# Supplementary Material

for

# Recognition of $tRNA^{Gln}$ by *Helicobacter pylori* GluRS2 – a $tRNA^{Gln}$-Specific Glutamyl-tRNA Synthetase

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**Table S1 – Oligonucleotide sequences for construction of reported tRNA chimeras and mutants.**

| | | |
|---|---|---|
| Chimera 1 | KM1F | CCGGAATTCGGCTCCTTCGCCAAGCGGTAAGGCACCAGGTTTTGGTCCTGGCATTC |
| | KM1R | CGCGGATCCTGGTGACTCCTCAAGGATTCGAACCTCGGAATGCCAGGACCAAAAC |
| Chimera 2 | KM2F | CCGGAATTCTGGGGTGTCATCTAGTGGTTAGGATACCAGGTTTTGGTCCTGGCATTC |
| | KM2R | CGCGGATCCTGGCTGGGGTGCAAGGATTCGAACCTCGGAATGCCAGGACCAAAAC |
| V-loop | KM34F | CCAGGTTTTGGTCCTGGTTACCGAGGTTCGAATCCTTGCACCCC |
| | KM34R | GGGGTGCAAGGATTCGAACCTCGGTAACCAGGACCAAAACCTGG |
| Chimera 3 | KM3F | CCGGAATTCTGGGGTGTCGCCAAGCGGTAAGGCACCACCCTTTGACGGTGGCATTCC |
| | KM3R | CGCGGATCCTGGCTGGGGTGCAAGGATTCGAACCTCGGAATGCCACCGTCAAAG |
| Chimera 4 | KM4F | CCGGAATTCTGGGGTGTCGCCAAGCGGTAAGGCACCAGGTTTTGGTCCTGGTTAC |
| | KM4R | CGCGGATCCTGGCTGGGGTGAGGGGATTTGAACCCCTGTAACCAGGACCAAAACC |
| D-stem | KM18F | GGAAACAGAATTCTGGGGTGTCATCCAAGCGGTAAGG |
| | KM18R | CCTTACCGCTTGGATGACACCCCAGAATTCTGTTTCC |
| | KM19F | CCAAGCGGTAAGGATACCAGGTTTTGGTCCTGGC |
| | KM19R | GCCAGGACCAAAACCTGGTATCCTTACCGCTTGG |
| | KM20F | GAATTCTGGGGTGTCATCTAAGCGGTAAGGATACCAGG |
| | KM20R | CCTGGTATCCTTACCGCTTAGATGACACCCCAGAATTC |
| D-loop | KM21F | GAATTCTGGGGTGTCGCCAGTGGTTAGGCACCAGGTTTTGGTCCTGGC |
| | KM21R | GCCAGGACCAAAACCTGGTGCCTAACCACTGGCGACACCCCAGAATTC |
| G36C | KM17F | GTAAGGCACCAGGTTTTCGTCCTGGCATTCCGAGG |
| | KM17R | CCTCGGAATGCCAGGACGAAAACCTGGTGCCTTAC |
| G37A | KM43F | GTAAGGCACCAGGTTTTGATCCTGGCATTCCGAGG |
| | KM43R | CCTCGGAATGCCAGGATCAAAACCTGGTGCCTTAC |
| U38C | KM44F | GTAAGGCACCAGGTTTTGGCCCTGGCATTCCGAGG |
| | KM44R | CCTCGGAATGCCAGGGCCAAAACCTGGTGCCTTAC |
| U1:A72--G1:C72 | KM36F1 | GGAAACAGAATTCGGGGGTGTCGCCAAGCGGTAAGG |
| | KM36R1 | CCTTACCGCTTGGCGACACCCCCGAATTCTGTTTCC |
| | KM36F2 | GTTCGAATCCTTGCACCCCCGCCAGGATCCGTC |
| | KM36R2 | GACGGATCCTGGCGGGGGTGCAAGGATTCGAAC |
| G2:C71--G2:U71 | KM37F1 | GTTCGAATCCTTGCACCCTAGCCAGGATCCGTC |
| | KM37R1 | GACGGATCCTGGCTAGGGTGCAAGGATTCGAAC |
| G3:C70--C3:G70 | KM38F1 | GGAAACAGAATTCTGCGGTGTCGCCAAGCGGTAAGG |

| | | |
|---|---|---|
| | KM38R1 | CCTTACCGCTTGGCGACACCGCAGAATTCTGTTTCC |
| | KM38F2 | GTTCGAATCCTTGCACCGCAGCCAGGATCCGTC |
| | KM38R2 | GACGGATCCTGGCTGCGGTGCAAGGATTCGAAC |
| G4:C69--U4:A69 | KM39F1 | GGAAACAGAATTCTGGTGTGTCGCCAAGCGGTAAGG |
| | KM39R1 | CCTTACCGCTTGGCGACACACCAGAATTCTGTTTCC |
| | KM39F2 | GTTCGAATCCTTGCACACCAGCCAGGATCCGTC |
| | KM39R2 | GACGGATCCTGGCTGGTGTGCAAGGATTCGAAC |
| G5:C68--C5:G68 | KM40F1 | GGAAACAGAATTCTGGGCTGTCGCCAAGCGGTAAGG |
| | KM40R1 | CCTTACCGCTTGGCGACAGCCCAGAATTCTGTTTCC |
| | KM40F2 | GTTCGAATCCTTGCAGCCCAGCCAGGATCCGTC |
| | KM40R2 | GACGGATCCTGGCTGGGCTGCAAGGATTCGAAC |
| U6:A67--C6:G67 | KM41F1 | GGAAACAGAATTCTGGGGCGTCGCCAAGCGGTAAGG |
| | KM41R1 | CCTTACCGCTTGGCGACGCCCCAGAATTCTGTTTCC |
| | KM41F2 | GTTCGAATCCTTGCGCCCCAGCCAGGATCCGTC |
| | KM41R2 | GACGGATCCTGGCTGGGGCGCAAGGATTCGAAC |
| G7:C66--U7:A66 | KM42F1 | GGAAACAGAATTCTGGGGTTTCGCCAAGCGGTAAGG |
| | KM42R1 | CCTTACCGCTTGGCGAAACCCCAGAATTCTGTTTCC |
| | KM42F2 | GTTCGAATCCTTGAACCCCAGCCAGGATCCGTC |
| | KM42R2 | GACGGATCCTGGCTGGGGTTCAAGGATTCGAAC |
| G73A | KM43F1 | GAACCGCACCCCAACCAGGATCCGTCGACCTGC |
| | KM43F2 | GCAGGTCGACGGATCCTGGTTGGGGTGCGGTTC |

**Table S2 – Final Sequences of all tRNA Chimeras and tRNA Mutants.** Mutations are shown in red. Native $tRNA^{Gln}$ is shown in black.

| | |
|---|---|
| Chimera 1 | GGCUCCUUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGAGGAGUCACCA |
| Chimera 2 | UGGGGUGUCAUCUAGUGGUUAGGAUACCAGGUUUUGGUCCUGGUUACCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| Chimera 3 | UGGGGUGUCGCCAAGCGGUAAGGCACCACCCUUUGACGGUGGCAUUCCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| Chimera 4 | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGUUACAGGGGUUCAAAUCCCCUCACCCCAGCCA |
| D-stem | UGGGGUGUCAUCUAAGCGGUAAGGAUACCAGGUUUUGGUCCUGGUUACCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| D-loop | UGGGGUGUCGCCAGUGGUUAGGCACCAGGUUUUGGUCCUGGUUACCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| G36C | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUCGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| G37A | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGAUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| U38C | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGCCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCCCAGCCA |
| U1:A72→G1:C72 | GGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCCCCGCCA |
| G2:C71→G2:U71 | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCCUAGCCA |
| G3:C70→C3:G70 | UGCGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACCGCAGCCA |
| G4:C69→U4:A69 | UGGUGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCACACCAGCCA |
| G5:C68→C5:G68 | UGGGCUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCAGCCCAGCCA |
| U6:A67→C6:G67 | UGGGGCGUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGCGCCCCAGCCA |
| G7:C66→U7:A66 | UGGGGUUUCGCCAAGCGGUAAGGCACCAGGUUUUGGUCCUGGCAUUCCGAGGUUCGAAUCCUUGAACCCCAGCCA |
| G73A | UGGGGUGUCGCCAAGCGGUAAGGCACCAGGUUUUGGTCCTGGCAUUCCGAGGUUCGAAUCCUUGCACCCCAACCA |

**Table S3 – Quantification of tRNA expression levels using GluRS1 and GluRS2.** The values shown are the average of triplicate aminoacylation assays. Error analyses represent the standard error. The values in red were used to establish the concentrations of each tRNA. See accompanying manuscript for details. These tRNA mutants were not quantifiable using either GluRS1 and GluRS2; see accompanying manuscript for details.

| **Mutant tRNAs** | **GluRS1 (pmoles/$OD_{260}$)** | **GluRS2 (pmoles/$OD_{260}$)** |
|---|---|---|
| H. pylori $tRNA^{Glu1}$ | 280 ± 50 | 70 ± 20 |
| H. pylori $tRNA^{Glu2}$ | 730 ± 150 | 20 ± 10 |
| H. pylori $tRNA^{Gln}$ | 50 ± 10 | 250 ± 40 |
| Chimera 1 | 210 ± 20 | 50 ± 20 |
| Chimera 2 | 670 ± 140 | 620 ± 110 |
| Chimera 3 | 80 ± 20 | 330 ± 10 |
| Chimera 4 | 80 ± 10 | 300 ± 10 |
| D-stem | 260 ± 40 | 170 ± 60 |
| D-loop | 130 ± 30 | 160 ± 10 |
| G36C | 100 ± 50 | 290 ± 100 |
| G37A | 40 ± 10 | 250 ± 10 |
| U38C | 30 ± 5 | 310 ± 30 |
| G1:C72 | 20 ± 10 | 120 ± 10 |
| G2:U71* | 40 ± 20 | 40 ± 5 |
| C3:G70 | 40 ± 10 | 130 ± 10 |
| U4:A69* | 40 ± 20 | 40 ± 5 |
| C5:G68 | 40 ± 20 | 100 ± 30 |
| C6:G67 | 60 ± 10 | 170 ± 40 |
| U7:A66 | 60 ± 5 | 110 ± 10 |
| G73A | 50 ± 10 | 550 ± 70 |

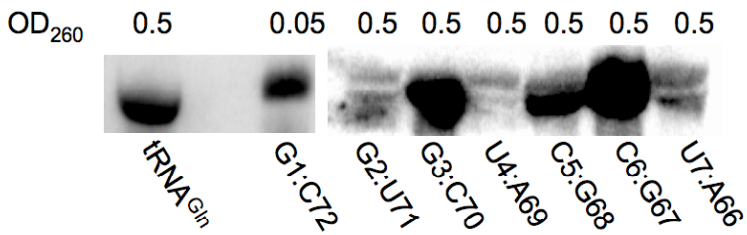


**Figure S1 – Northern blot of the acceptors stem mutants.** Each tRNA mutant has been blotted with one specific $^{32}$P-labelled oligonucleotide. The amount of the each tRNA used in this experiment was measured by $OD_{260}$. Each lane contains 0.5 or 0.05 $OD_{260}$ units of the corresponding tRNA. See Figure 6 and accompanying text for details.