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SUPPLEMENTARY INFORMATION

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An aminoacylation ribozyme evolved from a natural tRNA-sensing T-box riboswitch

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Supplementary Table 1| Primers

Name	Sequence (5′-3′)	Used for
Oligo 1	GTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGAAAGGCATTCTTGAGCCCTTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACC	Library construction
Oligo 2	CCACCTTGGCAAGGTGGTGTTCTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTAGGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGTTCCACCCTAGTTGCTGA	Library construction
Oligo 3	CCACCTTGGCAAGGTGGTGTTCTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGTTCCACCCTAGTTGCTGA	Library construction
Oligo 4	GGCGTAATACGACTCACTATAGGGGCAGTGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAG	Library construction
Oligo 5	TG(M)GAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTGGCAAGGTGGT	Library construction
Oligo 6	TG(M)GAGCGGAAGACGGG	RT PCR
Oligo 7	GGCGTAATACGACTCACTATAGGGGCAGTGAGAGAAAGAA	PCR amplification
Oligo 8	GCCTCTTCGCTATTACGCCAGC	Sequencing
Oligo 9	TGTTGTGTGGAATTGTGAGCGG	Sequencing
Oligo 10	CTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGAAAGGCAT	Tx1.3 and Tx2.1
Oligo 11	GCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCT	Tx1.3 and Tx2.1
Oligo 12	GCAGTGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGC	Tx1.3 and Tx2.1
Oligo 13	GGCGTAATACGACTCACTATAGGGGCAGTGAGAGAAAGAAGTAC	Tx1.3 and Tx2.1
Oligo 14	GCCTCTTTTTTAAAAGGGCTCAAGAATGCCTTTCGTTGCGTGCT	Tx1.3 and Tx2.1
Oligo 15	TAGTTGCTGAGAACAAAATCCCAGCCTCTTTTTTAAAAGGGCTC	Tx1.3 and Tx2.1
Oligo 16	CGCTTGCGTAGGGGTTCCACCCTAGTTGCTGAGAACAAAATCCC	Tx1.3
Oligo 17	CGCCGGTGCTAACCTTGTCGGTTCGCTTGCGTAGGGGTTC	Tx1.3
Oligo 18	TCCTGTCGCCGGTGCTAACCTTG	Tx1.3
Oligo 19	TGGGAATTTAGTGGTTCCACCCTAGTTGCTGAGAACAAAATCCC	Tx2.1
Oligo 20	CGGTCGGTCTGAGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1
Oligo 21	TAGGGCTCCCTTAGACGGTCGGTCTGAGTGTCA	Tx2.1
Oligo 22	AGAGTTCTCCCGCGGTTCCACCCTAGTTGCTGAGAACAAAATCCC	glyQS T-box riboswitch
Oligo 23	TAGGGACGAGAGTTCTCCCGCGGTTC	glyQS T-box riboswitch
Oligo 24	CTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGAAATTAAT	Tx2.1 (UUA)
Oligo 25	GCCTCTTTTTTAAAAGGGCTCAAGAATTAATTTCGTTGCGTGCT	Tx2.1 (UUA)
Oligo 26	GGCGTAATACGACTCACTATAG	Tx, tRNA and DNA template
Oligo 27	GAAGTAGTTCAGTGGTAGAACACCACCTTGCCAAGGTGGGGGTC	$tRNA^{Gly}_{GCC}$
Oligo 28	GGCGTAATACGACTCACTATAGCGGAAGTAGTTCAGTGGTAGAA	$tRNA^{Gly}$
Oligo 29	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTGGCA	$tRNA^{Gly}_{GCC}$
Oligo 30	TG(M)GAGCGGAAGACGGGA	$tRNA^{Gly}$
Oligo 31	GAAGTAGTTCAGTGGTAGAACACCACCTTTAAAAGGTGGGGGTC	$tRNA^{Gly}_{UAA}$
Oligo 32	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTTTAA	$tRNA^{Gly}_{UAA}$
Oligo 33	GAAGTAGTTCAGTGGTAGAACACCACCTTGCAAAGGTGGGGGTC	$tRNA^{Gly}_{GCA}$
Oligo 34	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTTGCA	$tRNA^{Gly}_{GCA}$
Oligo 35	GAAGTAGTTCAGTGGTAGAACACCACCTTGACAAGGTGGGGGTC	$tRNA^{Gly}_{GAC}$
Oligo 36	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTGTCA	$tRNA^{Gly}_{GAC}$
Oligo 37	GAAGTAGTTCAGTGGTAGAACACCACCTTTCCAAGGTGGGGGTC	$tRNA^{Gly}_{UCC}$
Oligo 38	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTGGAA	$tRNA^{Gly}_{UCC}$
Oligo 39	GAAGTAGTTCAGTGGTAGAACACCACCTTGAAAAGGTGGGGGTC	$tRNA^{Gly}_{GAA}$
Oligo 40	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTTTCA	$tRNA^{Gly}_{GAA}$
Oligo 41	GAAGTAGTTCAGTGGTAGAACACCACCTTTCAAAGGTGGGGGTC	$tRNA^{Gly}_{UCA}$
Oligo 42	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTTGAA	$tRNA^{Gly}_{UCA}$

Name	Sequence (5′-3′)	Used for
Oligo 43	GAAGTAGTTCAGTGGTAGAACACCACCTTTACAAGGTGGGGGTC	$tRNA^{Gly}_{UAC}$
Oligo 44	TGGAGCGGAAGACGGGATTCGAACCCGCGACCCCCACCTTGTAA	$tRNA^{Gly}_{UAC}$
Oligo 45	AAGTAGTTCAGCTGGTAGAACACCACCTTGCCAAGGTGGGGGTC	$tRNA^{Gly}_{GCC}$ 8nt d-loop
Oligo 46	GGCGTAATACGACTCACTATAGCGGAAGTAGTTCAGCTGGTAGA	$tRNA^{Gly}_{GCC}$ 8nt d-loop
Oligo 47	AGTAGTTCAGCCTGGTAGAACACCACCTTGCCAAGGTGGGGGTC	$tRNA^{Gly}_{GCC}$ 9nt d-loop
Oligo 48	GGCGTAATACGACTCACTATAGCGGAAGTAGTTCAGCCTGGTAG	$tRNA^{Gly}_{GCC}$ 9nt d-loop
Oligo 49	GGTCGGTCTGAGTGTCATGGGAATTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 50	GGTCGGTCTGAGTGTCATGGGAATTTAGVGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 51	GGTCGGTCTGAGTGTCATGGGAATTTAHTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 52	GGTCGGTCTGAGTGTCATGGGAATTTBGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 53	GGTCGGTCTGAGTGTCATGGGAATTVAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 54	GGTCGGTCTGAGTGTCATGGGAATVTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 55	GGTCGGTCTGAGTGTCATGGGAAVTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 56	GGTCGGTCTGAGTGTCATGGGABTTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 57	GGTCGGTCTGAGTGTCATGGGBATTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 58	GGTCGGTCTGAGTGTCATGGHAATTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 59	GGTCGGTCTGAGTGTCATGHGAATTTAGTGGTTCCACCCTAGTTGCTGA	scanning (library construct)
Oligo 60	CTCCCTTAGACGGTCGGTCTGAGTGTCATHGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 61	CTCCCTTAGACGGTCGGTCTGAGTGTCAVGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 62	CTCCCTTAGACGGTCGGTCTGAGTGTCBTGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 63	CTCCCTTAGACGGTCGGTCTGAGTGTDATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 64	CTCCCTTAGACGGTCGGTCTGAGTGVCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 65	CTCCCTTAGACGGTCGGTCTGAGTHTCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 66	CTCCCTTAGACGGTCGGTCTGAGVGTCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 67	CTCCCTTAGACGGTCGGTCTGAHTGTCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 68	CTCCCTTAGACGGTCGGTCTGBGTGTCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 69	CTCCCTTAGACGGTCGGTCTHAGTGTCATGGGAATTTAGTGGTTCCACCC	scanning (library construct)
Oligo 70	TTTTTTTTAGGGCTCCCTTAGACGGTCGGTCVGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 71	TTTTTTTTAGGGCTCCCTTAGACGGTCGGTDTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 72	TTTTTTTTAGGGCTCCCTTAGACGGTCGGVCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 73	TTTTTTTTAGGGCTCCCTTAGACGGTCGHTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 74	TTTTTTTTAGGGCTCCCTTAGACGGTCHGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 75	TTTTTTTTAGGGCTCCCTTAGACGGTDGGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 76	TTTTTTTTAGGGCTCCCTTAGACGGVCGGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 77	TTTTTTTTAGGGCTCCCTTAGACGHTCGGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 78	TTTTTTTTAGGGCTCCCTTAGACHGTCGGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 79	TTTTTTTTAGGGCTCCCTTAGADGGTCGGTCTGAGTGTCATGGGAATTTAGTGG	scanning (library construct)
Oligo 80	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTTAGBCGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 81	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTTAHACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 82	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTTBGACGGT CGGTCTGAGTGTCAT	scanning (library construct)

Name	Sequence (5′-3′)	Used for
Oligo 83	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTVAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 84	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCVTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 85	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCDTTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 86	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCDCTTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 87	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTDCCTTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 88	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCVCCCTTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 89	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGDTCCCTTAGACGGT CGGTCTGAGTGTCAT	scanning (library construct)
Oligo 90	TCAGCAACTAGGGTGGAACCACTAAATTCC	scanning (library construct)
Oligo 91	TCAGCAACTAGGGTGGAACCACTAAATTCCCATGACACTC	scanning (library construct)
Oligo 92	TCAGCAACTAGGGTGGAACCACTAAATTCCCATGACACTCAGACCGACCG	scanning (library construct)
Oligo 93	TACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTTAGACGGTC GGTCTGAGTGTCATG	scanning (library construct)
Oligo 94	CTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTTAGGGCTCCCTTAGACGGT CGGTCT	scanning (library construct)
Oligo 95	CCTTGGCAAGGTGGTGTTCTACCACTGAACTACTTCCGCTTTTTTTTTTTTTTTT AGGGCTCCCTTAGA	scanning (library construct)
Oligo 96	CCTTGGCAAGGTGGTGTTCTACCACTGAACTACTTCCGC	scanning (library construct)
Oligo 97	GAGGCTGGGATTTTGTTCTCA	scanning (RT PCR)
Oligo 98	GGTGGTGTTCTACCACTGAACTACTT	scanning (RT-PCR)
Oligo 99	CACTCTTTCCCTACACGACGCTCTTCCGATCTGAGGCTGGGATTTTGTTCTCA GCA	scanning (for miseq)
Oligo 100	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGGTGTTCTACCACTGAAC TACTTCCG	scanning (for miseq)
Oligo 101	GGTGGAACCCCTACGCAAGCGAACCGACAAG	$Tx1.3_{140-190}$
Oligo 102	GGCGTAATACGACTCACTATAGGGGTGGAACCCCTACGCAA	$Tx1.3_{140-190}$
Oligo 103	TCCTGTCGCCGGTGCTAACCTTGTCGGTTCGCTTGC	$Tx1.3_{140-190}$
Oligo 104	TCCTGTCGCCGGTGCT	$Tx1.3_{140-190}$
Oligo 105	GTGGAACCACTAAATTCCCATGACACTCAGACCG	$Tx2.1_{140-194}$
Oligo 106	GGCGTAATACGACTCACTATAGGGTGGAACCACTAAATTCCCA	$Tx2.1_{140-194}$
Oligo 107	TAGGGCTCCCTTAGACGGTCGGTCTGAGTGTCATGGG	$Tx2.1_{140-194}$
Oligo 108	TAGGGCTCCCTTAGACGG	$Tx2.1_{140-194}$
Oligo 109	TGCCAATTTAGTGGTTCCACCCTAGTTGCTGAGAACAAAATCCC	Tx2.1 C158C159/G187G186
Oligo 110	CGGTCGGTCTGAGTGTCATGCCAATTTAGTGGTTCCAC	Tx2.1 C158C159/G187G186
Oligo 111	TAGGGCTGGCTTAGACGGTCGGTCTGAGTGTCA	Tx2.1 C158C159/G187G186
Oligo 112	CGGTCGGTCTGAGTGAGATGGGAATTTAGTGGTTCCAC	Tx2.1 G163A164/C181U180
Oligo 113	TAGGGCTCCCTTAGACGGTCGGTCTGAGTGAGA	Tx2.1 G163A164/C181U180
Oligo 114	TAGGGCTCCCTTACTCGGTCGGTCTGAGTGAGA	Tx2.1 G163A164/C181U180
Oligo 115	TAGGGCTCCCTTACTCGGTCGGTCTGAGTGTCA	Tx2.1 G163A164/C181U180
Oligo 116	CGGACGGTCTGAGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 U168–A176
Oligo 117	TAGGGCTCCCTTAGACGGACGGTCTGAGTGTCA	Tx2.1 U168–A176
Oligo 118	CGGACGGTCTGTGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 U168–A176
Oligo 119	TAGGGCTCCCTTAGACGGACGGTCTGTGTGTCA	Tx2.1 U168–A176
Oligo 120	CGGTGGGTCTGAGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 C169–G175
Oligo 121	TAGGGCTCCCTTAGACGGTGGGTCTGAGTGTCA	Tx2.1 C169–G175
Oligo 122	CGGTGGGTCTCAGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 C169–G175
Oligo 123	TAGGGCTCCCTTAGACGGTGGGTCTCAGTGTCA	Tx2.1 C169–G175
Oligo 124	TGGGAATTTATTGGTTCCACCCTAGTTGCTGAGAACAAAATCCC	Tx2.1 C151A

Name	Sequence (5′-3′)	Used for
Oligo 126	CGGTCTGTCTGAGTGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 C174A
Oligo 127	TAGGGCTCCCTTAGACGGTCTGTCTGAGTGTCA	Tx2.1 C174A
Oligo 128	CGGTCGGTCTGAGGGTCATGGGAATTTAGTGGTTCCAC	Tx2.1 A166C
Oligo 129	TAGGGCTCCCTTAGACGGTCGGTCTGAGGGTCA	Tx2.1 A166C
Oligo 130	TAGGGCTCCCCTAGACGGTCGGTCTGAGTGTCA	Tx2.1 A166C
Oligo 131	TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAACGGC	DNA template
Oligo 132	GTCGTCGTCCTTGTAGTCCTTCTTCTTCGGGTTTTTCTC	DNA template
Oligo 133	CGAAGCTCACTTGTCGTCGTCGTCCTTGTAGTC	DNA template
Oligo 134	GTAATACGACTCACTATAGGATCGAAAGATTTCCGC	eFx
Oligo 135	ACCTAACGCTAATCCCCTTTCGGGGCCGCGGAAATCTTTCGATCC	eFx
Oligo 136	GGCGTAATACGACTCACTATAG	eFx
Oligo 137	ACCTAACGCTAATCCCCT	eFx

G(M) indicates 2′-*O*-methylated guanosine. N denotes for all possible nucleotides. B denotes for all possible nucleotides except for adenosine. D denotes for all possible nucleotides except for cytidine. H denotes for all possible nucleotides except for guanosine. V denotes for all possible nucleotides except for thymidine.

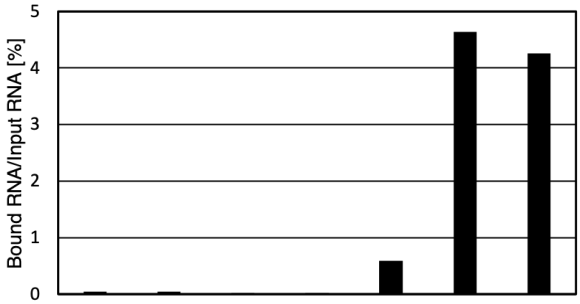
Supplementary Table 2| Transcripts.

Name	Transcript (5′-3′)
Library1	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAACACCAC CUUGCCAAGGUGGGGGUCGCGGGUUCGAAUCCCGUCUUCCGCUCCA
Library2	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNCCCUAAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAAC ACCACCUUGCCAAGGUGGGGGUCGCGGGUUCGAAUCCCGUCUUCCGCUCCA
Tx1.1-tRNA	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCAGAGGACCGGCCAAUGCGCAGCCUAC AACCGCACACUCUAAAAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAACACCACCU UGCCAAGGGGGGGUCGCGGGUUCGAAUCCCGUCUUCCGCUCCA
Tx1.2-tRNA	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCCAAAAAUGCAAGGGGCCUCUUUUAAG ACGCCCCAUCGGCUAAAAAAAAAAAAAGAAGCGGAAGUAGUUCAGUGGUAGAACACCACC UUGCCAAGGUGGGGGUCGCGGGUUCGAAUCCCGUCUUCCGCUCCA
Tx1.3-tRNA	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCCCUACGCAAGCGAACCGACAAGGUUA GCACCGGCGACAGGAAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAACACCACC UUGCCAAGGUGGGGGUCGCGGCUUCGAAUCCCGUCUUCCGCUCCA
Tx2.1-tRNA	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACACUCAGACCG ACCGUCUAAGGGAGCCCUAAAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAACAC CACCUUGCCAAGGUGGGGGUCGCGGGUUCGAAUCCCGUCUUCCGCUCCA
Tx2.2-tRNA	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUCCUCAGCAACUAGGGUGGAACCGGCAGAAGAAAAUAACUGGAAACCGGC GCGGCCAAGAGCACCCUAAAAAAAAAAAAAAAAAGCGGAAGUAGUUCAGUGGUAGAACACC ACCUUGCCAAGGUGGGGGGUCGCGGGUUCGAAUCCCGUCUUUCGCUCCA
Tx1.1	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCAGAGGACCGGCCAAUGCGCAGCCUAC AACCGCACACUCUA
Tx1.2	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCCAAAAAUGCAAGGGGCCUCUUUUAAG ACGCCCCAUCGGCU
Tx1.3	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCCCUACGCAAGCGAACCGACAAGGUUA GCACCGGCGACAGGA
Tx2.1	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACACUCAGACCG ACCGUCUAAGGGAGCCCUA
Tx2.2	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCGGCAGAAGAAAAUAACUGGAAACCGGC GCGGCCAAGAGCACCCUA

Name	Transcript (5′-3′)
$Tx2.1_{UUA}$	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAUUAAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACACUCAGACCG ACCGUCUAAGGGAGCCCUA
$tRNA^{Gly}_{GCC}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUGCCAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{GCA}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUGCAAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{GAC}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUGACAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{UCC}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUGCCAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{GAA}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUGAAAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{UCA}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUUCAAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{UAC}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUUACAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{UAA}$	GCGGAAGUAGUUCAGUGGUAGAACACCACCUUUAAAAGGUGGGGGUCGCGGGUUCGAA UCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{GCC}$ 8nt d-loop	GCGGAAGUAGUUCAGCUGGUAGAACACCACCUUGCCAAGGUGGGGGUCGCGGGUUCGA AUCCCGUCUUCCGCUCCA
$tRNA^{Gly}_{GCC}$ 9nt d-loop	GCGGAAGUAGUUCAGCCUGGUAGAACACCACCUUGCCAAGGUGGGGGUCGCGGGUUCG AAUCCCGUCUUCCGCUCCA
$Tx1.3_{140\text{-}190}$	GGGUGGAACCCCUACGCAAGCGAACCGACAAGGUUAGCACCGGCGACAGGA
$Tx2.1_{140\text{-}194}$	GGGUGGAACCACUAAAUUCCCAUGACACUCAGACCGACCGUCUAAGGGAGCCCUA
Tx2.1 G186C/G187C	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACUCAGACCGACCGUCUAAGCCAGCCCUA
Tx2.1 C158G/C159G/G186C/ G187C	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUGGCAUGACACUCAGACCGACCGUCUAAGCCAGCCCUA
Tx2.1 U180A/C181G	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACUCAGACCGACCGAGUAAGGGAGCCCUA
Tx2.1 G163C/A164U/U180A/ C181G	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUCUCACUCAGACCGACCGAGUAAGGGAGCCCUA
Tx2.1 A176U	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACUCAGACCGUCCGUCUAAGGGAGCCCUA
Tx2.1 U168A/A176U	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACACAGACCGUCCGUCUAAGGGAGCCCUA
Tx2.1 G175C	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACUCAGACCCACCGUCUAAGGGAGCCCUA

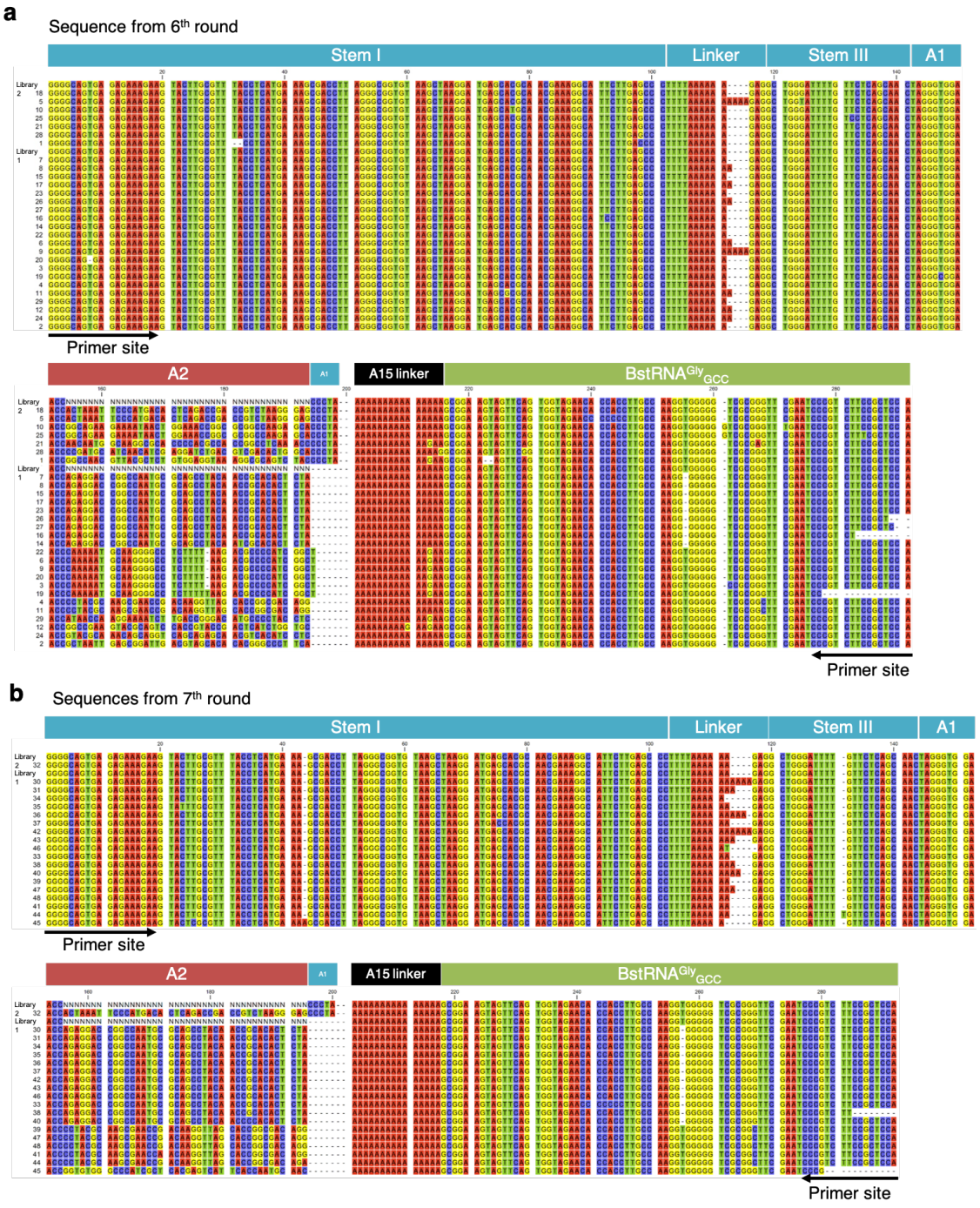
Name	Transcript (5′-3′)
Tx2.1 C169G/G175C	GGCGUAAUACGACUCACUAUAGGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCA UGAAAGCGACCUUAGGGCGGUGUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUU GAGCCCUUUUAAAAAAGAGGCUGGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUA AAUUCCCAUGACACUGAGACCCACCGUCUAAGGGAGCCCUA
Tx2.1 C151A	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCAAUAAAUUCCCAUGACACUCAGACCG ACCGUCUAAGGGAGCCCUA
Tx2.1 C174A	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACACUCAGACAG ACCGUCUAAGGGAGCCCUA
Tx2.1 A166C	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACCCUCAGACCG ACCGUCUAAGGGAGCCCUA
Tx2.1 A184G	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCACUAAAUUCCCAUGACACUCAGACCG ACCGUCUAGGGGAGCCCUA
glyQS T-box riboswitch	GGGGCAGUGAGAGAAAGAAGUACUUGCGUUUACCUCAUGAAAGCGACCUUAGGGCGGU GUAAGCUAAGGAUGAGCACGCAACGAAAGGCAUUCUUGAGCCCUUUUAAAAAAGAGGCU GGGAUUUUGUUCUCAGCAACUAGGGUGGAACCGCGGGAGAACUCUCGUCCCUA
mRNA for translation	GGGUUAACUUUAACAAGGAGAAAAACGGCAAGAAGAAGGACUACAAGGACGACGACGAC AAGUGAGCUUCG
eFx	GGAUCGAAAGAUUUCCGCGGCCCCGAAAGGGGAUUAGCGUUAGGU

N denotes for all possible nucleotides.

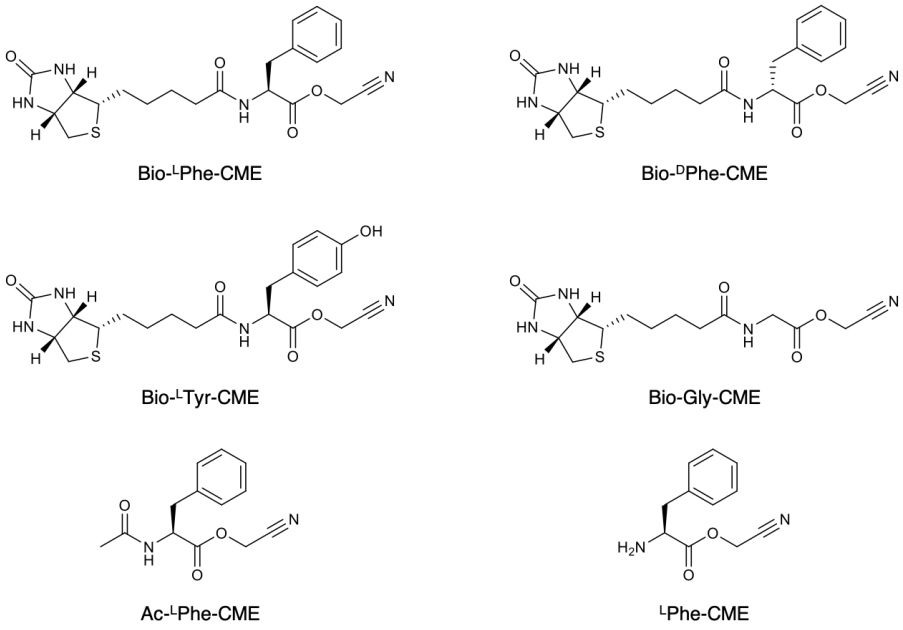


Round	1st	2nd	3rd	4th	5th	6th	7th
Bio-ᴸPhe-CME [mM]	5	5	5	5	5	5	5
Acylation volume [µL]	200	100	100	100	100	100	100
RNA in acylation [µM]	10	1	1	1	1	1	1
SAv beads volume [µL]	200	20	20	20	20	20	20
Acylation time [hr]	2	2	2	2	2	2	0.5

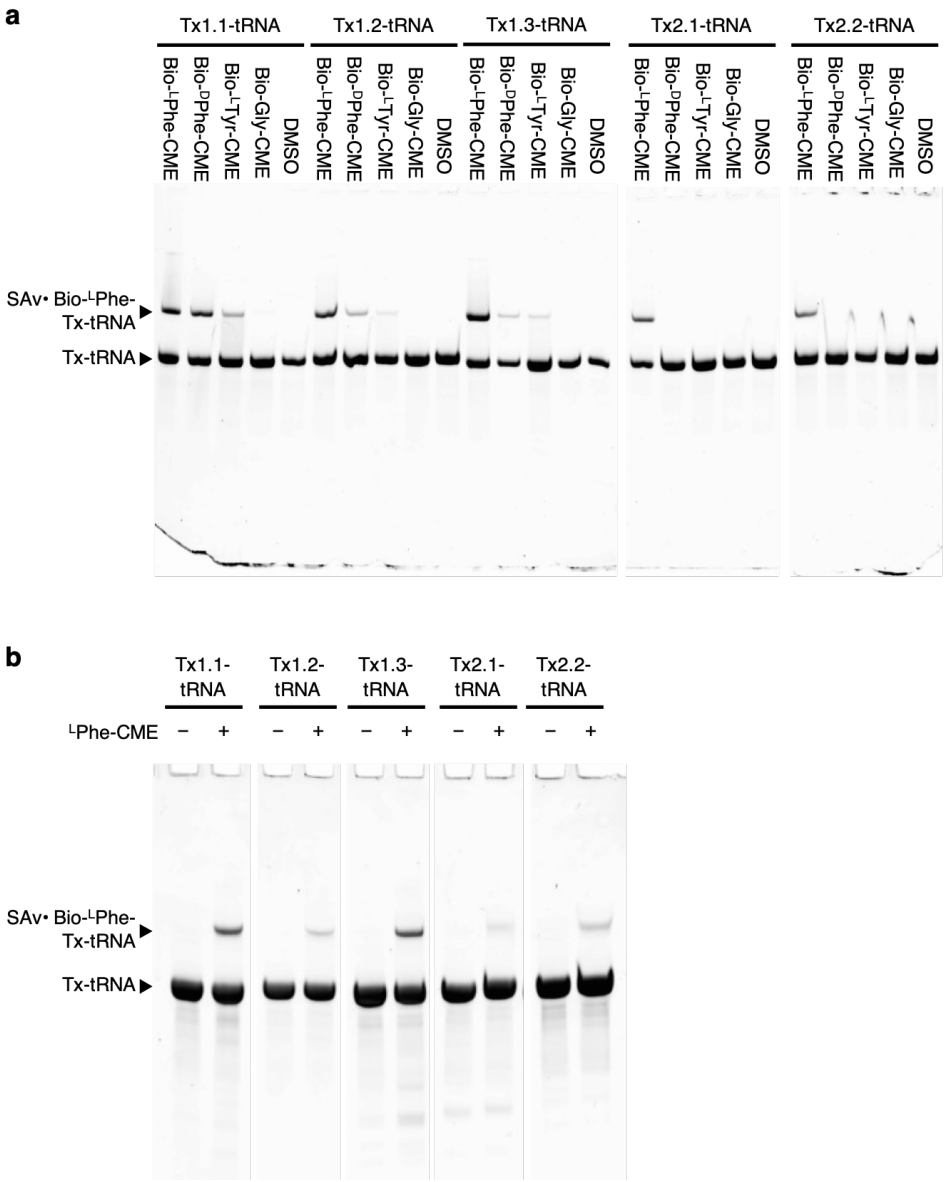
Supplementary Figure 1| The progress of *in vitro* selection. The conditions are listed on the bottom of the figure.



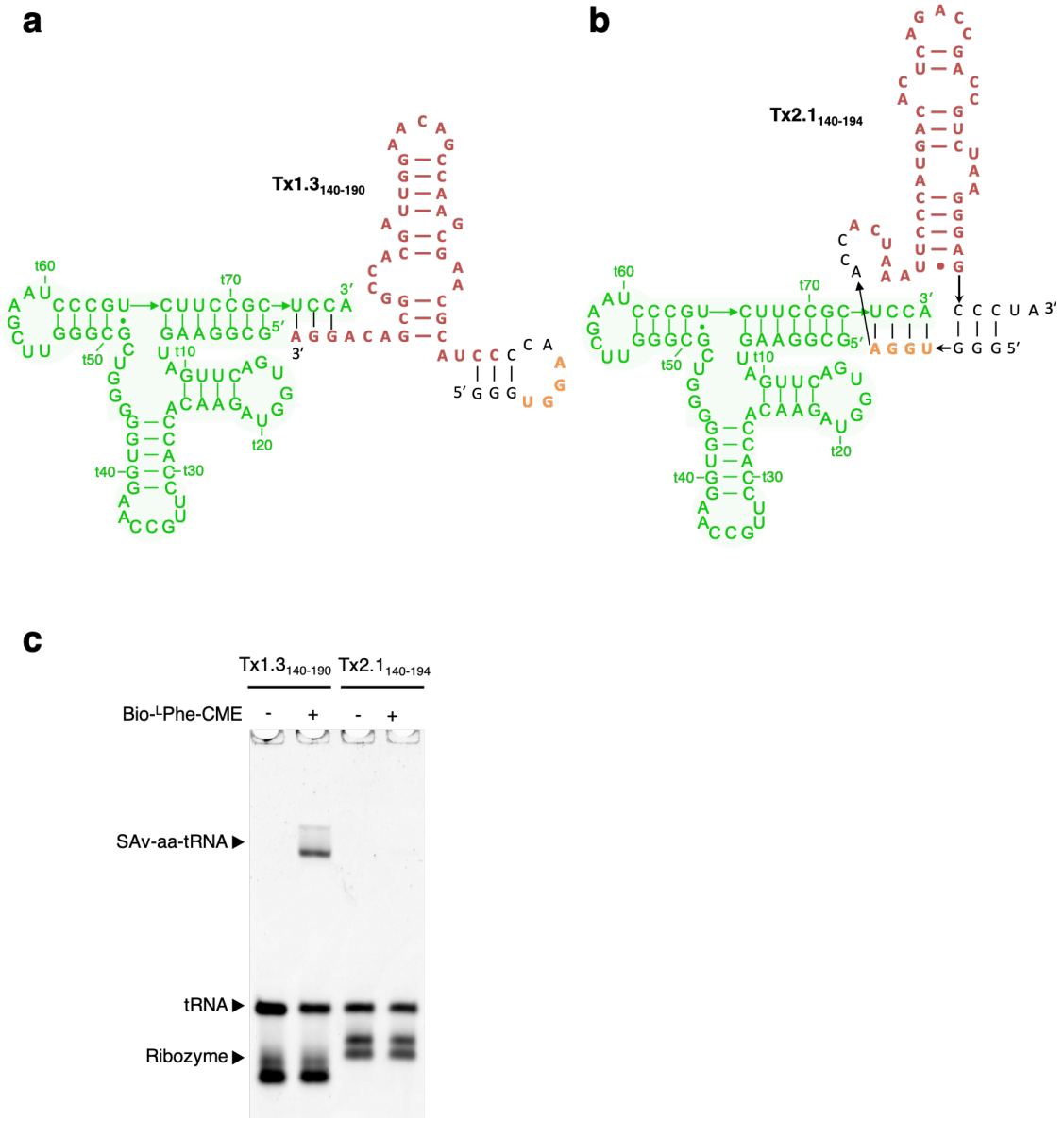
Supplementary Figure 2| Full sequences recovered after 6th and 7th round. (**a**) The complete sequences from the library after round 6th. (**b**) The complete sequences from the library after round 7th. Top lane indicates the corresponding positions within the designed library.



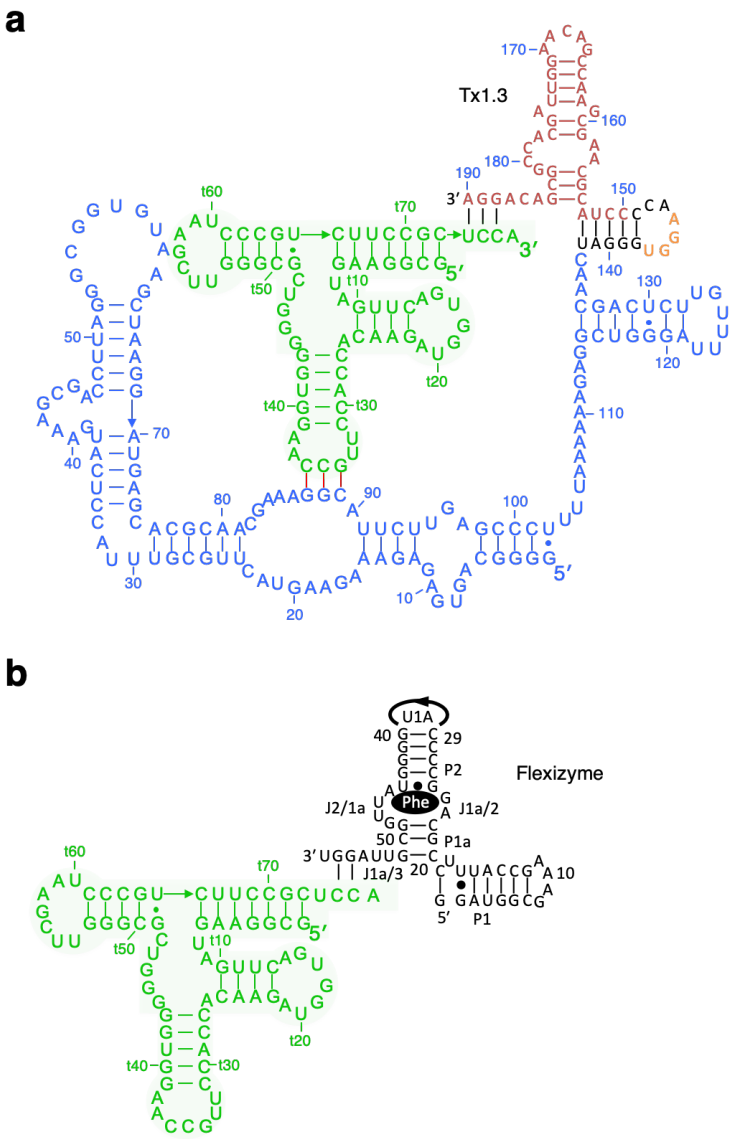
Supplementary Figure 3| Chemical structures of amino acid substrates used in this study. Complete structure of amino acids substrates used in this study.



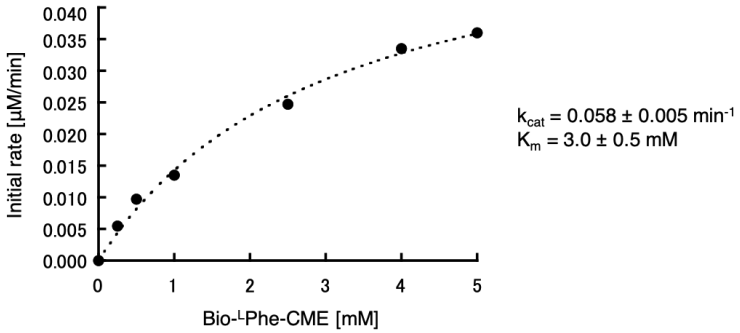
Supplementary Figure 4| Amino acid specificity of five clones in self-aminoacyaltion. (**a**) Self-aminoacylation activity of five clones against biotinylated substrates. (**b**) Self-aminoacylation activity of five clones against ^{L}Phe-CME. The aminoacylation reaction was followed by biotinylation of ^{L}Phe using Biotin-3-sulfo-*N*-hydroxylsuccinimide ester.



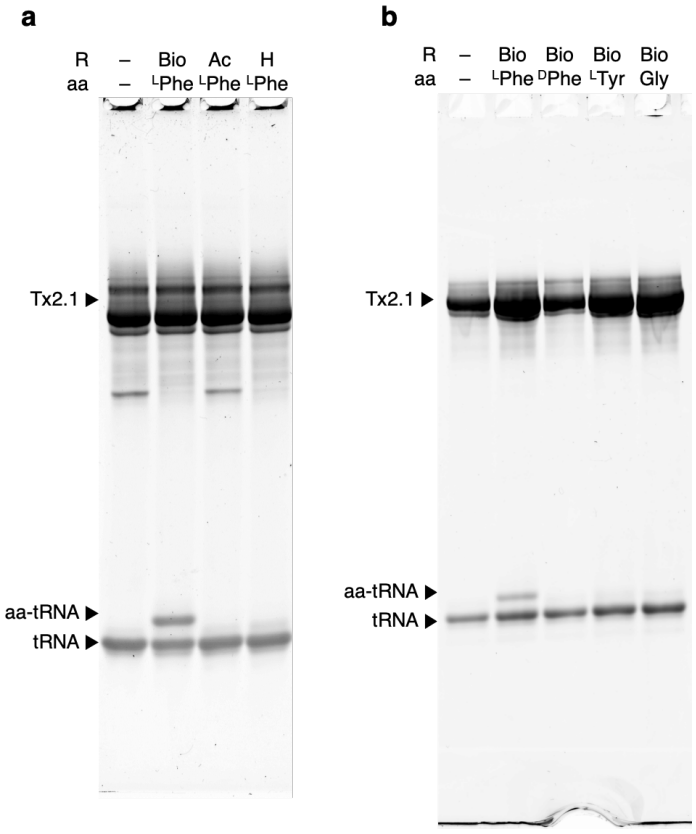
Supplementary Figure 5| *Trans*-aminoacylation experiment with truncated Tx1.3 and Tx2.1. (**a**) Possible secondary structure of truncated Tx1.3. Green letter denotes the tRNA sequence. Red letter denotes the random region in the designed library. Orange letter denotes the sequence that interacts with the tRNA 3′-end in the original T-box riboswitch. (**b**) Possible secondary structure of truncated Tx2.1. Color-coding as in (**a**). (**c**) *Trans*-aminoacylation activity of truncated Tx1.3 and Tx2.1.



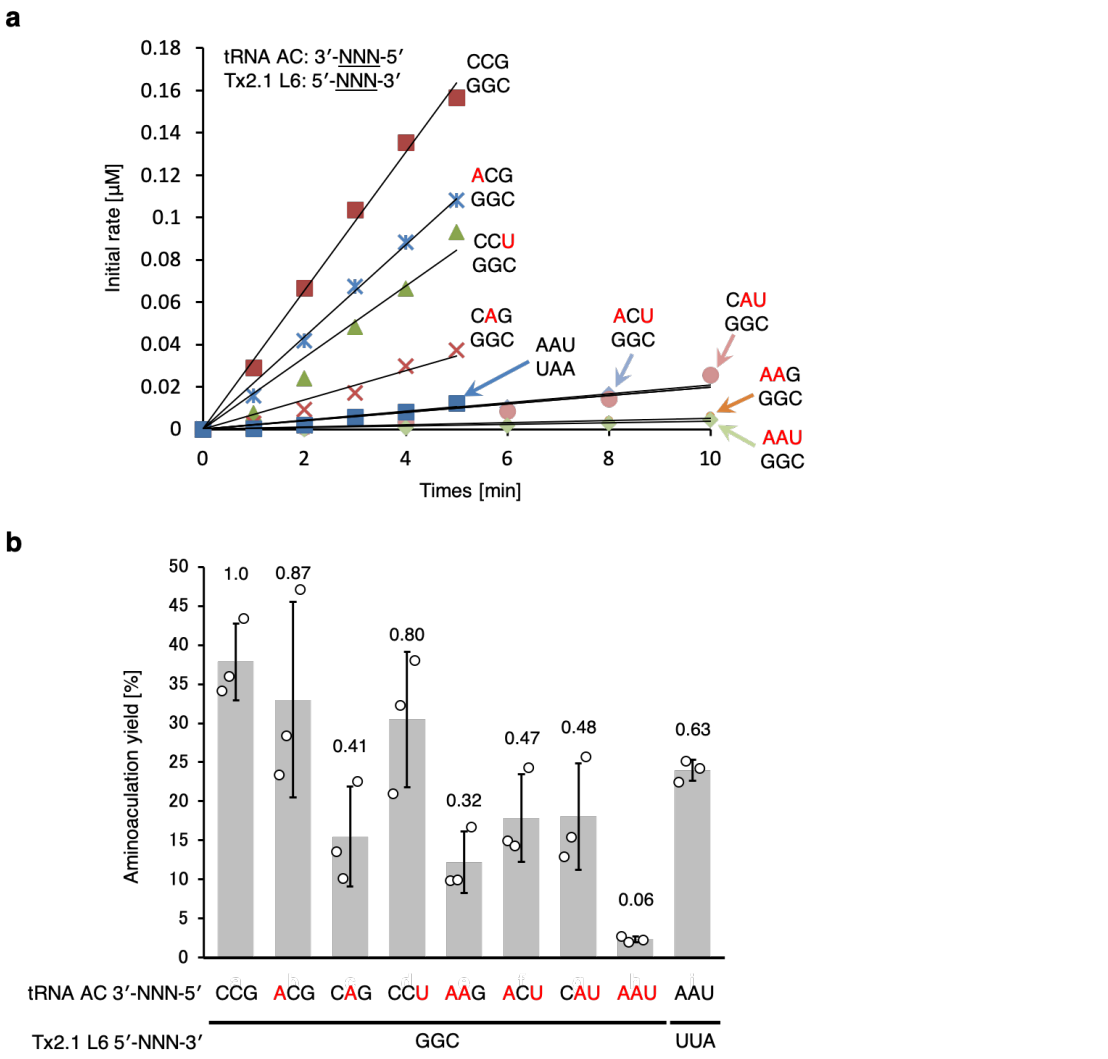
Supplementary Figure 6| Predicted secondary structures of Tx1.3. (**a**) Predicted secondary structure of Tx1.3. This structure was predicted from U138 of Tx1.3 and matched with the previously identified G1-C137 structure. Color-coding as in Supplementary Fig. 9. (**b**) Secondary structure of previously discovered aminoacylation ribozyme, flexizyme revealed by Xiao, H., Murakami, H., Suga, H. & Ferré-D'Amaré, A.R. Structural basis of specific tRNA aminoacylation by a small in vitro selected ribozyme. *Nature* 454, 358-361 (2008).



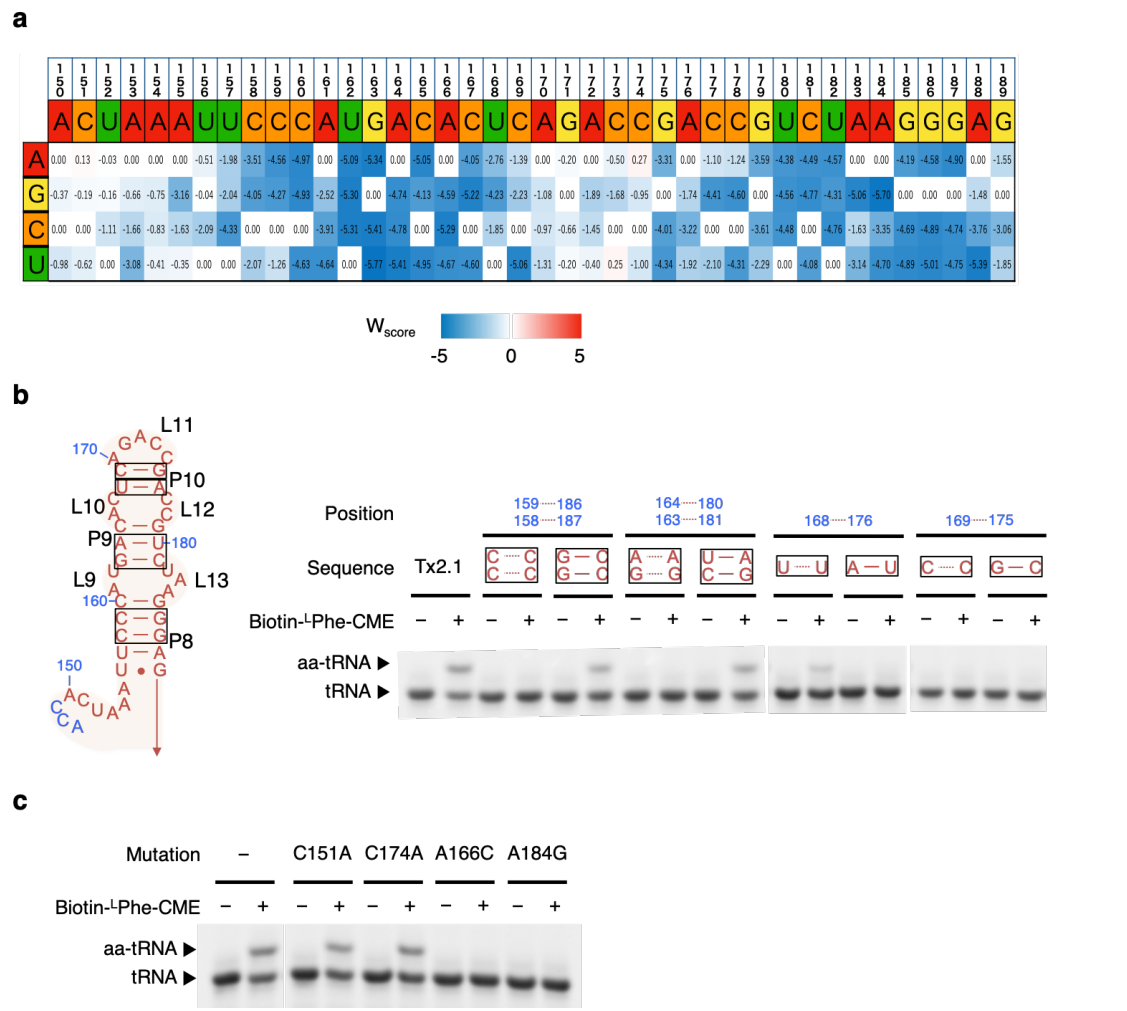
Supplementary Figure 7| Plot of self-aminoacylation activity initial rates. Each data point represents the mean of three independent experiments. The plot was fitted to the Michaelis-Menten non-linear curve by KaleidaGraph (HULINKS)



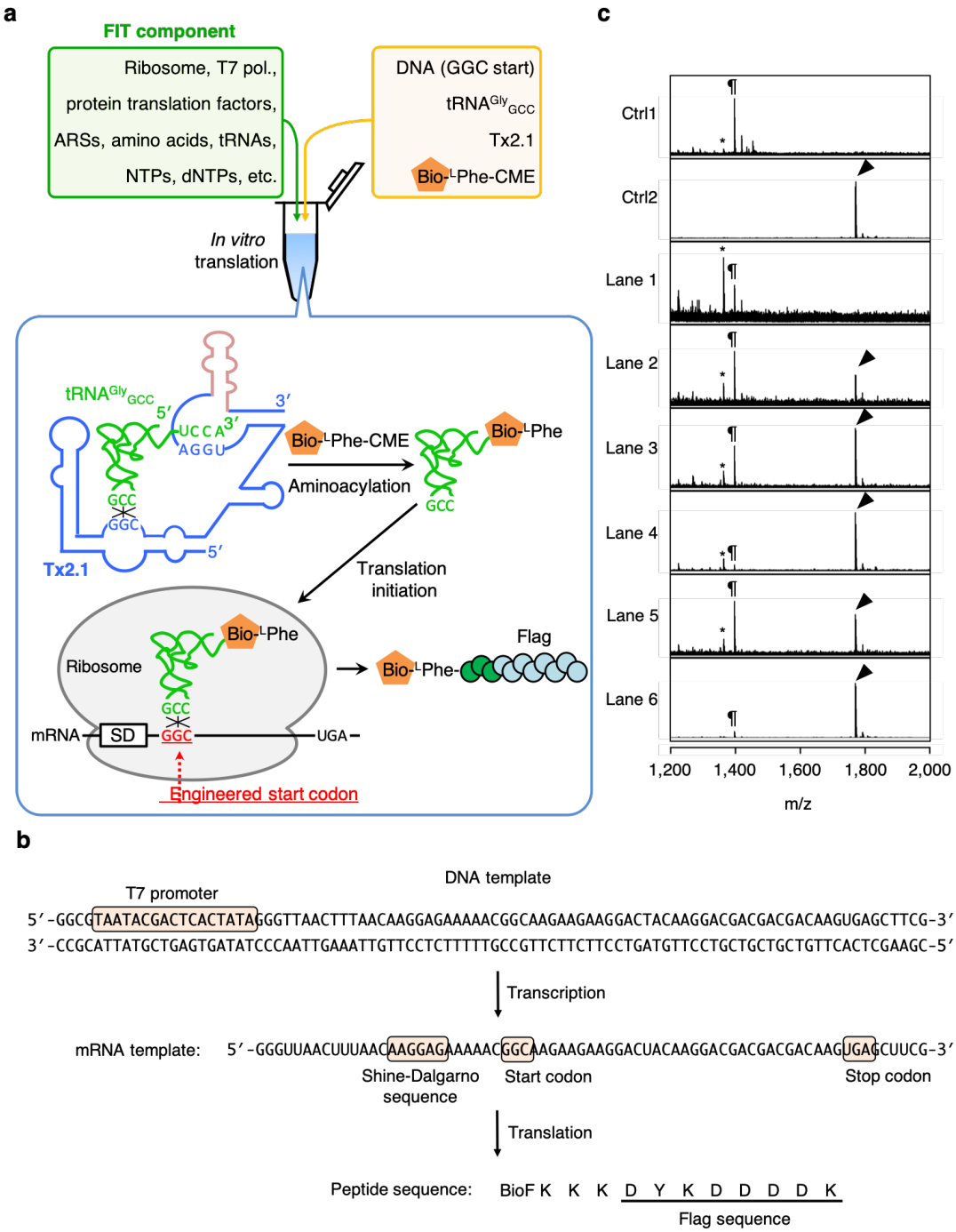
Supplementary Figure 8| Amino acid specificity of Tx2.1. (a) *Trans*-aminoacylation activity against ^{L}Phe derivatives. (b) *Trans*-aminoacylation activity against biotinylated substrates.



Supplementary Figure 9| *Trans*-aminoacylation activity of Tx2.1 against mutant tRNAs. (**a**) The initial rate of aminoacylation reaction against various mutant tRNAs. Each data point represents the mean of three independent experiments. Red letter denotes the position of base pair mismatch. (**b**) Endpoint analysis of *trans*-aminoacylation activity against various tRNA mutants. The values are the mean of n = 3 independent experiments and each measurement presented as an open circle. Error bars represent the standard deviation and the reaction was conducted for 2 hrs.



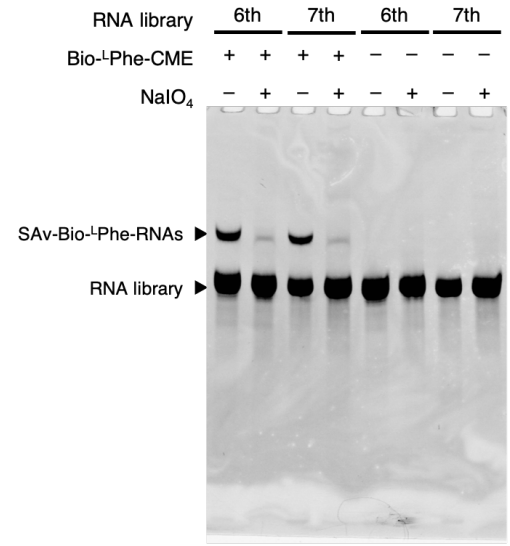
Supplementary Figure 10| Mutational studies for Tx2.1 structure determination. (**a**) W score mapping of all mutants tested in the deep mutational scanning. Top lane corresponds to the nucleotide position in Tx2.1, followed by the actual nucleotide sequence in each position. (**b**) Mutational study to check the base paring in P8, P9 and P10. The base paring positions studied are highlighted with black rectangles in the predicted secondary structure on the left. (**c**) *Trans*-aminoacylation activity of selected clones from (**a**).



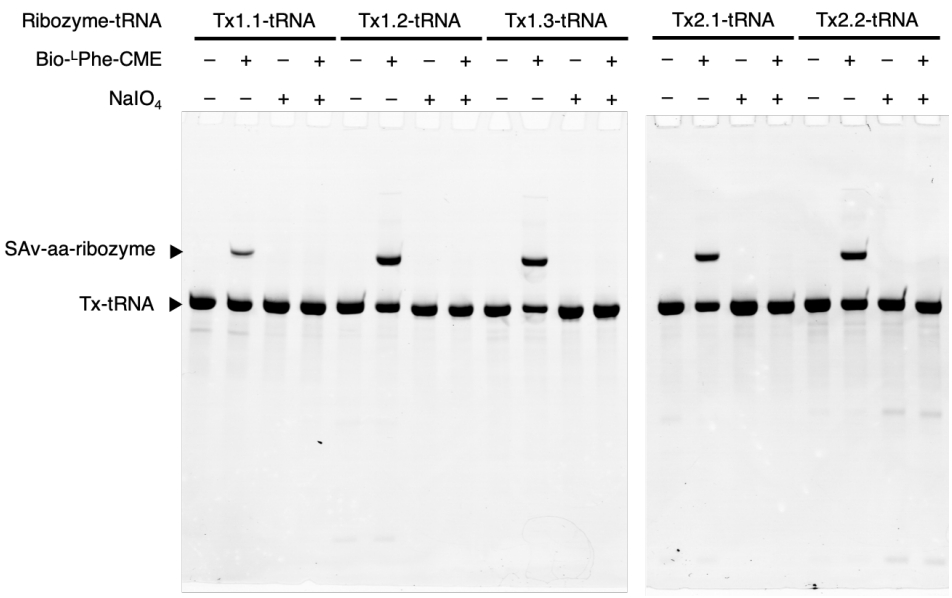
Supplementary Figure 11| *In situ* aminoacylation and translation experiments. (a) Detailed schematic illustration of *in situ* aminoacylation and translation experiment conducted in this study. Rectangle SD indicates the Shine-Dalgarno sequence in the mRNA. (**b**) The design of the DNA template used in the translation experiment. The transcribed mRNA as well as the translated peptide sequence is also shown. (**c**) MALDI-TOF-MS spectra of translation products of each lane from Fig. 5b. Black

triangle indicates the full-length product. ¶ denotes the peptide initiating from the second amino acid. * denotes background peak present in the translation system.

a


RNA library
6th
7th
6th
7th
Bio-LPhe-CME
+ + + + − − − −
NaIO4
− + − + − + − +
SAv-Bio-LPhe-RNAs
RNA library

b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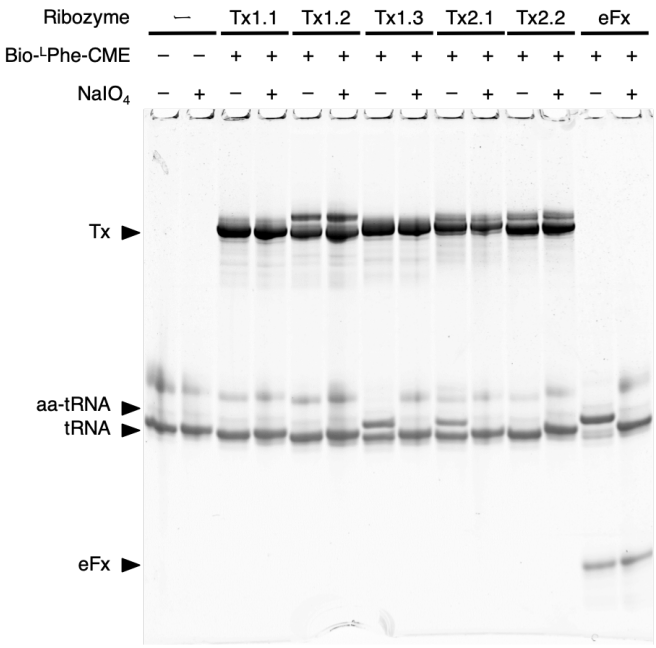
Tx-tRNA

c


Ribozyme
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Tx1.2
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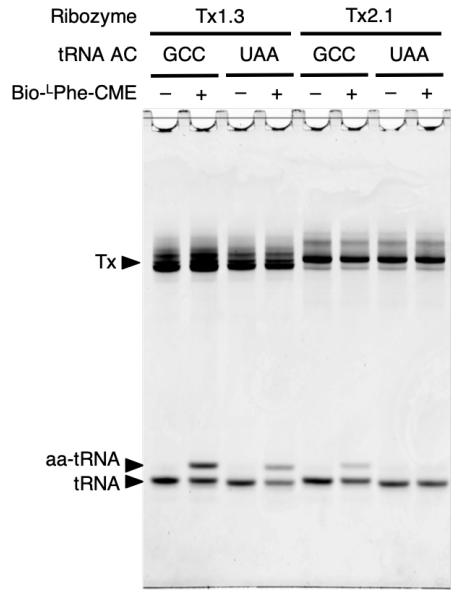
eFx

d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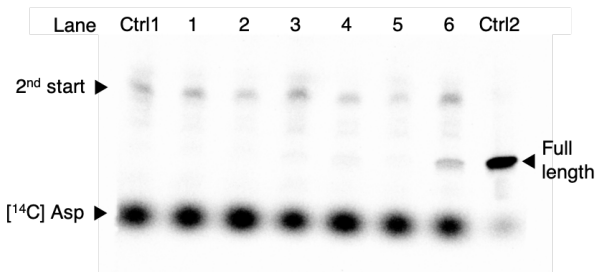
Lane
Ctrl1
1
2
3
4
5
6
Ctrl2
2nd start
Full length
[14C] Asp

Supplementary Figure 12| Full image of the gel used in the main figures. (**a**) Full gel image of Fig. 2a. (**b**) Full gel image of Fig. 3a. (**c**) Full gel image of Fig. 3b. (**d**) Full gel image of Fig. 3c. (**e**) Full gel image of Fig. 5b.