

## Supplementary Tables and Figures

**Bacterial/archaeal protein-only RNase P: complementation in *Escherichia coli* uncovers coevolution of protein-only RNase P and precursor tRNA structures in *Aquifex aeolicus***

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**Table S1**

| Assignment in ref. 1                                                                 | Merged assignment in the present study                                                   | Gene names                                               | Idiosyncrasies                                                                                                                                      |
|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| tRNA-Ala--GGC_1/2<br>tRNA-Ala--GGC_2/2                                               | tRNA-Ala--GGC_1/2<br>tRNA-Ala--GGC_2/2                                                   | <i>alaX</i><br><i>alaW</i>                               |                                                                                                                                                     |
| tRNA-Ala--UGC_1/3<br>tRNA-Ala--UGC_2/3<br>tRNA-Ala--UGC_3/3                          | tRNA-Ala--UGC_multi<br>tRNA-Ala--UGC_multi<br>tRNA-Ala--UGC_multi                        | <i>alaT</i><br><i>alaU</i><br><i>alaV</i>                | 5'-leader identical up to position -25 in all three tRNA genes                                                                                      |
| tRNA-Arg--ACG_1/4<br>tRNA-Arg--ACG_2/4<br>tRNA-Arg--ACG_3/4<br>tRNA-Arg--ACG_4/4     | tRNA-Arg--ACG_1/2<br>tRNA-Arg--ACG_2/2<br>tRNA-Arg--ACG_2/2<br>tRNA-Arg--ACG_2/2         | <i>argV</i><br><i>argY</i><br><i>argZ</i><br><i>argQ</i> |                                                                                                                                                     |
| tRNA-Arg--CCG                                                                        | tRNA-Arg--CCG                                                                            | <i>argX</i>                                              |                                                                                                                                                     |
| tRNA-Arg--CCU                                                                        | tRNA-Arg--CCU                                                                            | <i>argW</i>                                              |                                                                                                                                                     |
| tRNA-Arg--UCU                                                                        | tRNA-Arg--UCU                                                                            | <i>argU</i>                                              |                                                                                                                                                     |
| tRNA-Asn--GUU_1/4<br>tRNA-Asn--GUU_2/4<br>tRNA-Asn--GUU_3/4<br>tRNA-Asn--GUU_4/4     | tRNA-Asn--GUU_multi<br>tRNA-Asn--GUU_multi<br>tRNA-Asn--GUU_multi<br>tRNA-Asn--GUU_multi | <i>asnV</i><br><i>asnU</i><br><i>asnW</i><br><i>asnT</i> | <i>asnV</i> , <i>T</i> : 5'-leader identical up to position -17                                                                                     |
| tRNA-Asp--GUC_1/3<br>tRNA-Asp--GUC_2/3<br>tRNA-Asp--GUC_3/3                          | tRNA-Asp--GUC_1/2_multi<br>tRNA-Asp--GUC_2/2<br>tRNA-Asp--GUC_1/2_multi                  | <i>aspT</i><br><i>aspV</i><br><i>aspU</i>                | <i>aspT</i> , <i>U</i> : 5'-leader identical up to position > -25                                                                                   |
| tRNA-Cys--GCA                                                                        | tRNA-Cys--GCA                                                                            | <i>cysT</i>                                              |                                                                                                                                                     |
| tRNA-fMet--CAU_1/4<br>tRNA-fMet--CAU_2/4<br>tRNA-fMet--CAU_3/4<br>tRNA-fMet--CAU_4/4 | tRNA-fMet--CAU_1/3<br>tRNA-fMet--CAU_1/3<br>tRNA-fMet--CAU_2/3<br>tRNA-fMet--CAU_3/3     | <i>metV</i><br><i>metW</i><br><i>metY</i><br><i>metZ</i> | <i>metV</i> , <i>W</i> : identical up to position -15<br><br><i>metY</i> : 1 nt in the variable loop differs from <i>metV</i> , <i>W</i> , <i>Z</i> |
| tRNA-Gln--CUG_1/2                                                                    | tRNA-Gln--CUG_multi                                                                      | <i>glnV</i>                                              |                                                                                                                                                     |

|                    |                         |             |                                                                                                                    |
|--------------------|-------------------------|-------------|--------------------------------------------------------------------------------------------------------------------|
| tRNA-Gln--CUG_2/2  | tRNA-Gln--CUG_multi     | <i>glnX</i> |                                                                                                                    |
| tRNA-Gln--UUG_1/2  | tRNA-Gln--UUG_1/2       | <i>glnU</i> |                                                                                                                    |
| tRNA-Gln--UUG_2/2  | tRNA-Gln--UUG_2/2       | <i>glnW</i> |                                                                                                                    |
| tRNA-Glu--UUC_1/4  | tRNA-Glu--UUC_1/2_multi | <i>gltV</i> | <i>gltV</i> , <i>U</i> : identical up to position -25<br><br><i>gltT</i> , <i>W</i> : identical up to position -25 |
| tRNA-Glu--UUC_2/4  | tRNA-Glu--UUC_1/2_multi | <i>gltU</i> |                                                                                                                    |
| tRNA-Glu--UUC_3/4  | tRNA-Glu--UUC_2/2_multi | <i>gltT</i> |                                                                                                                    |
| tRNA-Glu--UUC_4/4  | tRNA-Glu--UUC_2/2_multi | <i>gltW</i> |                                                                                                                    |
| tRNA-Gly--CCC      | tRNA-Gly--CCC           | <i>glyU</i> |                                                                                                                    |
| tRNA-Gly--GCC_1/4  | tRNA-Gly--GCC_1/3       | <i>glyY</i> | <i>glyY</i> , <i>X</i> : identical up to position -15                                                              |
| tRNA-Gly--GCC_2/4  | tRNA-Gly--GCC_1/3       | <i>glyX</i> |                                                                                                                    |
| tRNA-Gly--GCC_3/4  | tRNA-Gly--GCC_2/3       | <i>glyV</i> |                                                                                                                    |
| tRNA-Gly--GCC_4/4  | tRNA-Gly--GCC_3/3       | <i>glyW</i> |                                                                                                                    |
| tRNA-Gly--UCC      | tRNA-Gly--UCC           | <i>glyT</i> |                                                                                                                    |
| tRNA-His--GUG      | tRNA-His--GUG           | <i>hisR</i> |                                                                                                                    |
| tRNA-Ile1--GAU_1/3 | tRNA-Ile1--GAU_multi    | <i>ileT</i> | all three identical sequence up position -25                                                                       |
| tRNA-Ile1--GAU_2/3 | tRNA-Ile1--GAU_multi    | <i>ileU</i> |                                                                                                                    |
| tRNA-Ile1--GAU_3/3 | tRNA-Ile1--GAU_multi    | <i>ileV</i> |                                                                                                                    |
| tRNA-Ile2--CAU_1/2 | tRNA-Ile2--CAU_1/2      | <i>ileX</i> |                                                                                                                    |
| tRNA-Ile2--CAU_2/2 | tRNA-Ile2--CAU_2/2      | <i>ileY</i> |                                                                                                                    |
| tRNA-Leu--CAA      | tRNA-Leu--CAA           | <i>leuX</i> |                                                                                                                    |
| tRNA-Leu--CAG_1/4  | tRNA-Leu--CAG_1/3       | <i>leuQ</i> | <i>leuP</i> : 1 nt in the variable stem differs from variants 1/4, 2/4 & 4/4                                       |
| tRNA-Leu--CAG_2/4  | tRNA-Leu--CAG_1/3       | <i>leuT</i> |                                                                                                                    |
| tRNA-Leu--CAG_3/4  | tRNA-Leu--CAG_2/3       | <i>leuP</i> |                                                                                                                    |
| tRNA-Leu--CAG_4/4  | tRNA-Leu--CAG_3/3       | <i>leuV</i> |                                                                                                                    |
| tRNA-Leu--GAG      | tRNA-Leu--GAG           | <i>leuU</i> |                                                                                                                    |
| tRNA-Leu--UAA      | tRNA-Leu--UAA           | <i>leuZ</i> |                                                                                                                    |
| tRNA-Leu--UAG      | tRNA-Leu--UAG           | <i>leuW</i> |                                                                                                                    |
| tRNA-Lys--UUU_1/6  | tRNA-Lys--UUU_1/5       | <i>lysV</i> |                                                                                                                    |

|                   |                     |             |                                        |
|-------------------|---------------------|-------------|----------------------------------------|
| tRNA-Lys--UUU_2/6 | tRNA-Lys--UUU_1/5   | <i>lysY</i> |                                        |
| tRNA-Lys--UUU_3/6 | tRNA-Lys--UUU_2/5   | <i>lysZ</i> |                                        |
| tRNA-Lys--UUU_4/6 | tRNA-Lys--UUU_3/5   | <i>lysQ</i> |                                        |
| tRNA-Lys--UUU_5/6 | tRNA-Lys--UUU_4/5   | <i>lysW</i> |                                        |
| tRNA-Lys--UUU_6/6 | tRNA-Lys--UUU_5/5   | <i>lysT</i> |                                        |
| tRNA-Met--CAU_1/2 | tRNA-Met--CAU_1/2   | <i>metT</i> |                                        |
| tRNA-Met--CAU_2/2 | tRNA-Met--CAU_2/2   | <i>metU</i> |                                        |
| tRNA-Phe--GAA_1/2 | tRNA-Phe--GAA_1/2   | <i>pheU</i> | 5'-leader identical up to position -18 |
| tRNA-Phe--GAA_2/2 | tRNA-Phe--GAA_2/2   | <i>pheV</i> |                                        |
| tRNA-Pro--CGG     | tRNA-Pro--CGG       | <i>proK</i> |                                        |
| tRNA-Pro--GGG     | tRNA-Pro--GGG       | <i>proL</i> |                                        |
| tRNA-Pro--UGG     | tRNA-Pro--UGG       | <i>proM</i> |                                        |
| tRNA-Sec--UCA     | tRNA-Sec--UCA       | <i>selC</i> |                                        |
| tRNA-Ser--CGA     | tRNA-Ser--CGA       | <i>serU</i> |                                        |
| tRNA-Ser--GCU     | tRNA-Ser--GCU       | <i>serV</i> |                                        |
| tRNA-Ser--GGA_1/2 | tRNA-Ser--GGA_multi | <i>serX</i> | 5'-leader identical up to position -7  |
| tRNA-Ser--GGA_2/2 | tRNA-Ser--GGA_multi | <i>serW</i> |                                        |
| tRNA-Ser--UGA     | tRNA-Ser--UGA       | <i>serT</i> |                                        |
| tRNA-Thr--CGU_1/2 | tRNA-Thr--CGU_1/2   | <i>thrX</i> |                                        |
| tRNA-Thr--CGU_2/2 | tRNA-Thr--CGU_2/2   | <i>thrW</i> |                                        |
| tRNA-Thr--GGU_1/2 | tRNA-Thr--GGU_1/2   | <i>thrT</i> |                                        |
| tRNA-Thr--GGU_2/2 | tRNA-Thr--GGU_2/2   | <i>thrV</i> |                                        |
| tRNA-Thr--UGU     | tRNA-Thr--UGU       | <i>thrU</i> |                                        |
| tRNA-Trp--CCA     | tRNA-Trp--CCA       | <i>trpT</i> |                                        |
| tRNA-Tyr--GUA_1/3 | tRNA-Tyr--GUA_1/3   | <i>tyrU</i> |                                        |
| tRNA-Tyr--GUA_2/3 | tRNA-Tyr--GUA_2/3   | <i>tyrT</i> |                                        |
| tRNA-Tyr--GUA_3/3 | tRNA-Tyr--GUA_3/3   | <i>tyrV</i> |                                        |
| tRNA-Val--GAC_1/2 | tRNA-Val--GAC_1/2   | <i>valW</i> |                                        |

|                   |                   |             |                                                                                      |
|-------------------|-------------------|-------------|--------------------------------------------------------------------------------------|
| tRNA-Val--GAC_2/2 | tRNA-Val--GAC_2/2 | <i>valV</i> |                                                                                      |
| tRNA-Val--UAC_1/5 | tRNA-Val--UAC_1/3 | <i>valY</i> | <i>valZ</i> , <i>T</i> : identical up to nt -22 except for 1 mismatch at position -7 |
| tRNA-Val--UAC_2/5 | tRNA-Val--UAC_1/3 | <i>valX</i> |                                                                                      |
| tRNA-Val--UAC_3/5 | tRNA-Val--UAC_2/3 | <i>valU</i> |                                                                                      |
| tRNA-Val--UAC_4/5 | tRNA-Val--UAC_3/3 | <i>valZ</i> |                                                                                      |
| tRNA-Val--UAC_5/5 | tRNA-Val--UAC_3/3 | <i>valT</i> |                                                                                      |

**Table S1.** Assignment code for tRNA isoacceptors in a related previous study (first column from left; ref. 1: Gößringer et al., 2017, *Nucleic Acids Res.* **45**, 7441), in the present study (second column from left), their gene names in *E. coli* strain MG1655 (genome NCBI NZ\_CP012868.1; third column from left) and noteworthy commonalities or differences between isoacceptor genes. Sequence-identical tRNAs were combined in the second column because RNA-seq reads representing 5'-mature tRNAs could not be assigned to a single tRNA gene transcript. In some cases, sequence identity extended into the 5'-flanking sequences. In all cases where reads perfectly matched more than one tRNA locus, the number of reads was divided equally between those loci.

**Table S2. pAaT substrate variants, single turnover cleavage kinetics**

| enzyme                     | rate constants<br>(min <sup>-1</sup> ) | -1U_0B                | -1U_3B                   | -1A_3B                   | -1U_6B                | -1U_9B                   | -1A_9B                   |
|----------------------------|----------------------------------------|-----------------------|--------------------------|--------------------------|-----------------------|--------------------------|--------------------------|
| <i>Aae</i> HARP            | $k_{\text{obs } C_0}$                  | $0.12 \pm 0.05$       | $0.25 \pm 0.03$          | $1.71 \pm 0.65$          | $0.35 \pm 0.06$       | $0.63 \pm 0.21$ (n=6)    | $1.58 \pm 0.44$ (n=6)    |
|                            | $k_{\text{obs } C_{-1}}$               | $0.13 \pm 0.05$       | $0.12 \pm 0.02$          | $0.37 \pm 0.09$          | $0.18 \pm 0.04$       | $0.29 \pm 0.05$ (n=6)    | $0.34 \pm 0.03$ (n=6)    |
|                            | overall $k_{\text{obs}}$               | $0.23 \pm 0.08$ (n=4) | $0.46 \pm 0.23$ (n=7)    | $1.89 \pm 0.63$ (n=7)    | $0.52 \pm 0.18$ (n=4) | $0.88 \pm 0.33$ (n=7)    | $1.67 \pm 0.44$ (n=6)    |
| <i>Tin</i> HARP            | $k_{\text{obs } C_0}$                  | $0.15 \pm 0.07$       | $0.11 \pm 0.01$          | $0.89 \pm 0.38$          | $0.18 \pm 0.04$       | $0.24 \pm 0.09$          | $0.37 \pm 0.25$          |
|                            | $k_{\text{obs } C_{-1}}$               | $0.09 \pm 0.06$       | $0.02 \pm 0.01$          | $0.04 \pm 0.01$          | $0.06 \pm 0.02$       | $0.08 \pm 0.02$          | $0.04 \pm 0.05$          |
|                            | overall $k_{\text{obs}}$               | $0.21 \pm 0.04$ (n=3) | $0.17 \pm 0.06$ (n=5)    | $1.01 \pm 0.18$ (n=6)    | $0.21 \pm 0.04$ (n=3) | $0.28 \pm 0.1$ (n=3)     | $0.38 \pm 0.26$ (n=3)    |
| <i>Mth</i> HARP            | $k_{\text{obs } C_0}$                  | n.d.                  | n.d.                     | n.d.                     | n.d.                  | $2.14 \pm 1.23$ (n=4)    | $7.39 \pm 0.66$ (n=4)    |
|                            | $k_{\text{obs } C_{-1}}$               | n.d.                  | n.d.                     | n.d.                     | n.d.                  | $0.91 \pm 0.63$ (n=4)    | $0.94 \pm 0.43$ (n=4)    |
|                            | overall $k_{\text{obs}}$               | n.d.                  | n.d.                     | n.d.                     | n.d.                  | $2.55 \pm 1.41$ (n=4)    | $7.54 \pm 0.69$ (n=4)    |
| <i>Eco</i> RNA/Bsu<br>RnpA | $k_{\text{obs } C_0}$                  | n.d.                  | $1.91 \pm 0.79$<br>(n=5) | $0.04 \pm 0.02$<br>(n=6) | n.d.                  | $5.82 \pm 0.55$<br>(n=3) | $0.08 \pm 0.03$<br>(n=3) |

**Table S2.** Rate constants ( $k_{\text{obs}}$ ) for cleavage of pAaT variants by *A. aeolicus* (*Aae*), *T. indicus* (*Tin*) and *M. thermautotrophicus* (*Mth*) HARP

(500 nM each) under single turnover conditions at the canonical ( $c_0$ , between nt  $-1$  and  $+1$ ) or aberrant site ( $c_{-1}$ , between nt  $-2$  and  $-1$ );

overall  $k_{\text{obs}}$  ( $\text{min}^{-1}$ ) is the rate constant when both 5'-leader cleavage products were treated as one product; n.d., not determined. *Eco*

RNA/*Bsu* RnpA: holoenzyme assembled from *E. coli* RNase P RNA and *B. subtilis* RnpA protein. We also tested *E. coli* RNase P RNA with an

*E. coli* RnpA preparation (*Eco* RNA/*Eco* RnpA), which showed a comparable hierarchy of cleavage efficiencies, although with lower overall

activity (Fig. S11), owing to activity losses during long-term storage. All values ( $\pm$  sample standard deviation) are based on 3 or more

independent experiments; higher numbers of experiments for overall  $k_{\text{obs}}$  relative to  $k_{\text{obs } c_0}$  and  $k_{\text{obs } c_{-1}}$  are due to inclusion of experiments

where separation of  $c_0$  and  $c_{-1}$  cleavage products was insufficient and both products were treated as one product.

**Table S3. pAaT substrate variants, cleavage site selection**

| enzyme                          | cleavage site selection | -1U_0B       | -1U_3B       | -1A_3B       | -1U_6B       | -1U_9B       | -1A_9B       |
|---------------------------------|-------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| <i>Aae</i> HARP                 | % cleaved at $c_0$      | $48 \pm 1.3$ | $78 \pm 1.3$ | $93 \pm 0.5$ | $75 \pm 0.8$ | $74 \pm 1.9$ | $92 \pm 1$   |
|                                 | % cleaved at $c_{-1}$   | $52 \pm 1.3$ | $22 \pm 1.3$ | $7 \pm 0.5$  | $25 \pm 0.8$ | $26 \pm 1.9$ | $8 \pm 1$    |
| <i>Tin</i> HARP                 | % cleaved at $c_0$      | $64 \pm 0.8$ | $82 \pm 0.3$ | $98 \pm 0.1$ | $82 \pm 1.5$ | $82 \pm 0.6$ | $97 \pm 0.9$ |
|                                 | % cleaved at $c_{-1}$   | $36 \pm 0.8$ | $18 \pm 0.3$ | $2 \pm 0.1$  | $18 \pm 1.5$ | $18 \pm 0.6$ | $3 \pm 0.9$  |
| <i>Mth</i> HARP                 | % cleaved at $c_0$      | n.d.         | n.d.         | n.d.         | n.d.         | $75 \pm 1$   | $96 \pm 0.4$ |
|                                 | % cleaved at $c_{-1}$   | n.d.         | n.d.         | n.d.         | n.d.         | $25 \pm 1$   | $4 \pm 0.4$  |
| <i>Eco</i> RNA/ <i>Eco</i> RnpA | % cleaved at $c_0$      | n.d.         | 100          | (100)        | n.d.         | 100          | (100)        |
|                                 | % cleaved at $c_{-1}$   | n.d.         | —            | (—)          | n.d.         | —            | (—)          |
| <i>Eco</i> RNA/ <i>Bsu</i> RnpA | % cleaved at $c_0$      | n.d.         | 100          | 100          | n.d.         | 100          | 100          |
|                                 | % cleaved at $c_{-1}$   | n.d.         | —            | —            | n.d.         | —            | —            |

**Table S3.** Percent (%) cleavage at the canonical ( $c_0$ , between nt -1 and +1) or aberrant site ( $c_{-1}$ , between nt -2 and -1) by *A. aeolicus* (*Aae*), *T. indicus* (*Tin*) and *M. thermautotrophicus* (*Mth*) HARP under single turnover conditions (enzyme concentrations as specified in Table 2), compared with bacterial holoenzymes. *Eco* RNA/*Eco* RnpA: holoenzyme assembled from *E. coli* RNase P RNA and *E. coli* RnpA protein; *Eco* RNA/*Bsu* RnpA: holoenzyme assembled from *E. coli* RNase P RNA and *B. subtilis* RnpA protein. n.d., not determined; —, no cleavage product detectable; (—), no cleavage product detectable yet based on very low overall cleavage; (100), only cleavage at the  $c_0$  site visible, but cleavage at the  $c_{-1}$  site cannot be ruled out due to very low overall cleavage activity. All values ( $\pm$  sample standard deviation) are based on 3 or more independent experiments (for exact number see Table 2) with evaluation of 4 time points in each experiment.

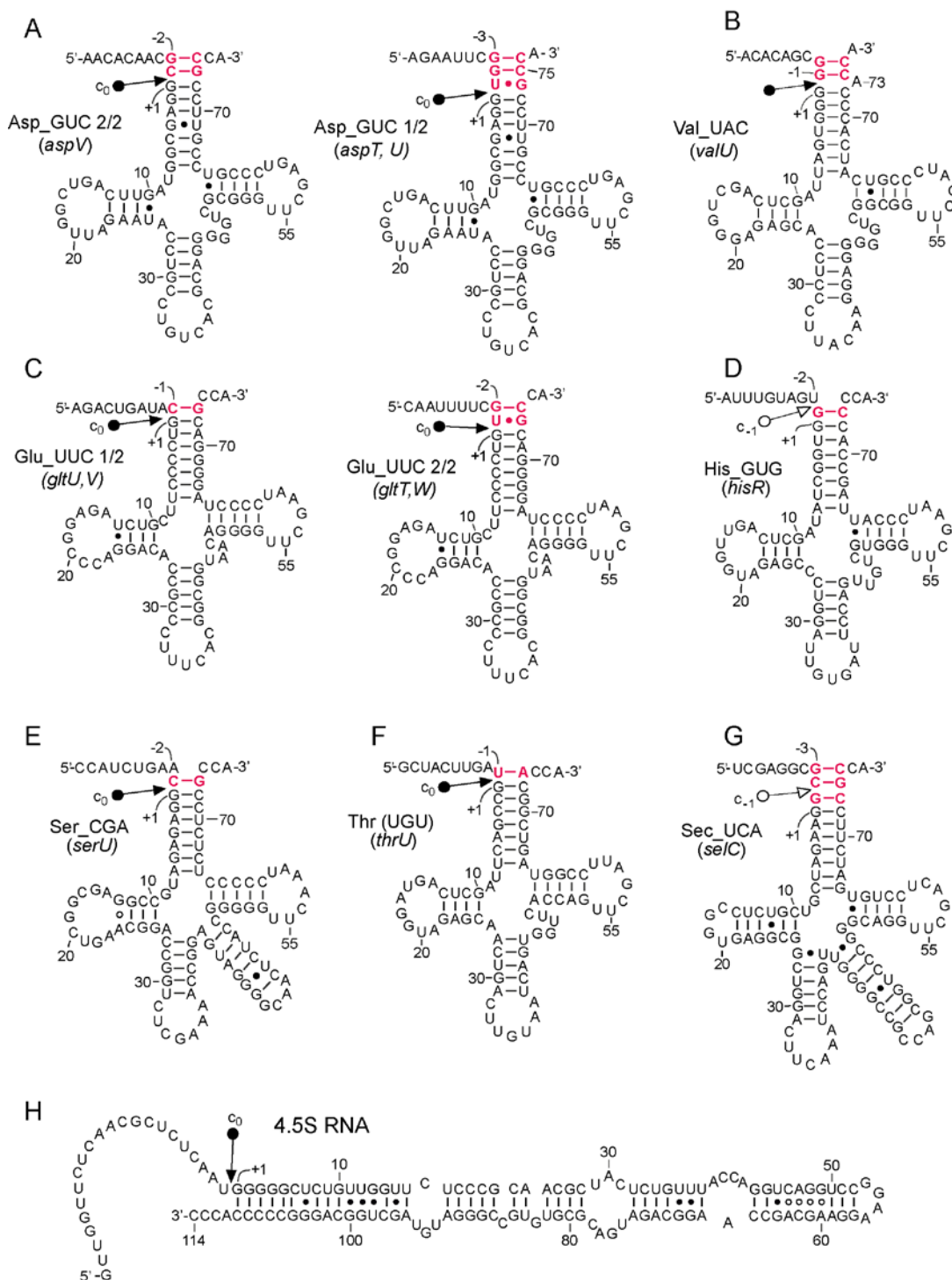
**Table S4: DNA oligonucleotides / primers**

| <b>vector construction</b>                                       |                                                                             |
|------------------------------------------------------------------|-----------------------------------------------------------------------------|
| <i>Mth</i> (a)-for                                               | 5'-TTT <b>CCA TGG</b> ATG CTT GCA AAG CAG AGA TTC<br>GTA C                  |
| <i>Mth</i> (a)-rev                                               | 5'-TTT <b>CTC GAG</b> TTC ATA ATA TTT AAG GTA TTC<br>CTT GAG CAT CTT GGG G  |
| <i>Mth</i> (b)-for                                               | 5'-TTT <b>CCC GGG</b> ATG CTT GCA AAG CAG AGA TTC<br>GTA C                  |
| <i>Mth</i> (b)-rev                                               | 5'-TTT <b>TCT AGA</b> TCA TTC ATA ATA TTT AAG GTA<br>TTC CTT GAG CAT CTT GG |
| <i>Tin</i> (a)-for                                               | 5'-TTT <b>CCA TGG</b> ATG GCT TTT GAA TTA GAA GTT<br>GAA AG                 |
| <i>Tin</i> (a)-rev                                               | 5'-TTT <b>CTC GAG</b> AGG ACT GTA AAT AAG GCC TTC T                         |
| <i>Tin</i> (b)-for                                               | 5'-TTT <b>CCC GGG</b> ATG GCT TTT GAA TTA GAA GTT<br>GAA AG                 |
| <i>Tin</i> (b)-rev                                               | 5'-TTT <b>GCA TGC</b> TTA AGG ACT GTA AAT AAG GCC                           |
| <b>primer extension analysis</b>                                 |                                                                             |
| Asp-tRNA ( <i>aspV</i> ), primer                                 | 5'-TAA CCG ACT GAA CTA CCG C                                                |
| Asp-tRNA ( <i>aspV</i> ) marker for<br>cleavage at site $c_0$    | 5'-TAA CCG ACT GAA CTA CCG CTC C                                            |
| Asp-tRNA ( <i>aspV</i> ) marker for<br>cleavage at site $c_{-2}$ | 5'-TAA CCG ACT GAA CTA CCG CTC CGC                                          |
| Glu-tRNA ( <i>gltU/V/T/W</i> ), primer                           | 5'-GGG CCT CTA GAC GAA GGG                                                  |
| Glu-tRNA ( <i>gltU/V/T/W</i> ) marker $c_0$                      | 5'-GGG CCT CTA GAC GAA GGG GAC                                              |
| Glu-tRNA ( <i>gltUV</i> ) marker $c_{-1}$                        | 5'-GGG CCT CTA GAC GAA GGG GAC G                                            |
| His-tRNA ( <i>hisR</i> ), primer                                 | 5'-GGC TCT ACC AAC TGA GCT ATA GC                                           |
| His-tRNA ( <i>hisR</i> ) marker $c_0$                            | 5'-GGC TCT ACC AAC TGA GCT ATA GCC AC                                       |

|                                                 |                                                                      |
|-------------------------------------------------|----------------------------------------------------------------------|
| His-tRNA ( <i>hisR</i> ) marker c <sub>-1</sub> | 5'-GGC TCT ACC AAC TGA GCT ATA GCC ACC                               |
| Sec-tRNA ( <i>selC</i> ), primer                | 5'-CTC ACC GGA GAC GAC GAT C                                         |
| Sec-tRNA ( <i>selC</i> ) marker c <sub>0</sub>  | 5'-CTC ACC GGA GAC GAC GAT CTT C                                     |
| Sec-tRNA ( <i>selC</i> ) marker c <sub>-1</sub> | 5'-CTC ACC GGA GAC GAC GAT CTT CC                                    |
| Ser-tRNA ( <i>serU</i> ), primer                | 5'-TCA GCC GCT CCG GCA TCT C                                         |
| Ser-tRNA ( <i>serU</i> ) marker c <sub>0</sub>  | 5'-TCA GCC GCT CCG GCA TCT CTC C                                     |
| Ser-tRNA ( <i>serU</i> ) marker c <sub>-1</sub> | 5'-TCA GCC GCT CCG GCA TCT CTC CG                                    |
| Thr-tRNA ( <i>thrU</i> ), primer                | 5'-GTT GCT CTA CCT ACT GAG CTA AGT C                                 |
| Thr-tRNA ( <i>thrU</i> ) marker c <sub>0</sub>  | 5'-GTT GCT CTA CCT ACT GAG CTA AGT CGG C                             |
| Thr-tRNA ( <i>thrU</i> ) marker c <sub>-1</sub> | 5'-GTT GCT CTA CCT ACT GAG CTA AGT CGG CA                            |
| 4.5S RNA ( <i>ffs</i> ), primer                 | 5'-GGA GAA CCA ACA GAG CCC                                           |
| 4.5S RNA ( <i>ffs</i> ) marker c <sub>0</sub>   | 5'-GGA GAA CCA ACA GAG CCC CC                                        |
| 4.5S RNA ( <i>ffs</i> ) marker c <sub>-1</sub>  | 5'-GG AGA ACC AAC AGA GCC CCC A                                      |
| <b>Northernblot analysis</b>                    |                                                                      |
| Asp-tRNA ( <i>aspV</i> )-for                    | 5'-ATT AAT ACG ACT CAC TAT AGG CGG AAC GGA<br>CGG GAC TCG AAC CCG    |
| Asp-tRNA ( <i>aspV</i> )-rev                    | 5'-GGA GCG GTA GTT CAG TCG GTT AGA ATA CCT<br>GC                     |
| Glu-tRNA ( <i>gltU/V/T/W</i> )                  | 5'-DIG-GTT ACC GCC GTG AAA GGG CGG                                   |
| His-tRNA ( <i>hisR</i> )-for                    | 5'-TAA TAC GAC TCA CTA TAG GGG TGG CTA ATG<br>GGA TTC                |
| His-tRNA ( <i>hisR</i> )-rev                    | 5'-GTG GCT ATA GCT CAG TTG GTA G                                     |
| Sec-tRNA ( <i>selC</i> )-for                    | 5'-ATT AAT ACG ACT CAC TAT AGG CGG AAG ATC<br>ACA GGA GTC GAA CCT GC |
| Sec-tRNA ( <i>selC</i> )-rev                    | 5'-GAT CGT CGT CTC CGG TGA GGC GGC                                   |
| Ser-tRNA ( <i>serU</i> )                        | 5'-DIG-GTA GAG TTG CCC CTA CTC CGG TTT TCG<br>AGA CCG                |

|                           |                                                                  |
|---------------------------|------------------------------------------------------------------|
| Thr-tRNA ( <i>thrU</i> )  | 5'-DIG-TGG TGA CCT ACT GAT TAC AAG TCA GTT<br>GC                 |
| 5S-RNA ( <i>rrf</i> )-for | 5'-TAA TAC GAC TCA CTA TAG GCA GTT CCC TAC<br>TCT CGC ATG GGG AG |
| 5S-RNA ( <i>rrf</i> )-for | 5'-TGC CTG GCG GCC GTA GCG CGG TGG TC                            |

T7 promoter sequence italicized; restriction sites relevant for cloning are shown in bold; DIG: digoxigenin label.



**Fig. S1.** Secondary structures of substrates for RNase P in *E. coli* that attracted our attention based on the results shown in Figs. 2 and 3. Base pairs in red depict potential acceptor stem extensions in the corresponding pre-tRNAs; all pre-tRNAs are arbitrarily shown with 5'-flanking sequences up to position  $-10$ . For each RNA, the native RNase P cleavage site is indicated, with cleavage between nt  $-1$  and  $+1$  denoted as  $c_0$  site (filled arrow), and that between nt  $-2$  and  $-1$  as  $c_{-1}$  site (open arrow). For the classification of *E. coli* tRNA isoacceptors and their gene names (italicized in parentheses) see Table S1.

NCBI Reference Sequence: NZ\_CP012868.1

Colour code:

- pink: 5'-precursor sequence
- grouped by blue frame: tRNAs with identical sequence in the mature domain
- grouped by orange frame: 5'-leaders and mature tRNAs sequence-identical
- highlighted in green: potential base pairing between nt -1, -2 and N73, C74
- red letters in the alignments: nucleotides used to differentiate subgroups of mature isoacceptors
- red letters highlighted in yellow: nucleotide differences between isodecoders

Ala tRNAs

```
>tRNA-Ala--GGC_1/2 CP012868.1|2123540..2123615|+
CAAACTTGGTACGTAACGCATCGGGGCTATAGCTCAGCTGGGAGAGCGCTTGCATGGCATGCAAGAGGTCAGCGGTTTCGATCCCGCTTAGCTCCACCA
>tRNA-Ala--GGC_2/2 CP012868.1|2123425..2123500|+
GTCAACTCGACAAGCTTTACGTGACGGGGCTATAGCTCAGCTGGGAGAGCGCTTGCATGGCATGCAAGAGGTCAGCGGTTTCGATCCCGCTTAGCTCCACCA
```

```
>tRNA-Ala--UGC_1/3 CP012868.1|604320..604395|-
TGAAGAGGTTTTAACTACATGTTAGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGGAGGTCTGCGGTTTCGATCCCGCATAGCTCCACCA
>tRNA-Ala--UGC_2/3 CP012868.1|1214623..1214698|+
TGAAGAGGTTTTAACTACATGTTAGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGGAGGTCTGCGGTTTCGATCCCGCATAGCTCCACCA
>tRNA-Ala--UGC_3/3 CP012868.1|4416091..4416166|-
TGAAGAGGTTTTAACTACATGTTAGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGGAGGTCTGCGGTTTCGATCCCGCATAGCTCCACCA
```

```
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<....
GGGGCTATAGCTCAGCTGGGAGAGCGCTTGCATGGCATGCAAGAG.....GtCAGCGGTTTCGATCCCGCTTAGCTCCACCA tRNA-Ala-GGC-1-2
GGGGCTATAGCTCAGCTGGGAGAGCGCTTGCATGGCATGCAAGAG.....GtCAGCGGTTTCGATCCCGCTTAGCTCCACCA tRNA-Ala-GGC-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<....
GGGGCTATAGCTCAGCTGGGAGAGCGCTGCTTTGCACGCAGGAG.....GtCTGCGGTTTCGATCCCGCATAGCTCCACCA tRNA-Ala-TGC-1-3
GGGGCTATAGCTCAGCTGGGAGAGCGCTGCTTTGCACGCAGGAG.....GtCTGCGGTTTCGATCCCGCATAGCTCCACCA tRNA-Ala-TGC-1-2
GGGGCTATAGCTCAGCTGGGAGAGCGCTGCTTTGCACGCAGGAG.....GtCTGCGGTTTCGATCCCGCATAGCTCCACCA tRNA-Ala-TGC-1-1
```

## Arg tRNAs

```
>tRNA-Arg--ACG_1/4 CP012868.1|1823107..1823183|+
TCGAATCCCCTCACCCTTGCATCCGTAGCTCAGCTGGATAGAGTACTCGGCTACGAACCGAGCGGTCGGAGGTTCAATCCTCCCGGATGCACCA
>tRNA-Arg--ACG_2/4 CP012868.1|1823382..1823458|+
TCTGCAGTAAAGTAAGTTTCCGATGCATCCGTAGCTCAGCTGGATAGAGTACTCGGCTACGAACCGAGCGGTCGGAGGTTCAATCCTCCCGGATGCACCA
>tRNA-Arg--ACG_3/4 CP012868.1|1823521..1823597|+
TCAGCAGTAAATAAATTTCCGATGCATCCGTAGCTCAGCTGGATAGAGTACTCGGCTACGAACCGAGCGGTCGGAGGTTCAATCCTCCCGGATGCACCA
>tRNA-Arg--ACG_4/4 CP012868.1|1823796..1823872|+
CAGCAGTAGAATAAGTTTCCGATGCATCCGTAGCTCAGCTGGATAGAGTACTCGGCTACGAACCGAGCGGTCGGAGGTTCAATCCTCCCGGATGCACCA
```

```
>tRNA-Arg--CCG CP012868.1|659204..659280|-
TTATCCACCCCGCAACGGCGTAAAGCGCCGTAGCTCAGCTGGATAGAGCGCTGCCCTCCGGAGGCAGAGGTTCTCAGGTTCAATCCTGTTCGGGCGCGCCA
```

```
>tRNA-Arg--CCU CP012868.1|2175273..2175347|-
TTAGCGGGTATACCTCATGCCGATGTCCTCTTAGTTAAATGGATATAACGAGCCCTCCTAAGGGCTAATTGCAGGTTTCGATTCTGCAGGGGACACCA
```

```
>tRNA-Arg--UCU CP012868.1|4076866..4076942|-
GATTACACCGCATTCGCGTATCAAAGCGCCCTTAGCTCAGTTGGATAGAGCAACGACCTTCTAAGTCGTGGGCCGAGGTTTCGAATCCTGCAGGGCGCGCCA
```

```
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<.<<<<<<....
GCATCCGTAGCTCAGCTGGATa.GAGTACTCGGCTACGAACCGAGCG.....GtCGGAGGTTTCGAATCCTCC.CGGATGCACCA tRNA-Arg-ACG-1-4
GCATCCGTAGCTCAGCTGGATa.GAGTACTCGGCTACGAACCGAGCG.....GtCGGAGGTTTCGAATCCTCC.CGGATGCACCA tRNA-Arg-ACG-1-3
GCATCCGTAGCTCAGCTGGATa.GAGTACTCGGCTACGAACCGAGCG.....GtCGGAGGTTTCGAATCCTCC.CGGATGCACCA tRNA-Arg-ACG-1-2
GCATCCGTAGCTCAGCTGGATa.GAGTACTCGGCTACGAACCGAGCG.....GtCGGAGGTTTCGAATCCTCC.CGGATGCACCA tRNA-Arg-ACG-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<.<<<<<<....
GCGCCCTAGCTCAGCTGGATa.GAGCGCTGCCCTCCGGAGGCAGAG.....GtCTCAGGTTTCGAATCCTGT.CGGGCGCGCCA tRNA-Arg-CCG-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<.<<<<<<....
GTCTCTTAGTTAAAT.GGATa.TAACGAGCCCTCCTAAGGGCTA.....AtTGCAGGTTTCGATTCCTGC.AGGGGACACCA tRNA-Arg-CCT-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<.<<<<<<....
GCGCCCTTAGCTCAGTTGGATa.GAGCAACGACCTTCTAAGTCGTGG.....GcCGCAGGTTTCGAATCCTGC.AGGGCGCGCCA tRNA-Arg-TCT-1-1
```

## Asn tRNAs

```
>tRNA-Asn--GUU_1/4 CP012868.1|2579319..2579394|-
TTAATATGCGCCCGTTACACGATTCCTCTGTAGTTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTATGTCACTGGTTCGAGTCCAGTCAGAGGAGCCA
>tRNA-Asn--GUU_2/4 CP012868.1|2581728..2581803|-
CGTTAAGATGTCCTCAACAACGATTCCTCTGTAGTTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTATGTCACTGGTTCGAGTCCAGTCAGAGGAGCCA
>tRNA-Asn--GUU_3/4 CP012868.1|2583552..2583627|+
CTAATATTCGCTCGTTCTCACGATTCCTCTGTAGTTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTATGTCACTGGTTCGAGTCCAGTCAGAGGAGCCA
>tRNA-Asn--GUU_4/4 CP012868.1|2597030..2597105|-
GTAATATTCGCCCGTTACACGATTCCTCTGTAGTTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTATGTCACTGGTTCGAGTCCAGTCAGAGGAGCCA
```

>>>>>>..>>>>.....<<<<.>>>>>.....<<<<<.....>>>>>.....<<<<<<<<<<<<....  
TCCTCTGTAGTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTAT.....GtCACTGGTTCGAGTCCAGTCAGAGGAGCCA tRNA-Asn-GTT-1-4  
TCCTCTGTAGTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTAT.....GtCACTGGTTCGAGTCCAGTCAGAGGAGCCA tRNA-Asn-GTT-1-3  
TCCTCTGTAGTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTAT.....GtCACTGGTTCGAGTCCAGTCAGAGGAGCCA tRNA-Asn-GTT-1-2  
TCCTCTGTAGTTCAGTCGGTAGAACGGCGGACTGTTAATCCGTAT.....GtCACTGGTTCGAGTCCAGTCAGAGGAGCCA tRNA-Asn-GTT-1-1

#### Asp tRNAs

>tRNA-Asp--GUC\_1/3 CP012868.1|694707..694783|-  
ACCGGTGGTTGTAAAGAATTCTGGAGCGGTAGTTCAGTCGGTTAGAATACCTGCCTGTACACGAGGGGGTGCGGGTTTCGAGTCCCGTCCGTTCCGCCA  
>tRNA-Asp--GUC\_2/3 CP012868.1|4404659..4404735|-  
CCTCGTTCCCAACGGAACACAACCGGAGCGGTAGTTCAGTCGGTTAGAATACCTGCCTGTACACGAGGGGGTGCGGGTTTCGAGTCCCGTCCGTTCCGCCA  
>tRNA-Asp--GUC\_3/3 CP012868.1|4412662..4412738|-  
ACCGGTGGTTGTAAAGAATTCTGGAGCGGTAGTTCAGTCGGTTAGAATACCTGCCTGTACACGAGGGGGTGCGGGTTTCGAGTCCCGTCCGTTCCGCCA

>>>>>>..>>>>.....<<<<.>>>>>.....<<<<<.....>>>>>.....<<<<<<<<<<<<....  
GGAGCGGTAGTTCAGTCGGTtAGAATACCTGCCTGTACACGAGGGG.....GtCGCGGGTTCGAGTCCCGTCCGTTCCGCCA tRNA-Asp-GTC-1-3  
GGAGCGGTAGTTCAGTCGGTtAGAATACCTGCCTGTACACGAGGGG.....GtCGCGGGTTCGAGTCCCGTCCGTTCCGCCA tRNA-Asp-GTC-1-2  
GGAGCGGTAGTTCAGTCGGTtAGAATACCTGCCTGTACACGAGGGG.....GtCGCGGGTTCGAGTCCCGTCCGTTCCGCCA tRNA-Asp-GTC-1-1

#### Cys tRNAs

>tRNA-Cys--GCA CP012868.1|2649667..2649740|+  
TCTGGCTGAAGGCCTGAAGAATTGGCGCGTTAACAAGCGGTTATGTAGCGGATTGCAATCCGTCTAGTCCGGTTCGACTCCGGAACGCGCCTCCA

>>>>>>..>>>>.....<<<<.>>>>>.....<<<<<.....>>>>>.....<<<<<<<<<<<<....  
GGCGCGTTAACAAGCGGTT.ATGTAGCGGATTGCAATCCGTCT.....AGTCCGGTTCGACTCCGGAACGCGCCTCCA tRNA-Cys-GCA-1-1

#### Gln tRNAs

>tRNA-Gln--CUG\_1/2 CP012868.1|3945049..3945123|+  
TTCGGCAAATTCAAACCAATTCTTGGGGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTTTCAATCCTCGTACCCCAGCCA  
>tRNA-Gln--CUG\_2/2 CP012868.1|3945161..3945235|+  
CGCTTACCTTGTAAAGTGCACCACTTGGGGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTTTCAATCCTCGTACCCCAGCCA

>tRNA-Gln--UUG\_1/2 CP012868.1|3944726..3944800|+  
CATTCACCAGAAAGCGTTGTACGGATGGGGTATCGCCAAGCGGTAAGGCACCGGTTTTTGATACCGGCATTCCCTGGTTTCAATCCAGGTACCCCAAGCCA  
>tRNA-Gln--UUG\_2/2 CP012868.1|3944835..3944909|+  
AGTAAGCGGTTACCGCCCGTTATTGGGGTATCGCCAAGCGGTAAGGCACCGGTTTTTGATACCGGCATTCCCTGGTTTCAATCCAGGTACCCCAAGCCA

>>>>>>..>>>>.....<<<<.>>>>>.....<<<<<.....>>>>>.....<<<<<<<<<<<<....  
TGGGGTATCGCCAAGCGGTA.AGGCACCAGATTCTGATTCCGGCA.....TtCCGAGGTTTCAATCCTCGTACCCCAAGCCA tRNA-Gln-CTG-1-2  
TGGGGTATCGCCAAGCGGTA.AGGCACCAGATTCTGATTCCGGCA.....TtCCGAGGTTTCAATCCTCGTACCCCAAGCCA tRNA-Gln-CTG-1-1

>>>>>>..>>>>.....<<<<.>>>>>>.....<<<<<<.....>>>>>>.....<<<<<<<<<<<<....  
TGGGGTATCGCCAAGCGGTA.AGGCACC GGTTT TTGATACCGGCA.....TtCCCTGGTTTCAATCCAGGTACCCAGCCA tRNA-Gln-TTG-1-2  
TGGGGTATCGCCAAGCGGTA.AGGCACC GGTTT TTGATACCGGCA.....TtCCCTGGTTTCAATCCAGGTACCCAGCCA tRNA-Gln-TTG-1-1

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### Glu tRNAs

```
>tRNA-Glu--UUC_1/4 CP012868.1|431806..431881|-  
GGCGTCTTGCGAAGCAGACTGATA GTCCCTTCGTCTAGAGGCCAGGACACCGCCCTTTCACGGCGGTAACAGGGGTTCAATCCCCTAGGGGACGCCA  
>tRNA-Glu--UUC_3/4 CP012868.1|473208..473283|-  
GCAACGCGTGATAAGCAATTTTC GTCCCTTCGTCTAGAGGCCAGGACACCGCCCTTTCACGGCGGTAACAGGGGTTCAATCCCCTAGGGGACGCCA  
>tRNA-Glu--UUC_2/4 CP012868.1|698145..698220|-  
GGCGTCTTGCGAAGCAGACTGATA GTCCCTTCGTCTAGAGGCCAGGACACCGCCCTTTCACGGCGGTAACAGGGGTTCAATCCCCTAGGGGACGCCA  
>tRNA-Glu--UUC_4/4 CP012868.1|1912212..1912287|+  
GCAACGCGTGATAAGCAATTTTC GTCCCTTCGTCTAGAGGCCAGGACACCGCCCTTTCACGGCGGTAACAGGGGTTCAATCCCCTAGGGGACGCCA
```

>>>>>>..>>>>.....<<<<.>>>>>>.....<<<<<<.....>>>>>>.....<<<<<<<<<<<<....  
GTCCCTTCGTCTAGAGGCCcA.GGACACCGCCCTTTCACGGCGGTA.....ACAGGGGTTCAATCCCCTAGGGGACGCCA tRNA-Glu-TTC-1-4  
GTCCCTTCGTCTAGAGGCCcA.GGACACCGCCCTTTCACGGCGGTA.....ACAGGGGTTCAATCCCCTAGGGGACGCCA tRNA-Glu-TTC-1-3  
GTCCCTTCGTCTAGAGGCCcA.GGACACCGCCCTTTCACGGCGGTA.....ACAGGGGTTCAATCCCCTAGGGGACGCCA tRNA-Glu-TTC-1-2  
GTCCCTTCGTCTAGAGGCCcA.GGACACCGCCCTTTCACGGCGGTA.....ACAGGGGTTCAATCCCCTAGGGGACGCCA tRNA-Glu-TTC-1-1

---

### Gly tRNAs

```
>tRNA-Gly--GCC_1/4 CP012868.1|248995..249070|-  
GTGCTGTAAGGCACAGACCACCCA GCGGGAATAGCTCAGTTGGTAGAGCACGACCTTGCCAAGGTCGGGGTCGCGAGTTCGAGTCTCGTTTCCCGCTCCA  
>tRNA-Gly--GCC_2/4 CP012868.1|249106..249181|-  
GTGCTGCAAAGCACAGACCACCCA GCGGGAATAGCTCAGTTGGTAGAGCACGACCTTGCCAAGGTCGGGGTCGCGAGTTCGAGTCTCGTTTCCCGCTCCA  
>tRNA-Gly--GCC_3/4 CP012868.1|249218..249293|-  
TTACGAAAGCAAATAAGTAGTAC GCGGGAATAGCTCAGTTGGTAGAGCACGACCTTGCCAAGGTCGGGGTCGCGAGTTCGAGTCTCGTTTCCCGCTCCA  
>tRNA-Gly--GCC_4/4 CP012868.1|2649537..2649612|+  
GTGATTAAGTATTGCTTGATGAAT GCGGGAATAGCTCAGTTGGTAGAGCACGACCTTGCCAAGGTCGGGGTCGCGAGTTCGAGTCTCGTTTCCCGCTCCA
```

>tRNA-Gly--CCC CP012868.1|1642599..1642672|+  
TTTATAATGCCTTACGCATCTCGA GCGGGCGTAGTTCAATGGTAGAACGAGAGCTTCCCAAGCTCTATACGAGGGTTCGATTCCCTTCGCCCGCTCCA

>tRNA-Gly--UCC CP012868.1|465908..465982|-  
AAATCAGGTAGCCGAGTTCAGGAT GCGGGCATCGTATAATGGCTATTACCTCAGCCTTCCAAGCTGATGATGCGGGTTCGATTCCCGCTGCCCGCTCCA

>>>>>>..>>>>.....<<<<.>>>>>>.....<<<<<<.....>>>>>>.....<<<<<<<<<<<<....  
GCGGGAAATAGCTCAGTTGGT.A.GAGCAGACCTTGCCAAGGTCGGG.....GtCGCGAGTTTCAGTCTCGTTTCCCGCTCCA tRNA-Gly-GCC-1-4  
GCGGGAAATAGCTCAGTTGGT.A.GAGCAGACCTTGCCAAGGTCGGG.....GtCGCGAGTTTCAGTCTCGTTTCCCGCTCCA tRNA-Gly-GCC-1-3  
GCGGGAAATAGCTCAGTTGGT.A.GAGCAGACCTTGCCAAGGTCGGG.....GtCGCGAGTTTCAGTCTCGTTTCCCGCTCCA tRNA-Gly-GCC-1-2

```
GCGGGGAATAGCTCAGTTGGT.A.GAGCAGCACCTGCCAAGGTCGGG.....GtCGCGAGTTTCAGTCTCGTTTCCCGCTCCA tRNA-Gly-GCC-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....<<<<<<<<<<<<....
GCGGGCGTAGTTCAAT.GGT.A.GAACGAGAGCTTCCCAAGCTCTAT.....A.CGAGGGTTTCGATTCCTTCGCCCCGCTCCA tRNA-Gly-CCC-1-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<<....
GCGGGGCATCGTATAAT.GGCTA.TTACCTCAGCCTTCCAAGCTGATG.....A.TGCGGGTTTCGATTCCTCGTCCCCGCTCCA tRNA-Gly-TCC-1-1
```

---

### His tRNAs

```
>tRNA-His--GUG CP012868.1|659070..659145|-
ATACCGCGTAACAAGATTTGTAGTGTGGCTATAGCTCAGTTGGTAGAGCCCTGGATTGTGATTCCAGTTGTCGTGGGTTTCAATCCCATTAGCCACCCCA
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<<....
GTGGCTATAGCTCAGTTGGTAGAGCCCTGGATTGTGATTCCAGTT.....GtCGTGGGTTTCAATCCCATTAGCCACCCCA tRNA-His-GTG-1-1
```

---

### Ile tRNAs

```
>tRNA-Ile1--GAU_1/3 CP012868.1|604438..604514|-
TGAAAATGAGCAGTAAACCTCTACAGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAGGTCGGTGGTTCAAGTCCACTCAGGCCTACCA
>tRNA-Ile1--GAU_2/3 CP012868.1|1214504..1214580|+
TGAAAATGAGCAGTAAACCTCTACAGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAGGTCGGTGGTTCAAGTCCACTCAGGCCTACCA
>tRNA-Ile1--GAU_3/3 CP012868.1|4416209..4416285|-
TGAAAATGAGCAGTAAACCTCTACAGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAGGTCGGTGGTTCAAGTCCACTCAGGCCTACCA
```

```
>tRNA-Ile2--CAU_1/2 CP012868.1|1425983..1426058|-
GAGTTACTTTATAATCCGCTACCAAGGCCCTTAGCTCAGTTGGTTAGAGCAGGCGACTLATAATCGCTTGGTCGCTGGTTCAAGTCCAGCAAGGGCCACCA
(falsely annotated as tRNA-Met)
>tRNA-Ile2--CAU_2/2 CP012868.1|1855819..1855894|+
ATAAGTATTGTTATTGCACACTCAAGGCCCTTAGCTCAGTTGGTTAGAGCAGGCGACTLATAATCGCTTGGTCGCTGGTTCAAGTCCAGCAAGGGCCACCA
(falsely annotated as tRNA-Met); L = Lysidine
```

```
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<<....
AGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAG.....GtCGGTGGTTCAAGTCCACTCAGGCCTACCA tRNA-Ile-GAT-1-3 Sc: 80.6
AGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAG.....GtCGGTGGTTCAAGTCCACTCAGGCCTACCA tRNA-Ile-GAT-1-2 Sc: 80.6
AGGCTTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAAGGGTGAG.....GtCGGTGGTTCAAGTCCACTCAGGCCTACCA tRNA-Ile-GAT-1-1 Sc: 80.6
```

```
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<<....
GGCCCTTAGCTCAGT..GGTtA.GAGCAGGCGACTCATAATCGCTTG.....GtCGCTGGTTCAAGTCCAGCAGGGGCCACCA tRNA-Ile2-CAT-1-1
GGCCCTTAGCTCAGT..GGTtA.GAGCAGGCGACTCATAATCGCTTG.....GtCGCTGGTTCAAGTCCAGCAGGGGCCACCA tRNA-Ile2-CAT-2-1
```

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## Leu tRNAs

>tRNA-Leu--GAG CP012868.1|1319498..1319584|+  
TCCCGAACTAAAAAGTAGTATCCGTCGCGAGGTGGTGAATTGGTAGACACGCTACCTTGAGGTGGTAGTGCCCAATAGGGCTTACGGGTTCAAGTCCCGTCCTCGGTACCA

>tRNA-Leu--CAG\_1/4 CP012868.1|35252..35338|+  
AATTCCTTTTAAAGATTGATGGTAGCGAAGGTGGCGGAATTGGTAGACGCGCTAGCTTCAGGTGTTAGTGTCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCACCA  
>tRNA-Leu--CAG\_2/4 CP012868.1|658963..659049|-  
CCCCATTATTAGAAGTTGTGACAAAGCGAAGGTGGCGGAATTGGTAGACGCGCTAGCTTCAGGTGTTAGTGTCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCACCA  
>tRNA-Leu--CAG\_4/4 CP012868.1|35488..35574|+  
ATATCAAAAAAGTAAGATGACTGTCGCGAAGGTGGCGGAATTGGTAGACGCGCTAGCTTCAGGTGTTAGTGTCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCACCA

>tRNA-Leu--CAG\_3/4 CP012868.1|35367..35453|+  
ACCACGTTGATATTGCTCGCACTGGCGCGAAGGTGGCGGAATTGGTAGACGCGCTAGCTTCAGGTGTTAGTGTCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCACCA

>tRNA-Leu--UAG CP012868.1|3944618..3944702|+  
CCCGTCGTAGCCACCATCTTTTTTGGCGGAGTGGCGAAATTGGTAGACGCACCAGATTTAGGTTCTGGCGCCGAAGGTGTGCGAGTTCAAGTCTCGCCTCCCGCACCA

>tRNA-Leu--CAA CP012868.1|145164..145248|-  
AACGTTTTCCGCATACCTCTTCATGCGCGAAGTGGCGAAATCGGTAGACGCAGTTGATTCAAATCAACCGTAGAAATACGTGCCGGTTCGAGTCCGGCCTTCGGGCACCA

>tRNA-Leu--UAA CP012868.1|2649753..2649839|+  
GAACGCGCTCCACTTTCTCCCGAGCCCGGATGGTGAATCGGTAGACACAAGGGATTTAAATCCCTCGGCGTTCGCGCTGTGCGGGTTCAAGTCCCGCTCCGGGTACCA

>>>>>>. .>>>. . . . . <<<. >>>>. . . . . <<<<. >>>. . . . . <. <<<. >>>>>. . . . . <<<<. <<<<<<. . . . .  
GCCGAGGTGGTGAATTGGTaGACACGCTACCTTGAGGTGGTAGTGCCCAA. . . . . TAGGGCTACGGGTTCAAGTCCCGT.CCTCGGTACCA tRNA-Leu-GAG-1-1  
>>>>. .>>>. . . . . <<<. >>>>. . . . . <<<<. >>>>. . . . . <<<<. >>>>>. . . . . <<<<. <<. <<<<. . . . .  
GC GAAGGTGGCGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGT.GTCC.TTAC. . . . . GGACG.TGGGGGTTCAAGTCCCGC.CCCTCGCACCA tRNA-Leu-CAG-1-3  
GC GAAGGTGGCGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGT.GTCC.TTAC. . . . . GGACG.TGGGGGTTCAAGTCCCGC.CCCTCGCACCA tRNA-Leu-CAG-1-2  
GC GAAGGTGGCGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGT.GTCC.TTAC. . . . . GGACG.TGGGGGTTCAAGTCCCGC.CCCTCGCACCA tRNA-Leu-CAG-1-1  
GC GAAGGTGGCGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGT.GTTC.TTAC. . . . . GGACG.TGGGGGTTCAAGTCCCGC.CCCTCGCACCA tRNA-Leu-CAG-2-1  
>>>>>>. .>>>. . . . . <<<. >>>>. . . . . <<<<. >>>. . . . . <<<. >>>>>. . . . . <<<<. <<<<<<. . . . .  
GCCGGAGTGGCGAATTGGTaGACGACCAAGATTAGGTTCTGGC.GCC.GCAA. . . . . GGTG.TGCGAGTTCAAGTCTCGC.CTCCCGCACCA tRNA-Leu-TAG-1-1  
>>>>>>. .>>>. . . . . <<<. >>>>. . . . . <<<<. >>>. . . . . <<<. >>>>>. . . . . <<<<. <<<<<<. . . . .  
GCCGAAGTGGCGAATCGGTaGACGAGTTGATTCAAATCAACC.GTA.GAAA. . . . . TACG.TGCCGGTTCAAGTCCGGC.CTTCCGGCACCA tRNA-Leu-CAA-1-1  
>>>>>>. .>>>. . . . . <<<. >>>>. . . . . <<<<. >>>. . . . . <<<. >>>>>. . . . . <<<<. <<<<<<. . . . .  
GCCCGGATGGTGAATCGGTaGACACAGGGGATTAAATCCCTC.GGCG.TTCG. . . . . CGCTG.TGCGGGTTCAAGTCCCGC.TCCGGGTACCA tRNA-Leu-TAA-1-1

## Lys tRNAs

```

>tRNA-Lys--UUU_1/6 CP012868.1|2120328..2120403|-
CGATCCCGTCATCACCACCACCTTCGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA
>tRNA-Lys--UUU_4/6 CP012868.1|3860013..3860088|-
GATTAAGGCAGCATAATCCGCAAGGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA
>tRNA-Lys--UUU_3/6 CP012868.1|3860221..3860296|-
AAAATCTTTCAGGTAACACCCGTAAGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA
>tRNA-Lys--UUU_2/6 CP012868.1|3860443..3860518|-
TCGATCCCGTCATCACCACCACCTTCGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA
>tRNA-Lys--UUU_5/6 CP012868.1|3860747..3860822|-
TTCGATCCCGTCATCACCACCACCTTCGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA
>tRNA-Lys--UUU_6/6 CP012868.1|3861036..3861111|-
AACCAACCGAAAGCAACGAAATTCGGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTGGTCGCAGGTTCAATCCTGCACGACCCACCA

```

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>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<....
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-6
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-5
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-4
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-3
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-2
GGGTCGTTAGCTCAGTTGGTAGAGCAGTTGACTTTTAAATCAATTG.....GtCGCAGGTTCAATCCTGCACGACCCACCA tRNA-Lys-TTT-1-1

```

## Met tRNAs

```

>tRNA-Met--CAU_1/2 CP012868.1|3944532..3944608|+
GATAAGGTATCGCGAAAAAAAGATGGCTACGTAGCTCAGTTGGTTAGAGCACATCACTCATAATGATGGGGTCACAGGTTCAATCCCCTCGTAGCCACCA
>tRNA-Met--CAU_2/2 CP012868.1|3944925..3945001|+
ACCCAGCCATCGAAGAAACAATCAGGCTACGTAGCTCAGTTGGTTAGAGCACATCACTCATAATGATGGGGTCACAGGTTCAATCCCCTCGTAGCCACCA

```

```

>tRNA-fMet--CAU_3/4 CP012868.1|1323367..1323443|+
CGAGTAGAATTTGCCACGTTTCAGGCGCGGGGTGGAGCAGCCTGGTAGCTCGTCGGGCTCATAACCCGAAGATCGTCGGTTCAAATCCGGCCCCCGCAACCA

```

```

>tRNA-fMet--CAU_1/4 CP012868.1|1693973..1694049|-
TGATGAAGTAAAGCAGTACGGTACGCGGGGTGGAGCAGCCTGGTAGCTCGTCGGGCTCATAACCCGAAGGTCGTCGGTTCAAATCCGGCCCCCGCAACCA
>tRNA-fMet--CAU_2/4 CP012868.1|1694083..1694159|-
TTGATGAAGTAAAGCAGTACGGTACGCGGGGTGGAGCAGCCTGGTAGCTCGTCGGGCTCATAACCCGAAGGTCGTCGGTTCAAATCCGGCCCCCGCAACCA
>tRNA-fMet--CAU_4/4 CP012868.1|1694193..1694269|-
GCGGAGTATAGTGCATCCACGGACGCGGGGTGGAGCAGCCTGGTAGCTCGTCGGGCTCATAACCCGAAGGTCGTCGGTTCAAATCCGGCCCCCGCAACCA

```

>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
GGCTACGTAGCTCAGTT.GGTtA.GAGCACATCACTCATAATGATGGG.....GtCACAGGTTCAATCCCGTCGTAGCCACCA tRNA-Met-CAT-2-2  
GGCTACGTAGCTCAGTT.GGTtA.GAGCACATCACTCATAATGATGGG.....GtCACAGGTTCAATCCCGTCGTAGCCACCA tRNA-Met-CAT-2-1

..>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
CGCGGGGTGGAGCAGCctGGT.A.GCTCGTCGGGCTCATAACCCGAAG.....GtCGTCGGTTCAATCCGGCCCCCGCAACCA tRNA-fMet-CAT-1-3  
CGCGGGGTGGAGCAGCctGGT.A.GCTCGTCGGGCTCATAACCCGAAG.....GtCGTCGGTTCAATCCGGCCCCCGCAACCA tRNA-fMet-CAT-1-2  
CGCGGGGTGGAGCAGCctGGT.A.GCTCGTCGGGCTCATAACCCGAAG.....GtCGTCGGTTCAATCCGGCCCCCGCAACCA tRNA-fMet-CAT-1-1  
CGCGGGGTGGAGCAGCctGGT.A.GCTCGTCGGGCTCATAACCCGAAG.....AtCGTCGGTTCAATCCGGCCCCCGCAACCA tRNA-fMet-CAT-2-1

### Phe tRNAs

```
>tRNA-Phe--GAA_1/2 CP012868.1|279027..279102|+  
AGATTACGGTTTAAATGCGCCCGTGGCCGGATAGCTCAGTCGGTAGAGCAGGGGATTGAAAATCCCCGTGTCCTTGTTTCGATTCCGAGTCCGGGCACCA  
>tRNA-Phe--GAA_2/2 CP012868.1|1531215..1531290|-  
CGAATCAGGTTTAAATGCGCCCGTGGCCGGATAGCTCAGTCGGTAGAGCAGGGGATTGAAAATCCCCGTGTCCTTGTTTCGATTCCGAGTCCGGGCACCA
```

>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
GCCCGGATAGCTCAGTCGGTAGAGCAGGGGATTGAAAATCCCCGT.....GtCCTTGTTTCGATTCCGAGTCCGGGCACCA tRNA-Phe-GAA-1-2  
GCCCGGATAGCTCAGTCGGTAGAGCAGGGGATTGAAAATCCCCGT.....GtCCTTGTTTCGATTCCGAGTCCGGGCACCA tRNA-Phe-GAA-1-1

### Pro tRNAs

```
>tRNA-Pro--GGG CP012868.1|2355369..2355445|-  
AACGGTAGTATTAGCAGCCACGATCGGCACGTAGCGCAGCCTGGTAGCGCACCGTCATGGGGTGTCGGGGGTCGGAGGTTCAAATCCTCTCGTGCCGACCA  
>tRNA-Pro--CGG CP012868.1|932963..933039|+  
TGCGCAGTAAGATGCGCCCGCATCGGTGATTGGCGCAGCCTGGTAGCGCACTTCGTTCCGGGACGAAGGGGTCGGAGGTTCAATCCTCTATCACCGACCA  
>tRNA-Pro--UGG CP012868.1|658844..658920|-  
ACTAAAATTCAAAAAGCAGTATTGGGCGAGTAGCGCAGCTTGGTAGCGCAACTGGTTTGGGACCAGTGGGTCGGAGGTTCAATCCTCTCTCGCCGACCA
```

>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
CGGCACGTAGCGCAGCctGGTAGCGCACCGTCATGGGGTGTCTGGGGTTCGAGGTTCAAATCCTCTCGTGCCGACCA tRNA-Pro-GGG-1-1  
>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
CGGTGATTGGCGCAGCctGGTAGCGCACTTCGTTCCGGGACGAAGGGGTCGAGGTTCAATCCTCTATCACCGACCA tRNA-Pro-CGG-1-1  
>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>.....<<<<<<<<<<<<.....  
CGGCGAGTAGCGCAGCtGGTAGCGCACTGGTTGGGACCAAGTGGGTCGAGGTTCAATCCTCTCTCGCCGACCA tRNA-Pro-TGG-1-1

### Sec tRNAs

```
>tRNA-Sec--UCA CP012868.1|805339..805433|-  
CGCCTCCATGTAGCAATCGAGGCGGAAGATCGTCGTCTCCGGTGAGGCGGCTGGACTTCAAATCCAGTTGGGGCCGCCAGCGGTCCCGGGCAGGTTCGACTCCTGTGATCTTCGCGCA
```

..>>>>>>..>>>>.....<<<<.>>>>.....<<<<<<.....>>>>>>>>.....<<<<<<<<<<<<.....  
gGAAGATCGTCGTCTCCGGTgA.GGCGGCTGGACTTCAAATCCAGTTGGGGCCGCCAGCGGTCCCGGGCAGGTTCGACTCCTGTGATCTTCC... tRNA-Sec-TCA-1-1 Sc: 142.9

>tRNA-Ser--GGA\_1/2 CP012868.1|3544013..3544100|+  
 TCCAGCAGACAGGAACGACAATTGGTGAGGTGTCCGAGTGGCTGAAGGAGCAGCGCTGGAAAGTGTGTATACGGCAACGTATCGGGGGTTTCAATCCCCCCTCACCGCCA  
 >tRNA-Ser--GGA\_2/2 CP012868.1|3715694..3715781|+  
 ATGAGGTTTGGTAGCAAAACAATTGGTGAGGTGTCCGAGTGGCTGAAGGAGCAGCGCTGGAAAGTGTGTATACGGCAACGTATCGGGGGTTTCAATCCCCCCTCACCGCCA

>>>>>>. .>>>. . . . . <<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<<. >>>>>. . . . .  
 GGTGAGGTGTCCGAGTGGCtgA.AGGAGCACGCCTGGAAGTGTGT.ATACG. .GCAA. .CGTAT.CGGGGGTTCTGAATCCCC. .CCTACCGCCA tRNA-Ser-GGA-1-2  
 GGTGAGGTGTCCGAGTGGCtgA.AGGAGCACGCCTGGAAGTGTGT.ATACG. .GCAA. .CGTAT.CGGGGGTTCTGAATCCCC. .CCTACCGCCA tRNA-Ser-GGA-1-1  
 >>>>>>. .>>>. . . . . <<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<<. >>>>>. . . . .  
 GGAGAGATGCCGGAGCGGctgA.ACGACCGGTCTCGAAACCGGA.GTAGGG. .GCAA. .CTCTAC.CGGGGGTTCAATCCCC. .TCTCTCCGCCA tRNA-Ser-CGA-1-1  
 >>>>>>. .>>>. . . . . <<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<<. >>>>>. . . . .  
 GGAAGTGTGCCGAGCGGttgA.AGGCACCGGTCTTGAAACCGCGACCC. .GAAA. . . . . GGGTTCCAGAGTTCTGAATCTCTG. .CGCTTCCGCCA tRNA-Ser-TGA-1-1  
 >>>>>>. .>>>. . . . . <<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<. >>>>>. . . . . <<<<<. >>>>>. . . . .  
 GGTGAGGTG6CCGAGAGGctgA.AGGCGTCCCCTGCTAAAGGAGT.ATGCGGTCAAAaGCTGCAT.CGGGGGTTCTGAATCCCCG. .CCTACCGCCA tRNA-Ser-GCT-1-1

>tRNA-Thr--GGU\_1/2 CP012868.1|465826..465901|-  
 ATTC**CCGCTGCCCGCTCCA**AGAT**GT**GTCTGATAT**AG**CTCAGTTGGTAGAGCGCACCC**TTGG**TAAGGGTGAGGTC**GG**CAGTTCGA**A**TCTG**CC**TATCAGC**ACCA**  
 >tRNA-Thr--GGU\_2/2 CP012868.1|1218001..1218076|+  
 AACT**GCCAGGCATCAAATTTAGC****GT**GTCTGATAT**GG**CTCAGTTGGTAGAGCGCACCC**TTGG**TAAGGGTGAGGTC**CC**CAGTTCGA**A**TCTG**GG**TATCAGC**ACCA**  
 >tRNA-Thr--CGU\_1/2 CP012868.1|4344411..4344487|-  
 ATAAAGTATGTTGTTT**GATTGAT****T**GTCTCAAGTAGTAAAAATGCATTAACATCGCATT**CG**TAATGCGAAGGTCGTAGGTT**CG**ACTCCTATTATCGGC**A**CCA  
 >tRNA-Thr--CGU\_2/2 CP012868.1|4378719..4378794|-  
 ACGTT**CGTGAACATGTCCTTT**CAG**G**GCCGATATAGCTCAGTTGGTAGAGCAGCGCATT**CG**TAATGCGAAGGTCGTAGGTT**CG**ACTCCTATTATCGGCACCA  
 >tRNA-Thr--UGU CP012868.1|466192..466267|-  
 CTCCATAGAA**TGCGCGCTACTTGA****T**GCCGACTTAGCTCAGTAGGTAGAGCAACTGACT**TG**TAATCAGTAGGTCACCAGTTCGATTCCGGTAGTCGGC**A**CCA  
 >>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<<<....  
 GCTGATAT**AGCTCAGTTGGT**.AGAG**CGCACCCCTT**GGTAAG**GGGTG**AG.....Gt**GGCA**GTTCGA**ATCTGCCTATC**AGC**ACCA** tRNA-Thr-GGT-1-1  
 GCTGATAT**GGCTCAGTTGGT**.AGAG**CGCACCCCTT**GGTAAG**GGGTG**AG.....Gt**CCCCA**GTTCGA**CTCTGGGTA**TCAGC**ACCA** tRNA-Thr-GGT-2-1

### Trp tRNAs

>>>>>>>. .>>>>. . . . . <<<<.>>>>>. . . . . <<<<<. . . . . >>>>>. . . . . <<<<<<<<<<. . . . .  
AGGGGCGTAGTTCAATTGGTAGAGCACCGGTCTCCAAACCGGGT. . . . . GtTGGGAGTTCGAGTCTCTCCGCCCTGCCA tRNA-Trp-CCA-1-1

TCCGGTAGTCGGCACCATCAAGTCCGGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACTGTAAATCTGCCGTCA**CA**GACTTCGAAGGTTGAATCCTTCCCCACCACCA.

>>>>>>..>>>.....<<<.>>>>.....<<<<<.>>>.....<<<..>>>>>>.....<<<<<<.<<<<<<<....  
 GGTGGGGTTCCCGAGCGGCcA.AGGGAGCAGACTGTAAATCTGCCGTCA**CA**... GACTtCGAAGGTTCTGAATCCTTC.CCCCACCACCA tRNA-Tyr-GTA-1-1  
 GGTGGGGTTCCCGAGCGGCcA.AGGGAGCAGACTGTAAATCTGCCGTCA**TC**... GACTtCGAAGGTTCTGAATCCTTC.CCCCACCACCA tRNA-Tyr-GTA-2-2  
 GGTGGGGTTCCCGAGCGGCcA.AGGGAGCAGACTGTAAATCTGCCGTCA**TC**... GACTtCGAAGGTTCTGAATCCTTC.CCCCACCACCA tRNA-Tyr-GTA-2-1

CGAGTCCAATTGAACGCACCATCCTGCGTCCGTAGCTCAGTTGGTTAGAGCACCACTTGACATGGTGGGGGTCGGTGGTTCGAGTCCACTCGGACGCACCA

CGTCCCGTTGTACCTACAACGTTCGCTTCAATAGCTCAGTTGGTTAGAGCACCACTTGACATGGTGGGGGTCGTTGGTTCGAGTCCAATTGAACGCACCA

>tRNA-Val--UAC\_1/5 CP012868.1|2120408..2120483| -  
 TTGTAAAAATGTGAAGTACCGAAATGGGTGATTAGCTCAGCTGGGAGAGCACCTCCCTTACAAGGAGGGGGTTCGGCGTTTCGATCCCGTCATCACCCACCA  
 >tRNA-Val--UAC\_2/5 CP012868.1|2120530..2120605| -

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GCCGTGTAGCAAGAAATTGAGAAAGGGTGATTAGCTCAGCTGGGAGAGCACCTCCCTTACAAGGAGGGGGTCGGCGGTTTCGATCCCGTCATCACCCACCA
>tRNA-Val--UAC_3/5 CP012868.1|2120650..2120725|-
CCTATAATGCGACTCCACACAGCGGGGGTGATTAGCTCAGCTGGGAGAGCACCTCCCTTACAAGGAGGGGGTCGGCGGTTTCGATCCCGTCATCACCCACCA
>tRNA-Val--UAC_4/5 CP012868.1|3860522..3860597|-
AGTTTGTAGTATCCAGCGCAGTATCGGGTGATTAGCTCAGCTGGGAGAGCACCTCCCTTACAAGGAGGGGGTCGGCGGTTTCGATCCCGTCATCACCCACCA
>tRNA-Val--UAC_5/5 CP012868.1|3860825..3860900|-
TAGTTGTAGTATCCAGCGTAGTATCGGGTGATTAGCTCAGCTGGGAGAGCACCTCCCTTACAAGGAGGGGGTCGGCGGTTTCGATCCCGTCATCACCCACCA

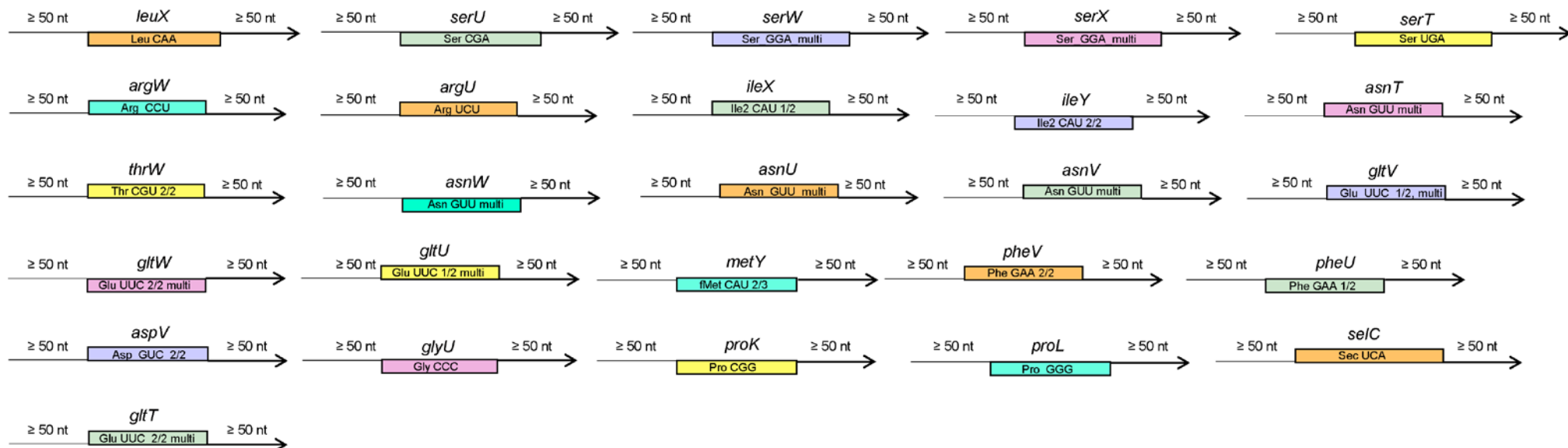
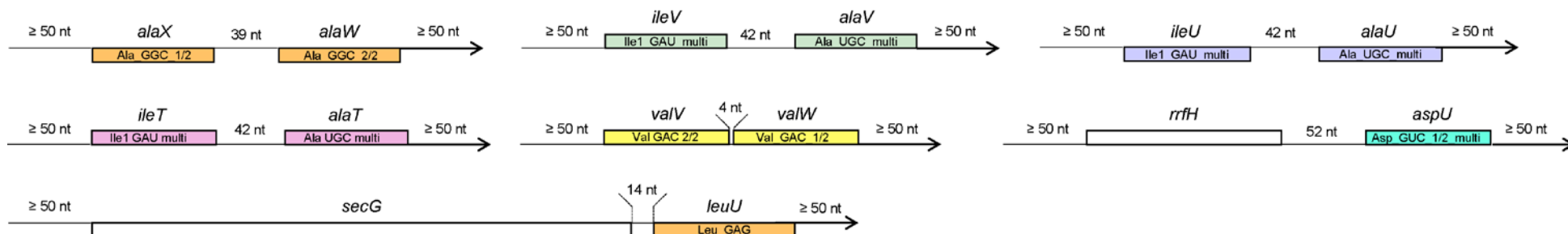
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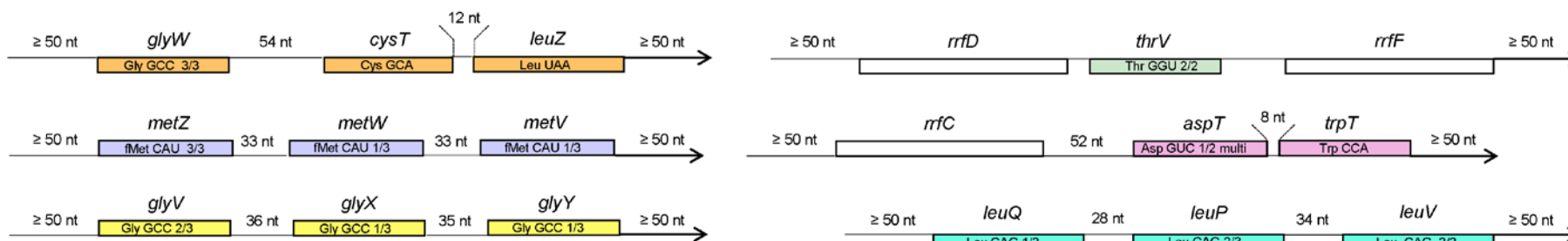
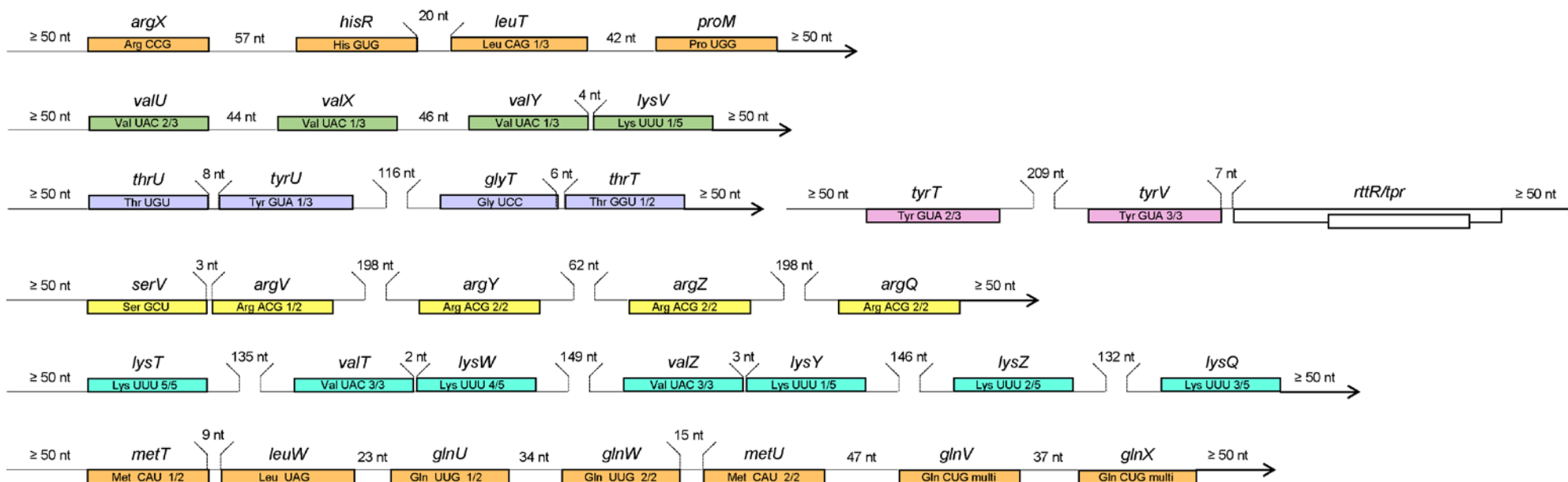
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>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<....
GCGTCCGTAGCTCAGTTGGTtAGAGCACCACTTGACATGGTGGGG.....GtCGGTGGTTCGAGTCCACTCGGACGCACCA tRNA-Val-GAC-1-1
GCGTTCATAGCTCAGTTGGTtAGAGCACCACTTGACATGGTGGGG.....GtCGTTGGTTCGAGTCCAATTGAACGCACCA tRNA-Val-GAC-2-1
>>>>>>..>>>>.....<<<<.>>>>.....<<<<.....>>>>.....<<<<<<<<<<....
GGGTGATTAGCTCAGCTGGG.AGAGCACCTCCCTTACAAGGAGGGG.....GtCGCGGTTTCGATCCCGTCATCACCCACCA tRNA-Val-TAC-1-5
GGGTGATTAGCTCAGCTGGG.AGAGCACCTCCCTTACAAGGAGGGG.....GtCGCGGTTTCGATCCCGTCATCACCCACCA tRNA-Val-TAC-1-4
GGGTGATTAGCTCAGCTGGG.AGAGCACCTCCCTTACAAGGAGGGG.....GtCGCGGTTTCGATCCCGTCATCACCCACCA tRNA-Val-TAC-1-3
GGGTGATTAGCTCAGCTGGG.AGAGCACCTCCCTTACAAGGAGGGG.....GtCGCGGTTTCGATCCCGTCATCACCCACCA tRNA-Val-TAC-1-2
GGGTGATTAGCTCAGCTGGG.AGAGCACCTCCCTTACAAGGAGGGG.....GtCGCGGTTTCGATCCCGTCATCACCCACCA tRNA-Val-TAC-1-1

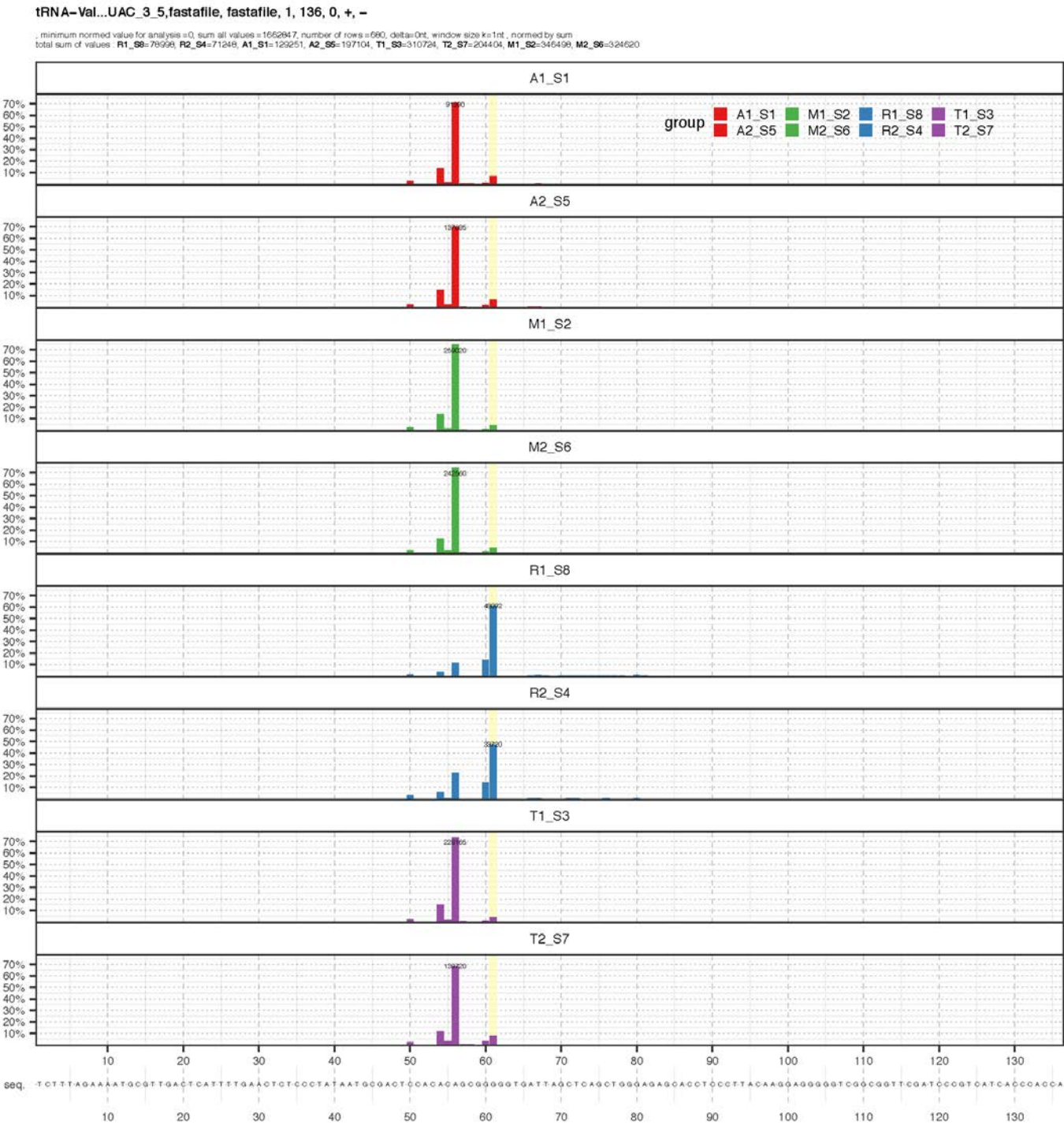
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**Fig. S2:** The sequences of *E. coli* tRNAs (genome NCBI NZ\_CP012868.1) including 25 nt of 5' precursor sequence, isoacceptor alignments and potential acceptor stem extensions. For color coding, see top of figure.

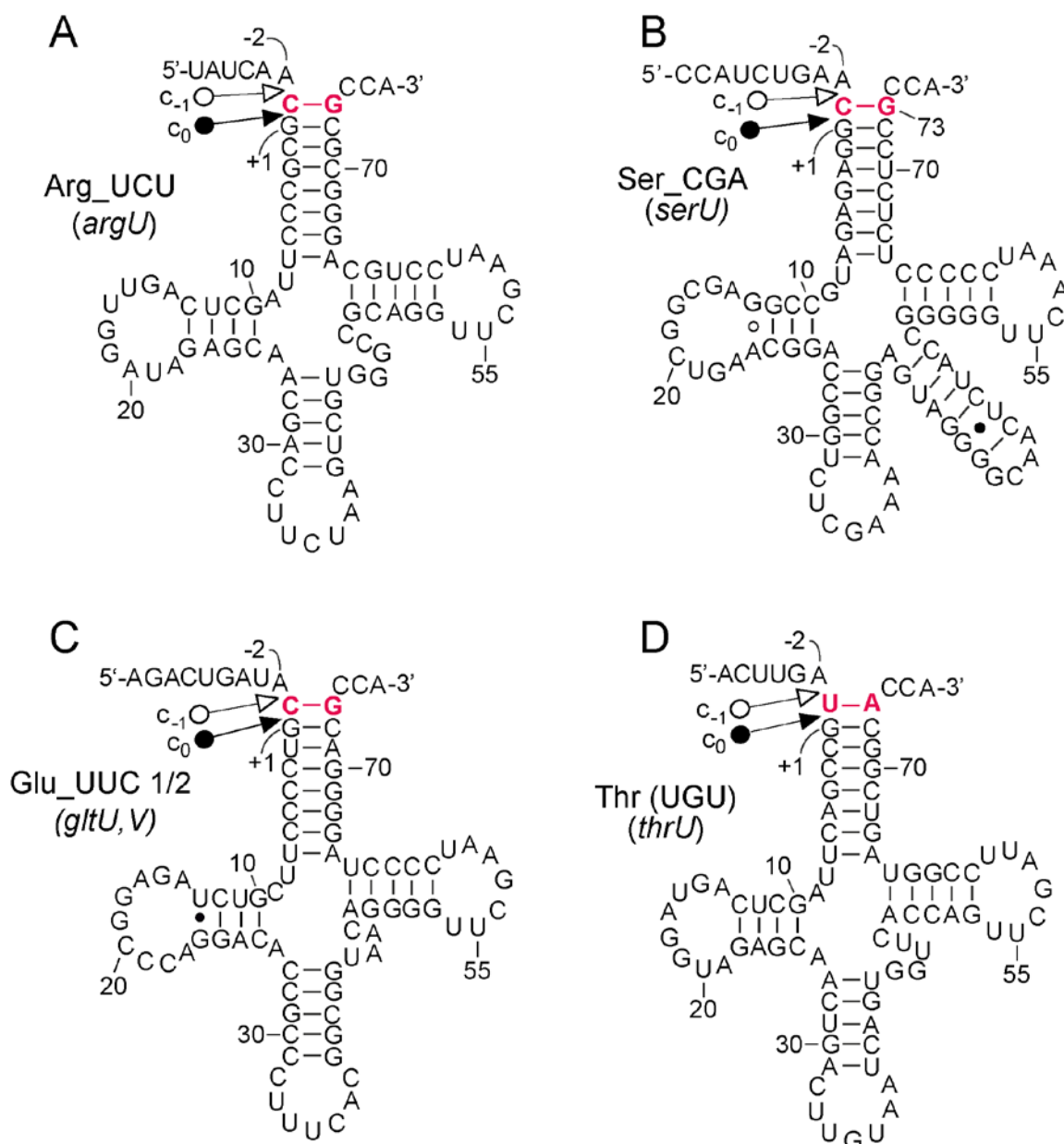
monocistronic tRNAs *E. coli* MG1655dicistronic tRNAs *E. coli* MG1655

tracistronic tRNAs *E. coli* MG1655polycistronic tRNAs *E. coli* MG1655

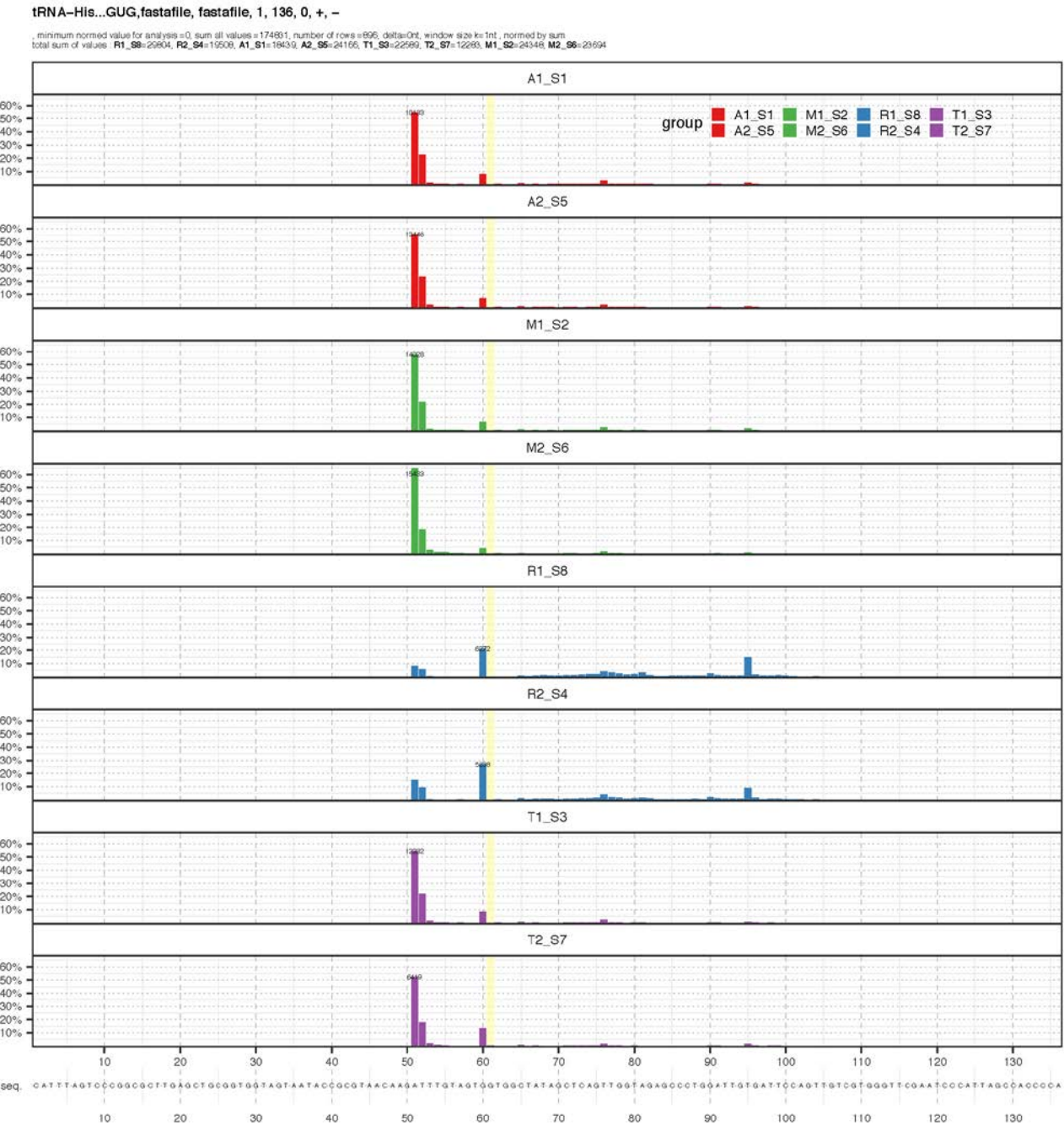
**Fig. S3:** Organization and genetic context of tRNA genes in *E. coli* strain MG1655 (genome NCBI NZ\_CP012868.1). Colored boxes above lines are encoded on one genome strand, those below lines on the other strand. Gene abbreviations are given above boxes and tRNA assignments according to the second column of Table S1 are given within boxes; *secG*, protein-export membrane protein SecG; *rrfC/D/F/H*, 5S rRNA; *rttR*, non-coding RtT RNA; *tpv*, protamine-like protein. Intercistronic nucleotide distances between genes of the same transcription unit are indicated. Flanking sequences of tRNA genes and operons are blanketly given as  $\geq 50$  nt.



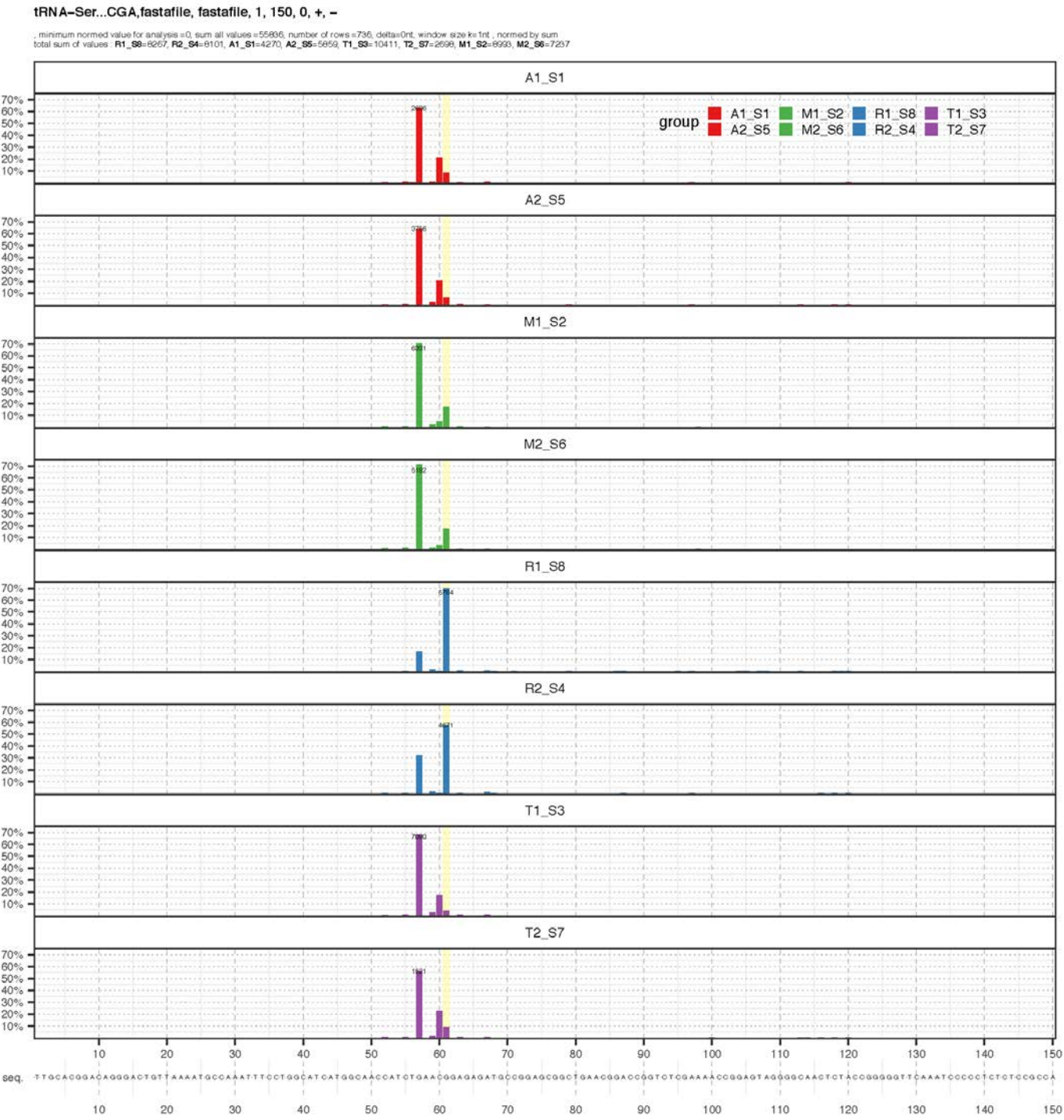
**Fig. S4:** 5'-ends in RNA-seq libraries at the *valU* [tRNA<sup>Val</sup> (UAC)] locus in *E. coli* BW bacteria complemented with *Aae harp* (red bars), *Mth harp* (green bars), *Tin harp* (magenta bars) and *E. coli rnpB* (blue bars). For each strain, results from two independent RNA-seq experiments are shown.



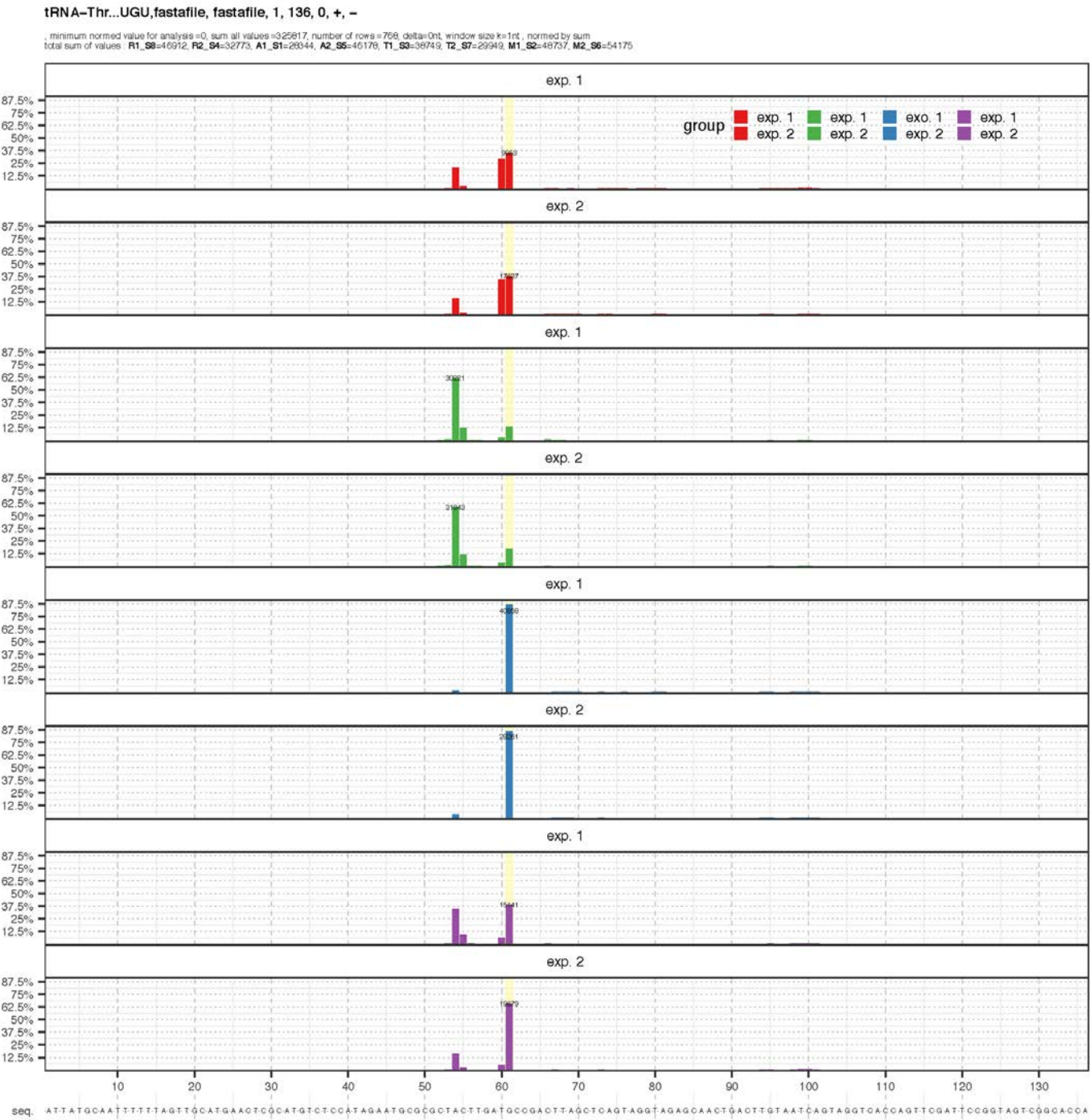
**Fig. S5.** Secondary structure of *E. coli* precursor tRNAs encoded by the genes **(A)** *argU*, **(B)** *serU*, **(C)** *gltU, V* and **(D)** *thrU*. Red nucleotides mark acceptor stem extensions in precursor transcripts. All four pre-tRNAs share the feature of an A residue at position -2, which favored cleavage at the c<sub>-1</sub> site by *Aae* and *Tin* HARP (see main text). Arbitrary numbers of 5'-leader nucleotides are shown for the individual pre-tRNAs. Filled arrows mark the canonical cleavage site c<sub>0</sub> (between nt -1 and +1) and open arrows the aberrant c<sub>-1</sub> site (between nt -2 and -1).



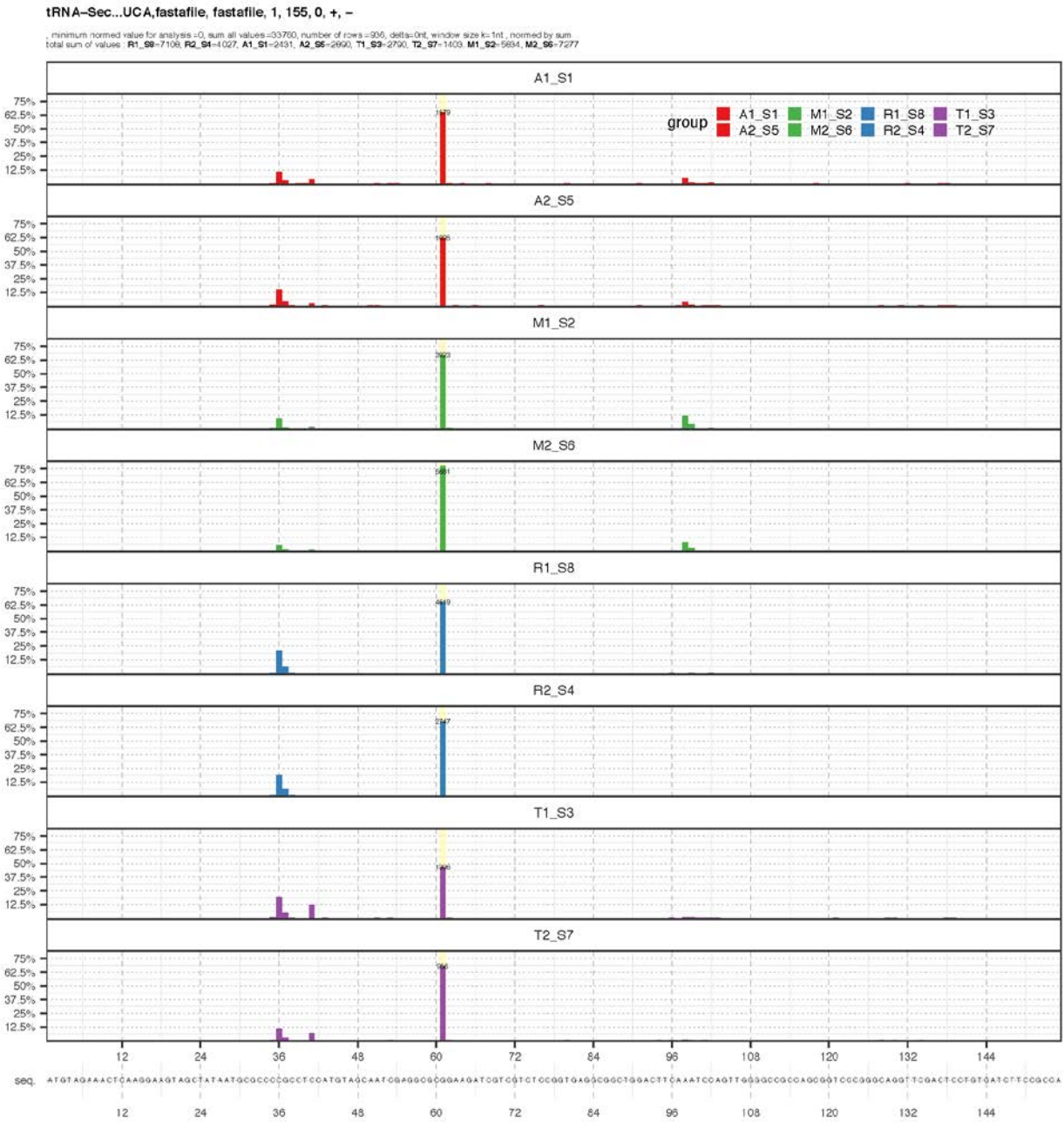
**Fig. S6:** 5'-ends in RNA-seq libraries at the *hisR* [tRNA<sup>His</sup> (GUG)] locus in *E. coli* BW bacteria complemented with *Aae harp* (red bars), *Mth harp* (green bars), *Tin harp* (magenta bars) and *E. coli rnpB* (blue bars). For each strain, results from two independent RNA-seq experiments are shown.



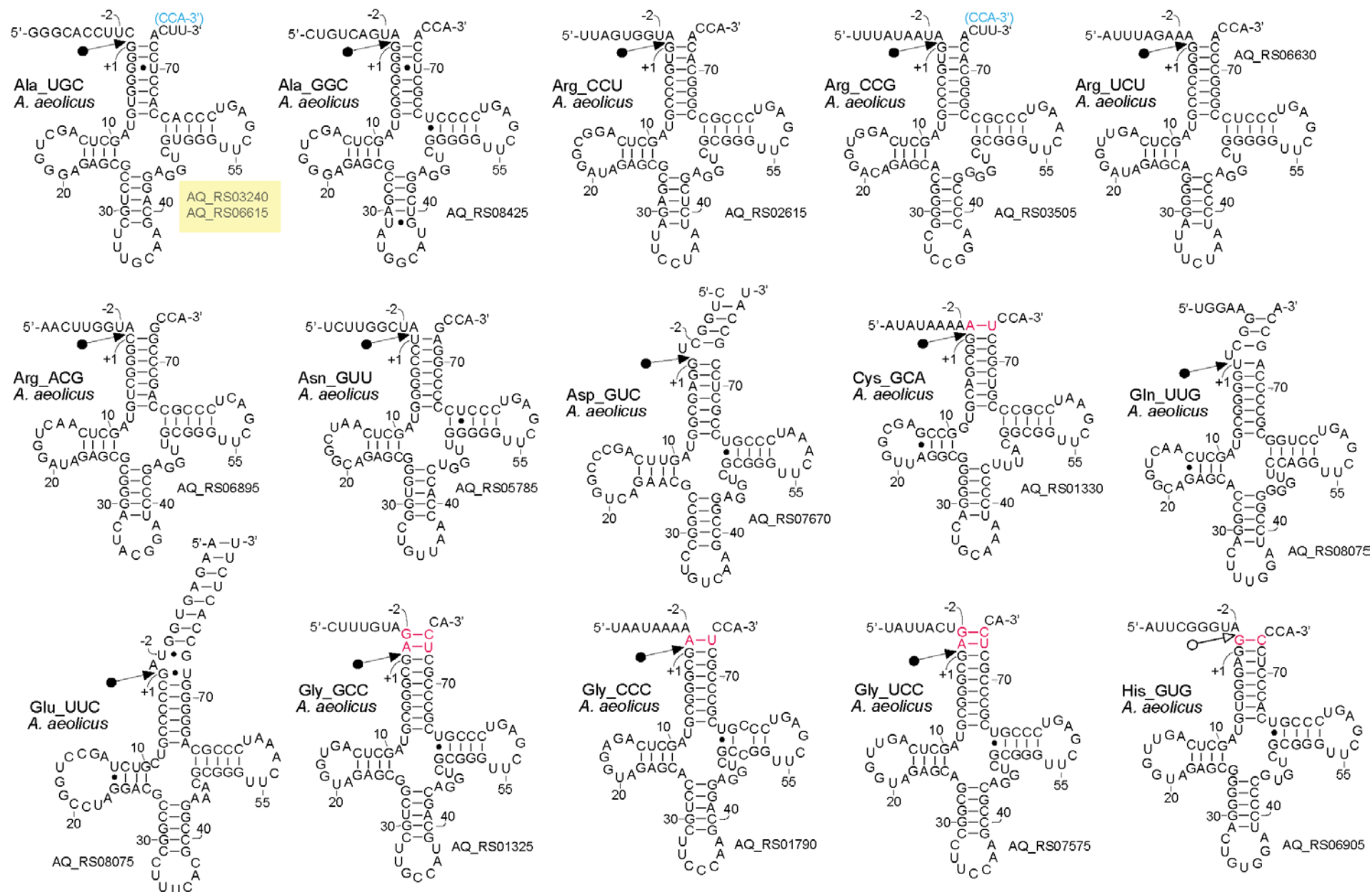
**Fig. S7:** 5'-ends in RNA-seq libraries at the *serU* [tRNA<sup>Ser</sup> (CGA)] locus in *E. coli* BW bacteria complemented with *Aae harp* (red bars), *Mth harp* (green bars), *Tin harp* (magenta bars) and *E. coli rnpB* (blue bars). For each strain, results from two independent RNA-seq experiments are shown.

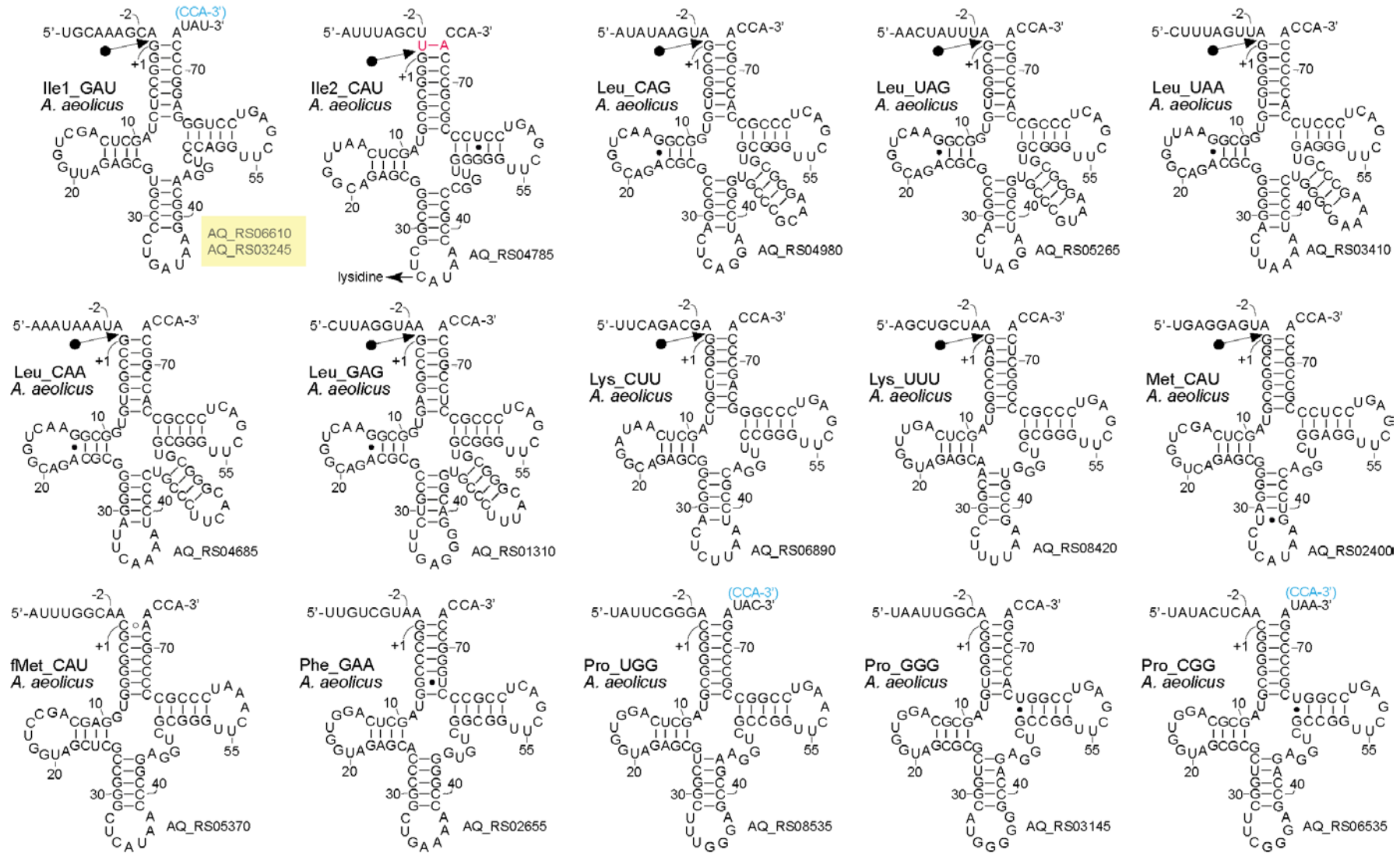


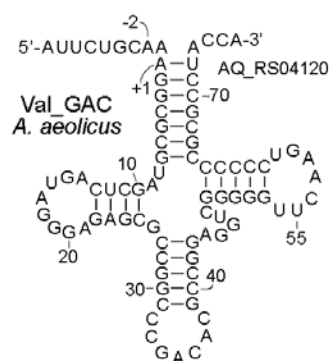
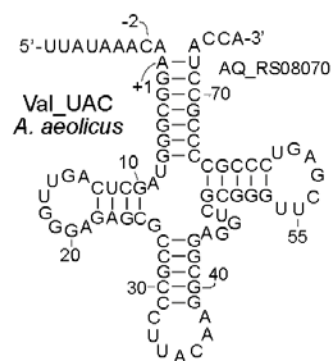
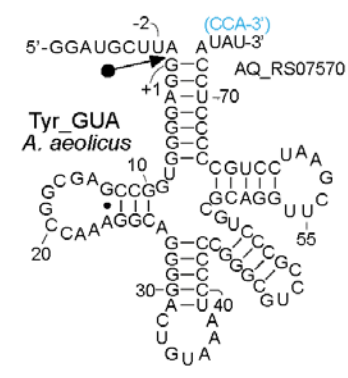
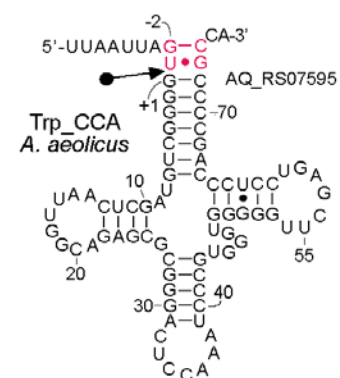
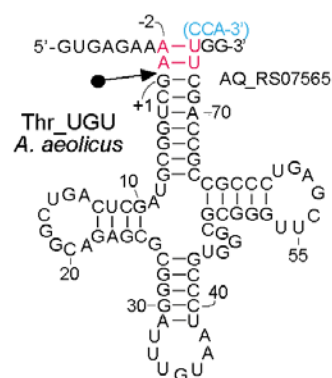
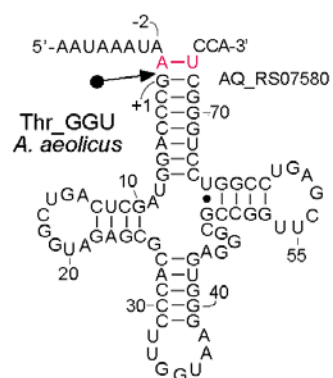
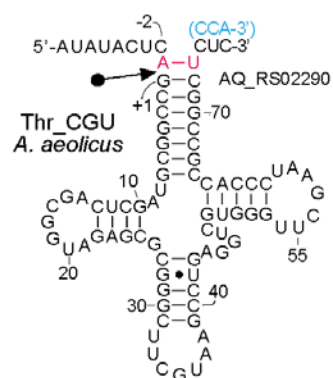
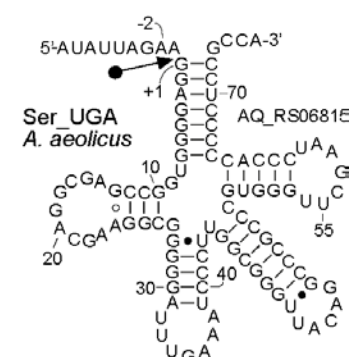
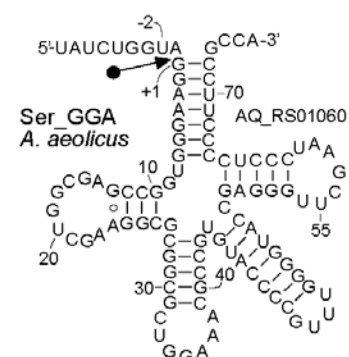
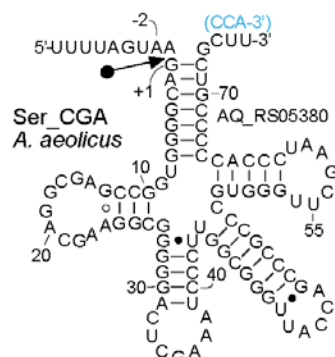
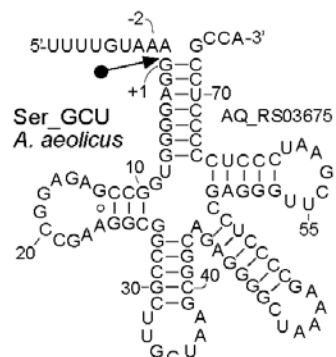
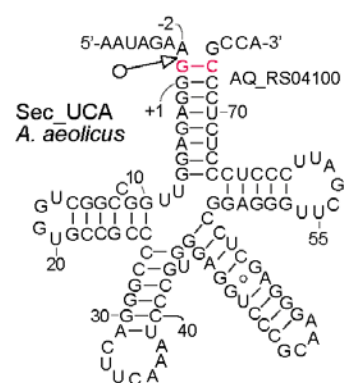
**Fig. S8:** 5'-ends in RNA-seq libraries at the *thrU* [tRNA<sup>Thr</sup> (UGU)] locus in *E. coli* BW bacteria complemented with *Aae harp* (red bars), *Mth harp* (green bars), *Tin harp* (magenta bars) and *E. coli rnpB* (blue bars). For each strain, results from two independent RNA-seq experiments are shown.



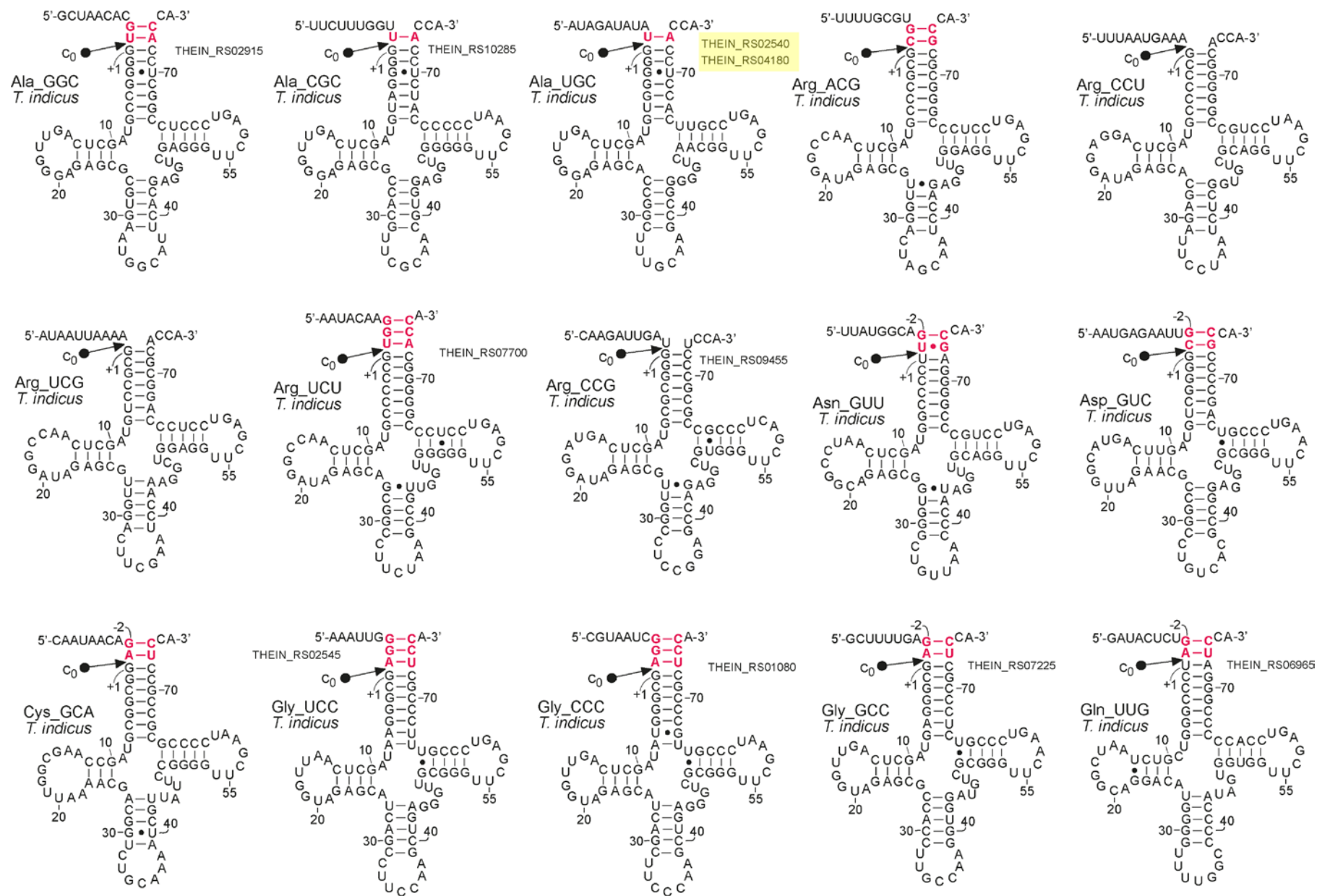
**Fig. S9:** 5'-ends in RNA-seq libraries at the *selC* [tRNA<sup>Sec</sup> (UCA)] locus in *E. coli* BW bacteria complemented with *Aae harp* (red bars), *Mth harp* (green bars), *Tin harp* (magenta bars) and *E. coli rnpB* (blue bars). For each strain, results from two independent RNA-seq experiments are shown.

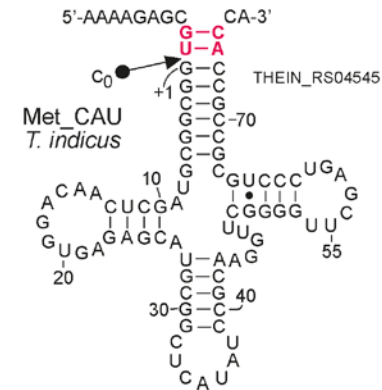
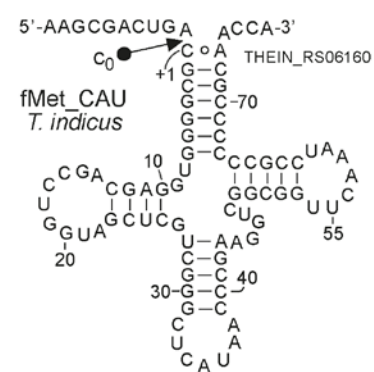
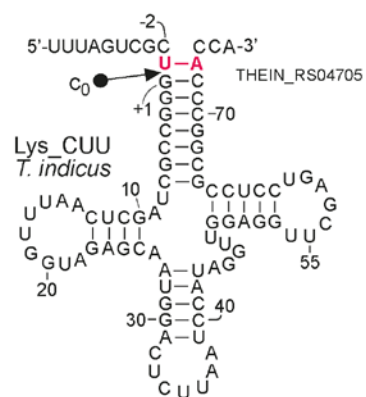
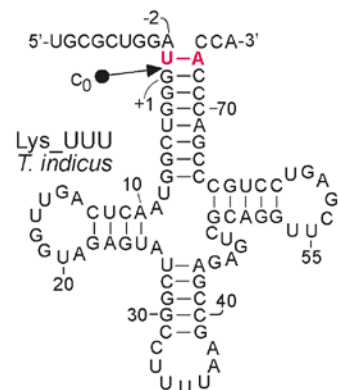
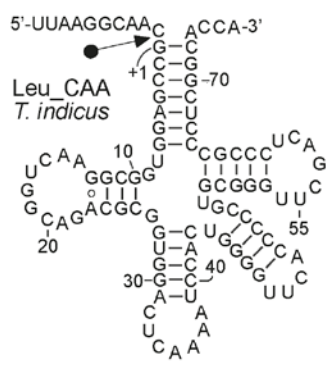
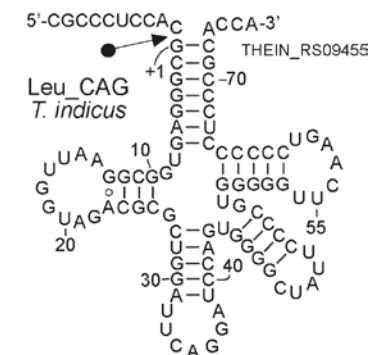
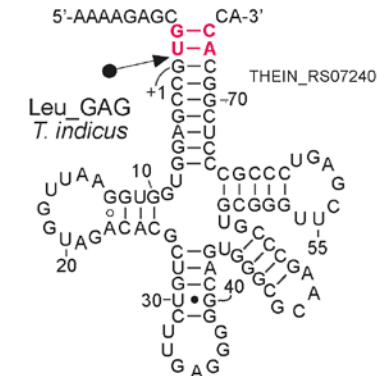
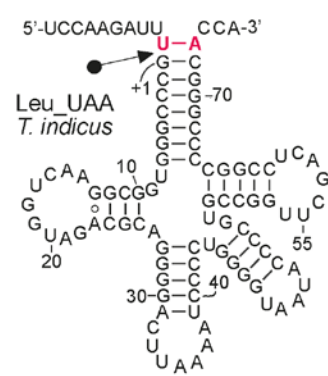
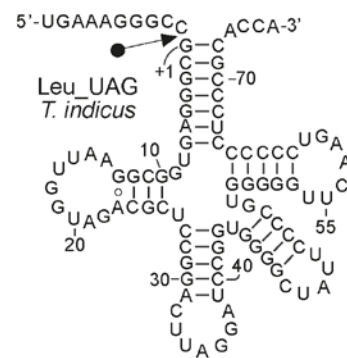
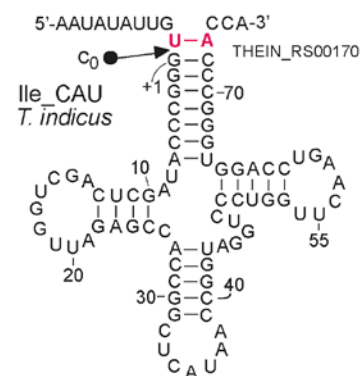
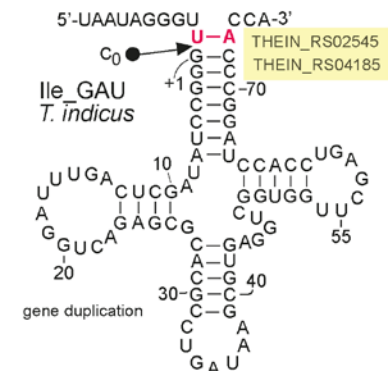
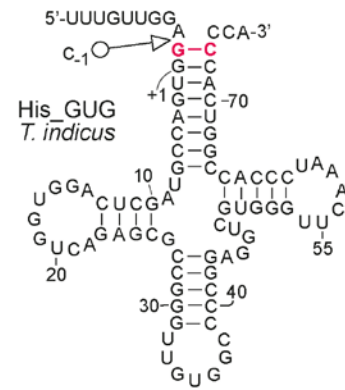
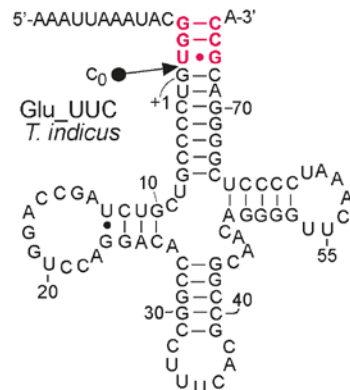
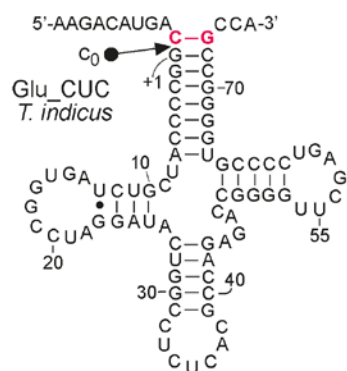
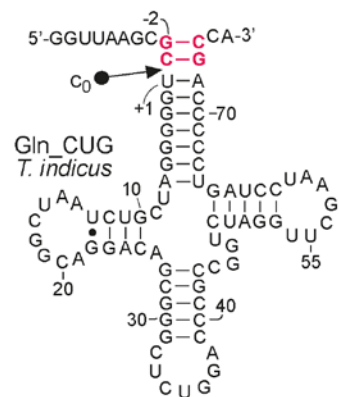


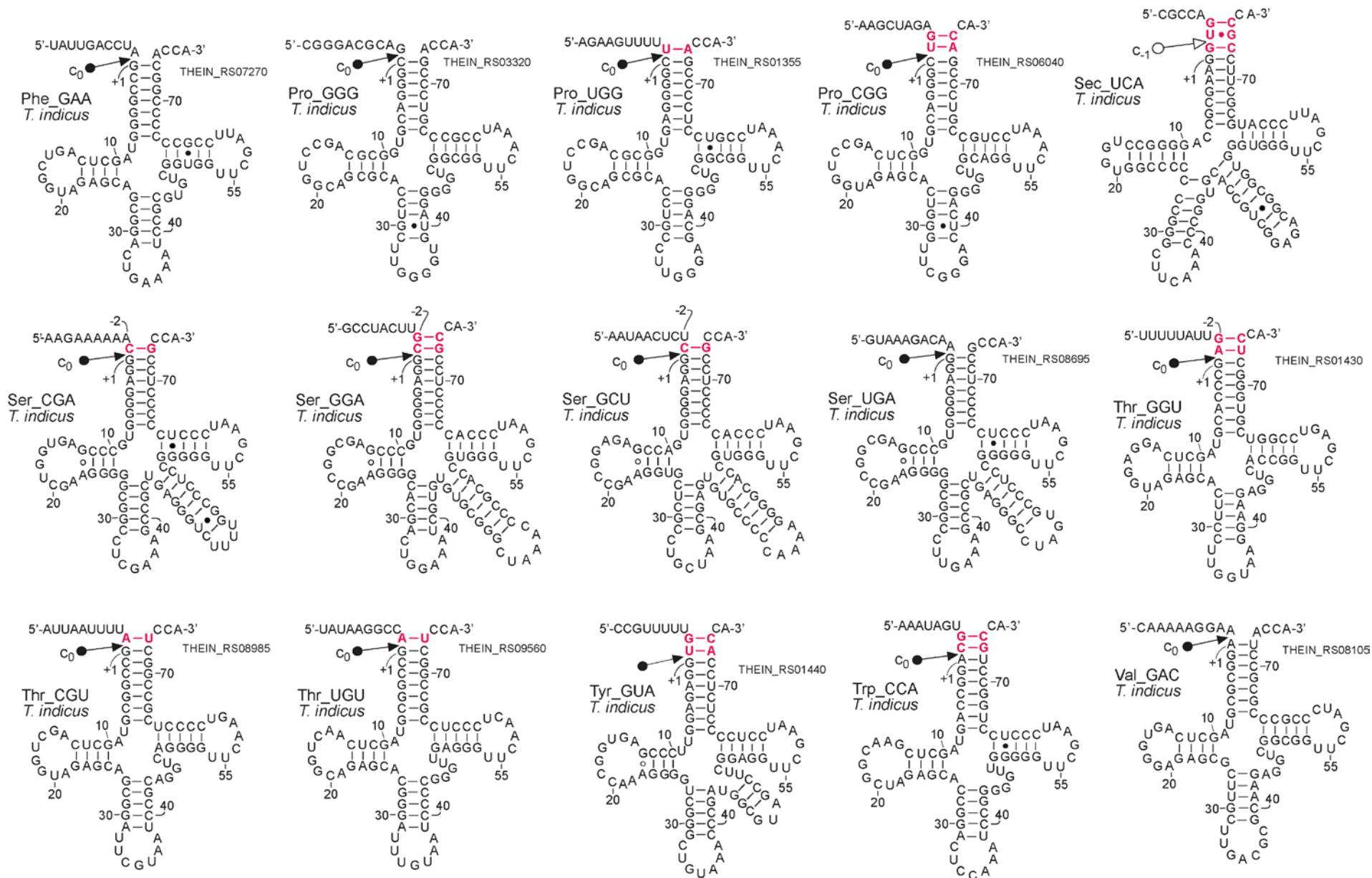


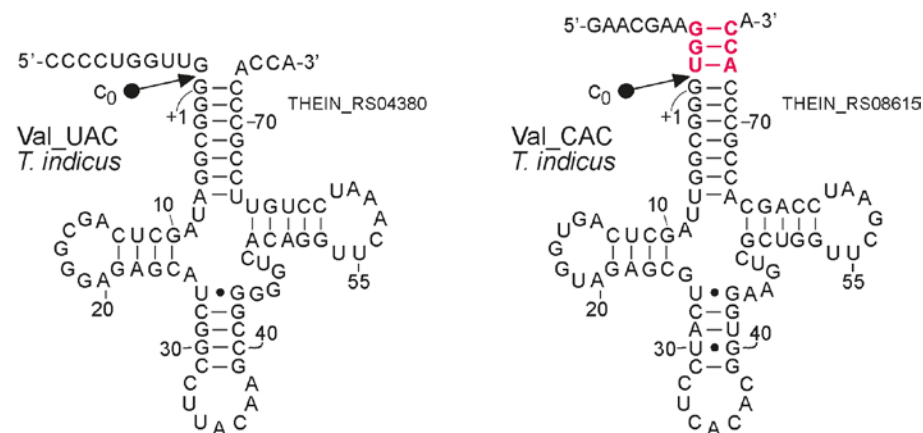


**Fig. S10:** 2D structures of the 44 tRNAs encoded in the *A.aeolicus* genome with several 5'-flanking nucleotides. Potential acceptor stem extensions are indicated in red letters. The 3'-CCA terminus is given in light blue letters in parentheses above the genetically encoded 3'-terminal sequence in cases where the CCA triplet is not encoded in the gene. Canonical RNase P cleavage sites ( $c_0$  sites) are marked by filled arrows, alternative cleavage sites ( $c_{-1}$  sites) in pre-tRNA<sup>His; Sec</sup> by open arrows. The 5'-C of the tRNA<sup>Ile2</sup> (CAU) anticodon is converted to lysidine *in vivo* to enable decoding of 5'-AUA codons (Nakanishi et al., 2005).

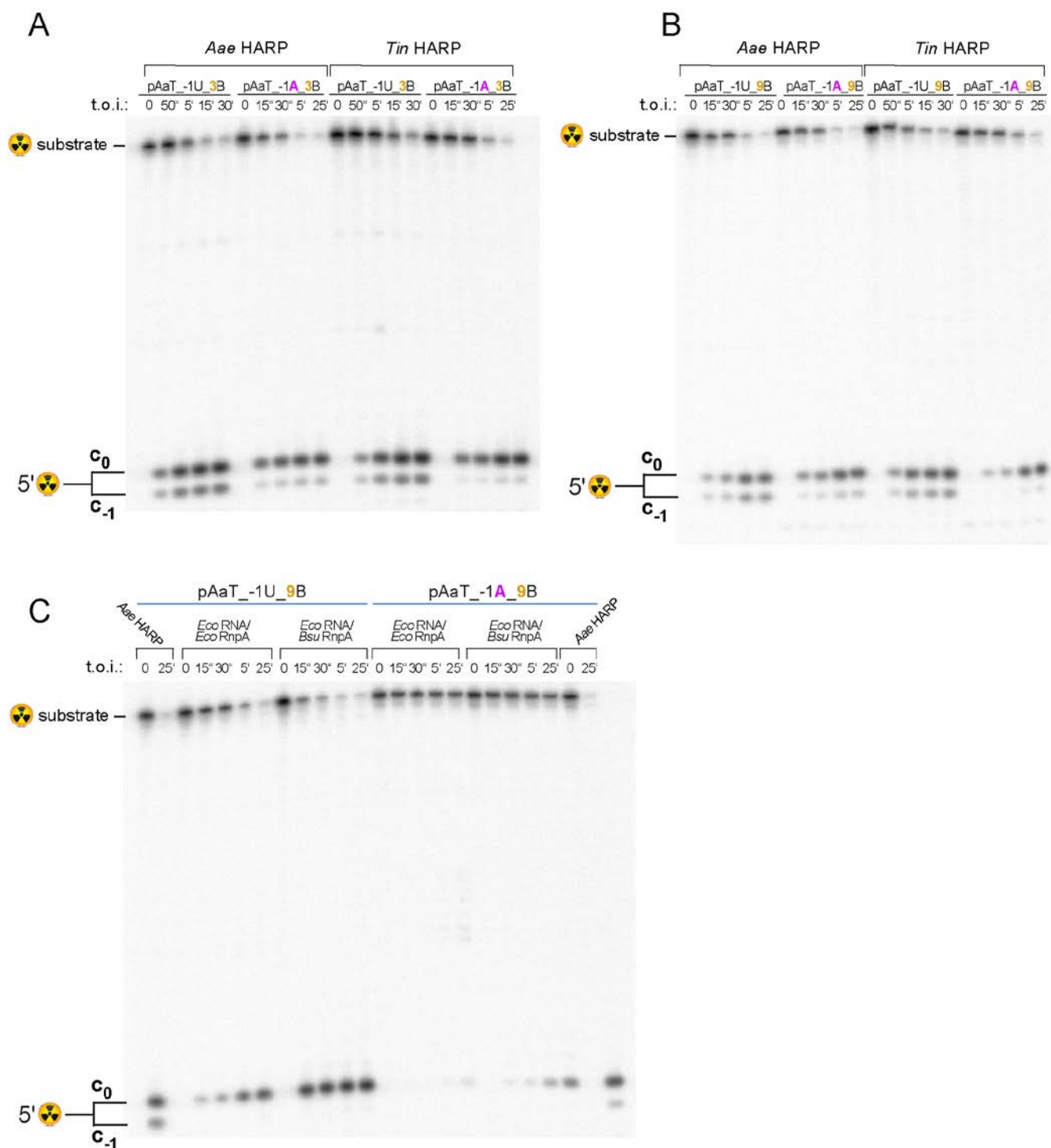








**Fig. S11:** 2D structures of some tRNAs from *T. indicus* DSM 15286 including several 5'-flanking nucleotides. Potential acceptor stem extensions are indicated in red letters. Canonical RNase P cleavage sites ( $c_0$  sites) are marked by filled arrows, the alternative cleavage site ( $c_{-1}$  site) in pre-tRNA<sup>Sec</sup> by an open arrow.



**Fig. S12:** Representative 20% denaturing (8 M urea) PAA gels analyzing the single turnover kinetics and cleavage site selection for the indicated pAaT substrate variants in reactions catalyzed by **(A, B)** Aq880 and *T. indicus* HARP or **(C)** *E. coli* RNase P RNA in complex with the *E. coli* RnpA protein (*Eco* RNA/*Eco* RnpA) or the *B. subtilis* RnpA protein (*Eco* RNA/*Bsu* RnpA). The 5'-<sup>32</sup>P-labeled substrates and 5'-leader products derived from cleavage at the canonical ( $c_0$ ) or aberrant ( $c_{-1}$ ) site are marked at the left margin of the phosphor images. For details, see Materials & Methods and Fig. 11 of the main manuscript, as well as Tables S2 and S3.

**References**

Nakanishi K, Fukai S, Ikeuchi Y, Soma A, Sekine Y, Suzuki T, Nureki O. 2005. Structural basis for lysidine formation by ATP pyrophosphatase accompanied by a lysine-specific loop and a tRNA-recognition domain. *Proc Natl Acad Sci USA* **102**: 7487-7492. doi: 10.1073/pnas.0501003102