Supplementary Information

Supplementary Figures

Phylum 4:1	4:1	6:1	8:1	
Acidobacteria	0	3	0	٠
Actinobacteria	6	130	0	6
Aquificae	0	11	0	
Bacteroidetes	3	91	0	F
Chlamydiae	0	8	0	
Chlorobi	0	10	0	
Chloroflexi	0	12	2	
Chrysiogenetes	0	1	0	•
Cyanobacteria	0	30	15	
Deferribacteres	0	2	0	•
Deinococcus-Thermus	3	3	0	•
Dictyoglomi	0	2	0	•
Elusimicrobia	0	2	0	•
Fibrobacteres	1	0	0	٠
Firmicutes	191	52	0	
Fusobacteria	13	0	0	
Gemmatimonadetes	1	0	0	٠
Lentisphaerae	0	2	0	•
Nitrospirae	0	3	0	•
Planctomycetes	0	6	0	
Proteobacteria	277	282	0	
Alphaproteobacteria	7	172	0	Ē
Betaproteobacteria	71	15	0	
Gammaproteobacteria	171	54	0	
Deltaproteobacteria	0	38	0	
Epsilonproteobacteria	28	1	0	•
Zetaproteobacteria	0	1	0	•
Spirochaetes	11	6	0	
Synergistetes	0	7	0	
Tenericutes	26	0	0	
Thermotogae	0	11	0	
Verrucomicrobia	3	5	0	

Supplementary Figure S1 | L12:L10 stoichiometry distribution in different phyla



4:1

8:1

6:1

Supplementary Figure S2 | Phylogenetic tree of bacteria based on the sequences of 16S rRNA



4:1 6:1 8:1



4:1

6:1

8:1



Supplementary Figure S5 | Binding segment sequence clustering

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Thermosynechococcus elongatus
Prochlorococcus marinus
Nodularia spumigena CCY9414
Acaryochloris_marina_MBIC11017
Synechococcus_sp._WH_8102
Synechocystis sp. PCC 6803
Nostoc_sp._PCC_7120
Synechococcus_elongatus_PCC_63
Anabaena variabilis ATCC 29413
Synechococcus sp. PCC 7002
Cyanothece_sp._PCC_7822
Synechococcus_sp._JA-2-3B'a_2-
Lyngbya_sp._PCC_8106
Cyanothece_sp._CCY_0110
Gloeobacter_violaceus_PCC_7421
Trichodesmium erythraeum IMS10
Crocosphaera watsonii WH 8501
Cyanothece_sp._PCC_8801
Cyanothece_sp._ATCC_51142
Cyanothece_sp._PCC_8802
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Thermosynechococcus elongatus Prochlorococcus marinus Nodularia spumigena CCY9414 Acaryochloris marina MBIC11017 Synechococcus_sp._WH_8102 Synechocystis sp. PCC 6803 Nostoc_sp._PCC_7120 Synechococcus_elongatus_PCC_63 Anabaena variabilis ATCC 29413 Synechococcus sp. PCC 7002 Cyanothece_sp._PCC_7822 Synechococcus_sp._JA-2-3B'a 2-Lyngbya_sp._PCC_8106 Cyanothece_sp._CCY_0110 Gloeobacter_violaceus_PCC_7421 Trichodesmium erythraeum IMS10 Crocosphaera watsonii WH 8501 Cyanothece_sp._PCC_8801 Cyanothece_sp._ATCC_51142 Cyanothece_sp._PCC_8802

TTCTGCTTCCTTGGCGCGGGCAACGCAGG-----TTCTTCTGGTCTAGCTAGATCACTTAAAC-----TTCCAGTTCTTTGGCTCGTGCTTTACAAG------TTCCGCTTCACTGGCCCGAGCCTTACAAG-----TTCCTCCGGTCTCGCGCGCGCGCGCTCAAGC-----TTCAGCTTCCGTGGCCCGTGGTCTCCAAG------TTCTGGTGGCTTGGCGCGCGCGCTTTGCAAG------TCCGAATTCTTTGGCACGCGGTATCAAAG-----TTCTGGTGGCTTGGCGCGGGCTTTGCAAG------TACGGGTTCTTTGGCCAGAGCCATCAAAG-----TTCCTCTTCTATTGCACGCGGTATCCAAG------TGCCACGCAAGTGGCGCGCGGTATCCACGAGATCCCAGCCTCTCTGGGGC TCCCACAAAACTGGCGGTTGGTATCAAAGAAGTACCTGCCTCCTTGGTTC TACGACTTCTATAGGACGGGGTATCAACGACATCCCCAACTCTTTGGGGC TGCGACGAAGGTGGCCGTGGGCATCAACGAAGTGCCCGCCGGCCTGGCCC TACTACCCAGCTTGCTACTGGTATTAATGAGGTTCCAGCTTCTGTAAACA TGCGAGTTCTTTGGGACGGGGTATCAACGACATCCCCAACTCTTTTGGGC TGCTTCGTCCGTGGCACGGGGGGGGATCAACGAGGTTCCTGGGTCTTTGGGTC TACGACTTCTATCGGACGGGGTATCAACGACATCCCCAACTCATTGGGGC TTCTTCGTCCGTGGCACGGGGGGATCAACGAGGTTCCTGGGTCTTTGGGTC

-----CGATCGCCGACAAAGGT -----AACATGCTGAGAACAGT -----CTGTGGCTGATAAAGAG ----CGCATTCAGACTCTGAT -----AGCACGCCGAAGGCGGC -----CCCATGTGGACAAAGAA ----CTGTCGCTGACAAAGAA -----CCGTTTCCGAACAAACC -----CTGTCGCTGACAAAGAA -----CTGTTTCCGAAAAAGAA ----CTTATTCCGAAAAGGAA GGGCCATCCGGGCCATTGCCGACAAGGAG GTGTTGTCAAAGCGGTTTCCGAGAAGGAA GTGTTATGGCTGCCATGGCCGCTAGAGAA GGGTCCTCGAAGCGCTCAGAAAACAAAAA GAGCAATTAAAGCTATCTCAGATAAAGAG GTGTTATGGCGGCTATGGCGGCCAAAGAA GTGTCGTCAACGCGATCGCCAACAAAGAA GTGTTATGGCCGCCATGGCCGCCAAAGAA GTGTCGTCAACGCGATCGCCAACAAAGAA

Substituting the state $SV + 1000$ set structure any initial to transport	Supplementary	Figure	S6	rplJ	gene	sequence	alignment	for	cvanobacter
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Supplementary Figure S7 | Optimization of digestion conditions

(a) Comparison of different denaturating agents: urea, acetonitrile, and Rapigest. 50S subunits (20 pmol, digested as described in Methods) were loaded on a 4-20 % acrylamide SDS page prior and after enzymatic digestion. The digestion in the presence of urea and Rapigest was

b

complete, whereas digestion in the presence of acetonitrile failed due to precipitation of the sample.

(b) Qualitative comparison of the completeness of digestion by evaluating the charge state of peptides. Aliquots of 50S subunits (*A. platensis*) digested at different conditions were analyzed by ESI MS/MS on a hybrid Linear Ion Trap-Orbitrap mass spectrometer (LTQ-Orbitrap XL, ThermoFisherScientific). The data were analyzed for the number of spectra recorded and their respective charge state by the Rawmeat software (version 2.1; ThermoFisherScientific). Rapigest and urea digestion led to comparable numbers of MS spectra but Rapigest digestion revealed a slightly larger fraction of doubly charged ions. This indicates that the peptides in the Rapigest digestion were on average shorter than in urea implying a more complete digestion or less missed cleavages.

(c) Qualitative comparison of the completeness of digestion by comparing the percentage of spectra of missed cleaved peptides. 50S subunits (*A. platensis*) digested at different conditions were analyzed on the LTQ Orbitrap and the data were processed by Mascot Daemon (Matrix Science) and searched against the NCBI non redundant database. The mass precision was set to 5 ppm for precursors and 0.5 Da for product ions. Carbamidomethylation was used as fixed and methionine oxidation and lysine carbamylation were used as variable modification. In the presence of Rapigest, 75% of all recorded MSMS spectra showed no missed cleavage site and 25 % of all spectra showed one missed cleavage site. Notably, these numbers do not reflect the absolute amount of missed cleaved peptides in the sample, as this analysis is not quantitative. For example, due to the dynamic exclusion (i.e. selection time for a precursor to be excluded from another fragmentation) of the mass spectrometric analysis, highly abundant precursors with no missed cleavage sites are excluded from further analysis and thus do not contribute further to the number of spectra with no missed cleavage site.

(d) Time course of trypsin digestion of 50S subunits (*A. platensis*). The ribosomal subunits were predigested with RNase A and LysC and then incubated with trypsin (0.005 μ g/ μ l).

Aliquots were quenched after different incubation times and spiked with one Aqua peptide for L10 (•, peptide 10) and L12 (•, peptide 8) and analyzed by SRM analysis (material and methods). Solid lines are visual guides. Error bars represent the standard deviation of technical triplicates.

(d) Trypsin titration. 50S subunits (*A. platensis*) were predigested with RNase A and LysC and then incubated with varying concentrations of trypsin for 8h. The samples were spiked with Aqua peptides for L10 (•, peptide 10) and L12 (•, peptide 8). Solid lines are visual guides. Error bars represent the standard deviation of technical triplicates.



Supplementary Figure S8 | L12:L10 stoichiometry determination by SRM analysis

(a) Broad range titration of Aqua peptides (*E. coli*). 70S ribosomes (40 fmol) in the presence of varying amounts of Aqua peptides were analyzed. The combined integrated areas for the respective transitions are plotted on the Y-axis. L10: peptide 3, ■; peptide 4, •; peptide 5, ▼.
L12: peptide 1, •; peptide 2, ▲. The dash lines indicate the amounts of Aqua peptides that equal the respective endogenous peptide. Error bars (not visible on the graph) represent the standard deviation of technical triplicates.

(b) Broad range titration of Aqua peptides (A. platensis). 70S ribosomes (40 fmol) in the presence of varying amounts of Aqua peptides were analyzed. The combined integrated areas for the respective transitions are plotted on the Y-axis. L10: peptide 9, \diamond ; peptide 10, $\mathbf{\nabla}$;

peptide 11, ●. L12: peptide 6, ▲; peptide 7, ▲; peptide 8, ■. Error bars represent the standard deviation of technical triplicates.

(c) Narrow range titration of Aqua peptides (*E. coli*, \bullet and *A. platensis*, \blacksquare) Constant amounts of 70S ribosomes in the presence of varying amounts of Aqua peptides (mixed (1:4 L10:L12)) for *E. coli* and (1:8 L10:L12)) for *A. platensis* were analyzed. Error bars represent the standard deviation of three independent biological replicates combined with three technical replicates. For all titrations the amount of ribosomes are within the titrated range. The titrations show no deviating trend suggesting that the measurement was performed in the linear part of the signal response curve.

(d) Matrix titration (*E. coli*, \bullet and *A. platensis*, \blacksquare): Aqua peptides (60 fmol L10 peptides, 4-or 8-fold excess L12 peptides) in the presence of varying amounts of digested ribosomes were injected. The error bars represent the standard deviation of three independent biological replicates and for each three technical replicates. For all titrations the amount of ribosomes are within the titrated range.

Supplementary Table S1 | Experimentally confirmed L12:L10 stoichiometries

Species	Stoichiometry	Method	Paper
Escherichia coli	4:1	Western blotting, MS	[9,21]
Thermotoga maritima	6:1	Western blotting, structure-based prediction	[9,23]
Thermus thermophilus	6:1	MS	[23]
Agrobacterium tumefaciens	6:1	Immunochemical	[22]
Bacillus subtilis	4:1	MS	[21,23]
Thermus aquaticus	6:1	MS	[23]
Bacillus stearothermophilus	4:1	MS	[23]
Arthrospira platensis	8:1	MS	This paper

		NCBI taxonomy
Organism	Phylum	ID
Arthrospira maxima CS-328	Cyanobacteria	513049
Arthrospira platensis NIES-39	Cyanobacteria	696747
Arthrospira sp. PCC 8005	Cyanobacteria	376219
Crocosphaera watsonii WH 8501	Cyanobacteria	165597
Cyanobacterium UCYN-A	Cyanobacteria	713887
Cyanothece sp. ATCC 51142	Cyanobacteria	43989
Cyanothece sp. CCY0110	Cyanobacteria	391612
Cyanothece sp. PCC 8801	Cyanobacteria	41431
Cyanothece sp. PCC 8802	Cyanobacteria	395962
Gloeobacter violaceus	Cyanobacteria	33072
Lyngbya sp. PCC 8106	Cyanobacteria	313612
Microcoleus chthonoplastes PCC 7420	Cyanobacteria	118168
Roseiflexus castenholzii DSM 13941	Chloroflexi	383372
Roseiflexus sp. RS-1	Chloroflexi	357808
Synechococcus sp. JA-2-3B'a(2-13)	Cyanobacteria	321332
Synechococcus sp. JA-3-3Ab	Cyanobacteria	321327
Trichodesmium erythraeum IMS101	Cyanobacteria	203124

Supplementary Table S2 | Organisms with predicted four L12 binding segments on L10

Supplementary Table S3 | Peptide selection

(a) Sequence of L12 from *E. coli* (MRE600). Aqua peptides for L12 are indiated red.

1 Acetylserine(2)60MSITKDQIIE AVAAMSVMDV VELISAMEEK FGVSAAAAVA VAAGPVEAAE EKTEFDVILK61Methyllysine(82)AAGANKVAVI KAVRGATGLG LKEAKDLVES APAALKEGVS KDDAEALKKA LEEAGAEVEV K.

(**b**) Tryptic peptides >600 Da from *E. coli* L12 that could be detected by MS. Peptides were inspected according to the criteria [28] for standard AQUA peptides.

$[M+H]^+$	Position	Peptide sequence	Missed	Remarks
			cleavages ^a	
2721.34	6-30	DQIIEAVAAMSVM	0	too long, contains
		DVVELISAMEEK		methionines, no signal
2015.04	31-52	FGVSAAAAVAVAA	3	too long, low signal intensity
		GPVEAAEEK		
964.53	53-60	TEFDVILK	0	high signal intensity
716.43	75-82	GATGLGLK	0	no signal
1113.61	86-96	DLVESAPAALK	0	high signal intensity
761.37	102-108	DDAEALK	3	no signal
1244.64	110-121	ALEEAGAEVEVK ^b	0	high signal intensity

^aProteolyzed ribosomes were analyzed for precursors of the target peptide that include nonprocessed trypsin cleavage sites. Number of spectra of missed cleaved peptides as indicated.

^bALEEAGAEVEVK is preceded by a bibasic sequence which could lead to a missed tryptic cleaved product (KALEEAGAEVEVK). However, in ribosome digests the missed cleaved peptide was 200-fold less abundant than the correctly processed (judged on the basis of the intensities of the doubly charged peptides in the extracted ion chromatogram). When both L10 peptides (*E. coli*) were compared, the amounts differed by less than 15 % (Supplementary Dataset S3). If these differences were due to the missed cleaved KALEEAGAEVEVK this could raise the stoichiometry to 4.4 instead of 4.1.

(c) Sequence of L10 from *E. coli* (MRE600). Aqua peptides for L10 are indiated blue.

1Acetyllysine(37)60MALNLQDKQAIVAEVSEVAKGALSAVVADSRGVTVDKMTELRKAGREAGVYMRVVRNTLL61Acetlylysine(105)120RRAVEGTPFECLKDAFVGPTLIAYSMEHPGAAARLFKEFAKANAKFEVKAAAFEGELIPA121165SQIDRLATLPTYEEAIARATMKEASAGKLVRTLAAVRDAKEAA

(d) Tryptic peptides >600 Da from *E. coli* L10 that could be detected by MS. Peptides were

$[M+H]^+$	Position	Peptide sequence	Missed cleavages ^a	Remarks
932.49	1-8	MALNLQDK	0	contains methionines
1243.69	9-20	QAIVAEVSEVAK	0	contains N- terminal glutamine high intensity
1045.56	21-31	GALSAVVADSR	0	high intensity
618.35	32-37	GVTVDK	0	too short
649.33	38-42	MTELR	0	too short, contains methionine
825.39	47-53	EAGVYMR	0	contains methionines
616.38	57-61	NTLLR	0	too short, contains N-terminal asparagine
1193.59	63-73	AVEGTPFECLK	2	contains cysteine
2174.07	74-94	DAFVGPTLIAYSMEHPGAAAR	0	too long, contains methionine
1687.86	110-125	AAAFEGELIPASQIDR	0	high intensity
1447.78	126-138	LATLPTYEEAIAR	0	high intensity
694.36	139-144	LMATMK	0	too short, contains methionines

inspected according to the criteria [28] for standard AQUA peptides.

630.39	154-159	TLAAVR	0	too short

^aProteolyzed ribosomes were analyzed for precursors of the target peptide that include nonprocessed trypsin cleavage sites. Number of spectra of missed cleaved peptides as indicated.

(e) Sequence of L12 from *A. platensis*. Aqua peptides for L12 are indiated red.

1 60 <u>MSEKTDQILE QLKSLSLLEA SELVK</u>QIEEA FGVDASASAG GGMMMAAPG MAAPGAEAAA 61 120 EEEKTEFDVI LTEFPADKKI AVLKVVRSIT GLGLKEAKDL VESAPKAIKE TTTKEDAESI 121 134 KKQLEEAGAK VEVK

(f) Tryptic peptides >600 Da from *A. platensis* L12 that could be detected by MS. Peptides were inspected according to the criteria [28] for standard AQUA peptides.

$[M+H]^+$	Position	Peptide sequence	Missed	Remarks
			cleavages	
1087.60	5-13	TDQILEQLK	2	high signal intensity
1288.73	14-25	SLSLLEASELVK	0	high signal intensity
3741.62	26-64	QIEEAFGVDASASAGGGMMM MAAPGMAAPGAEAAAEEEK	0	too long, contains methionines, no signal
1624.81	65-78	TEFDVILTEFPADK	0	no signal
788.49	88-95	SITGLGLK	0	medium signal intensity
858.46	99-106	DLVESAPK	0	low signal intensity
791.38	115-121	EDAESIK	0	low signal intensity
845.44	123-130	QLEEAGAK	0	N-terminal glutamine, low signal intensity

^aProteolyzed ribosomes were analyzed for precursors of the target peptide that include nonprocessed trypsin cleavage sites. Number of spectra of missed cleaved peptides as indicated.

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(g) Sequence of L10 from A. platensis. Aqua peptides for L10 are indiated blue.
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(h) Tryptic peptides >600 Da from *E. coli* L10 that could be detected by MS. Peptides were inspected according to the criteria [28] for standard AQUA peptides.

$[M+H]^+$	Position	Peptide sequence	Missed	Remarks
			cleavages ^a	
1057.59	9-17	ENIVVDIQK	0	medium signal
				intensity
2721.47	18-42	QLSSAQLTLVIDYTGLSVSQ	0	too long, no signal
		ITDLR		
723.33	47-53	ETGSVCK	0	contains cysteine
734.39	57-62	NTLMQK	0	contains N-terminal
				methionine
1518.69	63-75	AVEGDDNWKPMEK	0	contains methionine
				and tryptophan
2134.09	76-95	FLSGSSAFIFIQDDFGAAIK	0	too long
855.44	96-102	AYQAFQK	0	low signal intensity
705.33	111-117	GGVMEGR	0	contains methionine
872.47	118-125	ALSPDEIK	0	low signal intensity
872.51	126-133	AITELPTK	6	medium signal
				intensity
1			1	

748.37	134-139	EELMAR	6	contains methionine
1084.64	140-150	IAGAINSIPTK	0	high signal intensity
1054.63	151-161	VAVGVNAVPTK	0	high signal intensity
870.50	168-175	EVPASLVR	0	medium signal intensity
606.24	184-189	DDSQAA	1	too short

^aProteolyzed ribosomes were analyzed for precursors of the target peptide that include nonprocessed trypsin cleavage sites. Number of spectra of missed cleaved peptides as indicated.

(i) Summary of SRM transitions and optimized collision energies.

Protein	– Peptide	Q1	Q2	Fragment	CE
L12 (E.coli)	ALEEAGAVEVK light	622.822	731.393	у7	18
Peptide 1		622.822	802.431	y8	18
		622.822	931.473	y9	18
		622.822	1060.516	y10	18
	ALEEAGAVEVK* heavy	626.829	739.408	у7	18
		626.829	810.445	y8	18
		626.829	939.487	y9	18
		626.829	1068.53	y10	18
L12 (E.coli)	DLVESAPAALK light	557.311	499.324	y5	21
Peptide 2		557.311	657.393	у7	17
		557.311	786.436	y8	16
		557.311	885.504	y9	16
	DLVESAPAALK* heavy	561.318	507.338	y5	21
		561.318	665.407	у7	17
		561.318	794.450	y8	16
		561.318	893.518	y9	16
L10 (<i>E.coli</i>)	GALSAVVADSR light	523.285	547.283	y5	16
Peptide 3		523.285	646.352	y6	16
		523.285	717.389	у7	17
		523.285	804.421	y8	17
	GALSAVVADSR* heavy	528.290	557.292	y5	16
		528.290	656.360	y6	16
		528.290	727.397	у7	17
		528.290	814.429	y8	17
L10 (E.coli)	AAAFEGELIPASQIDR light	844.436	786.410	у7	24
Peptide 4		844.436	899.495	y8	24
		844.436	1012.579	y9	24
		844.436	1198.643	y11	24
	AAAFEGELIPASQIDR* heavy	849.440	796.419	у7	24

		849.440	909.503	y8	24
		849.440	1022.587	y9	24
		849.440	1208.651	y11	24
L10 (E.coli)	LATLPTYEEAIAR light	724.393	559.320	y5	28
Peptide 5	C C	724.393	851.426	y7	25
		724.393	952.473	y8	24
		724.393	1049.526	y9	21
	LATLPTYEEAIAR* heavy	729.397	569.328	y5	28
	-	729.397	861.434	y7	25
		729.397	962.482	y8	24
		729.397	1059.534	y9	21
L12	TDQILEQLK light	544.303	517.298	y4	18
(A.platensis)	-	544.303	630.382	y5	19
Peptide 6		544.303	743.466	y6	18
		544.303	871.525	y7	18
	TDQILEQLK* heavy	548.310	525.312	ý4	18
		548.310	638.396	y5	19
		548.310	751.480	y6	18
		548.310	879.539	, у7	18
		• ·		_	
Protein	Peptide	Q1	Q2	Fragment	CE
L12	SEKTDQILEQLK light	477.928	630.382	y5	16
(A.platensis)		477.928	689.310	b6	16
Peptide 7		477.928	743.466	y6	16
	SEKTDQILEQLK* heavy	480.599	638.396	y5	16
		480.599	689.310	b6	16
		480.599	751.480	у6	16
L12	SLSLLEASELVK light	644.872	646.377	y6	19
(A.platensis)		644.872	775.420	у7	19
Peptide 8		644.872	888.504	y8	20
		644.872	1088.62	y10	20
	SLSLLEASELVK* heavy	648.879	654.391	y6	19
		648.879	783.434	y7	19
		648.879	896.518	y8	20
		648.879	1096.634	y10	20
L10	IAGAINSIPTK light	542.822	345.213	у3	24
(A. platensis)		542.822	659.372	y6	18
Peptide 9		542.822	772.456	у7	18
		542.822	900.515	y9	18
	IAGAINSIPTK* heavy	546.829	353.227	у3	24
		546.829	667.386	y6	18
		546.829	780.471	у7	18
		546.829	908.529	y9	18
L10	VAVGVNAVPTK light	527.816	345.213	у3	18
(A.platensis)		527.816	629.362	y6	18
Peptide 10		527.816	785.452	y8	19
		527.816	884.520	y9	19
	VAVGVNAVPTK* heavy	531.823	353.227	у3	18
		531.823	637.376	y6	18
		531.823	793.466	y8	19
		531.823	892.534	у9	19

L10	EVPASLVR light	435.756	474.303	y4	21
(A.platensis)		435.756	545.341	y5	21
Peptide 11		435.756	642.393	y6	15
	EVPASLVR* heavy	440.760	484.312	y4	21
		440.760	555.349	y5	21
		440.760	652.402	y6	15

^a The L10 and L12 sequences of both organisms were digested *in silico* with endoproteinase trypsin. Tryptic peptides shorter than 6 or longer than 17 amino acids were excluded. Peptides containing chemically reactive residues, such as Trp, Cys, Met or N-terminal Asn were excluded. When possible, peptides with residues that might be deamidated (Asn, Gln) were avoided. If this was not possible, the extent of deamidation of the endogenous peptide was tested by comparing the integrated extracted ion chromatograms of the deamidated and non deamidated peptide form. Deamidation was always less than 0.1% (data not shown). In addition, peptides with consecutive lysine or arginine residues were avoided (see *E. coli* L10,) since they are prone to produce missed cleavages. The resulting tryptic peptides were analyzed for their behavior in mass spectrometry, signal intensity and chromatographic behavior by SRM analysis on a triple quadrupole. For all peptides the *m/z* values of the double charge state were used as Q1 mass and Q3 *m/z* values for all y-fragments larger than the precursor *m/z* values were analyzed. The most intense peptides were selected as reference peptides. Peptide 7 represents a missed cleaved version of peptide 6 and which was co-quantified.

Species	GI	Accession
Aedes aegypti	157123189	XP_001660051.1
Ailuropoda melanoleuca	281344364	EFB19948.1
Anolis carolinensis	327275544	XP_003222533.1
Ashbya gossypii ATCC 10895	45201289	NP_986859.1
Bos taurus	300798419	NP_001179324.1
Camponotus floridanus	307172023	EFN63617.1
Candida albicans SC5314	68477303	XP_717278.1
Candida albicans WO-1	238881387	EEQ45025.1
Candida dubliniensis CD36	241954754	XP_002420098.1
Candida glabrata CBS 138	50291399	XP_448132.1
Candida orthopsilosis	380354527	CCG24043.1
Candida parapsilosis	354544529	CCE41253.1
Candida tenuis ATCC 10573	344231766	EGV63648.1
Canis lupus familiaris	73966176	XP_850888.1
Capsaspora owczarzaki ATCC 30864	320169345	EFW46244.1
Ciona intestinalis	198433696	XP_002130196.1
Cricetulus griseus	354474798	XP 003499617.1
Culex quinquefasciatus	170033260	XP 001844496.1
Danio rerio	61806488	NP 001013476.1
Debaryomyces hansenii CBS767	50412995	XP 457192.1
Dicentrarchus labrax	317419666	CBN81703.1
Drosophila ananassae	194766415	XP_001965320.1
Drosophila erecta	194853286	XP_001968135.1
Drosophila grimshawi	195035279	XP_001989105.1
Drosophila melanogaster	17647667	NP_523440.1
Drosophila mojavensis	195114440	XP_002001775.1
Drosophila pseudoobscura pseudoobscura	125987413	XP_001357469.1
Drosophila sechellia	195350027	XP_002041543.1
Drosophila simulans	195575473	XP 002077602.1
Drosophila virilis	195386412	XP_002051898.1
Drosophila willistoni	195433106	XP 002064556.1
Drosophila yakuba	195470188	XP 002087390.1
Equus caballus	149723906	XP 001501924.1
Eremothecium cymbalariae DBVPG#7215	363751601	XP 003646017.1
Heterocephalus glaber	351711267	EHB14186.1
Homo sapiens	22547114	NP 660298.2
Hydra magnipapillata	221130972	XP 002164923.1
Kazachstania africana CBS 2517	372465302	CCF59578.1
Kazachstania naganishii CBS 8797	403213432	CCK67934.1
Kluyveromyces lactis NRRL Y-1140	302309931	XP_451143.2
Komagataella pastoris GS115	254572473	XP 002493346.1

Supplementary Table S4 | List of mitochondrial MRPL10 sequences

Lachancea thermotolerans	255715297	XP_002553930.1
Lodderomyces elongisporus NRRL YB-4239	149234497	XP_001523128.1
Loxodonta africana	344285947	XP_003414721.1
Macaca mulatta	386781444	NP_001247880.1
Meyerozyma guilliermondii ATCC 6260	190344935	EDK36729.2
Millerozyma farinosa CBS 7064	358249662	CCE72728.1
Mus musculus	13385658	NP_080430.1
Mustela putorius furo	355704273	AES02174.1
Naumovozyma castellii CBS 4309	366991705	XP_003675618.1
Naumovozyma dairenensis CBS 421	365987121	XP_003670392.1
Nematostella vectensis	156387640	XP_001634311.1
Nomascus leucogenys	332221773	XP_003260039.1
Ogataea parapolymorpha DL-1	320581777	EFW95996.1
Oreochromis niloticus	348522383	XP_003448704.1
Ornithorhynchus anatinus	149444133	XP_001515004.1
Oryctolagus cuniculus	291405903	XP_002719372.1
Otolemur garnettii	395826582	XP 003786496.1
Pan paniscus	397512997	XP 003826816.1
Pan troglodytes	114666402	XP 001173485.1
Papio anubis	402899434	XP 003912702.1
Pongo abelii	297715892	XP_002834282.1
Rattus norvegicus	157819877	NP 001103090.1
Saccharomyces arboricola H-6	401626507	EJS44451.1
Saccharomyces cerevisiae EC1118	259145044	CAY78308.1
Saccharomyces cerevisiae Kyokai no. 7	349576882	GAA22051.1
Saccharomyces cerevisiae Lalvin QA23	323349389	EGA83613.1
Saccharomyces cerevisiae S288c	6319999	NP_010079.1
Saccharomyces cerevisiae Vin13	323338381	EGA79606.1
Saccharomyces cerevisiae x Saccharomyces		
kudriavzevii VIN7	365757792	EHM99669.1
Saccoglossus kowalevskii	291233451	XP_002736666.1
Salpingoeca sp. ATCC 50818	326428236	EGD73806.1
Scheffersomyces stipitis CBS 6054	150864883	XP_001383885.2
Spathaspora passalidarum NRRL Y-27907	344305334	EGW35566.1
Sus scrofa	311267458	XP_003131579.1
Tetrapisispora phaffii CBS 4417	367000103	XP_003684787.1
Torulaspora delbrueckii	367010052	XP_003679527.1
Trichoplax adhaerens	196001151	XP_002110443.1
Vanderwaltozyma polyspora DSM 70294	156836771	XP_001642431.1
Xenopus (Silurana) tropicalis	301621740	XP_002940198.1
Xenopus laevis	51858967	AAH82225.1
Zygosaccharomyces rouxii	254577625	XP 002494799.1

Species	GI	Accession
Arabidopsis lyrata subsp. lyrata	297807377	XP 002871572.1
Arabidopsis thaliana	15240644	NP_196855.1
Bigelowiella natans	32307548	AAP79179.1
Brachypodium distachyon	357112780	XP_003558185.1
Chlamydomonas reinhardtii	159476750	XP_001696474.1
Chlorella variabilis	307110553	EFN58789.1
Coccomyxa subellipsoidea C-169	384246088	EIE19579.1
Glycine max	356562595	XP_003549555.1
Guillardia theta	57335911	CAH25357.1
Hordeum vulgare subsp. vulgare	326507838	BAJ86662.1
Medicago truncatula	357480187	XP_003610379.1
Micromonas pusilla CCMP1545	303273588	XP_003056154.1
Nannochloropsis gaditana CCMP526	387219517	AFJ69467.1
Nicotiana tabacum	75223179	O80362.1
Oryza sativa Indica Group	218192573	EEC75000.1
Oryza sativa Japonica Group	115452321	NP_001049761.1
Ostreococcus lucimarinus CCE9901	145343056	XP_001416282.1
Paulinella chromatophora	194476827	YP_002049006.1
Phaeodactylum tricornutum CCAP 1055/1	219124466	XP_002182524.1
Physcomitrella patens subsp. patens	168025872	XP_001765457.1
Picea sitchensis	116784063	ABK23199.1
Populus trichocarpa	224098099	XP_002311119.1
Ricinus communis	255555289	XP_002518681.1
Selaginella moellendorffii	302800123	XP_002981819.1
Sorghum bicolor	242036081	XP_002465435.1
Thalassiosira oceanica	397626921	EJK68284.1
Thalassiosira pseudonana CCMP1335	223997696	XP_002288521.1
Vitis vinifera	225448737	XP_002281167.1
Zea mays	226491231	NP_001142362.1

Supplementary Table S5 | List of chloroplastic L10 sequences