# Supplementary Information

## Mutagenesis and redox partners analysis of the P450 fatty acid decarboxylase $OleT_{JE}$

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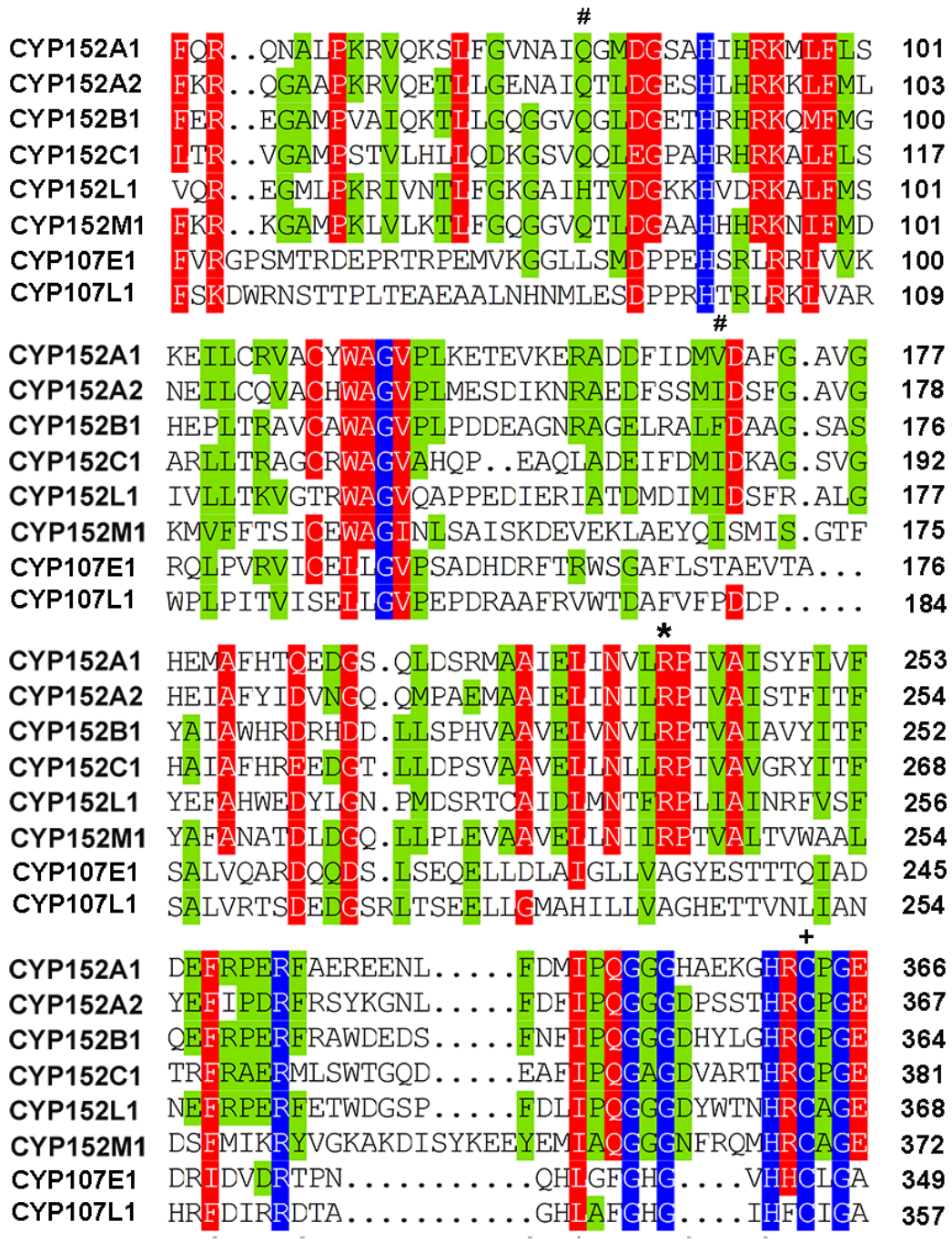
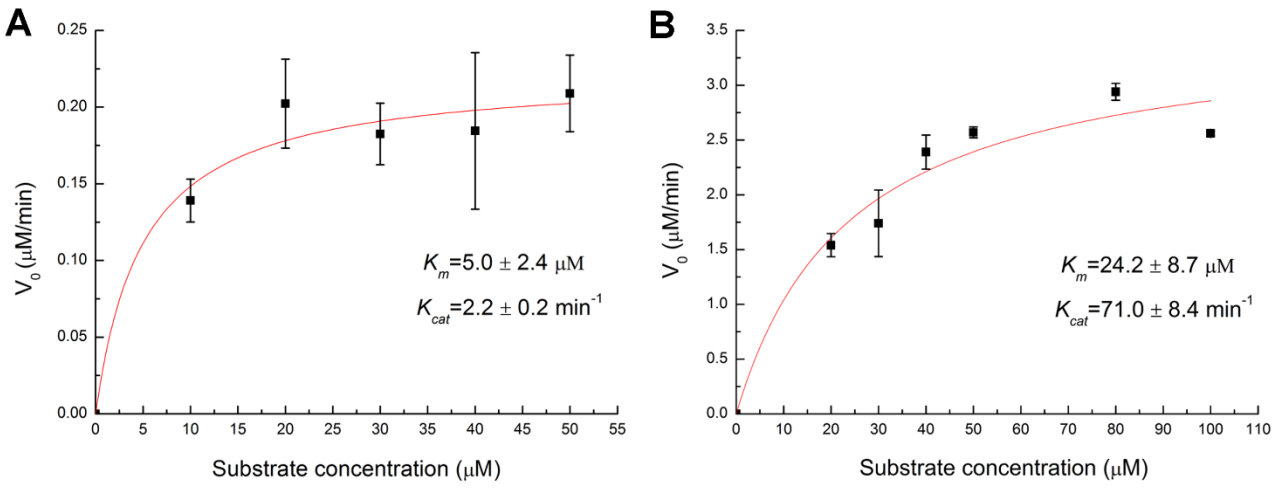



**Supplementary Figure S1** Protein sequence alignment of the select group of CYP152 family members along with two P450 monooxygenase sequences from the CYP107 family. CYP152A1: $P450_{BS\beta}$ from *Bacillus subtilis*; CYP152A2: $P450_{CLA}$ from *Clostridium acetobutylicum*; CYP152B1: $P450_{SP\alpha}$ from *Sphingomonas paucimobilis*; CYP152C1: from *Rhodobacter sphaeroides*; CYP152L1: $OleT_{JE}$ from *Jeotgalicoccus* sp. ATCC 8456; CYP152M1: from *Enterococcus faecium*; CYP107E1: monooxygenase P450 MycG from *Micromonospora griseorubida*; CYP107L1: monooxygenase P450 PikC from *Streptomyces venezuelae*; #: Residues important for product distribution; *: the key catalytic residue; +: the absolutely conserved Cys in P450s.




**Supplementary Figure S2** The kinetic curves of $OleT_{JE}$ toward myristic acid using (A) the $O_2$/NADPH/*Cg*FdR-2/*Se*Fdx-6 system, and (B) $H_2O_2$ as the sole oxygen and electron donor .

**Supplementary Table S1** Primers used for site-directed mutagenesis (The bold nucleotides denote the mutated codons.)

| Primer names | Sequence 5→3 |
|---|---|
| R245A-F | *GACTTAATGAACACATTC**GCC**CCATTAATCGCAATCAAC* |
| R245A-R | *GTTGATTGCGATTAATGG**GGC**GAATGTGTTCATTAAGTC* |
| R245L-F | *GACTTAATGAACACATTC**CTC**CCATTAATCGCAATCAAC* |
| R245L-R | *GTTGATTGCGATTAATGG**GAG**GAATGTGTTCATTAAGTC* |
| R245K-F | *GACTTAATGAACACATTC**AAG**CCATTAATCGCAATCAAC* |
| R245K-R | *GTTGATTGCGATTAATGG**CTT**GAATGTGTTCATTAAGTC* |
| R245H-F | *GACTTAATGAACACATTC**CAC**CCATTAATCGCAATCAAC* |
| R245H-R | *GTTGATTGCGATTAATGG**GTG**GAATGTGTTCATTAAGTC* |
| R245E-F | *GACTTAATGAACACATTC**GAG**CCATTAATCGCAATCAAC* |
| R245E-R | *GTTGATTGCGATTAATGG**CTC**GAATGTGTTCATTAAGTC* |
| R245Q-F | *GACTTAATGAACACATTC**CAG**CCATTAATCGCAATCAAC* |
| R245Q-R | *GTTGATTGCGATTAATGG**CTG**GAATGTGTTCATTAAGTC* |
| C365H-F | *GGACAAATCACCGT**CAT**GCAGGTGAATGGATC* |
| C365H-R | *GATCCATTCACCTGC**ATG**ACGGTGATTTGTCC* |
| I170X-F[a] | *CAGACATGGACATCATG**NNN**GATTCATTTAGAGCAC* |
| I170X-R[a] | *GTGCTCTAAATGAATC**NNN**CATGATGTCCATGTCTG* |
| I170R-F[b] | *CAGACATGGACATCATG**CGT**GATTCATTTAGAGCAC* |
| I170R-R[b] | *GTGCTCTAAATGAATC**ACG**CATGATGTCCATGTCTG* |
| I170D-F[b] | *CAGACATGGACATCATG**GAC**GATTCATTTAGAGCAC* |
| I170D-R[b] | *GTGCTCTAAATGAATC**GTC**CATGATGTCCATGTCTG* |
| I170S-F[b] | *CAGACATGGACATCATG**AGC**GATTCATTTAGAGCAC* |
| I170S-R[b] | *GTGCTCTAAATGAATC**GCT**CATGATGTCCATGTCTG* |
| H85X-F[a] | *GTAAAGGTGCAATC**NNN**ACGGTAGATGGT* |
| H85X-R[a] | *TACCATCTACCGT**NNN**GATTGCACCTTTAC* |
| H85Y-F[b] | *GTAAAGGTGCAATC**TAT**ACGGTAGATGGT* |
| H85Y-R[b] | *TACCATCTACCGT**ATA**GATTGCACCTTTAC* |
| H85F-F[b] | *GTAAAGGTGCAATC**TTC**ACGGTAGATGGT* |
| H85F-R[b] | *TACCATCTACCGT**GAA**GATTGCACCTTTAC* |
| H85P-F[b] | *GTAAAGGTGCAATC**CCT**ACGGTAGATGGT* |
| H85P-R[b] | *TACCATCTACCGT**AGG**GATTGCACCTTTAC* |
| H85W-F[b] | *GTAAAGGTGCAATC**TGG**ACGGTAGATGGT* |
| H85W-R[b] | *TACCATCTACCGT**CCA**GATTGCACCTTTAC* |
| H85M-F[b] | *GTAAAGGTGCAATC**ATG**ACGGTAGATGGT* |
| H85M-R[b] | *TACCATCTACCGT**CAT**GATTGCACCTTTAC* |

[a]for saturation mutagenesis

[b]for the mutants that were not obtained from screening

**Supplementary Table S2** Primers used for cloning (The underlined bases denote the restriction site.)

| Primer names | Sequence 5'→3' |
|---|---|
| *Cg*Fdx-3-NdeI | *GCAATATTCATATGATGACCTACACAATCGCC* |
| *Cg*Fdx-3-XhoI | *GAATTCTCGAGCTAGTTCTGGTTCTGTGG* |
| *Cg*Fdx-2-NdeI | *GGCCGGCCATATGATGTCTACTATTCATTTC* |
| *Cg*Fdx-2-XhoI | *GAATTCTCGAGTCACACTTGCGTTTCTGG* |
| *Cg*Fdx-1-NdeI | *GGCCGGCCATATGATGACATACACAATCGCA* |
| *Cg*Fdx-1-XhoI | *GAATTCTCGAGTTATGCCTGAGGCGGCAG* |
| *Cg*FdR-2-NdeI | *GAATTATTCATATGATGTCTCGCCCTTTGCGT* |
| *Cg*FdR-2-XhoI | *GGCTTCTCGAGTTAGATGTCGTATTCTGG* |
| *Cg*FdR-1-NdeI | *GAATTATTCATATGATGACAACTCCCCTGCGC* |
| *Cg*FdR-1-XhoI | *GGCTTCTCGAGTTAGACAATTGCTGGAGC* |

**Supplementary DNA sequences of redox partners**

**1. Ferredoxins**

(1) Codon-optimized *Se*Fdx-1 (GenBank Accession No. SYNPCC7942_2581)

ATGAGCGATACCTATACCGTTCGCATTCGCGATCGCCGCACCGATGA
AGAATTTACCGTGCAGGTGCCGCCGGATCGCTATATTCTGCAGACCGCCG
AAGAACAGGGCTATGAACTGCCGTTTAGCTGCCGTAATGGCGCCTGCAC
CGCATGTGCCGTTCGTGTGCTGGGTGGTGCCATTGAACAGACCGAAGCA
ATGGGTCTGAGTGCACCGCTGCGTCAGCGTGGTTATGCCCTGCTGTGTG
TGAGCTATCCGCGCAGTGATGTGATTGTGGAGACCCAGGACGAAGATGA
GGTGTACATGCTGCAGTTCGGTCGCTACTTTGGTCAGGGCAAGGTGAGC
TTTGGTCTGCCGCTGGATGAAGAATAA

(2) Codon-optimized *Se*Fdx-2 (GenBank Accession No. SYNPCC7942_1749)

ATGACCACCCCGGATCGTAGCGGTCTGGAGCCGGAACTGGGCGGTA
GTCTGCGTCATGGTCAGGCACGCAGTGGTCTGGAACCGGAGCTGGGTG
GCGAACTGCGTCAGAAACTGGTGTGGGTGGATGAGGTGACCTGCATTGG
CTGCCGTTATTGCAGCCATGTGGCCACCAACACCTTCTACATCGAGCCGG
ATTATGGCCGTAGCCGCGTGGTGCGCCAGAATGGTGATCCGGAAGAACT
GGTGCAGGAAGCCATTGATACCTGCCCGGTGGATTGCATCCATTGGGTG
AATCCGAGCGAACTGCGCCAGCTGGAAGCCGAACGCCGCAATCAGGTG
ATTATGCCGCTGGGTTTTCCGCAGGAACGCAGCAAACAGCGCCGCCGTA
CCTAA

(3) Codon-optimized *Se*Fdx-3 (GenBank Accession No. SYNPCC7942_1499)

ATGGCCACCTATAAAGTGACCCTGGTGAACGCCGCCGAAGGCCTGA
ATACCACCATTGATGTGGCCGACGACACCTATATTCTGGATGCCGCCGAA
GAACAGGGCATTGATCTGCCGTATAGCTGCCGCGCAGGTGCCTGTAGTA
CCTGCGCAGGTAAAGTGGTGAGCGGTACCGTGGATCAGAGCGATCAGAG
CTTCCTGGATGATGATCAGATTGCCGCCGGTTTTGTGCTGACCTGCGTGG
CCTATCCGACCAGCGATGTGACCATCGAAACCCACAAAGAAGAAGATCTG
TATTAA

(4) Codon-optimized *Se*Fdx-4 (GenBank Accession No. SYNPCC7942_0898)

ATGACCCTGGCCGAAACCCTGAGCGTTCGTGTTCGCAGCCTGGGCC
TGGACCAGATTGACCGTCATCTGTTTCTGTGCGCCGATCAGACCAAACC
GCTGTGCTGCGATCGCGATCGCAGCCTGGAAAGCTGGGAGTATCTGAAA
CGCCGCCTGCGTGAACTGGATCTGGATCGCCCGGATACCGGTAAACCGC
TGGTGTTTCGCACCAAAGCCAATTGCCTGCGCGTGTGCCAGGAAGGTCC
GATTCTGCTGGTGTATCCGGAAGGCATTTGGTATGGTCGCGTGACCCCG
GAAGCCATTGAACGCATTCTGCAGGAACACCTGCTGGGTGGTCAGCCGG
TGCAGGAACTGATTCTGCATCAGCATGCCCTGCCGGCAGTGGATCCGCT
GTAA

(5) Codon-optimized *Se*Fdx-5 (GenBank Accession No. SYNPCC7942_0814)

ATGGCCCATACCATTGTGACCAACACCTGCGAAGGTGTGGCAGATTG
CGTGGATGCCTGCCCGGTGGCCTGCATTCAGGAAGGTCCGGGTCGCAA
TCAGAAAGGCACCACCTGGTACTGGATCGATTTCAGCACCTGCATCGATT
GCGGCATTTGCCTGCAGGTGTGCCCGGTGGAAGGTGCCATTCTGCCGG

AAGAACGCCCGGAACTGCAGCAGACCCCGTAA

(6) Codon-optimized *Se*Fdx-6 (GenBank Accession No. SYNPCC7942_0698)

ATGCCGAGCATTCGCTTCATTCGCGAGGATAAAGAAGTTTTTGCCGC
CGATGGCGCAAACCTGCGCTTTAAAGCCGTGGAAAACCAGGTGGACCTG
TATACCTTCGGCGGCAAGATGATGAACTGCGGCGGTTATGGTCAGTGCG
GTACCTGCATTGTGGAGATTGTGCAGGGCGCCGAAAATCTGAGCCCGCG
CACCAGCTTCGAAGAACGCAAGCTGAAACGTAAGCCGGATAGCTATCGC
CTGGCCTGTCAGGCCACCGTGAATGGTCCGGTGACCGTTCTGACCAAAC
CGAACCCGAAAGAAGCCCAGCGCGAAACCCTGATTGCACAGGATCTGGC
CCGCCCGATTCCGGTTACCGCACCCCCTGCCCTGCCGCAGACCGAAAC
CGAGGTGGCAGGTGATCCGCCGAGCATTGCCACCGCCGAAACCTAA

(7) Codon-optimized *Se*Fdx-7 (GenBank Accession No. SYNPCC7942_0338)

ATGGCCACCTATCAGGTGGAAGTGATTTACCAGGGCCAGAGCCAGA
CCTTTACCGCCGATAGCGATCAGAGCGTGCTGGATAGCGCACAGGCCGC
AGGTGTTGATCTGCCGGCCAGCTGCCTGACCGGTGTGTGCACCACCTGT
GCCGCACGCATTCTGAGCGGCGAAGTGGATCAGCCGGATGCCATGGGT
GTGGGTCCGGAACCGGCCAAACAGGGTTATACCCTGCTGTGCGTGGCAT
ATCCGCGCAGCGACCTGAAAATCGAGACCCACAAAGAAGATGAGCTGTA
TGCCCTGCAGTTTGGTCAGCCGGGTTAA

(8) *Cg*Fdx-1 (GenBank Accession No. NCgl1057)

ATGACATACACAATCGCACAGCCCTGCGTTGACGTCTTGGATCGTGC
CTGCGTTGAAGAATGCCCAGTAGATTGCATCTACGAAGGTAAGCGCATGC
TGTACATCCACCCGGATGAGTGCGTTGACTGTGGTGCATGTGAGCCTGC
TTGCCCAGTTGAGGCAATCTTCTACGAGGACGATGTCCCAGACGAATGG
CTTGACTACAACGATGCCAACGCTGCATTCTTCGATGATCTGGGCTCCCC
AGGTGGTGCGGCTAAGCTTGGACCACAAGATTTTGATCACCCAATGATCG
CTGCGCTGCCGCCTCAGGCATAA

(9) *Cg*Fdx-2 (GenBank Accession No. NCgl0526)

TCACACTTGCGTTTCTGGCGTGGTCAACGAAAGATCCATGCCTTCGG
TGACCTTGATTTGGCAAGACAAACGGGAGCAATCCTCACGGTCCACGGC
AGCACCCCACAGCATTTCATCTTCCATCTCCTCCATTGGGGGAAGCGCAT
CATACTGTGCAGGGTCAACAAACACATGGCAGGTTGCACACGATAAGGAA
CCGCCGCATTCAGCAACAATTCCAGGCACTCCGTTTCGGACTGCGGTCT
CCATTACTGAATCACCAACAGTCGCCTCGATGGTGCGGGTTTTGCCAGCA
TGATCAATGAAATGAATAGTAGACAT

(10) *Cg*Fdx-3 (GenBank Accession No. NCgl2856)

CTAGTTCTGGTTCTGTGGCGGCAGCACCGCGACGAGCTGGGCGTC
GAAGTCCTGCGGACCCAGGCTGGCGGCACCGCCTGGCGAACCGAGGT
CGTCGAAAAAGGCGGCGTTAGCGCCGGTGTAGTCCCACCATTCGTGGG
GAACATCATCTTCGTAGAAGATGGCTTCAACCGGGCAGACGGGCTCGCA
GGCACCGCAGTCGACGCACTCATCGGGGTGGATGTAGAGCATCCGTTTG
CCCTCGTAGATGCAGTCCACGGGACATTCCTCGACGCAGGCTCGATCCA
GGACATCAACGCAGGGCTGGGCGATTGTGTAGGTCAT

**2. Ferredoxin reductases**

(1) *Cg*FdR-1 (GenBank Accession No. NCgl2719)

ATGACAACTCCCCTGCGCGTAGCCGTCATCGGAGCTGGCCCTGCTG
GCATTTACGCATCCGACCTCCTCATCCGCAATGAAGAGCGCGAAGTGTTC
GTTGACCTTTTCGAGCAAATGCCTGCACCGTTCGGACTCATCCGTTACGG
CGTTGCTCCAGACCACCCACGCATCAAGGGCATCGTTAAGTCCCTGCAC
AACGTGTTGGACAAGCCACGCCTGCGCCTGCTCGGTAACATTGAAATCG
GCAAAGACATCACCGTCGAAGAACTCCGCGACTACTACGATGCAGTCGT
GTTCTCCACCGGCGCAGTTGCAGACCGCGACCTCAACATCCCCGGAATT
GAAGCAGAAGGCTCCTTCGGTGCCGGCGAGTTCGTTGGCTTCTACGACG
GCAACCCACGCTTCGAGCGCTCCTGGGATCTGTCTGCACAGTCCGTCGC
TGTTATCGGCGTTGGTAACGTCGGCCTCGACGTAGCCCGCATCCTGGCT
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ACTCCCTCAAAGAAAACAAGGCCACTGAAGTGCACGTTTTCGGACGTCG
TGGCCCAGCACAGGTCAAGTTCACCCCACAGGAACTCAAAGAACTCGAC
CACTCCCCCACCATCAACGTGGTTGTTGATCCAGAAGACATCGACTACGA
CGGCGCCTCTGAAGAAGCCCGCCGCGCATCCAAGTCCCAGGACCTGGT
CTGCCAGATCCTGGAACAGTACGCAATCCGCGAGCCAAAGGACGCTCCG
CACACCCTGCAGATCCACCTCTTTGAAAACCCAGTTGAGGTTCTTCAAAA
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CAACGGCGGCGTAAACGGAACCGGCGAATTCAAGGACTGGCCAGTCCA
GGCTGTCTACCGCGCAGTCGGCTACAAGTCCGACCCCATCGACGGCGT
CCCATTCGATGAGAACAAGCACGTCATCCCTAATGACGGCGGACATGTCC
TCACCGCTCCAGGCGCAGAACCAGTACCAGGCCTCTATGCAACCGGCTG
GATCAAGCGTGGACCAATCGGTCTAATCGGCAACACCAAGTCCGACGCC
AAGGAAACCACCGACATCCTCATCAAGGATGCCGTCGCCGGTGTACTTG
AAGCTCCAAAGCACCAGGGCGAAGAAGCCATCATCGAGCTTCTCGATTC
CCGCAACATCCCATTCACCACCTGGGAAGGCTGGTACAAACTCGACGCA
GCAGAGCGCGCACTCGGTGAAGCCGAAGGCCGCGAGCGCAAGAAGATT
GTTGATTGGGAAGAAATGGTCCGCCAGGCCCGCGAAGCTCCAGCAATTG
TCTAA

(2) *Cg*FdR-2 (GenBank Accession No. NCgl2658)

ATGTCTCGCCCTTTGCGTGTTGCCGTTGTCGGTGCAGGTCCAGCAG
GAATCTACGCGTCTGATTTGTTGATGAAATCCGACACGGACGTGCAGATT
GATCTTTTTGAACGTATGCCAGCGCCTTTCGGTTTGATCCGTTATGGTGTT
GCGCCTGATCACCCTCGCATCAAGGGCATCGTGAAGTCCCTGCACAATG
TGATGGACAAGGAGCAGCTGCGTTTCTTGGGCAACATTGAGGTCGGCAA
GGACATCACTGTTGAGGAGTTGCGTGAGTTTTATGACGCGATCGTGTTCT
CCACTGGCGCTACTGGCGACCAGGATCTTCGGGTTCCAGGTTCTGATCT
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TGTTCATTCACTTCTTTGAGTCCCCAGTGGAGATCCTCGGTGAGGACGG
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ACCGCGCGGTAGGTTACCGTTCTGATGCGATCGAGGGTGTTCCTTTTGA
CGATGAGCGCGCGGTTGTCCCCAACGACGGCGGCCACATCATCGATCCT
GAGGTCGGCTCCCCCATCACTGGCCTGTACGCCACTGGCTGGATCAAGC
GTGGCCCAATTGGACTGATCGGCAACACCAAGTCCGACGCCAAGGAAAC
CACTGAGATGCTGCTTGCTGATCACGCTGCTGGTTCTTTGCCTGCGCCT
GCAAAGCCTGAGTTGGAGTCCATCATTGAGTTCCTCGATGAGCGCAAGG
TTGCGTTCACCACATGGGATGGCTGGCACCTGCTGGATGCTGCGGAGCG
CGCGCTGGGTGAGCCTGAGGGCCGCGAGCGCAAGAAGATCGTTGAGTG
GAATGACATGGTGCGCCATGCTCGTCCAGAATACGACATCTAA

(3) *Se*FdR-1 (GenBank Accession No. SYNPCC7942_0978)

ATGTTGAATGCGAGTGTGGCTGGCGGAGCAGCTACCACCACCTATG
GCAACCGGCTCTTTATCTATGAAGTGATCGGTCTGCGCCAAGCCGAGGG
CGAACCGTCCGACAGCTCAATCCGCCGTAGTGGCAGCACCTTCTTCAAG
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CGGCAAAATCGTTAGCATCCGGCCTGCGGAGGAAGCAGCCGCGAATAAT
GGTGCGGCTCCTCTACAGGCAGCAGCTGAAGAACCTGCTGCAGCACCA
ACCCCCGCTCCGGCTGCCAAAAAACATTCAGCCGAAGACGTGCCTGTCA
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CCCTTGGTTCAAGAAGGCGGGATTGGTGTTGTGCAGCACCTCACCTTCG
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TCCCGGATGGCACCGATGACAAAGGCAAGCCGCACAAGCTCCGTCTTTA
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TCGCTGTGCGTGCGCCAGCTGCAGTACCAGAACGAAGCCGGCGAAACG
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CGTCAAGATCACCGGTCCTGTGGGCAAAGAAATGCTCCTACCGGCGGAC
ACAGACGCCAACGTGATCATGATGGGTACTGGCACCGGGATTGCTCCGT
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AGCGAGTATCAATTCAACGGCAAGGCTTGGTTGATCTTCGGGATTCCGAC
GACCGCCAACATCCTCTACAAAGAGGAGCTGGAAGCGCTGCAGGCTCAG
TATCCAGATAACTTCCGCCTGACCTACGCGATCAGCCGCGAGCAGAAAAA
TGAAGCGGGCGGCCGGATGTACATCCAAGACCGCGTCGCTGAACATGCT
GACGAGATCTGGAACCTACTCAAGGACGAAAAAACCCACGTCTATATCTG
TGGTTTGCGTGGCATGGAAGATGGGATCGATCAAGCCATGACCGTCGCA
GCTGCCAAGGAAGATGTGGTTTGGTCTGACTACCAACGCACCCTCAAGA
AAGCGGGTCGTTGGCATGTTGAAACCTACTAG