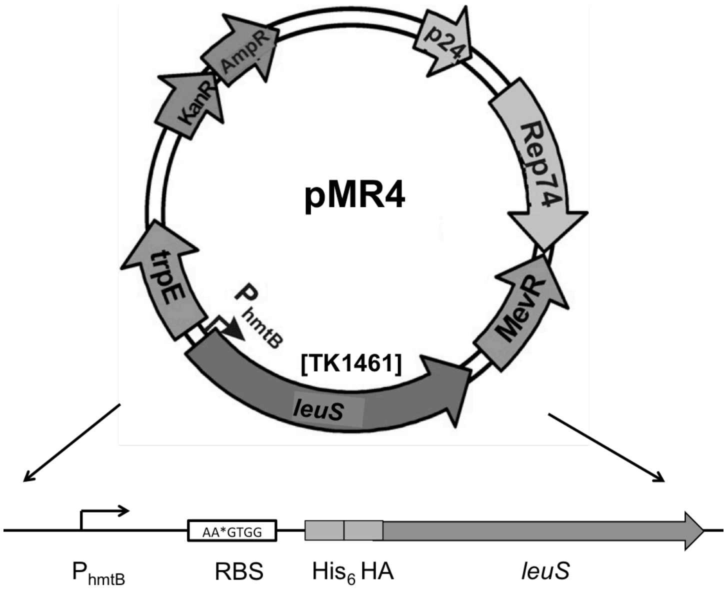
SUPPLEMENTARY INFORMATION

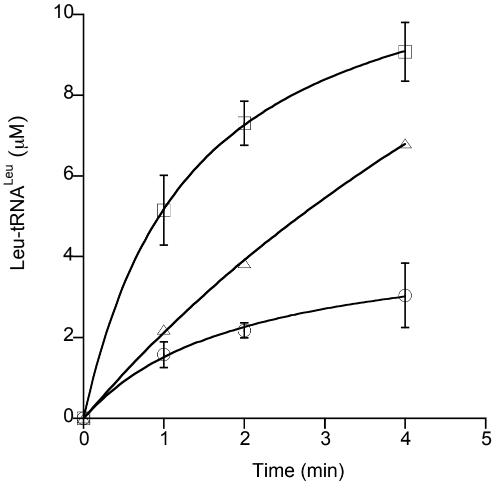
Plasmids and strain construction

Plasmids for the production of N-terminally tagged intein fusion derivatives of LeuRS, ProRS and EF1A (TK1461, TK0550 and TK 0308, respectively) were constructed by inserting the corresponding PCR amplified genes into pTYB11 between *Sap*I and *Xho*I. For the intein tagged LeuRS, ProRS and EF1A constructs the forward primer used were 5 'GTTGTTGTACAGAAC ATGGCTGAGCTTAACTTTAAG 3', 5' GTTGTTGTACAGAACATGGCGGTTGAGAGA AAGAA 3' and 5' GTTGTTGTACAGAACATGGCTAAGGAGAAG 3' and the reverse primers were 5 'CTGCAGTCACCCGGGTCACTCCACAAACACCGCGGG 3', 5' CTGCAGTCAC CCGGGTCAGTAAGTCCTCGC 3' and 5' CTGCAGTCACCCGGGTCAGTCGGCCTTCTG 3', respectively. A clone containing an N-terminally His_6 tagged TyrRS (TK0568) was construced by amplifying the gene from the *T. kodakarensis* DNA and ligating the purified amplicon into the *Bam*HI and *Hind*III digested pQE31 plasmid. The primers used were 5' CATACGGATCCG ATGGACATAGAAAGGAAG 3' and 5' ATTAAGCTTTTACCGGGTTATCTTTATCTC 3' flanked by *BamH*I and *Hind*III sites.

The plasmid to express the gene encoding the LeuRS-derived bait for interactome analysis was constructed as follows. The gene encoding LeuRS (*leuS*) was PCR amplified from *T. kodakarensis* DNA and the coding sequence was extended in frame by ligation of an oligonucleotide sequence that added the constitutive promoter from the *M. thermoautotrophicum* archaeal histone-encoding (hmtB) gene, a mutant RBS (AAGTGG), and also encoded a hemagglutinin (HA) epitope and six histidine residues (His_6) at the N terminus of LeuRS (Supplementary Fig. 1). This gene was then ligated into plasmid pLC70 between *Sal*I and *Not*I sites. Plasmid MR3 was similar to MR4 except that the RBS site was WT(AGGTGG). The mutant RBS was used to limit synthesis and thus mimic native expression of LeuRS in the cell (Supplementary Fig. 2).



Supplementary Figure 1. Map of plasmid pHis$_6$-HA-LeuRS (pMR4).



Supplementary Figure 2. Production of LeuRS in *T. kodakarensis* assessed by the aminoacylation activity of LeuRS . Aminoacylation activities of cell free extracts of *T. kodakarensis* strain KW128 transformed with pLC70 plasmid (a parent vector lacking a LeuRS coding sequence, open circle), pMR3 (open square) and pMR4 (open triangle) were measured with 5 μM tRNA^{Leu} after dialyzing the extracts against 25 Mm Tris-HCl pH7.5, 100 mM KCl and 10 mM beta-mercaptoethanol.

Supplementary Table 1

Complete list of proteins that co-purified with His_6-HA tagged LeuRS

Function	ORF	Description	Mascot Score
Translation	TK0568	tyrosyl-tRNA synthetase	953
	TK0978	glycyl-tRNA synthetase	435
	TK0550	prolyl-tRNA synthetase	291
	TK1461	leucyl-tRNA synthetase	117
	TK0444	cysteinyl-tRNA synthetase	110
	TK1049	methionyl-tRNA synthetase	102
	TK0308	elongation factor 1-alpha	13099
	TK0556	translation initiation factor IF-2B subunit beta	618
	TK1305	translation initiation factor IF-2	120
	TK0309	elongation factor EF-2	214
	TK0506	translation-associated GTPase	151
	TK1254	30S ribosomal protein S3Ae	369
	TK1529	30S ribosomal protein S4e	364
	TK1538	30S ribosomal protein S19P	336
	TK1276	30S ribosomal protein S19e	326
	TK1504	30S ribosomal protein S11P	319
	TK1505	30S ribosomal protein S4	299
	TK1496	30S ribosomal protein S2	294
	TK0307	30S ribosomal protein S10P	248
	TK1521	30S ribosomal protein S5P	212
	TK1526	30S ribosomal protein S8P	180
	TK1099	30S ribosomal protein S27e	178
	TK1500	30S ribosomal protein S9P	171
	TK1078	30S ribosomal protein S12P	161
	TK1251	30S ribosomal protein S15P	143
	TK1532	30S ribosomal protein S17P	131
	TK1951	30S ribosomal protein S6e	106
	TK1239	peptide chain release factor 1	331
	TK1671	tRNA-modifying enzyme	172
	TK0981	N2, N2-dimethylguanosine tRNA methyltransferase	170
	TK0970	N(2),N(2)-dimethylguanosine tRNA methyltransferase	145
	TK0704	SAM-dependent methyltransferase	144
	TK1114	ribonuclease Z	122
Proteasome	TK2252	proteasome-activating nucleotidase	474
	TK1637	proteasome subunit alpha	174
Protein binding and modification	TK2303	chaperonin beta subunit	460
	TK1473	amino acid kinase	203
	TK1085	protein disulfide oxidoreductase	139
	TK1022	D-aminopeptidase	540
Metabolic enzymes	TK0914	3-hydroxy-3-methylglutaryl-CoA reductase (mevanolate Pathway)	7866
	TK1470	isopentenyl pyrophosphate isomerase (mevanolate Pathway)	500
	TK0180	acetyl-CoA acetyltransferase (mevanolate Pathway)	110
	TK0828	NAD(P)H:rubredoxin oxidoreductase (Fatty acid metabolism)	120
	TK0187	glutamine amidotransferase, class I	437
	TK1404	phosphomannomutase-related protein	321
	TK0217	pyridoxine biosynthesis protein	1183

	TK0890	thiamine monophosphate kinase	179
	TK1990	cysteine desulfurase	521
	TK1796	glutamine synthetase	120
	TK0193	GMP synthase subunit B	184
	TK0504	aspartate racemase	166
	TK0297	L-aspartate oxidase	322
	TK1548	serine--glyoxylate aminotransferase, class V (transferase-transaminase)	258
	TK1379	glycine dehydrogenase subunit 2	255
	TK0149	Pyruvoyl-dependent arginine decarboxylase	154
	TK2257	deoxycytidylate deaminase	163
	TK0305	uridylate kinase	164
	TK1517	adenylate kinase	109
	TK1514	cytidylate kinase	305
	TK2196	aspartate carbamoyltransferase catalytic subunit	158
	TK1895	5'-methylthioadenosine phosphorylase II	280
	TK1482	5'-methylthioadenosine phosphorylase	158
	TK1193	CTP synthetase	105
	TK2042	ATPase	260
	TK1326	NAD(P)H-flavin oxidoreductase	471
	TK1299	NADH oxidase	432
	TK1056	rubrerythrin-related protein	317
	TK0814	Type A Flavoprotein	911
	TK2290	ribulose bisophosphate carboxylase	1767
	TK1426	ribose-5-phosphate isomerase A	107
	TK2164	fructose-1,6-bisphosphatase	140
	TK0866	cofactor-independent phosphoglycerate mutase	909
	TK2106	phosphopyruvate hydratase	650
	TK1396	glycerol kinase	257
	TK1125	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	407
	TK1123	2-oxoglutarate ferredoxin oxidoreductase subunit gamma	352
	TK1009	putative 5-methylcytosine restriction system, GTPase subunit	947
	TK1325	Putative oxidoreductase	538
	TK1326	ferredoxin--NADP(+) reductase subunit alpha	471
	TK0793	GTP cyclohydrolase	398
	TK0893	pyruvate formate lyase family activating protein	298
	TK1984	pyruvate ferredoxin oxidoreductase subunit beta	282
	TK1205	PBP family phospholipid-binding protein	272
	TK2077	4Fe-4S cluster-binding protein	254
	TK1611	metal-dependent hydrolase	253
	TK1997	hydrogenase maturation protein HypF	235
	TK1234	lipoate-protein ligase A, C-terminal section	234
	TK0494	bifunctional carboxypeptidase/aminoacylase	214
	TK0296	quinolinate synthetase	206
	TK0868	bis(5'-adenosyl)-triphosphatase	205
	TK1549	predicetd ATPase	204
	TK1481	NADH:polysulfide oxidoreductase	203
	TK1863	N6-adenine-specific DNA methylase	194
	TK1457	phosphate transport regulator	193
	TK2016	iron-molybdenum cofactor-binding protein	192
	TK2225	molybdenum cofactor biosynthesis protein A	182
	TK0134	acetylpolyamine aminohydrolase	140
	TK1149	methylmalonyl-CoA mutase, N-terminus of large subunit	172

	TK1605	metallo-beta-lactamase superfamily hydrolase	134
	TK1716	cytidylyltransferase	128
	TK0354	putative molybdenum cofactor biosynthesis protein C	127
	TK0828	NAD(P)H:rubredoxin oxidoreductase	120
	TK1711	sugar-phosphate nucleotydyltransferase	108
Cell Division	TK2271	cell division protein FtsZ	821
Transcription	TK1331	Lrp/AsnC family transcriptional regulator	378
	TK1881	ArsR family transcriptional regulator	113
	TK1083	DNA-directed RNA polymerase subunit beta	373
	TK1503	DNA-directed RNA polymerase subunit D	302
	TK1769	transcriptional regulator	364
DNA repair	TK2213	Bipolar DNA helicase	448
	TK1899	DNA repair and recombination protein RadA	323
	TK0155	RecJ-like exonuclease	476
	TK1165	AP endonuclease (base excision repair pathway)	365
Ribosome Biogenesis	TKO566	DEAD/DEAH box RNA helicase	175
	TK0679	serine/threonine protein kinase	111
	TK1636	putative RNA-associated protein	167
Quorum sensing	TK1605	metallo-beta-lactamase superfamily hydrolase	134
Miscellaneous	TK1764	N-acetylchitobiose deacetylase	124
	TK0038	flagellin	118
	TK1037	calcineurin superfamily metallophosphoesterase	118
	TK0008	DNA methylase	115
	TK0798	DNA topoisomerase VI subunit A	209
	TK0799	DNA topoisomerase VI subunit B	192
	TK2213	bipolar DNA helicase	448
Hypothetical Proteins	TK1492		921
	TK2200		813
	TK0453		687
	TK0675		626
	TK1430		592
	TK2148		474
	TK0033		349
	TK0205		324
	TK1196		268
	TK1424		261
	TK0027		259
	TK0013		240
	TK1497		205
	TK0175		200
	TK0897		193
	TK1996		198
	TK0593		176
	TK0361		152
	TK0109		147
	TK0515		140
	TK1626		140
	TK0335		140
	TK0733		135
	TK0318		135
	TK0206		124
	TK0438		123
	TK0790		111

	TK2038		111
	TK2294		107
	TK1882		105