**SUPPLEMENTARY INFORMATION**

## Methods

### Construction of overlapping tRNA genes *in E. coli*

Constructs consisting of tRNA genes and/or HDV ribozyme sequence in overlapping and non-overlapping arrangement were amplified by overlap extension PCR (Schürer et al., 2002). Anticodons: bold, underlined characters; downstream tRNA and HDV ribozyme: italics. For diagnostic purposes, the first (overlapping) base (C) of $tRNA^{Cys}$ was replaced by G (underlined) to distinguish partial CCA addition from 3'-end processing downstream of the discriminator position of $tRNA^{Tyr}$ (see Fig. 1 and 2). Accordingly, position 72 of $tRNA^{Tyr}$ was replaced by C.

sup-$tRNA^{Tyr}$+A/T5 $tRNA^{Cys}$:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACCA*G*GACCGTTGGCTGAATGGCTTAGGC

*GAAGGATT**GCA**AATCCTTTTTATGTGAGTTCAAATCTCATGCGGTC*C*TCCA*

sup-$tRNA^{Tyr}$-1/T5 $tRNA^{Cys}$:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACC*G*GACCGTTGGCTGAATGGCTTAGGCG

*AAGGATT**GCA**AATCCTTTTTATGTGAGTTCAAATCTCATGCGGTC*C*TCCA*

sup-$tRNA^{Tyr}$+A/HDV:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACCA*GGGUCGGCAUGGCAUCUCCACCUC*

*CUCGCGGUCCGACCUGGGCUACUUCGGUAGGCUAAGGGAGAAGCTTGGCACTGGC*
*CGTCGTTT*

sup-tRNA$^{Tyr}$-1/HDV:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC
TTCGAAGGTTCGAATCCTTCCCCCACCACC*GGGUCGGCAUGGCAUCUCCACCUCC*
*UCGCGGUCCGACCUGGGCUACUUCGGUAGGCUAAGGGAGAAGCTTGGCACTGGCC*
*GTCGTTT*

PCR products were inserted into plasmid pBAD30 (atcc, USA) downstream of an arabinose promoter and verified by sequencing. Correct plasmids were introduced into *E. coli* CA244 (*E. coli* Stock Center, Yale, USA), and cells were grown on LB agar plates containing 30 µg/ml ampicillin and 0.2% arabinose at 37°C.

**Construction of overlapping tRNA genes in *S. cerevisiae***

Gene constructs for *S. cerevisiae* were generated by the same procedure as for the *E. coli* constructs. Anticodons: bold, underlined characters; downstream tRNA and HDV ribozyme: italics.

sup-tRNA$^{Tyr}$+A/*E. coli*-tRNA$^{Cys}$:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC
TTCGAAGGTTCGAATCCTTCCCCCACCACCA*GGCGCGTTAACAAAGCGGTTATGTAG*
*CGGATT**GCA**AATCCGTCTAGTCCGGTTCGACTCCGGAACGCGCCTCCA*

sup-tRNA$^{Tyr}$-1/*E. coli*-tRNA$^{Cys}$:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACC*GGCGCGTTAACAAAGCGGTTATGTAGC*

*GGATT**GCA**AATCCGTCTAGTCCGGTTCGACTCCGGAACGCGCCTCCA*

sup-tRNA$^{Tyr}$+A/HDV:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACCA*GGGUCGGCAUGGCAUCUCCACCUC*

*CUCGCGGUCCGACCUGGGCUACUUCGGUAGGCUAAGGGAGAAGCTTGGCACTGGC*

*CGTCGTTT*

sup-tRNA$^{Tyr}$-1/HDV:

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACT**CTA**AATCTGCCGTCATCGAC

TTCGAAGGTTCGAATCCTTCCCCCACCACC*GGGUCGGCAUGGCAUCUCCACCUCC*

*UCGCGGUCCGACCUGGGCUACUUCGGUAGGCUAAGGGAGAAGCTTGGCACTGGCC*

*GTCGTTT*

Products were inserted downstream of the galactose promoter of plasmid Y352_gal1, analyzed by sequencing and introduced into *S. cerevisiae* BY 4741 (Gietz and Woods, 2002). Expression was induced during growth for 48 h at 30°C in uracil-dropout medium containing 2% galactose.

## REFERENCES


Gietz RD, Woods RA (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Methods Enzymol.* **350:** 87-96

Schürer H, Lang K, Schuster J, Mörl M (2002) A universal method to produce in vitro transcripts with homogeneous 3' ends. *Nucleic Acids Res.* **30:** e56