

Distinct subcellular localization of tRNA-derived fragments in the infective metacyclic forms of *Trypanosoma cruzi*

Larissa Reifur^{1,3}, Maria Rosa Garcia-Silva², Saloê Bispo Poubel¹, Lysangela Ronalte Alves¹, Paulo Arauco¹, Diane Kelly Buiar³, Samuel Goldenberg¹, Alfonso Cayota², Bruno Dallagiovanna^{1/+}

¹Instituto Carlos Chagas-Fiocruz, Curitiba, PR, Brasil ²Functional Genomics Unit, Institut Pasteur de Montevideo, Mataojo, MV, Uruguay

³Departamento de Patologia Básica, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR, Brasil

Small non-coding RNAs derived from transfer RNAs have been identified as a broadly conserved prokaryotic and eukaryotic response to stress. Their presence coincides with changes in developmental state associated with gene expression regulation. In the epimastigote form of Trypanosoma cruzi, tRNA fragments localize to posterior cytoplasmic granules. In the infective metacyclic form of the parasite, we found tRNA-derived fragments to be abundant and evenly distributed within the cytoplasm. The fragments were not associated with polysomes, suggesting that the tRNA-derived fragments may not be directly involved in translation control in metacyclics.

Key words: *Trypanosoma cruzi* - tRNA-derived fragments - subcellular localisation

The non-infective epimastigote form of *Trypanosoma cruzi* undergoes metabolic and morphological adaptations to differentiate into the pathogenic metacyclic trypomastigote form, which causes Chagas disease in mammals (Figueiredo et al. 2000). Morphological changes are associated with and preceded by a shift in metabolism, which is correlated with the differential expression of several genes (Minning et al. 2009). Although the metabolism of the replicative epimastigote is fully active, with constitutive polycistronic transcription, starvation reduces the transcriptional rates of the RNA polymerases in metacyclics (Ferreira et al. 2008). Despite the reduced level of transcription, translation is detected in these non-replicative forms, indicating that gene expression control in metacyclics occurs primarily at the post-transcriptional level (reviewed in Haile & Papadopoulou 2007).

Considering the almost exclusive post-transcriptional control of gene expression in *T. cruzi*, it would be surprising if this organism did not have an alternative pathway to compensate for the absence of an RNA interference system. A myriad of small non-coding RNAs have been reported in trypanosomatids (Dumas et al. 2006, Garcia-Silva et al. 2010, Michaeli et al. 2012), canonical microRNAs, and siRNAs have not been detected in *T. cruzi* (Franzén et al. 2011). Short (20-35 nt) RNAs derived from tRNAs were first observed in cytoplasmic granules in the epimastigote form of *T. cruzi* after an ini-

tial fingerprint sequencing of 348 clones (Garcia-Silva et al. 2010). Although 26% of the sequenced clones represented tRNA-derived fragments, a more thorough sequencing of the small RNAs from epimastigotes showed that 65.3% of more than 282.000 clones represented tRNA-derived fragments (Franzén et al. 2011). The composition of the small RNA population in *T. cruzi* was strikingly different from that observed in *Trypanosoma brucei*, reflecting clear differences in the molecular biology of the two parasites (Michaeli et al. 2012). Nonetheless, the relative abundance of tRNA-derived fragments could be related to gene expression control under various types of cellular stress, as has been suggested for prokaryotes, yeast, mammalian cells, the protozoans *Giardia lamblia* and *Tetrahymena thermophila* (Lee & Collins 2005, Li et al. 2008, Pederson 2010).

In this study, we conducted further analyses of the relative abundance and subcellular localization of *T. cruzi* tRNA-derived fragments during the infective stage of the parasite. We used *T. cruzi* metacyclic trypomastigotes derived from Dm28c epimastigotes cultured in vitro, as described by Contreras et al. (1985). Total RNA was extracted using TRIzol (Invitrogen) and size fractionated on a denaturing 15% polyacrylamide gel electrophoresis (PAGE) gel. Subsequently, 18-40 nt RNAs were excised from the gel, purified and cloned as described by Garcia-Silva et al. (2010). To recover the small RNAs, specific oligonucleotide adaptors containing Ban I restriction sites were ligated to the 5' and 3' ends. The RNAs were then reverse transcribed, amplified by polymerase chain reaction for 10 cycles, Ban I-digested, concatamerized, cloned into the pGEM T-easy vector (Promega Corp) and sequenced. Analyses were performed using the public GenBank (ncbi.nlm.nih.gov/genbank), GeneDB ([genedb](http://genedb.org)) and TriTrypDB (tritrypdb.org/tritrypdb) databases.

From a total of 844 clones analysed, 509 sequences aligned with the *T. cruzi* genome, whereas 2.16% showed no matches, indicating that these sequences may correspond to regions of the genome that have not been se-

Financial support: FIOCRUZ, Araucaria Foundation (18.456) (to LR) SG, LRA and BD received fellowships from CNPq, SBP received a fellowship from CAPES and DKB received a fellowship from UFPR/TN.
+ Corresponding author: brunod@tepar.br

Received 20 December 2011

Accepted 10 May 2012

quenced, exogenous contaminating DNA or differences between the reference strain and the strain used in this study. No sequences matched small nuclear RNA sequences, but 0.98% mapped to small nucleolar RNAs, 2.95% mapped to intergenic regions, 6.09% mapped to mRNAs and 24.56% mapped to rRNAs. Most of the cloned sequences (63.26%) were fragments derived from tRNAs that appeared to be the result of a specific cleavage at or around the anticodon loop (Supplementary data). The tRNA fragments averaged 33 nt in length and were mostly derived from the 3' end (86.96%) of a restricted group of isoacceptors. In contrast with the RNAs obtained from an epimastigote population sequenced by Garcia-Silva et al. (2010), the tRNA-derived fragments obtained in this study from metacyclics exhibited differences with respect to abundance (tRNA fragments composed 63.26% of the small RNAs in metacyclics versus 26% in epimastigotes), orientation (most of the tRNA fragments were derived from the 3' side of the tRNAs in metacyclics vs. the 5' side in epimastigotes) and origin (in metacyclics, fragments were derived mostly from tRNA^{Glu}, tRNA^{Thr} and tRNA^{Val}; in epimastigotes, they were generally derived from tRNA^{Asp} and tRNA^{Glu}). The relative abundances of most fragments did not correspond to either tRNA gene copy number or codon usage (Horn 2008, Padilla-Mejia et al. 2009), consistent with previous reports (Franzén et al. 2011). As reported by Franzén et al. (2011), we observed that the majority of the fragments were derived from the 3' side of the tRNAs and a significant proportion of these fragments (24.53%) carried the 3' CCA sequence, indicating that both mature and pre-tRNAs undergo the cleavage process. The biological importance of these tRNA fragments is unknown, but we expected that a higher percentage of these fragments would be observed in metacyclics because stressed epimastigotes showed only a slight increase in tRNA-derived fragments (Garcia-Silva et al. 2010). This phenomenon is likely observed because tRNA cleavage is a conserved process in cells under various types of stress and metacyclic trypomastigotes are the product of epimastigote differentiation triggered by nutritional stress (Contreras et al. 1985). A comparison of our results with those of Garcia-Silva et al. (2010) demonstrates that the percentage of tRNA fragments is clearly higher in metacyclics. In contrast, this difference is not observed by Franzén et al. (2011). Moreover, the epimastigote tRNA-derived fragments sequenced by Franzén et al. (2011) are mostly derived from the 3' arm of tRNA^{His}. These discrepancies could be due to differences in the strains analysed in the two studies and to the cloning and sequencing methods used. Franzén et al. (2011) used a different strain (CL Brener) and analysed a much larger number of clones using RNAseq, which provided a higher coverage of the parasite genome.

The cloned RNA sequences were aligned using the LocARNA server (Will et al. 2007) and the secondary structures identified using the RNAalifold server (Bernhart et al. 2008) from the Vienna RNA package and were adjusted manually according to the secondary structural domains of canonical tRNAs. The predicted secondary structures adopted by the most abundant 3' tRNA frag-

ments revealed that, upon cleavage around the anticodon nucleotides, the tRNA fragment maintained the T ψ C loop conformation and was extended a few extra base pairs into the double-stranded stem, resulting in an energetically favourable structure (Supplementary data). Although this result is based only on computational calculations and does not confirm the true molecular structure *in vivo*, the fact that the most abundant tRNA-derived fragments can assume a similar, relatively stable configuration suggests a structural (or functional) significance.

The subcellular localization of the tRNA-derived fragments in the metacyclic forms was evaluated through fluorescence *in situ* hybridisation (FISH) using probes complementary to the 5' and 3' ends of tRNA^{Glu-UUC} (the most abundantly cloned tRNA fragment). FISH assays showed that these fragments are dispersed throughout the cytoplasm in metacyclics, whereas stressed epimastigotes exhibited the same posterior granular distribution as non-stressed epimastigotes (Fig. 1). To further analyze the cellular localization of the tRNA-derived fragments, metacyclic cells were gently lysed in lysis buffer (300 mM KCl, 10 mM MgCl₂, 10 mM Tris-HCl 7.4 pH and 0.5% NP40) for 5 min. Two fractions were collected: the supernatant, containing only the soluble cytosol contents (S), and the insoluble fraction, or pellet (P), containing insoluble organelles, vesicles and cellular membranes. Both fractions were loaded on a sodium dodecyl sulphate-PAGE (10%) and transferred onto Hybond-C membranes (Amersham). Western blots were performed using antibodies to TcPUF6 (1/250), a cytosolic RNA binding protein (Dallagiovanna et al. 2005), and dynamin (1/200), a membrane protein present in endocytic vesicles (Pucadyil & Schmid 2009) (Fig. 2F, G). Northern blots were conducted using total RNA extracted from the two cell fractions (S and P) and a ra-

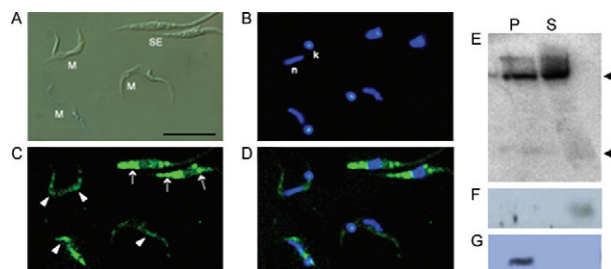


Fig. 1: subcellular localization of tRNA-derived RNAs in metacyclic trypomastigote forms. A: differential imaging contrast of metacyclic (M) and stressed epimastigote forms (SE); B: 4'-6-diamidino-2-phenylindole staining was used as a reference to identify nuclei (n) and kinetoplast (k); C: fluorescein isothiocyanate (FITC)-labeled probes recognizing the 3' fragment tRNA^{Glu-UUC} (5'TTCCGATACCGGAATCGA3') in SE (arrows) and M forms (arrowheads); D: merged images (Bar = 10 μ m). Cells were observed under an Olympus IX 81 microscope coupled to a Hamamatsu Orca-ER camera (Diagnostic Instruments); E: total RNA from soluble (S) and insoluble (P) metacyclic cell fractions were analyzed with the radioactively labelled probe 3'tRNA^{Glu-UUC}, as in Garcia-Silva et al. (2010). Western blot assays with F (anti TcPUF6) and G (anti-Dynamin) sera were performed to assess the identity of both fractions.

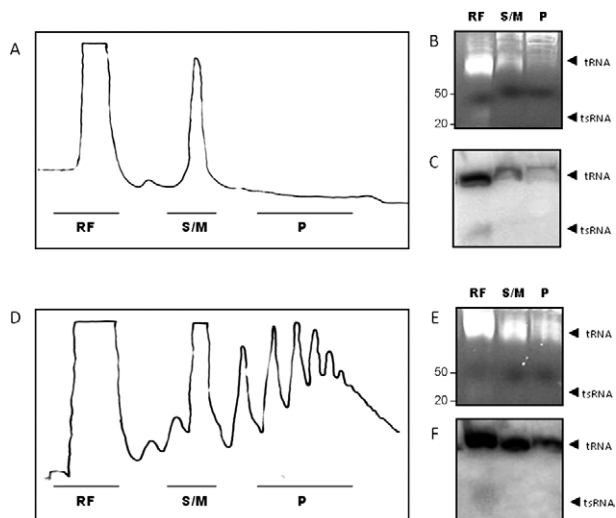


Fig. 2: tRNA-derived fragments are not associated with polysomes. Polysome profiles of (A) metacyclic trypomastigote and (D) epimastigote forms. Fractions were collected and pooled (RF: ribosome free; S/M: subunits and monomers; P: polysomes). RNA was purified and separated into 15% acrylamide gels (B, E). Northern blots of metacyclic (C) and epimastigote RNA (F) analyzed with the radioactively labelled probe tRNA^{Glu-UUC}, as in Fig. 1.

diolabeled probe specific to the tRNA^{Glu-UUC} fragment. The tRNA-derived fragments were detected in both fractions, supporting the dispersed pattern observed in the immunofluorescence assays (Fig. 2E). The signal observed for the insoluble cellular fraction could indicate the presence of these RNAs in smaller intracellular vesicles. Nonetheless, their dispersed pattern in the cytoplasm in metacyclics contrasts with the granular and posterior localization in epimastigotes, in which a partial co-localization with reservosomes has been inferred. The dismantling of the tRNA fragments in metacyclics could be correlated with the absence of reservosomes in this life stage (Figueiredo et al. 2000) and is consistent with the described cytoplasmic localization of the type II tRNA-derived fragments. Type II tRNA-derived fragments are most likely generated in the cytosol by RNaseZ cleavage and by RNA polymerase III termination and these fragments have been found to coimmunoprecipitate with Argonaute proteins (Elbarbary et al. 2009, Haussecker et al. 2010). This dramatic change in subcellular localization is puzzling and suggests distinct roles for these molecules during the different stages of the parasite life cycle.

To determine whether the dispersed tRNA-derived fragments were associated with translating polysomes, metacyclic and epimastigote forms of *T. cruzi* were treated with cycloheximide and the polysomes were purified and separated on sucrose gradients, as previously described (Nardelli et al. 2007) (Fig. 2A, D). Parasite extracts were also treated with puromycin as a negative control (not shown). The polysomes, monosomes and ribosome-free fractions were pooled and the total RNA was extracted. After acrylamide gel separation and staining, a high concentration of small RNAs was

observed in the ribosome-free pool for the metacyclic and epimastigote forms and a faint small RNA signal was observed in the ribosome-containing fractions (Fig. 2B, E). Northern blot analysis confirmed that tRNA-derived fragments from metacyclics and epimastigotes were concentrated in the ribosome-free fractions (Fig. 2C, F). Therefore, tRNA-derived fragments may not be related to the repression of the translational machinery. The knockdown of the tRNA-derived fragments and structural analysis should be used to help uncover the biological importance of these RNAs.

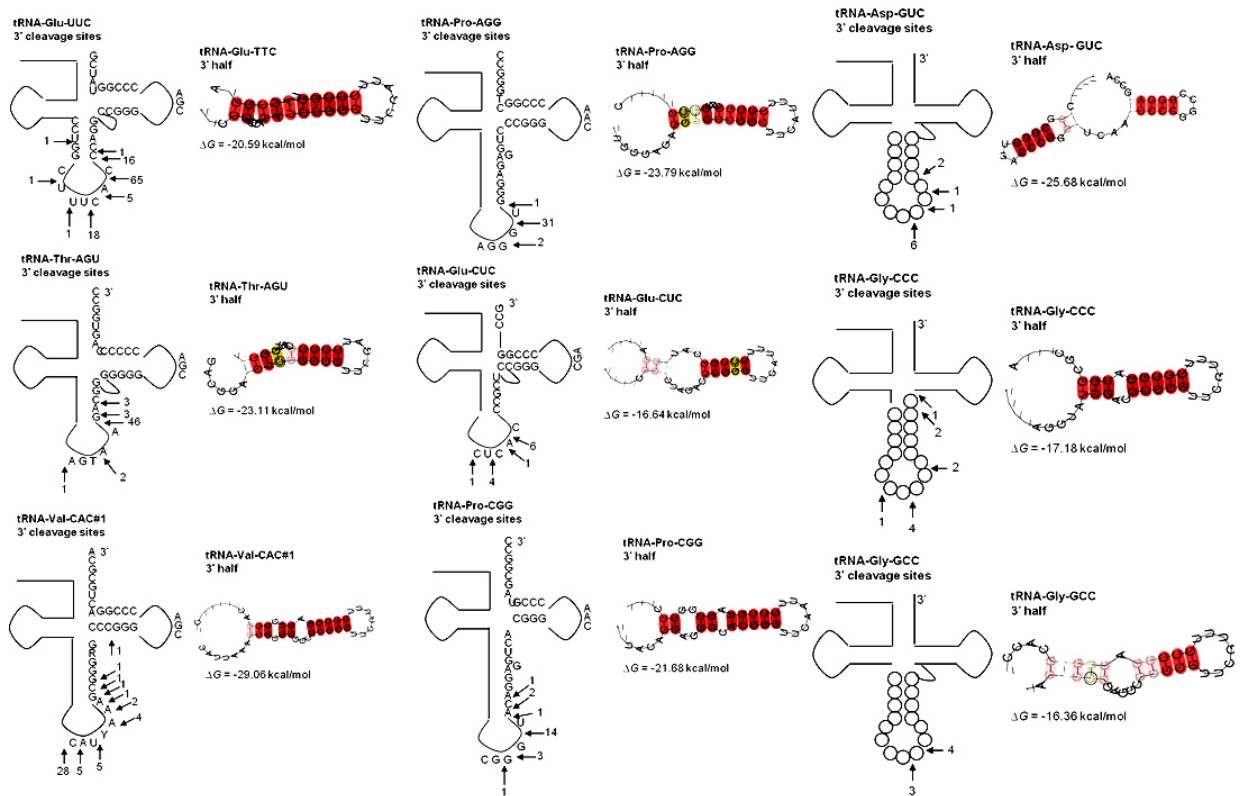
ACKNOWLEDGEMENTS

To Dr Donna J Koslowsky, Dr Laura E Yu and the reviewers, for critical reading of the manuscript, to Stenio Fragoso and Rosana Gonçalves, for the anti-Dynamin serum, and Nilsson Fidencio, for technical assistance.

REFERENCES

- Bernhart SH, Hofacker IL, Will S, Gruber AR, Stadler PF 2008. RNAalifold: improved consensus structure prediction for RNA alignments. *BMC Bioinformatics* 9: 474.
- Contreras VT, Salles JM, Thomas N, Morel CM, Goldenberg S 1985. *In vitro* differentiation of *Trypanosoma cruzi* under chemically defined conditions. *Mol Biochem Parasitol* 16: 315-327.
- Dallagiovanna B, Perez L, Sotelo-Silveira J, Smircich P, Duhagon MA, Garat B 2005. *Trypanosoma cruzi*: molecular characterization of TePUF6, a Pumilio protein. *Exp Parasitol* 109: 260-264.
- Dumas C, Chow C, Muller M, Papadopoulou B 2006. A novel class of developmentally regulated noncoding RNAs in *Leishmania*. *Eukaryot Cell* 5: 2033-2046.
- Elbarbary RA, Takaku H, Uchiumi N, Tamiya H, Abe M, Takahashi M, Nishida H, Nashimoto M 2009. Modulation of gene expression by human cytosolic tRNase Z(L) through 5'-half-tRNA. *PLoS ONE* 4: e5908.
- Ferreira LR, Dossin F de M, Ramos TC, Freymuller E, Schenkman S 2008. Active transcription and ultrastructural changes during *Trypanosoma cruzi* metacyclogenesis. *An Acad Bras Cienc* 80: 157-166.
- Figueiredo RC, Rosa DS, Soares MJ 2000. Differentiation of *Trypanosoma cruzi* epimastigotes: metacyclogenesis and adhesion to substrate are triggered by nutritional stress. *J Parasitol* 86: 1213-1218.
- Franzén O, Arner E, Ferella M, Nilsson D, Respuela P, Carninci P, Hayashizaki Y, Aslund L, Andersson B, Daub CO 2011. The short non-coding transcriptome of the protozoan parasite *Trypanosoma cruzi*. *PLoS Negl Trop Dis* 5: e1283.
- Garcia-Silva MR, Frugier M, Tosar JP, Correa-Dominguez A, Ronal-te-Alves L, Parodi-Talice A, Rovira C, Robello C, Goldenberg S, Cayota A 2010. A population of tRNA-derived small RNAs is actively produced in *Trypanosoma cruzi* and recruited to specific cytoplasmic granules. *Mol Biochem Parasitol* 171: 64-73.
- Haile S, Papadopoulou B 2007. Developmental regulation of gene expression in trypanosomatid parasitic protozoa. *Curr Opin Microbiol* 10: 569-577.
- Haussecker D, Huang Y, Lau A, Parameswaran P, Fire AZ, Kay MA, 2010. Human tRNA-derived small RNAs in the global regulation of RNA silencing. *RNA* 16: 673-695.
- Horn D 2008. Codon usage suggests that translational selection has a major impact on protein expression in trypanosomatids. *BMC Genomics* 9: 2.

- Lee SR, Collins K 2005. Starvation-induced cleavage of the tRNA anticodon loop in *Tetrahymena thermophila*. *J Biol Chem* 280: 42744-42749.
- Li Y, Luo J, Zhou H, Liao JY, Ma LM, Chen YQ, Qu LH 2008. Stress-induced tRNA-derived RNAs: a novel class of small RNAs in the primitive eukaryote *Giardia lamblia*. *Nucleic Acids Res* 36: 6048-6055.
- Michaeli S, Doniger T, Gupta SK, Wurtzel O, Romano M, Visnovsky D, Sorek R, Unger R, Ullu E 2012. RNA-seq analysis of small RNPs in *Trypanosoma brucei* reveals a rich repertoire of non-coding RNAs. *Nucleic Acids Res* 40: 1282-1298.
- Minning TA, Weatherly DB, Atwood J 3rd, Orlando R, Tarleton RL 2009. The steady-state transcriptome of the four major life-cycle stages of *Trypanosoma cruzi*. *BMC Genomics* 10: 370.
- Nardelli SC, Avila AR, Freund A, Motta MC, Manhaes L, de Jesus TC, Schenkman S, Fragoso SP, Krieger MA, Goldenberg S, Dal-lagiovanna B 2007. Small-subunit rRNA processome proteins are translationally regulated during differentiation of *Trypanosoma cruzi*. *Eukaryot Cell* 6: 337-345.
- Padilla-Mejia NE, Florencio-Martinez LE, Figueroa-Angulo EE, Manning-Cela RG, Hernandez-Rivas R, Myler PJ, Martinez-Calvillo S 2009. Gene organization and sequence analyses of transfer RNA genes in trypanosomatid parasites. *BMC Genomics* 10: 232.
- Pederson T 2010. Regulatory RNAs derived from transfer RNA? *RNA* 16: 1865-1869.
- Pucadyil TJ, Schmid SL 2009. Conserved functions of membrane active GTPases in coated vesicle formation. *Science* 325: 1217-1220.
- Will S, Reiche K, Hofacker IL, Stadler PF, Backofen R 2007. Inferring noncoding RNA families and classes by means of genome-scale structure-based clustering. *PLoS Comput Biol* 3: 680-691.



Predicted secondary structures adopted by the most abundant 3' tRNA fragments.

TABLE

List of cloned tRNA-derived fragments, their identity obtained by Basic Local Alignment Search Tool against database sequences from *Trypanosoma cruzi* and if the sequence matches either the 5', mid portion or 3' portion of the tRNA

Clone	tRNA	Cloned sequence
332	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
634	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
70	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA ACC
713	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA ACC
831	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA CCA
247	tRNA-Glu:anticodon TTC, 3'	ACCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA CCA
172	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTC
102	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTC
803	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCG
685	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGCA
133	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
150	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
513	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
334	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA
614	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CC
637	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CC
686	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CCA
5	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CCA
703	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTGGATTCCCAGGATCGGA
387	tRNA-Glu:anticodon TTC, 3'	CACCCAGGAGATCCGGGTTTGATTCCCAGGATCGGA
444	tRNA-Glu:anticodon TTC, 3'	CAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
124	tRNA-Glu:anticodon TTC, 3'	CCAGGAAATCCGGGTTTCGATTCCCAGGATCGGAA CC
487	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGG
397	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCG
352	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
456	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
481	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
516	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
319	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
380	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
399	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
791	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
792	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
833	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CCA
549	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAA CCA
311	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTTCGATTCCCAGGATCGGAT
722	tRNA-Glu:anticodon TTC, 3'	CCAGGAGATCCGGGTTGGATTCCCAGGATCGGAA CCA
702	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATAGGA
472	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
427	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCG
87	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
248	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
331	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
390	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
429	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
506	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
602	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
779	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
793	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
542	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGG
117	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA
151	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCAGGATCGGA



Clone	tRNA	Cloned sequence
164	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
252	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
354	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
402	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
403	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
407	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
408	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
416	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
419	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
424	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
439	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
452	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
466	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
480	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
483	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
485	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
492	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
494	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
505	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
507	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
511	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
515	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
519	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
701	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
727	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
734	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
749	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
751	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
776	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
790	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
834	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
840	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
141	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
25	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGA
357	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA
7	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA C
652	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA C
710	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
626	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
656	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
342	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
647	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
9	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGGAA CC
479	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
484	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
774	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
375	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
283	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
760	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
708	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
442	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
348	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
184	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG
314	tRNA-Glu:anticodon TTC, 3'	CCCAGGAGATCCGGGTTCGATTCCCAGGATCGG AA TTCACCAGGAGATCCGGGTTCGATTCCCAGGATCGG AA C

Clone	tRNA	Cloned sequence
339	tRNA-Glu:anticodon TTC, 5'	CACCCAGGAGATCCGGGTTTCGATCCCCGGTATCGGAA ACC
723	tRNA-Glu:anticodon TTC, 5'	CCGATATGGTATAACGGTTAGAACACCCGGC
860	tRNA-Glu:anticodon TTC, 5'	CCGATATGGTATAACGGTTAGAACACCTGG
778	tRNA-Glu:anticodon TTC, 5'	CCGATATGGTATAACGGTTAGAACACCTGGC
282	tRNA-Glu:anticodon TTC, 5'	CCGATATGGTATAACGGTTAGAACACCTGGC
520	tRNA-Glu:anticodon TTC, 5'	CCGATATGGTATAACGGTTAGAACACCTGGC
580	tRNA-Glu:anticodon TTC, 5'	CGATATGGTATAACGGTTAGAACACCTGG
471	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTT
623	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTTAGAACACCTGGC
358	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTTAGAACACCTGGC
425	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTTAGAACACCTGGC
495	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTTAGAACACCTGGC
568	tRNA-Glu:anticodon TTC, 5'	TCCGATATGGTATAACGGTTAGAACACCTGGC
225	tRNA-Thr:anticodon AGT, 3'	AAAGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
601	tRNA-Thr:anticodon AGT, 3'	AAGACGGAGGTCGGGGGTTTCGATCCCCCAGTGGC
817	tRNA-Thr:anticodon AGT, 3'	AAGACGGAGGTCGGGGGTTTCGATCCCCCAGTGGC CTCCA
355	tRNA-Thr:anticodon AGT, 3'	ACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
512	tRNA-Thr:anticodon AGT, 3'	ACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
251	tRNA-Thr:anticodon AGT, 3'	CGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
398	tRNA-Thr:anticodon AGT, 3'	CGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
720	tRNA-Thr:anticodon AGT, 3'	CGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
795	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCAGGGGTTTCGATCCCCCAGTGGCCT
781	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTAGATCCCCCAGCGGCCT
567	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGC
595	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGC
78	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
160	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
185	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
443	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
474	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
490	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
498	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
526	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
546	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
575	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
635	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
719	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
728	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
761	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
846	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
851	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
13	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
56	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
63	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
85	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
551	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCC
61	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
2	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
96	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
120	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
318	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
423	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
455	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
504	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT



Clone	tRNA	Cloned sequence
578	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
721	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
745	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
780	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
176	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
345	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCTC
295	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCTCCA
530	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCTCCA
785	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCTCCA
593	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCTGCGGA
799	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTCGGGGGTTTCGATCCCCCAGTGGCCT
818	tRNA-Thr:anticodon AGT, 3'	GACGGAGGTTGGGGGTTTCGATCCCCCAGTGGCC
271	tRNA-Val:anticodon CAC#1, 3'	AAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
784	tRNA-Val:anticodon CAC#1, 3'	AAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
737	tRNA-Val:anticodon CAC#1, 3'	AAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
321	tRNA-Val:anticodon CAC#1, 3'	AAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCACCA
577	tRNA-Val:anticodon CAC#1, 3'	AAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
532	tRNA-Val:anticodon CAC#1, 3'	ACGCGGAGGCCCGGGTTCGTTTCCCGGACTGCGCAC
246	tRNA-Val:anticodon CAC#1, 3'	CAAAGCGGGGGGCCCGGGTTCGATTCCCGGACTGCGCAC
197	tRNA-Val:anticodon CAC#1, 3'	CAAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
581	tRNA-Val:anticodon CAC#1, 3'	CAAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
137	tRNA-Val:anticodon CAC#1, 3'	CAAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
261	tRNA-Val:anticodon CAC#1, 3'	CAAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
564	tRNA-Val:anticodon CAC#1, 3'	CATCAAAGCGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCACCA
356	tRNA-Val:anticodon CAC#1, 3'	CGCGGGAGGCCCGGGTTCGTTTCCCGGACTGCGCA
679	tRNA-Val:anticodon CAC#1, 3'	CGGGGGGCCCGGGTTTTTCCCGGACTGCGCAC
540	tRNA-Val:anticodon CAC#1, 3'	GCGGGGGGCCCGGGTTCGATTCCCGGACTGCGCAC
289	tRNA-Val:anticodon CAC#1, 3'	GGGCCCGGGTTCGTTTCCCGGACTGCGCACCAT
717	tRNA-Val:anticodon CAC#1, 3'	GGGGGGGCCCGGGTTCGTTTCCCGGACTGCGCAC
450	tRNA-Pro:anticodon AGG, 3'	GTGGGAGAGGTCCCGGGTTCGTTTCCCGGCTGGGCC
861	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTTTTCCCGGCTGGGCC
393	tRNA-Pro:anticodon AGG, 3'	ACAGGAGGTACCGGGTTCATTTCCCGGCTGGGCC
335	tRNA-Pro:anticodon AGG, 3'	GGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
410	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGCTTCAGTTCCCGGCTGGATCC
92	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
180	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
527	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
250	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
67	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
69	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
337	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
361	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
370	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
379	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
384	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
400	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
517	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
651	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
665	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
762	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
775	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
191	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
349	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC
422	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCCGGGTTCATTTCCCGGCTGGGCC

Clone	tRNA	Cloned sequence
431	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
458	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
664	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
189	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
272	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
814	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
824	tRNA-Pro:anticodon AGG, 3'	TGGGAGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
396	tRNA-Pro:anticodon AGG, 3'	TGGGGGAGGTCCC GGTTCAATTTCCCGGCTGGGCCCC
496	tRNA-Glu:anticodon CTC, 3'	ACCCGCTAGACCCGGGTTCAATTTCCCGGCATCGGA
159	tRNA-Glu:anticodon CTC, 3'	CACCCAGGAGATCCGGGTTTCGATTCCCGGTATCGG
128	tRNA-Glu:anticodon CTC, 3'	CACCCGCTAGACCCGGGTTCAATTTCCCGGCATCGGAA
669	tRNA-Glu:anticodon CTC, 3'	CACCCGCTAGACCCGGGTTCAATTTCCCGGCATCGGA CCA
765	tRNA-Glu:anticodon CTC, 3'	CACCCGGTAGACCCGGGTTCAATTTCCCGTCATCGGA CCA
338	tRNA-Glu:anticodon CTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCGGTATCGGA
378	tRNA-Glu:anticodon CTC, 3'	CCCAGGAGATCCGGGTTTCGATTCCCGGTATCGGA
453	tRNA-Glu:anticodon CTC, 3'	CCCGTAGACCCGGGTTCAATTTCCCGGCATCGG
486	tRNA-Glu:anticodon CTC, 3'	CCCGTAGACCCGGGTTCAATTTCCCGGCATCGGA
571	tRNA-Glu:anticodon CTC, 3'	CCCGTAGACCCGGGTTCAATTTCTCGGCATC
820	tRNA-Glu:anticodon CTC, 3'	CCCGTAGACCCGGGTTCAATTTCTCGGCATC
536	tRNA-Glu:anticodon CTC, 5'	CCGGTGTGGTATAGTGGTTAGAACAAGCGGC
692	tRNA-Glu:anticodon CTC, 5'	CCGGTGTGGTATAGTGGTTAGAACAAGCGG
598	tRNA-Glu:anticodon CTC, 5'	CCGGTGTGGTATAGTGGTTAGAACAAGCGGC
340	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
543	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
259	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
426	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
454	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
508	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGCTC
343	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
437	tRNA-Glu:anticodon CTC, 5'	TCCGGTGTGGTATAGTGGTTAGAACAAGCGGC
459	tRNA-Glu:anticodon CTC, mid	TAGTGGTTAGAACAAGCGGC
666	tRNA-Asp:anticodon GTC, 3'	ACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CCA
239	tRNA-Asp:anticodon GTC, 3'	CACGCGGGAGACCCGGGTTTCCTT
612	tRNA-Asp:anticodon GTC, 3'	CACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGA
752	tRNA-Asp:anticodon GTC, 3'	CACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CCA
77	tRNA-Asp:anticodon GTC, 3'	CACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CCA
131	tRNA-Asp:anticodon GTC, 3'	CGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CC
430	tRNA-Asp:anticodon GTC, 3'	GCGGGTGACCCGGGTTCAATTTCCCGGCCGGGA
447	tRNA-Asp:anticodon GTC, 3'	GCGGGTGACCCGGGTTCAATTTCCCGGCCGGGA
155	tRNA-Asp:anticodon GTC, 3'	CACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CCA
156	tRNA-Asp:anticodon GTC, 3'	CACGCGGGTGACCCGGGTTCAATTTCCCGGCCGGGAAG CCA
620	tRNA-Asp:anticodon GTC, 5'	CTCGGTAGTATAATGGTAAGTATACCCGCC
4	tRNA-Asp:anticodon GTC, 5'	CTCGGTAGTATAGTGGTAAGTATACCCGCC
57	tRNA-Asp:anticodon GTC, 5'	CTCGGTAGTATAGTGGTAAGTATACCCGCC
500	tRNA-Asp:anticodon GTC, 5'	CTCGGTAGTATAGTGGTAAGTATACCCGCC
46	tRNA-Asp:anticodon GTC, 5'	TCTCGGTAGTATAGTGGTAAGTATACCCGCC
254	tRNA-Asp:anticodon GTC, 5'	TCCGGTAGTATAGTGGTAAGTATACCCGCC
320	tRNA-Asp:anticodon GTC, 5'	TCCGGTAGTATAGTGGTAAGTATACCCGCC
88	tRNA-Asp:anticodon GTC, 5'	TCTCGGTAGTATAGTGGTAAGTATACCCGC
678	tRNA-Asp:anticodon GTC, 5'	TCTCGGTAGTATAGTGGTAAGTATACCCGC
363	tRNA-Asp:anticodon GTC, 5'	TCTCGGTAGTATAGTGGTAAGTATACCCGCCT
741	tRNA-Pro:anticodon CGG, 3'	AGGAGGTCACGGGTTCAATTTCCCGTAGCGGCC
368	tRNA-Pro:anticodon CGG, 3'	CAGGAGGTCACGGGTTCAATTTCCCGTAGCGGCC
470	tRNA-Pro:anticodon CGG, 3'	CAGGAGGTCACGGGTTCAATTTCCCGTAGCGGCC



Clone	tRNA	Cloned sequence
697	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGC
140	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC
71	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
351	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
636	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
350	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
420	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
457	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCC
465	tRNA-Pro:anticodon CGG, 3'	GTACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC
521	tRNA-Pro:anticodon CGG, 3'	GTACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC
84	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCCC
256	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCCCC
385	tRNA-Pro:anticodon CGG, 3'	ACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC CC
523	tRNA-Pro:anticodon CGG, 3'	GGTACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC
497	tRNA-Pro:anticodon CGG, 3'	GTACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC CC
369	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC CC
802	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC CC
449	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC CCA
460	tRNA-Pro:anticodon CGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC T
341	tRNA-Pro:anticodon TGG, 3'	TACAGGAGGTCACGGGTTCAATTCCCCTAGCGGCC A
138	tRNA-Gly:anticodon CCC, 3'	AGGTATTGACCCGGGTCGTTTCCCGGACGGCGC CA
152	tRNA-Gly:anticodon CCC, 3'	AGGTATTGACCCGGGTTTCGTTTCCCGGACGGCGC A
16	tRNA-Gly:anticodon CCC, 3'	CAAGGTATTGACCCGGGTTTCGATTCCCGGACGGCGC A
34	tRNA-Gly:anticodon CCC, 3'	CAAGGTATTGACCCGGGTTTCGATTCCCGGACGGCGC A
590	tRNA-Gly:anticodon CCC, 3'	CAAGGTATTGACCCGGGTTTCGATTCCCGGACGGCGC A
114	tRNA-Gly:anticodon CCC, 3'	CAAGGTATTGACCCGGGTTTCGTTTCCCGGACGGCGC CA
223	tRNA-Gly:anticodon CCC, 3'	CCCAAGGTATTGACCCGGGTTTCGTTTCCCGGACGGCGC A
473	tRNA-Gly:anticodon CCC, 3'	TGACCCGGGTTTCGTTTCCCGGACGGCGC
40	tRNA-Gly:anticodon CCC, 3'	TTGACCCGGGTTTCGTTTCCCGGACGGCGC
325	tRNA-Gly:anticodon CCC, 3'	TTGACCCGGGTTTCGTTTCCCGGACGGCGC
296	tRNA-Gly:anticodon GCC, 3'	ACCCAGGAGATCCGGGTTTCGATTCCCGGACTGCGC ACC
233	tRNA-Gly:anticodon GCC, 3'	ACGCGGGAGGCCCGGGTTCGTTTCCCGGACTGCGC ACC
51	tRNA-Gly:anticodon GCC, 3'	ACGCGGGAGGCCCGGGTTCGTTTCCCGGACTGCGC ACC
857	tRNA-Gly:anticodon GCC, 3'	CACGCGGGAGGCCCGGGTTCGATTCCCGGACTGCGC CCA
236	tRNA-Gly:anticodon GCC, 3'	CACGCGGGAGGCCCGGGTTCGTTTCCCGGACTGCGC ACC
74	tRNA-Gly:anticodon GCC, 3'	CACGCGGGAGGCCCGGGTTCGTTTCCCGGACTGCGC CCA
53	tRNA-Gly:anticodon GCC, mid	ACGAGGGAGGCCCGGGTTCGTTTCCCGGACTGCGC ACC
798	tRNA-His:anticodon GTG, 3'	CTCTGAATACCCGGGTTTCGATTCCCGGTCTTCCCT CCA
418	tRNA-His:anticodon GTG, 3'	CTGAATACCCGGATTTCGATTCCCGGTCTTCCCT C
336	tRNA-His:anticodon GTG, 3'	GTTTCGATTCCCGGTCTTCCCT
260	tRNA-Lys:anticodon TTT, 3'	AACCGTGTGGTCGTTGGGTTTCGATCCCCACGGATGGC ACC
801	tRNA-Ala:anticodon AGC, 3'	TGCGGGAGGTATTGGGATCGTTACCCAACTTCTCC
10	tRNA-Ala:anticodon CGC, 3'	TACGGAAGGCCTAGGGTTCGATCCCCTACTCGTCCA
301	tRNA-Ala:anticodon CGC, 3'	TACGGAAGGCCTAGGGTTCGATCCCCTACTCGTCCA
208	tRNA-Val:anticodon CAC#2, 5'	GCGATGGTCGTCTAGGGGTTAGGACATTCTC
446	tRNA-Val:anticodon CAC#2, 5'	GCGATGGTCGTCTAGTGGTTAGGACATTCTGCC
856	tRNA-Val:anticodon CAC#2, 5'	GCGATGGTCGTCTAGTGGTTAGGACATTCTGC
395	tRNA-Arg:anticodon TCG, 3'	CAGAGGGTTGCAGGTTTCGGATCCTGTCACGGAT
381	tRNA-Gly:anticodon TCC, 5'	GCAATCGTGGTCCAACGGTTAAGATCCCCGCC
428	tRNA-Gly:anticodon TCC, 5'	GCAATCGTGGTCCAACGGTTAAGATCCCCGCC
573	tRNA-Val:anticodon AAC, 5'	GCGCTGATGGTCTAGGTGGTTATGACGTCGCTTTG
421	tRNA-Val:anticodon AAC, 3'	GACACGGCGAAGGTCTCGGGTTCGGGTCCCCTAGCGGC
518	tRNA-Trp:anticodon CCA, 3'	TCCAGGGTTCGAGGTTCAATCCCTGCAGTCTCTC
475	tRNA-Tyr:anticodon GTA, 5'	CCTTCTGTAGCTCAATTGGTAGAGCATGTGACTGTAG

the non-encoded nucleotides are in red.