
</tr>
<tr>
<td>His</td>
<td>0.184</td>
<td>Pro 0.927</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>0.069</td>
<td>Ala 0.495</td>
</tr>
<tr>
<td>Asp</td>
<td>0.151</td>
<td>Ala 0.139</td>
</tr>
<tr>
<td>Glu</td>
<td>0.569</td>
<td>Ala 0.056</td>
</tr>
<tr>
<td>Asp</td>
<td>0.157</td>
<td>Ala 0.140</td>
</tr>
<tr>
<td>U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop</td>
<td>Ser 0.829</td>
<td>Stop</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.216</td>
<td>Ser 0.118</td>
</tr>
<tr>
<td>Stop</td>
<td>Ser 1.372</td>
<td>Trp 0.067</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.226</td>
<td>Ser 0.121</td>
</tr>
</tbody>
</table>
Supplemental Table 2. Recombinant sequences used to generate data in figure 1.

>MinCFLuc

ATGGAGGACGCAAAAAACATAAAAAAAGGGCCCTGCAGCCCTCTAACATCTCAGGATGGGAGCGGGGGA
GCAGCTCCATAAAAGGCATGAAAAAGCTCGAATTCGATTCCTGGGAGATGATCGGTCTTTGGCTGAACATAG
ATGGAACATTAAGGCGAGATTCTTCGAGATGCTGGGAGGTGAGCTTTGGCGAGATATGGGAGAACATATGG
GTCAGACTAGGGATGATGGGGAGGTGAGCTTTGGCGAGATATGGGAGAACATATGG

>StaAFLuc

ATGGAGGACGCAAAAAACATAAAAAAAGGGCCCTGCAGCCCTCTAACATCTCAGGATGGGAGCGGGGGA
GCAGCTCCATAAAAGGCATGAAAAAGCTCGAATTCGATTCCTGGGAGATGATCGGTCTTTGGCTGAACATAG
ATGGAACATTAAGGCGAGATTCTTCGAGATGCTGGGAGGTGAGCTTTGGCGAGATATGGGAGAACATATGG
GTCAGACTAGGGATGATGGGGAGGTGAGCTTTGGCGAGATATGGGAGAACATATGG

AAGAGCTCTTCCGATCAAGGACGGACCGATAATGGGAAATACCTGAAATTTGACGGGAGATGATCGGTCTTTGGCTGAACATAG

GTCTACTTTTGGATTCAGAGTACGACTTGTCAACTTTGCACGAAATTGCTTCTGGCGGCGCCATTTGTCTAGG
AAATGGGCGGAAAGCTGTGGTCTAAGAGATAATTCCACTTGCACGAGGAGGTACGACTTGTGACTGAAACGA
CGTCGGCGATATCATCCTAAGCCGGCAGGGAGATGATAAACCTGCGGGAGACAGAGATGTGAGCACTGAGCTACG
GTCGGCGATACTCATAACGCCCGAGGGGGATGATAA

ACCTGGGGCGGTAGGGAAAGTGGTGCCCTTTTTTG

AGGCGAAAGTGGTGGATCTCGATACGGGGAAAACGCTCGGGGTGAATCAGAGGGGGGAGCTCTGTGTAAG
GGGGCCTATGATAATGTCGGGGTATGTAAACAATCCTGAGGCGACGAACGCACTCATAGACAAAGATGGGTG
GCTCCATTCGGGGGACATAGCGTACTGGGACGAGGACGAGCACTTCTTCATAGTGGACCGGCTCAAATCGCT
CATAAAATAAACAGGGTATGTTGACCGCACCCTGCGAGCCTGAGCTGATACTCTCCAGACCCCCAACATATCC
GACCCGGGTTGGCAGGCGCTCCGGCGACTGAGCGACGGAGAGGAGACCTCCCCGCGACAGTGGTGGTGCTCGAGCA
CGGGAAAAAACGATGAGGGAAAGAGAGATATGGGATTCCATACGATATTTTTTCAATAGGCGCAGCGGCAAAACTCC
GGGGGGGTTGGGTGGTCTGGACGAGATCCTACTTAAGAGGGCTACGGGGGAACCTGACAGCGAAAGAAAATAG
GGAGATATCCTAAAGAAGAGGGGAAATAA

>minHIS3

ATGACAGAGCAGAAGGGCACTCGTAAAGAGGATAACAAATGAGACAAAGATACAGATAGCAATATCGCTCAA
GGGGGGGCTCTCCGAAATAGAGCACCTGATATTTCTCGAGAAGAGGGCGAGGCAACAGGGCGCGAA
CACAGCTCCGAGTAAATTTAAATTCTACACAGAGGATAGGCTCTCTCGTCATACGATACCGCGGCAAGAC
CTCGGGGTTGGTCGCTCATAGTATGAGTGCTACAGGGGATCTCCACATAGTATGACCAACACACAAACAGAGAAT
GGGATAGCACTCCGGAGGACGCGTTAAGGAGGCGTGAAGGAGGGGCTAAGAGATGTGAGCAGGTCGGTGC
GGGTTGCACACTTGCAAGGAGACGACTCTCCTCAGGCTATGAGTATCTCCTGAAATAGCGTACTATTAG
AGCTCGGGCTCCAGAGGGAGAAGGTAGGGGTGTGATCCTCTGTCGAGATGATACCTCCTACCTCCTGAGTCTGTC
CAGAGCAGCTCAGGAAACTCAGTACTTAGAATAGGGGGAAAAAACATCGGCTAATGGGACAAATGATGTACCTTCGACA
AAGGGGGTACTCATGTACTCTTATGATGATCTGATTATGCTAG

>staHIS3

ATGACAGAGCAGAAGGGCACTCGTAAAGAGGATAACAAATGAGACAAAGATACAGATAGCAATATCGCTCAA
GGGGGGGCTCTCCGAAATAGAGCACCTGATATTTCTCGAGAAGAGGGCGAGGCAACAGGGCGCGAA
CACAGCTCCGAGTAAATTTAAATTCTACACAGAGGATAGGCTCTCTCGTCATACGATACCGCGGCAAGAC
CTCGGGGTTGGTCGCTCATAGTATGAGTGCTACAGGGGATCTCCACATAGTATGACCAACACACAAACAGAGAAT
GGGATAGCACTCCGGAGGACGCGTTAAGGAGGCGTGAAGGAGGGGCTAAGAGATGTGAGCAGGTCGGTGC
GGGTTGCACACTTGCAAGGAGACGACTCTCCTCAGGCTATGAGTATCTCCTGAAATAGCGTACTATTAG
AGCTCGGGCTCCAGAGGGAGAAGGTAGGGGTGTGATCCTCTGTCGAGATGATACCTCCTACCTCCTGAGTCTGTC
CAGAGCAGCTCAGGAAACTCAGTACTTAGAATAGGGGGAAAAAACATCGGCTAATGGGACAAATGATGTACCTTCGACA
AAGGGGGTACTCATGTACTCTTATGATGATCTGATTATGCTAG

>maxHIS3

ATGACTGAACAAAAAGCGTTGGTCAAAAGAATTACTAACAAATGACAAAGATACAGATAGCAATATCGCTCAA
GGGGGGGCTCTCCGAAATAGAGCACCTGATATTTCTCGAGAAGAGGGCGAGGCAACAGGGCGCGAA
CACAGCTCCGAGTAAATTTAAATTCTACACAGAGGATAGGCTCTCTCGTCATACGATACCGCGGCAAGAC
CTCGGGGTTGGTCGCTCATAGTATGAGTGCTACAGGGGATCTCCACATAGTATGACCAACACACAAACAGAGAAT
GGGATAGCACTCCGGAGGACGCGTTAAGGAGGCGTGAAGGAGGGGCTAAGAGATGTGAGCAGGTCGGTGC
GGGTTGCACACTTGCAAGGAGACGACTCTCCTCAGGCTATGAGTATCTCCTGAAATAGCGTACTATTAG
AGCTCGGGCTCCAGAGGGAGAAGGTAGGGGTGTGATCCTCTGTCGAGATGATACCTCCTACCTCCTGAGTCTGTC
CAGAGCAGCTCAGGAAACTCAGTACTTAGAATAGGGGGAAAAAACATCGGCTAATGGGACAAATGATGTACCTTCGACA
AAGGGGGTACTCATGTACTCTTATGATGATCTGATTATGCTAG
>mCherryv4

ATGTTTCAAAGGGCGAAGAAGACAAATATGGCTTTTATTAAGGAATTTACATGAGATTCAAGGGTCCACATGG
GTTTCTTCAACGGTACGAAATTGAAGGTGAGGGAAAGGTAGACCAACAGGAAGGTACCCAAACC
GCTAAGGTGAGTCAACCAAGGGTGGTCCTTTGAGATATTTTGCTCCACAATTCATGTACGG
TTCTAAGGGCTTACGTGAAGCCACCCAGCTGATAGGGAAAGGTGTTGCTTCTTGCAAGAGATGG
GGAAAGGAGTCAACTTCGAAGATGTGCTGTCACCGTCACCCAAGATTCTCTGGCAAGATGGTGA
ATTCACTTACAAGGCTAAGGGTACGACCAACTCTCGATAGGTCGACGTGCAAGATGGTCAAGAAGTGTGCTTTGAAGGGTGAAATTAAGCAAAGATTGAAGTTGAAGGATGGTGGTCGTACCGTACGGTCAAGACCAATA
CCAGGTGCATAACGTTAATATTAAAGCTTGATATCACCTCATAACGAAGATTATATTGTGAGCAATA
CGAAAGAGCTGAAGGATGACACTCCACTGGCGGTATGGACGAATTGTGACAAAGTAA
**HIS3 codon variants: codon substitutions and resulting decoding speed changes**

- faster 16x
- no change
- 16x slower

Fold speed change compared to staHIS3

maxHIS3
staHIS3
minHIS3
RLuc codon variants: codon substitutions and resulting decoding speed changes

- **更快 16x**
- **无变化**
- **更慢 16x**

**Fold speed change compared to staRLuc**

**staRLuc**
- ATGACTTCCAGAGTTTATGACCCAGACAAAGGAAACGGATGATAACTGGTCCGCAGTGGTGGGCCAGATGTAAACAAATGAATGTTCTTGATTCATTTATTAATTATTATGATTCAGAAAAACATGCAGAAAATGCTGTTATTTTTTTAT

**minRLuc**
- ATGACATCGAAGGTATATGATCCTGAGCAGAGGAAGAGGATGATAACAGGGCCTCAGTGGTGGGCAAGGTGCAAGCAGATGAATGTACTCGATTCGTTCATAAATTATTATGATTCGGAGAAGCACGCAGAGAAATGACTTCGAAAGTTTAT

**Fold**
- faster slower
- no change

**Speed**
- STA RLUC
- MIN RLUC

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>210</td>
<td>300</td>
<td>450</td>
<td>600</td>
<td>750</td>
<td>900</td>
</tr>
</tbody>
</table>

**RLuc codon variants:**
- **STA RLUC**
- **MIN RLUC**

**Codon variants:**
- **RLuc codon**
- **variants:**
- **codon substitutions**
- **resulting decoding speed changes**
mCherry codon variants: codon substitutions and resulting decoding speed changes

Fold speed change compared to mCherryv3

mCherryv3
mCherryv4

ATGTTTCAAGGGCGAAGACAAATATGCCATTATATTAGAATTCCAGAGATTTAAAGTTCATATGGAGGCTATTATAGATCTACCTGACGCAGACATTGCAGATTACATCATCAGAGGGTACCCAAACTGCTAAG

ATGTTTCAAGGGCGAAGACAAATATGCCATTATATTAGAATTCCAGAGATTTAAAGTTCATATGGAGGCTATTATAGATCTACCTGACGCAGACATTGCAGATTACATCATCAGAGGGTACCCAAACTGCTAAG

TTGAAAGTGCAAAAGGGGCTTTTACATTGCTCTGGGCACTTTTGCTCTCAAAAATTGCTACATTGCTACATTGCAGATTCCAGATTCCTTTCTCCAGAAAGGTTTTATGAGTTGAGGAAAGG
TTGAAAGTGCAAAAGGGGCTTTTACATTGCTCTGGGCACTTTTGCTCTCAAAAATTGCTACATTGCTACATTGCAGATTCCAGATTCCTTTCTCCAGAAAGGTTTTATGAGTTGAGGAAAGG

GTTATGAAATTTTGAGACGTGTTGTGCTACTAACAGATGGCCAATTCATATACAAAAACTGAAGTGAAGGAGGTGGAGACATTTCCCAGACCGTACGGTGCATGGAACTTCGAAGATGGTGGTCACTACGAC
GTTATGAAATTTTGAGACGTGTTGTGCTACTAACAGATGGCCAATTCATATACAAAAACTGAAGTGAAGGAGGTGGAGACATTTCCCAGACCGTACGGTGCATGGAACTTCGAAGATGGTGGTCACTACGAC

TCTAGCGAAAGAATGTATCCAGAAGATGGTGCTCTGAAAGGAGAAATCAAGCAACGTTTGAAATTAAAGGATGGTGGTCACTACGACGCTGAAGTTAAAACTACATATAAGGCCAAAAAGCCTGTCCAATTGCCAGGTGCATACAACGTT
TCTAGCGAAAGAATGTATCCAGAAGATGGTGCTCTGAAAGGAGAAATCAAGCAACGTTTGAAATTAAAGGATGGTGGTCACTACGACGCTGAAGTTAAAACTACATATAAGGCCAAAAAGCCTGTCCAATTGCCAGGTGCATACAACGTT

AATATTAAGCCTGATATCACCCTTCTCATAACGAAGATTATACTATTTGTCAGCAATACAAAGAAGCTGAAGGTGAAGACACTCCACTGGCGGTATGGACGAATTGTACAAGTAA
AATATTAAGCCTGATATCACCCTTCTCATAACGAAGATTATACTATTTGTCAGCAATACAAAGAAGCTGAAGGTGAAGACACTCCACTGGCGGTATGGACGAATTGTACAAGTAA

mCherry codon variants: codon substitutions and resulting decoding speed changes

faster 16x no change 16x slower

Fold speed change compared to mCherryv3
### 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).

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Alt Name</th>
<th>Description</th>
<th>Ref.</th>
<th>Addgene Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTH644</td>
<td>CENBEVY-U</td>
<td>Centromeric URA3 marker plasmid containing bidirectional expression cassette, for simultaneous expression of genes from TDH3 and ADH1 promoters.</td>
<td>(Chu et al., 2011)</td>
<td>29695</td>
</tr>
<tr>
<td>pTH645</td>
<td>CEN_R</td>
<td>CENBEVY-U with Renilla luciferase (RLuc) expressed from the ADH1 promoter.</td>
<td>(Chu et al., 2011)</td>
<td>29694</td>
</tr>
<tr>
<td>pTH726</td>
<td>CEN_R/minCFLuc</td>
<td>pTH645 with a slow codon variant of cytoplasmic Fluc expressed from the TDH3 promoter.</td>
<td>this study</td>
<td>38210</td>
</tr>
<tr>
<td>pTH727</td>
<td>CEN_R/staCFLuc</td>
<td>pTH645 with normal codon variant of cytoplasmic Fluc expressed from the TDH3 promoter.</td>
<td>this study</td>
<td>38211</td>
</tr>
<tr>
<td>pTH728</td>
<td>CEN_R/maxCFLuc</td>
<td>pTH645 with fast codon variant of cytoplasmic Fluc expressed from the TDH3 promoter.</td>
<td>this study</td>
<td>38212</td>
</tr>
<tr>
<td>pTH786</td>
<td>CEN_R/GAA10maxCFLuc</td>
<td>pTH728 including a run of 10 GAA codons following the maxCFLuc start codon</td>
<td>this study</td>
<td>45556</td>
</tr>
<tr>
<td>pTH787</td>
<td>CEN_R/GAG10maxCFLuc</td>
<td>pTH728 including a run of 10 GAG codons following the maxCFLuc start codon</td>
<td>this study</td>
<td>45557</td>
</tr>
<tr>
<td>pTH747</td>
<td>CEN_R/min4maxCFLuc</td>
<td>As pTH728, but first 4 codons replaced by slow codons</td>
<td>this study</td>
<td>38213</td>
</tr>
<tr>
<td>pTH748</td>
<td>CEN_R/min8maxCFLuc</td>
<td>As pTH728, but first 8 codons replaced by slow codons</td>
<td>this study</td>
<td>38214</td>
</tr>
<tr>
<td>pTH749</td>
<td>CEN_R/min12maxCFLuc</td>
<td>As pTH728, but first 12 codons replaced by slow codons</td>
<td>this study</td>
<td>38215</td>
</tr>
<tr>
<td>pTH750</td>
<td>CEN_R/min16maxCFLuc</td>
<td>As pTH728, but first 16 codons replaced by slow codons</td>
<td>this study</td>
<td>38216</td>
</tr>
<tr>
<td>pTH751</td>
<td>CEN_R/min53maxCFLuc</td>
<td>As pTH728, but first 53 codons replaced by slow codons</td>
<td>this study</td>
<td>38217</td>
</tr>
</tbody>
</table>
**Basic CFLuc codon variants with slow initiation region**

- **pTH752**
  - **CEN_R/ min103maxCFLuc**
  - As pTH728, but first 103 codons replaced by slow codons

- **pTH753**
  - **CEN_R/ min346maxCFLuc**
  - As pTH728, but first 346 codons replaced by slow codons

- **pTH754**
  - **CEN_R/ max346minCFLuc**
  - As pTH728, but last 201 codons replaced by slow codons

- **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.

**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.

**pTH742**
- **CENslow-R/ minCFLuc**
  - pTH741 with a slow codon variant of cytoplasmic Fluc expressed from the TDH3 promoter via a uORF-containing 5'-UTR.

**pTH743**
- **CENslow-R/ staCFLuc**
  - pTH741 with normal codon variant of cytoplasmic Fluc expressed from the TDH3 promoter via a uORF-containing 5'-UTR.

**pTH744**
- **CENslow-R/ maxCFLuc**
  - pTH741 with fast codon variant of cytoplasmic Fluc expressed from the TDH3 promoter via a uORF-containing 5'-UTR.

**pTH729**
- **CEN_minRLuc**
  - pTH644 with slow codon variant of RLuc expressed from the TDH3 promoter.

**pTH730**
- **CEN_staRLuc**
  - pTH644 with normal codon variant of RLuc expressed from the TDH3 promoter.

**pTH735**
- **CEN_minHIS3**
  - pTH644 with slow codon variant of HA-tagged yeast HIS3 expressed from the TDH3 promoter.

**pTH736**
- **CEN_staHIS3**
  - pTH644 with normal codon variant of HA-tagged yeast HIS3 expressed from the TDH3 promoter.

**pTH737**
- **CEN_maxHIS3**
  - pTH644 with fast codon variant of HA-tagged yeast HIS3 expressed from the TDH3 promoter.

**pTH760**
- **CEN_mCherry_v3**
  - Centromeric plasmid expressing v3 variant of mCherry fluorescent protein.

**pTH761**
- **CEN_mCherry_v4**
  - Centromeric plasmid expressing v4 variant of mCherry fluorescent protein.
Centromeric plasmid, contains genes for the five essential tRNAs encoded by a single chromosomal gene in S. cerevisiae. (Chu et al., 2011)
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.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Purpose</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>qMaxFLuc_f</td>
<td>Target nucleotides 1263-1351 of the MaxCFLuc ORF</td>
<td>CGACATTGCTTACTGGGACG</td>
</tr>
<tr>
<td>qMaxFLuc_r</td>
<td></td>
<td>GAGCAACTTGTTAGCCCTTG</td>
</tr>
<tr>
<td>qMaxFLuc5_f</td>
<td>Target nucleotides 600-734 of the MaxCFLuc ORF</td>
<td>CTCTACTGGCTTGCAAGGG</td>
</tr>
<tr>
<td>qMaxFLuc5_r</td>
<td></td>
<td>TGGGGAATGGAAACAACAGA</td>
</tr>
<tr>
<td>qMinFLuc5_f</td>
<td>Target nucleotides 32-137 of the MinCFLuc ORF</td>
<td>CTGCGCCCTTCTATCTCTC</td>
</tr>
<tr>
<td>qMinFLuc5_r</td>
<td></td>
<td>TGTGATCCGTAACCGCTAT</td>
</tr>
<tr>
<td>qREN_f</td>
<td>Target nucleotides 758-880 of the RLuc ORF</td>
<td>TGTTATTGAAATCGGACCA</td>
</tr>
<tr>
<td>qREN_r</td>
<td></td>
<td>CATCAGGTGCATCTCTTGC</td>
</tr>
<tr>
<td>qADH1_f</td>
<td>Target a 70 nt long sequence in the ( ADH1 )-derived 3’-UTR present in the recombinant protein expression constructs.</td>
<td>TGGCAAGCTTGGACTTTCTTC</td>
</tr>
<tr>
<td>qADH1_r</td>
<td></td>
<td>CAAGGTAGACAAAGCCGACAA</td>
</tr>
<tr>
<td>qHIS3_f</td>
<td>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</td>
<td>ATGTAAGTGAACCGATTATTTA</td>
</tr>
<tr>
<td>qHIS3_r</td>
<td></td>
<td>TACATACCTACTGACATTCAG</td>
</tr>
<tr>
<td>qLEU3_f</td>
<td>Target a 134 nt-long sequence in the ( LEU3 ) mRNA, comprising nucleotides 2125-2259 of the ORF</td>
<td>CAGCAACTAAGGACAAGG</td>
</tr>
<tr>
<td>qLEU3_r</td>
<td></td>
<td>GGTCGTTAATGAGCTTCC</td>
</tr>
</tbody>
</table>

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 \( \text{OD}_{600} \) 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 \( \text{OD}_{600} \) 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.

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