Supplementary material for:

Identification and characterization of genes required for 5-hydroxyuridine synthesis in *Bacillus subtilis* and *Escherichia coli* tRNA

Author:

Charles T. Lauhon[1,*]

Affiliation:

[1] Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI, 53705, USA

* To whom correspondence should be addressed. Tel: 608-262-3083; Email: clauhon@wisc.edu

Table S1. List of primers used in this study. All sequences are written from 5' to 3' end.

YegQpBAD33.N	GCCGGTACCATGTTTAAACCGGAACTCCTT
YegQpBAD33.C	GCCAAGCTTTCACTTACCGTGGGGATTACG
HisYegQ.N	CATCATCACCACCATCACTTTAAACCGGAACTCCTTTCC
HisYegQ.C	GTGGCGGCCGCTCTATTACTTACCGTGGGGATTACGCGT
YhbUpBAD33.N	GCCGGTACCATGGAGCTGCTCTGCCCTGCC
YhbUpBAD33.C	GCCAAGCTTTCACTGCCATTTACGGTGATA
HisYhbU.N	CATCATCACCACCATCACGAGCTGCTCTGCCCTGCCGGA
HisYhbU.C	GTGGCGGCCGCTCTATTACTGCCATTTACGGTGATATGC
HisYhbV.N	CATCATCACCACCATCACAAATATTCCTTAGGGCCAGTG
HisYhbV.C	GTGGCGGCCGCTCTATTAGGCTTGCAGCTCCAGTCCTGC
HisRlhA.N	CATCATCACCACCATCACACCGTATCTTCTCATCGACTT
HisRlhA.C	GTGGCGGCCGCTCTATTACCCTTTTCGCTTCGGCAATGT
pKD3*cat*.N	GTGTAGGCTGGAGCTGCTTC

pKD3*cat*.C	ATGGGAATTAGCCATGGTCC
pKD3*cat*primer2.C	CATATGAATATCCTCCTTAG
Delta*yegQcat*40.N	AGGTGAAGCGGATCTGACCTGTCATCAGAACGAGAGAATTGTGT AGGCTGGAGCTGCTTC
Delta*yegQcat*40.C	TTCTAAGAATTTTCCATCCGGGAAAAATAATCGAAATTAAATGGGAATT AGCCATGGTCC
Delta*yhbUcat*.N	ACATTTTTGCGTTTTGATAGCGCAACCTTCAGGAAAAATTGTGTAGGCTG GAGCTGCTTC
Delta*yhbUcat*.C	TACCACAGCACTGGCCCTAAGGAATATTTCATTGCTTTTCATGGGAATTA GCCATGGTCC
Delta*yhbVcat*.N	CTCTTGGCGCATATCACCGTAAATGGCAGTGAGAAAAGCAGTGTAGG CTGGAGCTGCTTC
Delta*yhbVcat*.C	TAAAGAGTAGTTAAAGTTGTTAACAAAGTGAGCTATTTAC ATGGGAATTAGCCATGGTCC
Delta*rlhAkan*.N	GGCTAAAATAGCCGCCATTTTTCAGCTACTGGATAAGAATGTGTAGGCT GGAGCTGCTTC
Delta*rlhAkan*.C	GCAGATTCAATAAGTTGTGAGTAACCAGAAAACTGGCGTTATGGGAA TTAGCCATGGTCC
*yegQ*up100	ATTGCCTCCGTTAAAGCCAATGG
*yegQ*down100	AAATCCTTTTATTTCATTGTATTACG
*yhbU*up77	TTGTCGCAGCAAGGTTAACT
*yhbU*down89	GCTGGTGGCGGCCTGCTGAT
*yhbV*up100	TTGTACCGCAAAGCGCGTGG
*yhbV*down100	GCAATGGTTTTATCAGTCAT
*rlhA*up100	TTCCCTCATCCATTACCCGC
*rlhA*down100	CAACGTTGAGTCATTTGATG
C1	TTATACGCAAGGCGACAAGG
C2	GATCTTCCGTCACAGGTAGG
C4	CGCCACATCTTGCGAATATATG
K1	CAGTCATAGCCGAATAGCCT
K2	CGGTGCCCTGAATGAACTGC

*yhbU*internal.N	TGCCGGAAATCTCCCGGCGC
*yhbU*internal.C	CGGTGATATGCGCCAAGAGT
*yhbV*internal.N	GGGCCAGTGCTGTGGTACTG
*yhbV*internal.C	AGCTCCAGTCCTGCCAGCCG
*yfhL*pBAD33.N	GCCGGTACCATGGCGTTGTTAATCACTAAAAAATGC
*yfhL*pBAD33.C	GCCAAGCTTTTAAATTTTATCCGCGTGGTGCAT
HisYfhL.N	CATCATCACCACCATCACGCGTTGTTAATCACTAAAAAATGC
HisYfhL.C	GTGGCGGCCGCTCTATTAAATTTTATCCGCGTGGTGCAT
HisYrrM.N	CATCATCACCACCATCACACTGACCGGTATGAACAAATA
HisYrrM.C	GTGGCGGCCGCTCTATTACCTCTTCTTTTTACTAATCGC
HisYrrN.N	CATCATCACCACCATCACAAAAAACCAGAGCTCTTAGTGACG
HisYrrN.C	GTGGCGGCCGCTCTATTAATAAACCGTTTCCTTGAAGAAGAA
HisYrrO.N	CATCATCACCACCATCACACTGCCGTAAATGATAAAATATCC
HisYrrO.C	GTGGCGGCCGCTCTATTACTTCCCCTTTCTCATCATGTTGCT
*yrrM*upstream	AAAATATATCGGGTTGTTTACCGA
*yrrM*downstream	TGTTCCCCAACTAAAAACGCAGTT
*yrrN*upstream	ATGAATATAATCATTGGCTGATGA
*yrrN*downstream	CAGGTGCGAGAAGCTCCGGCT
*yrrO*upstream	CTGGATCGAACGCATTGAAAG
*yrrO*downstream	GTCCTTTAAACTGTTCATAAATGG
erm internal	GTTGATCACGATAATTTCCAAGTT
YegQpLIKE.N	GCCTCTAGATGTTTAAACCGGAACTCCTTTCC
YegQpLIKE.C	GCCAAGCTTTCACTTACCGTGGGGATTACG
YrrNpLIKE.N	GCCTCTAGATGAAAAAACCAGAGCTCTTAGTG
YrrNpLIKE.C	GCCCTGCAGTTAATAAACCGTTTCCTTGAA
YrrOpLIKE.N	GCCTCTAGATGACTGCCGTAAATGATAAAATATCC
YrrOpLIKE.C	GCCAAGCTTTTACTTCCCCTTTCTCATCAT
pLIKErepseq.fwd	GATTCGTTTTGCATATCTTCC
pLIKErepseq.rev	GGAAAGCGGGCAGTGAGCGCA
BSPROBIOTIN	5’-TCCCAAACCATGTGCTCTACCAAGCT-BIOTIN

BSALABIOTIN	5'-GTGCAAA GCAGG C GCTC TCCCAGCT-BIOTIN
BSTHRBIOTIN	5'-TTACAAG TCAGT T GCTC TACCAATT-BIOTIN
BSVALBIOTIN	5'-TTGTAAG GCAGA T GCTC TCCCAGCT-BIOTIN
BSARGBIOTIN	5'-TTAGAAG GCCGTTGCTC TATCCAGCT-BIOTIN
BSSERBIOTIN	5'-TTTCAAG ACCGA T CCCT TCAGCCAGACT-BIOTIN

Supplementary Figure Legends

FIGURE S1. PCR confirmation of *E. coli yegQ*<frt>, *yhbUV*::*cat*, *rlhA*::*kan* quadruple mutant construction. **A**: PCR analysis showing gene deletion or replacements (expected DNA size shown below lane). Lane M, MW markers; lane 1, mutant *yegQ* region with primers *yegQ*up100 + *yegQ*down100; lane 2, wt *yegQ* region with *yegQ*up100 + *yegQ*down100; lane 3, mutant *yhbUV* region with primers *yhbU*up77 + *yhbV*down100; lane 4 wt *yhbUV* region with *yhbU*up77 + *yhbV*down100; lane 5, mutant *rlhA* region with primers *rlhA*up100 + *rlhA*down100; lane 6, wt *rlhA* region with primers *rlhA*up100 + rlhAdown100. **B**: PCR analysis of mutant showing accurate replacement of *yhbUV* with *cat* gene. Lane M, MW markers; lane 1, primer *yhbU*up77 + internal cat primer C1; lane 2, primers *yhbV*down100 + internal *cat* primer C2; lane 3, primers *yhbU*up77 + *yhbV*down100; lane 4, wt strain with primers *yhbU*up77 + *yhbV*down100. **C:** PCR analysis of mutants with internal primers showing absence of peptidase U32 genes in genomic DNA. Lane M, MW markers; lane 1, mutant strain with HisYhbU primers; lane 2, wt with HIsYhbU primers; lane 3, mutant with HisYhbV primers; lane 4, wt with HIsYhbV primers; lane 5, mutant with HisRlhA primers; lane 6, wt with HisRlhA primers; lane 7 mutant with HisYegQ primers (wt with HisYegQ primers not shown).

FIGURE S2. Denaturing PAGE analysis of individual tRNAs purified from *B. subtilis yrrO* deletion strain BKK27340 (BGSC). Purified unmodified transcripts prepared using T7 RNA polymerase are shown for reference. Lane 1, AX-500 purified total tRNA; lane 2, purified

$tRNA^{Ala}$(5GC); lane 3, $tRNA^{Ala}$(5GC) T7 transcript; lane 4, $tRNA^{Val}$(5AC); lane 5, $tRNA^{Val}$(5AC) T7 transcript; lane 6, purified $tRNA^{Thr}$(5GU); lane 7, $tRNA^{Thr}$(5GU) T7 transcript; lane 8, purified $tRNA^{Pro}$(5GG). (5 = mo^5U)

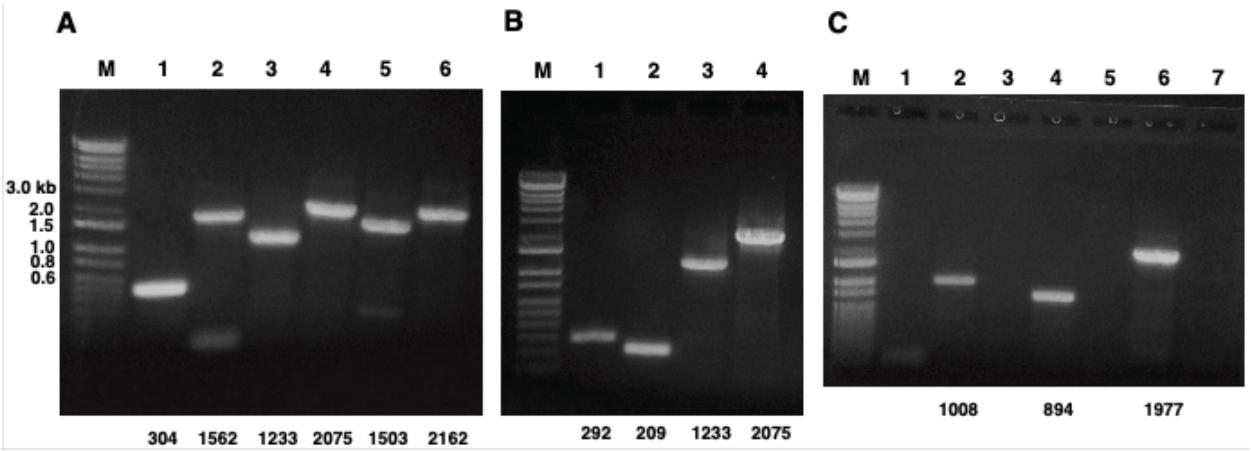
A
M 1 2 3 4 5 6
3.0 kb
2.0
1.5
1.0
0.8
0.6
304 1562 1233 2075 1503 2162
B
M 1 2 3 4
292 209 1233 2075
C
M 1 2 3 4 5 6 7
1008 894 1977

Figure S1

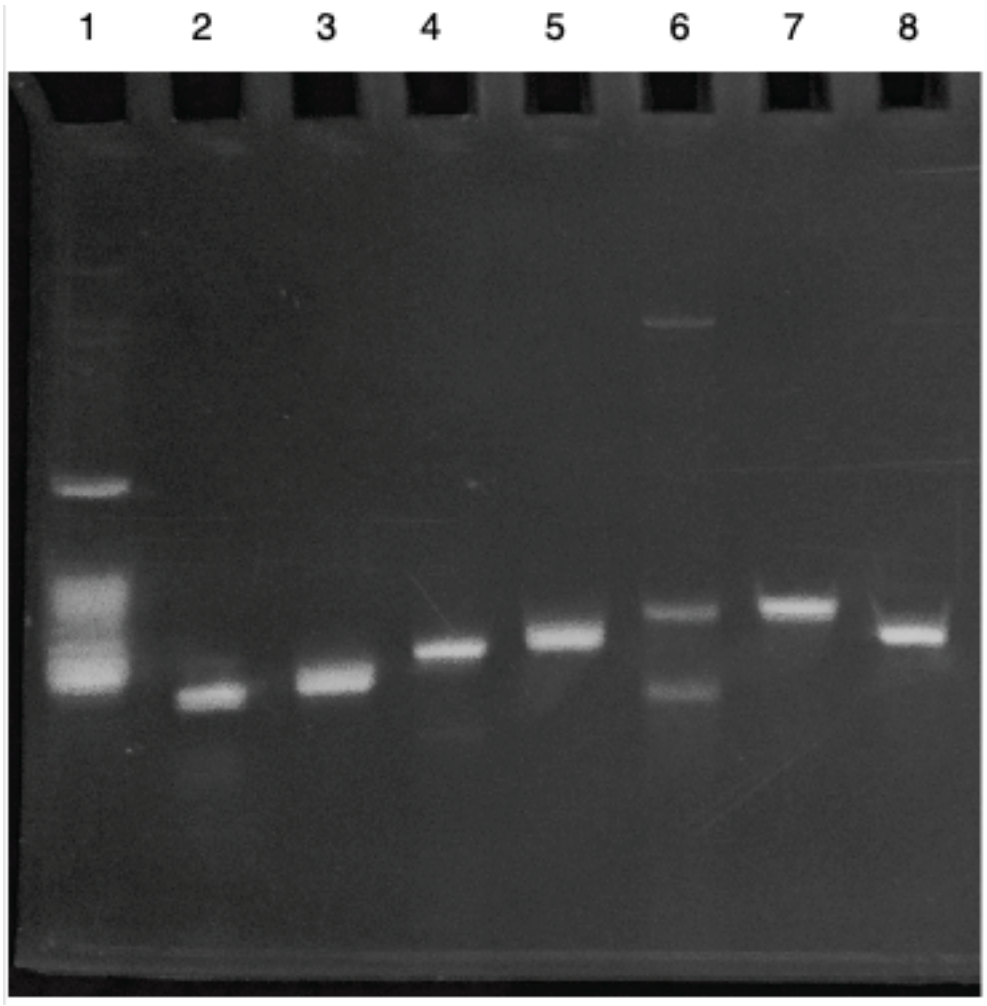

Figure S2