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Supplemental Material to:

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Molecular reconstruction of a fungal genetic code alteration

2013; 10(6)

http://dx.doi.org/10.4161/rna.24683

www.landesbioscience.com/journals/rnabiology/article/24683/

Supplementary Material

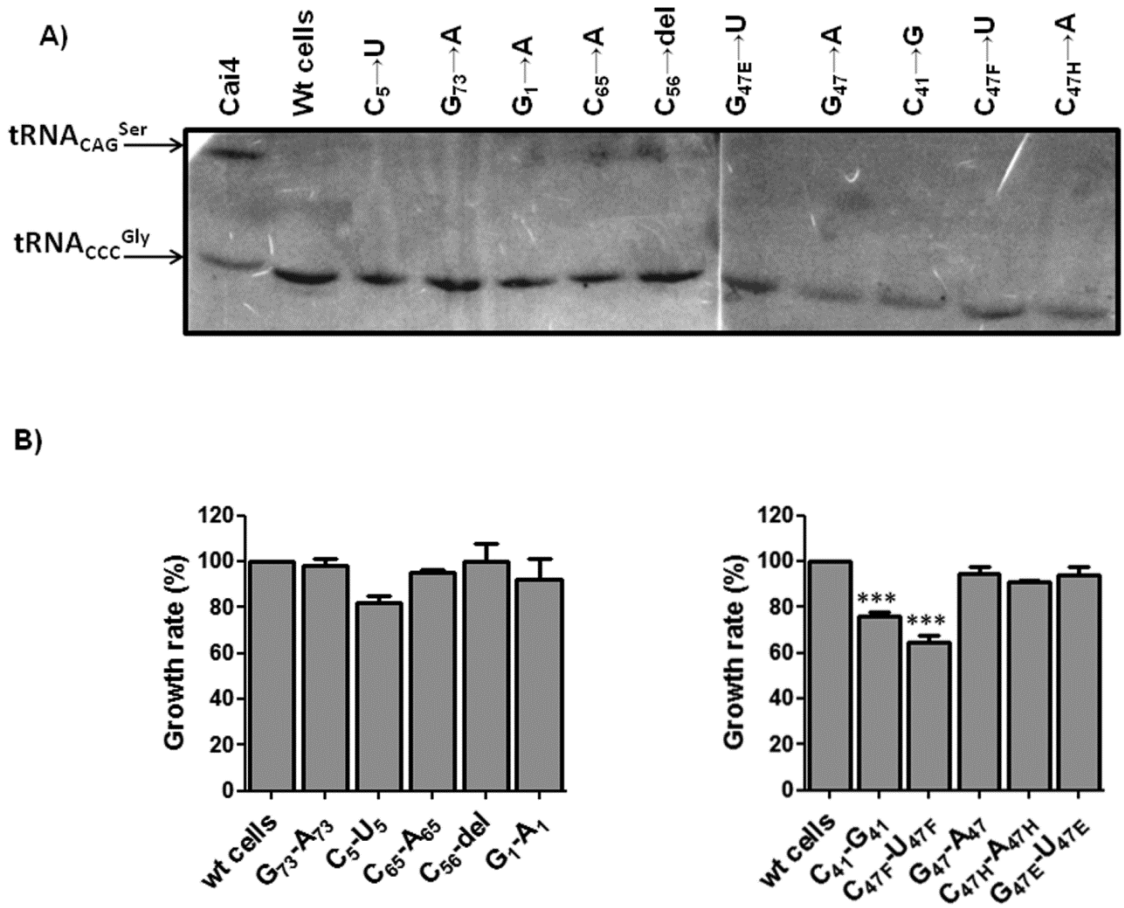

Figure S1: Mutations identified by forced evolution studies inactivate the *C. albicans* $tRNA_{CAG}^{Ser}$. (A) Northern blot analysis of mutant *C. albicans* $tRNA_{CAG}^{Ser}$ expressed in yeast haploid cells. 50 µg of tRNAs extracted from viable haploid spores were purified under acidic conditions and fractionated on 12% polyacrylamide gels containing 8M urea at room temperature. Detection of $tRNA_{CAG}^{Ser}$ and $tRNA_{CCC}^{Gly}$ was carried out using ɣ-^{32}P-ATP-$tRNA_{CAG}^{Ser}$ and ɣ-^{32}P-ATP-$tRNA_{CCC}^{Gly}$ probes. Cai4 corresponds to *C. albicans* total tRNA extract and WT corresponds to tRNAs extracted from yeast cells containing KanMx4 cassette only. Membranes were exposed for 24 hours to a K-screen and were visualized using Bio-Rad Molecular Imager FX. (B)

Mutations in the *C. albicans* $tDNA_{CAG}^{Ser}$ gene restored yeast growth. Yeast cultures were inoculated at an initial OD_{600} of 0.02 and were grown at 30ºC, 180 rpm in YPD+geneticin until late stationary phase. WT corresponds to cells containing the KanMX4 cassette only. The relative growth rate of cells transformed with mutant $tRNA_{CAG}^{Ser}$ was determined using exponential growth phase values, relative to the control cells (WT). Data represent the mean ± s.e.m. of 3-5 independent experiments. (***p < 0.001 one-way Anova post Bonferroni's multiple comparison test with CI of 95%, relative to the WT control cells).

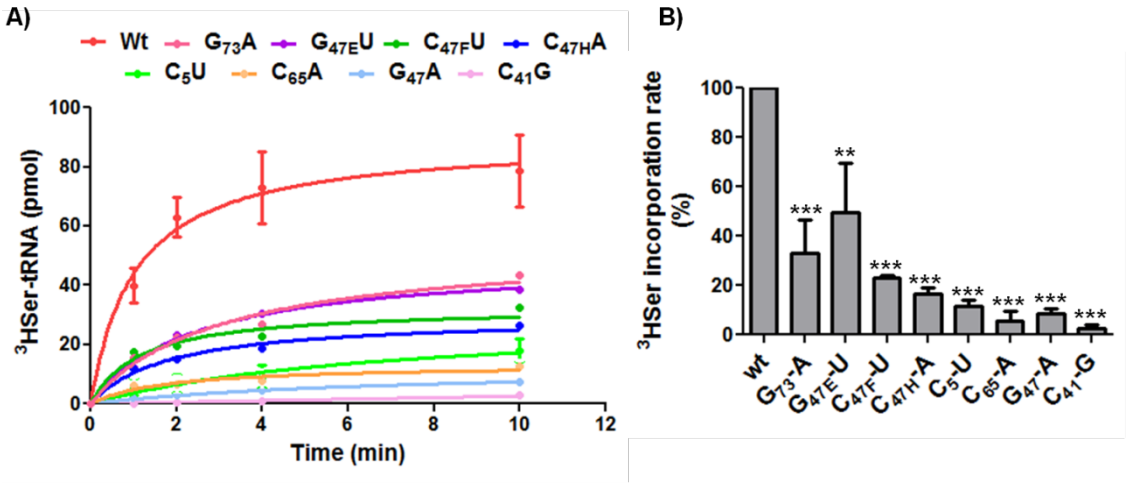

Figure S2: Serylation of *C. albicans* WT and mutant $tRNA_{CAG}^{Ser}$ with *C. albicans* SerRS. *In vitro* aminoacylation assays were carried out with *in vitro* synthesized wild-type and mutant $tRNA_{CAG}^{Ser}$. Aminoacylation reactions were carried out using purified *C. albicans* SerRS overexpressed in *E. coli*. Data represent the mean ± SD of 3 independent experiments (**p < 0.01, ***p < 0.001 one-way Anova post Bonferroni's multiple comparison test wit CI 95%, relatively to WT tRNA).

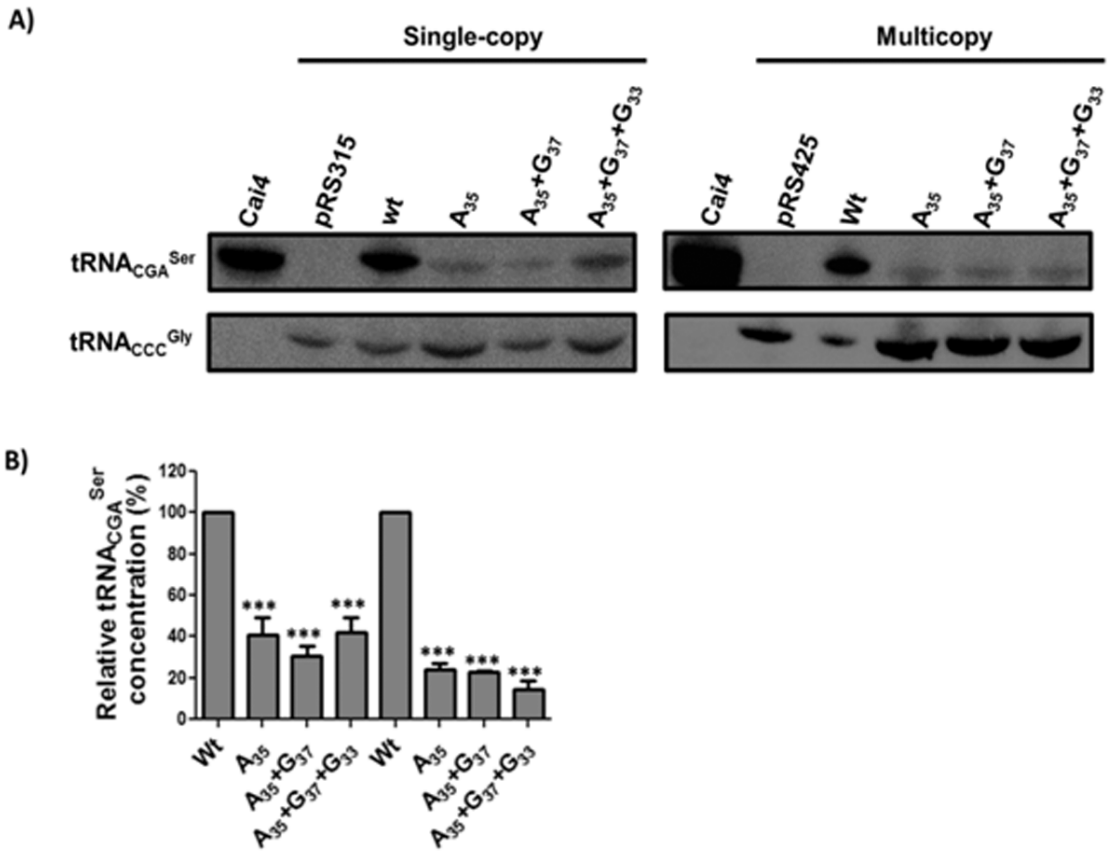

Figure S3: Expression of mutants $tRNA_{CGA}^{Ser}$ from multicopy plasmids do not restore tRNAs level in yeast cells. (A) Northern blot analysis of mutant *C. albicans* $tRNA_{CGA}^{Ser}$ expressed in from single copy and multicopy plasmids in diploid yeast cells. 50 µg of total tRNA extracted and purified under acidic conditions were fractionated on 15% polyacrylamide gels containing 8M urea at room temperature. Detection of $tRNA_{CGA}^{Ser}$ and $tRNA_{CCC}^{Gly}$ was carried out with ɣ -^{32}P-ATP-$tRNA_{CGA}^{Ser}$ and ɣ -^{32}P-ATP-$tRNA_{CCC}^{Gly}$ probes. Membranes were exposed for 24 hours to a K-screen and were visualized using Bio-Rad Molecular Imager FX. (B) Quantification of mutant tRNAs expression from single copy and multicopy plasmids relative to the WT tRNA expression (WT $tRNA_{CGA}^{Ser}$ expressed from single copy or multicopy plasmids). Data represent the mean ± s.e.m. of 3 independent experiments (***$p < 0.001$, one-way Anova post Bonferroni's test with CI 95% relative to WT $tRNA_{CGA}^{Ser}$).

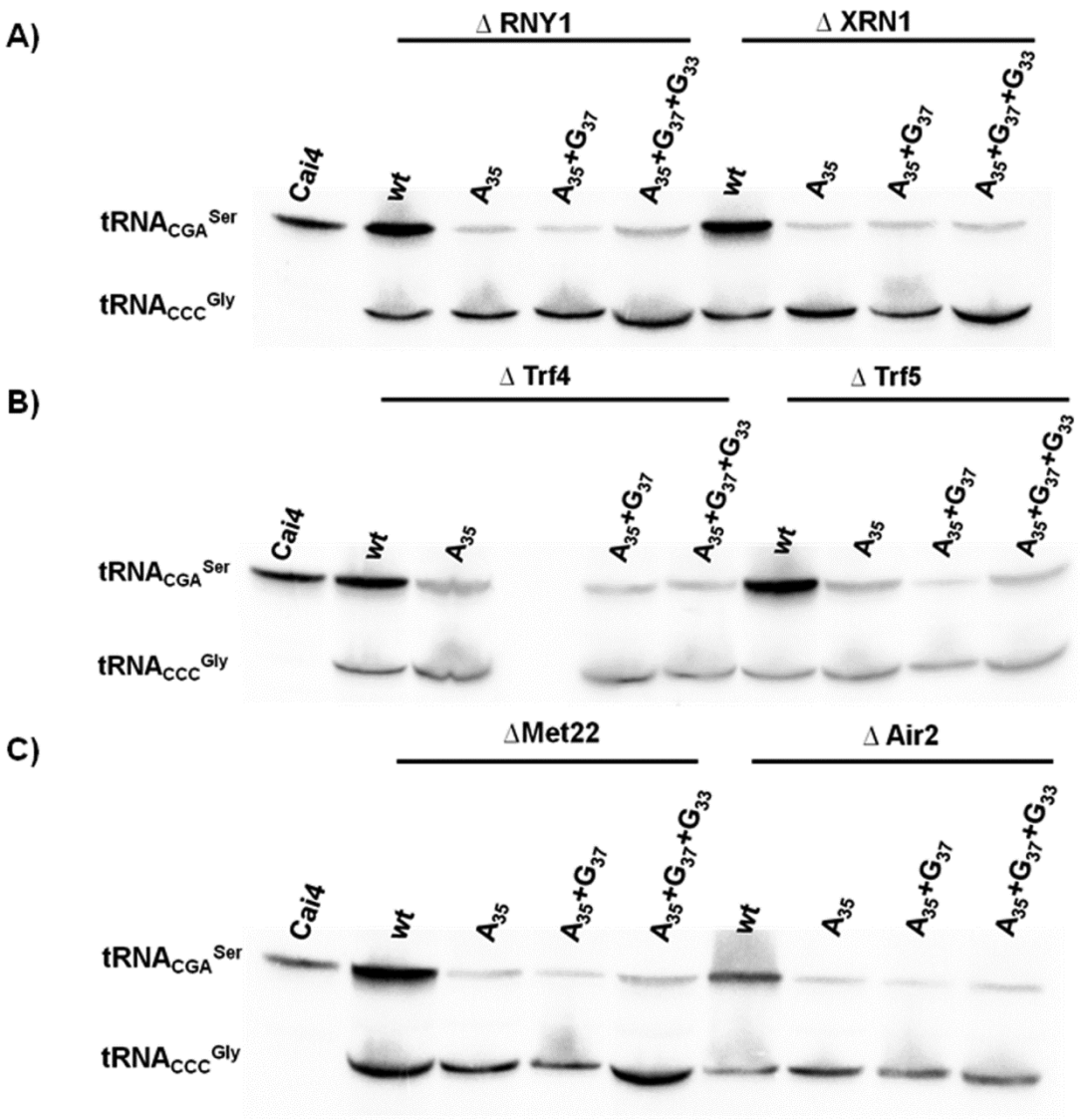

Figure S4: Down regulation of the mutant $tRNA_{CGA}^{Ser}$ is not mediated by known tRNA degradation pathways. In order to restore the levels of mutant tRNAs, WT and mutant $tRNA_{CGA}^{Ser}$ were expressed in yeast strains deleted in the RNY1, XRN1, TRF4, TRF5, MET22 and AIR2 genes, respectively, which encode proteins involved in tRNA degradation. 50 µg of total tRNA extracted and purified under acidic conditions were fractionated on 15% polyacrylamide gels containing 8M urea at room temperature. Detection of $tRNA_{CGA}^{Ser}$ and $tRNA_{CCC}^{Gly}$ was carried out with ɣ -^{32}P-ATP-$tRNA_{CGA}^{Ser}$

and ɣ -^{32}P-ATP-tRNA$_{CCC}^{Gly}$ probes. Membranes were exposed for 24 hours to a K-screen and were visualized using Bio-Rad Molecular Imager FX.

Table S1: List of the oligonucleotides used

Oligo	Tm (ºC)	Sequence 5'-3'
Integration of tRNA$_{CGA}^{Ser}$ in pRS315		
oUA2177	60ºC	CGCGTCGACAAATTTGACAGTGTGGCCGAGC
oUA2178	60ºC	CGCGGATCCGTGGGAAAAAAATATTCAAGAAAC
SDM of tRNA$_{CGA}^{Ser}$ for A$_{35}$ insertion		
oUA2105	55ºC	GTTAAGGCGTCTGACTCAGAATCTTATTCGCG
oUA2106	55ºC	CGCGAATAAGATTCTGAGTCAGACGCCTTAAC
SDM of tRNA$_{CGA}^{Ser}$ with A$_{35}$ inserted for A$_{37}$→G$_{37}$ transition		
oUA2107	55ºC	GTTAAGGCGTCTGACTCAGGATCTTATTCGCGTTATCAG
oUA2108	55ºC	CTGATAACGCGAATAAGATCCTGAGTCAGACGCCTTAAC
SDM of tRNA$_{CGA}^{Ser}$ with A$_{35}$+G$_{37}$ inserted for U$_{33}$→G$_{33}$ transversion		
oUA2109	55ºC	GTTAAGGCGTCTGACGCAGGATCTTATTCGCGTTATCAG
oUA2110	55ºC	CTGATAACGCGAATAAGA TCCTGCGTCAGACGCCTTAAC
KanMX4-tRNA$_{CAG}^{Ser}$ integration in the Leu2 locus		
oUA243	59ºC	CTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTAAG ATGCAAGAGTTCGCCGGGTTAATTAAGGCGCGC
oUA244	59ºC	GGGGCAGACATTAGAATGGTATATCCTTGAAATATATATAT ATATATTGCTGTAGTTGAAACACCAAACAAAAGATG
tRNA$_{CAG}^{Ser}$ integration confirmation		
oUA219	47ºC	CTCAATCTCGAGCCCACAGATGATTGAC
oUA222	47ºC	AATTTACCGCGGACTAGTTGAAACACC
KanMx4 integration in the Leu2 locus		
oUA611	59ºC	GGGGCAGACATTAGAATGGTATATCCTTGAAATATATATAT ATATATTGCTGGCATGTAATAAAGTCAATCATCTG
oUA243	59ºC	CTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTAAG ATGCAAGAGTTCGCCGGGTTAATTAAGGCGCGC
KanMX4 integration confirmation		
oUA622	59ºC	CCG GGT TAA TTA AGG CGC GC
oUA623	59ºC	GCA TGT AAT AAA GTC AAT CAT CTG

Cloning of $tRNA_{CGA}^{Ser}$ in pUC19 plasmid		
oUA2165		AGCTTAATACGACTCACTATAGACAGTGTGGCCGAG-
oUA2166		CGGTTAAGGCGTCTGACTCGAAATCAGTTGGGCTTTG
oUA2167		CCCGCGCA GGTTCGAATCCTGCTGCTGTCGCCAGGG
oUA2168		TTAACCGCTCGGCCACACTGTCTATAGTGAGTCGTATTA
oUA2169		GCGCGGGCAAAGCCCAACTGATTTCGAGTCAGACGCC
oUA2170		GATCCCCTGGCGACAGCAGCAGGATTCGAACCT
SDM of $tRNA_{CGA}^{Ser}$ gene cloned in pUC19 for A_{35} insertion		
oUA2171	55ºC	CGGTTAAGGCGTCTGACTCAGAATCAGTTGGGCTTTG
oUA2172	55ºC	CAAAGCCCAACTGATTCTGAGTCAGACGCCTTAACCG
SDM of $tRNA_{CGA}^{Ser}$ with A_{35} inserted cloned in pUC19 plasmid for $A_{37}\rightarrow G_{37}$ transition		
oUA2173	55ºC	CGGTTAAGGCGTCTGACTCAGGATCAGTTGGGCTTTG
oUA2174	55ºC	CAAAGCCCAACTGATCCTGAGTCAGACGCCTTAACCG
SDM of $tRNA_{CGA}^{Ser}$ with A_{35}+G_{37} inserted cloned in pUC19 for $U_{33}\rightarrow G_{33}$ transversion		
oUA2175	55ºC	CGGTTAAGGCGTCTGACGCAGGATCAGTTGGGCTTTG
oUA2176	55ºC	CAAAGCCCAACTGATCCTGCGTCAGACGCCTTAACCG
SDM of pUK1302 for $C_{41}\rightarrow G_{41}$ transition		
oUA2185	55ºC	GAAGGATTCAGGTTCGTTTGGGCATTGCC
oUA2186	55ºC	GGCAATGCCCAAACGAACCTGAATCCTTC
SDM of pUK1302 for $G_{47}\rightarrow A_{47}$ transition		
oUA2115	55ºC	GATTCAGGTTCCTTTGGACATTGCCCGCGCAGG
oUA2116	55ºC	CCTGCGCGGGCAATGTCCAAAGGAACCTGAATC
SDM of pUK1302 for $G_{47E}\rightarrow U_{47E}$ transition		
oUA2187	55ºC	GTTCCTTTGGGCATTTCCCGCGCAGGTTC
oUA2188	55ºC	GAACCTGCGCGGGAAATGCCCAAAGGAAC
SDM of pUK1302 for $C_{47F}\rightarrow U_{47F}$ transition		
oUA2119	55ºC	GGTTCCTTTGGGCATTGTCCGCGCAGGTTCGAACC
oUA2120	55ºC	GGTTCGAACCTGCGCGGACAATGCCCAAAGGAACC
SDM of pUK1302 for $C_{47H}\rightarrow A_{47H}$ transition		
oUA2121	55ºC	CCTTTGGGCATTGCCAGCGCAGGTTCGAACCC
oUA2122	55ºC	GGGTTCGAACCTGCGCTGGCAATGCCCAAAGG
SDM of pUK1302 for $C_{65}\rightarrow U_{65}$ transition		
oUA2123	55ºC	CGCAGGTTCGAACCCTGATCGTGTCGCCAGGCC
oUA2124	55ºC	GGCCTGGCGACACGATCAGGGTTCGAACCTGCG
SDM of pUK1302 for $G_{73}\rightarrow A_{73}$ transition		
oUA2125	55ºC	CTGCTCGTGTCACCAGGCCC

oUA2126	55ºC	GGGCCTGGTGACACGAGCAG
$tRNA_{CCC}^{Gly}$ detection by northern blot		
oUA2195	52-55ºC	GCGGAAGCCGGGAATCGAAC
$tRNA_{CGA}^{Ser}$ detection by northern blot		
oUA2194	55ºC	GCGACAGCAGCAGGATTCG
$tRNA_{CAG}^{Ser}$ detection by northern blot		
oUA2193	55ºC	GCGACACGAGCAGGGTTC

Table S2: Description of the plasmids constructed

Name	Description
WT	pRS315 plasmid containing one copy of *C. albicans* $tRNA_{CGA}^{Ser}$ cloned into BamHI and SalI restriction sites
A_{35}	Plasmid containing the $tRNA_{CGA}^{Ser}$ sequence with A_{35} insertion. Obtained by SDM of the WT plasmid after A_{35} insertion in the middle of the anticodon of $tRNA_{CGA}^{Ser}$ gene.
A_{35}+G_{37}	Plasmid containing the $tRNA_{CGA}^{Ser}$ sequence with A_{35}+G_{37}. Obtained by SDM of the A_{35} plasmid after insertion of the A_{37}→G_{37} mutation.
A_{35}+G_{37}+G_{33}	Plasmid containing the $tRNA_{CGA}^{Ser}$ sequence with A_{35}+G_{37}+G_{33}. Obtained by SDM of the A_{35}+G_{37} plasmid after insertion of the A_{37}→G_{37} mutation.
pUC19-WT	pUC19 containing C. albicans $tRNA_{CGA}^{Ser}$ inserted between BamHI and HindIII for in vitro transcription of tRNA
pUC19-A_{35}	Variant of pUC19-WT containing the insertion of A_{35} and replacement of A_{37} of tRNA gene sequence, ($tRNA_{CGA}^{Ser}$ with A_{35})
pUC-19-A_{35}+G_{37}	Variant of pUC19-A_{35} containing the mutation A_{37}→G_{37} in tRNA gene, ($tRNA_{CGA}^{Ser}$ with A_{35}+G_{37})
pUC19-A_{35}+G_{37}+G_{33}	Variant of pUC19-A_{35}+G_{37} containing the mutation U_{33}→G_{33} in tRNA gene, ($tRNA_{CGA}^{Ser}$ with A_{35}+G_{37}+G_{33})

C_{41}→A	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation C_{41}→A_{41} in tRNA gene.
C_5→U	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation C_5→U_5 in tRNA gene.
G_{47}→A	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation G_{47}→A_{47} in tRNA gene.
G_{47E}→U	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation G_{47E}→U_{47E} in tRNA gene.
C_{47F}→U	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for "in vitro" transcription, plus the mutation C_{47F}→U_{47F} in tRNA gene.
C_{47H}→A	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation C_{47H}→A_{47H} in tRNA gene.
C_{65}→A	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene with U_{33} for in vitro transcription, plus the mutation C_{65}→A_{65} in tRNA gene.
G_{73}→A	Variant of pUK1302: plasmid containing the $tRNA_{CAG}^{Ser}$ gene U_{33} for in vitro transcription, plus the mutation G_{73}→A_{73} in tRNA gene.

* Mutant $tRNA_{CAG}^{Ser}$ genes for *in vitro* transcription were prepared by SDM using the plasmid pUKC1302 ($tRNA_{CAG}^{Ser}$) constructed in a previous work.[1]

Reference List

1. Perreau VM, Keith G, Holmes WM, Przykorska A, Santos MA, Tuite MF. The Candida albicans CUG-decoding ser-tRNA has an atypical anticodon stem-loop structure. J Mol Biol 1999: 1039-53