Electronic Supplementary Information

For

Improvement of *In Vitro*-Transcribed Amber Suppressor tRNAs toward Higher Suppression Efficiency in Wheat Germ Extract

Atsushi Ogawa, Yasunori Doi, Nobuto Matsushita*

[a]*Senior Research Fellow Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan*

CONTENTS:

Primers and Templates for PCR.

Forward primer for the amber and amber-free templates

CATACGATTTAGGTGACACT

Reverse primer for the amber and amber-free templates

TTAGCGGCTTTATTGATTGC

Plasmid sequences as PCR templates for the amber and amber-free templates (only the region to be amplified is shown)

(XXX = TAG for pHis-TAG-RY-YPet, TCT for pHis-SRY-YPet; shaded: SP6 promoter; underlined: priming sites; red: start and stop codons)

…CATACGATTTAGGTGACACTATAGAACTCACCTATCTCCCCAACACCTAATAACAT
TCAATCACTCTTTCCACTAACCACCTATCTACATCACCAAGATATCACTAGTATGGC
CCATCACCATCACCATCATXXXAGATACAGCAGCGGCCTGGTGCCGCGCGGCAGC
CACATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG
AGCTGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGG
GCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGCTGTGCACCACCGGCAAGCT
GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCGTGCAGTGCTTC
GCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGA
AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGG
GCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTA
CAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCC
AACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACT
ACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTA
CCTGAGCTACCAGTCCGCCCTGTTCAAAGACCCCAACGAGAAGCGCGATCACATG
GTCCTGCTGGAGTTCCTGACCGCCGCCGGGATCACTGAGGGCATGAACGAGCTGT
ACAAGTAACTCGAGCTCCTGGGCCTCATGGGCCTTCCTTTCACTGCCCGCTTTCCA
GTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTTTCCTTGCGT
ATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGGTA
AAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAA
GGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAA
ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC
GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCG
GATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGC
TGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGA
ACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA

ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC
AGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG
GCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTG
GTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGG
GATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAA
ATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTATTAG
AAAAATTCATCCAGCAGACGATAAAACGCAATACGCTGGCTATCCGGTGCCGCAAT
GCCATACAGCACCAGAAAACGATCCGCCCATTCGCCGCCCAGTTCTTCCGCAATAT
CACGGGTGGCCAGCGCAATATCCTGATAACGATCCGCCACGCCCAGACGGCCGCA
ATCAATAAAGCCGCTAA…

Forward primer for S1, S3, S1-2V, S1-2AC[a]-X except for S1-2AC[a]-$G_{27}C_{43}$-2A-G_{73}, S1-2AC[b], S1-U_{26}, S1-$U_{31}A_{39}$, dc6[a], and dc6[b]
GAAATTAATACGACTCACTATAGTCGATATGTCCGAGTG
Reverse primer for S1, S1-2AC[a]-X except for S1-2AC[a]-$G_{27}C_{43}$-2A-G_{73}, S1-2AC[b], S1-U_{26}, and S1-$U_{31}A_{39}$
TGGCGTCGACAGCAGGATTCGAACCTGCGCAGGCAAAG
Template for S1
GTCGATATGTCCGAGTGGTTAAGGAGACAGACTCTAAATCTGTTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Forward primer for S2, S7, S2-1V, S2-1AC[a], S2-1AC[b], S2-G_{73}, S2-$G_{27}C_{43}$-G_{73}, dc1[a], and dc1[b]
GAAATTAATACGACTCACTATAGGAGAGATGGCTGAGTG
Reverse primer for S2, S2-1AC[a], and S2-1AC[b]
TGGAGGAGAGAGAGGGATTCGAACCCTCGATAGTTC
Template for S2 and S2-G_{73}
GGAGAGATGGCTGAGTGGTTGATAGCTCCGGTCTCTAAAACCGGTATAGTTCTAGG
AACTATCGAGGGTTCGAATCCCT

Reverse primer for S3
TGGCGTCGACAGCAGGGTTCGAACCTGCGCGGGCGAAG
Template for S3
GTCGATATGTCCGAGTGGTTAAGGAGACAGACTCTAAATCTGTTGGGCTTCGCCCG

CGCAGGTTCGAACCCTGCTGTCG

Forward primer for S4

GAAATTAATACGACTCACTATAGTGGTCGTGCCGGAG

Reverse primer for S4

TGGCGTGGTCGGCAGGATTCGAACCTGCGCGGGCAAAG

Template for S4

GTGGTCGTGCCGGAGTGGTTATCGGGCATGACTCTAAATCATGTGGGCTTTGCCCG
CGCAGGTTCGAATCCTGCCGACC

Forward primer for S5

GAAATTAATACGACTCACTATAGGAGAGATGGCCGAG

Reverse primer for S5

TGGCGGAAAGAGAGGGATTCGAACCCTCGGTAAAC

Template for S5

GGAGAGATGGCCGAGCGGTTCAAGGCGTAGCATTCTAACTGCTATGTAGACTTTTG
TTTACCGAGGGTTCGAATCCCTC

Forward primer for S6

GAAATTAATACGACTCACTATAGACGCTTTGGCCGAGTGG

Reverse primer for S6

TGGCGACGCCTGAGAGATTCGAAC

Template for S6

GACGCTTTGGCCGAGTGGTTAAGGCGTGTGCCTCTAAAGTACATGGGGTTTCCCCG
CGAGAGTTCGAATCTCTCAGGCG

Reverse primer for S7

TGGCGGAAAGAGAGGGATTCGAACCCTCGGTACAAAAAAATTG

Template for S7

GGAGAGATGGCTGAGTGGACTAAAGCGGCGGATTCTAAATCCGTTGTACAATTTTT
TTGTACCGAGGGTTCGAATCCCT

Forward primer for L1 and A4

GAAATTAATACGACTCACTATAGTCAAGATGGCCGAG

Reverse primer for L1 and A4

TGGTGTCAAGAATGGGATTCGAACCCATG

Template for L1

GTCAAGATGGCCGAGTTGGTCTAAGGCGCCAGTTTCTAGTACTGGTCCGAAAGGG
CATGGGTTCGAATCCCATTCTTGA

Forward primer for L2

GAAATTAATACGACTCACTATAGCCGCCATGGTGAAATTG

Reverse primer for L2

TGGTGCCGCCACTCGGACTCGAACCGAGATGCTTG

Template for L2

GCCGCCATGGTGAAATTGGTAGACACGCTGCTCTCTAGAAGCAGTGCTCAAGCATC
TCGGTTCGAGTCCGAGTGGCGGC

Forward primer for L3

GAAATTAATACGACTCACTATAGACAGTTTGGCCGAG

Reverse primer for L3

TGGTGACAGCTGTGGGATTTGAACCCACGCCCTTTC

Template for L3

GACAGTTTGGCCGAGTGGTCTAAGGCGCCAGATTCTAGCTCTGGTCCGAAAGGGC
GTGGGTTCAAATCCCACAGCTGTC

Forward primer for L4

GAAATTAATACGACTCACTATAGTTGAGATGGCCGAG

Reverse primer for L4

TGGTGTTGAGAGTGGGATTTGAACCCACGCCCTTTC

Template for L4

GTTGAGATGGCCGAGTTGGTCTAAGGCGCCAGATTCTAGTTCTGGTCCGAAAGGG
CGTGGGTTCAAATCCCACTCTCAA

Forward primer for L5

GAAATTAATACGACTCACTATAGCCTTGATGGTGAAATG

Reverse primer for L5

TGGTGCCTTGAAGAGGACTCGAACCTCCACGCTC

Template for L5

GCCTTGATGGTGAAATGGTAGACACGCGAGACTCTAAATCTCGTGCTAAAGAGCGT
GGAGGTTCGAGTCCTCTTCAAGG

Forward primer for L6

GAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTG

Reverse primer for L6

TGGTGTCAGAAGTGGGATTTGAACCCACGCCCTCGTTAG

Template for L6

GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTCTGGTCCTCTAACGA
GGGCGTGGGTTCAAATCCCACTTC

Forward primer for L7

GAAATTAATACGACTCACTATAGCTGGTTTGCCCGAGTG

Reverse primer for L7

TGGTGCTGGCTGTGGGGTTCGAACCCACGCGCACTTC

Template for L7

GCTGGTTTGCCCGAGTGGTTAAGGGGGAAGACTCTAGATCTTCTGCACTGAAGTG
CGCGTGGGTTCGAACCCCACAGCC

Forward primer for A1

GAAATTAATACGACTCACTATAGGGGACGTAGCTCATATG

Reverse primer for A1

TGGTGGAGACGCGGGGAATCGAACCCCGTGCCTCTC

Template for A1

GGGGACGTAGCTCATATGGTAGAGCGCTCGCTTCTAATGCGAGAGGCACGGGGTT
CGATTCCCCGCGTCTCCACCA

Forward primer for A2

GAAATTAATACGACTCACTATAGGGGATGTAGCTCAAATG

Reverse primer for A2

TGGTGGAGATGCGGGGGATCGAACCCCGTG

Template for A2

GGGGATGTAGCTCAAATGGTAGAGCGCTCGCTTCTAATGCGAGAGGCACGGGGTT
CGATCCCCCGCATCTCCACCA

Forward primer for A3

GAAATTAATACGACTCACTATAGGGGATGTAGCTCAG

Reverse primer for A3

TGGTGGAGATGCGGGGTATCGATCCCCGTAC

Template for A3
GGGGATGTAGCTCAGATGGTAGAGCGCTCGCTTCTAATGCGAGAGGTACGGGGAT
CGATACCCCGCATCTCCACCA

Template for A4
GTCAAGATGGCCGAGTTGGTCTAAGGCGCCAGTTTCTAAAATTTCATTGGCATGGG
TTCGAATCCCATTCTTGACACCA

Forward primer for S1-2A, S2-1DT[a], S2-1DT[b], S1-2AC[a]-$G_{27}C_{43}$-2A-G_{73}, dc2[a], dc2[b], and dc3
GAAATTAATACGACTCACTATAGGAGAGATGTCCGAGTG
Reverse primer for S1-2A, dc2[a], and dc2[b]
TGGAGGAGAGAGCAGGATTCGAACCTGCGCAGGCAAAG
Template for S1-2A
GGAGAGATGTCCGAGTGGTTAAGGAGACAGACTCTAAATCTGTTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTCTCT

Reverse primer for S1-2V, dc6[a], and dc6[b]
TGGCGTCGACAGCAGGATTCGAACCTGCGATAGTTC
Template for S1-2V
GTCGATATGTCCGAGTGGTTAAGGAGACAGACTCTAAATCTGTTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Forward primer for S1-2DT[a], S1-2DT[b], S2-1A, S2-1A-U_{73}, dc4, dc5[a], and dc5[b]
GAAATTAATACGACTCACTATAGTCGATATGGCTGAGTG
Reverse primer for S1-2DT[a], S1-2DT[b], and dc4
TGGCGTCGACAGAGGGATTCGAACCCTCGCAGGCAAAG
Template for S1-2DT[a]
GTCGATATGGCTGAGTGGTTGATAGCGACAGACTCTAAATCTGTTGGGCTTTGCCT
GCGAGGGTTCGAATCCCTCTGTC
Template for S1-2DT[b]
GTCGATATGGCTGAGTGGTTGATAGCTACAGACTCTAAATCTGTTGGGCTTTGCCT
GCGAGGGTTCGAATCCCTCTGTC

Template for S1-2AC[a]
GTCGATATGTCCGAGTGGTTAAGGATCCGGTCTCTAAAACCGGTGGGCTTTGCCTG

CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC[b]

GTCGATATGTCCGAGTGGTTAAGGAGCCGGTCTCTAAAACCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Reverse primer for S2-1A, dc5[a], and dc5[b]

TGGCGTCGACAGAGGGATTCGAACCCTCGATAGTTC

Template for S2-1A and S2-1A-U_{73}

GTCGATATGGCTGAGTGGTTGATAGCTCCGGTCTCTAAAACCGGTATAGTTCTAGG
AACTATCGAGGGTTCGAATCCCT

Reverse primer for S2-1V, dc1[a], and dc1[b]

TGGAGGAGAGAGAGGGATTCGAACCCTCGCAGGCAAAG

Template for S2-1V

GGAGAGATGGCTGAGTGGTTGATAGCTCCGGTCTCTAAAACCGGTGGGCTTTGCC
TGCGAGGGTTCGAATCCCTCTCTC

Reverse primer for S2-1DT[a], S2-1DT[b], and dc3

TGGAGGAGAGAGCAGGATTCGAACCTGCGATAGTTC

Template for S2-1DT[a]

GGAGAGATGTCCGAGTGGTTAAGGATCCGGTCTCTAAAACCGGTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Template for S2-1DT[b]

GGAGAGATGTCCGAGTGGTTAAGGAGCCGGTCTCTAAAACCGGTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Template for S2-1AC[a]

GGAGAGATGGCTGAGTGGTTGATAGCGACAGACTCTAAATCTGTTATAGTTCTAGG
AACTATCGAGGGTTCGAATCCCT

Template for S2-1AC[b]

GGAGAGATGGCTGAGTGGTTGATAGCTACAGACTCTAAATCTGTTATAGTTCTAGG
AACTATCGAGGGTTCGAATCCCT

Template for S1-2AC[a]-$G_{27}C_{43}$

GTCGATATGTCCGAGTGGTTAAGGATGCGGTCTCTAAAACCGCTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC$_{[a]}$-C$_{29}$G$_{41}$

GTCGATATGTCCGAGTGGTTAAGGATCCCGTCTCTAAAACGGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC$_{[a]}$-G$_{27}$C$_{43}$-C$_{29}$G$_{41}$

GTCGATATGTCCGAGTGGTTAAGGATGCCGTCTCTAAAACGGCTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC$_{[a]}$-U$_{27}$A$_{43}$-U$_{29}$A$_{41}$

GTCGATATGTCCGAGTGGTTAAGGATTCTGTCTCTAAAACAGATGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC$_{[a]}$-A$_{27}$U$_{43}$-A$_{29}$U$_{41}$

GTCGATATGTCCGAGTGGTTAAGGATACAGTCTCTAAAACTGTTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC$_{[a]}$-A$_{31}$U$_{39}$

GTCGATATGTCCGAGTGGTTAAGGATCCGGACTCTAAATCCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-U$_{26}$

GTCGATATGTCCGAGTGGTTAAGGATACAGACTCTAAATCTGTTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-U$_{31}$A$_{39}$

GTCGATATGTCCGAGTGGTTAAGGAGACAGTCTCTAAAACTGTTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Reverse primer for S2-G$_{73}$ and S2-G$_{27}$C$_{43}$-G$_{73}$

TGGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for S2-1A-U$_{73}$

TGGAGTCGACAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for S1-2AC$_{[a]}$-G$_{27}$C$_{43}$-2A-G$_{73}$

TGGCGGAGAGAGCAGGATTCGAACCTGCGCAGGCAAAG

Template for S1-2AC$_{[a]}$-G$_{27}$C$_{43}$-2A-G$_{73}$

GGAGAGATGTCCGAGTGGTTAAGGATGCGGTCTCTAAAACCGCTGGGCTTTGCCT
GCGCAGGTTCGAATCCTGCTCTCT

Template for S2-G$_{27}$C$_{43}$-G$_{73}$

GGAGAGATGGCTGAGTGGTTGATAGCTGCGGTCTCTAAAACCGCTATAGTTCTAGG
AACTATCGAGGGTTCGAATCCCT

Reverse primer for S2-G$_{27}$C$_{43}$-G$_{73}$ with 3' tuning

TXGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTC
(X = 2'-OMe-G)

Template for dc1$_{[a]}$

GGAGAGATGGCTGAGTGGTTGATAGCGACAGACTCTAAATCTGTTGGGCTTTGCCT
GCGAGGGTTCGAATCCCTCTCTC

Template for dc1$_{[b]}$

GGAGAGATGGCTGAGTGGTTGATAGCTACAGACTCTAAATCTGTTGGGCTTTGCCT
GCGAGGGTTCGAATCCCTCTCTC

Template for dc2$_{[a]}$

GGAGAGATGTCCGAGTGGTTAAGGATCCGGTCTCTAAAACCGGTGGGCTTTGCCT
GCGCAGGTTCGAATCCTGCTCTCT

Template for dc2$_{[b]}$

GGAGAGATGTCCGAGTGGTTAAGGAGCCGGTCTCTAAAACCGGTGGGCTTTGCCT
GCGCAGGTTCGAATCCTGCTCTCT

Template for dc3

GGAGAGATGTCCGAGTGGTTAAGGAGACAGACTCTAAATCTGTTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Template for dc4

GTCGATATGGCTGAGTGGTTGATAGCTCCGGTCTCTAAAACCGGTGGGCTTTGCCT
GCGAGGGTTCGAATCCCTCTGTC

Template for dc5$_{[a]}$

GTCGATATGGCTGAGTGGTTGATAGCGACAGACTCTAAATCTGTTATAGTTCTAGGA
ACTATCGAGGGTTCGAATCCCT

Template for dc5[b]

GTCGATATGGCTGAGTGGTTGATAGCTACAGACTCTAAATCTGTTATAGTTCTAGGA
ACTATCGAGGGTTCGAATCCCT

Template for dc6[a]

GTCGATATGTCCGAGTGGTTAAGGATCCGGTCTCTAAAACCGGTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Template for dc6[b]

GTCGATATGTCCGAGTGGTTAAGGAGCCGGTCTCTAAAACCGGTATAGTTCTAGGA
ACTATCGCAGGTTCGAATCCTGC

Template for S1-2AC[a]-$G_{31}C_{39}$

GTCGATATGTCCGAGTGGTTAAGGATCCGGGCTCTAAACCCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC[a]-$C_{31}G_{39}$

GTCGATATGTCCGAGTGGTTAAGGATCCGGCCTCTAAAGCCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC[a]-$G_{31}U_{39}$

GTCGATATGTCCGAGTGGTTAAGGATCCGGGCTCTAAATCCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Template for S1-2AC[a]-$U_{31}G_{39}$

GTCGATATGTCCGAGTGGTTAAGGATCCGGTCTCTAAAGCCGGTGGGCTTTGCCTG
CGCAGGTTCGAATCCTGCTGTCG

Forward primer for eRF aptamer

GGAAATTAATACGACTCACTATAGGGAGCTCAGAATAAACGCTCA

Reverse primer for eRF aptamer

GCAGGATCCGTGTCTCATGTCG

Template for eRF aptamer

GGGAGCTCAGAATAAACGCTCAAGTACCTGAAAATGGGAAGCAGAGCGAGCCTTT
CGACATGAGACACGGATCCTGC

tRNA sequences in the first generation.

S1:GUCGAUAUGUCCGAGUGGUUAAGGAGACAGACUCUAAAUCUGUUGGGCUU
UGCCUGCGCAGGUUCGAAUCCUGCUGUCGACGCCA
S2:GGAGAGAUGGCUGAGUGGUUGAUAGCUCCGGUCUCUAAAACCGGUAUAGU
UCUAGGAACUAUCGAGGGUUCGAAUCCCUCUCUCUCCUCCA
S3:GUCGAUAUGUCCGAGUGGUUAAGGAGACAGACUCUAAAUCUGUUGGGCUU
CGCCCGCGCAGGUUCGAACCCUGCUGUCGACGCCA
S4:GUGGUCGUGCCGGAGUGGUUAUCGGGCAUGACUCUAAAUCAUGUGGGCUU
UGCCCGCGCAGGUUCGAAUCCUGCCGACCACGCCA
S5:GGAGAGAUGGCCGAGCGGUUCAAGGCGUAGCAUUCUAACUGCUAUGUAGAC
UUUUGUUUACCGAGGGUUCGAAUCCCUCUCUUUCCGCCA
S6:GACGCUUUGGCCGAGUGGUUAAGGCGUGUGCCUCUAAAGUACAUGGGGUU
UCCCCGCGAGAGUUCGAAUCUCUCAGGCGUCGCCA
S7:GGAGAGAUGGCUGAGUGGACUAAAGCGGCGGAUUCUAAAUCCGUUGUACAA
UUUUUUUGUACCGAGGGUUCGAAUCCCUCUCUUUCCGCCA

L1:GUCAAGAUGGCCGAGUUGGUCUAAGGCGCCAGUUUCUAGUACUGGUCCGAA
AGGGCAUGGGUUCGAAUCCCAUUCUUGACACCA
L2:GCCGCCAUGGUGAAAUUGGUAGACACGCUGCUCUCUAGAAGCAGUGCUCAA
GCAUCUCGGUUCGAGUCCGAGUGGCGGCACCA
L3:GACAGUUUGGCCGAGUGGUCUAAGGCGCCAGAUUCUAGCUCUGGUCCGAAA
GGGCGUGGGUUCAAAUCCCACAGCUGUCACCA
L4:GUUGAGAUGGCCGAGUUGGUCUAAGGCGCCAGAUUCUAGUUCUGGUCCGA
AAGGGCGUGGGUUCAAAUCCCACUCUCAACACCA
L5:GCCUUGAUGGUGAAAUGGUAGACACGCGAGACUCUAAAUCUCGUGCUAAAG
AGCGUGGAGGUUCGAGUCCUCUUCAAGGCACCA
L6:GUCAGGAUGGCCGAGUGGUCUAAGGCGCCAGACUCUAGUUCUGGUCCUCUA
ACGAGGGCGUGGGUUCAAAUCCCACUUCUGACACCA
L7:GCUGGUUUGCCCGAGUGGUUAAGGGGGAAGACUCUAGAUCUUCUGCACUG
AAGUGCGCGUGGGUUCGAACCCCACAGCCAGCACCA

A1:GGGGACGUAGCUCAUAUGGUAGAGCGCUCGCUUCUAAUGCGAGAGGCACGG
GGUUCGAUUCCCCGCGUCUCCACCA
A2:GGGGAUGUAGCUCAAAUGGUAGAGCGCUCGCUUCUAAUGCGAGAGGCACGG
GGUUCGAUCCCCCGCAUCUCCACCA

A3:GGGGAUGUAGCUCAGAUGGUAGAGCGCUCGCUUCUAAUGCGAGAGGUACG
GGGAUCGAUACCCCGCAUCUCCACCA
A4:GUCAAGAUGGCCGAGUUGGUCUAAGGCGCCAGUUUCUAAAAUUUCAUUGG
CAUGGGUUCGAAUCCCAUUCUUGACACCA

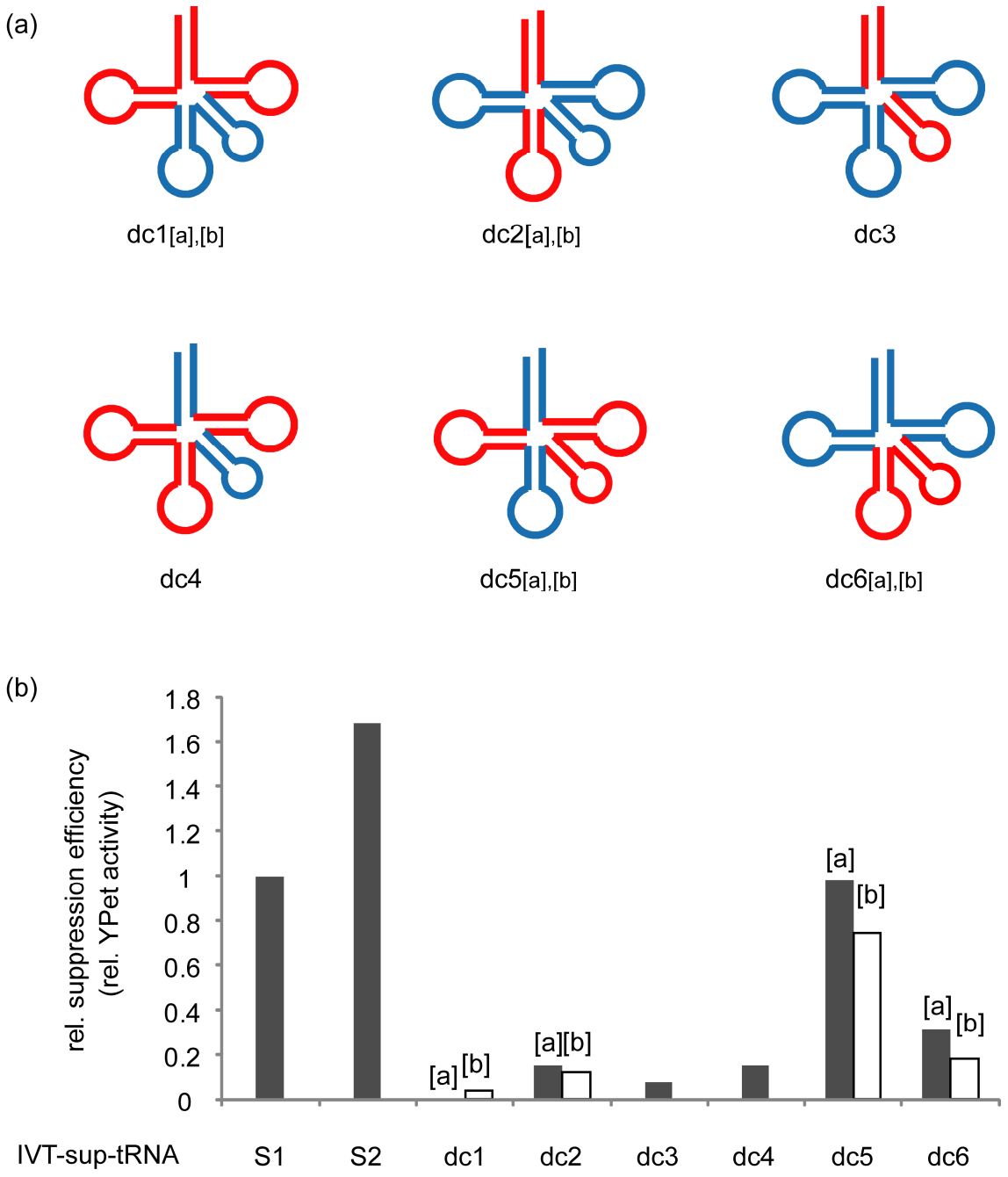

Fig. S1 Doubly chimeric IVT-sup-tRNAs. (a) Schematic diagrams of the IVT-sup-tRNAs. The blue and red lines show bases derived from S1 and S2, respectively. (b) Relative suppression efficiency of doubly chimeric IVT-sup-tRNAs to S1.

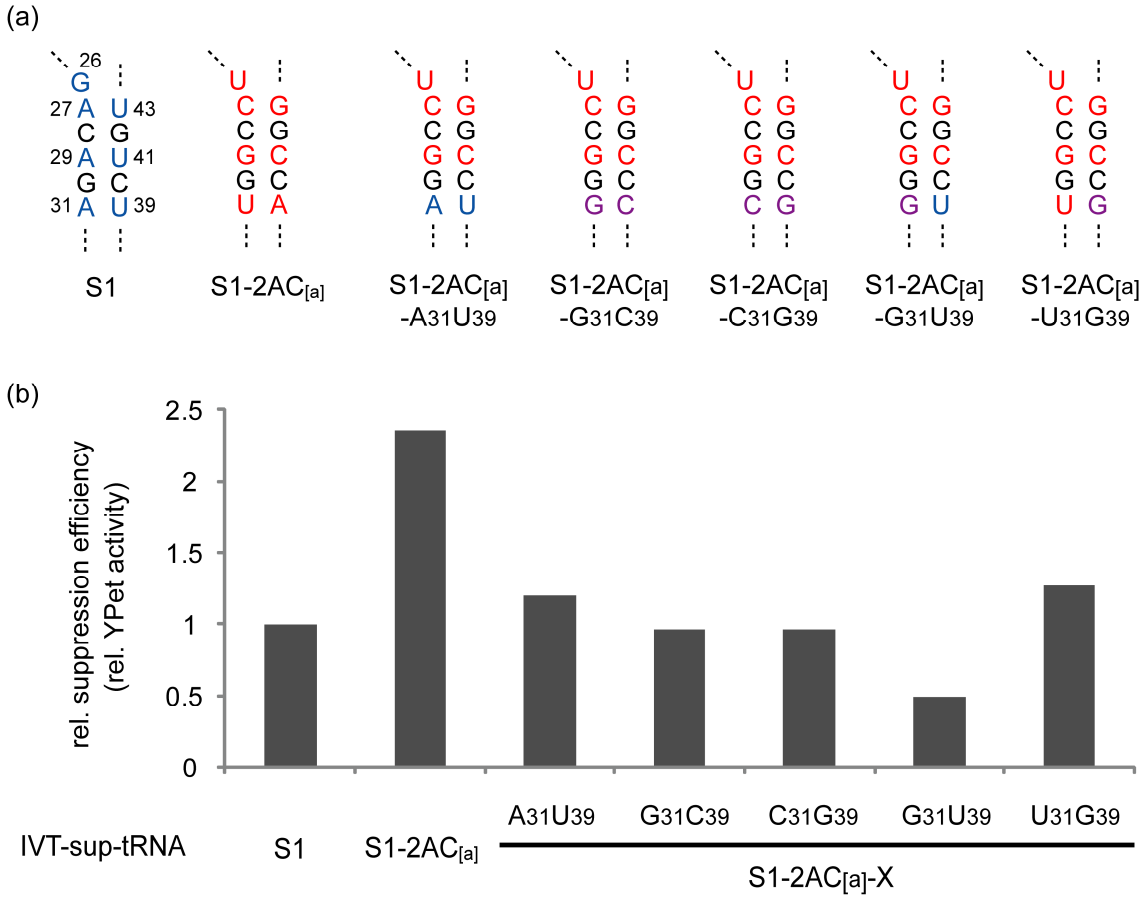

Fig. S2 Effect of the 31-39 base pair in S1-2AC[a] on suppression efficiency. (a) The sequences of the anticodon-stem and the 26th base in various S1-2AC[a]-based IVT-sup-tRNAs. The letter colours have the same meaning as in Fig. 3. (b) Relative suppression efficiency of IVT-sup-tRNAs to S1.