Species names acronyms used in this file: Scer – *Saccharomyces cerevisiae*, Hvol – *Haloferax volcanii*, Ecol – *Escherichia coli*, Bsub – *Bacillus subtilis*, Mcap – *Mycoplasma capricolum*, Llac – *Lactococcus lactis.*

Table S 1 Sanity-check predictions results using only predicted enzymes data

	TP	**FP**	**FN**	**Precision**	**Recall**	**F-measure**
Bsub	131	283	19	0.316	0.873	0.465
Ecol	374	690	18	0.352	0.954	0.514
Hvol	379	503	59	0.430	0.865	0.574
Mcap	171	235	0	0.421	1.000	0.593
Scer	588	774	19	0.432	0.969	0.597
			Avg	**0.390**	**0.932**	**0.548**

Table S 2 Sanity-check predictions results with phylogeny filter

	TP	**FP**	**FN**	**Precision**	**Recall**	**F-measure**
Bsub	131	187	19	0.412	0.873	0.560
Ecol	374	632	18	0.372	0.954	0.535
Hvol	379	289	59	0.567	0.865	0.685
Mcap	171	155	0	0.525	1.000	0.688
Scer	588	685	19	0.462	0.969	0.626
			Avg	**0.468**	**0.932**	**0.619**

Table S 3 Cross-validation predictions results using only predicted enzymes data

	TP	**FP**	**FN**	**Precision**	**Recall**	**F-measure**
Bsub	128	355	22	0.265	0.853	0.404
Ecol	209	339	156	0.381	0.573	0.458
Hvol	354	488	84	0.420	0.808	0.553
Llac	148	423	19	0.259	0.886	0.401
Mcap	171	335	0	0.338	1.000	0.505
Scer	497	505	110	0.496	0.819	0.618
			Avg	**0.360**	**0.823**	**0.490**

Table S 4 Cross-validation predictions results with phylogeny filter

	TP	**FP**	**FN**	**Precision**	**Recall**	**F-measure**
Bsub	128	214	22	0.374	0.853	0.520
Ecol	209	182	154	0.535	0.576	0.554
Hvol	354	274	84	0.564	0.808	0.664
Llac	145	276	22	0.344	0.868	0.493
Mcap	171	192	0	0.471	1.000	0.640
Scer	497	398	110	0.555	0.819	0.662
			Avg	**0.474**	**0.821**	**0.589**

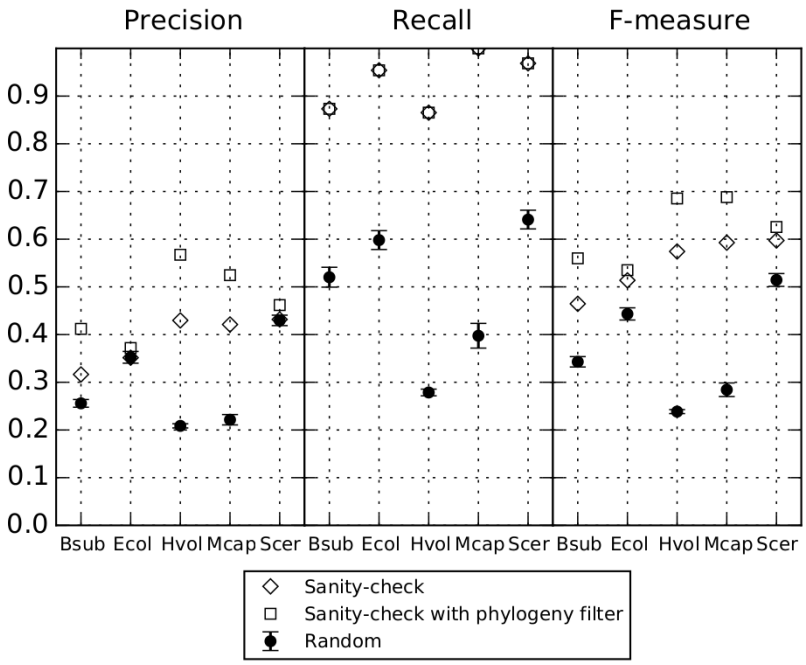

Figure S 1 Comparison of the tRNAmodpred performance to results obtained by random assignment of modified residues to tRNA sequences from the target species. Scores for tRNAmodpred predictions done in the sanity-check setup are shown. Error bars for random probes depict scores variation between five random modifications sets.

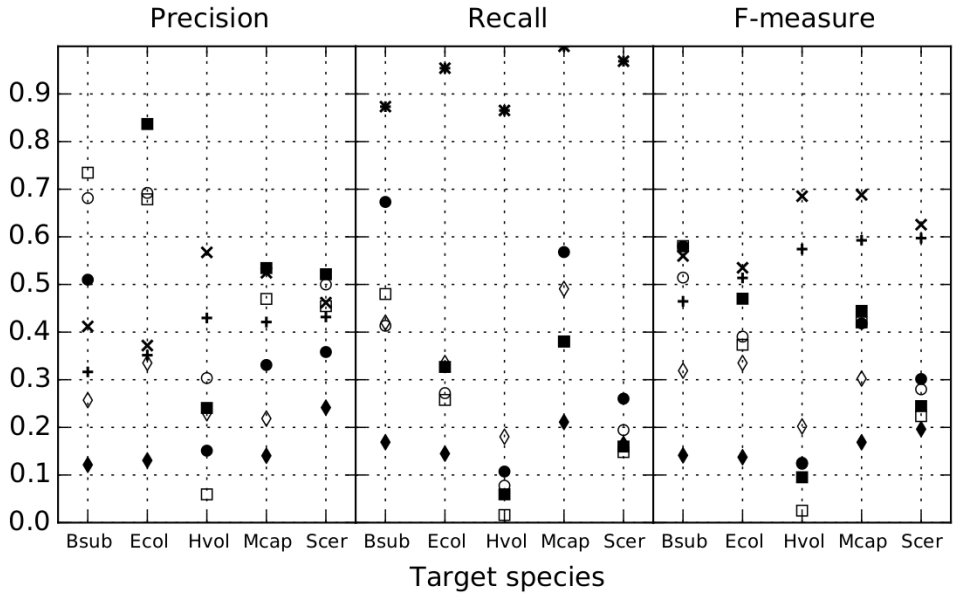

Figure S 2 Comparison of precision, recall and F-measure calculated for tRNAmodpred predictions and results of mapping modifications from different species, for all tRNAs from the target species. Black and white points with different shapes represent different species used as source of modifications: ■ – *Bacillus subtilis*, ● – *Escherichia coli*, ♦ – *Haloferax volcanii*, □ – *Lactococcus lactis*, ○ – *Mycoplasma capricolum*, ◊ – *Saccharomyces cerevisiae*; "x" and "+" – predictions done by tRNAmodpred in the sanity-check setup with phylogeny filter or without it, respectively.

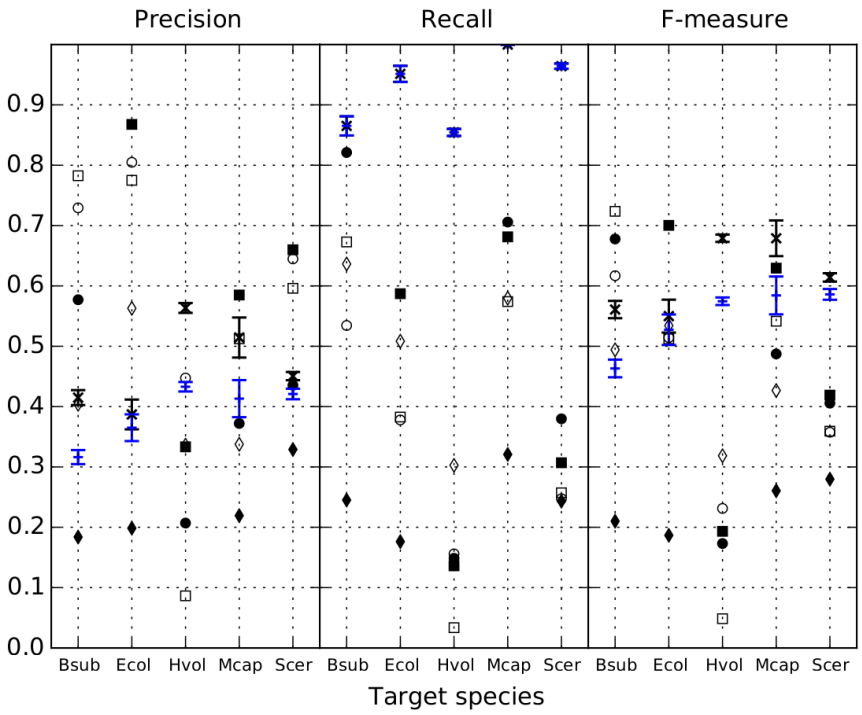

Figure S 3 Comparison of precision, recall and F-measure calculated for tRNAmodpred predictions and results of mapping modifications from different species, for those tRNAs from the target species for which an isoacceptor with the same anticodon sequence exists in the source species. Black and white points with different shapes represent different species used as source of modifications: ■ – *Bacillus subtilis*, ● – *Escherichia coli*, ♦ – *Haloferax volcanii*, □ – *Lactococcus lactis*, ○ – *Mycoplasma capricolum*, ◊ – *Saccharomyces cerevisiae*. Black "x" and blue "-" markers with error bars depict predictions done by tRNAmodpred in the sanity-check setup with phylogeny filter or without it, respectively. Error bars represent variation of scores obtained for different sets of tRNAs (depending on the source species).

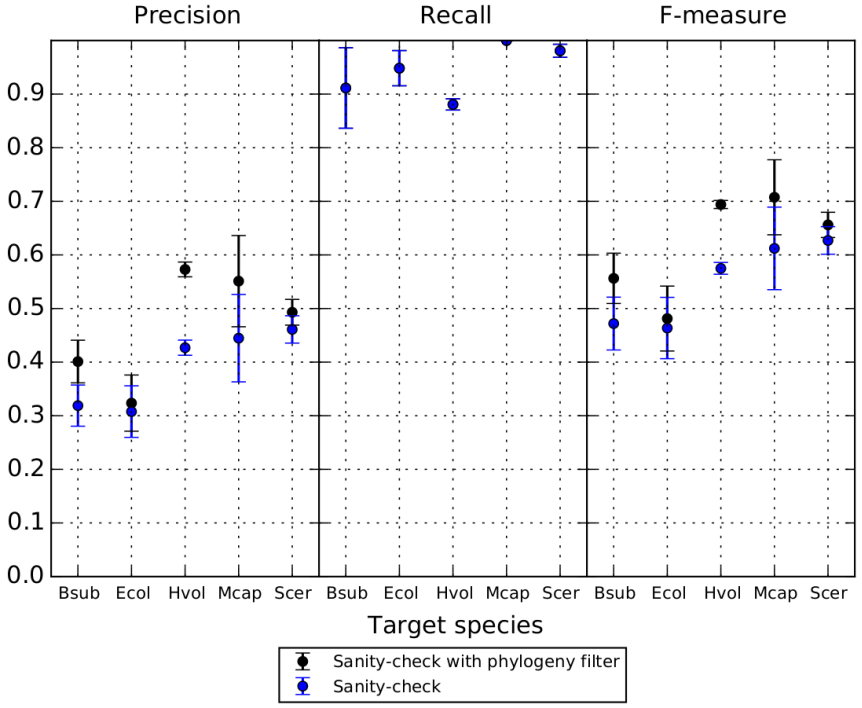

Figure S 4 Precision, recall and F-measure calculated for tRNAmodpred predictions for those tRNAs from the target species for which an isoacceptor with the same anticodon sequence does not exist in the source species. Error bars represent variation of scores obtained for different sets of tRNAs (depending on the source species).

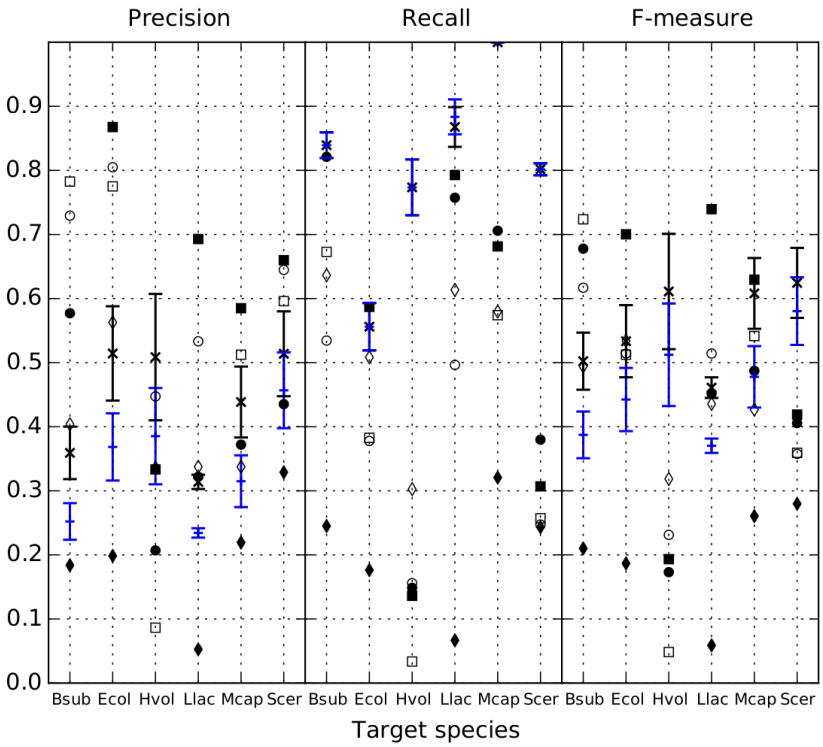

Figure S 5 Comparison of precision, recall and F-measure calculated for tRNAmodpred predictions and results of mapping modifications from different species, for those tRNAs from the target species for which an isoacceptor with the same anticodon sequence exists in the source species. Black and white points with different shapes represent different species used as source of modifications: ■ – *Bacillus subtilis*, ● – *Escherichia coli*, ♦ – *Haloferax volcanii*, □ – *Lactococcus lactis*, ○ – *Mycoplasma capricolum*, ◊ – *Saccharomyces cerevisiae*. Black "x" and blue "-" markers with error bars depict predictions done by tRNAmodpred in cross validation setup with phylogeny filter or without it, respectively. Error bars represent variation of scores obtained for different sets of tRNAs (depending on the source species).

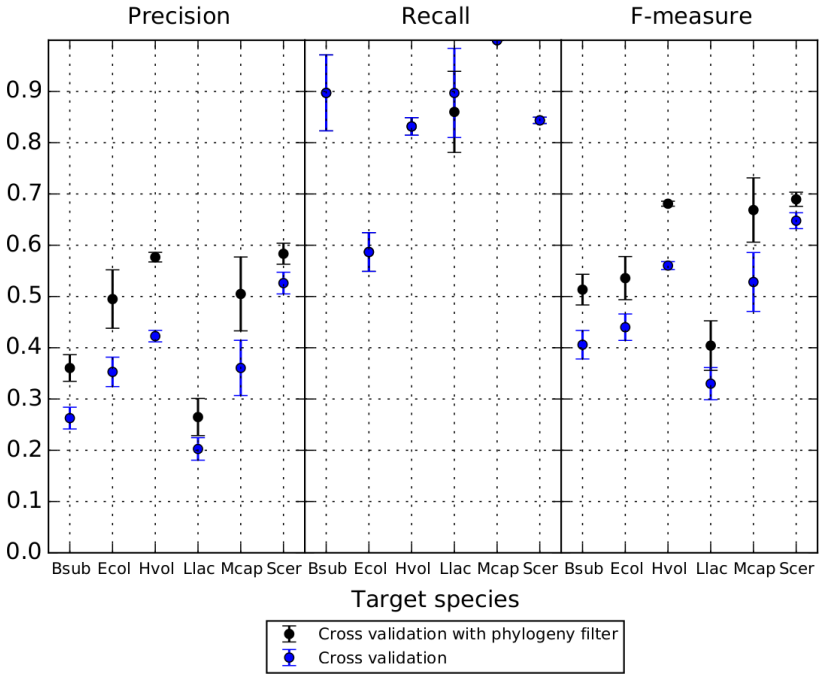

Figure S 6 Precision, recall and F-measure calculated for tRNAmodpred predictions for those tRNAs from the target species for which an isoacceptor with the same anticodon sequence does not exist in the source species. Error bars represent variation of scores obtained for different sets of tRNAs (depending on the source species).

Comparison of tRNAmodpred and tRNAmod

Results of checking whether combining tRNAmod and tRNAmodpred results improves the accuracy of predictions. tRNAmod was run with the common model. "Intersection" – from all U modifications reported by tRNAmodpred only those were kept, which were also predicted by tRNAmod. "Sum" – predictions of pseudouridine, dihydrouridine and 5-methyluridine which were present in results from tRNAmod but missing in the results from tRNAmodpred were added to the final results file.

Table S 5 Influence of incorporating tRNAmod predictions on the results of sanity-check predictions results using only predicted enzymes data in tRNAmodpred

	Intersection			**Sum**		
	Precision change	**Recall change**	**F change**	**Precision change**	**Recall change**	**F change**
Bsub	0.039	0.000	0.040	0.003	0.020	0.006
Ecol	0.019	-0.026	0.016	-0.002	0.003	-0.002
Hvol	0.036	-0.063	0.015	-0.004	0.007	-0.002
Mcap	0.080	-0.006	0.074	-0.003	0.000	-0.003
Scer	0.042	-0.082	0.020	-0.005	0.002	-0.004
Avg	**0.043**	**-0.035**	**0.033**	**-0.002**	**0.006**	**-0.001**

Table S 6 Influence of incorporating tRNAmod predictions on the results of sanity-check predictions results with phylogeny filter in tRNAmodpred

	Intersection			**Sum**		
	Precision change	**Recall change**	**F change**	**Precision change**	**Recall change**	**F change**
Bsub	0.043	0.000	0.038	-0.005	0.020	0.000
Ecol	0.018	-0.026	0.014	-0.003	0.003	-0.002
Hvol	0.011	-0.063	-0.013	-0.019	0.007	-0.012
Mcap	0.070	-0.006	0.056	-0.013	0.000	-0.011
Scer	0.043	-0.082	0.018	-0.005	0.002	-0.005
Avg	**0.037**	**-0.035**	**0.023**	**-0.009**	**0.006**	**-0.006**

Table S 7 Influence of incorporating tRNAmod predictions on the results of cross validation predictions results using only predicted enzymes data in tRNAmodpred

	Intersection			**Sum**		
	Precision change	**Recall change**	**F change**	**Precision change**	**Recall change**	**F change**
Bsub	0.027	0.000	0.031	0.003	0.020	0.006
Ecol	0.033	-0.005	0.021	0.024	0.121	0.054
Hvol	0.036	-0.067	0.012	-0.004	0.007	-0.002
Llac	0.004	-0.078	-0.003	-0.004	0.000	-0.005
Mcap	0.049	-0.006	0.052	-0.002	0.000	-0.002
Scer	0.039	-0.056	0.011	-0.001	0.036	0.009
Avg	**0.032**	**-0.035**	**0.021**	**0.003**	**0.031**	**0.010**

Table S 8 Influence of incorporating tRNAmod predictions on the results of cross validation predictions results with phylogeny filter in tRNAmodpred

	Intersection			**Sum**		
	Precision change	**Recall change**	**F change**	**Precision change**	**Recall change**	**F change**
Bsub	0.036	0.000	0.034	-0.003	0.020	0.001
Ecol	0.026	-0.009	0.010	0.007	0.117	0.054
Hvol	0.011	-0.067	-0.017	-0.020	0.007	-0.012
Llac	0.008	-0.072	-0.004	-0.016	0.006	-0.015
Mcap	0.055	-0.006	0.048	-0.010	0.000	-0.009
Scer	0.041	-0.056	0.008	-0.004	0.036	0.009
Avg	**0.030**	**-0.035**	**0.013**	**-0.008**	**0.031**	**0.004**

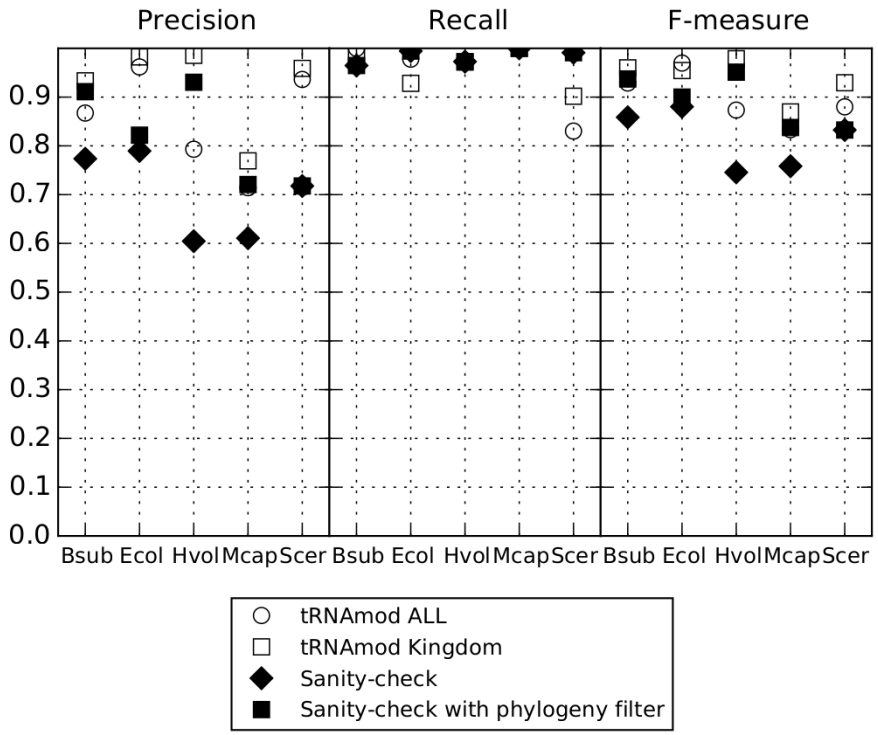

Figure S 7 Precision, recall and F-measure calculated for pseudouridine, dihydrouridine and 5-methyluridine predictions done by tRNAmod and tRNAmodpred. Sanity-check with phylogeny filter, sanity-check – predictions by tRNAmodpred in the sanity-check setup with and without phylogeny filter, tRNAmod ALL, tRNAmod Kingdom – predictions done by tRNAmod with common and kingdom-specific models.

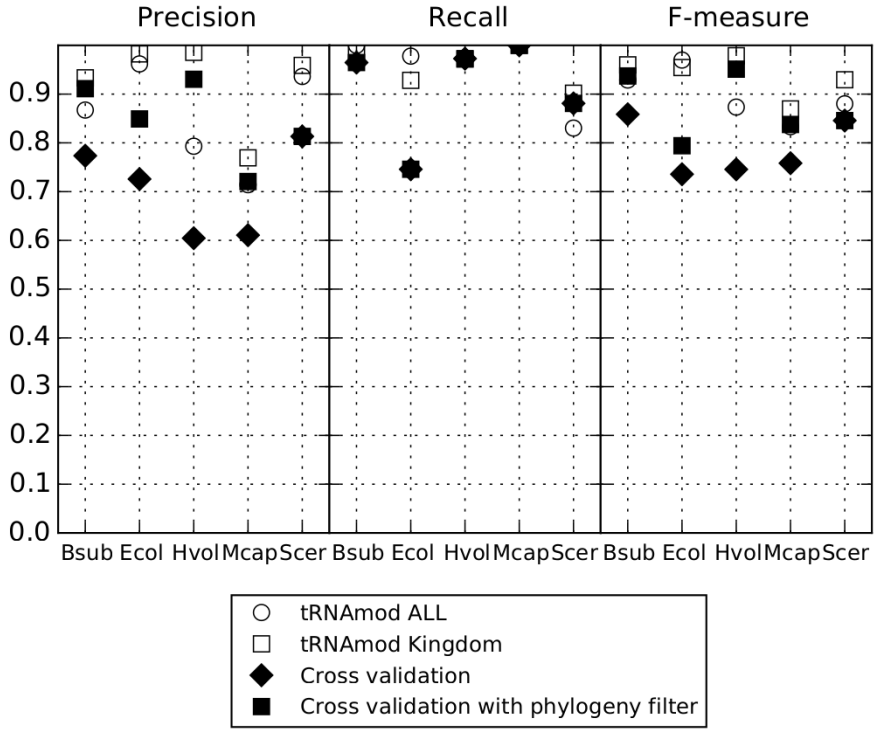

Figure S 8 Precision, recall and F-measure calculated for pseudouridine, dihydrouridine and 5-methyluridine predictions done by tRNAmod and tRNAmodpred. Cross validation with phylogeny filter, cross validation – predictions by tRNAmodpred in the cross validation setup with and without phylogeny filter, tRNAmod ALL, tRNAmod Kingdom – predictions done by tRNAmod with common and kingdom-specific models.

Table S 9 Detailed analysis of predictions for three chosen *L. lactis* tRNA sequences. Modifications expected based on *B. subtilis* and *E. coli* data represent all possible pathway intermediates of modifications which were experimentally identified.

tRNA	Anticodon	Position	Possible modifications expected based on experimental data	Possible modifications predicted by tRNAmodpred with phylogeny filter	Possible modifications expected based on B. subtilis data	Possible modifications expected based on E. coli data
Ala	UGC	8	s4U	s4U	None	None
Ala	UGC	17	D	D	D	D
Ala	UGC	34	ho5U, mo5U	s2U, Um, s2Um, nm5U, nm5s2U, cmnm5U, cmnm5s2U, cmnm5Um	ho5U, mo5U	ho5U, mo5U, cmo5U
Ala	UGC	37	t6A	i6A, m2A, m6A, t6A, ct6A	m6A	None
Ala	UGC	46	None	m7G	m7G	m7G
Ala	UGC	54	m5U	m5U	m5U	m5U
Leu 5	UAA	8	None	s4U	data unavailable	s4U
Leu 5	UAA	17	D	D	data unavailable	D
Leu 5	UAA	18	None	None	data unavailable	Gm
Leu 5	UAA	22	None	m1A	data unavailable	None
Leu 5	UAA	34	s2U, cmnm5U, cmnm5s2U	s2U, Um, s2Um, nm5U, nm5s2U, cmnm5U, cmnm5s2U, cmnm5Um	data unavailable	Um, cmnm5U, cmnm5Um
Leu 5	UAA	37	i6A	i6A, m2A, m6A, t6A, ct6A	data unavailable	i6A, ms2i6A
Leu 5	UAA	46	None	m7G	data unavailable	None
Leu 5	UAA	54	m5U	m5U	data unavailable	m5U
Phe	GAA	8	s4U	s4U	None	s4U
Phe	GAA	16	None	D	None	D
Phe	GAA	17	D	D	D	None
Phe	GAA	20	D	D	D	D
Phe	GAA	34	None	None	Gm	None
Phe	GAA	37	m1G	m1G	None	None
Phe	GAA	46	m7G	m7G	m7G	m7G
Phe	GAA	47	None	None	None	acp3U
Phe	GAA	54	m5U	m5U	m5U	m5U
Arg	ACG	8	None	s4U	None	s4U
Arg	ACG	17	D	D	None	D
Arg	ACG	20a	D	D	D	D
Arg	ACG	32	None	None	None	s2C
Arg	ACG	34	I	I	I	I
Arg	ACG	37	m2A	i6A, m2A, m6A, t6A, ct6A	None	m2A
Arg	ACG	46	m7G	m7G	m7G	m7G
Arg	ACG	47	None	None	None	acp3U
Arg	ACG	54	m5U	m5U	m5U	m5U

Lys 2	UUU	8	None	s4U	None	None
Lys 2	UUU	16	None	D	None	D
Lys 2	UUU	17	D	D	D	D
Lys 2	UUU	20	D	D	D	D
Lys 2	UUU	34	s2U, cmnm5U, cmnm5s2U	s2U, Um, s2Um, nm5U, nm5s2U, cmnm5U, cmnm5s2U, cmnm5Um	s2U, cmnm5U, cmnm5s2U	s2U, Um, s2Um, nm5U, nm5s2U, cmnm5U, cmnm5s2U, cmnm5Um, mnm5s2U
Lys 2	UUU	37	t6A	i6A, m2A, m6A, t6A, ct6A	t6A, ms2t6A	t6A
Lys 2	UUU	46	m7G	m7G	m7G	m7G
Lys 2	UUU	47	None	None	None	acp3U
Lys 2	UUU	54	m5U	m5U	m5U	m5U

Prediction of modifications in S. cerevisiae mitochondrial tRNAs

Predictions of modifications for yeast mitochondrial tRNAs were performed as follows:

1. A set of sequences of proteins reported to be present in mitochondria was collected based on the Supplementary Table 2 provided in: Wiederhold E, Veenhoff LM, Poolman B, Slotboom DJ. *Proteomics of Saccharomyces cerevisiae Organelles*. Mol Cell Proteomics. 2010 Mar;9(3):431-45.

2. tRNAmodpred was used to predict modifications in 17 *S. cerevisiae* mitochondrial tRNAs which have been sequenced and are available in the MODOMICS database. Predictions without any phylogenetic filter and with phylogenetic filter set to mitochondria were performed.

Table S 10 Scores obtained for the prediction of modifications in S. cerevisiae mitochondrial tRNAs.

Phylogenetic filter	**TP**	**FP**	**FN**	**Precision**	**Recall**	**F-measure**
None	61	108	61	0.36	0.5	0.42
Mitochondria	61	65	61	0.48	0.5	0.49

Example score calculations for hypothetical prediction of mnm^{5}s^{2}U modification

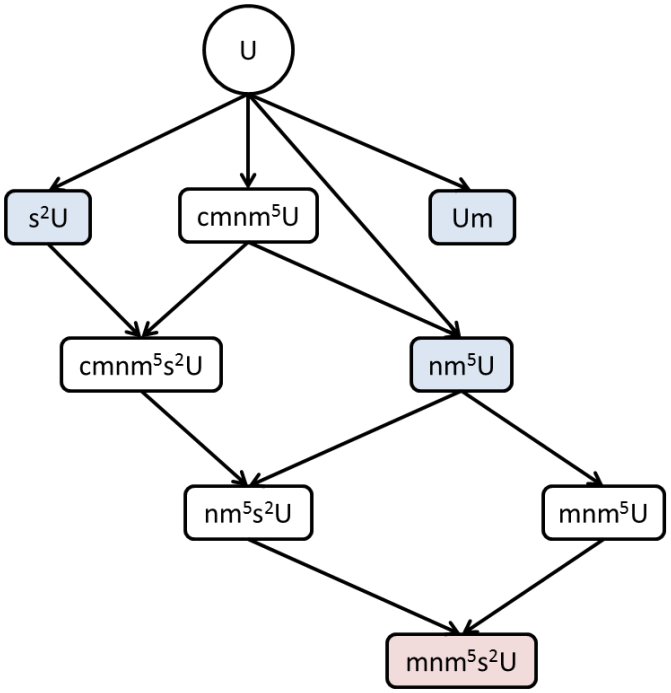

Figure S 9 Alternative pathways leading to the mnm^{5}s^{2}U modification; blue - modifications predicted by tRNAmodpred, red – modification supported by experimental data.

If experimental data supported the presence of mnm5s2U in the position of interest but tRNAmodpred predicted Um, s^{2}U and nm^{5}U for this position, then the following scores values would be calculated:

tp = 2 (because both s^{2}U and nm^{5}U belong to alternative pathways leading to experimentally supported mnm^{5}s^{2}U)

fp = 1 (because Um does not belong to any alternative pathway leading to mnm^{5}s^{2}U)

fn = 2 (because the minimal number of modifications missing in the pathway leading to mnm^{5}s^{2}U equals 2: mnm^{5}U and mnm^{5}s^{2}U itself)