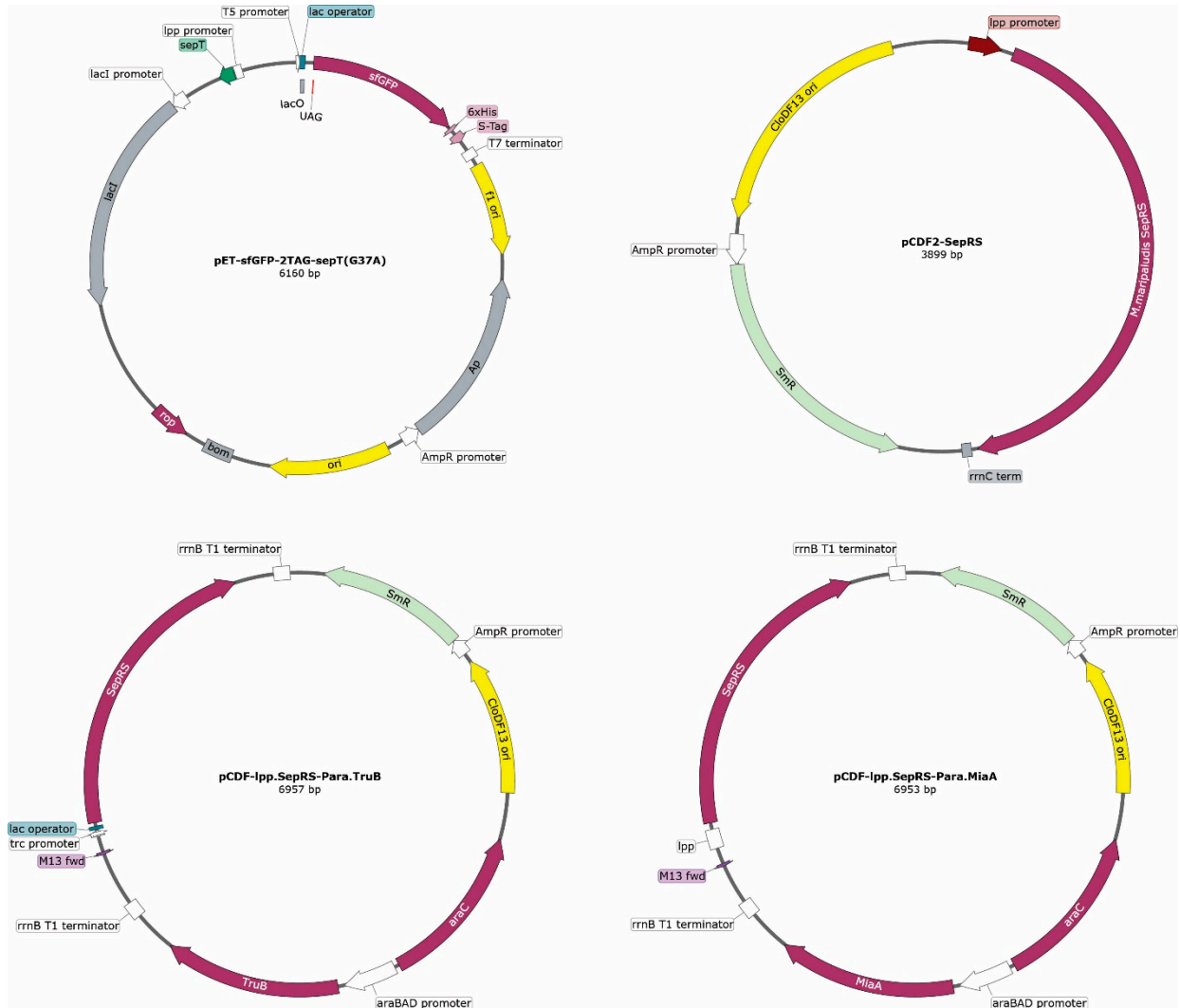
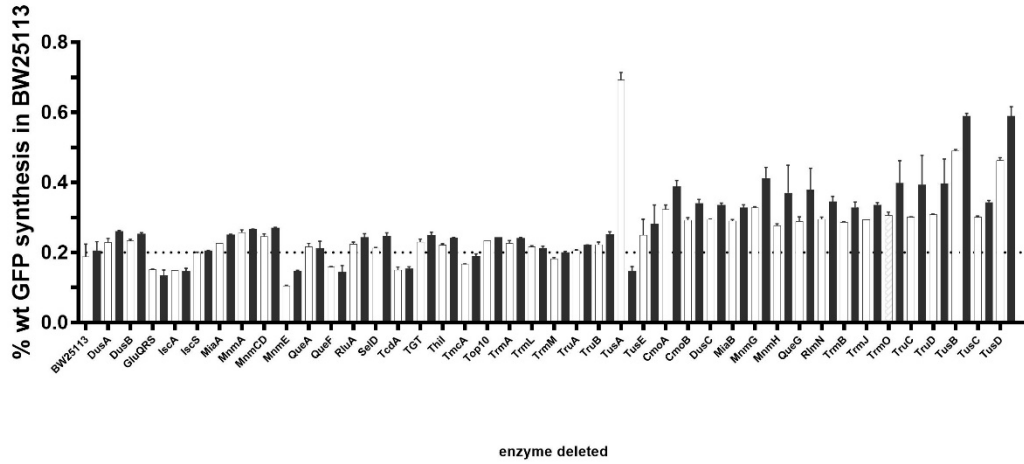


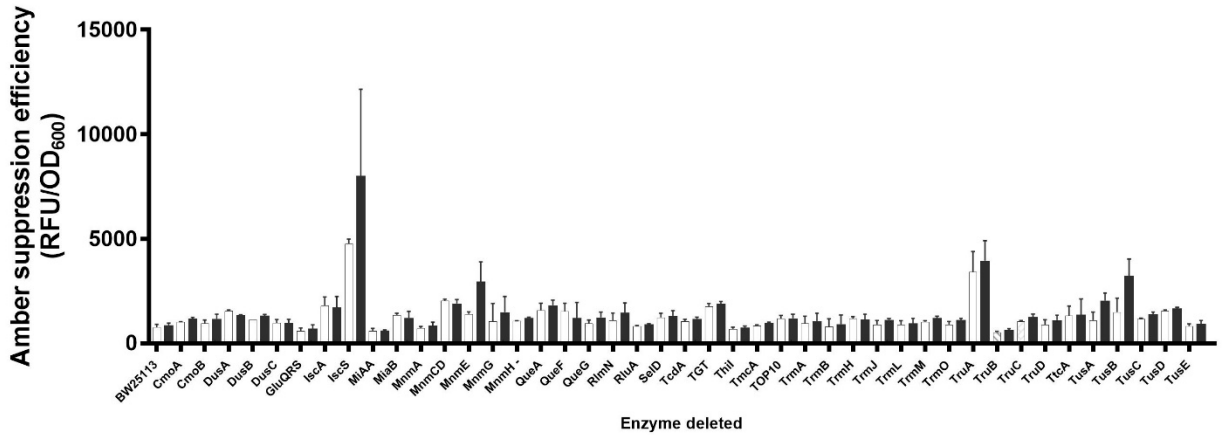
Supplementary material for Heterologous tRNA modifications influence o-tRNA orthogonality and OTS efficiency by Ana Crnković, Oscar Vargas-Rodriguez, Anna Merkurjev and Dieter Söll



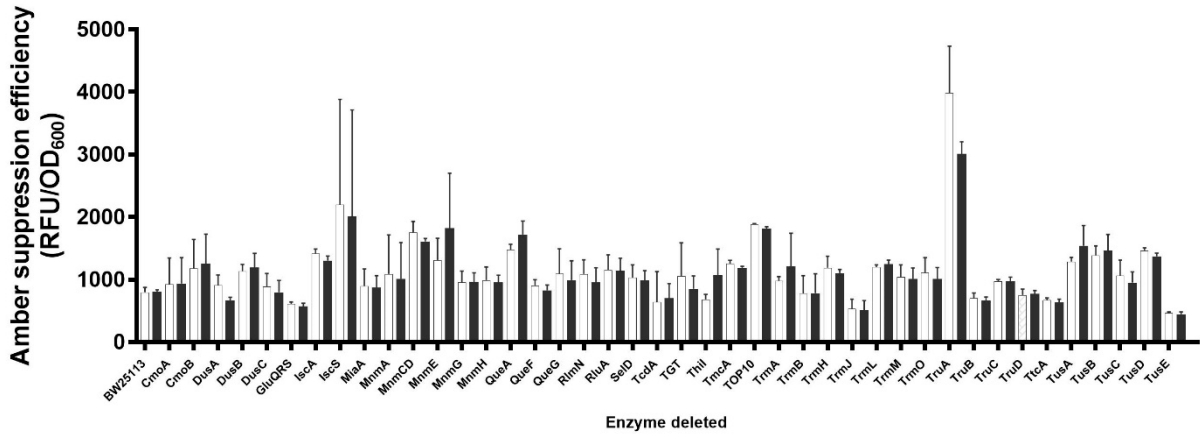
Supplementary Figure S1. Maps of plasmids used in this study.



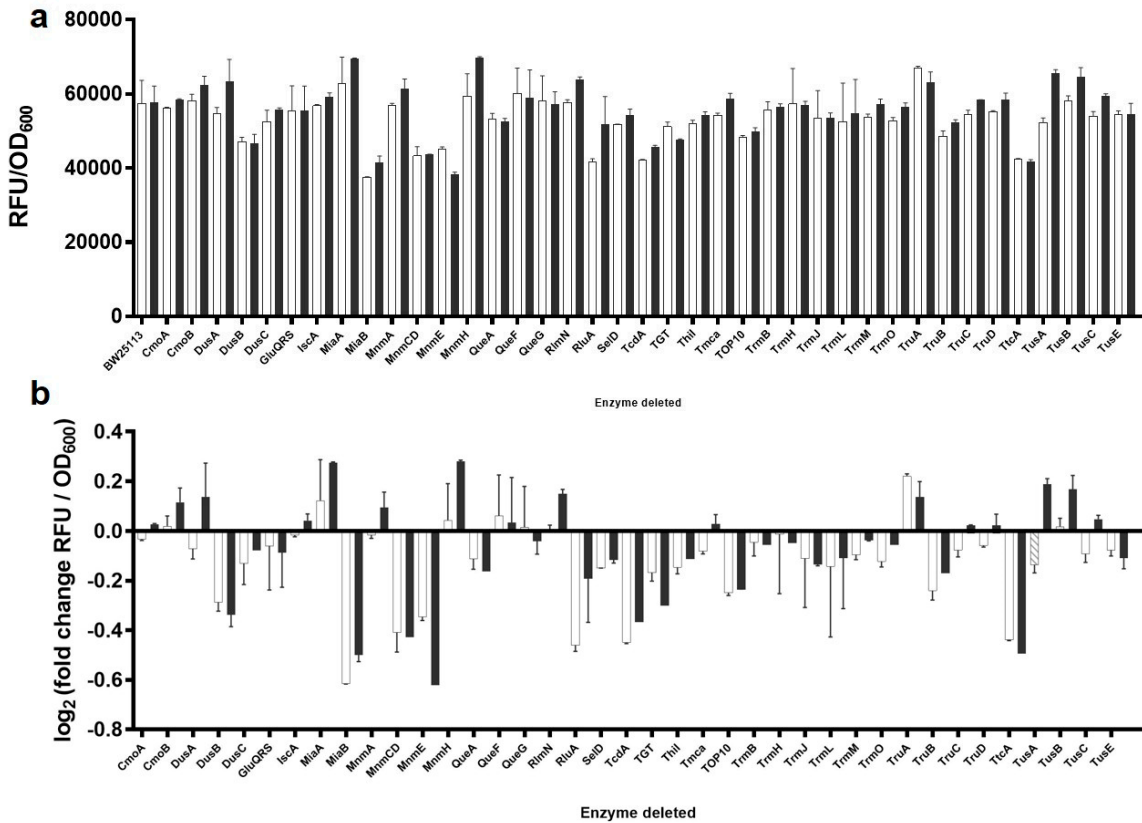
Supplementary Figure S2. Levels of near cognate suppression (measured as sfGFP-2TAG synthesis in the absence of tRNA^{Sep}) in the investigated Keio deletion strains are not markedly higher than those of commonly employed strains. The levels of suppression are expressed relative to the mean wild-type GFP fluorescence obtained from the parental BW25113 strain. Cells were grown in LB medium without supplementation (empty columns) or with 5 mM Sep added (black columns). Values are mean +/- S.D., N=3.



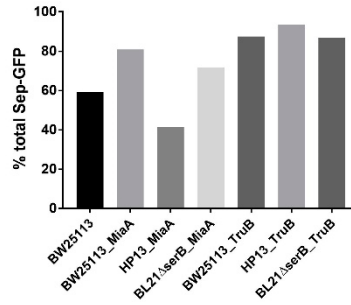
Supplementary Figure S3. Levels of amber codon suppression efficiency (measured as sfGFP-2TAG synthesis) in the selected Keio deletion strains in presence of Sep-OTS. Cells were grown in LB medium without supplementation (empty columns) or with 5 mM Sep added (black columns). Values are mean +/- S.D., N=4-8.



Supplementary Figure S4. Levels of amber codon suppression efficiency (measured as sfGFP-2TAG synthesis) in the selected Keio deletion in presence of nonsense suppressor tRNA^{SepCUA}^{G37A}. Cells were grown in LB medium without supplementation (empty columns) or with 5 mM Sep added (black columns). Values are mean +/- S.D., N=3-6.



Supplementary Figure S5. Levels of wild type sfGFP synthesis in the tested Keio collection strains expressed in relative fluorescence units (a) and as fold change with respect to the synthesis in parental strain BW25113 (b). Cells were grown in LB medium without supplementation (empty columns) or with 5 mM Sep added (black columns). Values are mean +/- S.D., N=2-3.



Supplementary Figure S6. Quantification of the shifted bands corresponding to Sep-GFP yields observed in presence of increased amounts of MiaA and TruB (Fig. 5B). Percentages of Sep-modified GFP in the corresponding isolate are shown.

Supplementary Table S1. List of Keio knockout strains employed in this study. Adapted according to MODOMICS database ([1], <http://modomics.genesisilico.pl>).

	Synonym	Full name	RNA substrate	Modification type	Position in tRNA	Substrate base present in Mj <i>tRNA</i> ^{SEP_{CUA}G37A}
CmoA	YecO	tRNA (uridine-5-oxyacetic acid methyl ester)(34) synthase	tRNA	cmo ⁵ U, mcmo ⁵ U	34	no
CmoB	YecP	tRNA 5-methoxyuridine(34) synthase	tRNA	cmo ⁵ U, mcmo ⁵ U	34	no
DusA	YjbN	tRNA dihydrouridine synthase A	tRNA	D	20	
DusB	YhdG	tRNA dihydrouridine synthase B	tRNA	D	16, 17, 20, 20a	yes (U20)
DusC	YohI	tRNA dihydrouridine synthase C	tRNA	D	16, 17, 20, 20a	yes (U20)
GluQRS	YadB	tRNA glutamyl-Q(34) synthetase	tRNA	gluQtRNA	34	no
IscA	YfhF	Iron-binding protein IscA	none	none	none	-
IscS	NuvC	Cysteine desulfurase IscS subfamily	none	none	none	-
MiaA	TrpX	tRNA (adenosine(37)-N6)-dimethylallyltransferase	tRNA	i ⁶ A	37	yes
MiaB	YleA	tRNA (N6-isopentenyl adenosine(37)-C2)-methylthiotransferase	tRNA	ms ² i ⁶ A	37	yes
MnmA	TrmU, AsuE, YcfB	tRNA 2-thiouridine(34) synthase	tRNA	s ² U, mnm ⁵ s ² U	34	no
MnmCD	TrmC, MnmC	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase / FAD-dependent cmnm(5)s(2)U34 oxidoreductase	tRNA	mnm ⁵ s ² U, mnm ⁵ U	34	no

MnmE	ThdF, TrmE	tRNA uridine(34) 5- carboxymethylaminomethyl synthesis GTPase	tRNA	mnm ⁵ s ² U, cmnm ⁵ s ² U, cmnm ⁵ Um, mnm ⁵ U	34	no
MnmG	GidA, TrmF	tRNA uridine(34) 5- carboxymethylaminomethyl synthesis enzyme	tRNA	mnm ⁵ s ² U, cmnm ⁵ s ² U, cmnm ⁵ Um, mnm ⁵ U	34	no
MnmH	SelU, YbbB	tRNA 2-selenouridine(34) synthase	tRNA	cmnm ⁵ ges ² U, mnm ⁵ se ² U, mnm ⁵ ges ² U	34	no
QueA		tRNA preQ1(34) S- adenosylmethionine ribosyltransferase-isomerase	tRNA	QtRNA	34	no
QueF	YgcD	preQ1 synthase	tRNA	QtRNA	34	no
QueG	YjeS	tRNA epoxyqueuosine(34) reductase	tRNA	QtRNA	34	no
RlmN	TrmG, YfgB	tRNA (adenosine(37)-C2)- methyltransferase / Ribosomal RNA large subunit methyltransferase N	tRNA,rRNA	m ² A	37	yes
RluA	YabO	tRNA pseudouridine(32) synthase / Ribosomal large subunit pseudouridine synthase A	tRNA,rRNA	Y	32	no
SelD	FdhB	Selenide,water dikinase	none	none	none	-
TcdA	YgdL, CsdL	tRNA threonylcarbamoyladenine dehydratase A	tRNA	ct ⁶ A	37	yes
Tgt	JW0396	tRNA guanosine(34) transglycosylase	tRNA	QtRNA	34	no
ThiI	NuvA	tRNA 4-thiouridine(8) synthase	tRNA	s ⁴ U	8	yes
TmcA	YpfI, JW2459	tRNA cytidine(34) acetyltransferase	tRNA	ac ⁴ C	34	yes
TrmA	RumT	tRNA (uridine(54)-C5)- methyltransferase	tRNA	m ⁵ U	54	yes
TrmB	YggH	tRNA (guanine(46)-N7)- methyltransferase	tRNA	m ⁷ G	46	yes
TrmH	SpoU	tRNA (guanosine(18)-2'-O)- methyltransferase	tRNA	Gm	18	yes
TrmJ	YfhQ, JW2516	tRNA (cytidine(32)/uridine(32)-2'- O)-methyltransferase	tRNA	Um, Cm	32	yes(C32)
TrmL	YibK	tRNA (cytidine(34)-2'-O)- methyltransferase	tRNA	Cm, cmnm ⁵ Um	34	yes
TrmM	YfiC, TrmN6	tRNA (adenosine(37)-N6)- methyltransferase	tRNA	m ⁶ A	37	yes
TrmO	TsaA, YaeB	tRNA-methyltransferase O	tRNA	m ⁶ t ⁶ A	37	yes

TruA	HisT, AsuC, HisT, LeuK	tRNA pseudouridine(38,39,40) synthase	tRNA	Y	38, 39, 40	yes(U39)
TruB	YhbA	tRNA pseudouridine(55) synthase	tRNA	Y	55	yes
TruC	YqcB	tRNA pseudouridine(65) synthase	tRNA	Y	65	no
TruD	YgbO	tRNA pseudouridine(13) synthase	tRNA	Y	13	yes
TtcA	YdaO	tRNA 2-thiocytidine(32) synthetase	tRNA	s ² C	32	yes
TusA	sirA, yhhP	tRNA 2-thiouridine(34) synthase	tRNA	none	none	-
TusB	yheL	tRNA 2-thiouridine(34) synthase	tRNA	none	none	-
TusC	yheM	tRNA 2-thiouridine(34) synthase	tRNA	none	none	-
TusD	yheN	tRNA 2-thiouridine(34) synthase	tRNA	none	none	-
TusE	yccK	tRNA 2-thiouridine synthesizing protein E	tRNA	none	none	-

Supplementary Table S2. Plasmids used in this study.

Name	Description	Antibiotic resistance	* Source
pET-sfGFP-2TAG -sepT ^a	pET-Duet1 containing the gene encoding sfGFP with S2TAG mutation and <i>sepT</i> encoding amber suppressor derived from <i>Methanocaldococcus jannaschii</i> tRNA ^{Cys}	Amp	This study
pET-sfGFP-2TAG	identical to pET-sfGFP-2TAG -sepT but without the <i>sepT</i> gene		
pET-sfGFP	identical to pET-sfGFP-2TAG but with a wild type sfGFP sequence		
pET-sfGFP-2TAG -sepT(G37A)	pET-Duet1 containing the gene encoding sfGFP with S2TAG mutation and <i>sepT</i> with a G37A mutation	Amp	This study
pET-sfGFP-2TAG -sepT-Trm5	pET-Duet1 containing the gene encoding sfGFP with S2TAG mutation, <i>Methanococcus maripaludis</i> Trm5 gene under <i>lpp</i> promoter and <i>sepT</i> encoding amber suppressor derived from <i>M. jannaschii</i> tRNA ^{Cys}		
pET-sfGFP151TAG-sepT ^{G37A}	pET-Duet1 containing the gene encoding sfGFP with Y151TAG mutation and <i>sepT</i> gene with a G37A mutation	Amp	This study
pCDF2-SepRS	pCDF2 containing gene encoding SepRS ^b	Sp	This study

pCDF-lpp.SepRS-Para.MiaA	pCDF containing the gene encoding SepRS under lpp and MiaA under arabinose promoter	Sp	This study
pCDF-lpp.SepRS-Para.TruB	pCDF containing the gene encoding SepRS under lpp and TruB under arabinose promoter	Amp	This study

^a In all pET-sfGFP constructs, GFP gene was placed under the control of T5, and tRNA (if present) under an lpp promoter

^bGene for SepRS was under lpp promoter

Supplementary Table S3. Statistical analysis of the fluorescence measurements obtained for the amber codon suppression efficiency (sfGFP-2TAG synthesis) in the selected Keio deletion strains in presence of Sep-OTS. The fluorescence measurements were calculated as a log₂ fold change relative to the BW25113 strain. The results of the Student's t-test are shown. Theoretical mean value is 0. Supplementation with 5 mM Sep is indicated by "+".

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
cmoA	0.4212	0.01414	0.006325	0.4212	0.4212	0.4036 to 0.4387	t=66.59 df=4	<0.0001	Yes
cmoA +	0.6328	0.06042	0.03488	0.6328	0.6328	0.4828 to 0.7829	t=18.14 df=2	0.003	Yes
cmoB	0.2968	0.2406	0.1076	0.2968	0.2968	-0.001901 to 0.5956	t=2.759 df=4	0.0509	No
cmoB +	0.5878	0.2933	0.1312	0.5878	0.5878	0.2237 to 0.952	t=4.482 df=4	0.011	Yes
dusA	1.025	0.02839	0.01639	1.025	1.025	0.9545 to 1.096	t=62.53 df=2	0.0003	Yes
dusA +	0.8149	0.0113	0.006525	0.8149	0.8149	0.7869 to 0.843	t=124.9 df=2	<0.0001	Yes
dusB	0.5722	0.001129	0.000652	0.5722	0.5722	0.5694 to 0.575	t=877.8 df=2	<0.0001	Yes
dusB +	0.7879	0.04469	0.0258	0.7879	0.7879	0.6769 to 0.8989	t=30.54 df=2	0.0011	Yes
dusC	0.329	0.2333	0.1043	0.329	0.329	0.03931 to 0.6187	t=3.153 df=4	0.0344	Yes
dusC +	0.3225	0.2566	0.1148	0.3225	0.3225	0.003804 to 0.6411	t=2.81 df=4	0.0483	Yes
qluQRS	-0.3944	0.3295	0.1647	-0.3944	-0.3944	-0.9188 to 0.1299	t=2.394 df=3	0.0964	No
gluQRS +	-0.1599	0.3728	0.1864	-0.1599	-0.1599	-0.7532 to 0.4333	t=0.8579 df=3	0.454	No
iscA	1.209	0.3327	0.1664	1.209	1.209	0.6791 to 1.738	t=7.265 df=3	0.0054	Yes
iscA +	1.111	0.4526	0.2263	1.111	1.111	0.3913 to 1.832	t=4.912 df=3	0.0162	Yes
iscS	2.641	0.06597	0.02693	2.641	2.641	2.572 to 2.71	t=98.06 df=5	<0.0001	Yes
iscS +	3.249	0.682	0.2784	3.249	3.249	2.533 to 3.965	t=11.67 df=5	<0.0001	Yes
miAA	-0.3791	0.2525	0.1262	-0.3791	-0.3791	-0.7808 to 0.02259	t=3.003 df=3	0.0575	No
miaA +	-0.3474	0.0793	0.03965	-0.3474	-0.3474	-0.4736 to -0.2213	t=8.762 df=3	0.0031	Yes
miaB	0.8201	0.08743	0.0391	0.8201	0.8201	0.7115 to 0.9286	t=20.97 df=4	<0.0001	Yes
miaB +	0.6187	0.4018	0.1797	0.6187	0.6187	0.1198 to 1.118	t=3.443 df=4	0.0262	Yes
mnmA	-0.09548	0.1624	0.08121	-0.09548	-0.09548	-0.3539 to 0.163	t=1.176 df=3	0.3245	No

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
mnmA +	0.105	0.3079	0.1539	0.105	0.105	-0.3849 to 0.5949	t=0.6823 df=3	0.544	No
mnmC D	1.415	0.04914	0.02457	1.415	1.415	1.337 to 1.494	t=57.6 df=3	<0.0001	Yes
mnmC D +	1.316	0.1308	0.0654	1.316	1.316	1.108 to 1.524	t=20.12 df=3	0.0003	Yes
mnmE	0.8683	0.1062	0.04748	0.8683	0.8683	0.7365 to 1	t=18.29 df=4	<0.0001	Yes
mnmE +	1.887	0.4985	0.2229	1.887	1.887	1.268 to 2.506	t=8.466 df=4	0.0011	Yes
mnmG 1	0.02361	1.238	0.5536	0.02361	0.02361	-1.513 to 1.561	t=0.04264 df=4	0.968	No
mnmG +	0.7424	1.016	0.4545	0.7424	0.7424	-0.5194 to 2.004	t=1.634 df=4	0.1777	No
mnmH	0.4887	0.01468	0.008477	0.4887	0.4887	0.4522 to 0.5252	t=57.65 df=2	0.0003	Yes
mnmH +	0.6717	0.02601	0.01502	0.6717	0.6717	0.6071 to 0.7363	t=44.73 df=2	0.0005	Yes
queA	1.033	0.2964	0.1482	1.033	1.033	0.5608 to 1.504	t=6.966 df=3	0.0061	Yes
queA +	1.231	0.2062	0.1031	1.231	1.231	0.9033 to 1.56	t=11.94 df=3	0.0013	Yes
queF	0.9549	0.3596	0.1608	0.9549	0.9549	0.5084 to 1.402	t=5.937 df=4	0.004	Yes
queF +	0.3354	1.144	0.5115	0.3354	0.3354	-1.085 to 1.756	t=0.6557 df=4	0.5478	No
queG	0.2985	0.2533	0.1266	0.2985	0.2985	-0.1045 to 0.7015	t=2.357 df=3	0.0996	No
queG +	0.6772	0.2897	0.1449	0.6772	0.6772	0.2162 to 1.138	t=4.675 df=3	0.0185	Yes
rlmN	0.4471	0.4562	0.204	0.4471	0.4471	-0.1193 to 1.014	t=2.192 df=4	0.0935	No
rlmN +	0.874	0.4922	0.2201	0.874	0.874	0.2629 to 1.485	t=3.971 df=4	0.0165	Yes
rluA	0.1163	0.03216	0.01608	0.1163	0.1163	0.06512 to 0.1675	t=7.231 df=3	0.0055	Yes
rluA +	0.2611	0.03212	0.01606	0.2611	0.2611	0.21 to 0.3122	t=16.26 df=3	0.0005	Yes
selD	0.6873	0.2211	0.1106	0.6873	0.6873	0.3354 to 1.039	t=6.217 df=3	0.0084	Yes
selD +	0.7591	0.2774	0.1387	0.7591	0.7591	0.3176 to 1.201	t=5.472 df=3	0.012	Yes
tcdA	0.4659	0.124	0.062	0.4659	0.4659	0.2686 to 0.6632	t=7.514 df=3	0.0049	Yes
tcdA +	0.617	0.08613	0.04307	0.617	0.617	0.48 to 0.7541	t=14.33 df=3	0.0007	Yes
tgt	1.205	0.1109	0.05546	1.205	1.205	1.028 to 1.381	t=21.72 df=3	0.0002	Yes
tgt +	1.309	0.0794	0.0397	1.309	1.309	1.183 to 1.436	t=32.99 df=3	<0.0001	Yes
thiI	-0.1781	0.1869	0.09343	-0.1781	-0.1781	-0.4754 to 0.1193	t=1.906 df=3	0.1527	No
thiI +	-0.04168	0.1574	0.0787	-0.04168	-0.04168	-0.2921 to 0.2088	t=0.5296 df=3	0.6331	No
tmcA	0.1532	0.04364	0.02182	0.1532	0.1532	0.08379 to 0.2227	t=7.022 df=3	0.0059	Yes
tmcA +	0.3442	0.05134	0.02567	0.3442	0.3442	0.2625 to 0.4259	t=13.41 df=3	0.0009	Yes
TOP10	0.6162	0.173	0.0865	0.6162	0.6162	0.3409 to 0.8915	t=7.124 df=3	0.0057	Yes
TOP10 +	0.625	0.23	0.115	0.625	0.625	0.259 to 0.9911	t=5.434 df=3	0.0122	Yes

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
trmA	0.2308	0.5573	0.2786	0.2308	0.2308	-0.656 to 1.117	t=0.8282 df=3	0.4683	No
trmA +	0.393	0.5327	0.2663	0.393	0.393	-0.4546 to 1.241	t=1.476 df=3	0.2365	No
trmB	-0.07429	0.724	0.362	-0.07429	-0.07429	-1.226 to 1.078	t=0.2052 df=3	0.8505	No
trmB +	0.1082	0.7623	0.3811	0.1082	0.1082	-1.105 to 1.321	t=0.284 df=3	0.7949	No
trmH	0.6263	0.1095	0.04895	0.6263	0.6263	0.4904 to 0.7622	t=12.79 df=4	0.0002	Yes
trmH +	0.5318	0.3477	0.1555	0.5318	0.5318	0.1 to 0.9635	t=3.42 df=4	0.0268	Yes
trmJ	0.1963	0.3267	0.1633	0.1963	0.1963	-0.3235 to 0.7161	t=1.202 df=3	0.3156	No
trmJ +	0.5501	0.08019	0.04009	0.5501	0.5501	0.4225 to 0.6777	t=13.72 df=3	0.0008	Yes
trmL	0.1738	0.3256	0.1628	0.1738	0.1738	-0.3443 to 0.692	t=1.068 df=3	0.364	No
trmL +	0.2891	0.3565	0.1783	0.2891	0.2891	-0.2782 to 0.8564	t=1.622 df=3	0.2033	No
trmM	0.4305	0.06643	0.02971	0.4305	0.4305	0.348 to 0.513	t=14.49 df=4	0.0001	Yes
trmM +	0.656	0.1057	0.04729	0.656	0.656	0.5246 to 0.7873	t=13.87 df=4	0.0002	Yes
trmO	0.2074	0.2415	0.108	0.2074	0.2074	-0.09253 to 0.5073	t=1.92 df=4	0.1273	No
trmO +	0.5309	0.1116	0.04993	0.5309	0.5309	0.3923 to 0.6695	t=10.63 df=4	0.0004	Yes
truA	2.108	0.4166	0.1473	2.108	2.108	1.759 to 2.456	t=14.31 df=7	<0.0001	Yes
truA +	2.333	0.3483	0.1232	2.333	2.333	2.042 to 2.625	t=18.95 df=7	<0.0001	Yes
truB	-0.562	0.1208	0.0604	-0.562	-0.562	-0.7542 to -0.3698	t=9.305 df=3	0.0026	Yes
truB +	-0.2878	0.1469	0.06571	-0.2878	-0.2878	-0.4702 to -0.1053	t=4.379 df=4	0.0119	Yes
truC	0.46	0.04539	0.02269	0.46	0.46	0.3878 to 0.5322	t=20.27 df=3	0.0003	Yes
truC +	0.6966	0.1795	0.08974	0.6966	0.6966	0.411 to 0.9823	t=7.763 df=3	0.0044	Yes
truD	0.1721	0.3759	0.1681	0.1721	0.1721	-0.2946 to 0.6388	t=1.024 df=4	0.3638	No
truD +	0.5062	0.2874	0.1285	0.5062	0.5062	0.1494 to 0.863	t=3.939 df=4	0.017	Yes
ttaA	0.7246	0.4769	0.2133	0.7246	0.7246	0.1324 to 1.317	t=3.397 df=4	0.0274	Yes
ttaA +	0.6751	0.7312	0.327	0.6751	0.6751	-0.2327 to 1.583	t=2.065 df=4	0.1079	No
tusA	0.4458	0.524	0.2139	0.4458	0.4458	-0.104 to 0.9957	t=2.084 df=5	0.0916	No
tusA +	1.394	0.2742	0.1119	1.394	1.394	1.107 to 1.682	t=12.46 df=5	<0.0001	Yes
tusB	0.7278	1.057	0.3997	0.7278	0.7278	-0.2502 to 1.706	t=1.821 df=6	0.1185	No
tusB +	2.039	0.3713	0.1516	2.039	2.039	1.649 to 2.429	t=13.45 df=5	<0.0001	Yes
tusC	0.6023	0.05913	0.02957	0.6023	0.6023	0.5082 to 0.6964	t=20.37 df=3	0.0003	Yes
tusC +	0.859	0.1088	0.05439	0.859	0.859	0.6859 to 1.032	t=15.79 df=3	0.0006	Yes
tusD	1.015	0.04516	0.02019	1.015	1.015	0.9588 to 1.071	t=50.26 df=4	<0.0001	Yes

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
tusD +	1.12	0.05405	0.02417	1.12	1.12	1.053 to 1.187	t=46.32 df=4	<0.0001	Yes
tusE	0.08858	0.1851	0.09257	0.08858	0.08858	-0.206 to 0.3832	t=0.9568 df=3	0.4092	No
tusE +	0.2664	0.2414	0.1207	0.2664	0.2664	-0.1178 to 0.6506	t=2.207 df=3	0.1144	No

Supplementary Table S4. Statistical analysis of the fluorescence measurements obtained for the amber codon suppression efficiency (sfGFP-2TAG synthesis) in the selected Keio deletion strains in presence of tRNA^{Sep} only. The fluorescence measurements were calculated as a log₂ fold change relative to the BW25113 strain. The results of the Student's t-test are shown. Theoretical mean value is 0. Supplementation with 5 mM Sep is indicated by "+".

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
cmoA	0.0906	0.698	0.349	0.0906	0.0906	-1.02 to 1.201	t=0.2596 df=3	0.812	No
cmoA +	0.1184	0.6709	0.3354	0.1184	0.1184	-0.9491 to 1.186	t=0.3529 df=3	0.7475	No
cmoB	0.4771	0.5895	0.2948	0.4771	0.4771	-0.4609 to 1.415	t=1.619 df=3	0.2039	No
cmoB +	0.5727	0.562	0.281	0.5727	0.5727	-0.3215 to 1.467	t=2.038 df=3	0.1343	No
dusA	0.1713	0.2544	0.1272	0.1713	0.1713	-0.2336 to 0.5762	t=1.347 df=3	0.2708	No
dusA +	-0.2667	0.1154	0.05769	-0.2667	-0.2667	-0.4503 to -0.0831	t=4.623 df=3	0.0191	Yes
dusB	0.5075	0.128	0.064	0.5075	0.5075	0.3038 to 0.7112	t=7.93 df=3	0.0042	Yes
dusB +	0.5607	0.2821	0.1411	0.5607	0.5607	0.1118 to 1.01	t=3.975 df=3	0.0285	Yes
dusC	0.124	0.3548	0.1774	0.124	0.124	-0.4406 to 0.6885	t=0.6989 df=3	0.5349	No
dusC +	-0.03174	0.341	0.1705	-0.03174	-0.03174	-0.5744 to 0.5109	t=0.1862 df=3	0.8642	No
gluQRS	-0.3779	0.0566	0.03268	-0.3779	-0.3779	-0.5185 to -0.2373	t=11.57 df=2	0.0074	Yes
gluQRS +	-0.4843	0.09237	0.05333	-0.4843	-0.4843	-0.7137 to -0.2548	t=9.081 df=2	0.0119	Yes
iscA	0.8254	0.04885	0.0282	0.8254	0.8254	0.7041 to 0.9468	t=29.27 df=2	0.0012	Yes
iscA +	0.7044	0.05809	0.03354	0.7044	0.7044	0.5601 to 0.8487	t=21 df=2	0.0023	Yes
iscS	0.9832	1.504	0.7519	0.9832	0.9832	-1.41 to 3.376	t=1.308 df=3	0.2822	No
iscS +	0.7606	1.625	0.8123	0.7606	0.7606	-1.825 to 3.346	t=0.9363 df=3	0.4182	No
miAA	0.1147	0.4237	0.173	0.1147	0.1147	-0.3299 to 0.5594	t=0.6634 df=5	0.5364	No
miA +	0.1088	0.2987	0.1219	0.1088	0.1088	-0.2046 to 0.4223	t=0.8925 df=5	0.413	No
miAB	0.3088	0.01587	0.009162	0.3088	0.3088	0.2693 to 0.3482	t=33.7 df=2	0.0009	Yes
miAB +	0.2149	0.01236	0.007138	0.2149	0.2149	0.1842 to 0.2456	t=30.1 df=2	0.0011	Yes
mnmA	0.2491	0.8941	0.4471	0.2491	0.2491	-1.174 to 1.672	t=0.5572 df=3	0.6163	No
mnmA +	0.1474	0.8971	0.4485	0.1474	0.1474	-1.28 to 1.575	t=0.3287 df=3	0.764	No
mnmCD	1.134	0.1347	0.06733	1.134	1.134	0.92 to 1.349	t=16.85 df=3	0.0005	Yes
mnmCD +	1.013	0.04154	0.02077	1.013	1.013	0.9466 to 1.079	t=48.76 df=3	<0.0001	Yes
mnmE	0.672	0.4086	0.1827	0.672	0.672	0.1647 to 1.179	t=3.678 df=4	0.0212	Yes
mnmE +	1.014	0.8384	0.3749	1.014	1.014	-0.02653 to 2.056	t=2.706 df=4	0.0538	No
mnmG	0.2405	0.1924	0.1111	0.2405	0.2405	-0.2375 to 0.7185	t=2.164 df=2	0.1629	No
mnmG +	0.2252	0.1695	0.09787	0.2252	0.2252	-0.1959 to 0.6463	t=2.301 df=2	0.1481	No
mnmH	0.2885	0.3093	0.1547	0.2885	0.2885	-0.2038 to 0.7807	t=1.865 df=3	0.159	No

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
mnmH +	0.2525	0.1761	0.08803	0.2525	0.2525	-0.02769 to 0.5326	t=2.868 df=3	0.0642	No
queA	0.8851	0.08516	0.04258	0.8851	0.8851	0.7496 to 1.021	t=20.79 df=3	0.0002	Yes
queA +	1.096	0.1821	0.09106	1.096	1.096	0.8066 to 1.386	t=12.04 df=3	0.0012	Yes
queF	0.176	0.1473	0.07363	0.176	0.176	-0.05831 to 0.4103	t=2.391 df=3	0.0967	No
queF +	0.05339	0.1429	0.07146	0.05339	0.05339	-0.174 to 0.2808	t=0.7471 df=3	0.5092	No
queG	0.3834	0.5552	0.2776	0.3834	0.3834	-0.5001 to 1.267	t=1.381 df=3	0.2612	No
queG +	0.252	0.4748	0.2374	0.252	0.252	-0.5035 to 1.007	t=1.061 df=3	0.3664	No
rlmN	0.4231	0.3082	0.1541	0.4231	0.4231	-0.06726 to 0.9134	t=2.746 df=3	0.071	No
rlmN +	0.2313	0.3535	0.1767	0.2313	0.2313	-0.3311 to 0.7937	t=1.309 df=3	0.2818	No
rluA	0.5111	0.3028	0.1514	0.5111	0.5111	0.0292 to 0.9929	t=3.375 df=3	0.0432	Yes
rluA +	0.4956	0.2493	0.1246	0.4956	0.4956	0.09891 to 0.8922	t=3.976 df=3	0.0285	Yes
selD	0.3499	0.2883	0.1442	0.3499	0.3499	-0.1089 to 0.8087	t=2.427 df=3	0.0936	No
selD +	0.2951	0.2266	0.1133	0.2951	0.2951	-0.0655 to 0.6558	t=2.604 df=3	0.0801	No
tcdA	-0.9499	1.73	0.7063	-0.9499	-0.9499	-2.766 to 0.8657	t=1.345 df=5	0.2364	No
tcdA +	-0.2518	0.5033	0.2055	-0.2518	-0.2518	-0.78 to 0.2764	t=1.225 df=5	0.275	No
tgt	0.2598	0.7691	0.3846	0.2598	0.2598	-0.964 to 1.484	t=0.6756 df=3	0.5477	No
tgt +	0.06248	0.3862	0.1931	0.06248	0.06248	-0.552 to 0.677	t=0.3236 df=3	0.7675	No
thiI	-0.2298	0.1638	0.08189	-0.2298	-0.2298	-0.4904 to 0.03076	t=2.807 df=3	0.0675	No
thiI +	0.3367	0.5895	0.2948	0.3367	0.3367	-0.6013 to 1.275	t=1.142 df=3	0.3362	No
tmcA	0.6546	0.04383	0.02531	0.6546	0.6546	0.5457 to 0.7635	t=25.87 df=2	0.0015	Yes
tmcA +	0.5745	0.02093	0.01208	0.5745	0.5745	0.5225 to 0.6265	t=47.55 df=2	0.0004	Yes
TOP10	1.241	0.009848	0.005686	1.241	1.241	1.216 to 1.265	t=218.2 df=2	<0.0001	Yes
TOP10 +	1.18	0.01977	0.01142	1.18	1.18	1.131 to 1.229	t=103.4 df=2	<0.0001	Yes
trmA	0.3005	0.0975	0.04875	0.3005	0.3005	0.1454 to 0.4557	t=6.165 df=3	0.0086	Yes
trmA +	0.5086	0.5625	0.2812	0.5086	0.5086	-0.3864 to 1.404	t=1.808 df=3	0.1683	No
trmB	-0.1125	0.5475	0.2738	-0.1125	-0.1125	-0.9838 to 0.7587	t=0.411 df=3	0.7087	No
trmB +	-0.1175	0.6008	0.3004	-0.1175	-0.1175	-1.073 to 0.8384	t=0.3912 df=3	0.7218	No
trmH	0.5643	0.2134	0.1067	0.5643	0.5643	0.2247 to 0.904	t=5.288 df=3	0.0132	Yes
trmH +	0.4695	0.07603	0.03802	0.4695	0.4695	0.3485 to 0.5904	t=12.35 df=3	0.0011	Yes
trmJ	-0.6247	0.4524	0.2262	-0.6247	-0.6247	-1.345 to 0.09519	t=2.762 df=3	0.0701	No
trmJ +	-0.6937	0.4639	0.2319	-0.6937	-0.6937	-1.432 to 0.04449	t=2.991 df=3	0.0581	No
trmL	0.5893	0.04341	0.0217	0.5893	0.5893	0.5203 to 0.6584	t=27.15 df=3	0.0001	Yes
trmL +	0.6431	0.07241	0.03621	0.6431	0.6431	0.5279 to 0.7583	t=17.76 df=3	0.0004	Yes
trmM	0.3675	0.2686	0.1343	0.3675	0.3675	-0.05989 to 0.7949	t=2.737 df=3	0.0715	No
trmM +	0.3262	0.2504	0.1252	0.3262	0.3262	-0.07226 to 0.7247	t=2.605 df=3	0.08	No
trmO	0.4556	0.3113	0.1557	0.4556	0.4556	-0.03981 to 0.9509	t=2.927 df=3	0.0612	No
trmO +	0.3205	0.2749	0.1374	0.3205	0.3205	-0.1168 to 0.7579	t=2.332 df=3	0.1019	No
truA	2.304	0.251	0.1255	2.304	2.304	1.905 to 2.703	t=18.36 df=3	0.0004	Yes
truA +	1.912	0.09473	0.04736	1.912	1.912	1.762 to 2.063	t=40.38 df=3	<0.0001	Yes
truB	-0.1864	0.1698	0.0849	-0.1864	-0.1864	-0.4566 to 0.08379	t=2.196 df=3	0.1157	No
truB +	-0.2649	0.1201	0.06006	-0.2649	-0.2649	-0.456 to -0.07374	t=4.41 df=3	0.0216	Yes

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
truC	0.2865	0.04022	0.02011	0.2865	0.2865	0.2225 to 0.3505	t=14.25 df=3	0.0007	Yes
truC +	0.2881	0.09082	0.04541	0.2881	0.2881	0.1436 to 0.4326	t=6.344 df=3	0.0079	Yes
truD	-0.09493	0.1901	0.09505	-0.09493	-0.09493	-0.3974 to 0.2076	t=0.9987 df=3	0.3915	No
truD +	-0.05004	0.1037	0.05186	-0.05004	-0.05004	-0.2151 to 0.115	t=0.9651 df=3	0.4057	No
ttcA	-0.2315	0.05636	0.03254	-0.2315	-0.2315	-0.3715 to -0.09152	t=7.115 df=2	0.0192	Yes
ttcA +	-0.1381	0.3297	0.1903	-0.1381	-0.1381	-0.957 to 0.6809	t=0.7253 df=2	0.5436	No
tusA	0.692	0.07203	0.03601	0.692	0.692	0.5774 to 0.8066	t=19.21 df=3	0.0003	Yes
tusA +	0.9288	0.2984	0.1492	0.9288	0.9288	0.4541 to 1.404	t=6.226 df=3	0.0084	Yes
tusB	0.7939	0.1534	0.07668	0.7939	0.7939	0.5499 to 1.038	t=10.35 df=3	0.0019	Yes
tusB +	0.858	0.2492	0.1246	0.858	0.858	0.4614 to 1.255	t=6.884 df=3	0.0063	Yes
tusC	0.3857	0.3307	0.1653	0.3857	0.3857	-0.1405 to 0.9119	t=2.333 df=3	0.1019	No
tusC +	0.239	0.2618	0.1309	0.239	0.239	-0.1776 to 0.6556	t=1.826 df=3	0.1654	No
tusD	0.8727	0.02977	0.01719	0.8727	0.8727	0.7987 to 0.9466	t=50.77 df=2	0.0004	Yes
tusD +	0.7785	0.03836	0.02215	0.7785	0.7785	0.6832 to 0.8738	t=35.15 df=2	0.0008	Yes
tusE	-0.7681	0.04562	0.02281	-0.7681	-0.7681	-0.8407 to -0.6955	t=33.67 df=3	<0.0001	Yes
tusE +	-0.8365	0.1193	0.05967	-0.8365	-0.8365	-1.026 to -0.6466	t=14.02 df=3	0.0008	Yes

Supplementary Table S5. Statistical analysis of the fluorescence measurements obtained for the wild type sfGFP synthesis in the selected Keio deletion strains in presence of Sep-OTS. The fluorescence measurements were calculated as a log₂ fold change relative to the BW25113 strain. The results of the Student's t-test are shown. Theoretical mean value is 0. Supplementation with 5 mM Sep is indicated by "+".

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05)?
CmoA	-0.03225	0.005784	0.00409	-0.03225	-0.03225	-0.08422 to 0.01972	t=7.885 df=1	0.0803	No
CmoA+Sep	0.02755	0.002376	0.00168	0.02755	0.02755	0.006207 to 0.0489	t=16.4 df=1	0.0388	Yes
CmoB	0.01941	0.04072	0.02879	0.01941	0.01941	-0.3464 to 0.3852	t=0.6742 df=1	0.6224	No
CmoB+Sep	0.1157	0.05793	0.04096	0.1157	0.1157	-0.4048 to 0.6362	t=2.824 df=1	0.2167	No
DusA	-0.07001	0.04225	0.02988	-0.07001	-0.07001	-0.4496 to 0.3096	t=2.343 df=1	0.2568	No
DusA+Sep	0.1366	0.1367	0.09664	0.1366	0.1366	-1.091 to 1.364	t=1.413 df=1	0.392	No
DusB	-0.2868	0.03661	0.02589	-0.2868	-0.2868	-0.6157 to 0.04206	t=11.08 df=1	0.0573	No
DusB+Sep	-0.3054	0.08038	0.05684	-0.3054	-0.3054	-1.028 to 0.4168	t=5.374 df=1	0.1171	No
DusC	-0.1306	0.08521	0.06025	-0.1306	-0.1306	-0.8961 to 0.635	t=2.167 df=1	0.2753	No
DusC+Sep	-0.04587	0.01509	0.01067	-0.04587	-0.04587	-0.1814 to 0.08971	t=4.299 df=1	0.1455	No
GluQRS	-0.05919	0.1785	0.1262	-0.05919	-0.05919	-1.663 to 1.545	t=0.4689 df=1	0.7209	No
GluQRS+Sep	-0.05466	0.1717	0.1214	-0.05466	-0.05466	-1.597 to 1.488	t=0.4502 df=1	0.7307	No
IscA	-0.01573	0.00787	0.005565	-0.01573	-0.01573	-0.08644 to 0.05499	t=2.826 df=1	0.2165	No
IscA+Sep	0.04171	0.0268	0.01895	0.04171	0.04171	-0.1991 to 0.2825	t=2.201 df=1	0.2715	No
MiaA	0.123	0.1643	0.1162	0.123	0.123	-1.353 to 1.599	t=1.059 df=1	0.4818	No
MiaA+Sep	0.2757	0.002455	0.001736	0.2757	0.2757	0.2536 to 0.2977	t=158.8 df=1	0.004	Yes
MiaB	-0.6134	0.002878	0.002035	-0.6134	-0.6134	-0.6393 to -0.5876	t=301.4 df=1	0.0021	Yes

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05) ?
MiaB+Sep	-0.4675	0.05858	0.04143	-0.4675	-0.4675	-0.9938 to 0.05889	t=11.28 df=1	0.0563	No
MnmA	-0.01481	0.01512	0.01069	-0.01481	-0.01481	-0.1506 to 0.121	t=1.385 df=1	0.398	No
MnmA+Sep	0.09416	0.06264	0.04429	0.09416	0.09416	-0.4686 to 0.6569	t=2.126 df=1	0.2799	No
MnmCD	-0.4076	0.08048	0.05691	-0.4076	-0.4076	-1.131 to 0.3155	t=7.162 df=1	0.0883	No
MnmCD+Sep	-0.3945	0.000743	0.000525	-0.3945	-0.3945	-0.4012 to -0.3879	t=751.5 df=1	0.0008	Yes
MnmE	-0.3455	0.01546	0.008928	-0.3455	-0.3455	-0.3839 to -0.3071	t=38.7 df=2	0.0007	Yes
MnmE+Sep	-0.5877	0.02386	0.01687	-0.5877	-0.5877	-0.8021 to -0.3733	t=34.84 df=1	0.0183	Yes
MnmH	0.04349	0.1469	0.1039	0.04349	0.04349	-1.277 to 1.364	t=0.4186 df=1	0.7476	No
MnmH+Sep	0.2796	0.006213	0.004393	0.2796	0.2796	0.2238 to 0.3354	t=63.65 df=1	0.01	Yes
QueA	-0.1117	0.04272	0.03021	-0.1117	-0.1117	-0.4955 to 0.2721	t=3.699 df=1	0.1681	No
QueA+Sep	-0.1301	0.02525	0.01786	-0.1301	-0.1301	-0.3569 to 0.09681	t=7.284 df=1	0.0869	No
QueF	0.0614	0.164	0.1159	0.0614	0.0614	-1.412 to 1.535	t=0.5296 df=1	0.6899	No
QueF+Sep	0.03325	0.1822	0.1288	0.03325	0.03325	-1.603 to 1.67	t=0.2582 df=1	0.8392	No
QueG	0.01521	0.1641	0.116	0.01521	0.01521	-1.459 to 1.49	t=0.1311 df=1	0.917	No
QueG+Sep	-0.00789	0.08583	0.06069	-0.00789	-0.00789	-0.779 to 0.7632	t=0.13 df=1	0.9177	No
RlmN	0.004665	0.01914	0.01353	0.004665	0.004665	-0.1673 to 0.1766	t=0.3446 df=1	0.7887	No
RlmN+Sep	0.1499	0.01796	0.0127	0.1499	0.1499	-0.01147 to 0.3113	t=11.8 df=1	0.0538	No
RluA	-0.4601	0.02504	0.01771	-0.4601	-0.4601	-0.6851 to -0.2352	t=25.99 df=1	0.0245	Yes
RluA+Sep	-0.1594	0.2086	0.1043	-0.1594	-0.1594	-0.4914 to 0.1725	t=1.529 df=3	0.2238	No
SelD	-0.1485	0.001089	0.00077	-0.1485	-0.1485	-0.1583 to -0.1387	t=192.9 df=1	0.0033	Yes
SelD+Sep	-0.08434	0.04494	0.03178	-0.08434	-0.08434	-0.4881 to 0.3195	t=2.654 df=1	0.2294	No
TcdA	-0.4478	0.006392	0.00452	-0.4478	-0.4478	-0.5053 to -0.3904	t=99.08 df=1	0.0064	Yes
TcdA+Sep	-0.3353	0.01882	0.01331	-0.3353	-0.3353	-0.5044 to -0.1662	t=25.19 df=1	0.0253	Yes
TGT	-0.1667	0.03516	0.02487	-0.1667	-0.1667	-0.4827 to 0.1492	t=6.705 df=1	0.0942	No
TGT+Sep	-0.2674	0.004964	0.00351	-0.2674	-0.2674	-0.312 to -0.2228	t=76.18 df=1	0.0084	Yes
ThiI	-0.1453	0.02666	0.01885	-0.1453	-0.1453	-0.3848 to 0.09421	t=7.708 df=1	0.0821	No
ThiI+Sep	-0.07967	0.02105	0.01489	-0.07967	-0.07967	-0.2688 to 0.1095	t=5.352 df=1	0.1176	No
Tmca	-0.0804	0.01222	0.00864	-0.0804	-0.0804	-0.1902 to 0.02938	t=9.306 df=1	0.0682	No
Tmca+Sep	0.02813	0.03782	0.02675	0.02813	0.02813	-0.3117 to 0.368	t=1.052 df=1	0.4839	No
TOP10	-0.248	0.01224	0.006119	-0.248	-0.248	-0.2675 to -0.2285	t=40.53 df=3	<0.0001	Yes
TOP10+Sep	-0.2041	0.02981	0.01491	-0.2041	-0.2041	-0.2515 to -0.1566	t=13.69 df=3	0.0008	Yes
TrmB	-0.04462	0.05638	0.03987	-0.04462	-0.04462	-0.5512 to 0.462	t=1.119 df=1	0.4642	No
TrmB+Sep	-0.02259	0.02017	0.01427	-0.02259	-0.02259	-0.2038 to 0.1587	t=1.583 df=1	0.3586	No
TrmH	-0.01267	0.2396	0.1694	-0.01267	-0.01267	-2.165 to 2.14	t=0.07479 df=1	0.9525	No
TrmH+Sep	-0.01482	0.02867	0.02027	-0.01482	-0.01482	-0.2724 to 0.2428	t=0.7309 df=1	0.5982	No
TrmJ	-0.1095	0.1995	0.1411	-0.1095	-0.1095	-1.902 to 1.683	t=0.7759 df=1	0.5799	No
TrmJ+Sep	-0.1026	0.03742	0.02646	-0.1026	-0.1026	-0.4388 to 0.2336	t=3.878 df=1	0.1607	No
TrmL	-0.1421	0.2854	0.2018	-0.1421	-0.1421	-2.706 to 2.422	t=0.7043 df=1	0.6094	No
TrmL+Sep	-0.07548	0.2376	0.168	-0.07548	-0.07548	-2.211 to 2.06	t=0.4492 df=1	0.7312	No
TrmM	-0.09532	0.02042	0.01444	-0.09532	-0.09532	-0.2788 to 0.08816	t=6.601 df=1	0.0957	No
TrmM+Sep	-0.0052	0.03484	0.02463	-0.0052	-0.0052	-0.3182 to 0.3078	t=0.211 df=1	0.8676	No

Gene deleted	Mean	Std. Deviation	Std. Error of Mean	Actual mean	Discrepancy	95% CI of discrepancy	t, df	P value (two tailed)	Significant (alpha=0.05) ?
TrmO	-0.1219	0.02289	0.01619	-0.1219	-0.1219	-0.3276 to 0.0837	t=7.534 df=1	0.084	No
TrmO+Sep	-0.02313	0.02652	0.01875	-0.02313	-0.02313	-0.2614 to 0.2151	t=1.234 df=1	0.4337	No
TruA	0.2209	0.009734	0.006883	0.2209	0.2209	0.1335 to 0.3084	t=32.1 df=1	0.0198	Yes
TruA+Sep	0.1364	0.06268	0.04432	0.1364	0.1364	-0.4267 to 0.6996	t=3.078 df=1	0.2	No
TruB	-0.2392	0.03931	0.0278	-0.2392	-0.2392	-0.5923 to 0.114	t=8.605 df=1	0.0737	No
TruB+Sep	-0.1366	0.02193	0.01551	-0.1366	-0.1366	-0.3336 to 0.06038	t=8.812 df=1	0.0719	No
TruC	-0.07562	0.02865	0.02026	-0.07562	-0.07562	-0.333 to 0.1818	t=3.732 df=1	0.1666	No
TruC+Sep	0.02293	0.001341	0.000949	0.02293	0.02293	0.01088 to 0.03498	t=24.18 df=1	0.0263	Yes
TruD	-0.05717	0.007729	0.005465	-0.05717	-0.05717	-0.1266 to 0.01227	t=10.46 df=1	0.0607	No
TruD+Sep	0.02265	0.04479	0.03167	0.02265	0.02265	-0.3798 to 0.4251	t=0.7152 df=1	0.6047	No
TtcA	-0.4377	0.004059	0.00287	-0.4377	-0.4377	-0.4742 to -0.4013	t=152.5 df=1	0.0042	Yes
TtcA+Sep	-0.4615	0.01819	0.01286	-0.4615	-0.4615	-0.6249 to -0.2981	t=35.89 df=1	0.0177	Yes
TusA	-0.1359	0.03326	0.02352	-0.1359	-0.1359	-0.4348 to 0.1629	t=5.778 df=1	0.1091	No
TusA+Sep	0.1893	0.02198	0.01554	0.1893	0.1893	-0.008112 to 0.3868	t=12.18 df=1	0.0521	No
TusB	0.01747	0.03315	0.02344	0.01747	0.01747	-0.2804 to 0.3153	t=0.7453 df=1	0.5922	No
TusB+Sep	0.1687	0.05544	0.0392	0.1687	0.1687	-0.3294 to 0.6668	t=4.303 df=1	0.1454	No
TusC	-0.09136	0.03504	0.02478	-0.09136	-0.09136	-0.4062 to 0.2234	t=3.687 df=1	0.1686	No
TusC+Sep	0.04732	0.01534	0.01085	0.04732	0.04732	-0.09052 to 0.1852	t=4.362 df=1	0.1435	No
TusE	-0.07592	0.0249	0.01437	-0.07592	-0.07592	-0.1378 to -0.01408	t=5.282 df=2	0.034	Yes
TusE+Sep	-0.07581	0.07636	0.04409	-0.07581	-0.07581	-0.2655 to 0.1139	t=1.72 df=2	0.2276	No

Supplementary references

1. Boccaletto, P.; Machnicka, M.A.; Purta, E.; Piatkowski, P.; Baginski, B.; Wirecki, T.K.; de Crecy-Lagard, V.; Ross, R.; Limbach, P.A.; Kotter, A., *et al.* MODOMICS: a database of RNA modification pathways. 2017 update. *Nucleic Acids Res* **2018**, *46*, D303-D307.