Supplementary Information for

In vitro regeneration of twenty aminoacyl-tRNA synthetases coupled with replication of artificial genomic DNAs

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This file includes:
Figs. S1-10
Tables S1-S4

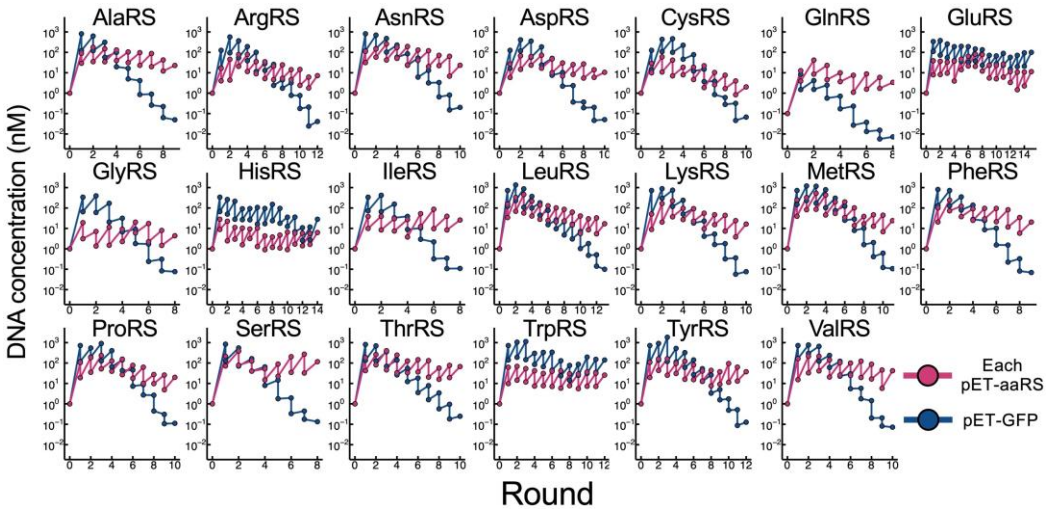

Figure S1. Raw data of Fig. 2b.

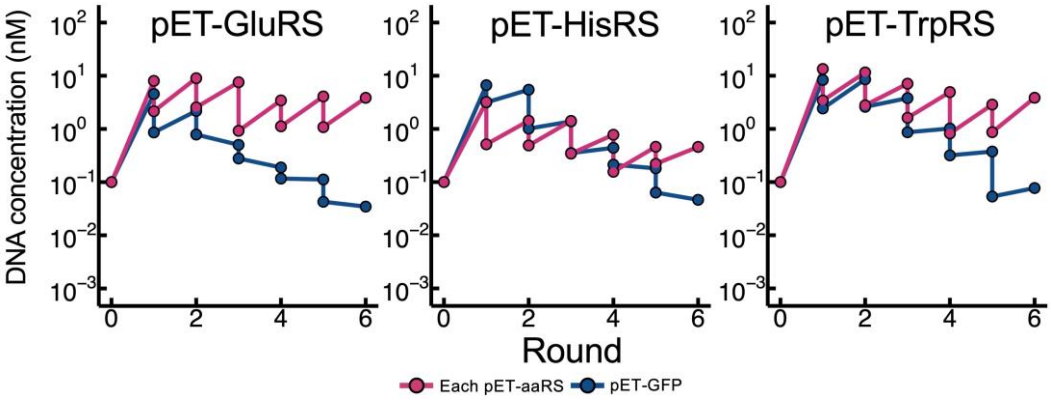

Figure S2. Raw data of Fig. 3d.

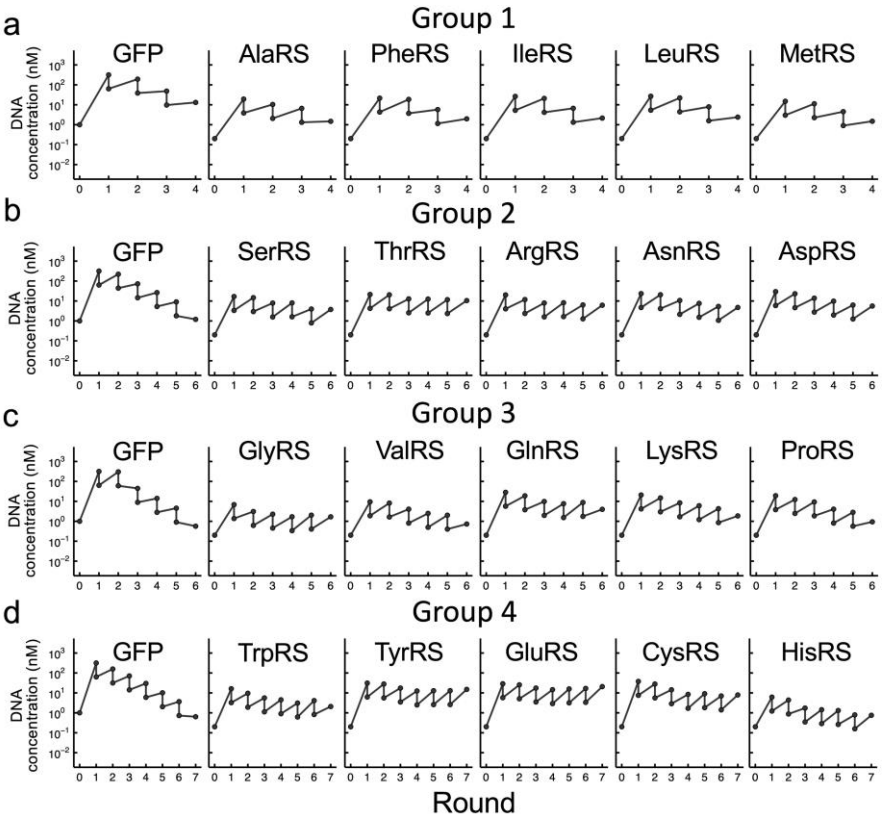

Figure S3. Raw data of Fig. 4b.

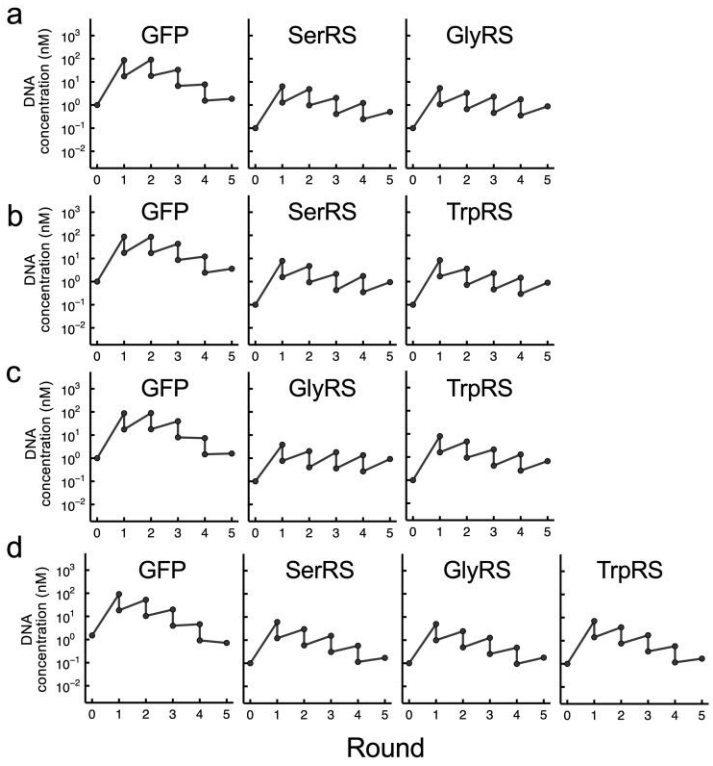

Figure S4. Raw data of Fig. 4c.

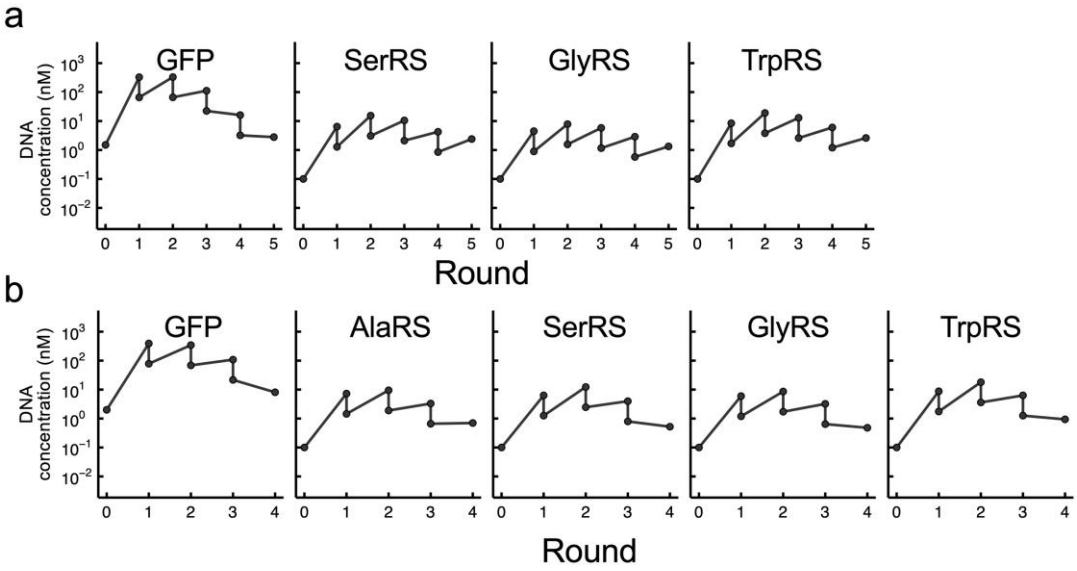

Figure S5. Raw data of Figs. 5c and 5d.

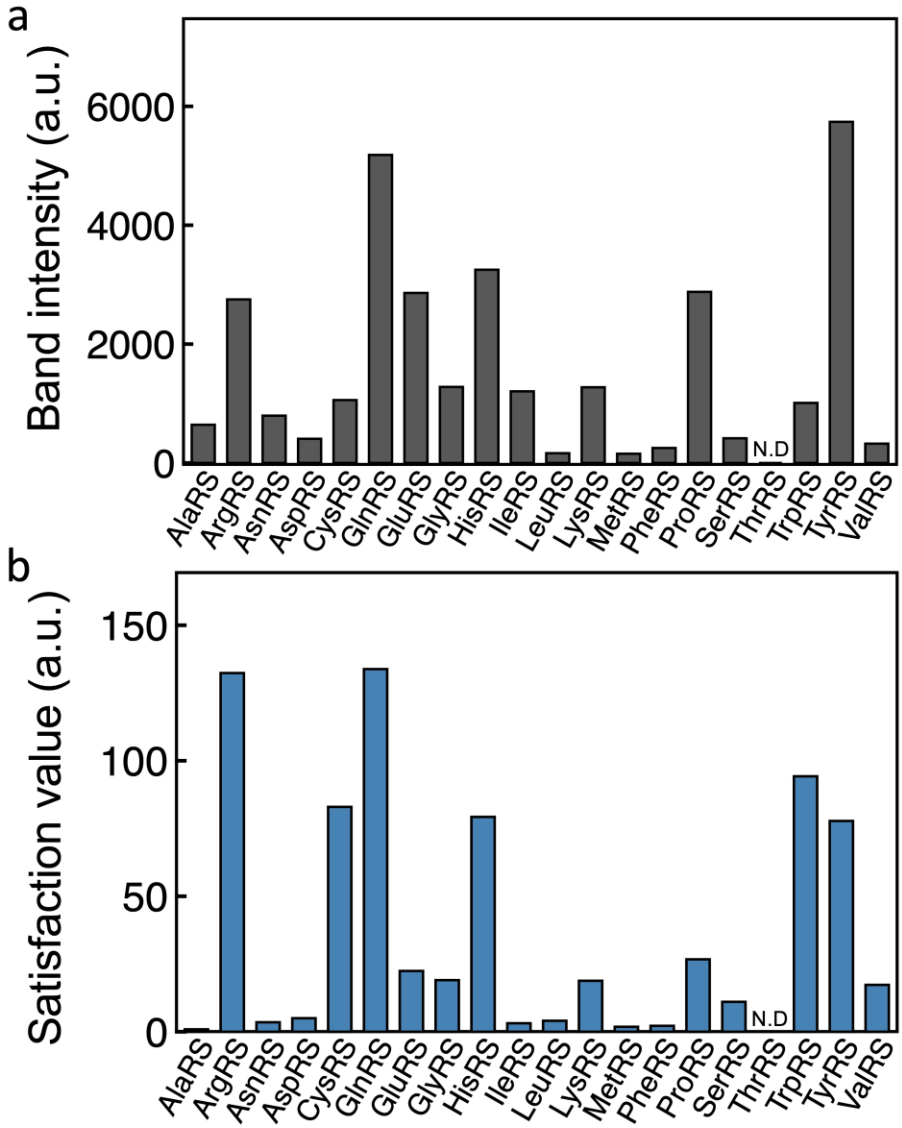

Figure S6. Band intensities of Fig. 6a and the satisfaction values.
(a) The band intensities in Fig. 6a. (b) The "satisfaction values" by dividing the band intensity by the concentration in the PURE system according to the following formula.

$$S = \frac{I \times 100}{MW \times [aaRS]}$$

S: Satisfaction value.
I: Band intensity.
MW: Molecular weight of each aaRS (kDa).
[aaRS]: Concentration of each aaRS in the PURE system (nM).

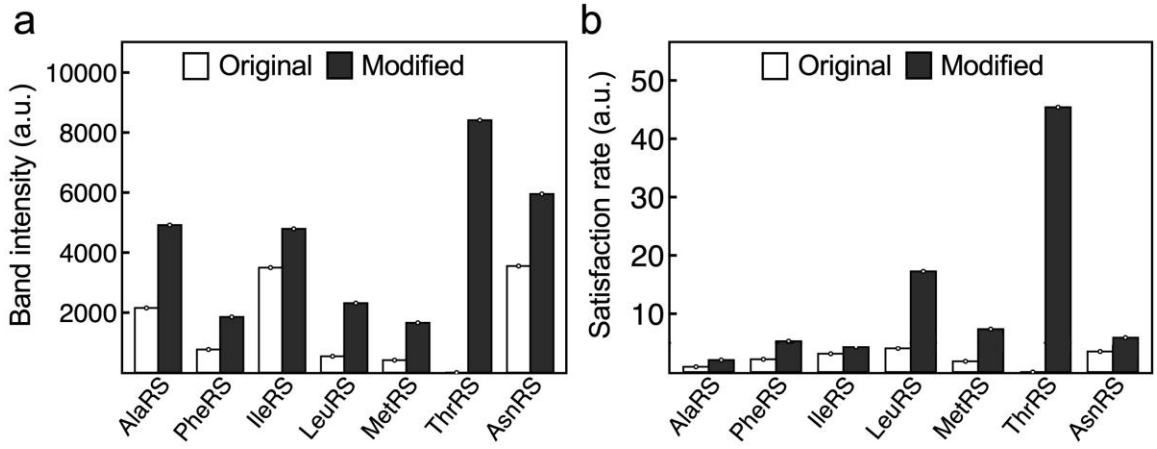

Figure S7. Band intensities of Fig. 6b and the satisfaction values.
(a) The band intensities in Fig. 6b. (b) Thee "satisfaction values" by dividing the intensity by the concentration in the PURE system according to the formula shown in the legend of Fig. S6.

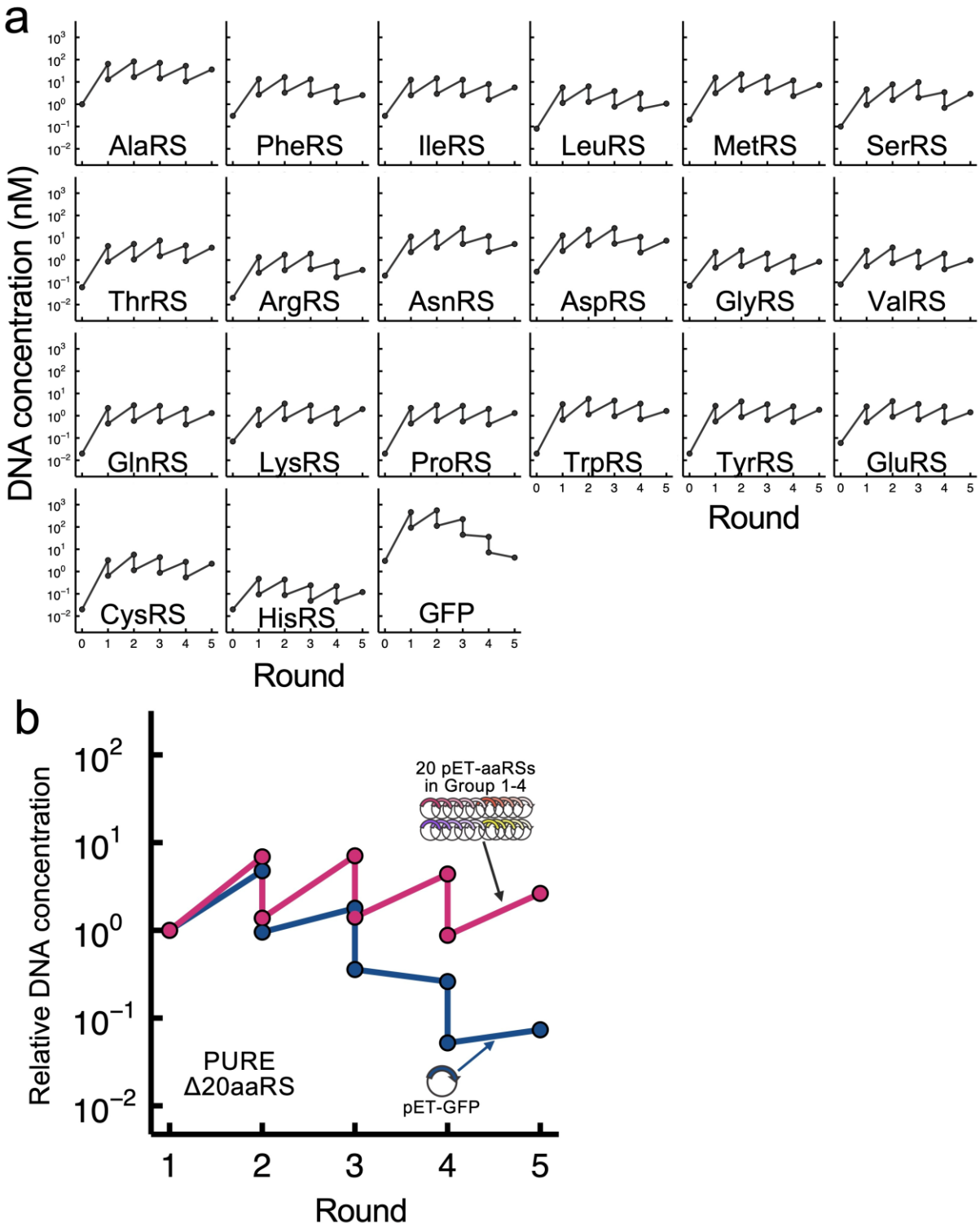

Figure S8. Raw data plots of simultaneously 20 aaRS regeneration.
a) Raw data of Fig. 7b. b) Another simultaneous 20 aaRS regeneration experiment coupled with DNA replication. The same experiment as Fig. 7b was performed again to examine the reproducibility. The DNA concentration of only pET-AlaRS was measured for the experiment with 20 pET-aaRSs.

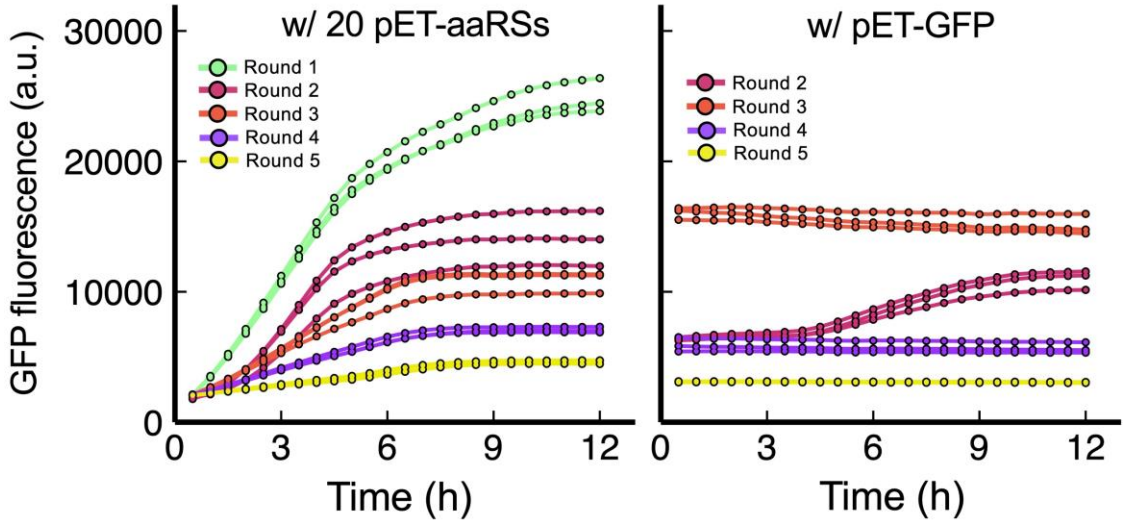

Figure S9. Time-course data of GFP fluorescence.
The raw time-course data of GFP fluorescence obtained in Fig. 7c are shown. The slopes of the five measured points in 2-4 h for the experiment with 20 pET-aaRSs and in 6-8 h for that with pET-GFP and were defined as translational activity.

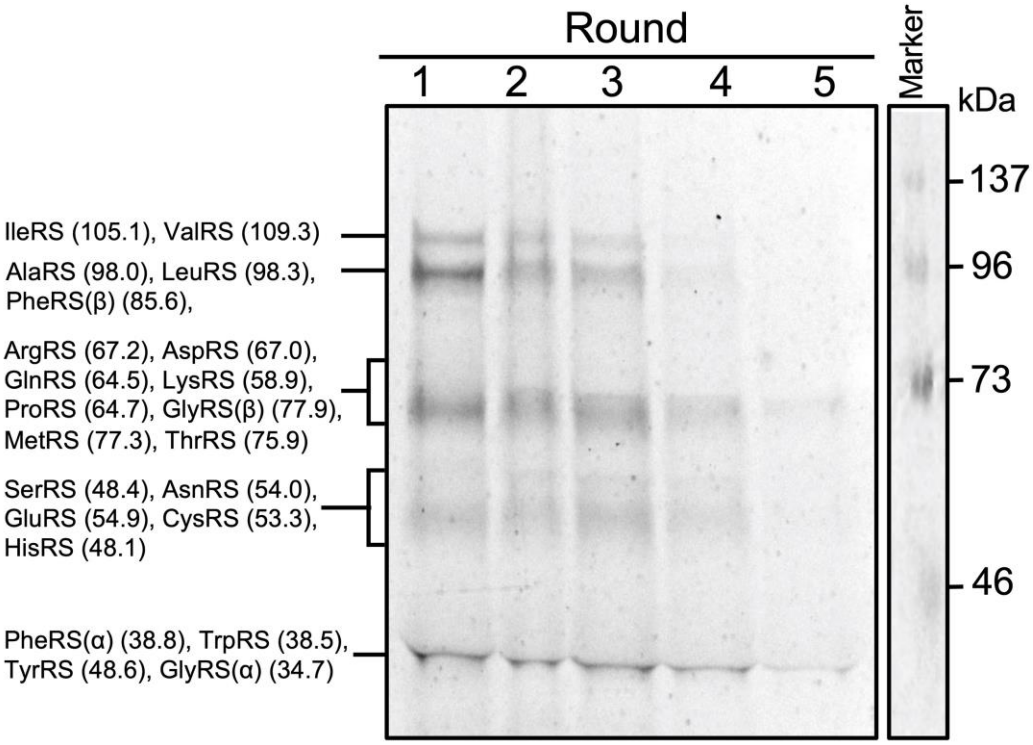

Figure S10. SDS-PAGE analysis of the regenerated 20 aaRSs.
The same dialyzed serial dilution experiment as Fig. 7b was performed in the presence of fluorescent-labeled lysyl-tRNA to label newly synthesized aaRSs. The reaction mixture at each round was subject to SDS-PAGE, followed by fluorescent imaging.

Table S1. Composition of the customized PURE system

Initiation factor 1	25 μM	ValRS	17 nM
Initiation factor 2	1 μM	Methionyl-tRNA formyltransferase	590 nM
Initiation factor 3	4.9 μM	Myokinase	1.4 μM
Elongation factor G	1.1 μM	Creatine kinase	250 nM
Elongation factor Tu	80 μM	Nucleoside diphosphate kinase	16 nM
Elongation factor Ts	3.3 μM	Pyrophosphatase	41 nM
Release factor 1	49 nM	Trigger factor	1 μM
Release factor 2	48 nM	*E. coli* DEAH type RNA helicase A	100 nM
Release factor 3	170 nM	70S ribosome	1 μM
Ribosome recycling factor	3.9 nM		
AlaRS	730 nM	Tyrosine	0.3 mM
ArgRS	31 nM	Cysteine	0.3 mM
AsnRS	420 nM	18 other amino acids	0.36 mM
AspRS	120 nM	tRNA mix (Roche, *E. coli*)	0.52 mg/mL
CysRS	24 nM	ATP	0.375 mM
GlnRS	60 nM	GTP	0.25 mM
GluRS	230 nM	CTP	0.125 mM
GlyRS	86 nM	UTP	0.125 mM
HisRS	85 nM	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7.6)	100 mM
IleRS	370 nM	Glutamate acid potassium salt	70 mM
LeuRS	41 nM	Spermidine	0.375 mM
LysRS	120 nM	Magnesium acetate	10.7 mM
MetRS	110 nM	Creatine phosphate	25 mM
PheRS	130 nM	Dithiothreitol	6 mM
ProRS	170 nM	10-formyl-5,6,7,8-tetrahydro folic acid	10 μg/mL
SerRS	78 nM	Each dNTP	0.6 mM
ThrRS	84 nM	Yeast inorganic pyrophosphatase (NEB)	0.2 mU/μL
TrpRS	28 nM	RNase inhibitor (Promega)	0.1 U/μL
TyrRS	150 nM	T7 RNA polymerase (Takara)	0.42 U/μL

Table S2. Dilution rate of each aaRS in the activity assay conducted in Fig. 1

Name	Dilution rate
AlaRS	1
ArgRS	30
AsnRS	30
AspRS	30
CysRS	10000
GlnRS	100
GluRS	2000
GlyRS	30
HisRS	10000
IleRS	10
LeuRS	10
LysRS	300
MetRS	10
PheRS	5
ProRS	300
SerRS	10
ThrRS	20
TrpRS	300
TyrRS	300
ValRS	30

Table S3. Composition of the dialysis buffer

Component	Concentration
Tyrosine	0.3 mM
Cysteine	0.3 mM
18 other amino acids	0.36 mM
ATP	0.375 mM
GTP	0.25 mM
CTP	0.125 mM
UTP	0.125 mM
N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7.6)	100 mM
Glutamate acid potassium salt	70 mM
Spermidine	0.375 mM
Magnesium acetate	6.36 mM
Creatine phosphate	25 mM
Dithiothreitol	6 mM
10-formyl-5,6,7,8-tetrahydro folic acid	10 μg/mL
Each dNTP	0.06 mM
Stock buffer*	10 % (v/v)
Fosfomycin	100 μg/mL
Ampicillin	50 μg/mL

* Stock buffer: Hepes-KOH (pH7.6): 50 mM, KCl: 100 mM, $MgCl_2$: 10 mM, 2-mercaptoethanol: 7 mM, glycerol: 30%

Table S4 Initial concentration of 20 pET-aaRSs

Name	Concentration (nM)
AlaRS	1.00
ArgRS	0.02
AsnRS	0.20
AspRS	0.30
CysRS	0.02
GlnRS	0.02
GluRS	0.06
GlyRS	0.07
HisRS	0.02
IleRS	0.30
LeuRS	0.08
LysRS	0.07
MetRS	0.20
PheRS	0.30
ProRS	0.06
SerRS	0.10
ThrRS	0.06
TrpRS	0.02
TyrRS	0.02
ValRS	0.08

Table S5. Sequence modification of the selected 7 aaRS

aaRS	Original sequence (GC%) (AA sequence)	Modified sequence (GC%) (AA Sequence)
AlaRS	ATGAGAGGATCGCATCAC (50) (MRGSHH)	ATGCATCATCATCATCAT (33) (MHHHHH)
PheRS (α)	ATGAGAGGATCGCATCAC (50) (MRGSHH)	ATGCATCATCATCATCAT (33) (MHHHHH)
IleRS	ATGCACCACCACCACCAC (61) (MHHHHH)	ATGCATCATCATCATCAC (39) (MHHHHH)
LeuRS	ATGCAAGAGCAATACCGC (50) (MQEQYR)	ATGCAAGAACAATACCGA (39) (MQEQYR)
MetRS	ATGACTCAAGTCGCGAAG (50) (MTQVAK)	ATGACTCAAGTAGCAAAG (39) (MTQVAK)
ThrRS	ATGAGAGGATCGCATCAC (50) (MRGSHH)	ATGCATCATCATCATCAT (33) (MHHHHH)
AsnRS	ATGAGAGGATCGCATCAC (50) (MRGSHH)	ATGCATCATCATCATCAT (33) (MHHHHH)

Table S6. Primer list

Primer name	Sequence	Purpose
Primer 1	AGGCATGCTAATACGACTCACTATAGGG	Amplify phi29
Primer 2	GCATGCGCTAGTTATTGCTCAGCGG	Amplify phi29
Primer 3	GGAGATATACATATGAGAGGATCGCATCACC	Transfer to pET
Primer 4	AGCTCGAATTCATTATTGCAATTTCGCGCTG	Transfer to pET
Primer 5	AGCTCGAATTCATTAGAAGCTGGCGTTACGC	Transfer to pET
Primer 6	AGCTCGAATTCATTAATCCCTCAATGATGCCTGG	Transfer to pET
Primer 7	AGCTCGAATTCATTATTCCTCCAATTGTTTAAGACTGCG	Transfer to pET
Primer 8	TAATGAATTCGAGCTCCGTCG	pET vector
Primer 9	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTC	pET vector
Primer 10	ATGCATCATCATCATCATCATCACCATCACGGATCC	Seq modification
Primer 11	ATGATGATGATGCATATGTATATCTCCTTCTTAAAGTTAA	Seq modification
Primer 12	ATGCATCATCATCATCATCACAGTGACTATAAATCAACCC	Seq modification
Primer 13	ATGCAAGAACAATACCGACCGGAAGAGATAGAATCCA	Seq modification
Primer 14	GTATTGTTCTTGCATGGCAGCATCTCCTTCTAGAG	Seq modification
Primer 15	ATGACTCAAGTAGCAAAGAAAATTCTGGTGACGTGCG	Seq modification
Primer 16	TGCTACTTGAGTCATAGTAGGATCTCCTTCTAGAG	Seq modification
Primer 17	GCGAAATTAATACGACTCACTATAGGG	Amplify GFP
Primer 18	CCGCTGAGCAATAACTAGCATAACC	Amplify GFP
Primer 19	AGGGTATGGGCGTATGGTTATATG	qPCR for phi29
Primer 20	TGTCCCATGCGAGATATGATCG	qPCR for phi30
Primer 21	ACAACGACCTGGAAAACGTC	qPCR for AlaRS
Primer 22	CATGCAAACTGAATGGCATC	qPCR for AlaRS
Primer 23	CAGGTTCGTCAGTCAGCAAA	qPCR for ArgRS
Primer 24	CCGGATCAAGGAAAATGTTG	qPCR for ArgRS
Primer 25	CGTTTATGACGGTTCCTGCT	qPCR for AsnRS
Primer 26	ACCCAACCAGCAACTTCAAC	qPCR for AsnRS
Primer 27	ATGTTCTGCCGCTTGACTCT	qPCR for AspRS
Primer 28	GGAGTTTCGATGTCGAGGAA	qPCR for AspRS
Primer 29	TGATATGGCGCGTGAAGTAA	qPCR for CysRS
Primer 30	ACGCTTCAATCTCAGCCACT	qPCR for CysRS
Primer 31	ATAAAGGCCAGTGCAACCTG	qPCR for GlnRS
Primer 32	AACGGACGTTACCAGACCAG	qPCR for GlnRS

Primer 33	CCGTGTGTTGTACGTTTTGC	qPCR for GluRS
Primer 34	CGTGGGTGATTTCCATATCC	qPCR for GluRS
Primer 35	TTACCGGCGAGATCACCTAC	qPCR for GlyRS
Primer 36	GTGGACTGCTCCACTTCGTT	qPCR for GlyRS
Primer 37	ATGGCATTAGCTGAGCGTCT	qPCR for HisRS
Primer 38	CACTTCAGACTCACCCAGCA	qPCR for HisRS
Primer 39	ACAAAACGCCGATCATCTTC	qPCR for IleRS
Primer 40	ACTGCACGCCTTTGATCTCT	qPCR for IleRS
Primer 41	AAACCGACACTTTCGACACC	qPCR for LeuRS
Primer 42	ATCACGCATCAGTTTGTGGA	qPCR for LeuRS
Primer 43	GGGTTGGGACGTATTGTCAC	qPCR for LysRS
Primer 44	CCGGGTTAACATCATTACGG	qPCR for LysRS
Primer 45	TTACCTGAAGCCGGTACTGC	qPCR for MetRS
Primer 46	TAGAGGCTTCCACCAGTGCT	qPCR for MetRS
Primer 47	ACAGGCAAGCCTGATTATGG	qPCR for PheRS
Primer 48	GGCAGCAGTAAGGCTTCAAC	qPCR for PheRS
Primer 49	TAGGCTGCTGATCAAACGTG	qPCR for ProRS
Primer 50	GATGCCTCGTTCGTCGTAGT	qPCR for ProRS
Primer 51	GATCTCTTCCTGCTCCAACG	qPCR for SerRS
Primer 52	ACCAACAGCCAGACCAGAAC	qPCR for SerRS
Primer 53	GGCACGCGATTAAACAACTT	qPCR for ThrRS
Primer 54	GGTTAACGTGCGGTCAAGAT	qPCR for ThrRS
Primer 55	GCTGGGCACTGAACTGCTAT	qPCR for TrpRS
Primer 56	CGAGGTGCTGTTTCTGGTCT	qPCR for TrpRS
Primer 57	CTATCGCGGCGAACAACTAT	qPCR for TyrRS
Primer 58	GCTGCTTAACCGCTTCTTTG	qPCR for TyrRS
Primer 59	CGCTGGTTAACCGTCGTATT	qPCR for ValRS
Primer 60	ATATCGCCGTCAAAGGTCAG	qPCR for ValRS
Primer 61	TCACATGAAACGGCATGACT	qPCR for GFP
Primer 62	TGTGTCCGAGAATGTTTCCA	qPCR for GFP