SUPPLEMENTARY DATA

The MRPP1/MRPP2 complex is a tRNA-maturation platform in human mitochondria

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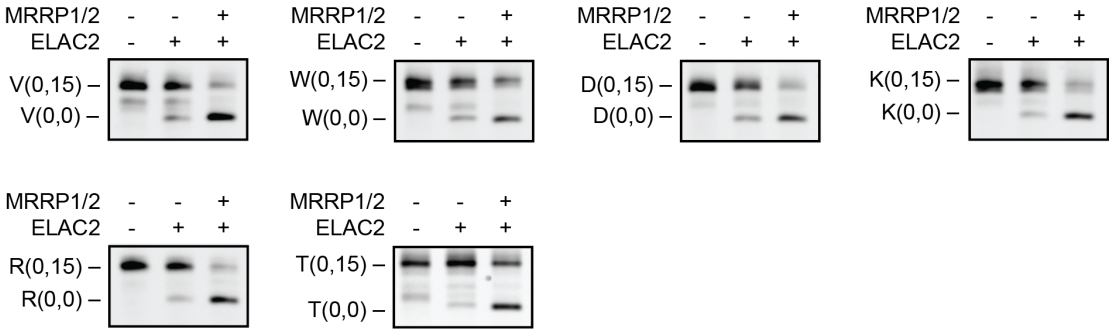
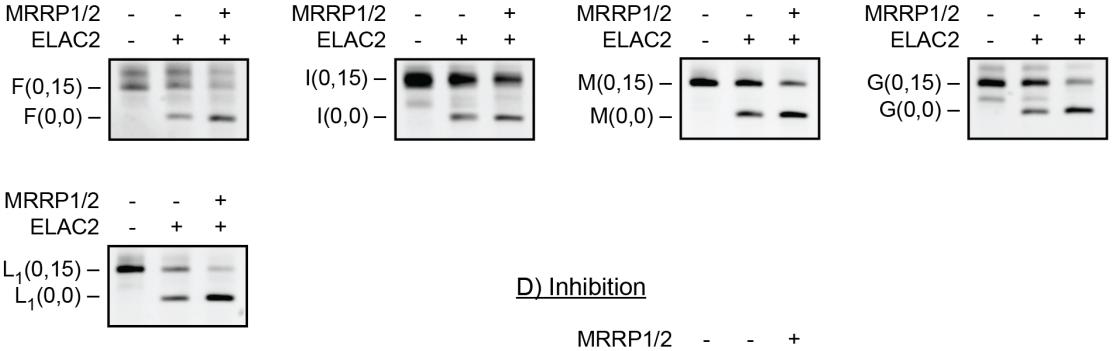
SUPPLEMENTARY FIGURES

Heavy-strand

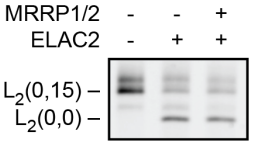
A) Strong support



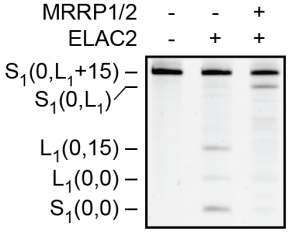
B) Moderate support



C) No effect

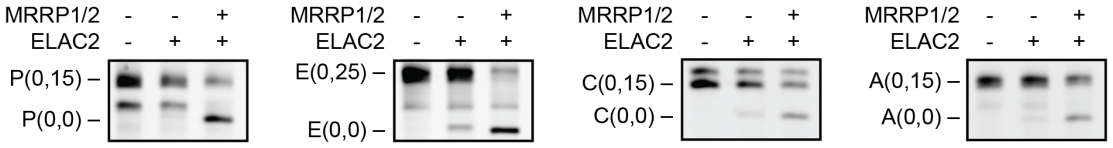


D) Inhibition



Light-strand

E) Strong support



F) No effect

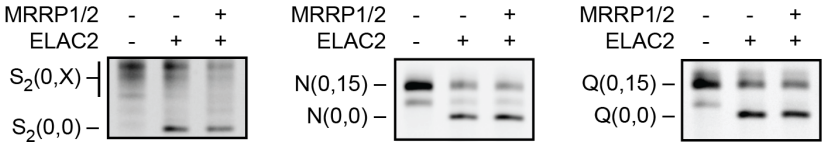


Figure S1. Effect of MRPP1/2 on ELAC2 based 3´-tail removal of human mt-tRNAs. Urea PAGE images showing the activity of 50 nM ELAC2 on 200 nM pre-tRNA substrates in the absence and presence of 800 nM MRPP1/2. The reaction mixes were incubated for 20 min at 30°C. The tRNA substrates form the heavy strand (**A-D**) and light strand (**E-F**) are shown. The following abbreviations are used: L_1 for $tRNA^{Leu(CUN)}$ (in B and D), L_2 for $tRNA^{Leu(UUR)}$ (in C), S_1 for $tRNA^{Ser(AGY)}$ (in D), and S_2 for $tRNA^{Ser(UCN)}$ (in F). The tRNA substrates were produced *in vitro* by run-off transcription, and in several cases this leads to additional shorter and/or longer transcripts, which are also visible on the urea PAGE image. These tRNA products can potentially also serve as substrates in the ELAC2 cleavage reaction, explaining why some "additional" bands disappear after ELAC2 or MRPP1/2:ELAC2 treatment. For S_2 no main product corresponding to a 15 nucleotide long 3´-tail could be obtained, nonetheless, treatment with ELAC2 and MRRP1/2:ELAC2 resulted in formation of the mature S_2 product.

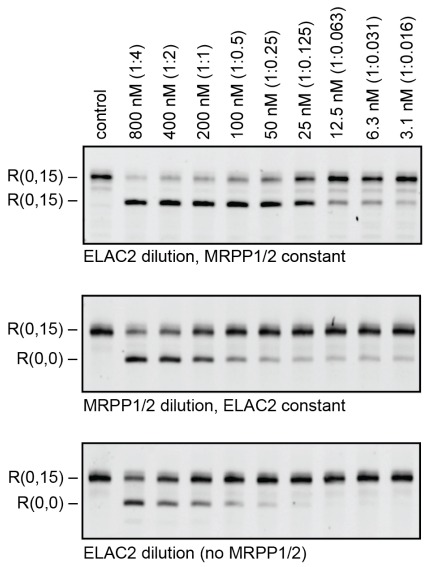


Figure S2. RNase Z dilution experiments on pre-$tRNA^{Arg}$(0,15) (R(0,15)). *Top:* ELAC2 dilution series (800 to 3.1 nM) in the presence of 800 nM MRPP1/2. *Middle:* MRPP1/2 dilution series (800 to 3.1 nM) in the presence of 50 nM ELAC2. *Bottom:* ELAC2 dilution series (800 to 3.1 nM) in the absence of MRPP1/2. The reactions were incubated for 1 h. The molar ratio of tRNA to protein is shown in parentheses.

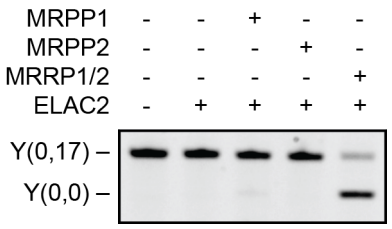


Figure S3. The full MRPP1/2 complex is required to support ELAC2 based 3´-tail removal. Urea PAGE showing the activity of 50 nM ELAC2 on 200 nM pre-tRNA^{Tyr}(0,17) in the absence and presence of MRPP1, MRPP2, and the MRPP1/2 complex (each at 800 nM).

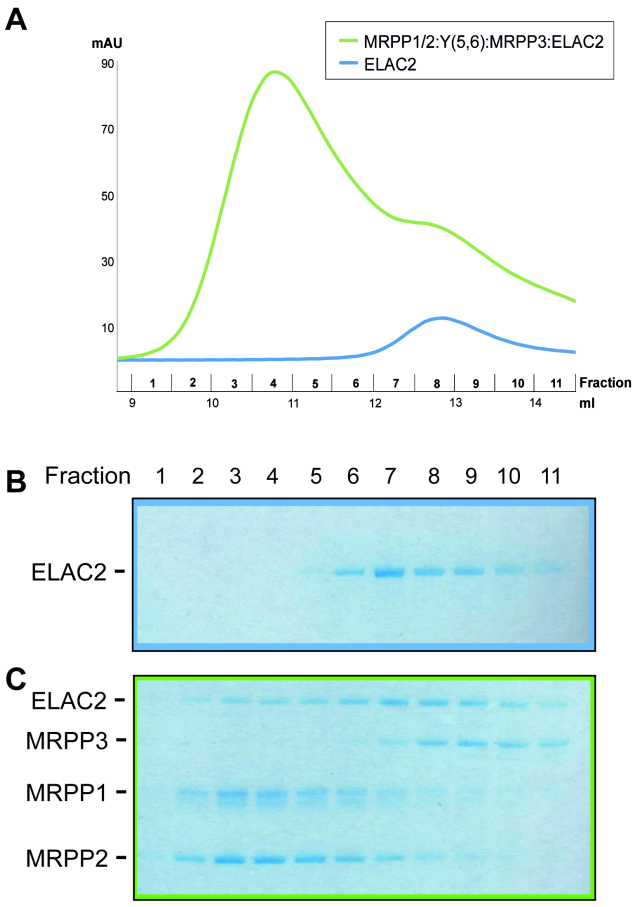


Figure S4. Association of ELAC2 with MRPP1/2:pre-tRNA^{Tyr}(0,0) during gel filtration. (**A**) Gel filtration chromatograms of ELAC2 alone (in blue) and MRPP1/2:pre-tRNA^{Tyr}(5,6) after treatment with MRPP3 and ELAC2 resulting in the formation of MRPP1/2:pre-tRNA^{Tyr}(0,0) (in green). (**B, C**) The corresponding SDS-PAGE images for ELAC2 alone (B) and MRPP1/2:pre-tRNA^{Tyr}(5,6) after treatment with MRPP3 and ELAC2 (C) are shown below such that the individual fractions are aligned to the chromatogram. A clear shift of ELAC2 towards earlier elution volumes in the presence of MRPP1/2: pre- tRNA^{Tyr}(0,0) is visible indicating an interaction of ELAC2 with MRPP1/2: pre- tRNA^{Tyr}(0,0).

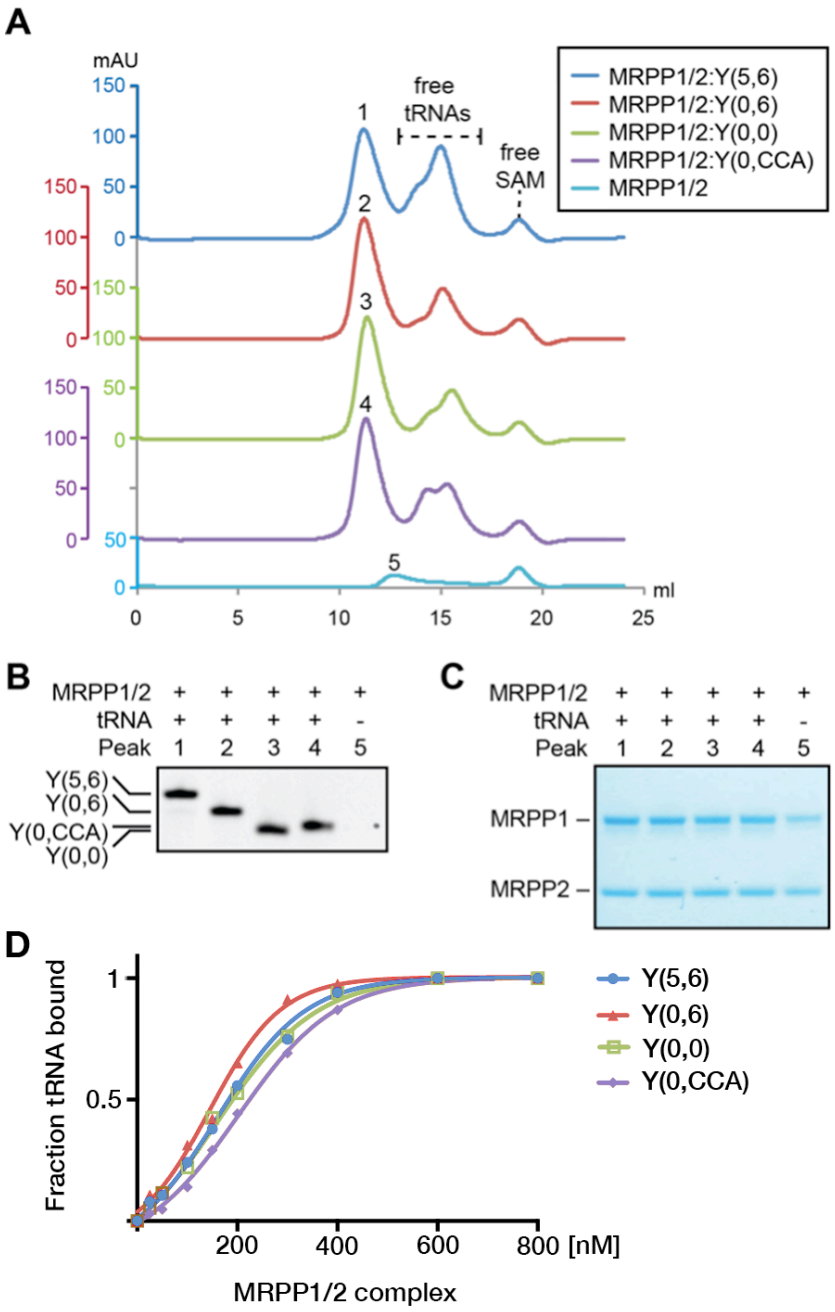


Figure S5. MRPP1/2 recognizes $tRNA^{Tyr}$ of different processing status. **(A-D)** Gel filtration chromatograms of MRPP1/2 in complex with $tRNA^{Tyr}$ variants of different processing status (A). Peaks of interest are numbered, and the content of tRNA and protein peaks is visualized by urea PAGE (B) and SDS-PAGE (C), respectively. MRPP1/2 is able to form stable complexes with all provided $tRNA^{Tyr}$ substrates. (D) Results from electron-mobility shift assays of binding by MRPP1/2 to the four different $tRNA^{Tyr}$ substrates performed in triplicates, lines from non-linear regression analysis. Estimated Kd values for the different tRNAs: ~180 nM for Y(5,6); ~155 nM for Y(0,6); ~190 nM for Y(0,0); ~225 nM for Y(0,CCA).

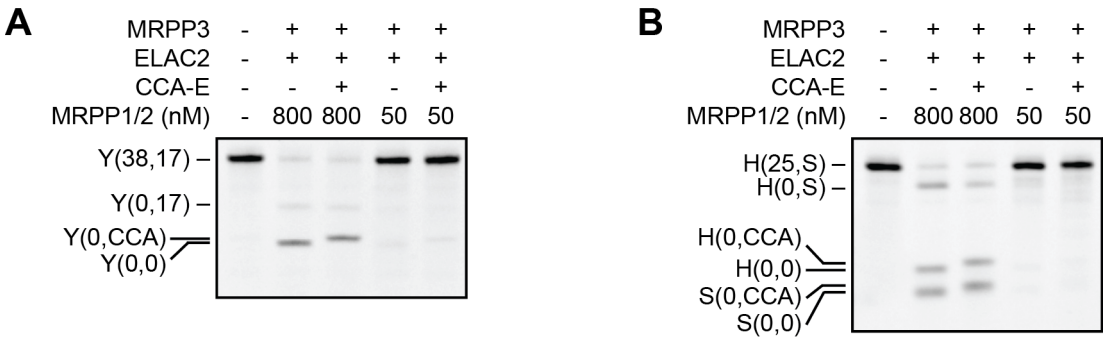


Figure S6. CCA-addition does not trigger MRPP1/2 to turn-over. (**A, B**) Full core processing of pre-tRNA^{Tyr}(38,17) (A) and pre-tRNA^{His}(25,S) (B) using 50 or 800 nM MRPP1/2, 50 nM MRPP3, and 50 nM ELAC2 in the absence and presence of 50 nM CCA-adding enzyme (CCA-E). The reaction mixes were incubated for 1h. When an excess of MRPP1/2 was supplied, RNase P and RNase Z were able to remove the 5´-leader and 3´-tail from the tRNA substrate. Further supplementation with CCA-adding enzyme resulted in 3´-CCA addition. Under the experimental conditions, nearly all tRNA was processed. However, if submolar amounts of MRPP1/2 were used, little or no end-product formation is observed, indicating that 3´-CCA addition is not responsible for releasing the 5´- and 3´-mature pre-tRNA from MRPP1/2.

SUPPLEMENTARY TABLES

Table S1. mt-tRNA constructs from the heavy strand directly produced by run-off transcription. The tRNA sequence is presented in bold; 5´-leader and 3´-tail are colored in red and blue, respectively. For T7 RNA polymerase based transcription a starting "G" is required, and therefore, tRNA(0,X) constructs which started with a non-G nucleotide were mutated to G to form a GC base pair (underlined).

Name of tRNA	Location in mt-DNA	DNA sequence
$tRNA^{Phe}$(0,15)	577-662	**GTTTATGTAGCTTACCTCCTCAAAGCAATACACTGAAAATGTTTAGACGG GCTCACATCACCCCATAAACA**AATAGGTTTGGTCCT
$tRNA^{Val}$(0,15)	1602-1685	**G**AGAGTGTAGCTTAACACAAAGCACCCAACTTACACTTAGGAGATTTCA ACTTAACTTGACCGCTCT**C**AGCTAAACCTAGCCCC
$tRNA^{Leu(UUR)}$(0,15)	3230-3319	**GTTAAGATGGCAGAGCCCGGTAATCGCATAAAACTTAAAACTTTACAGT CAGAGGTTCAATTCCTCTTCTTAACA**ACATACCCATGGCCA
$tRNA^{Ile}$(0,15)	4263-4346	**G**GAAATATGTCTGATAAAAGAGTTACTTTGATAGAGTAAATAATAGGAG CTTAAACCCCCTTATTTC**C**AGGACTATGAGAATCG
$tRNA^{Met}$(0,15)	4402-4484	**G**GTAAGGTCAGCTAAATAAGCTATCGGGCCCATACCCCGAAAATGTTGG TTATACCCTTCCCGTAC**C**AATTAATCCCCTGGCC
$tRNA^{Trp}$(0,15)	5512-5594	**G**GAAATTTAGGTTAAATACAGACCAAGAGCCTTCAAAGCCCTCAGTAAG TTGCAATACTTAATTTC**C**GTAACAGCTAAGGACT
$tRNA^{Asp}$(0,15)	7518-7600	**G**AGGTATTAGAAAAACCATTTCATAACTTTGTCAAAGTTAAATTATAGGC TAAATCCTATATATCT**C**AATGGCACATGCAGCG
$tRNA^{Lys}$(0,15)	8295-8379	**G**ACTGTAAAGCTAACTTAGCATTAACCTTTTAAGTTAAAGATTAAGAGAA CCAACACCTCTTTACAGT**C**AAATGCCCCAACTAAA
$tRNA^{Gly}$(0,15)	9991-10073	**G**CTCTTTTAGTATAAATAGTACCGTTAACTTCCAATTAACTAGTTTTGACA ACATTCAAAAAAGAGC**C**AATAAACTTCGCCTTA
$tRNA^{Arg}$(0,15)	10405-10484	**G**GGTATATAGTTTAAACAAAACGAATGATTTCGACTCATTAAATTATGAT AATCATATTTACC**C**AATGCCCCTCATTTAC
$tRNA^{His}$(0,0)	12138-12206	**GTAAATATAGTTTAACCAAAACATCAGATTGTGAATCTGACAACAGAGG CTTACGACCCCTTATTTACC**
$tRNA^{His}$(0,S)	12138-12265	**GTAAATATAGTTTAACCAAAACATCAGATTGTGAATCTGACAACAGAGG CTTACGACCCCTTATTTACCGAGAAAGCTCACAAGAACTGCTAACTCAT GCCCCCATGTCTAACAACATGGCTTTCTCA**
$tRNA^{His}$(25,S)	12113-12265	GACATCATTACCGGGTTTTCCTCTT**GTAAATATAGTTTAACCAAAACATC AGATTGTGAATCTGACAACAGAGGCTTACGACCCCTTATTTACCGAGAA AGCTCACAAGAACTGCTAACTCATGCCCCCATGTCTAACAACATGGCTT TCTCA**
$tRNA^{Ser(AGU)}$(0, $tRNA^{Leu(CUN)}$+15)	12207-12351	**GAGAAAGCTCACAAGAACTGCTAACTCATGCCCCCATGTCTAACAACAT GGCTTTCTCAACTTTTAAAGGATAACAGCTATCCATTGGTCTTAGGCCCC AAAAATTTTGGTGCAACTCCAAATAAAAGTA**ATAACCATGCACACT
$tRNA^{Ser(AGY)}$(0,15)	12207-12280	**GAGAAAGCTCACAAGAACTGCTAACTCATGCCCCCATGTCTAACAACAT GGCTTTCTCA**ACTTTTAAAGGATAA
$tRNA^{Leu(CUN)}$(0,15)	12266-12351	**G**CTTTTAAAGGATAACAGCTATCCATTGGTCTTAGGCCCCAAAAATTTTG GTGCAACTCCAAATAAAAGC**C**AATAACCATGCACACT
$tRNA^{Thr}$(0,15)	15888-15968	**GTCCTTGTAGTATAAACTAATACACCAGTCTTGTAAACCGGAGATGAAA ACCTTTTTCCAAGGACA**AATCAGAGAAAAAGT

Table S2. mt-tRNA constructs from the light strand directly produced by run-off transcription. The tRNA sequence is presented in bold; 5´-leader and 3´-tail are colored in red and blue, respectively. For T7 RNA polymerase based transcription a starting "G" is required. Therefore tRNA(0,X) constructs which started with a non-G nucleotide were mutated to G to form a GC base pair (underlined).

Name of tRNA	Location in mt-DNA	DNA sequence
$tRNA^{Pro}$(0,15)	15941-16023	**GAGAGAATAGTTTAAATTAGAATCTTAGCTTTGGGTGCTAATGGTGGAG TTAAAGACTTTTTCTCTCA**TTTGTCCTTGGAAAA
$tRNA^{Glu}$(0,15)	14659-14742	**GTTCTTGTAGTTGAAATACAACGATGGTTTTTCATATCATTGGTCGTGGT TGTAGTCCGTGCGAGAATA**ATGATGTATGCTTTG
$tRNA^{Ser(UCN)}$(0,15)	7431-7514	**GAAAAAGTCATGGAGGCCATGGGGTTGGCTTGAAACCAGCTTTGGGGG GTTCGATTCCTTCCTTTTTTG**TCTAGATTTTATGTA
$tRNA^{Tyr}$(38,17)	5809-5929	GAGAATAGTCAACGGTCGGCGAACATCAGTGGGGGTGA**GGTAAAATGGC TGAGTGAAGCATTGGACTGTAAATCTAAAGACAGGGGTTAGGCCTCTTT TTACCA**GCTCCGAGGTGATTTTC
$tRNA^{Tyr}$(0,17)	5809-5891	**GGTAAAATGGCTGAGTGAAGCATTGGACTGTAAATCTAAAGACAGGGG TTAGGCCTCTTTTTACCA**GCTCCGAGGTGATTTTC
$tRNA^{Cys}$(0,15)	5746-5826	**GGCTCCGAGGTGATTTTCATATTGAATTGCAAATTCGAAGAAGCAGCTT CAAACCTGCCGGGGCCT**CTCCCGCCTTTTTTC
$tRNA^{Asn}$(0,15)	5642-5729	**GAGATTGAAGCCAGTTGATTAGGGTGCTTAGCTGTTAACTAAGTGTTTGT GGGTTTAAGTCCCATTGGTCTCG**TAAGGGCTTAGCTTA
$tRNA^{Ala}$(0,15)	5572-5655	**GAGGGCTTAGCTTAATTAAAGTGGCTGATTTGCGTTCAGTTGATGCAGA GTGGGGTTTTGCAGTCCTCA**GCTGTTACAGAAATT
$tRNA^{Gln}$(0,15)	4314-4400	**GAGGATGGGGTGTGATAGGTGGCACGGAGAATTTTGGATTCTCAGGGA TGGGTTCGATTCTCATAGTCCTCG**AAATAAGGGGGTTTA

Table S3. mt-tRNA constructs produced fused 5´ to a GlmS ribozyme. The tRNA sequence is presented in bold; 5´-leader and 3´-tail are colored in red and blue, respectively. The 3´-CCA is not encoded in the mt-DNA. Hence, it was added in the appropriate tRNA constructs (underlined). In case of $tRNA^{Tyr}$(X,6) constructs, position six of the 3´-tail was mutated to an A (in italics) in order to initiate cleavage by the GlmS ribozyme.

Name of tRNA	Location in mt-DNA	DNA sequence
$tRNA^{His}$(25,17)	12113-12223	GACATCATTACCGGGTTTTCCTCTT**GTAAATATAGTTTAACCAAAACATC AGATTGTGAATCTGACAACAGAGGCTTACGACCCCTTATTTACC**GAGAA AGCTCACAAGAA
$tRNA^{His}$(0,17)	12138-12223	**GTAAATATAGTTTAACCAAAACATCAGATTGTGAATCTGACAACAGAGG CTTACGACCCCTTATTTACC**GAGAAAGCTCACAAGAA
$tRNA^{His}$(0,CCA)	12138-12209	**GTAAATATAGTTTAACCAAAACATCAGATTGTGAATCTGACAACAGAGG CTTACGACCCCTTATTTACC**CCA
$tRNA^{Tyr}$(5,6)	5820-5896	GGTGA**GGTAAAATGGCTGAGTGAAGCATTGGACTG TAAATCTAAAGACAGGGGTTAGGCCTCTTTTTACCA**GCTCC*A*
$tRNA^{Tyr}$(0,6)	5820-5891	**GGTAAAATGGCTGAGTGAAGCATTGGACTG TAAATCTAAAGACAGGGGTTAGGCCTCTTTTTACCA**GCTCC*A*
$tRNA^{Tyr}$(0,0)	5826-5891	**GGTAAAATGGCTGAGTGAAGCATTGGACTGTAAATCTAAAGACAGGGG TTAGGCCTCTTTTTACCA**
$tRNA^{Tyr}$(0,CCA)	5823-5891	**GGTAAAATGGCTGAGTGAAGCATTGGACTG TAAATCTAAAGACAGGGGTTAGGCCTCTTTTTACCA**CCA