**Supplemental Table S1** Catalytic efficiency of FADS2s from various species with LA and ALA substrates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Rank*a* | Preference index*b* | Strain names | Catalytic efficiency of FADS2 on LA（%）*c* | Catalytic efficiency of FADS2 on ALA（%）***c*** | Locus*c* |
| +2 | 1 | ***Thalassiosira pseudonana*** | 68 | **80** | AY817155 |
| -1 | -1 | *Ostreococcus tauri* | 73 | **71** | AY746357 |
| +8 | 3 | *Primula vialii* | 20.9 | **63.6** | AY234127 |
| +12 | 13 | *Micromonas pusilla* | 4.8 | **63** | EEH58637 |
| +7 | 2 | *Psetta maximus* | 31.2 | **59.5** | AY546094 |
| +10 | 6 | *Mantoniella squamata* | 6.6 | **38.8** | DAA34893 |
| +3 | 1 | *Primula farinosa* | 28 | **38.7** | AY234125 |
| +13 | 114 | *Mantoniella squamata* | 0.3 | **34.2** | CAQ30479 |
| +11 | 9 | *Oncorhynchus mykiss* | 3.6 | **31.5** | AF301910 |
| -2 | -1 | *Phaeodactylum tricornutum* | 27.8 | **26.7** | AY082393 |
| +6 | 2 | *Sparus aurata* | 12.2 | **23.1** | AY055749 |
| +5 | 2 | *Mucor rouxii* | 12 | **20** | AF296076 |
| -4 | -1 | *Ceratodon purpureus* | 16 | **13.8** | AJ250735 |
| -5 | -1 | *Rhizopus arrhizus* | 23.4 | **11.9** | AAS93682 |
| +9 | 5 | *Cyprinus carpio* | 1.5 | **7** | AF309557 |
| +1 | 1 | ***Glossomastix chrysoplasta*** | 6 | **7** | AAU11445 |
| +4 | 1 | *Conidiobolus obscurus* | 4.25 | **6.3** | HQ656805 |
| -7 | -8 | *Pythium irregulare* | 40d | **5** | AF419296 |
| -3 | -1 | *Parietochloris incisa* | 5.1 | **4.5** | GU390532 |
| -10 | -29 | *Mortierella fungus* ATCC32222 | 58.4 | **2** |  |
| -8 | <-1 | *Mortierella fungus 1S-4* | 40.4 |  | AB020032 |
| -6 | <-1 | *Cunninghamella echinulata* | 3.1d | **-** | ABA06503 |
| -9 | <-1 | *Mortierella alpina W15* | 51.2 | **-** | EF413024 |

a The “Rank” numbers represent the level of corresponding FADS2 substrate preference. E.g.: “+13” represents the strongest ω-3 substrate preference; “-10” represents the strongest ω-6 substrate preference or the weakest ω-3 substrate preference.

b Preference indexes equal [Catalytic efficiencyALA/ Catalytic efficiencyLA] or -[Catalytic efficiencyLA/ Catalytic efficiencyALA].

c These data indicated fatty acid (w/w %) content GLA and SDA respectively in corresponding strains. “-” indicated not to catalyze LA or ALA.

d The blank spaces represent not mentioned in corresponding reference, but its preference is known, or represent its locus is not found.

Based on previous summary of catalytic efficiency of FADS2s from various species with LA and ALA substrates, their catalytic efficiency was reordered according to catalytic efficiency of ALA substrate.

**Supplemental Table S2** Primers used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| Primer name | Restriction enzyme | Oligonucleotide sequence (5'-3')*a* | Function |
| FTp | *Eco*R I | TACCGGAATTCATGGGAAAAGGAGGAGACGC | TpFADS6 amplification for expression in *S. cerevisiae* |
| RTp | *Xho* I | CGGGGCCTCGAGTTACATGGCAGGGAAATCCT |
| FGc | *Eco*R I | TACCGGAATTCATGCGTGGATGGTGCACCAC | GcFADS6 amplification for expression in *S. cerevisiae* |
| RGc | *Not* I | CGAAGGAAAAAAGCGGCCGCTTACATGGCTGGGAACTCGT |
| T7 | - | TAATACGACTCACTATAGGG | Target genes insert detection for yeast expression construction |
| pYES2.R | - | TCGGTTAGAGCGGATGTG |
| FA | *Eco*R I | TACCGGAATTCATGGGAAAAGGAGGAGACGC | Overlap extension PCR for eighteen kinds of fusion genes |
| RA | - | CATGTCATCGCCCGCGTGGCTAAAGATCACCAGACCGCCG |
| FB | - | TATGGACCACCCCGGCGGTCTGGTGATCTTTAGCCACGCG |
| RB | *Not* I | CGAAGGAAAAAAGCGGCCGCTTACATGGCTGGGAACTCGT |
| FC | *Eco*R I | TACCGGAATTCATGCGTGGATGGTGCACCAC |
| RC | - | AATCAAGAACTTGTCCATGAAGTTGAACGCCGAGGGCGGG |
| FD | - | GGCCAGCTTCGAGAAGGCATACCGCAATCTGCGAGTCCAG |
| RD | - | CGAAGTGGTCCGACCCCAGCACGAGGCCCCAGGACACGAG |
| FE | - | GCTGTGCCTCGTGTCCTGGGGCCTCGTGCTGGGGTCGGAC |
| RE | - | GCGCGAGGAAGAGAGCGCCCACGAGGTGCACA |
| FF | - | GCAGGGCTTCTCCGTGGCCTGGTGGAAGAACAAGCA |
| RF | - | CCATGCGCAGCGACCAGGCGAGGAGGGGCAT |
| FG | - | TCCTTGGCCTCGCGCGGCTCTCGTGGCTCGAGGGGT |
| RG | - | ACCCCTCGAGCCACGAGAGCCGCGCGAGGCCAAGGA |
| FH | - | AGCAGCTCGTGACCAACCCCCTGTTGGAG |
| RH | - | CCCAGGCGTAGTGGACCAGGAGGCCGGCCT |
| FI | - | TGGTGCGCGCCCTGGCGTTCTTCTTCGTGGCCACCT |
| RI | - | GGTGCAGGTGGCCACGAAGAAGAACGCCA |
| FJ | - | GGTGCAGGTGGCCACGAAGAAGAACGCCA |
| RJ | - | CTGGAGCTTCCAGAAGTCCGGCCGGGCGTTGGCCT |
| FK | - | GGTGGAGCACCACCTCTTCCCCTCGCTGCCCCGGCA |
| RK | - | CTCGTGGGCCCGGGGGAGGTTGTGCCGGGGCAGCG |
| FL | - | CACGAGGCCGACCTCCTTACCGGAACCAAGGAGATT |
| RL | - | GTCGAGGAACTCCGTGGTTACCTCCGACAAGCAC |
| FM | - | CTTTCGTGCTTGTCGGAGGTAACCACGGAGTTCCT |
| RM | *Xho* I | CGGGGCCTCGAGTTACATGGCAGGGAAATCCT |
| FN | - | AAGCGTAGTGGAGCATTGAAATTGGCGGAGAAGC |
| RN | - | CTTCTTCACCTCCTGCCAAGTGTACTTCTG |
| FO | - | AACAAAGTCTACGACGTCTCCAACTGGTACGACCAC |
| RO | - | GTGAACACCACGGCTCCACCGGGGTGGTCGTACCAGTTG |
| FP | - | CATGACGGACATCTTCGCCGCCTTCCACGCCCAAGG |
| RP | - | TGAGAGCCTTGGGCGTGGAAGGCGGCGAAGATG |
| FQ | - | GAGCATAAGGATCAAAGACAGTTGGATTT |
| RQ | - | ATCCCTTCTCGAAATCCAACTGTCTTTGATC |
| FR | - | CTACTCGGACAGTTTGGCAATGCACATTGGA |
| RR | - | AAGAGAGCCGATCCAATGTGCATTGCCAAACTGT |
| FS | - | GGGATTGTTCTGGCAGCAGTGTGGATGGCTTGCGC |
| RS | - | ACTTGGTGGTGAAGAAAGTCGTGCGCAAGCCATCCA |
| FT | - | GATCTCATGCAGGGGTTCTCGATGCAGT |
| RT | - | GGTGGCCATTGTGCTTGTTCTTCCACC |  |
| FU | - | TTGCACAACTCTTCCTTGGACAGTC |  |
| RU | - | GAGTGGCATGGTATCAATATCGGGATCACCAT |  |
| FV | - | AAGTACGCTATCAAATTCCAGGCATTCACATAC |  |
| RV | - | AAGAGATGCGAGCCAAGAGGAGGATGGGGAAG |  |
| FW | - | CTCGGAGCTGCCTCGGAGAATGCCAAGTTGGAG |  |
| RW | - | AGCTTCTCCAAAAGTGGGTACTGAAGTCCACGC |  |
| FX | - | TGTTCGTCCTCTCTTCCGGATTTGGAAGGTGGTCTCTTC |  |
| RX | - | AAGTCCCGAGGAGCATGTGGCAGTGAAG |  |
| FY | - | TGGTGGCATCGTACACTGACATACCGTTGTGTC |  |
| RY | - | TAGTGGTGACTTGGAGTTGCCAGAAGTCAGGT |  |
| FZ | - | TTGGTTCTGCGGTGGATTGCAATACCAAGTGGATCA |  |
| RZ | - | ATATCAGCCTCATGGTACTTCACACCCCACTCCTTAC |  |
| F7-1 |  | CCCCTCGCTGCCCCGGCACAACCTCCCCCGGGC |  |
| R7-1 |  | CACGATCTCGTGGGCCCGGGGGAGGTTGTGCCGGGG |  |
| F9-1 |  | CCAGGTGGAGCACCACCTCTTCCCCTCGCTGCCCCGG |  |
| R9-1 |  | GAGGTTGTGCCGGGGCAGCGAGGGGAAGAGGT |  |
| F9-2 |  | CCGGGCCCACGAGATCGTGACGGCCTTCTGCAAGG |  |
| R9-2 |  | GCCCTGCTCCTTGCAGAAGGCCGTCACGATCTCG |  |
| F9-3 |  | GCCCTGCTCCTTGCAGAAGGCCGTCACGATCTCG |  |
| R9-3 |  | CACGGAGTTCCTCGACGAGTTCCCAGCCATGTAA |  |
| F-V295T |  | GGCGACCTCGCCGGTAACCTGATCGGC | Targeted mutagenesis for constructing GcFADS6 mutants |
| R-V295T |  | TTGCCGATCATGAGGTCGGCGAGGTCGCC |
| F-T299S  |  | CTCGCCGGTATCTTGATCGGCAACGTGTGGCA |
| R-T299S |  | TGCCACACGTTGCAGATCATGATACCGGCGAG |
| F-L303F  |  | ATCATGATCGGCAACGTTTGGCAGGGCTTCTC |
| R-L303F  |  | GAGAAGCCCTGCCAAACGTTGCCGATCATGAT |
| F-I306L |  | TCGGCAACGTTAGGCAGGGCTTCTCC |
| R-I306L |  | GGAGAAGCCCTGCCTAACGTTGCCGA |
| F-A315S  |  | CGTGTGGCAGGGCTTCTCCGTAGCCTGGTGGAAGAAC |
| R-A315S |  | GTTCTTCCACCAGGCTACGGAGAAGCCCTGCCACACG |
| F-H360D  |  | GAACAAGCACAAGATCCACCACTCGGTGCC |
| R-H360D |  | GGCACCGAGTGATCGGTGTTGTGCTTGTTC |
| F-F368Y  |  | AACACCCACCACTACGTGCCGAACCTCTACG |
| R-F368Y |  | CGTAGAGGTTCGGCACGTAGTGGTGGGTGTT |
| F-S377M/L378M  |  | GCCCTGGCGTTCTTCTATGATGCCACCTGCACCAGCGG |
| R-S377M/L378M |  | CCGCTGGTGCAGGTGGCATCATAGAAGAACGCCAGGGC |
| F-H381N  |  | GCGTTCTTCTTCGTGAACACCTGCACCAGCGGCCTCCTCC |  |
| R-H381N |  | GGAGGAGGCCGCTGGTGCAGGTGTTCACGAAGAAGAACGC |  |
| F-L383I |  | TTCTTCGTGGCCACCTGCATTAGCGGCCTCCTCCTCG |  |
| R-L383I |  | CGAGGAGGAGGCCGCTAATGCAGGTGGCCACGAAGAA |  |

a Underlined sequences indicate the additional restriction sites or mutation sites.