Supplementary Table 2. Ln fitness relative to *S. cerevisiae* of tRNA sequences from the six extant species.

S. cerervisiae tRNA has a ln fitness of 0. One sample t-test performed to assess differences in fitness relative to *S. cerevisiae*. FDR considering all variants in the library (n=4,176 tests).

Species	*ln(fitness)*	*q-value*	*Nt distance to S. cerevisiae*
K. africana	*0.011*	*0.018*	*1*
C. glabrata	*-0.006*	*0.152*	*2*
N. dairenensis	*0.001*	*0.921*	*2*
S. castellii	*-0.023*	*0.202*	*3*
K. naganishii	*-0.024*	*0.043*	*4*
T. blattae	*-0.010*	*0.087*	*5*

Supplementary Table 3. Primers used in this study

Illumina indexes of adapters written in lower case.

Primer name	*Strand*	*Sequence (5' – 3')*	*Usage*
JD007	*Fwd*	*CGCGGATCCCGAGGAGAACT TCTAGTATATCTACATACC*	*Cloning genomic sequence of HSX1 into pRS413 to get pJD001*
JD008	*Rev*	*GCTCTAGATTATATTTACCA TCAACTCCGCGAC*	*Cloning genomic sequence of HSX1 into pRS413 to get pJD001*
JD027	*Fwd*	*TCTTAGAGTTCAACCAAGTT G*	*Amplify synthetized oligonucleotide*
JD028	*Rev*	*ACGAAAAAAAAAAAATAAT CAA*	*Amplify synthetized oligonucleotide*
JD029	*Rev*	*CAACTTGGTTGAACTCTAAG A*	*Linearizing by PCR pRS413 or pJD001*
JD030	*Fwd*	*TTGATTATTTTTTTTTTTTC GT*	*Linearizing by PCR pRS413 or pJD001*
JD116		*GCCTACATACCTCGCTCTGC*	*Plasmid library quantification by real-time quantitative PCR*
JD117		*CAACCCGGTAAGACACGACT*	*Plasmid library quantification by real-time quantitative PCR*

JD126	*Fwd*	*ACACTCTTTCCCTACACGAC GCTCTTCCGATCTTCTTAGA GTTCAACCAAGTTG*	*Amplify tRNA library adding part of Illumina adapter*
JD127	*Rev*	*GTGACTGGAGTTCAGACGT GTGCTCTTCCGATCTACGAA AAAAAAAAAATAATCAA*	*Amplify tRNA library adding part of Illumina adapter*
TS-HT-D7x-51-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT gacggatt GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-55-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT gcaactaa GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-57-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT gcatcgac GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-61-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT ggcgttgc GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-63-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT ggtagttg GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-65-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT gtaattat GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-70-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT gttcaggc GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
TS-HT-D7x-73-r	*Rev*	*CAA GCA GAA GAC GGC ATA CGA GAT taagcctg GTG ACT GGA GTT CAG ACG TGT GCT CTT C*	*Add index to Illumina adapter*
Illumina adapter 1.0 f	*Fwd*	*AATGATACGGCGACCACCGA GATCTACACTCTTTCCCTAC ACGACGCTCTTCCGATCT*	*Add rest of adapter to Illumina forward universal*