SUPPORTING ONLINE MATERIAL

Materials and Methods

In vitro CCA addition assays. His-tagged versions of the CCA-adding enzymes from human (*13*), *E. coli* (*14*), and *S. shibatae* (*15*) were expressed in *E. coli* strain BL21(DE3) and purified using the QIAexpress Ni-NTA Fast Start kit (Qiagen) under native conditions using the manufacturer's protocol. Unlabeled RNA substrates were *in vitro* transcribed using the MAXIscript kit (Ambion) and gel purified. To assay if the RNAs are substrates for the CCA-adding enzyme, 5 pmol of RNA was incubated in a 10 µl reaction containing 100 mM glycine, pH 9.0, 10 mM MgCl₂, 1 mM DTT, 50 µM ATP, 50 µM CTP, 0.05 µM [α -³²P] CTP or [α -³²P] ATP, and 50 ng of purified protein. Reactions were incubated at 37 degrees (human or *E. coli* enzymes) or 70 degrees (*S. shibatae* enzyme) for 30 minutes (unless otherwise indicated). The sequences of all RNA substrates and Northern probes are provided in **Table S5**.

In vitro decay assays. *In vitro* decay assays were performed using substrates that had a single radioactive phosphate on their 5' ends. Decay assays using HeLa nuclear extracts were performed as previously described (*30*). *E. coli* RNase R was obtained from Epicentre Biotechnologies and decay assays were performed using 20 U of enzyme according to the manufacterer's protocol. Yeast Rrp44 was purified as previously described (*27*) and yeast Xrn1 was obtained from New England Biolabs. Assays were carried out in 30 µl reaction mixtures containing 10 µM Tris-HCl, pH 8.0, 75 mM NaCl, 100 µM MgCl₂, and 1 mM β-mercaptoethanol.

Northern blots and 3' RACE using miRNA Cloning Linker 1 or 3 (Integrated DNA Technologies) were performed as previously described (9).

Multiplex deep sequencing of 3' ends of yeast tRNAs.

Replicates of yeast strains were grown in YPD medium at 28°C until $OD_{600} \sim 1.5$ and then shifted to 37°C. At the indicated time points after shift, 2 mL of culture was harvested, pelleted, rinsed with 1 mL of cold sterile water, pelleted, and frozen on dry ice. RNA was purified from cells using hot phenol as previously described (*22*).

Strain name	Genotype	Source
(Derivative)		
JMW 007	BY4741	Whipple et al. 2011
AA0527	BY4741 trm8- Δ ::natMX trm4- Δ ::kanMX	Alexandrov et al. 2006
(JMW 009)		
JMW 119	BY4741 $trm44-\Delta::natMX$ $tan1-\Delta::kanMX$	Whipple et al. 2011
JMW 316	JMW 119 sup61- Δ ::bleR ade2::tS(CGA) G ₅₁ :C ₆₃	Whipple et al. 2011
JMW 510	BY4741 <i>met22-</i> Δ:: <i>hphMX</i>	This study
JMW 561	JMW 119 sup61- Δ ::bleR ade2::tS(CGA) U ₆ :A ₆₇	Whipple et al. 2011
JMW 1251	JMW 119 sup61- Δ ::bleR ade2::tS(CGA) C ₆ :G ₆₇	Whipple et al. 2011
	$G_{51}:C_{63} G_2:C_{71}$	
ISC 725	BY4742 xrn1- Δ ::bleR	This study
ISC 850	BY4741 $trm44-\Delta::natMX$ $tan1-\Delta::kanMX$ $xrn1-$	Whipple et al. 2011
	Δ :: $bleR$	
LY 1729	$MATa trm44-\Delta::natMX tan1-\Delta::kanMX met22-$	Chernyakov et al. 2008
	Δ :: $hphMX$	

Yeast strains used in this study:

The overall sequencing approach used is diagrammed in **Fig. S16**. A preadenylated oligo (miRNA Linker 3, IDT; 5'-rAppTTTAACCGCGAATTCCAG/3ddC/) was ligated to the 3' ends of 1.5 µg of total RNA using the truncated form of T4 RNA Ligase 2 (New England Biolabs). Ligation reactions were incubated at room temperature for 2 hr followed by a phenol/chloroform extraction and ethanol precipitation. Reverse transcription was then performed using Superscript III (Invitrogen) as per the manufacturer's instructions and 5 pmol of primer complementary to the 3' linker sequence (5'-GACTAGCTGGAATTCGCGGTTAAA). cDNAs were amplified by PCR using AmpliTaq DNA Polymerase (Applied Biosystems).

To sequence the 3' ends of the endogenous tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} transcripts (due to their high similarity, this sequencing approach can not distinguish between them), the following forward (5'-

CAAGCAGAAGACGGCATACGAGGxxxxxx<u>GGCTCTGCCCGCGCTGG</u>, where xxxxxx is a unique 7-nucleotide barcode for each RNA prep so that multiple samples can be run together in a single lane of an Illumina flow cell) and reverse (5'-

AATGATACGGCGACCACCGAGGACTAGCTGGAATTCGCGGTTAAA) primers were used. These forward and reverse primers were also used to amplify the $U_6:A_{67}$ tRNA^{Ser(CGA)} variant in **Fig. 4B** and **Fig. S17B** – however, only reads that contained the A₆₇ mutation were included in the downstream analysis.

To sequence the 3' ends of the G_{51} : C_{63} , C_6 : G_{67} , G_2 : C_{71} tRNA^{Ser(CGA)} variant or the G_{51} : C_{63} tRNA^{Ser(CGA)} variant, the following forward (5'-

CAAGCAGAAGACGGCATACGAGGxxxxxxGCGGGGTTCAAATCCCGC, where xxxxxx is a unique 7-nucleotide barcode) and reverse (5'-

AATGATACGGCGACCACCGAGGACTAGCTGGAATTCGCGGTTAAA) primers were used to avoid amplification of the endogenous tRNA^{Ser(UGA)} loci.

The 7-nucleotide barcodes used were:

ACTGCGC	TCGTCAG	ATCGACG	CTCACAT
ATATCAC	TGTACGC	AGAGTAG	CTCTGCA
ATGCTAC	TGTGCAG	AGTCGAG	ACGTAGC

CACGTCA	AGTATAC	TCGTGTC	ATAGAGC
CATCAGA	TAGCACG	AGATATC	TGATGAC
CGAGACA	ACACGAC	TAGTCGC	ACAGCTG
CGCTCGT	ATCAGAC	TCTAGAC	ATGCATG
CTAGTGA	CGTCTCA	AGTCAGC	TACGTCG
TACTATG	CGTGTGT	TACGAGC	TAGATAG
TATAGCG	CGCATGA	TCGATGC	TGAGTGC
TATCATC	CGACAGT	TGCTCTC	TATGTAC
TCACAGC	AGCTGCG	CAGTGCT	

PCR reactions were incubated at 94° for 2 min, followed by 21 cycles of 94° for 15 sec, 58° for 30 sec, and 72° for 30 sec. All PCR reactions were then combined in one tube and extracted with phenol/chloroform and ethanol precipitated. Using a 2% low melting point agarose gel, the PCR products (~110 to 120 bp) were purified and then cleaned up using a phenol extraction, followed by a phenol/chloroform extraction, followed by a chloroform extraction. The purified DNA was resuspended in water and subjected to deep sequencing using the Illumina HiSeq 2000 Sequencing System and the following sequencing primer (5'-CCGAGGACTAGCTGGAATTCGCGGTTAAA). 58 nucleotide reads from the 3' ends of each transcript were obtained. At least three biological replicates were performed for each strain/time point as shown in **Tables S1-4**. This required the use of multiple sequencing lanes.

The resulting sequencing files were processed by collapsing identical reads and reverse complementing the sequences (as this sequencing strategy actually sequences the reverse complement of a given transcript). The reads were then separated into the individual libraries by only using reads that contained perfect 7-nucleotide barcodes and perfect gene-specific forward primer sequences (the underlined regions in the primer). For the CCACCA addition analysis (**Table S1**), we only included sequences that were represented by 2 or more reads in the downstream analysis. For the tRNA^{Ser(CGA)} variants, however, we included all reads as these libraries generally had much lower overall read counts (**Table S2**). To calculate the percentage of reads that ended in an extended CCA motif in a given sample, we used the following formula:

of reads ending in CCAC, CCACC, or CCACCA # of reads ending in CCA x 100

Although we did detect a handful of reads ending in CCACCAC, CCACCACC, or CCACCACCA (**Tables S1 and S2**), these reads were not included in the analysis.

For the poly(A) tail analysis (**Tables S3 and S4**), we only included sequences that were represented by 10 or more reads in the downstream analysis. For libraries with low overall read counts (*xrn1*- Δ Replicates #2, 3 and the tRNA^{Ser(CGA)} variants), we included sequences that were represented by 2 or more reads in the analysis. To calculate the percentage of reads ending in polyadenylated CCA motifs in a given sample, we used the following formula:

Due to the length of our sequencing reads, only transcripts with 12 or fewer A's were included in our analysis as a complete 7-nt barcode would not be sequenced if the transcript ended in 13 or more A's. Although we did detect tRNAs with up to 19 A's on

their 3' ends (data not shown), all sequences with more than 12 A's were not included in our analysis as we could not unambiguously assign them to their proper library.

Supporting Text

CCACCA addition requires isomerization of the tRNA acceptor stem

Only those MEN β tRNA-like small RNA homologs that have unstable acceptor stems were targets for CCACCA addition in vitro (Fig. 2D) or in vivo (Fig. S1 and fig. S7). However, the presence of instability is not sufficient to generate a CCACCA target, as a mouse mascRNA mutant (Mut 7, Fig. S8A) that has a C-A mismatch in its acceptor stem remained a CCA target (Fig. S8, B and C). Other single point mutations in mascRNA (Mut 5-9, Fig. S8A) also had no effect on the specificity of the *E. coli* (Fig. S8B) or human (Fig. S8C) CCA-adding enzymes. Introduction of four mutations (Mut 10, Fig. S8A), all located at the end of the acceptor stem, was sufficient to convert mascRNA from a CCA to a CCACCA target in vitro (Fig. S8, D and E). A similar transcript (Mut 11, Fig. S8A) containing three mutations but no predicted instability only had CCA added to its 3' end (Fig. S8, D and E), indicating that both instability and specific nucleotides at the end of the acceptor stem were required for CCACCA addition. The distal base pairs of the tRNA acceptor stem are critical for the accuracy of the genetic code as this structure commonly contains identity elements that determine which amino acid will be used to charge the tRNA (31, 32).

To address how the CCA-adding enzyme monitors nucleotide identity at specific positions in the acceptor stem, we generated the Mut 10A mascRNA transcript, which is identical to the Mut 10 RNA except that the C-A mismatch has been swapped across the stem (Fig. S8A). Unlike the Mut 10 substrate, the Mut10A transcript was a CCA target (Fig. S8, F and G). Thus, the CCA-adding enzyme either requires specific nucleotides at the swapped positions or these nucleotides need to form base pairs with other nucleotides

in an alternative RNA conformation for CCACCA addition to occur. In support of the latter, we recognized that after the first CCA is added, the instability in the acceptor stem would facilitate RNA isomerization, such that the right side of the acceptor stem is shifted by three nucleotides (Fig. 2F). The A of the CCA sequence would thus be the new discriminator base and be positioned in the enzyme's catalytic pocket, ready to undergo a second round of CCA addition using the well-established polymerization mechanisms (*8*, *17-19*).

Pursuing this model, the mascRNA Mut 10, but not Mut 10A, transcript can form a base pair in the shifted conformation between nucleotide 3 and the last encoded nucleotide (the original discriminator base) (Fig. S8A). Therefore, it might be possible to convert the Mut 10A transcript to a CCACCA target by mutating the last encoded nucleotide to a U (Mut 10AD, Fig. S8A), thus establishing an A-U base pair in the shifted conformation. Indeed, the Mut 10AD construct was a CCACCA target (Fig. S8, F and G), providing strong support for the RNA isomerization model (Fig. 2E). Interestingly, additional mutational analysis revealed that CCACCA addition can occur even when a base pair can not form between nucleotide 3 and the last encoded nucleotide (Fig. S9). In particular, CCACCA addition to the Mut 10A construct occurs if the end of the acceptor stem is further weakened by the presence of a G-U wobble base pair (Mut 10AU, Fig. S8A and fig. S9E). This is likely because the shifted conformation of the Mut10AU, but not the Mut10A, substrate is predicted to be thermodynamically more stable than the nonshifted conformation. We, therefore, conclude that CCACCA addition requires the transcript to isomerize to a shifted conformation that is, at minimum, of similar predicted stability as the non-shifted conformation.

Combining the results from all of the mutational analysis suggests rules for addition of CCACCA by the CCA-adding enzyme. For CCACCA addition, the RNA must have (1) an unstable acceptor stem, which allows isomerization; (2) guanosines at the first and second positions of the RNA, which form base pairs with the Cs of the first CCA in the shifted conformation; and (3) the ability to isomerize to a stable shifted conformation, e.g. due to a base pair between the third nucleotide and the last encoded nucleotide. We tested and confirmed these rules by mutating the mouse MEN β tRNAlike small RNA, converting it from a CCACCA to a CCA target (Fig. S10), and by defining a minimal set of mutations that convert the canonical tRNAs used in Fig. S5 to CCACCA targets (Fig. S11). In human, 238 annotated tRNAs begin with GG and 66 of these can form a base pair between nucleotide 3 and the last encoded nucleotide (35 form a G-C or A-U base pair, 31 a G-U wobble).

Supplementary Figure Legends

Fig. S1. CCACC(A) is added to the 3' end of the human and mouse MEN β tRNAlike small RNA transcripts. The 3' end of the MEN β tRNA-like small RNA was cloned using a ligation-based approach (3' linker oligo is designated) and RNA from human HeLa cervical carcinoma cells (A), mouse C2C12 myoblast cells (B), or mouse EpH4-A6 mammary epithelial cells transformed by ErbB2 over-expression (*33*) (C). * denotes a common nucleotide modification site observed in mascRNA (*9*) and several of the cDNA clones.

Fig. S2. Purification and validation of His-tagged CCA-adding enzymes from all three kingdoms of life. (A to C) His-tagged versions of the CCA-adding enzymes from human (A), *E. coli* (B), and *S. shibatae* (C) were expressed and purified from *E. coli*. 5 µl of each fraction was loaded on a SDS-PAGE gel that was subsequently stained with Coomasssie blue. Eluate 2 of each prep was used for all subsequent *in vitro* CCA addition assays. (D to F) *In vitro* CCA addition assays using a threonine tRNA as a substrate to verify the specificity of the purified enzymes. The unlabeled tRNA substrates ended in CCA, CC, C or simply the discriminator base (designated No CCA) and were incubated with the CCA-adding enzymes from human (D), *E. coli* (E), or *S. shibatae* (F) in the presence of an excess of cold CTP and ATP as well as a trace amount of either $[\alpha^{-32}P]$ CTP or $[\alpha^{-32}P]$ ATP. The reactions were then run on 6% polyacrylamide gels and exposed to a PhorphorImager screen. As the input RNA substrates are not labeled, a band is only observed when the CCA-adding enzyme incorporated the hot nucleotide onto the 3' end of the transcript. In (F), CTP reactions were incubated for only 5 min.

Fig. S3. CCACC(A) is added to the 3' end of the MEN β tRNA-like small RNA *in vitro*. (A) *In vitro* CCA addition assays using a threonine tRNA (top), the human MEN β tRNA-like small RNA (middle), or the mouse MEN β tRNA-like small RNA (bottom). The unlabeled substrates ended in the discriminator base (designated No CCA) or CCA and were incubated with the *E. coli* enzyme in the presence of an excess of cold CTP and ATP as well as a trace amount of either $[\alpha$ -³²P] CTP or $[\alpha$ -³²P] ATP. As expected, the threonine tRNA with CCA already on its end was unable to accept any additional nucleotides. In contrast, both the human and mouse MEN β tRNA-like transcripts with CCA already on their 3' ends were efficiently extended. (B) *In vitro* transcribed mouse MEN β tRNA-like small RNA lacking CCA at its 3' end was used as a substrate for the *E. coli* CCA-adding enzyme. A ligation-based 3' RACE approach (3' linker oligo is designated) was then used to clone the 3' ends of the *in vitro* reaction products.

Fig. S4. CCA-adding enzymes from all three kingdoms of life are able to build and repair a 3'-terminal CCACCA motif. (A) Generation of mouse mascRNA (denoted mmascRNA) chimeras in which one of the arms was swapped with that of the mouse MEN β tRNA-like small RNA. All sequences derived from mascRNA are in uppercase, whereas all sequences derived from the MEN β tRNA-like small RNA are in lowercase. (B) *In vitro* transcribed mmascRNA or mmascRNA Mut 1 lacking CCA at its 3' end were used as substrates for the *E. coli* CCA-adding enzyme. A ligation-based 3' RACE approach (3' linker oligo is designated) was then used to verify that the *E. coli* enzyme adds CCACCA to the 3' end of the mmascRNA Mut 1 transcript *in vitro*. (C) Swapping the mmascRNA acceptor stem with that of the mouse MEN β tRNA-like small RNA

(Mut 1) converts the transcript to a CCACCA target for the *E. coli* enzyme. (**D** to **F**) Wild-type mouse mascRNA or mmascRNA Mut 1 substrates with different 3' termini were incubated *in vitro* with the CCA-adding enzymes from human (**D**), *E. coli* (**E**), or *S. shibatae* (**F**). All three enzymes are able to repair the 3'-terminal CCA or CCACCA motifs on mmascRNA and mmascRNA Mut 1, respectively. Consistent with a previous report that demonstrated that the *E. coli* CCA-adding enzyme is able to add A to the 3' end of any RNA ending in CC *in vitro* (*34*), we observed that the *E. coli* CCA-adding enzyme efficiently adds A to the mmascRNA substrate ending in CCACC.

Fig. S5. Swapping the acceptor stems of canonical tRNAs with that of the MEN β tRNA-like small RNA converts the transcripts to CCACCA targets *in vitro*. Wild-type tRNAs or ones with their acceptor stem swapped with that of mouse mascRNA or mouse MEN β tRNA-like small RNA [denoted at top in (A)] were used as substrates. RNA substrates either lacked CCA on their 3' ends (A) or had various 3' termini as denoted at the top (B). Whereas wild-type tRNAs and tRNAs with the mascRNA acceptor stem were substrates for CCA addition, all tRNAs tested that had the MEN β acceptor stem were substrates for CCACCA addition.

Fig. S6. Conservation of mascRNA and the MEN β tRNA-like small RNA across species. The acceptor stems of the two transcripts are shaded in blue. Compared to mascRNA, the right side of the MEN β tRNA-like small RNA acceptor stem has been very poorly conserved throughout evolution, suggesting that it may be becoming a pseudogene.

Fig. S7. The MEN β tRNA-like small RNA is a CCA target and stable transcript in African Green Monkey COS7 cells. (A) Northern blot analysis using 15 µg of total RNA from human HeLa cells, human MCF7 cells, or African Green Monkey COS7 cells. Unlike the human homolog, expression of the African Green Monkey MEN β tRNA-like small RNA was easily detected. U6 snRNA was used as a loading control. (B) The 3' ends of mascRNA and the MEN β tRNA-like small RNA were cloned using a ligationbased approach (3' linker oligo is designated) and RNA from COS7 cells.

Fig. S8. The tRNA acceptor stem is isomerized to allow for CCACCA addition. (A) Predicted secondary structures of the acceptor stems from wild-type and mutant mouse mascRNA transcripts. Mut 1 is equivalent to the acceptor stem of the mouse MEN β tRNA-like small RNA and was used as a guide to generate the other mutants. Nucleotides in blue are conserved between mouse mascRNA and the mouse MEN β tRNA-like small RNA, while nucleotides in black or red are specific to mascRNA or the MEN β transcript, respectively. (B to G) *In vitro* assays using the *E. coli* or human CCA-adding enzyme (as noted at the top of each figure part) were performed using WT or mutant mascRNA substrates. We obtained highly similar results with both enzymes, indicating that the human and *E. coli* enzymes recognize the same features in the tRNA acceptor stem to determine whether to add CCA or CCACCA.

Fig. S9. A base pair between the third nucleotide and the discriminator base in the shifted conformation is not absolutely required for CCACCA addition. (A) In contrast to Fig. S8 where we swapped the mouse mascRNA acceptor stem with that of the mouse MEN β tRNA-like small RNA, here we swapped the mouse mascRNA

acceptor stem with that of the human MEN β tRNA-like small RNA (generating the Mut 14 substrate). The human MEN β acceptor stem has no mismatches but does have multiple G-U wobble base pairs at the end of the stem. We then generated additional mutants to determine which nucleotide changes are required to convert mascRNA from a CCA to a CCACCA target. (B) Wild-type mouse mascRNA or the Mut 14-17 substrates were incubated with the E. coli CCA-adding enzyme. Of particular interest, CCACCA was efficiently added to the 3' end of the Mut 16 substrate despite the fact that it can not form a base pair between the third nucleotide and the discriminator base (it instead forms a C-U mismatch). (C) Analogous to the strategy used to generate the mascRNA Mut 10A and Mut 10AD constructs from Mut 10 in Fig. S8A, we swapped the third base pair of Mut 15 across the stem to generate the Mut 15A substrate. Whereas Mut 15 can form a C-G base pair between the third nucleotide and the discriminator base, Mut 15A forms a G-G mismatch and Mut 15AD re-establishes the potential for this base pair. Interestingly, Mut 15, 15A, and 15AD are all efficient substrates for CCACCA addition, likely because the shifted conformations of all three substrates are predicted to be of similar stability as the non-shifted conformations. (D) Comparison of the acceptor stems of mascRNA Mut 10A, a CCA target, and Mut 15A, a CCACCA target. There are only three point mutations between the two substrates (denoted in red), which we used as a guide to generate the Mut10AU construct in Fig. S8A. (E) CCACCA addition to the Mut10A construct can be rescued by the presence of a G-U wobble at the end of the acceptor stem (Mut 10AU).

Fig. S10. Mutational analysis to convert the mouse MEN β tRNA-like small RNA from a CCACCA to a CCA target *in vitro*. (A) Predicted secondary structures of the

acceptor stems from the wild-type and mutant mouse MEN β tRNA-like transcripts. Mut 1 is equivalent to the acceptor stem of mouse mascRNA and was used as a guide to generate the other mutants. Below the structures is a chart that describes if each substrate follows the rules for CCA or CCACCA addition and if the prediction matches the experimental data in **(B)**. **(B)** *In vitro* CCA addition assays using the *E. coli* enzyme. We found that the wild-type mouse MEN β tRNA-like small RNA transcript consistently migrated anomalously (it is expected to run at 63 nucleotides, but generally ran as a doublet at ~55 and ~60 nucleotides, possibly suggesting different RNA conformations). The addition of a single nucleotide in the D-arm (Mut 2 substrate) had no effect on CCACCA addition but caused the transcript to migrate as a single band at the expected size, so all other mutant substrates also have this change in their D-arms and are thus designated Mut #+D. All mutant substrates followed the rules for CCA versus CCACCA addition with the possible exception of Mut 6+D which was weakly able to accept an additional C when it already had CCA on its end.

Fig. S11. Mutational analysis to convert canonical tRNAs from CCA to CCACCA targets *in vitro*. (A) Predicted secondary structures of the acceptor stems from wild-type and mutant tRNA-Thr-ACY transcripts. Mut 1 and Mut 2 are equivalent to the acceptor stems of mouse mascRNA and the mouse MEN β tRNA-like small RNA, respectively. The MEN β acceptor stem was used as a guide to generate the other mutants. Below the structures is a chart that describes if each substrate follows the rules for CCA or CCACCA addition and if the prediction matches the experimental data. (B) *In vitro* CCA addition assays using the *E. coli* enzyme and wild-type or mutant tRNA-Thr-ACY substrates that lack CCA at their 3' termini. (C) Similar mutational analysis was

performed using a tRNA-Leu-CTY transcript. **(D)** *In vitro* CCA addition assays using the *E. coli* enzyme and wild-type or mutant tRNA-Leu-CTY substrates that lack CCA at their 3' termini. All mutant threonine and leucine tRNAs followed the rules for CCA versus CCACCA addition.

Fig. S12. GG is enriched at the 5' ends of tRNA genes from all three kingdoms of life. The sequences of all tRNA genes (including potential pseudogenes, if present) encoded in the genomes of 16 species were obtained from the Genomic tRNA Database (http://gtrnadb.ucsc.edu). As GG is required at the 5' end of a tRNA for CCACCA addition to occur, we analyzed the sequences to determine how common GG is at the 5' end of tRNAs. For each species, the actual number of tRNAs that have a given dinucleotide combination at their 5' end and the corresponding percentage of the total are shown in the # and % columns, respectively. The numbers in red indicate the most abundant 5' end combination in each species. In the Summary table, we combined the data from all 16 species to show that, in general, GG is the most common dinucleotide combination at the 5' ends of tRNAs. One would only expect 6.25% of tRNAs to start with GG if the sequences were completely random. Sequences are from Arabidopsis thaliana (Feb 2004), Caenorhabditis elegans (Jan 2007), Drosophila melanogaster (Release 5 Apr 2006), Homo sapiens (hg18 - NCBI Build 36.1 Mar 2006), Mus musculus (mm9 July 2007), Saccharomyces cerevisiae, Zea mays (Version 4a.53), Escherichia coli K12, Haemophilus influenzae, Mycobacterium tuberculosis CDC1551, Mycoplasma pneumoniae, Vibrio cholerae, Candidatus Korarchaeum cryptofilum OPF8, Methanosarcina acetivorans, Nanoarchaeum equitans, and Sulfolobus solfataricus.

Fig. S13. Single point mutations can convert arginine tRNAs from CCA to

CCACCA targets *in vitro*. (A) We chose to mutate two unrelated arginine tRNAs that have GG at their 5' ends and the ability to form a base pair between the third nucleotide and the discriminator base. Nevertheless, the wild-type tRNAs have stable acceptor stems, making them targets for CCA addition. Single point mutations were introduced to destabilize the acceptor stem as shown. (B to C) *In vitro* CCA addition assays using the human or *E. coli* CCA-adding enzyme. All of the single point mutations tested were sufficient to efficiently convert the tRNA from CCA to CCACCA addition.

Fig. S14. Single point mutations can convert a cysteine tRNA from a CCA to a

CCACCA target *in vitro*. (A) We mutated a wild-type cysteine tRNA by introducing single point mutations that should destabilize the acceptor stem. The last mutant (G2A) creates an unstable acceptor stem but mutates the 5' end of the transcript from GG to GA and was thus expected to remain a target for CCA addition. (B) *In vitro* CCA addition assays using the *E. coli* enzyme. All mutants followed the rules for CCA versus CCACCA addition, including the G2A mutant, which remained a CCA target.

Fig. S15. Mutant tRNAs ending in CCACCA are rapidly degraded *in vitro*. The *in vitro* decay assay in **Fig. 3C** was repeated using a shorter time course. Radiolabeled wild-type or mutant arginine tRNAs with CCA or CCACCA at their 3' ends, respectively, were incubated in HeLa nuclear extracts for the indicated amounts of time. Arrow denotes a slight accumulation of wild-type tRNAs cleaved in the anticodon loop.

Fig. S16. Multiplex deep sequencing strategy used to analyze the 3' ends of specific tRNAs. (A) Using total RNA isolated from various conditions, a linker oligo (drawn in brown) is first ligated to the 3' ends, which is subsequently used as a priming site for first-strand cDNA synthesis. A gene-specific forward primer containing a unique barcode is then used for 3' RACE PCR. By combining all of the PCR products from each of the conditions together, multiplex deep sequencing can be performed. The barcodes are then used to determine the source of each read, allowing one to analyze the 3' end of a given tRNA under many conditions. **(B)** Diagram depicting the primers used for 3' RACE PCR on tRNA^{Ser(CGA)}. The gene-specific forward primer hybridizes to a region spanning the variable arm and T-stem.

Fig. S17. Short poly(A) tails are added to mature tRNAs being degraded by RTD *in vivo*. (A) In addition to increases in CCACCA addition levels during RTD, we noticed a significant number of mature tRNAs that had a short poly(A) tail added to their CCA end when the transcripts were being actively degraded. A low, but reproducible, level of polyadenylated CCA motifs (defined as CCAA, CCAAA, etc.) was detected on tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} in a wild type strain grown at 28°C or 37°C (**Table S3**). Upon switching the *trm44-* Δ *tan1-* Δ deletion strain to growth at 37°C, a progressive increase in polyadenylated CCA motifs was observed (41.6 +/- 8.8 fold over wild-type levels at the 6 hr time point), which mirrors the time course of tRNA degradation. Deleting Xrn1 or Met22 rescued this effect. (B) As in Fig. 4B, we replaced the endogenous tRNA^{Ser(CGA)} gene in the *trm44-* Δ *tan1-* Δ deletion strain with the tRNA^{Ser(CGA)} variants shown. Increasing the structural stability of the tRNAs made the transcripts resistant to polyadenylation and RTD. Error bars represent standard deviation. Fig. S18. Mature tRNAs ending in CCACCA are efficiently degraded in vitro. (A) Radiolabeled wild-type tRNAs with CCA or CCACCA at their 3' ends (as denoted at top) were incubated with E. coli RNase R, a 3'-5' exonuclease, for the indicated amounts of time. (B) An arginine tRNA ending in CCACCA, but not CCA, was efficiently degraded by yeast Rrp44 in vitro. Degradation is dependent on the 3'-5' exonuclease activity of Rrp44 as a point mutation in the active site (D551N) abrogated this effect. (C) Mature tRNAs (radioactively labeled at their 5' end) ending in CCACCA were incubated with yeast Rrp44, yeast Xrn1, or both enzymes for the indicated amounts of time. Increased degradation rates were observed when both 3'-5' and 5'-3' exonucleases were present. As the transcripts are radioactively labeled only at their 5' ends, the degradation intermediates observed in the Rrp44 alone reactions correspond to tRNAs with shortened 3' tails. This suggests that Rrp44 pauses once it nears the double-stranded tRNA acceptor stem. In the Xrn1-deficient strain, we propose that only the extended 3' tail of the transcript would be removed, resulting in a rescue of the phenotype at high temperatures and in the observed decrease in transcripts ending in CCACCA in Fig. 4A.

Fig. S19. The CCA-adding enzyme functions in tRNA maturation, repair, and

quality control. A primary tRNA transcript in eukaryotes is cleaved by RNase P and RNase Z near its 5' and 3' ends, respectively, to generate a transcript that is recognized by the CCA-adding enzyme (drawn in purple). For a normal tRNA with a stable structure, the CCA-adding enzyme catalyzes the addition of CCA to its 3' end. Once released from the CCA-adding enzyme, the tRNA is recognized by the appropriate aminoacyl tRNA synthetase, which charges it with an amino acid, thus completing the maturation of the tRNA. If the CCA terminus becomes damaged at any point, the CCA-

adding enzyme recognizes the damage and repairs it, thus re-generating a functional tRNA that can be aminoacylated and used by the ribosome. In addition to these roles in tRNA maturation and repair, our work has now shown that the CCA-adding enzyme functions in tRNA quality control. If the tRNA structure becomes destabilized (e.g. because of mistakes during transcription or the lack of post-transcriptional nucleotide modifications) and the other rules are followed, the CCA-adding enzyme recognizes an alternate conformation of the tRNA, such that a second CCA motif is added to the 3' end. Alternatively, a short poly(A) tail can be added to the CCA motif. These structurally unstable tRNAs are then targeted for degradation, preventing potential downstream errors in aminoacylation or translation.

Fig. S20. Identification of two tRNAs that are encoded in the genome as CCACCA targets reveals likely regulatory roles for CCACCA addition. (A) In the jakobid protist *Seculamonas ecuadoriensis*, the mitochondrial serine tRNA contains two mismatched base pairs in its acceptor stem (shown in red). Interestingly, these mismatches are corrected post-transcriptionally *in vivo* to generate canonical base pairs (*28*). The arrows show the nucleotide changes introduced by editing. Leigh and Lang proposed that that this occurs by exonucleolytic degradation of the 3' end of the tRNA acceptor stem followed by repair by a hypothetical enzyme whose activity resembles that of the CCA-adding enzyme because nearly all of the inserted nucleotides are either C or A (*28*). **(B)** *In vitro* CCA addition assays using the human or *E. coli* enzymes. Unlike the fully edited form, the genomic version of the *S. ecuadoriensis* mitochondrial serine tRNA is a CCACCA target. **(C)** As the hypothetical enzyme proposed by Leigh and Lang has never been identified, we instead propose a model in which the CCA-adding enzyme

adds CCACCA to the end of the tRNA, followed by a splicing-like event that removes the three bulged nucleotides. CCACCA addition by the CCA-adding enzyme may, therefore, be critical for the maturation of certain tRNAs. (**D and E**) In the amoeboid protozoan *Acanthamoeba castellanii*, the acceptor stem of the mitochondrial alanine tRNA contains a mismatch between U₃:U₇₀, causing the transcript to be subjected to CCACCA addition *in vitro*. However, this mismatch is corrected to an A-U base pair *in vivo* by a previously identified post-transcriptional RNA editing event (*29*), generating a functional tRNA that is a CCA target. We, therefore, propose that CCACCA addition serves as a checkpoint that ensures that this mismatch is corrected, an event that is especially critical for this tRNA as the third base pair is a major determinant of alanine aminoacylation specificity in many species.

Supplementary Table Legends

Table S1. Addition of extended CCA motifs to tRNA^{Ser(CGA)} **and tRNA**^{Ser(UGA)} **during RTD.** The data used in **Fig. 4A** is shown. For each sample, the total number of reads in the library as well as the number of reads that ended in CCA, CCAC, CCACC, CCACCA, etc. is given. As described in the Materials and Methods, we summed the number of reads ending in CCAC, CCACC, or CCACCA to determine the percentage of reads ending in an extended CCA motifs (Column 14).

Table S2. Stabilizing the structure of tRNA^{Ser(CGA)} **inhibits RTD and CCACCA addition.** The data used in **Fig. 4B** is shown. For each sample, the total number of reads in the library as well as the number of reads that ended in CCA, CCAC, CCACC, CCACCA, etc. is given. As described in the Materials and Methods, we summed the number of reads ending in CCAC, CCACC, or CCACCA to determine the percentage of reads ending in an extended CCA motifs (Column 14).

Table S3. Addition of poly(A) tails to mature tRNA^{Ser(CGA)} **and tRNA**^{Ser(UGA)} **transcripts during RTD.** The data used in **Fig. S17A** is shown. For each sample, the total number of reads in the library as well as the number of reads that ended in CCA, CCAA (denoted 2 A's), CCAAA (denoted 3 A's), CCAAAA (denoted 4 A's), etc. is given. As described in the Materials and Methods, we summed the number of reads ending in 2 to 12 A's to determine the percentage of reads ending in a polyadenylated CCA motif (Column 19). **Table S4. Stabilizing the structure of tRNA**^{Ser(CGA)} **inhibits RTD and the addition of poly(A) tails.** The data used in **Fig. S17B** is shown. For each sample, the total number of reads in the library as well as the number of reads that ended in CCA, CCAA (denoted 2 A's), CCAAA (denoted 3 A's), CCAAAA (denoted 4 A's), etc. is given. As described in the Materials and Methods, we summed the number of reads ending in 2 to 12 A's to determine the percentage of reads ending in a polyadenylated CCA motif (Column 19).

Table S5. Sequences of all RNA substrates and Northern blot probes used. The

sequences of all RNA substrates are given as the "No CCA" version. Additional nucleotides (C, CC, CCA, CCAC, CCACC, or CCACCA) were included at the 3' ends of the substrates as indicated in the figures and text. All substrates that start with GG at their 5' ends were transcribed using T7 RNA polymerase, whereas all substrates that start with GA were transcribed using SP6 RNA polymerase.

Supplementary References

- 30. L. P. Ford, P. S. Bagga, J. Wilusz, *Mol Cell Biol* 17, 398 (1997).
- 31. R. Giege, M. Sissler, C. Florentz, Nucleic Acids Res 26, 5017 (1998).
- 32. M. Ibba, D. Soll, Annu Rev Biochem 69, 617 (2000).
- V. R. Fantin, M. J. Berardi, L. Scorrano, S. J. Korsmeyer, P. Leder, *Cancer Cell* 2, 29 (2002).
- 34. E. Lizano, M. Scheibe, C. Rammelt, H. Betat, M. Morl, *Biochimie* 90, 762 (2008).

HeLa

hGenomic DNA	GGTGGCACGTCCAGCACGGCTGGGCCCGGGGTTCGAGTCCCCGCAGTGTTGCTGCTTCCTTC
17 Clones 4 Clones	GGTGGCACGTCCAGCACGGCTGGGCCCGGGGTTCGAGTCCCCGCAGTGTTGCCACCACtgtaggcaccatcaatc GGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGGGCCCCGCAGTGTTGCCCACCACCACtgtaggcaccatcaatc * Ligated 3' Linker
2 Clones 1 Clone	GGTGGCACGTCCAGCACGGCTGGGCCCGGGGTTCG G GTCCCCGCAGTGTTGCCACCCtgtaggcaccatcaatc GGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCGCAGTGTTGCCACCCCtgtaggcaccatcaatc
1 Clone	GGTGGCACGTCCAGCACGGCTGGGCCCGGGGTTCGAGTCCCCGCAGTGTTGCCctgtaggcaccatcaatc Ligated 3' Linker

В

Α

C2C12

mGenomic DNA	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCGTGCTTCCTTC
9 Clones	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG <mark>CCACC</mark> ctgtaggcaccatcaatc
	Ligated 3' Linker
1 Clone	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG <mark>CC</mark> ctgtaggcaccatcaatc
	Ligated 3' Linker

С

EpH4-A6

mGenomic DNA	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCGTGCTTCCTTC
1 Clone	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCGCCACCACtgtaggcaccatcaatc
	Ligated 3' Linker
12 Clones 2 Clones 1 Clone	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCGCCACCCCCGCACCTCGGGCCAGGGTTCGGGTCCCTGCAGTACCGCCACCCCCGCACCTCGGGCCAGGGTTCGGCGCCGCACCCCGCACCCCCGCACCCCGCACCCCGCCCCGCACCCCGGGCCAGGGTTCGGCCAGGGTCCGCACCCCGCACCCCCCCC
4 Clones	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG CCAC ctgtaggcaccatcaatc Ligated 3' Linker
3 Clones	GGCACGCCCGCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG CCA ctgtaggcaccatcaatc



Figure S2

Α



В

mMEN	β	Sul	Substrate	
mMEN	β	3′	RACE	1
mMEN	β	3′	RACE	2
mMEN	β	3′	RACE	3
mMEN	β	3′	RACE	4
mMEN	β	3′	RACE	5
mMEN	β	3′	RACE	6
mMEN	β	3′	RACE	7

GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCGCCACCA	-

GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG

Ligated 3' Linker
GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG CCACCA ttaaccgcgaattccagc
${\tt GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG} {\tt CCACCA} {\tt ttaaccgcgaattccagc}$
GtACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG <mark>CCACC</mark> tttaaccgcgaattccagc
GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG <mark>CCACC</mark> tttaaccgcgaattccagc
GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG CCACCA ttaaccgcgaattccagc
GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG <mark>CCACC</mark> tttaaccgcgaattccagc
GCACCTCGGGCCAGGGTTCGAGTCCCTGCAGTACCG CCACCA ttaaccgtgaattccagc

Α	
Mouse mascRNA	GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGAC <mark>GGGGTTCAAGTCCC</mark> TGCGGTGTCT
Mouse MEN eta tRNA	gcactggtggc*ggcacgcccgcacctcgggcc <mark>agggttcgagtccc</mark> tgcagtaccg
	Acceptor Stem D Arm Anticodon Arm T Arm
mmascRNA Mut 1	ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTG
mmascRNA Mut 2	GACGCTG <mark>gtggc*ggcac</mark> TCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCT
mmascRNA Mut 3	GACGCTGGTGGCTGGCAC <mark>gcccgcacctcgggcc</mark> GGGGTTCAAGTCCCTGCGGTGTCT
mmascRNA Mut 4	GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGAC <mark>agggttcgagtccc</mark> TGCGGTGTCT

В

mmascRNA 3' RACE

3' RACE 1 GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCTCCA Ligated 3' Linker

mmascRNA Mut 1 3' RACE

3′	RACE	1	ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg <mark>CCACCA</mark> tttaaccgcgaattccagc
3′	RACE	2	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	3	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	4	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	5	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	6	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	7	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
3′	RACE	8	${\tt ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg{\tt CACCA}{\tt ttaaccgcgaattccagc}$
			Ligated 3' Linker













mascRNA

Human	gatgctggtggttggcactcctgg-tttccaggacggggttcaaatccctgcggcgtct
Chimp	aaaa
Orangutan	
Rhesus	a
Baboon	a
Tree shrew	
Mouse	c
Kangaroo rat	
Guinea pig	
Rabbit	aa
Pika	at.gaa
Alpaca	aaa
Cow	
Horse	gg.
Cat	aaa
Dog	aaaa
Microbat	g.gt
Hedgehog	a.cccca.aa.gag.g
Elephant	t
Armadillo	
Wallaby	a
Opossum	ag.ga
Platypus	aaaa
X. tropicalis	a.c
Stickleback	
Medaka	
Zebrafish	act

MEN β tRNA-like small RNA

Human	ggcgctggtggtggcacgtccagcacggctgggccggggttcgagtccccg <mark>cagtgttg</mark>
Chimp	
Gorilla	
Orangutan	t
Rhesus	a
Baboon	at
Tree shrew	ta
Mouse	accc.tcat.
Rat	aactg
Kangaroo rat	
Guinea pig	aa.g.ta
Rabbit	
Pika	
Alpaca	atttt
Dolphin	aa
Cow	t
Horse	a
Dog	.aat.tat.t.t.tat.tg.ccc
Hedgehog	c
Elephant	
Wallaby	acgc



В

mascRNA

Genome GTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAATCCCTGCGGCATCTTTGCTTTG

RACE	#1	GTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCA T ATCCCTGCGGCATCT CCA tttaaccgcgaattccagctagtc
RACE	#2	${\tt GTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCA{\tt T}{\tt A}{\tt T}{\tt C}{\tt C}{\tt C}{\tt G}{\tt G}{\tt C}{\tt A}{\tt C}{\tt C}{\tt C}{\tt C}{\tt A}{\tt C}{\tt C}{\tt C}{\tt C}{\tt C}{\tt C}{\tt C}{\tt C$
RACE	#3	${\tt GTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCA} {\tt G} {\tt AGGTTGGCGCATCT} {\tt CCA} {\tt ttaaccgcgaattccagctagtc} {\tt ccagctagtc} {\tt ccagc$
RACE	#4	${\tt GTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCA{t}atccctGCGGCATCT}{\tt CCA}{\tt ttaaccgcgaattccagctagtc}{\tt ccagctagtc}{\tt ccag$

Ligated 3' Linker

MEN β tRNA-like small RNA

Genome GTGGTGGCACGTCCAGCACGGCTGGGCCCGGGGTTCGAGTCCCCCGCAGTGTCTCTGCTTCC

RACE	#1	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#2	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#3	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCG T GTCCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#4	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#5	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#6	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#7	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#8	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#9	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCG <mark>G</mark> GTCCCCGCAGTGTCT <i>CC</i> At	ttaaccgcgaattccagctagtc
RACE	#10	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCGAGTCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc
RACE	#11	GTGGTGGCACGTCCAGCACGGCTGGGCCGGGGTTCG <mark>G</mark> GTCCCCGCAGTGTCT	ttaaccgcgaattccagctagtc

Ligated 3' Linker







	Mut 10A
g - c g - c a c g - u c - g u - g g - c	g - u g - u g - u g - u c - g u - g g - c

Mut 15A

Mut 10A

Α

A	WT			mMEN β tRN/	Acceptor	Stem Mutan	its	
	mMEN β tRNA	Mut 1	Mut 5	Mut 5A	Mut 6	Mut 7	Mut 8	Mut 9
	5' g - c g - c c a a - u c - g u - a g - c	u g-c a-u c-g g-u c-g u-g g-c	u g - c c a a - u c - g u - a g - c	u g-c g-c a-u c-g u-a g-c	g - c a - u c - a u - a g - c	g - c g - c c - g a - u c - g u - a g - c	g - c g - c c a g - u c - g u - a g - c	g - c g - c c a a - u c - g u - g g - c
	I	mmascRNA						
Rule 1: Instability?	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Rule 2: GG at 5' end?	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Rule 3: +3-D base pair? -or-	Yes	No	No	Yes	Yes	Yes	Yes	Yes
G-U wobble near en	d?	No	No					
Prediction	CCACCA	CCA	CCA	CCACCA	CCA	CCA	CCACCA	CCACCA
Prediction Correct?	Yes	Yes	Yes	Yes	Yes*	Yes	Yes	Yes

Nucleotides shared by mmascRNA and mMEN β tRNA Nucleotides specific to mmascRNA Nucleotides specific to mMEN β tRNA

В

-	+ E. coli									adding Enzyme								
-	[α-32P] CTP									[α-32Ρ] ATP								
	mMEN ß tRNA	mMEN β tRNA Mut 2	mMEN β tRNA Mut 1+D	mMEN ß tRNA Mut 5+D	mMEN β tRNA Mut 5A+D	mMEN β tRNA Mut 6+D	mMEN β tRNA Mut 7+D	mMEN β tRNA Mut 8+D	mMEN β tRNA Mut 9+D	mmen ß tRNA	mMEN β tRNA Mut 2	mMEN eta tRNA Mut 1+D	mMEN β tRNA Mut 5+D	mMEN β tRNA Mut 5A+D	mMEN β tRNA Mut 6+D	mMEN β tRNA Mut 7+D	mMEN β tRNA Mut 8+D	mMEN β tRNA Mut 9+D
70 —	_		_	_		_												
60 —		•	•	•	•	•	•	•	•		•	•	•		•		-	*
50 —														Sub	strat	es: I	No C	СА
70 —								_	_									
60 —	-	-	5		•	-											-	
50 —														Sub	strat	es: (CCA	



WT	Acceptor Stem Mutants										
ACY	Mut 1	Mut 2	Mut 3	Mut 4	Mut 5	Mut 6					
u ³ g - c g - c c - g c - g g - c g - c	u g-c a-u c-g g-u c-g u-g g-c	g = c g = c c = a a = u c = g u = a g = c	g - c g - c c a a - u c - g c - g g - c	u g - c c a a - u c - g c - g g - c	g - c g - c c - g c - g c - g g - c	u g = c c = a u = g c = g g = c					
	mmascRNA	mMEN β tRNA									

Nucleotides present in wildtype tRNA-Thr-ACY Mutant nucleotides

Rule 1: Instability?	No	No	Yes	Yes	Yes	Yes	Yes
Rule 2: GG at 5' end?	Yes	No	Yes	Yes	Yes	Yes	Yes
Rule 3: +3-D base pair? -or-	No	No	Yes	Yes	No	Yes	No
G-U wobble near end?	No	No			No		No
Prediction	CCA	CCA	CCACCA	CCACCA	CCA	CCACCA	CCA
Prediction Correct?	Yes	Yes	Yes	Yes	Yes	Yes	Yes



С

D

		Acceptor S	tem Mutants	
CTY	Mut 1	Mut 2	Mut 3	Mut 4
a ³ 5 g - c g - c u - g a - u g - c c - g g - c	u g-c a-u c-g g-u c-g u-g g-c	g - c g - c c - a c - g u - a g - c	g - c g - c c a a - u g - c c - g g - c	a g = c g = c c = a g = c c = g g = c

Nucleotides present in wildtype tRNA-Leu-CTY Mutant nucleotides

			tRNA		
Rule 1: Instability?	No	No	Yes	Yes	Yes
Rule 2: GG at 5' end?	Yes	No	Yes	Yes	Yes
Rule 3: +3-D base pair? -or-	Yes	No	Yes	Yes	No
G-U wobble near end?		No			No
Prediction	CCA	CCA	CCACCA	CCACCA	CCA
Prediction Correct?	Yes	Yes	Yes	Yes	Yes

mmascRNA mMEN β



Eukaryotes

	<u>A. tha</u>	aliana	C. ele	egans	D. mela	nogaster	<u>H. sa</u>	piens	<u>M. mu</u>	sculus	S. cere	evisiae	<u>Z. n</u>	na <u>ys</u>
	#	%	#	%	#	%	#	%	#	%	#	%	#	%
AA	2	0.3	2	0.2	0	0.0	1	0.2	0	0.0	1	0.3	0	0.0
AC	1	0.2	7	0.9	0	0.0	12	1.9	8	1.8	0	0.0	70	6.0
AG	7	1.1	13	1.6	6	2.0	13	2.1	11	2.5	6	2.0	31	2.7
AU	9	1.4	1	0.1	0	0.0	2	0.3	0	0.0	2	0.7	20	1.7
CA	2	0.3	3	0.4	0	0.0	1	0.2	0	0.0	0	0.0	1	0.1
CC	70	11.0	20	2.4	9	3.0	21	3.3	10	2.3	0	0.0	15	1.3
CG	1	0.2	6	0.7	0	0.0	1	0.2	0	0.0	0	0.0	3	0.3
CU	2	0.3	2	0.2	1	0.3	4	0.6	1	0.2	8	2.7	0	0.0
GA	30	4.7	48	5.9	26	8.6	28	4.4	21	4.8	19	6.4	64	5.5
GC	152	23.8	346	42.2	103	33.9	128	20.3	93	21.5	92	31.2	330	28.3
GG	203	31.8	241	29.4	92	30.3	238	37.7	189	43.6	109	36.9	411	35.2
GU	130	20.3	39	4.8	28	9.2	105	16.6	60	13.9	11	3.7	149	12.8
UA	1	0.2	2	0.2	0	0.0	16	2.5	0	0.0	0	0.0	0	0.0
UC	27	4.2	80	9.8	39	12.8	50	7.9	38	8.8	39	13.2	67	5.7
UG	2	0.3	9	1.1	0	0.0	6	1.0	2	0.5	2	0.7	6	0.5
UU	0	0.0	1	0.1	0	0.0	5	0.8	0	0.0	6	2.0	1	0.1
Total	639		820		304		631		433		295		1168	

	Summary							
	All 16 9	Species						
	#	%						
AA	6	0.1						
AC	102	2.1						
AG	115	2.4						
AU	34	0.7						
CA	7	0.1						
CC	151	3.1						
CG	39	0.8						
CU	19	0.4						
GA	238	4.9						
GC	1448	30.1						
GG	1687	35.1						
GU	545	11.3						
UA	20	0.4						
UC	352	7.3						
UG	36	0.7						
UU	13	0.3						
Total	4812							

Eubacteria

	E. co	li K12	H. infl	uenzae	M. tube	rculosis	M. pneu	imoniae	V. cho	olerae
	#	%	#	%	#	%	#	%	#	%
AA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
AC	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
AG	4	4.5	1	1.7	1	2.2	1	2.7	6	6.1
AU	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
CA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
CC	1	1.1	1	1.7	0	0.0	1	2.7	0	0.0
CG	7	8.0	4	6.9	4	8.9	2	5.4	9	9.2
CU	0	0.0	0	0.0	0	0.0	1	2.7	0	0.0
GA	0	0.0	0	0.0	0	0.0	1	2.7	0	0.0
GC	29	33.0	17	29.3	17	37.8	11	29.7	39	39.8
GG	33	37.5	26	44.8	18	40.0	16	43.2	34	34.7
GU	6	6.8	4	6.9	2	4.4	2	5.4	6	6.1
UA	0	0.0	0	0.0	0	0.0	1	2.7	0	0.0
UC	4	4.5	2	3.4	2	4.4	0	0.0	4	4.1
UG	4	4.5	3	5.2	1	2.2	1	2.7	0	0.0
UU	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	88		58		45		37		98	

Archaea

	K. cryp	tofilum	M. acet	ivorans	N. equ	uitans	S. solfataricus			
	#	%	#	%	#	%	#	%		
AA	0	0.0	0	0.0	0	0.0	0	0.0		
AC	0	0.0	4	6.7	0	0.0	0	0.0		
AG	3	6.5	7	11.7	2	4.5	3	6.5		
AU	0	0.0	0	0.0	0	0.0	0	0.0		
CA	0	0.0	0	0.0	0	0.0	0	0.0		
CC	0	0.0	1	1.7	1	2.3	1	2.2		
CG	1	2.2	0	0.0	1	2.3	0	0.0		
CU	0	0.0	0	0.0	0	0.0	0	0.0		
GA	0	0.0	1	1.7	0	0.0	0	0.0		
GC	22	47.8	25	41.7	21	47.7	23	50.0		
GG	20	43.5	19	31.7	19	43.2	19	41.3		
GU	0	0.0	3	5.0	0	0.0	0	0.0		
UA	0	0.0	0	0.0	0	0.0	0	0.0		
UC	0	0.0	0	0.0	0	0.0	0	0.0		
UG	0	0.0	0	0.0	0	0.0	0	0.0		
UU	0	0.0	0	0.0	0	0.0	0	0.0		
Total	46		60		44		46			



tRNA-Arg-TCG WT

GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCGGATCAGAAGATTGCAGGTTCGAGTCCTGCCGCGGTCG

tRNA-Arg-TCG C72U (Mutation causes an additional G-U wobble base pair) GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCGGATCAGAAGATTGCAGGTTCGAGTCCTGCCGCGGTtG

tRNA-Arg-TCG G70A (Mutation causes a C-A mismatch)

GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCGGATCAGAAGATTGCAGGTTCGAGTCCTGCCGCGaTCG Acceptor Stem

tRNA-Arg-TCT WT

GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAGTCG

tRNA-Arg-TCT C72U(Mutation causes an additional G-U wobble base pair)GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGGTTCGAGTCCCGGCGGAGTtG

tRNA-Arg-TCT G70A (Mutation causes a C-A mismatch)

GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAaTCG Acceptor Stem





Α

trna-Cys-GCA WT GGGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCCCCTT

tRNA-Cys-GCA C70U (Mutation causes an additional G-U wobble base pair) GGGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCCCtTT

tRNA-Cys-GCA G3A (Mutation causes an A-C mismatch) GGaGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCCTGGTTCAAATCCAGGTGCCCCTT

tRNA-Cys-GCA C69A (Mutation causes a G-A mismatch) GGGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCCaCTT

tRNA-Cys-GCA C68A (Mutation causes a G-A mismatch) GGGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCaCCTT

 tRNA-Cys-GCA G2A
 (Mutation causes an A-C mismatch, but RNA has GA at 5' end)

 GaGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCCCCTT

 Acceptor Stem





Figure S15





Figure S17















Strain	Time at 37	Replicate #	Total Reads	<u>CCA</u>	CCAC	CCACC	CCACCA	CCACCAC	CCACCACC	CCA x3	Н	SUM	% Extended CCA Motifs	Average	Stdev	Relative Level	Stdev
WT (JMW007)	Unr	2	2,383,825 2,836,577	2,215,590	27	2	3		2			32	0.00163	0.00371	0.00261	1.00	0.70
		3	2,119,015	1,643,012	96	2	13					111	0.00676				
		4	2,147,038	1,740,055	0/	4	10					87	0.00300				
WT (JMW007)	0.5 hr	2	3,131,139 2,080,275	2,387,937	36	8	6				$\left \right $	50 46	0.00209 0.00264	0.00283	0.00058	0.76	0.16
		3	1,863,204	1,561,183	38	5	7					50	0.00320				
		4	2,382,333	1,954,507		3	0					00	0.00338				
WT (JMW007)	1.5 hr	1 2	1,763,183	1,457,121	30	8	4				\square	42	0.00288	0.00323	0.00136	0.87	0.37
		3	2,450,329	1,985,101	26	7	8					41	0.00207				
WT (JMW007)	3 hr	1	3,363,884	2,619,879	50	9	7	2			++	66	0.00252	0.00273	0.00037	0.73	0.10
		2	1,774,037	1,456,810	24	8	2					34	0.00233				
		4	2,238,661	1,788,351	30	13	5					52	0.00291				
WT (JMW007)	6 hr	1	2.145.677	1.511.288	59	41	11				\square	111	0.00734	0.01224	0.00527	3.30	1.42
		2	2,431,609	1,610,373	180	80	27		3			287	0.01782				
		3	2,522,427	1,790,013	141	51	15	4	4			207	0.01136				
trm44-Δ tan1-Δ (JMW119)	0 hr	1	2,553,264	1,713,750	101	26	10	2				137 40	0.00799	0.00782	0.00381	2.11	1.03
		3	1,954,117	1,405,172	48	2	8					58	0.00413				
		4	2,028,146	1,377,183	87 62	14	9	3	2		+	107 87	0.00777				
		6	2,279,391	1,451,951	101	26	6		2	2		133	0.00916				
		,	1,433,473	000,333		15			-			120	0.01404				
trm44-Δ tan1-Δ (JMW119)	1.5 hr	2	1,425,638	1,078,057	259 271	90	6				$\left \right $	355 387	0.03293 0.03346	0.02942	0.00655	7.93	1.76
		3	1,786,720	1,413,013	210	89	10					309	0.02187				
trm44-Δ tan1-Δ (JMW119)	3 hr	1	1,734,848	1,057,832	400	281	21					702	0.06636	0.04039	0.01349	10.89	3.64
		2	2,421,152	1,830,979	577	262	19				H	858	0.04686		+		
		4	2,731,860	2,208,457	342	229	24				Ħ	595	0.02694				
		5	1,608,371 2,606,893	1,298,975 1,995,353	242 419	118 215	16				+	376 653	0.02895				
		7	2,266,270	1,539,302	405	199	42				H	646	0.04197				
trm44-Δ tan1-Δ (JMW119)	6 hr	1	1,996,874	1,273,668	1,181	1,868	75				╓	3,124	0.24528	0.17440	0.03900	47.01	10.51
		2	2,507,487	1,630,090	1,142	1,492	77		2		H	2,711	0.16631		+		
		4	1,508,822	1,004,566	585	692	41				Ħ	1,318	0.13120				
		6	2,026,332	983,072 657,766	739 540	893 598	53	2			++	1,685	0.17140 0.18198				
trm44 A tan1 A mot22 A (1¥1720)	0.br	1	2 146 254	2 106 850	63	16	6					94	0.00383	0.00504	0.00288	1.60	1.05
and a tant is needed a (erryes)	0111	2	3,635,133	2,813,591	36	3	12	3				51	0.00181	0.00000	0.00300	1.00	1.05
		3 4	3,230,696 2,465,954	2,407,540	81	9	21	5	2		++	40	0.00461	_			
		5	2,010,837	1,391,668	131	7	11		2			142	0.01020				
		7	2,803,550	1,422,885	127	7	25		3	2		161	0.01134				
trm44-Δ tan1-Δ met22-Δ (LY1729)	1.5 hr	1	2.269.836	1.822.161	154	43	10				\square	207	0.01136	0.01275	0.00494	3.44	1.33
		2	2,289,474	1,606,948	207	71	15					293	0.01823				
		3	2,722,495	2,473,794	157	44	13				++	214	0.00865				
trm44-Δ tan1-Δ met22-Δ (LY1729)	3 hr	1	3,199,484	2,334,115	265	91	5	3	3			361	0.01547	0.01482	0.00204	3.99	0.55
		3	1,973,749	1,509,434	153	73	12	3	3			244	0.01616				
		4	2,334,696	1,831,187	146 226	62	19					227	0.01240				
		6	2,394,726	1,761,193	202	39	31					272	0.01544				
		,	2,411,522	1,654,700	115	01						201	0.01230				
trm44-Δ tan1-Δ met22-Δ (LY1729)	6 hr	2	2,169,513 2,242.083	1,333,937	217	278	23	2			$\left \right $	518 1.580	0.03883 0.10896	0.07434	0.02617	20.04	7.05
		3	2,479,796	1,664,930	828	841	20	3	3	2		1,689	0.10145				
		5	2,380,334 2,371,125	1,609,933	649	328	33			3		1,092	0.06547				
		6	1,835,683	1,159,536	382	371	5				\square	758	0.06537	_			
trm44-Δ tan1-Δ xrn1-Δ (ISC850)	0 hr	1	2,547,502	1,733,200	134	17	15					166	0.00958	0.01018	0.00245	2.74	0.66
		3	2,148,658	1,111,647	114	10	23		2	5		159	0.01288				
trm44-Δ tan1-Δ xrn1-Δ (ISC850)	3 hr	1	2,275,988	1,607,613	232	67	21				$\left \right $	320	0.01991	0.01532	0.00605	4.13	1.63
		2	2,282,082	1,618,636	73	62	2					137	0.00846				
		3	3,214,119	2,056,776	222	110	30					362	0.01760				
trm44-Δ tan1-Δ xrn1-Δ (ISC850)	6 hr	1	2,327,188	1,784,834	254	204	29		3	2		487	0.02729	0.02387	0.00299	6.43	0.81
		3	3,057,414	2,023,748	203	224	12			Ŭ	Ħ	439	0.02169				
trm8-Δ trm4-Δ (JMW009)	0 min	1	3,217,937	2,427,387	44	6	14	2	2		\vdash	64	0.00264	0.00295	0.00042	0.79	0.11
		2	2,057,657	1,688,303	45	4	3				H	52	0.00308				
		4	2,243,094 2,448,193	2,044,144	45	4	14				Ħ	53	0.00259				
trm8-Δ trm4-Δ (JMW009)	8 min	1	1,421.347	1,140.327	28	2	4				H	34	0.00298	0.00256	0.00075	0.69	0.20
		2	1,736,254	1,466,406	35	7	5				Ħ	47	0.00321				
		3	2,042,023 1,909,325	1,668,629	36	2	6		2			42 25	0.00252				
trm8-A trm4-A (IMW009)	30 min	1	3 119 846	2 393 799	53	21	14					88	0.00368	0.00298	0.00070	0.80	0.19
		2	1,724,412	1,436,222	45		5					50	0.00348				
		3	2,117,406	1,75,957 1,432,627	26	5	4				⊢	40 36	0.00225				
met22-A (IMW/510)	0.br	1	2 014 720	1 385 67/	126		10				H	145	0.01046	0.00977	0.00162	2.22	0.44
	0111	2	1,986,129	1,258,600	81	2	20				Ħ	145	0.00818	0.00865	0.00103	2.33	0.44
		3	1,342,082	890,977	40	4	13		3	5	\mathbb{H}	65	0.00730		+ $+$		
met22-Δ (JMW510)	3 hr	1	2,850,472	2,200,923	147	12	37				Ħ	196	0.00891	0.00649	0.00307	1.75	0.83
		3	3,211,401	2,274,591	52	3 14	3				\parallel	69	0.00753				
met22-Δ (JMW510)	6 hr	1	2,485,240	1,839,852	113	43	57		2		\mathbb{H}	213	0.01158	0.00835	0.00307	2.25	0.83
		2	1,900,637	1,401,918	67	15	30			-	Ħ	112	0.00799			-	
<u> </u>		3	3,543,066	2,376,635	93	29	3		2	3	⊢	130	0.00547				
xrn1-Δ (ISC725)	0 hr	1	2,576,281	1,620,697	34	10	41			2	H	85 4	0.00524	0.00485	0.00195	1.31	0.53
		3	927,599	403,350		3	8				Ħ	11	0.00273				
xrn1-Δ (ISC725)	3 hr	1	2,449,767	1,620,316	152	36	47		2	6	+	235	0.01450	0.01022	0.00389	2.75	1.05
		2	1,927,825	1,285,343	34	39	16			2	Ħ	89	0.00692				
		3	2,457,152	1,207,974	31	0/	1/				Ħ	11/	0.00923				
xrn1-A (ISC725)	6 hr	2	2,509,017 2,589.338	1,911,485	138	40	28		2	3	+	206	0.01078	0.00877	0.00238	2.36	0.64
		3	2.606.985	1.598.249	39	49	10					98	0.00613				

Strain	Time at 37	Replicate #	Total Reads	CCA	CCAC	CCACC	CCACCA	CCACCAC	CCACCACC	CCA x3	SUM	% Extended CCA Motifs	Average	Stdev	Relative Level	Stdev
WT (JMW007)	0 hr	1	2,383,825	1,720,410	14	8	6		2		28	0.00163	0.00371	0.00261	1.00	0.70
		2	2,836,577	2,215,590	27	2	3				32	0.00144				
		3	2,119,015	1,643,012	96	2	13				111	0.00676				
		4	2,147,058	1,740,053	67	4	16				87	0.00500				
WT (JMW007)	6 hr	1	2,145,677	1,511,288	59	41	11				111	0.00734	0.01224	0.00527	3.30	1.42
		2	2,431,609	1,610,373	180	80	27		3		287	0.01782				
		3	2,522,427	1,790,013	141	51	15	4	4		207	0.01156				
trm44-Δ tan1-Δ (JMW119)	0 hr	1	2,553,264	1,713,750	101	26	10	2			137	0.00799	0.00782	0.00381	2.11	1.03
		2	1,847,577	1,292,496	26	6	8				40	0.00309				
		3	1,954,117	1,405,172	48	2	8				58	0.00413				
		4	2,028,146	1,377,183	87	14	6	3	2		107	0.00777				
		5	1,724,791	1,118,440	62	16	9				87	0.00778				
		6	2,279,391	1,451,951	101	26	6		2	2	133	0.00916				
		7	1,453,475	808,359	95	13	7		2	3	120	0.01484				
trm44-Δ tan1-Δ (JMW119)	6 hr	1	1,996,874	1,273,668	1,181	1,868	75				3,124	0.24528	0.17440	0.03900	47.01	10.51
		2	2.507.487	1.630.090	1.142	1.492	77		2		2.711	0.16631				
		3	2,558,513	1.754.856	1.074	1.493	69				2.636	0.15021				
		4	1.508.822	1.004.566	585	692	41				1.318	0.13120				
		5	2.026.332	983.072	739	893	53				1.685	0.17140				
		6	1.332.707	657.766	540	598	57	2			1.197	0.18198				
			1													
trm44-Δ tan1-Δ (JMW1251)	0 hr	1	1.353.146	104.710		1	6				7	0.00669	0.00840	0.00187	2.26	0.50
tRNASer(CGA): G51:C63 C6:G67 G2:C71		2	782.805	73.836	2	2	2				6	0.00813				
		3	1.342.090	221.288	1	5	14		3		23	0.01039				
			1. 1													
trm44-Δ tan1-Δ (JMW1251)	6 hr	1	875.453	45.849			2				2	0.00436	0.00731	0.00540	1.97	1.46
tRNASer(CGA): G51:C63 C6:G67 G2:C71		2	1,752,710	49.623		1	1				2	0.00403				
		3	518.307	29.528			-		1	3	4	0.01355				
		-														
trm44-A tan1-A (IMW316)	0 hr	1	646.958	87.650	3		1				4	0.00456	0.00511	0.00190	1.38	0.51
tRNASer(CGA) G51:C63		2	466.026	69.244	3		2				5	0.00722				
		3	1.407.999	311,199	3	1	6			1	11	0.00353				
			-,,	011/100						-						
trm44-Δ tan1-Δ (JMW316)	6 hr	1	639.263	257.165	5	4	5			1	15	0.00583	0.00479	0.00270	1.29	0.73
tRNASer(CGA) G51:C63		2	829.520	88.087	-	2	4				6	0.00681				
		3	229,523	57,791		1					1	0.00173				
				0.7.02												
trm44-A tan1-A (IMW561)	0 hr	1	126,218	48.985	4						4	0.00817	0.00589	0.00291	1.59	0.78
tRNASer(CGA) LI6:A67	•	2	99.674	43 564	2		1				3	0.00689	0.0000		2.00	
		3	80 114	38 338	-		1					0.00261		1		-
		- '	00,114	30,330								0.00201				-
trm44-A tan1-A (IMW561)	0 hr	1	46 225	29 581	4	11	2				17	0.05747	0.07299	0.01949	19.67	5.25
tPNIASer(CGA) 116:467	1	2	26,690	24.012	7	0		-	1		16	0.06662	0.07255	5.01545	15.07	5.25
and beiled by bolker	-	3	29.879	18.973	5	9	4	-		-	18	0.09487		-		

					CCAA	CCAAA	CCAAAA	ССААААА	ССАААААА												
Strain	Time at 37	Replicate #	Total Reads	<u>CCA</u>	2 A's	3 A's	<u>4 A's</u>	<u>5 A's</u>	<u>6 A's</u>	<u>7 A's</u>	<u>8 A's</u>	<u>9 A's</u>	<u>10 A's</u>	11 A's	<u>12 A's</u>	SUM	% Reads with A tails	Average	Stdev	Relative Level	Stdev
WT (JMW007)	Unr	2	2,383,825 2,836,577	2,215,590	552	45	38 19	23								616	0.0278	0.03225	0.01239	1.00	0.38
		3	2,119,015	1,643,012	449	43	12									492	0.0299				
		*	2,147,038	1,740,033	510	33	15									370	0.0213				
WT (JMW007)	0.5 hr	1	3,131,139	2,387,937	982	58	31	23								1,150	0.0482	0.03535	0.00910	1.10	0.28
		3	1,863,204	1,561,183	414	51	12									477	0.0306				
		4	2,382,333	1,954,507	623	48	17									688	0.0352				
WT (JMW007)	1.5 hr	1	1,763,183	1,457,121	384	57										441	0.0303	0.03938	0.01100	1.22	0.34
		2	2,450,329	1,480,671 1,985,101	682 594	70	12 30	11								764	0.0516				
WT (JMW007)	3 hr	2	3,363,884	2,619,879	1,570	274	61 31	39 15	14	11	12					1,981	0.0756	0.05996	0.01456	1.86	0.45
		3	1,940,037	1,558,880	814	177	46	24	11							1,072	0.0688				
		4	2,238,661	1,788,351	665	157	46	23	11	-						902	0.0504				
WT (JMW007)	6 hr	1	2,145,677	1,511,288	788	322	84	56	26	21	17	27	13	12		1,366	0.0904	0.08663	0.01105	2.69	0.34
		2	2,431,609	1,610,373	838	341	76	71 48	38	28	23	33	34 26	24	13	1,535	0.0953				
				1 210 250	4 000	4.070	470	204									0.1010	0.10765	0.05460	0.05	
trm44-Δ tan1-Δ (JMW119)	Unr	2	2,553,264	1,713,750	538	1,273	66	281	43	15	15					3,116	0.1818	0.12755	0.05463	3.95	1.69
		3	1,954,117	1,405,172	627	453	88	55	47	- 10						1,223	0.0870				
		4	2,028,146	1,377,183	604	574	99 145	107	23	12	11					1,141	0.12982				
		6	2,279,391	1,451,951	590	664	168	117	26	11						1,576	0.10854				
		- '	1,453,475	606,359	966	505	139	122	24	12						1,790	0.22144				
trm44-Δ tan1-Δ (JMW119)	1.5 hr	1	1,425,638	1,078,057	2,793	1,421	269	172	58	71	24	19	12	14		4,839	0.4489	0.38305	0.09052	11.88	2.81
		3	1,548,559	1,156,582	2,516	1,547	31	372	63	54	36	22	1/	14		3,954	0.2798	_			
trm44-A tan1 A (IA6M/110)	2 h-	1	1 724 040	1.057.033	3 101	2 500	744	720	213	242	140	101	40	25	16	0.077	0.9550	0 4405 4	0.20747	12.04	6 42
2	3111	2	2,421,152	1,830,979	5,053	2,999	524	423	174	177	80	42	33	20	10	9,005	0.5208	0.44954	0.20/4/	13.94	0.43
		3	2,387,242	1,732,862	4,548	2,848	529 342	450	165	131	61	39	26	11	-	8,808	0.5083				
		5	1,608,371	1,298,975	1,884	888	150	81	20	22	14		20			3,059	0.23549			L	
		6	2,606,893	1,995,353	3,773	1,897	338	313	145	133 90	67	47	13	<u> </u>		6,726	0.33708	-	 −		\vdash
		, í	_,_00,270	2,000,002	5,510	-,-32	321	200	10/		5/		~			5,350		-		1	
trm44-Δ tan1-Δ (JMW119)	6 hr	1	1,996,874	1,273,668	9,590	7,295	1,591	1,433	737	619 438	492	445	425 206	369	242	23,238	1.8245	1.34262	0.28432	41.63	8.82
		3	2,558,513	1,754,856	10,450	5,905	1,284	1,096	553	438	386	357	355	356	256	21,436	1.2215			1	
		4	1,508,822	1,004,566 983 072	4,942	2,548	586 697	559 608	269 360	216	158	108	88 373	39		9,513	0.94698		[
		6	1,332,707	657,766	4,799	2,456	455	386	224	175	196	138	119	53		9,001	1.36842				
trm44-Δ tan1-Δ met22-Δ (LY1729)	0 hr	1	3.146.254	2.196.850	1.645	277	60	44	22	22		10				2.080	0.0947	0.04629	0.02487	1.44	0.77
		2	3,635,133	2,813,591	677	119	21	23								840	0.0299				
		3	3,230,696 2,465,954	2,407,540 2,085,947	610 361	102	31 28	23								766	0.0318 0.0224				
		5	2,010,837	1,391,668	457	79	15	10	10							561	0.04031				
		7	2,803,550	1,422,885	437	101	27	17	15							600	0.04224				
trm44 A tan1 A mot22 A (IV1720)	1.E.br	1	2 260 926	1 922 161	1.025	205	57	22	24	20	10	12	15	10		1.420	0.0795	0.09199	0.02716	2.54	0.84
um44-A tan1-A met22-A (c11723)	1.5 11	2	2,289,474	1,606,948	1,351	230	56	46	24	25	16	13	12	10		1,430	0.1106	0.00100	0.02710	2.34	0.84
		3	2,722,495	2,473,794	985	233	62	49	31	25	15					1,400	0.0566				
trm44-Δ tan1-Δ met22-Δ (LY1729)	3 hr	1	3,199,484	2,334,115	1,928	685	209	150	65	77	63	33	28	18	12	3,268	0.1400	0.09905	0.02211	3.07	0.69
		2	2,046,207	1,597,118	1,102	260	88	58	25	39	25	21	19	15		1,652	0.1034				
		4	2,334,696	1,831,187	1,010	248	54	62	29	44	16	17	19	10		1,514	0.0827				
		5	2,457,998	1,944,618	1,423	183	38	28		10						1,682	0.08650				
		7	2,334,720	1,634,700	906	168	32	24	17	19		10				1,176	0.07194				
trm44-A tan1-A met22-A (LY1729)	6 hr	1	2 169 513	1.333.937	3.904	1.516	494	478	382	459	430	382	328	248	163	8 784	0.6585	0 44946	0.16903	13.94	5.24
		2	2,242,083	1,450,098	3,721	1,566	592	545	381	476	490	450	313	279	147	8,960	0.6179				
		3	2,479,796	1,664,930	3,610	1,461	468 259	451 205	346	479	471	448 54	332	289	165	8,520	0.5117 0.32347				
		5	2,371,125	1,609,933	3,297	883	231	187	135	137	127	99	72	26		5,194	0.32262				
		6	1,835,683	1,159,536	1,870	512	158	122	75	71	95	61	62	18		3,044	0.26252				
trm44-Δ tan1-Δ xrn1-Δ (ISC850)	0 hr	1	2,547,502	1,733,200	677	181	29	17								904	0.05216	0.08543	0.02958	2.65	0.92
		2	1,887,586 2,148,658	1,111,647 1,234,743	840	169 218	24 32	27 36								1,060	0.09535				
			0.075.000	1.007.010	0.04	205		10									0.07046	0.00700	0.01.107	2.00	0.15
um44-Δ tan1-Δ xrn1-Δ (ISC850)	3 hr	2	2,275,988 2,282,082	1,618,636	891 635	205	34 24	19		11	-			-		1,160	0.07216	0.06702	0.01467	2.08	U.45
		3	3,214,119	2,056,776	1,243	298	37	35								1,613	0.07842				
trm44-Δ tan1-Δ xrn1-Δ (ISC850)	6 hr	1	2,327,188	1,784,834	1,311	262	47	21	16	L						1,657	0.09284	0.09601	0.00507	2.98	0.16
		2	2,706,605	1,890,755	1,353	493	44	36		12	11					1,926	0.10186				
		,	3,037,414	2,023,748	1,404	2/0	12			13	11					1,689	0.09534				
trm8-∆ trm4-∆ (JMW009)	0 min	1	3,217,937	2,427,387	677	298	113	75	24	12	10		_	-		1,209	0.0498	0.03613	0.00916	1.12	0.28
		3	2,057,657	1,008,303	419	75	30	26								534	0.0316				
		4	2,448,193	2,044,144	534	60	14	15							-	623	0.0305				
trm8-Δ trm4-Δ (JMW009)	8 min	1	1,421,347	1,140,327	577	139	32	21								769	0.0674	0.04444	0.01818	1.38	0.56
		2	1,736,254	1,466,406	393	111	38	20	13	-	-		_	-		562	0.0383		+		
		4	1,909,325	1,646,350	328	58	11	- 2								397	0.0241			1	
trm8-Δ trm4-Δ (JMW009)	30 min	1	3,119 846	2.393 799	2,524	464	111	72	19	15	11	\vdash		-		3.216	0.1343	0,06222	0.04817	1 93	1.49
		2	1,724,412	1,436,222	433	101	33	29		10						606	0.0422				
		3	2,117,406	1,775,957	529 407	83	16	16			<u> </u>	$\left \right $		<u> </u>		644	0.0363 0.0361		+ +	1	
			-,	-,,.																	
met22-Δ (JMW510)	0 hr	2	2,014,730	1,385,676	488 467	49 50	18			-	-			-	<u> </u>	555	0.04005	0.03582	0.00932	1.11	0.29
		3	1,342,082	890,977	198	26										224	0.02514				
met22-Δ (JMW510)	3 hr	1	2,850,472	2,200,923	1,471	63	32	10								1,576	0.07161	0.04138	0.02619	1.28	0.81
		2	2,058,311	1,566,640	339	52	18	16								425	0.02713	-			
		3	3,211,401	2,274,591	402	/3	19	24								5/8	0.02541				
met22-Δ (JMW510)	6 hr	1	2,485,240	1,839,852	475	83	24	10		-	-		_	-		592	0.03218	0.04063	0.01698	1.26	0.53
		3	3,543,066	2,376,635	1,074	232	36	18	13	17	13	12	15			1,430	0.06017				
xrn1-Δ (ISC725)	0 hr	1	2,576.281	1,620.697	336	38	10			<u> </u>	<u> </u>	\vdash		<u> </u>	<u> </u>	384	0.02369	0.03095	0.01163	0.96	0.36
		2	480,354	60,864	19	2	2	4								27	0.04436	2.03033		2.50	
		3	927,599	403,350	85	6	7	2			<u> </u>	\vdash		<u> </u>	<u> </u>	100	0.02479	-	+		
xrn1-Δ (ISC725)	3 hr	1	2,449,767	1,620,316	620	47	12									679	0.04191	0.03076	0.00966	0.95	0.30
		2 3	1,927,825 2,497,152	1,285,343 1,267,974	295 259	25 48	16			-	-			-		320	0.02490		++		
4.4.0000000																	0.007-77		0.00		0
xrn1-Δ (ISC725)	6 hr	2	2,509,017	1,911,485	406	39	13				-	$\left \right $		-		445	0.02328	0.03756	0.02655	1.16	0.82
		3	2 606 985	1.598.249	289	31	19			1	1			1		339	0.02121	-			

					CCAA	0000	00000	000000	COMMAN				1									
Strain	Time at 37	Replicate #	Total Reads	CCA	<u>2 A's</u>	<u>3 A's</u>	<u>4 A's</u>	<u>5 A's</u>	<u>6 A's</u>	<u>7 A's</u>	<u>8 A's</u>	<u>9 A's</u>	10 A's	11 A's	<u>12 A's</u>	SUM	% Reads with A tails	1	Average	Stdev	Relative Level	Stdev
WT (JMW007)	0 hr	1	2,383,825	1,720,410	729	70	38	23								860	0.0500	(0.03225	0.01239	1.00	0.38
		2	2,836,577	2,215,590	552	45	19									616	0.0278					
		3	2,119,015	1,643,012	449	43										492	0.0299					
		4	2,147,058	1,740,053	318	39	13									370	0.0213					
WT (JMW007)	6 hr	1	2,145,677	1,511,288	788	322	84	56	26	21	17	27	13	12		1,366	0.0904	(0.08663	0.01105	2.69	0.34
		2	2,431,609	1,610,373	838	341	76	71	38	28	39	33	34	24	13	1,535	0.0953					
		3	2,522,427	1,790,013	785	278	65	48	27	30	23	22	26	14	10	1,328	0.0742					
trm44-Δ tan1-Δ (JMW119)	0 hr	1	2,553,264	1,713,750	1,203	1,273	278	281	43	23	15					3,116	0.1818	(0.12755	0.05463	3.95	1.69
		2	1,847,577	1,292,496	538	344	66	74	14	15						1,051	0.0813					
		3	1,954,117	1,405,172	627	453	88	55								1,223	0.0870					
		4	2,028,146	1,377,183	476	419	99	107	17	12	11					1,141	0.0829					
		5	1,724,791	1,118,440	604	574	145	89	23	17						1,452	0.12982					
		6	2,279,391	1,451,951	590	664	168	117	26	11						1,576	0.10854					
		7	1.453.475	808.359	988	505	139	122	24	12						1.790	0.22144					
			,, .																			
trm44-Δ tan1-Δ (JMW119)	6 hr	1	1.996.874	1.273.668	9,590	7.295	1.591	1.433	737	619	492	445	425	369	242	23.238	1.8245		1.34262	0.28432	41.63	8.82
		2	2 507 487	1.630.090	10.871	6.780	1.436	1,200	407	438	314	220	206	125	73	22 070	1 3539					
		3	2 558 513	1,754,856	10,450	5,905	1.284	1.096	553	438	386	357	355	356	256	21.436	1 2215					
		4	1.508.822	1.004.566	4 942	2 548	586	559	269	216	158	108	88	39		9.513	0.94698					
		5	2 026 332	983.072	6.442	3 391	697	608	360	369	377	355	373	205		13 177	1 34039					
		6	1 332 707	657.766	4 799	2,456	455	386	224	175	196	138	119	53		9,001	1.34033					
		0	1,332,707	037,700	4,733	2,430	455	380	224	1/5	190	130	115	33		3,001	1.30042					
trm44 A tan1 A (IA44/12E1)	0.br	1	1 252 146	104 710	50	6			-			-				56	0.05249		0.02620	0.01509	1.12	0.47
ADMAG-(CCA), CE1, CC2 CC, CC3 C2, C2	0111	2	703.005	72.020	17	0			2			-				10	0.03548		0.03020	0.01308	1.12	0.47
(RNASER(CGA): G51:C63 C6:G67 G2:C71		2	1 242,000	/3,630	17	6		2				-	-			19	0.02573					
		3	1,542,090	221,200	57	0		2				-				60	0.02937					
America A. America A. (18414/12/21)	C ha	1	075 453	45.040	16							-	-			16	0.02400		0.02720	0.00730	0.07	0.22
U11144-A (J111-A (J1V1VV1251)	011	1	8/5,455	45,849	10											10	0.03490		0.02758	0.00738	0.85	0.23
tRNASer(CGA): G51:C63 C6:G67 G2:C71		2	1,752,710	49,623	10	-										10	0.02015					
		3	518,307	29,528	6	2										8	0.02709					
trm44-Δ tan1-Δ (JMW316)	0 hr	1	646,958	87,650	21	5										26	0.02966	(0.07007	0.07064	2.17	2.19
tRNASer(CGA) G51:C63		2	466,026	69,244	94	11										105	0.15164					
		3	1,407,999	311,199	72	11	5	2								90	0.02892					
trm44-Δ tan1-Δ (JMW316)	6 hr	1	639,263	257,165	78	9	3	3		3						96	0.03733	(0.06838	0.06704	2.12	2.08
tRNASer(CGA) G51:C63		2	829,520	88,087	111	7	2	3		3	2					128	0.14531					
		3	229,523	57,791	13											13	0.02249					
trm44-Δ tan1-Δ (JMW561)	0 hr	1	126,218	48,985	73	23	6	4			2					108	0.22048	(0.14055	0.07024	4.36	2.18
tRNASer(CGA) U6:A67		2	99,674	43,564	10	27	3	9								49	0.11248					
		3	80,114	38,338	13	15	2	4		1	1	1	1	1		34	0.08868					
			l í					1			1											
trm44-Δ tan1-Δ (JMW561)	0 hr	1	46.225	29.581	46	46	10	1	3							105	0.35496		0.35550	0.01845	11.02	0.57
tRNASer(CGA) U6:A67		2	36,690	24.012	42	30	6	3	<u> </u>							81	0.33733					
		2	20,030	10,072	27	25					-		+	1			0.03400					

Substrate (without CCA at 3' end)	Sequence	Figures where substrate is used
mmascRNA (WT)	GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCT	Fig. 2B-D; Supplementary Fig. 4B-F; Supplementary Fig. 8B-G; Supplementary Fig 9B-E
mmascRNA Mut 1	qqcactqGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcaqtaccq	Fig. 2B-C; Supplementary Fig. 4B-F; Supplementary Fig. 8B-G
mmascRNA Mut 2	GACGCTGqtqqcqqcacTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCT	Fig. 2B; Supplementary Fig. 4C
mmascRNA Mut 3	GACGCTGGCTGGCTGGCACGCccccccccccccccGGGGTTCAAGTCCCTGCGGTGTCT	Fig. 2B: Supplementary Fig. 4C
mmascRNA Mut 4	CACCOMPCCACCOMPCCACCAMACTER CONTRACTOR CONTR	Fig. 28: Supplementary Fig. 4C
mmascRNA Mut 5		
mmascRNA Mut 6		
mmaschink Hut 7		Supplementary Fig. 00-C
mmasckna mut 7	GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTATCT	Supplementary Fig. 88-C
mmascRNA Mut 8	GACaCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCT	Supplementary Fig. 8B-C
mmascRNA Mut 9	GACGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCaGTGTCT	Supplementary Fig. 8B-C
mmascRNA Mut 10	ggcGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTaccg	Supplementary Fig. 8D-G; Supplementary Fig. 9E
mmascRNA Mut 10A	ggaGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTcccg	Supplementary Fig. 8F-G; Supplementary Fig. 9E
mmascRNA Mut AD	ggaGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTccct	Supplementary Fig. 8F-G
mmascRNA Mut 10AU	qqaGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTcctq	Supplementary Fig. 9E
mmascRNA Mut 11	qqCGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGccq	Supplementary Fig. 8D-E
mmascRNA Mut 14	Gacgetgetgetgetgetetetetegettecetgegetteageteteagetetea	Supplementary Fig. 9B-C
mmascRNA Mut 15		Supplementary Fig. 9B-C
mmascRNA Mut 15A		Supplementary Fig. 9C
mmascRNA Mut 15AD		
mmaschia Hut 16		Supplementary Fig. 00
IIIIIIascrina Mut 17		Supplementary Fig. 9B
mmascRNA Mut 1/	GgCGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCGGTGTCg	Supplementary Fig. 98
mmascRNA with mouse MEN β acceptor stem	ggcactgGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGcagtaccg	Fig. 2D
mmascRNA with human MEN β acceptor stem	GgCGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCaGTGTtg	Fig. 2D
mmascRNA with Old World monkey MEN β acceptor stem	GgCaCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGCaGTGTCT	Fig. 2D
mmascRNA with dog MEN β acceptor stem	GaCGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGLGGcGTCc	Fig. 2D
mmascRNA with horse MEN β acceptor stem	GqCGCTGGTGGCTGGCACTCCTGGTTTCCAGGACGGGGTTCAAGTCCCTGLGGcGTCc	Fig. 2D
mMEN β tRNA-like small RNA (WT)	GGCACTGGTGGCGGCACGCCCGGACCTCGGGGCCAGGGTTCGAGTCCCTGCAGTACCG	Supplementary Fig. 3A: Supplementary Fig. 10B
mMEN 6 tRNA-like small RNA Mut 2	agaacta@TGCTGCTACCACaccaccaccaccaccaccaccaccaccaccaccaccacc	Supplementary Fig. 10B
mMEN 6 tRNA-like small RNA Mut 1+D	gyster system rearran a general start system and a start sta	Supplementary Fig. 10B
mMEN & tDNA-like email DNA Mut 5+D		Supplementary Fig. 100
Inmen p triva-like small Riva Mut 5+D	ggcactggtggclggcacgcccgcacctcgggccagggttcgagtccctgcagtaccT	Supplementary Fig. 10B
mMEN β tRNA-like small RNA Mut 5A+D	ggAactggtggcTggcacgcccgcacctcgggccagggttcgagtccctgcagtCccT	Supplementary Fig. 10B
mMEN β tRNA-like small RNA Mut 6+D	gAcactggtggcTggcacgccccgcacctcgggccagggttcgagtccctgcagtaTcg	Supplementary Fig. 10B
mMEN β tRNA-like small RNA Mut 7+D	ggcactggtggcTggcacgcccgcacctcggggccagggttcgagtccctgcagtGccg	Supplementary Fig. 10B
mMEN β tRNA-like small RNA Mut 8+D	ggcGctggtggcTggcacgcccgcacctcgggccagggttcgagtccctgcagtaccg	Supplementary Fig. 10B
mMEN β tRNA-like small RNA Mut 9+D	ggcactggtggcTggcacgcccgcacctcgggccagggttcgagtccctgcGgtaccg	Supplementary Fig. 10B
hMEN 6 tRNA-like small RNA (WT)	GC/CCTTGGTGGTGGC2CGCC2GGCTTGGGCCCGGGGTTGG2CTCCCCCCCC	Supplementary Fig. 3A
		Supplementary rig. SA
PANA THE ACY WE		Cumplementers Ein 20 Er Cumplementers Ein 24, Cumplementers Ein Er Cumplementers Ein 110
trna-inf-acy wi	GGCTCCGTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGCGGGGCCT	Supplementary Fig. 2D-F; Supplementary Fig. 3A; Supplementary Fig. 5; Supplementary Fig. 11B
tRNA-Thr-ACY with mascRNA acceptor stem	gacgctgTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGcggtgtct	Supplementary Fig. 5
tRNA-Thr-ACY with MEN β tRNA acceptor stem	ggcactgTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGcagtaccg	Supplementary Fig. 5
tRNA-Thr-ACY Mut 1	gacgetgTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGcggtgtet	Supplementary Fig. 11B
tRNA-Thr-ACY Mut 2	ggcactgTGGCTTAGCTGGTTAAAGCGCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGcagtaccg	Supplementary Fig. 11B
tRNA-Thr-ACY Mut 3	GGCaCCGTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGCGGtaCCg	Supplementary Fig. 11B
tRNA-Thr-ACY Mut 4	GGCaCCGTGGCTTAGCTGGTTAAAGCGCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGCGGtaCCT	Supplementary Fig. 11B
tRNA-Thr-ACY Mut 5	GGCTCCGTGGCTTAGCTGGTTAAAGCGCCCTGTCTAGTAAACAGGAGATCCTGGGTTCGAATCCCAGCGGGACCg	Supplementary Fig. 11B
tRNA-Thr-ACY Mut 6		Supplementary, G. 11B
adda fill Act fide o		Supplementary Fig. 110
ADNA Law CTV MT		Cumlementers En FA: Cumplementers En 11D
IRNA-LEU-CTF WT	GGTAGCGTGGCGAGCGGTCTAAGGCGCTGGATTAAGGCTCCAGTCTTCGGGGGGGG	Supplementary Fig. 5A, Supplementary Fig. 110
tRNA-Leu-CTY with mascRNA acceptor stem	gaegetgrosccgaccgottaaggcgcrogattaaggctccaGttTCTTCGgGggcGTGgGTTCGaatcccaceggtgtet	Supplementary Fig. 5A
trina-Leu-Ci i with Men p trina acceptor stem	ggeactgroupdgccgagcggretaaggcgcrogattaaggetecagtertergggggeggggtecgaateccagtaccg	Supplementary Fig. 5A
tRNA-Leu-CTY Mut 1	gacgetgTGGCCGAGCGGTCTAAGGCGCTGGATTAAGGCTCCAGTCTCTTCGGGGGCGTGGGTTCGAATCCCACcggtgtet	Supplementary Fig. 11D
tRNA-Leu-CTY Mut 2	ggcactgTGGCCGAGCGGTCTAAGGCGCTGGATTAAGGCTCCAGTCTCTCGGGGGCGTGGGTTCGAATCCCACcagtaccg	Supplementary Fig. 11D
tRNA-Leu-CTY Mut 3	GGcAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTAAGGCTCCAGTCTCTTCGGGGGGCGTGGGTTCGAATCCCACCGCTaCCg	Supplementary Fig. 11D
tRNA-Leu-CTY Mut 4	GGCAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTAAGGCTCCAGTCTCTCGGGGGGCGTGGGTTCGAATCCCACCGCTaCCA	Supplementary Fig. 11D
tRNA-Arg-CGY WT	GGGCCAGTGGCGAATGGATAACGCGTCTGACTACGGATCAGAAGATTCCAGGTTCGACTCCTGGCTGG	Supplementary Fig. 5A
tRNA-Arg-CGY with mascRNA acceptor stem	accenterGCCCC22TCCC2TCCCCCCCCCCCCCCCCCCCCCCCCCC	Supplementary, Eq. 5A
tRNA-Arg-CGY with MEN 6 tRNA acceptor stem		Supplementary Fig. 5A
, see the second second second		
tRNA-Are-TCG WT		Eig 2B: Supplementary Eig 12B: Supplementary Eig 19B C
IRNA Au TCO COUL	GGUGUGTATAGATAAGGUTTUGATTUGATTUGAGTUGAG	Fig. 36; Supplementary Fig. 136; Supplementary Fig. 166°C
tRNA-Arg-ICG C/2U	GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCGGATCAGAAGATTGCAGGTTCGAGTCCTGCCGCGGTtG	Fig. 3B; Supplementary Fig. 13B
tRNA-Arg-TCG G70A	GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCGGATCAGAAGATTGCAGGTTCGAGTCCTGCCGCGATCG	Fig. 3B; Supplementary Fig. 13B
tRNA-Arg-TCT WT	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAGTCG	Fig. 3C; Supplementary Fig. 13C; Supplementary Fig. 15; Supplementary Fig. 18C
tRNA-Arg-TCT C72U	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAGTLG	Fig. 3C; Supplementary Fig. 13C; Supplementary Fig. 15
tRNA-Arg-TCT G70A	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCTAGAGGCTGAAGGCATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAATCG	Fig. 3C: Supplementary Fig. 13C: Supplementary Fig. 15
tRNA-Cys-GCA WT	CCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	Supplementary Fig. 14B
tRNA-Cyc-GCA C701		Supplementary Hg. 110
HRNA-Cyc-GCA G2A		Supplementary Fig. 1/B
IDNA Cue CCA CCOA	STREAM IN A STREAM AND AND A STREAM AND A STREAM AND A ST	Supplementary Fig. 14D
IRNA-CUS-GCA COM	Georgetatrice tradegetacageta	Supplementary rig. 140
TKNA-LYS-GLA C68A	GGGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCaCCTT	Supplementary Fig. 148
tRNA-Lys-GCA G2A	GAGGGTATAGCTTAGGGGTAGAGCATTTGACTGCAGATCAAAAGGTCCCTGGTTCAAATCCAGGTGCCCCTT	Supplementary Fig. 14B
tRNA-Ser-GGA WT	GGTGAGGTGTCCGAGTGGC±gAAGGAGCACGCCTGGAAAGTGTGTATACGGCAACGTATCGGGGGTTCGAATCCCCCCCC	Fig. 4C-D, Supplementary Fig. 18A
tRNA-Tyr-GTA WT	GGTGGGGTTCCCGAGCGGCCCAAAGGGAGCAGACTGTAAATCTGCCGTCATCGACTTCGAAGGTTCGAATCCTTCCCCCAACA	Supplementary Fig. 18A
S. ecuadoriensis mito Ser tRNA (Genomic)	ana and a second s	Supplementary Fig. 20B
C ocuadoriansis mito Ser (NNA (Genomic)	gagana geograph gho a the gagang the set of the gamma sho has a sho ha a sh	Supplementary Fig. 200
S. ecuadoriensis mito Ser triva (cuited)	yyayaaatyytayaytyyttetettyögödöytttyyddygöytööttöytdatotdattytyyyttöjäätöööäötteTCCA	Supplementally Fig. 200
A statelling" softe the IDNA (Conserve)		Constant For 205
A. castellanii mito Ala tRNA (Genomic)	ggttgcatagtttaatggtaaaatcaataccttgcacgtattagatatcagttcgattctgattgcgtcca	Supplementary Fig. 2011
A. castellanii mito Ala tRNA (Edited)]ggAtgcatagtttaatggtaaaatcaataccttgcacgtattagatatcagttcgattctgattgcgtcca	Supplementary Fig. 20E

Northern Probes used in Fig. 1B, Supplementary Fig. 7A	Sequence
mascRNA	aaccccgtcctggaaaccagga
MEN β tRNA-like small RNA	aaccccggcccagccgtgctggac
	,