Supplementary Material

Physiological assembly of functionally active 30S ribosomal subunits from *in vitro* synthesized parts

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Supplementary Table S1. 54 *E. coli* ribosomal proteins, name designation, coding genes and protein sizes

30S subunit						50S subunit					
Protein	Gene	Protein Size	Protein	Gene	Protein size	Protein	Gene	Protein size	Protein	Gene	Protein size
S1	rpsA	61.2kD	S18	rpsR	9kD	L1	rplA	24.7kD	L20	rplT	13.5kD
S2	rpsB	27kD	S19	rpsS	10.1kD	L2	rplB	30kD	L21	rplU	11.6kD
S3	rpsC	25.6kD	S20	rpsT	9.7kD	L3	rplC	22.3kD	L22	rplV	12.1kD
S4	rpsD	23.5kD	S21	rpsU	8.5kD	L4	rplD	22.1kD	L23	rplW	11.2kD
S5	rpsE	17.6kD				L5	rplE	20.3kD	L24	rplX	11.3kD
S6	rpsF	15.2kD				L6	rplF	18.9kD	L25	rplY	10.7kD
S7	rpsG	20kD				L9	rplI	15.8kD	L27	rpmA	9.1kD
S8	rpsH	14.1kD				L10	rplJ	17.7kD	L28	rpmB	9kD
S9	rpsI	15.1kD				L11	rplK	14.9kD	L29	rpmC	7kD
S10	rpsJ	11.7kD				L12	rplL	12.3kD	L30	rpmD	6.5kD
S11	rpsK	13.8kD				L13	rplM	16kD	L31	rpmE	7.9kD
S12	rpsL	13.7kD				L14	rplN	13.5kD	L32	rpmF	6.4kD
S13	rpsM	13,1kD				L15	rplO	15kD	L33	rpmG	6.4kD
S14	rpsN	11.6kD				L16	rplP	15kD	L34	rpmH	5.4kD
S15	rpsO	10.3kD				L17	rplQ	14.4kD	L35	rpmI	7.3kD
S16	rpsP	9.2kD				L18	rplR	12.8kD	L36	rpmJ	4.4kD
S17	rpsQ	9.3kD				L19	rplS	13.1kD			

Supplementary Table S2. Summary of 50S ribosome assembly cofactors

Factor	Possible functions	Reference

CsdA	CsdA is an ATP-dependent RNA helicase that binds to large ribosomal subunit to mediate unwinding of 23S rRNA during assembly.	(1)
DbpA	DbpA is an ATP-dependent RNA helicase that mediates unwinding of 23S rRNA during assembly.	(2)
Der	Unique G-protein with tandem G-domains and RNA binding KH domain. Probably involved in assembly of 50S subunit.	(3)
SrmB	SrmB is involved in an early step of 50S assembly that is necessary for the binding of L13.	(4)

Supplementary Table S3. Modified nucleotides of *E. coli* 23S rRNA and their modification enzymes (essential ones (5) are marked by *, oh^{5}C is 5-hydroxycytidine (6))

Location	Enzyme	Modification	Reference	Location	Enzyme	Modification	Reference

745	RlmA(I) (YebH)	m^1G	(7)	2251	RlmB (YjfH)	Gm	(8)
746	RluA (YabO)	Ψ	(9)	2445*	RlmL (YcbY)	m^2G	(10)
747	RumB (YbjF)	m^5U	(11)	2449*	N/A	D	
955	RluC (YceC)	Ψ	(12)	2457*	RluE (YmfC)	Ψ	(13)
1618	RlmF (YbiN)	m^6A	(14)	2498*	RlmM (YgdE)	Cm	(15)
1835	RlmG (YgjO)	m^2G	(14)	2501*	N/A	oh^5C	
1911	RluD (Yfil)	Ψ	(16)	2503*	RlmN (YfgB)	m^2A	(17)
1915	RlmH (YbeA) RluD (Yfil)	$m^3Ψ$	(16,18)	2504*	RluC (YceC)	Ψ	(12)
1917	RluD (Yfil)	Ψ	(16)	2552	RrmJ (FtsJ)	Um	(19)
1939	RumA (YgcA)	m^5U	(20)	2580	RluC (YceC)	Ψ	(12)
1962	RlmI (YccW)	m^5C	(21)	2604	RluF (YjbC)	Ψ	(13)
2030	RlmJ (YhiR)	m^6A	(22)	2605	RluB (YciL)	Ψ	(13)
2069	RlmKL (YcbY)	m^7G	(10)				

Supplementary Table S4. Primers for 30S ribosome assembly cofactor cloning.

Gene	Vector	N-terminal primer	C-terminal primer	Tag and

				position
era	pET-24b	5’- GGAATTCCATATGAGCATCGATAAAAGTTACTGCGG-3’	5’-CCGCTCGAGTTACTTTTCGAACTGCGGGTGGCTCCAAAGATCGTCAACGTAACCGAGAC-3’	C terminal strep-tag
rimM	pET-24b	5’-GGAATTCCATATGAGCAAACAACTCACCGC-3’	5’-CCGCTCGAGTTAGTGGTGGTGGTGGTGGTGAAAACCAGGATCCCAATCTACTTCGA-3’	C terminal his-tag
rimN	pET-24b	5’-GGAATTCCATATGAATAATAACCTGCAAAGAGACGC-3’	5’-CCGCTCGAGTTAGTGGTGGTGGTGGTGCCCCTGTCGAAACAGTTCAC-3’	C terminal his-tag
rimP	pET-24b	5’-GGAATTCCATATGCACCACCACCACCACCACTCCACATTAGAGCAAAAATTAACAGAGATGATTACTG-3’	5’-CCGCTCGAGTTAAAAGTGGGGAACCAGGTTCGC-3’	N terminal his-tag
rfbA	pET-24b	5’-GGAATTCCATATGGCGAAAGAATTTGGTCGCC-3’	5’-CCGCTCGAGTTAGTGGTGGTGGTGGTGGTCCTCCTTGCTGTCGTCC-3’	C terminal his-tag
rsgA	pET-24b	5’-GGAATTCCATATGCACCACCACCACCACCACAGTAAAAATAAACTCTCCAAAGGCCAGCAG-3’	5’-CCGCTCGAGTTAGTCATCCGTATCAGAAAAGTTTTTACGCG-3’	N terminal his-tag

Supplementary Table S5. Primers for 16S rRNA modification enzyme cloning.

Gene	Vector	N-terminal primer	C-terminal primer	His-tag position
yejD	pET-24b	5’-GGAATTCCATATGCACCAC CACCACCACCACCGACTTGATA AATTTATCGCACAGCAAC-3’	5’-CCGCTCGAGTTAGACGAC GCTGGCAATTTCTTC-3’	N terminus
gidB	pET-24b	5’-GGAATTCCATATGCACC ACCACCACCACCACCTCAACAA ACTCTCCTTACTGCTGAAAG-3’	5’-CCGCTCGAGTTAAATTT TATTTGCTTTAATCACCACCA GATG-3’	N terminus
yhhF	pET-15b	NA	NA	C terminus
fmu	pET-24b	5’-GGAATTCCATATGCACCACC ACCACCACCACAAAAAACAACG TAATTTACGTAGCATGGC-3’	5’-CCGCTCGAGTTACTTTT TGATTAGCTTAGCGTAAAAGA AGCC-3’	N terminus
yjjT	pET-24b	5’-GGAATTCCATATGCACC ACCACCACCACCACTCTGCATT TACCCCGGCAAG-3’	5’-CCGCTCGAGTTAACCT TTCTTCGCCTGGCG-3’	N terminus
yabC	pET-24b	5’-GGAATTCCATATGCACCACC ACCACCACCACATGGAAAACTA TAAACATACTACGGTGCT-3’	5’-CCGCTCGAGTTATGCA TTCGTCCTCTCTGCAA-3’	N terminus
yraL	pET-24b	5’-GGAATTCCATATGCACC ACCACCACCACCACAAACAACA CCAATCGGCGG-3’	5’-CCGCTCGAGTTACCC CTGCTGCTCCAG-3’	N terminus
yebU	pET-24b	5’-GGAATTCCATATGGCCC AACACACCGTTTATTTC-3’	5’-CCGCTCGAGTTAGTGG TGGTGGTGGTGGTGGGCGTT ACCGGTAAAAAGTTTCC-3’	C terminus
yggJ	pET-32a	NA	NA	N terminus
yhiQ	pET-28a	NA	NA	N terminus
ksgA	pET-24b	5’-GGAATTCCATATGCACC ACCACCACCACCACAATAATCG AGTCCACCAGGGC-3’	5’-CCGCTCGAGTTAAC TCTCCTGCAAAGGCGC-3’	N terminus

Supplementary Table S6. PCR primers for 30S ribosomal proteins.

Gene	N-terminal	C-terminal
rpsA	5’-GGAATTCCATATGACTGAATCTTT TGCTCAACTCTTTG-3’	5’-CCGCTCGAGTTACTCGCCTTTAGCTG CTTTGAAAG-3’
rpsB	5’-GGAATTCCATATGGCAACTGTTTC CATGCG-3’	5’-CCGCTCGAGTTACTCAGCTTCTACGA AGCTTTC-3’
rpsC	5’-GGAATTCCATATGGGTCAGAAAGT ACATCC-3’	5’-CCGCTCGAGTTATTTACGGCCTTTAC GCTG-3’
rpsD	5’-GGAATTCCATATGGCAAGATATTT GGGTCC-3’	5’-CCGCTCGAGTTACTTGGAGTAAAGC TCGAC-3’
rpsE	5’-GGAATTCCATATGGCTCACATCGA AAAACAA-3’	5’-CCGCTCGAGTTATTTCCCCAGAATTT CTTCA-3’
rpsF	5’-GGAATTCCATATGCGTCATTACGA AATCGTT-3’	5’-CCGCTCGAGTTACTCTTCAGAATCCC CAG-3’
rpsG	5’-GGAATTCCATATGCCACGTCGTC GCGTC-3’	5’-CCGCTCGAGTCAATTTAAGTAGCCCA AAGC-3’
rpsH	5’-GGAATTCCATATGAGCATGCAAGA TCCGAT-3’	5’-CCGCTCGAGTTAGGCTACGTAGCAG ATAAT-3’
rpsI	5’-GGAATTCCATATGGCTGAAAATCA ATACTACG-3’	5’-CCGCTCGAGTTAACGTTTGGAGAAC TGCG-3’
rpsJ	5’- GGAATTCCATATGCAGAACCAA AGAATCCG-3’	5’-CCGCTCGAGTTAACCCAGGCTGATC TGCA-3’
rpsK	5’-GGAATTCCATATGGCAAAGGCA CCAATTCG-3’	5’-CCGCTCGAGTTATACGCGACGTTTT TTCGG-3’
rpsL	5’-GGAATTCCATATGATCCAAGAACA GACTATG-3’	5’-CCGCTCGAGTTAGAGTACTTCTGGT GCCA-3’
rpsM	5’-GGAATTCCATATGGCCCGTATA GCAGGCA-3’	5’-CCGCTCGAGTTATTTCTTGATCGGT TTGCG-3’
rpsN	5’-GGAATTCCATATGGCTAAGCAATC AATGAAAG-3’	5’-CCGCTCGAGTTACCAGCTAGCCTTTTT CAG-3’

rpsO	5’-GGAATTCCATATGTCTCTAAGTAC TGAAGCA-3’	5’-CCGCTCGAGTTAGCGACGCAGACCCAG - 3’
rpsP	5’-GGAATTCCATATGGTAACTATTCG TTTAGCAC-3’	5’-CCGCTCGAGTTAAGCTGCTTTGTTTAC TTCT-3’
rpsQ	5’-GGAATTCCATATGACCGATAAAAT CCGTACT-3’	5’-CCGCTCGAGTTACAGAACCGCTTTCTC TAC-3’
rpsR	5’-GGAATTCCATATGGCACGTTATTTC CGTCG-3’	5’-CCGCTCGAGTTACTGATGGCGATCAGT GT -3’
rpsS	5’-GGAATTCCATATGCCACGTTCTCTC AAGAA-3’	5’-CCGCTCGAGTTATTTCTTCTTCGCTTT TTTATCA-3’
rpsT	5’-GGAATTCCATATGGCTAATATCAA ATCAGCTAA-3’	5’-CCGCTCGAGTTAAGCCAGTTTGTTGAT CTGT-3’
rpsU	5’-GGAATTCCATATGCCGGTAATTAA AGTACGT-3’	5’-CCGCTCGAGTTAGTACAGACGAGTGCG G-3’

Supplementary Table S7. PCR primers for 50S ribosomal proteins.

Gene	N-terminal	C-terminal
rplA	5’-GGAATTCCATATGGCTAAACTGAC CAAGCG-3’	5’-CCGCTCGAGTTAGTTTACAGAAGCGCT CAG -3’
rplB	5’-CATGCCATGGCAGTTGTTAAATGT AAACCG-3’	5’-CCGCTCGAGTTATTTGCTACGGCGACG TACG-3’
rplC	5’-GGAATTCCATATGATTGGTTTAGT CGGTAAAAA-3’	5’-CCGCTCGAGTTACGCCTTCACAGCTGG TT-3’
rplD	5’-GGAATTCCATATGGAATTAGTATT GAAAGACG-3’	5’-CCGCTCGAGTCATGCCAGCATCTCCTC AA-3’
rplE	5’-GGAATTCCATATGGCGAAACTGCA TGATTAC-3’	5’-CCGCTCGAGTTACTTGCGGAACGGGAA GT-3’
rplF	5’-GGAATTCCATATGTCTCGTGTTGC TAAAGC-3’	5’-CCGCTCGAGTTACTTCTTCTTAGCCTC TTTG-3’
rplI	5’-GGAATTCCATATGCAAGTTATTCT GCTTGATA-3’	5’-CCGCTCGAGTTATTCAGCTACTACGTT TACG-3’
rplJ	5’-GGAATTCCATATGGCTTTAAATCT TCAAGACAA-3’	5’-CCGCTCGAGTTAAGCAGCTTCTTTCGC ATC-3’
rplK	5’-GGAATTCCATATGGCTAAGAAAGT ACAAGCC-3’	5’-CCGCTCGAGTTAGTCCTCCACTACCA GG-3’
rplL	5’-GGAATTCCATATGTCTATCACTAA AGATCAAAT-3’	5’-CCGCTCGAGTTATTTAACTTCAACTTC AGCG-3’
rplM	5’-GGAATTCCATATGAAAACTTTTAC AGCTAAACC-3’	5’-CCGCTCGAGTTAGATGTCAAGAACTTG CGG-3’
rplN	5’-GGAATTCCATATGATCCAAGAACA GACTATG-3’	5’-CCGCTCGAGTTAGAGTACTTCTGGTGC CA-3’
rplO	5’-GGAATTCCATATGCGTTTAAATAC TCTGTCTC-3’	5’-CCGCTCGAGTTATTCCTCGATTTTACC GCC-3’
rplP	5’-GGAATTCCATATGTTACAACCAAA GCGTACAA-3’	5’-CCGCTCGAGTTACATCACCGTCTTAGT TACA-3’

rplQ	5’-GGAATTCCATATGCGCCATCGTAA GAGTG-3’	5’-CCGCTCGAGTTACTCTGCAGCAGCTTC TG-3’
rplR	5’-GGAATTCCATATGGATAAGAAATC TGCTCGT-3’	5’-CCGCTCGAGTTAGAACTGAAGGCCAGC TT-3’
rplS	5’-GGAATTCCATATGAGCAACATTAT TAAGCAAC-3’	5’-CCGCTCGAGTTAGTTAAGACGCTCTTT GATA-3’
rplT	5’-GGAATTCCATATGGCTCGCGTAAA ACGTG-3’	5’-CCGCTCGAGTTATGCCAGAGCTGCTTT CG-3’
rplU	5’-GGAATTCCATATGTACGCGGTTTTC CAAAG-3’	5’-CCGCTCGAGTTAGGCGCTGATGCCAGT AA-3’
rplV	5’-GGAATTCCATATGGAAACTATCGC TAAACATC-3’	5’-CCGCTCGAGTCAGCGATCGGACACAAC C-3’
rplW	5’-GGAATTCCATATGATTCGTGAAGA ACGTCT-3’	5’-CCGCTCGAGTTACTCAGCGCCGCCAAC-3’
rplX	5’-GGAATTCCATATGGCAGCGAAAAT CCGTC-3’	5’-CCGCTCGAGTTACTTGATAGTTTCGCT GTTA-3’
rplY	5’-GGAATTCCATATGTTTACTATCAA CGCAGAAG-3’	5’-CCGCTCGAGTTAAGCGCGAACGAAGTC GA-3’

Supplementary Table S8. Mass spectrum analysis of co-expressed 30S ribosomal proteins in PURE system. Newly synthesized 30S ribosomal proteins are labelled with ^{13}C on lysine and arginine (heavy form), while the other protein components in PURE system are all in the ^{12}C light form. Samples are first alkylated by iodoacetamide, precipitated by trichloroacetic acid, and finally digested by trypsin before subjected to mass spectroscopy. Spectra count data of detected peptides are presented by their encoded genes.

Reference	assigned_MS/MS_spectra (L)	assigned_MS/MS_spectra (H)	median_log2_H/L_protein_ratio
AlaRS	120	0	<-6.44
ArgRS	24	0	<-3.77
AsnRS	42	0	<-7.07
AspRS	61	0	<-5.38
CysRS	13	0	<-4.06
GlnRS	34	0	<-4.78
GluRS	34	0	<-6.29
GlyRS	68	0	<-5.32
HisRS	55	0	<-5.18
IleRS	111	0	<-6.42
LeuRS	55	0	<-4.32
LysRS	15	0	<-6.13
MetRS	45	0	<-4.96
PheRS	29	0	<-5.56
ProRS	55	0	<-5.75
SerRS	14	0	-3.68
ThrRS	41	0	<-5.00
TrpRS	9	0	-3.76
TyrRS	14	0	-3.85
ValRS	30	0	<-3.95
IF1	18	0	<-5.41
IF2	94	0	<-5.83
IF3	32	0	<-5.20
EF-G	84	0	-7.05
EF-Ts	53	0	<-6.70
EF-Tu	216	1	<-6.30
RF1	29	0	<-5.75
RF2	27	0	-5.49
RF3	31	0	<-5.01
RRF	14	0	-6.00
T7	66	0	<-4.55
MTF	38	0	-5.84
myokinase	6	0	-5.46
nucleoside-diphosphate	8	0	-4.43
rplA	63	0	<-6.42

rplB	58	0	<-7.68
rplC	21	0	<-9.90
rplD	28	0	<-8.23
rplE	67	0	<-6.96
rplF	31	0	-7.91
rplI	27	0	<-7.50
rplJ	18	0	-7.94671
rplK	46	0	<-6.38
rplL	25	0	-7.61
rplM	20	0	<-6.05
rplN	28	0	-5.54
rplO	17	0	<-8.39
rplP	21	0	-6.68
rplQ	23	0	<-7.83
rplR	10	0	-7.69
rplS	19	0	<-7.92
rplT	12	0	-5.97
rplU	7	0	-7.47
rplV	31	0	<-7.62
rplW	4	0	-4.74
rplX	26	0	<-7.19
rplY	20	0	<-6.71
rpmA	3	0	<-4.48
rpmB	8	0	<-7.39
rpmC	10	0	-6.35
rpmD	10	0	<-7.02
rpmE	12	0	<-5.40
rpmF	4	0	<-4.34
rpmG	2	0	<-6.47
rpmH	n/a	n/a	n/a
rpmI	2	0	<-8.68
rpmJ	n/a	n/a	n/a
rpsA_S1	95	37	-3.58
rpsB_S2	50	15	-5.12
rpsC_S3	60	28	-1.14
rpsD_S4	34	12	-3.99
rpsE_S5	80	10	-5.08
rpsF_S6	19	16	-0.40
rpsG_S7	42	22	-2.24
rpsH_S8	19	11	-3.67
rpsI_S9	25	13	-1.28
rpsJ_S10	16	9	-2.70
rpsK_S11	10	16	0.00
rpsL_S12	8	2	-4.18
rpsM_S13	21	6	-5.52
rpsN_S14	5	2	-3.47
rpsO_S15	9	2	-5.67
rpsP_S16	16	13	-1.18

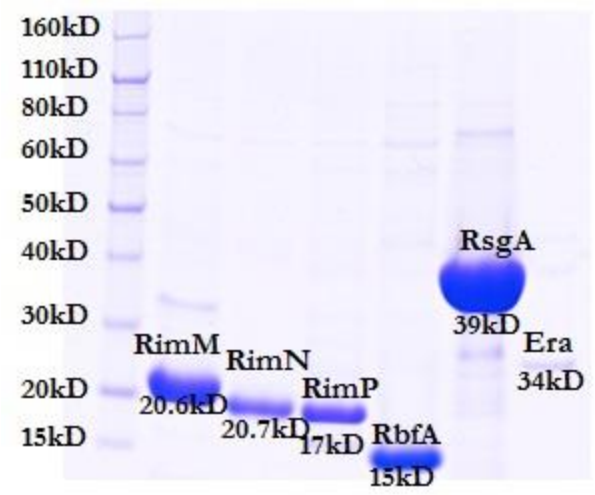
rpsQ_S17	8	1	-3.00
rpsR_S18	4	0	-8.13
rpsS_S19	7	0	-4.60
rpsT_S20	3	2	-0.40
rpsU_S21	3	2	-4.74

Supplementary Table S9. Mass spectrum analysis of co-expressed 50S ribosomal proteins in PURE system. Newly synthesized 50S ribosomal proteins are labelled with ^{13}C on lysine and arginine (heavy form), while the other protein components in PURE system are all in the ^{12}C light form. Samples are first alkylated by iodoacetamide, precipitated by trichloroacetic acid, and finally digested by trypsin before subjected to mass spectroscopy. Spectra count data of detected peptides are presented by their encoded genes.

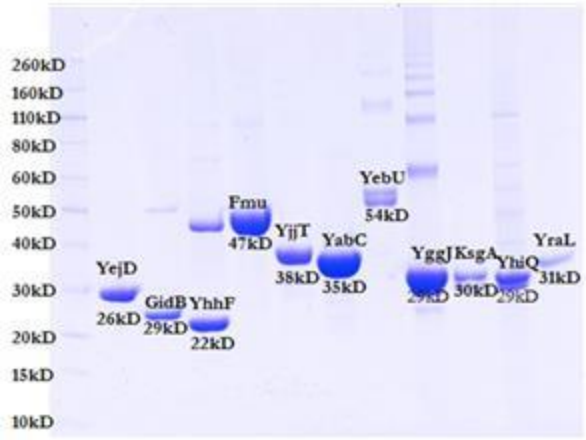
Reference	assigned_MS/MS_spectra (L)	assigned_MS/MS_spectra (H)	median_log2_H/L_protein_ratio
AlaRS	88	0	<-6.85
ArgRS	16	0	<-4.52
AsnRS	27	0	-6.63
AspRS	33	0	<-5.60
CysRS	5	0	-4.53
GlnRS	16	0	<-5.12
GluRS	21	0	<-6.17
GlyRS	35	0	<-5.69
HisRS	25	0	<-5.32
IleRS	69	0	<-6.54
LeuRS	39	0	<-5.26
LysRS	12	0	-5.98
MetRS	25	0	<-5.36
PheRS	16	0	<-5.64
ProRS	30	0	<-5.62
SerRS	16	0	<-5.66
ThrRS	17	0	<-5.02
TrpRS	3	0	<-4.65
TyrRS	5	0	<-4.11
ValRS	16	0	<-5.29
IF1	20	0	<-5.02
IF2	19	0	<-5.65
IF3	23	0	-6.17
EF-G	55	0	-6.58
EF-Ts	35	0	-6.43
EF-Tu	105	0	<-6.30
RF1	20	0	<-6.25
RF2	19	0	<-7.01
RF3	23	0	<-6.04
RRF	15	0	<-7.36
T7	33	0	<-5.12
MTF	21	0	-7.64
myokinase	5	0	<-6.77
nucleoside-diphosphate	5	0	-6.04
rplA	30	7	-5.99

rplB	48	32	-1.98
rplC	16	10	-4.69
rplD	22	8	-5.16
rplE	32	0	-6.14
rplF	31	4	-7.23
rplI	27	7	-4.71
rplJ	21	1	-6.33
rplK	35	0	-7.49
rplL	11	2	-5.07
rplM	18	6	-3.31
rplN	18	2	-5.55
rplO	18	7	-3.81
rplP	16	5	-3.61
rplQ	20	2	-6.68
rplR	9	0	-5.19
rplS	13	0	-5.31
rplT	11	0	-7.21
rplU	11	0	-4.35
rplV	27	4	-5.75
rplW	3	2	-2.59
rplX	15	4	-3.36
rplY	16	1	-4.77
rpmA	5	1	-5.64
rpmB	6	0	-1.94
rpmC	5	0	-3.40
rpmD	7	0	-6.30
rpmE	8	6	-2.37
rpmF	3	0	-2.96
rpmG	4	0	-5.87
rpmH	n/a	n/a	n/a
rpmI	1	0	-3.66
rpmJ	n/a	n/a	n/a
rpsA_S1	45	0	<-6.56
rpsB_S2	39	0	-8.40
rpsC_S3	30	0	<-8.03
rpsD_S4	25	0	<-8.84
rpsE_S5	30	0	-7.01
rpsF_S6	13	0	-5.22
rpsG_S7	27	0	<-5.32
rpsH_S8	13	0	-5.40
rpsI_S9	20	0	-6.04
rpsJ_S10	13	0	<-7.59
rpsK_S11	8	0	-7.67
rpsL_S12	6	0	-5.43
rpsM_S13	19	0	<-6.83
rpsN_S14	n/a	n/a	n/a
rpsO_S15	3	0	<-9.36
rpsP_S16	11	0	-4.93

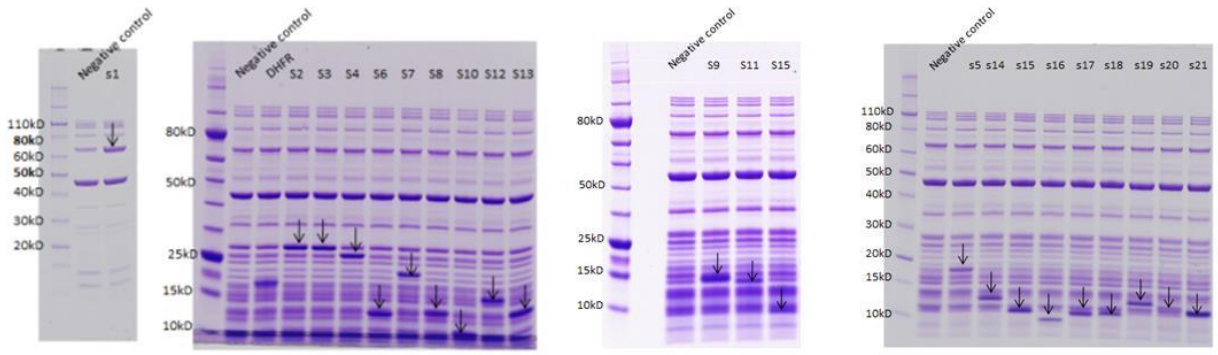
rpsQ_S17	3	0	-8.73
rpsR_S18	3	0	<-8.65
rpsS_S19	2	0	-6.70
rpsT_S20	3	0	<-7.51
rpsU_S21	5	0	-5.13



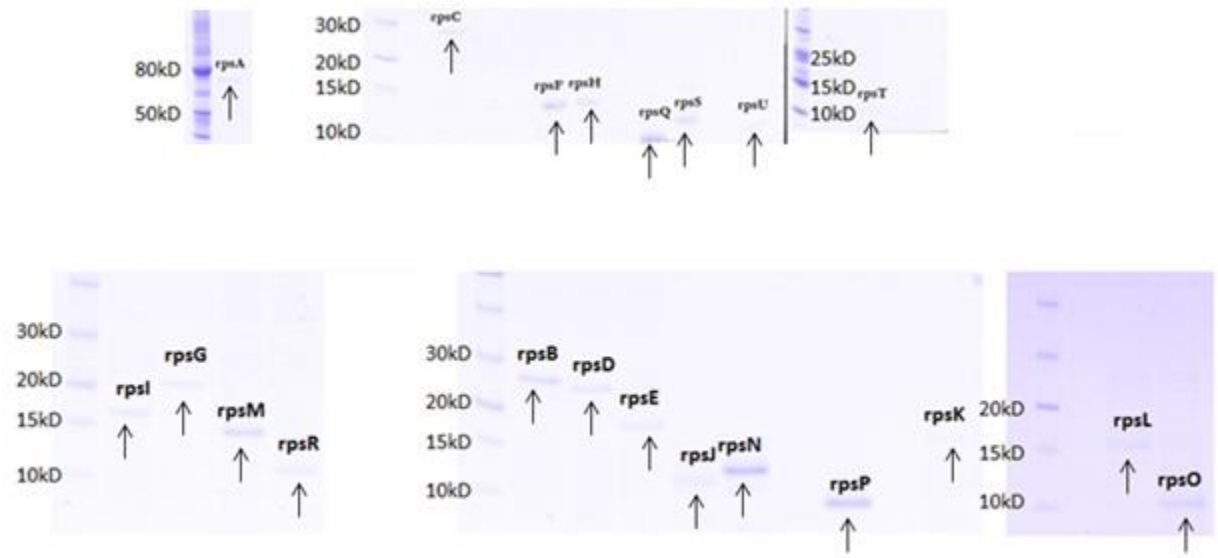
Supplementary Figure S1. **Assessment of purified 30S ribosome assembly cofactors.** RimM, RimN, RimP, RbfA, RsgA were his-tagged, overexpressed in *E. coli* and purified using Ni-NTA affinity purification. Strep-tagged Era was expressed in PURE system, purified using Strep-Tactin affinity purification. Purified factors were analyzed on 4-12% Bis-Tris PAGE gel, stained by Coomassie-blue.



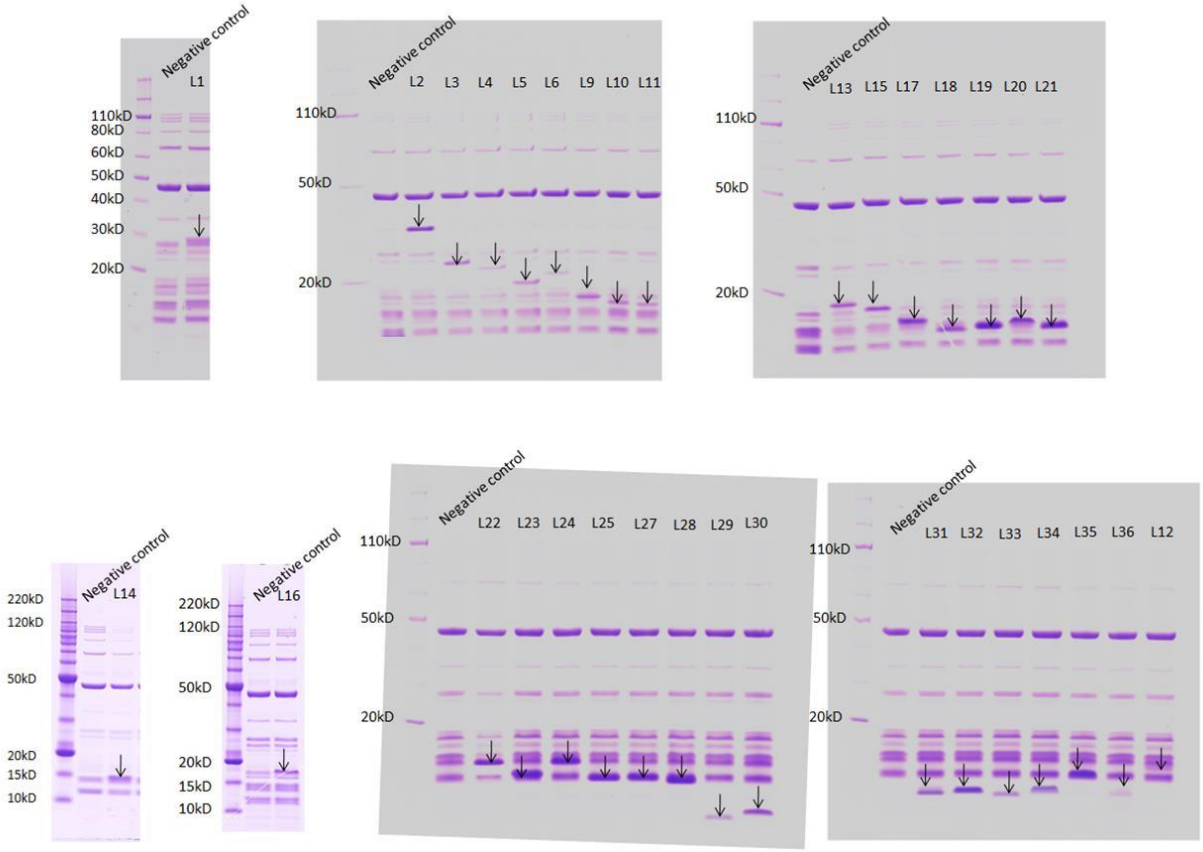
Supplementary Figure S2. Assessment of purified 16S rRNA modification enzymes. Enzymes were his-tagged, overexpressed in *E. coli* and purified using Ni-NTA affinity purification. Purified enzymes were analyzed on 4-12% Bis-Tris PAGE gel, stained by Coomassie-blue.



Supplementary Figure S3. SDS PAGE analysis of 21 30S ribosomal proteins expressed in PURE system by Coomassie Blue staining. Plasmids encoding 30S ribosomal proteins were added to PURE system for expression. After 2 hrs incubation, samples were analyzed directly on 4-12% Bis-Tris PAGE gel, stained by Coomassie-blue. PURE system reaction with no DNA template was taken as negative control. The expected migration of each protein is marked on the gel.



Supplementary Figure S4. **Assessment of purified 30S ribosomal proteins synthesized in PURE system.** 30S ribosomal proteins were expressed in PURE system and then purified using reverse histag purification method. Purified proteins were analyzed on 4-12% Bis-Tris PAGE gel, stained by Coomassie-blue. The expected migration bands are marked on the gel.



Supplementary Figure S5. SDS PAGE analysis of 33 50S ribosomal proteins expressed in PURE system by Coomassie Blue staining. Plasmids encoding 50S ribosomal proteins were added to PURE system for expression. After 2 hrs incubation, samples were analyzed directly on 4-12% Bis-Tris PAGE gel, stained by Coomassie-blue. PURE system reaction with no DNA template was taken as negative control. The expected migration of each protein is marked on the gel.

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