

Supplemental Data

Identification of Transport-Critical Residues in a Folate Transporter from the FBT Family

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Supplemental Figures and Tables

Legends for Interactive Figures

The interactive Figures are zipped and in pse format to be viewed by PyMol (Delano Scientific), publicly available at <http://www.pymol.org/>.

Supplemental Figure S2A. Interactive comparative structural model of *Synechocystis* Slr0642 showing amphipathy (accompanies Fig. S1A to C).

Supplemental Figure S2B. Interactive comparative structural model of *Synechocystis* Slr0642 showing residue conservation among its orthologs (accompanies Fig. S1D to F).

Supplemental Figure S6A. Interactive structural model of *Synechocystis* Slr0642 showing the location of essential (red) and non-essential residues (blue) of residues mutated in the first round (accompanies Fig. 4A).

Supplemental Figure S6B. Interactive structural model of *Synechocystis* Slr0642 showing the location of essential (red) and non-essential residues (blue) of residues mutated in the second round (accompanies Fig. 4B).

Supplemental Figure S6C. Interactive structural model of *Synechocystis* Slr0642 showing the location of essential (red) and non-essential residues (blue) of residues mutated in the third round (accompanies Fig. 4C).

FIGURE S1. Comparative structural model of *Synechocystis* Slr0642 showing amphipathy and residue conservation. Slr0642 is shown in cartoon representation, with a view into the central cavity from the cytoplasmic side (A, D) and lateral views from the membrane on opposite sides of the transporter (B, E and C, F). A–C, The hydropathy scores of Kyte and Doolittle projected onto the backbone of the Slr0642 model in cartoon representation. The residues are colored in a rainbow scale from hydrophobic (red) to hydrophilic (blue). D–F, The conservation of the residues in an alignment of Slr0642 orthologs, determined with ConSurf (35). The residues are colored in a rainbow scale from variable (red) to conserved (blue). Residues 1-26 from the N-terminus and 454-494 of the C-terminus of Slr0642 have been omitted, as they could not be modeled on the basis of the LacY structure. Supplemental Figures S1A and S1B are interactive versions of the hydropathy and conservation portions, respectively, of this figure. For the conservation analysis the accession numbers were as follows. Cyanobacteria: *Anabaena variabilis* (YP_321756), *Crocospaera watsonii* (ZP_00518975); *Cyanotheca* sp. (ZP_01728059); *Lyngbya* sp. (ZP_01619402); *Microcystis aeruginosa* (YP_001655933); *Nodularia spumigena* (ZP_01628557); *Synechococcus elongatus* (YP_399216); *Synechococcus* sp. (YP_476017); *Synechococcus* sp. (YP_477391); *Synechocystis* sp. (NP_442292); *Thermosynechococcus elongatus* (NP_680807); *Trichodesmium erythraeum* (YP_722803). Plants: *Arabidopsis thaliana* (NP_565734); *Micromonas pusilla* (scaffold_12000004); *Oryza sativa* (NP_001059000); *Ostreococcus lucimarinus* (XP_001418597); *Physcomitrella patens* (XP_001778772, XP_001773424); *Vitis vinifera* (CAO71191).

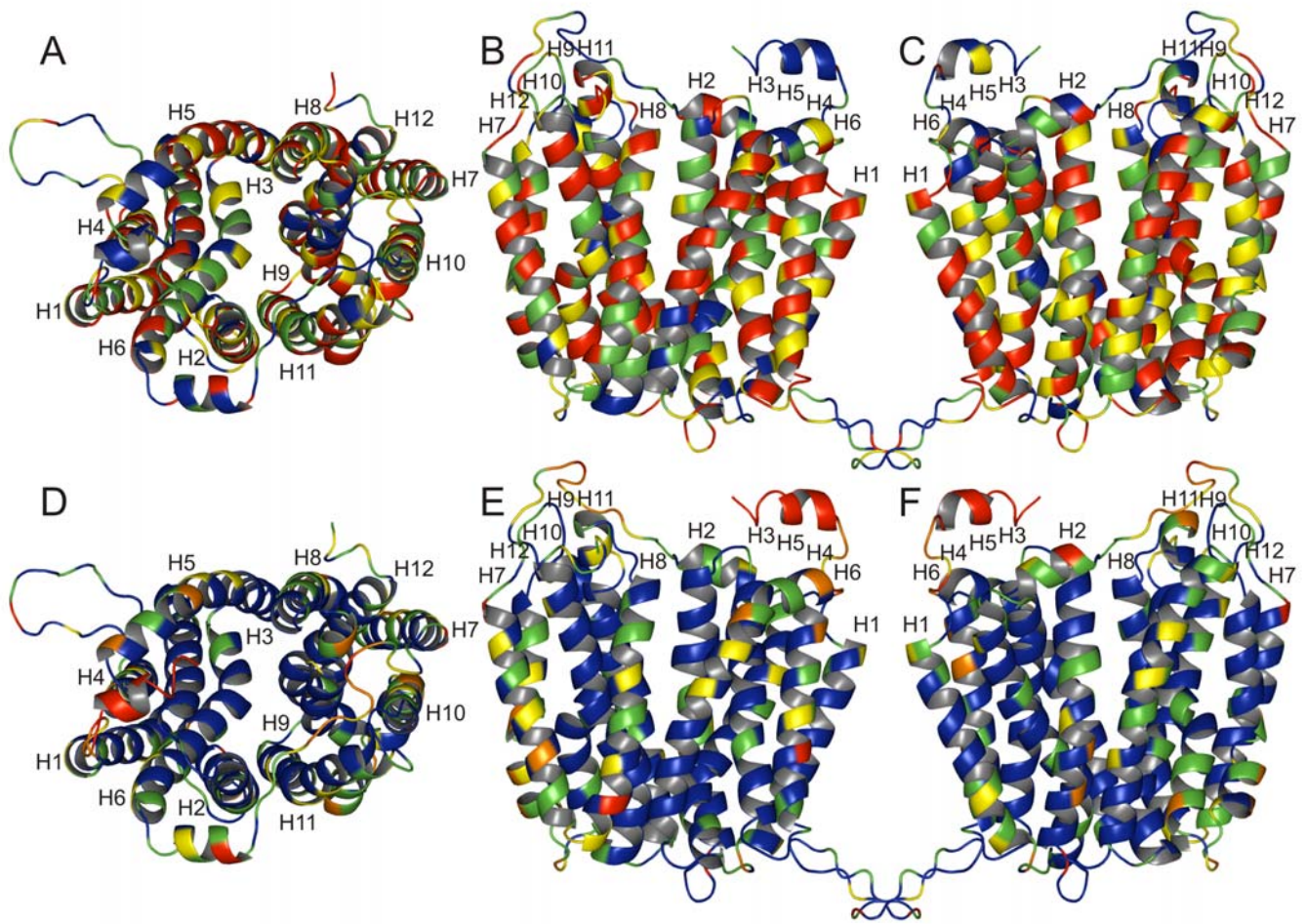


FIGURE S3. The interactions of amino acid side chains with the ligand tetrahydrofolate in structures deposited in the Protein Data Bank <http://www.ebi.ac.uk/pdbe-site/pdbesite/statistics> (as of August 2009). The total number of residue interactions for folate (FOL) was 209. The three moieties of tetrahydrofolate (pterin, *p*-aminobenzoate, and glutamate) are indicated. The amino acid residues that have specific interactions with folate are shown in red. The size of the font reflects the normalized percentage of all recorded interactions; the larger the font the larger the number of recorded interactions.

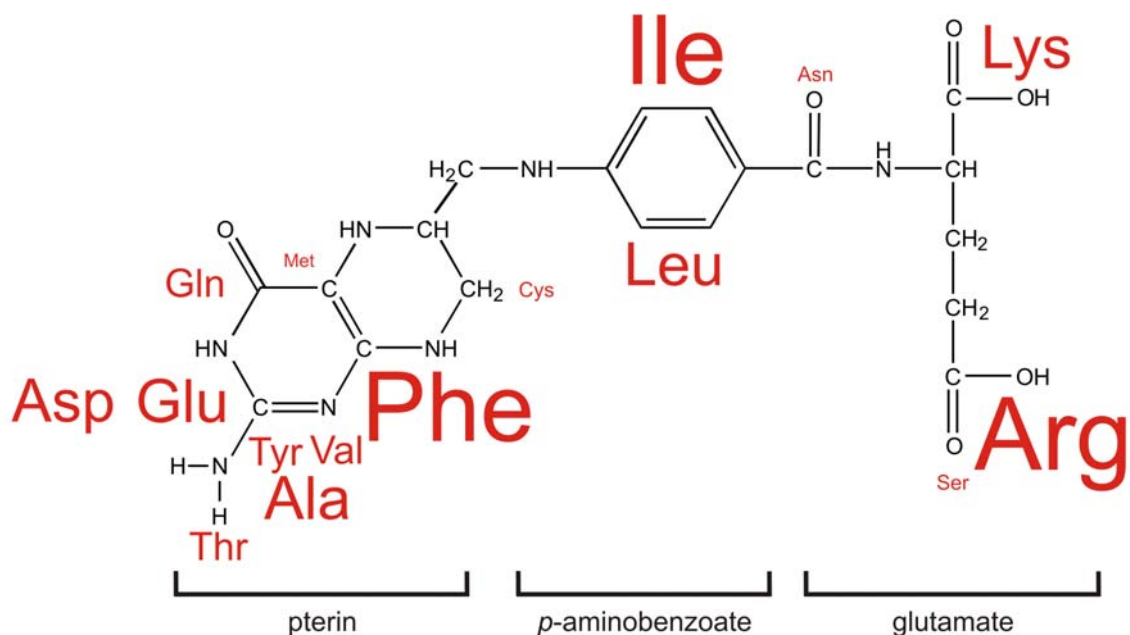


FIGURE S4. Second round of site-directed mutagenesis of Slr0642. A, *In-vivo* folate uptake assays. The *E. coli pabA abgT* strain was transformed with pLOI707HE with no insert (V) or containing native *slr0642* (WT) or 16 mutants thereof. Two independent clones of each mutant construct were streaked on minimal medium plus 0.5 mM IPTG alone (control) or containing 3.6 μ M *pABA* or 11 μ M 5-FTHF. Clones were streaked once on *pABA*-supplemented plates and three times in succession on the others. The *pABA* plates were photographed 1 day after streaking, others after 2 days. Transport-critical residues are colored red; non-critical residues are colored blue. B, Western blot analysis of Slr0642 protein levels in membrane preparations from the above clones. Membrane proteins (25 μ g per track) were separated by SDS-PAGE. The position of the 52-kDa molecular size marker is shown.

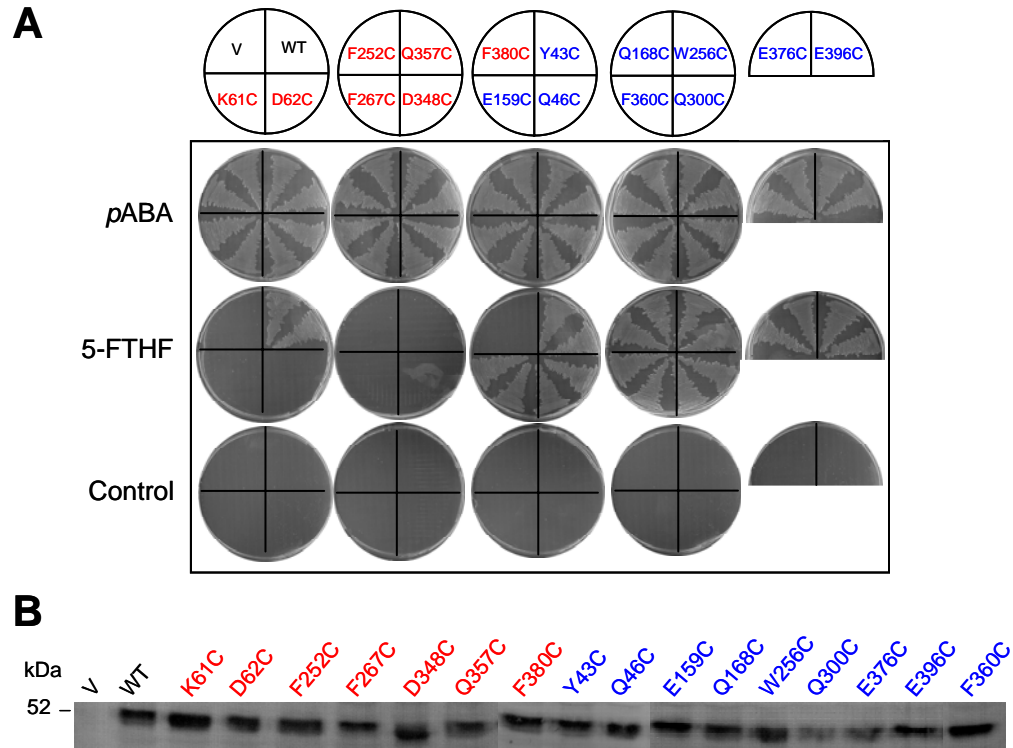
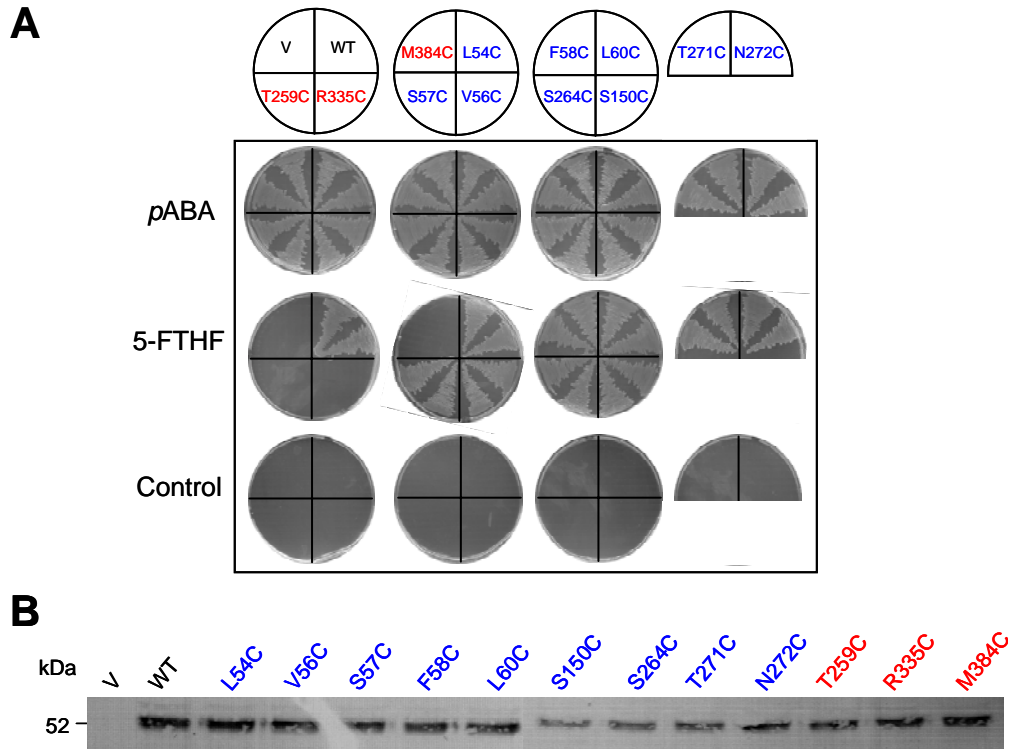


FIGURE S5. Third round of site-directed mutagenesis of Slr0642. A, *In-vivo* folate uptake assays. The *E. coli pabA abgT* strain was transformed with pLOI707HE with no insert (V) or containing native *slr0642* (WT) or 12 mutants thereof. Other details as in Figure S1. B, Western blot analysis of Slr0642 protein levels in membrane preparations from the above clones.



SUPPLEMENTAL TABLE S1

Synthetic oligonucleotides used in this study

Restriction sites are underlined and italicized. Mutated codons are underlined. Start codons are in green, stop anticodons in red, and Shine-Dalgarno sequences in blue.

| FBT full-length cDNAs | | |
|---------------------------------|--|---|
| | Forward (5'-3') | Reverse (5'-3') |
| At5g25040 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGGCAAGAGAAGAGCAA | <u>GAGCTCTA</u> GCTCCTATCAACATC |
| At5g25050 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGGTAGTAGAAGAGCAA | <u>GAGCTCTA</u> TCTTCTATCAACAGA |
| At1g79710 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGGCCAAATCGATCGG | <u>GAGCTCTA</u> AGCTTCATCACTGAT |
| At5g10820 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGGAAACACTAGAAACTG | <u>GAGCTCTA</u> CAGAAGCAAAAAGTTAA |
| At5g54860 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGATACATTGGTTGAAGC | <u>GAGCTCTA</u> TGATGCTGATACTCCT |
| At1g04570 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGGAAAGAATTAT | <u>GAGCTCTA</u> ACCTTTGTGCTTCTCTGAC |
| Slr0642-FBT chimeric constructs | | |
| | Truncated FBT cDNA | |
| | Forward (5'-3') | Reverse (5'-3') |
| (N-term)-Slr0642-At2g32040 | AAGGTGCTCTACGGTGTGAATTATCACCAG | <u>GAGCTCTA</u> ATTGGACTTCATCTCAACGTC |
| (N-term)-Slr0642-At5g25050 | AAGGTGCTCTTGTCTTCAGAGCTTCATTGG | <u>GAGCTCTA</u> TCTTCTATCAACAGA |
| (N-term)-Slr0642-At1g79710 | AAGGTGCTCTCATCGAAGAGCTTCAGTGG | <u>GAGCTCTA</u> AGCTTCATCACTGAT |
| (N-term)-Slr0642-At5g10820 | AAGGTGCTCAGTTCTAGGCTAAACCTGAGC | <u>GAGCTCTA</u> CAGAAGCAAAAAGTTAA |
| (N-term)-Slr0642-At5g54860 | GAAAAGGTGTTGGCGTCGGTCTTCTGTG | <u>GAGCTCTA</u> TGATGCTGATACTCCT |
| (N-term)-Slr0642-At1g04570 | AAGGTGCTCTTGAACAAGTGGTGGTAA | <u>GAGCTCTA</u> ACCTTTGTGCTTCTCTGAC |
| | N-terminal 27 residues of Slr0642 | |
| | Forward (5'-3') | Reverse (5'-3') |
| (N-term)-Slr0642-At2g32040 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGCTTGTGCTATGTCCATG | CAACACCGTAGAGCACCTTTTCCCGCAGGA |
| (N-term)-Slr0642-At5g25050 | " | CTGAAGACAAGAGCACCTTTTCCCGCAGG |
| (N-term)-Slr0642-At1g79710 | " | CTTCGATGAGGAGCACCTTTTCCCGCAGGA |
| (N-term)-Slr0642-At5g10820 | " | GCCTAGAAGTACGACCTTTTCCCGCAGG |
| (N-term)-Slr0642-At5g54860 | " | GCCAAACGCCACCTTTTCCCGCAGGAAACGCTTC |
| (N-term)-Slr0642-At1g04570 | " | CACTTGTCAAGAGCACCTTTTCCCGC |
| | Chimera (N-term)-Slr0642-FBT | |
| | Forward (5'-3') | Reverse (5'-3') |
| (N-term)-Slr0642-At2g32040 | <u>GCGGCCGC</u> CACAGGAAACAGCTATGCTTGTGCTATGTCCATG | <u>GAGCTCTA</u> ATTGGACTTCATCTCAACGTC |
| (N-term)-Slr0642-At5g25040 | " | <u>GAGCTCTA</u> TCTTCTATCAACAGA |
| (N-term)-Slr0642-At1g79710 | " | <u>GAGCTCTA</u> AGCTTCATCACTGAT |
| (N-term)-Slr0642-At5g10820 | " | <u>GAGCTCTA</u> CAGAAGCAAAAAGTTAA |
| (N-term)-Slr0642-At5g54860 | " | <u>GAGCTCTA</u> TGATGCTGATACTCCT |
| (N-term)-Slr0642-At1g04570 | " | <u>GAGCTCTA</u> ACCTTTGTGCTTCTCTGAC |
| Site-directed mutagenesis | | |
| | Forward (5'-3') | Reverse (5'-3') |
| Y43C | GGCCATCTCAGCATTGCTTTGTACAAGGGGTG | CACCCCTGTACAAAGCAAAATGCTGAGGATGGCC |
| Q46C | CTGGCCATCTCAGCATTACTTTGTATGCGGGGTGTGGGTCT | AGACCCAACACCCCGCATACAAAAGTAAATGCTGAGGATGGCCAG |
| R53C | GGGGTGTGGGTCTCTCCGTTGCTTAGCAGTGAGCTTTTTC | GAAAAAGTCACTGCTAAGCAGGAGAGACCCAACACCCC |
| L54C | GTGTGGGTCTCTCCGTTGCGCAGTGAGCTTTTTCCT | AGGAAAAAGTCACTGCGCAACGGGAGAGACCCAACAC |
| V56C | GTGTGGGTCTCTCCGTTTAGCATGCACTTTTTCCTCAAGGA | TCCTTGAGGAAAAAGCTGCATGCTAAACGGGAGAGACCCAACAC |
| S57C | TCTCCGTTTAGCAGTGTGCTTTTTCCTCAAGGAT | ATCCTTGAGGAAAAAGCAGCACTGCTAAACGGGAGA |
| F58C | TCCCGTTTAGCAGTGAGCTTCTCTCAAGGATGAAGTGG | CCAGTTCATCCTTGAGGAAAGCACTGCTAAACGGGA |
| L60C | TCCCGTTTAGCAGTGAGCTTTTTCGCAAGGATGAAGTGGGA | TCCAGTTCATCCTTGCAAAAAAGTCACTGCTAAACGGGA |

| | | |
|-------|--|--|
| K61C | TAGCAGTGAGCTTTTCTCTGCGATGAACTGGGACTGAGTCC | GGACTCAGTCCCAGTTCATCGCAGAGGAAAAAGCTCACTGCTA |
| D62C | CAGTGAGCTTTTCTCAAGTGCAGAACTGGGACTGAGTCCGGC | GCCGGACTCAGTCCCAGTTCGCACTTGTAGGAAAAAGCTCACTG |
| W82C | GGCGGCGCCCTGCATTCTGAAGCCAG | CTGGCTTCAGAATGCAGGGCGCCGC |
| K85C | GCGGGCCCTGGATTCTGTGCCAGTCTGGTTTAAATG | CATTAAACCAAGCACTGGGCAAGAAATCCAGGGCGCCGC |
| D93C | AGTGCTTGGTTTAAATGTCCTGCACGGTGCCTCTATTGGC | GCCAAATAGAGGCACCGTGCAGGACATTAACCAAGCACT |
| D145C | CCTATCCGTGGCGATCGGCTGCGTGATTGTGATTCCCTAG | CTAGGGAATCGACAATCAGCAGCCGATGCCACGGATAGG |
| D149C | GATCGGCGATGTGATTGCTGCCTCCTAGTGGTGAACGGG | CCCGTTCCACCCTAGGGAGCAGACAATCACATCGCCGATC |
| S150C | GGCGATGTGATTGTGATTCGCTAGTGGTGA | TCCACCCTAGGCAATCGACAATCACATCGCC |
| E154C | TCGATTCCCTAGTGGTGTGCCGGGCCAACGGGAATC | GATTCCCCTGGGCCGCACTCCACTAGGGAATCGA |
| R155C | GATTCCCTAGTGGTGGAAATGCCCAACGGG | CCCGTTGGGCGCATTCACCCTAGGGAATC |
| R158C | GTGGAACGGGCCAAATGCGAATCCTTAGCCAA | TTGGGCTAAGGATTCGCAATGGGCCGCTTCCAC |
| E159C | GGAACGGGCCAACGGTGCCTTAGCCCAAGTGG | CCACTTGGGCTAAGGACGACCGTTGGGCCGCTTCC |
| Q168C | GCCCAAGTGGGATCTTTGCTCCCTACCTGGGGAGC | GCTCCCAGGTGAGGGAGCAGAAAGATCCCACTTGGG |
| S169C | CCCAAGTGGGATCTTTGCAATGCTCACCTGGG | CCCAGGTGAGGCATTCGCAAGATCCCACTTGGG |
| W172C | TCCCTCACCTGCGGAGCCGCCG | GCGGGCGCTCCGAGGTGAGGGA |
| F252C | CGATTCTAATGCCACTTTGTCATTCTTTTGGCAGGCAACC | GTTTGCCTGCCAAAAAGAAATGCACAAAGTGGGCAATAGAAATCG |
| W256C | CACCTTGTTTATTTCTTTTGGCAGGCAACCCACG | GCTGGGGTGTGCTGCAAAAGAAAAATAACAAAGT |
| T259C | TGTTTATTTCTTTTGGCAGGCAATGCCCAAGCCGCA | TCGGCGCTGGGGCATGCTGCCAAAAAGAAAAATAACA |
| E263C | GCAGGCAACCCAGCGCCGCTCAGCCTTTTTTATTTCAC | GTGAAATAAAAAAGGCTGAGCAGGCGTGGGGTTGCTCG |
| S264C | AGGCAACCCAGCGCCGAGTGCCTTTTTTTA | TAAAAAAGGCGCACTCGGCGTGGGGTTGCT |
| F267C | GCGCCGAGTCAGCCTTTGCTATTTACCACCAACGAG | CTCGTTGGTGGTGAATAAGCAAAAGGCTGACTCGGCG |
| T271C | CAGCCTTTTTTATTTCACCTGCACGAGTTAGGCTTTGAGC | GCTCAAAGCCTAACTCGTTGCAAGGTGAAATAAAAAAGGCTG |
| N272C | CCTTTTTTATTTCACCACCTGCGAGTTAGGCTTTGAGCCGA | TCGGCTCAAAGCCTAACTCGCAGGTGGTGAATAAAAAAGG |
| Q300C | CCGGATTGATTGGGGTGGGACTTTACTGCGCTTTTTAAAAACCTTG | GCAAGTTTTTAAAAAGCGGCAAGTAAAGTCCACCCCAATCAATCCG |
| R301C | GATTGATTGGGGTGGGACTTTACCAAATGCTTTTTAAAAACCTTG | CAAGTTTTTAAAAAGCATTTGGTAAAGTCCACCCCAATCAATC |
| K304C | GGGGTGGGACTTTACCAACGCTTTTTATGCACTTGCCTTTCCG | CGGAAAGGCAAGGTGCAATAAAAAAGGCTGGTAAAGTCCACCC |
| R309C | TTTAAAAACCTTGCCTTTTGCCTGATCATGGGCTGGAGTA | TACTCCAGCCATGATCAGCAGAAAGGCAAGGTTTTTAA |
| R335C | TTTAATTACCACGCCAATGCGCCTAGGATTTGATGACC | GGTCATCAATACCCATGGCGCAGTTGGCGTGGGTAATTA |
| D348C | GACCAATTGGTTAGCTTGGGGTGCAGCATTATTCTCACCGTAACG | CGTTACGGTGAGAATAATGCTGCCACCCCAAGCTAAACCAATGGTC |
| Q357C | ATTATTCTACCGTAACGGGGTGCATTGCTTTATGCCGGTGTG | CAACACCGGCATAAAGGCAATGCACCCCGTTACGGTGAGAATAAT |
| F360C | GTAACGGGGCAAATGCCTGCAATGCGGTTGGTTTTGG | CCAAAACCAACACCGGCAATGCAGGCAATTTGCCCGTTAC |
| M361C | CACCGTAACGGGGCAAATGCCTTTTGCCTGGTGTGGTTT | AAACCAACACCGGGCAAAGGCAATTTGCCCGTTACGGTG |
| R369C | GTTTTGGCCGCTGCTCTGTCC | GGGGACAGAGGCACGCGCCAAAAC |
| C371A | CCGCGCCCTCGCTCCCGGGCA | TGCCCGGGGACGAGGCGCGCG |
| E376C | CGCCTGTGCCCCGGGCATTGCGGACTTTGTTG | GCAAACAAAGTCGCGCAATGCCCGGGGACAGAGGCG |
| F380C | CCCGGCATTGAAGCGACTTTGTCGCCTATTGATGT | ACATCAATAGGGCGCAAAAGTCGTTCAATGCCCGG |
| N388C | CCCTATTGATGTCGGTGATGTGCTTGGCCGGCGTATTGCTT | AAGGACAATACCGGCCAAGCAGCATACCGACATCAATAGG |
| M384C | GAAGCGACTTTGTTGCCCTATTGCTCGGTGATGAATTTGGCCG | CGGCCAAATTCATCACCAGCAATAGGGCAACAAAGTCGCTTC |
| E396C | GCCGGCGTATTGCTCTGCGTGGGGTCACTACTGACC | GGTCAGTAATGACCCACGCAAGGACAATACCGCGG |
