

Self-Re-direction of Metabolic Flux Toward Squalene and Ethanol Pathways by Engineered Yeast

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Supplementary Material

Supplementary table.S1a. List of the primers designed to amplify and construct the engineered promoters.

HHF2t (-300 to -669bp)

HHF2t-F: GTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGAAC GGACAACGTGTGGTCGT
HHF2t-R: TATTTTATTGTATTGATTG
LAC Z-F: ATGACCATGATTACGGATTC
LAC Z-R: TGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTG TTATTTTTGACACCAGACCA
TFBS_{HHF2}-COQ1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA ACCGTCTTTTACCTGTGTTT
TFBS_{HHF2}-COQ1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT CGTCCCAGTACTCAATTC
TFBS_{HHF2}-IRA1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA GCGAGCCGACACCGCCTGGA
TFBS_{HHF2}-IRA1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT TTTAGAAAGAGCCTTAATCT
TFBS_{HHF2}-MAK5-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TCCGCTGTATTAGTGGTATC
TFBS_{HHF2}-MAK5-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT GATGGAAAACTGATTATG
TFBS_{HHF2}-RER-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA GAATTGAGTACAGTGGGACG
TFBS_{HHF2}-RER-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT ACCGTCTTTTACCTGTGTTT
TFBS_{HHF2}-RHO1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TTCCCTCATTTCCAATAACA
TFBS_{HHF2}-RHO1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT CTTTCTAGTATAATTTTTAA
TFBS_{HHF2}-PET9-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TAACGAAATAATTTCTTTT
TFBS_{HHF2}-PET9-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG GGCTATTTGCTTATATGTAT
TFBS_{HHF2}-USA3-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TTTCCGTGTGATGGGCCTTC
TFBS_{HHF2}-USA3-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TTTCACAGTACAAATATTTT
TFBS_{HHF2}-ATP16-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TACTTTTCAGACGGGCAGTTT
TFBS_{HHF2}-ATP16-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TTTCTATCCTCACGGCTA
TFBS_{HHF2}-CMD1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA ATGTCCTAATGAATTCAGC
TFBS_{HHF2}-CMD1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TGTACTTTTTTATTTGTATT
TFBS_{HHF2}-RIM2-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TAAAAATAAATTTGTGTCTA
TFBS_{HHF2}-RIM2-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG CTTTATCTCCTTGAAAAATT
TFBS_{HHF2}-BRN-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA ATAGTGTGCTCCCTTAC
TFBS_{HHF2}-BRN-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TCTTTTAATCTTAACCGATT
TFBS_{HHF2}-HHF2-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TGTTTGCTTGGATCCTTTAG
TFBS_{HHF2}-HHF2-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TATTTTATTGTATTGATTGT
TFBS_{HHF2}-GRS1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA ATAGTGTGCTCCCTTAC

TFBS_{HHF2}-GRS1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TCTTTTAATCTTAACCGATT

TEF1t (-300 to -579bp)

TEF1t-F: GTATCGA TAAGCTTGAT ATCGAATTCC TGCAGCCCGG ACAATGCATA CTTTGTACGT

TEF1t-F: TTTGTAATTAATACTTAGAT

LAC Z-F: ATGACCATGATTACGGATT

LAC Z-R: TGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTG TTATTTTGGACACCAGACCA

TFBS_{TEF1}.COQ1-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA ACCGTCTTTTACCTGTGTTT

TFBS_{TEF1}.COQ1-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT CGTCCCACTGTACTCAATTC

TFBS_{TEF1}.IRA1-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA GCGAGCCGAC ACCGCCTGGA

TFBS_{TEF1}.IRA1-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TTAGAAAAGAGCCTTAATCT

TFBS_{TEF1}.MAK5-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TCCGCTGTAT TAGTGGTATC

TFBS_{TEF1}.MAK5-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT GATGGAAAACTGATTATG

TFBS_{TEF1}.RER-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA GAATTGAGTACAGTGGGACG

TFBS_{TEF1}.RER-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT ACCGTCTTTTACCTGTGTTT

TFBS_{TEF1}.RHO1-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TTCCCTCATT TCCAATAACA

TFBS_{TEF1}.RHO1-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT CTTTCTAGTATAATTTTTAA

TFBS_{TEF1}.PET9-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TAACGAAATA ATTTCTTTT

TFBS_{TEF1}.PET9-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT GGCTATTTGCTTATATGTAT

TFBS_{TEF1}.USA3-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TTTCCGTGTG ATGGGCCTTC

TFBS_{TEF1}.USA3-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TTTACAGTACAAATATTTT

TFBS_{TEF1}.ATP16-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TACTTTCAGA CGGGCAGTTT

TFBS_{TEF1}.ATP16-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TTTCTATCCTCACGGCTA

TFBS_{TEF1}.CMD1-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA ATGTCCTAAT GAATTCAAGC

TFBS_{TEF1}.CMD1-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TGTACTTTTTTATTGTATT

TFBS_{TEF1}.RIM2-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TAAAAAATAA ATTGTGTCTA

TFBS_{TEF1}.RIM2-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT CTTTATCTCCTTGAAAAAT

TFBS_{TEF1}.BRN-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA ATAGTGTTTG CTCCCCTTAC

TFBS_{TEF1}.BRN-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TCTTTTAATCTTAACCGATT

TFBS_{TEF1}.HHF2-F: AAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA TGTTTGCTTG GATCCTTTAG

TFBS_{TEF1}.HHF2-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TATTTTATTGTATTGATTGT

TFBS_{TEF1}.GRS1: FAAGAAAGCAT AGCAATCTA A TCTAAGTTTT AATTACAAA ATAGTGTTTG CTCCCCTTAC

TFBS_{TEF1}.GRS1-R: TTGTAAAACG ACGGCCAGTG AATCCGTAAT CATGGTCAT TCTTTTAATCTTAACCGATT

Supplementary table.S1b. List of the primers designed to amplify the metal ions repressible promoters.

Primers for amplification of AFT1p repressible promoters

AFT1-F: TATTACAACCTTTTTTACTTCTTGCTCATTAGAAAGAAAGATCACGTAAAACAACAACC

AFT1-R1: CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTGTCGTAGATTTTTCTGTGA

AFT1-R2: TTGTAATACGACGGCCAGTGAATCCGTAATCATAGTCATTGTCGTAGATTTTTCTGTGA

EGFP-F: ATTTTAAAAACAAAAAATAACAGAAAAATCTACGACAATGGTGAGCAAGGGCGAGGA

EGFP-R: TCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCTTACTTGTACAGCTCGTCCA

LacZ-F: ATTTTAAAAACAAAAAATAACAGAAAAATCTACGACAATGACTATGATTACGGATT

LacZ-R: TCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCTTATTTTGGACACCAGACCA

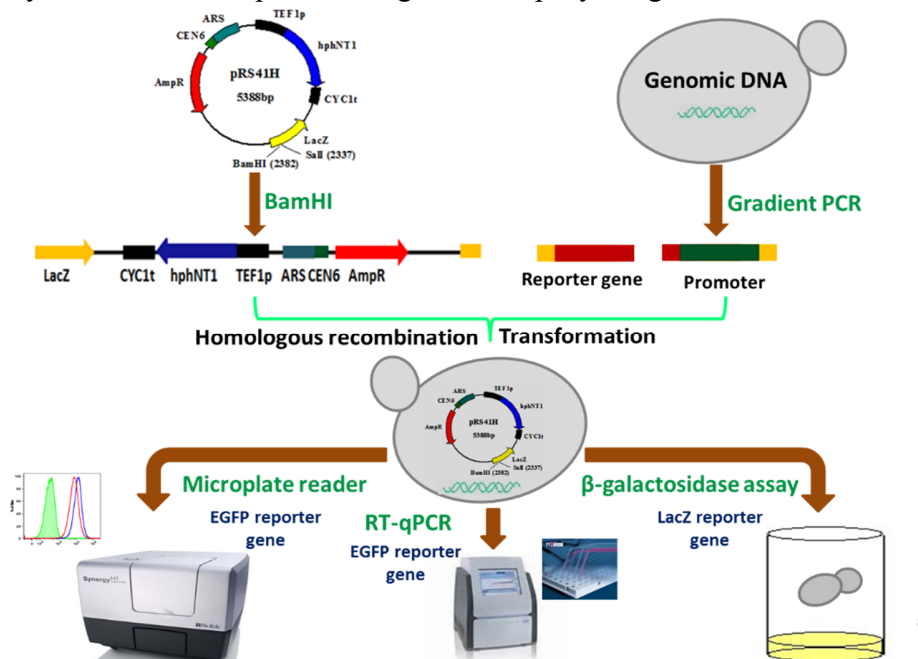
Primers for amplification of CTR1p repressible promoters

CTR1-F: GTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGACCGTATTTTGCTCGTTCCA

CTR1-R1: CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTTTGAATGTCAAATATAATA
 CTR1-R2: TTGTAATACGACGGCCAGTGAATCCGTAATCATAGTCATTTTGAATGTCAAATATAATA
 EGFP-F: TAGAAAAATAAAAAAAGTGTATTATATTTGACATTCAAATGGTGAGCAAGGGCGAGGA
 EGFP-R: TCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCTTACTTGTACAGCTCGTCCA
 LacZ-F: TAGAAAAATAAAAAAAGTGTATTATATTTGACATTCAAATGACTATGATTACGGATTC
 LacZ-R: TCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCTTATTTTGGACACCAGACCA

Note: R1 and R2 primers were designed to overlap with EGFP and LacZ reporter genes respectively.

Supplementary table.S2. List of primer designed to amplify the genes of module-1.



Supplementary figure.S1. Work flow of characterization of strength of engineered and repressible promoters.

Construction of Module-1 for engineering of FSE strain

Delta-F: GACTATTCCTCATTCCGTTT
 Delta-R: AGACTGTCAAGGAGGGTATTCTGGGCCTCCATGTCGCTGTTAGTAGTATACTATCACAT
 HbH-F: TGTTATCTTTTTACACCACATGTGATAGTATACTACTAACAGCGACATGGAGGCCGAGA
 HbH-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTCGTCCCAAAACCTTCTCAAG
 TFBS_{TEF1}-HHF2-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TGTTTGCTTGGATCCTTTAG
 TFBS_{TEF1}-HHF2-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TATTTTATTGTATTGATTGT
 HMG1-F: CCATGATTACGATTCACTGGCCGTCGTTTTACAA ATGACTGCTCCAAATAAAGA
 HMG1-R: ATAAATTGAAGCTTGGTCGTTCCGACCAATACCGTAGGGTTAGGATTTAATGCAGGTGACG
 ERG19t-F: CGTTTGAAAAGATGGGTCCGTCACCTGCATTAAATCCTAACCCTACGGTATTGGTCGGAA
 ERG19t-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTGGCTGCTGAGATCTTTAAG
 TFBS_{TEF1}-IRA1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATAGCGAGCCGACACCGCCTGGA
 TFBS_{TEF1}-IRA1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATTTTAGAAAGAGCCTTAATCT
 IDI1-F: ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAAATGACTGCCGACAACAATAG
 IDI1-R: CTTCTTTTTCAAGAGAATACCAATGACGTATGACTAAGTTTATAGCATTCTATGAATTT
 HMG1t-F: TTGACCATGAGAAAAATATACAGAATGTGAACCTTAGTCATACGTCATTGG
 HMG1t-R: GGAAGATTTTCACTCATAAGGGAAAGGAACCGCATGACTGGAAGATTTTCACTCATAAG

TFBS-^{TEF1}-RH01-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATATTCCCTCATTTCCTCAATAACA
 TFBS-^{TEF1}-RH01-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATCTTTCTAGTATAATTTTAA
 ERG20-F: ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAAATGGCTTCAGAAAAAGAAAT
 ERG20-R: TTACAAGAGAAGCAAATAGTAAATGTATATTCTTGTAATATTATAACATATATACGA
 MRH4t-F: ACTGCGTTCTTGAACAAAGTTTACAAGAGAAGCAAATAGTAAATGTATATTCTTGTAAA
 MRH4t-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTAAGCAATCGTTCTTTTATCC
 TFBS-^{TEF1}-PET9-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATATAACGAAATAATTTCTTTT
 TFBS-^{TEF1}-PET9-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGGGCTATTTGCTTATATGTAT
 ERG9-F: CCATGATTACGGATTCACTGGCCGTCGTTTTACAA ATGGGAAAGCTATTACAATT
 ERG9-R: CTTTAATGTTCTTTAGGTATATATTTAAGAGCGATTTGTTACGCTCTGTGTAAAGGT
 PGI1t-F: CTTGGGTTTTATTATATATACACTTTACACAGAGCGTGAACAAATCGCCTTAAATATA
 PGI1t-R: GGAATCCCAACAATTATCTCAAAACTCACTTATATCTCATTTTAAACAGTTGATGAGAA
 Delta1-2-F: CTTGAACTTGCGAAAAAGGTTCTCATCAACTGTTTAAATGAGATATAAGTGAGTTTG
 Delta1-2-R: GTATTATTAGTATGTATAGA

Construction of Module-2 for engineering of FSE strain

Delta6-1-F: TGTTAATATTCATTGATCCT
 Delta6-1-R: AGACTGTCAAGGAGGGTATTCTGGGCCTCCATGTCGCTGTGTTGGAATAAAATCAACT
 KamMX-F: ATACTAGTTAGTAGATGATAGTTGATTTTTATTCCAACACAGCGACATGGAGGCCCAGA
 KamMX-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTTTCGACACTGGATGGCGGC
 TFBS^{HHF2}-HHF2-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TGTTTGCTTGGATCCTTTAG
 TFBS^{HHF2}-HHF2-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG TATTTTATTGTATTGATTG
 PDC5-F: CTGTGCGATTCGATACTAACGCCCGCATCCAGTGTGCGAAA ATGTCTGAAA TAACCTTAGG
 PDC5-R: CTACTAACGCTAAACAATAACCCTACGGTATTGGTCGGAA CGACCAAGCTTCAATTTAT
 ERG19t-F: AGCTCAATTGACTGCCGCTACTAACGCTAAACAATAA CCCTACGGTA TTGGTCGGAA
 ERG19t-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTTTGCTGCTGAGATCTTTAAG
 TFBS^{HHF2}-IRA1-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA GCGAGCCGACACCGCCTGGA
 TFBS^{HHF2}-IRA1-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT TTTAGAAAGAGCCTTAATCT
 ADH1-F: ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAA ATGTCTATCC CAGAACTCA
 ADH1-R: CTTCTTTTTCAAGAGAATACCAATGACGTATGACTAAGTTTATTTAGAAGTGTCAACAA
 HMG1t-F: TCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAATAA ACTTAGTCAT ACGTCATTGG
 HMG1t-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTGGAAGATTTTCACTCATAAG
 TFBS^{HHF2}-RH01-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TTCCCTCATTTCCTCAATAACA
 TFBS^{HHF2}-RH01-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCAT CTTTCTAGTATAATTTTAA
 ADH4-F: ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAA ATGTCTTCCG TTACTGGGTT
 ADH4-R: TCGTATATATGTTATAATATTTACAAGAATATACATTTATTAATATTCATAGGCTTTCT
 MRH4-F: CAAGTGGTTG CCATTATCAA GAAAGCCTAT GAATATTAA TAAATGTATA TTCTTGTA
 MRH4-R: TATTTTATTGTATTGATTGTTGTTTTTGCTACTCTTTTAAGCAATCGTTCTTTTATCC
 TFBS^{HHF2}-PET9-F: AAAAGAGTAGCAAAAACAACAATCAATACAATAAAATA TAACGAAATAATTTCTTTT
 TFBS^{HHF2}-PET9-R: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG GGCTATTTGCTTATATGTAT
 ADH2-F: TTGTAAAACGACGGCCAGTGAATCCGTAATCATGG ATGTCTATTC CAGAACTCA
 ADH2-R: CTTTAATGTTCTTTAGGTATATATTTAAGAGCGATTTGTTTATTTAGAAGTGTCAACAA
 PGI1t-F: CCA AATTGCTGGT AGATACGTTG TTGACACTT TAAATAAACAAATCGCTCTTAAATATA
 PGI1t-R: GGAATCCCAACAATTACATCAAAATCCACATTCTCTTCATTTTAAACAGTTGATGAGAA
 Delta6-2-F: CTTGAACTTGCGAAAAAGGTTCTCATCAACTGTTTAAATGAAGAGAAT GTGGATTTTG
 Delta6-2-R: TATAAAATGATGATAATAAT

Supplementary table.S3. List of primer designed for qRT-PCR of overexpressed genes of the squalene biosynthesis pathway, ethanol production pathway and fusel alcohol pathway.

HMG1-F: TTTGCCCCGTTGGTGTTATAG
 HMG1-R: CATTGATTGCCTTACAGCCA
 IDI1-F: ATCGTGCATTCTCCGTCTTTA
 IDI1-R: ACCCTTCAAACCTAATTCGTC

ERG20-F: TTAATCACTGCACCTGAAGAC
 ERG20-R: CGACAGGCAAGTAGAAAAGAAT
 ERG9-F: GTGAAGCCAAATGAAACTCCA
 ERG9-R: AAACCCAAGAAGAACGGACA
 PDC5-F: GAACAACACCCAAGATACGG
 PDC5-R: GAAATCAGACAACAAAGCACC
 ADH1-F: GCTTCTACCAGATACGTTAGAG
 ADH1-R: CAACAATAGAGATGGACTTGAC
 ADH4-F: GACTAACCCAGTTCAATTACCC
 ADH4-R: ATAAGGCACACGCATAATTGAC
 ADH2-F: AAGGCTTTGAAGCTGCCAA
 ADH2-R: TCCTTCTCTTGGTGAAGTC

ARO8-F AAATACAACCTCAGACCTTACCAG
 ARO8-R AATCTCTTCAGACCTTCAGTCAG
 ARO9-F TTTCTTCTATAATCCGCAAGAG
 ARO9-R GTAGTAATGTCGGCCAAATCCA
 ARO10-F TGGTGATGTTGTCGTTTGTC
 ARO10-R ATTGATGTGAGCGTTTGAGTG
 BAT1-F ACTAGAAACCCAAATCCATCCA
 BAT1-R GGCAGACTTGTTACATCGGG
 BAT2-F GAAGCCACTGATTATGCCAC
 BAT2-R GAATCCCTAGTAACACCTTCCA
 GCN4-F: CCACTTCTACTGCCAAACCA
 GCN4-R: GAAAGATTCCACTACAGCGTC

Supplementary table.S4. List of primer designed to amplify the DNA fragments for modules given in supplementary figure-S1.

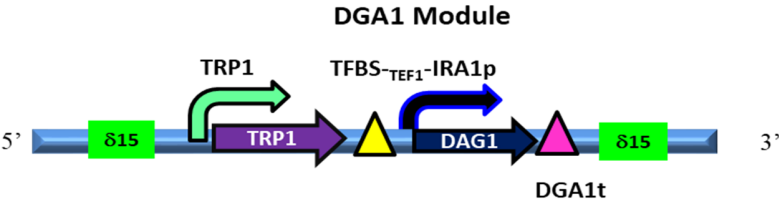
Module to swap the AFT1p with ERG1p

ERG1-UP-F: ATGTCTGCTGTTAACGTTGC
 ERG1-UP-R: AATCATTACCGAGGCATAAAAAAATATAGAGTGTACTAGAAATGTTTCTCAAGTTGTTCA
 HIS3-F: TTTGTTTCATGGTAGATTCTTGAACAACCTTGAGAAACATTCTAGTACACTCTATATTTTT
 HIS3-R: TGCAGTTCAACCTCACTCGCGTGC GGCGGTGTGAGGTGCCCTGATGCGGTATTTTCTCC
 AFT1-F: ATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGGCACCTCACACCGCCGCACG
 AFT1-R: GGCATTAATCAATTCAGGTGCAACGTTAACAGCAGACATTGTCGTAGATTTTCTGTTA
 ERG1-F: ATTTTTTAAAAACAAAAAATAACAGAAAAATCTACGACAATGTCTGCTGTTAACGTTGC
 ERG1-R: CAAAGTACACGTTAAAAATTCAGCTCGGGTATGTTAAGTATTAACCAATCAACTCACCAA
 YRA1t-F: GTATTACCCCATTTTTGTTTGGTGAGTTGATTGGTTAATACTTAACATACCCGAGCTG
 YRA1t-R: GACAGTCATACCACCACAGTCAATGGATGTCTCATATTTACCATTTGCAAGACATTGT
 ERG1-Dn-F: CTTTCAACAAAAATATTATACAATGTCTTGCAAATGGTAAATATGAGACATCCATTGAC
 ERG1-Dn-R: TTAACCAATCAACTCACCAA

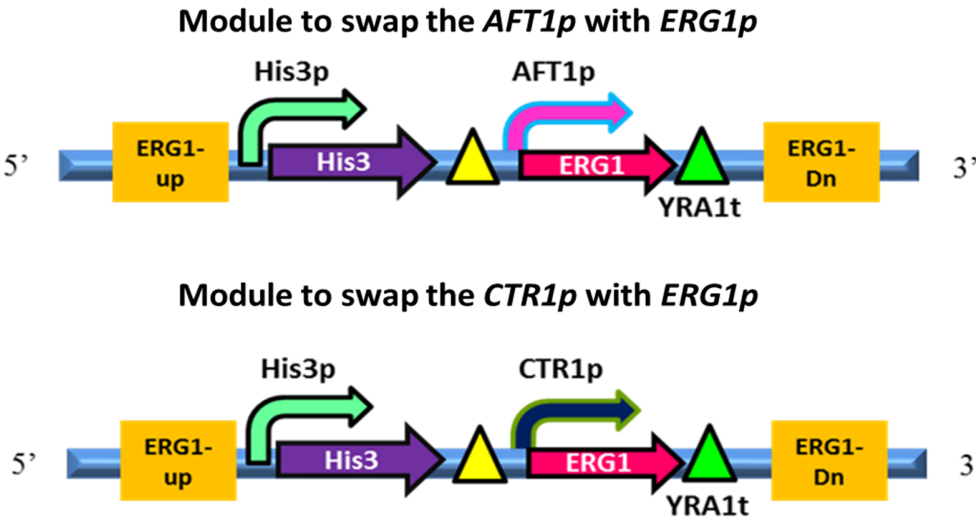
Module to swap the CTR1p with ERG1p

ERG1-UP-F: ATGTCTGCTGTTAACGTTGC
 ERG1-UP-R: AATCATTACCGAGGCATAAAAAAATATAGAGTGTACTAGAAATGTTTCTCAAGTTGTTCA
 HIS3-F: TTTGTTTCATGGTAGATTCTTGAACAACCTTGAGAAACATTCTAGTACACTCTATATTTTT
 HIS3-R: AACAAAAACGTGGAAGGCTGGAACGAGCAAAATACGGTCCTGATGCGGTATTTTCTCC
 CTR1-F: ATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGG ACCGTATTTT GCTCGTTCCA
 CTR1-R: GGCATTAATCAATTCAGGTGCAACGTTAACAGCAGACATTTTGAATGTCAAATATAATA
 ERG1-F: TAGAAAAATAAAAAAAGTGATTATATTTGACATTCAAATGTCTGCTGTTAACGTTGC
 ERG1-R: CAAAGTACACGTTAAAAATTCAGCTCGGGTATGTTAAGTATTAACCAATCAACTCACCAA
 YRA1t-F: GTATTACCCCATTTTTGTTTGGTGAGTTGATTGGTTAATACTTAACATACCCGAGCTG
 YRA1t-R: GACAGTCATACCACCACAGTCAATGGATGTCTCATATTTACCATTTGCAAGACATTGT

ERG1-Dn-F: CTTTCAACAAAAATATTATACAATGTCTTGCAAATGGTAAATATGAGACATCCATTGAC
ERG1-Dn-R: TTAACCAATCAACTCACCAA



Supplementary figure.S2. Design of the module used to overexpress the *DGA1* in engineered strain FSE.



Supplementary figure.3. Design of the module used to swipe the ERG1 promoter with AFT1p and CTR1p in engineered strain FSE.

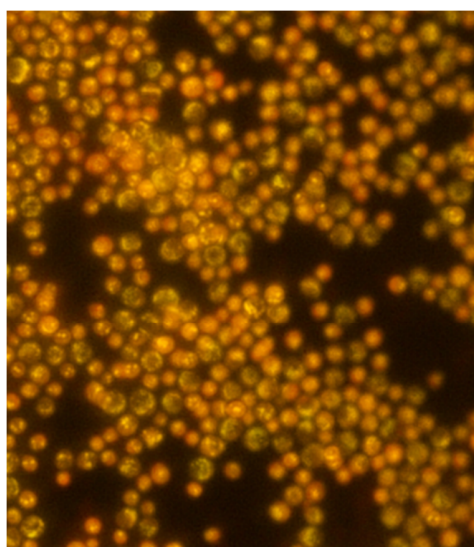
Supplementary table.S5. List of primer designed to amplify the DNA fragments of DGA1 module.

DELTA15-1-F: TATGTTTATATTCATTGATC
DELTA15-1-R: ATTAAATGCTTCCTATATTATATATATAGTAATGTCGTTTGTGGAATAAAAAATCCACT
TRP1-F: CTATTAGTTGATAGACGATAGTGGATTTTATTCCAACAAACGACATTACTATATATAT
TRP1-R: ACCACTTTCCCGGTGCCAAACGACCACAGTTGTCCGTTCCCTGATGCGGTATTTTCTCC
TFBS-^{TEF1}IRA1-F: ATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGGGGAACGGACAACGTGGTTCGT
TFBS-^{TEF1}-IRA1-R: TTCTAGCCTTCCCATTCAGTCAATGCTGACTGAATCTATTTTAGAAAGAGCCTTAATCT
DGA1-F: CAGCATATAACATACAACAAGATTAAGGCTCTTTCTAAAATAGATTCACTCAGTCAGCATTTGA
DGA1-R: GGAATCCCAACAATTATCTCAACATTCACATATTTCTCATTACCCAACATCTTCAATTC
DELTA15-2-F: TATGGGGTACCGGATGCAGAATTGAAGATAGTTGGGTAATGAGAAATATGTGAATGTTG
DELTA15-2-R: TAAAACGGAATGAGGAATAA

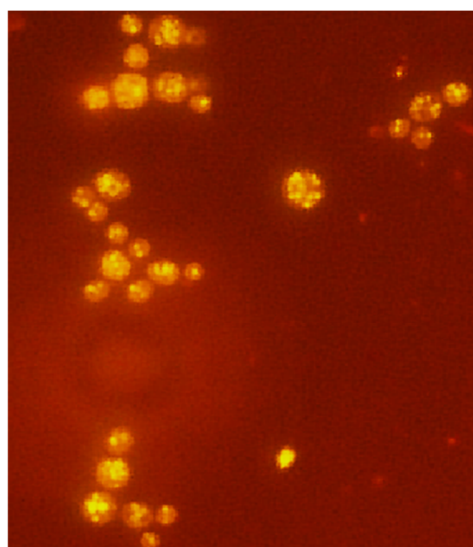
Supplementary table.S6. Fluorescence intensity of wild type promoters and engineered promoters.

#	wild type promoters	Size of Wild type	Average Fluorescence Intensity	Engineered promoters with	Average Fluorescence Intensity	% increase in fluorescence	Engineered promoters with	Average Fluorescence Intensity	% increase in fluorescence
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		promoters (bp)	(A.U) of wild type promoters	TFBS-TEF1 (-300 to -579 bp)	(A.U) of engineered promoters	relative intensity of engineered promoters	TFBS-HHF2	(A.U) of engineered promoters	relative intensity of engineered promoters
1	HHF2	669	402.2	HHF2-TEF1	662.39	56.39%	HHF2-HHF2	550.55	36.88%
2	IRA1	396	231.48	IRA1-TEF1	357.67	101.7%	IRA1-HHF2	369.65	59.69%
3	RHO1	441	177.2	RHO1-TEF1	343	95.51%	RHO1-HHF2	268.34	51.43%
4	PET9	490	140.31	PET9-TEF1	373.33	164.89%	PET9-HHF2	281.21	100.42%
5	CMD1	245	128.08	CMD1-TEF1	253.33	108.69%	CMD1-HHF2	246.58	92.52%
6	ATP16	719	118.81	ATP16-TEF1	231.67	97.63%	ATP16-HHF2	220.68	85.74%
7	USA3	385	110.95	USA3-TEF1	198.34	96.49%	USA3-HHF2	195.67	76.36%
8	RER2	240	100.99	RER2-TEF1	183.33	81.53%	RER2-HHF2	182.54	80.75%
9	COQ1	240	100.2	COQ1-TEF1	163.54	63.21%	COQ1-HHF2	148.83	48.53%
10	RIM1	428	98.01	RIM1-TEF1	122	24.48%	RIM1-HHF2	123.95	26.47%
11	GRS1	602	95.75	GRS1-TEF1	116.33	21.49%	GRS1-HHF2	118.62	23.89%
12	MAK5	278	87.37	MAK5-TEF1	106	21.32%	MAK5-HHF2	124.46	42.45%
13	BRN1	400	83.17	BRN1-TEF1	106	27.45%	BRN1-HHF2	125.23	50.57%

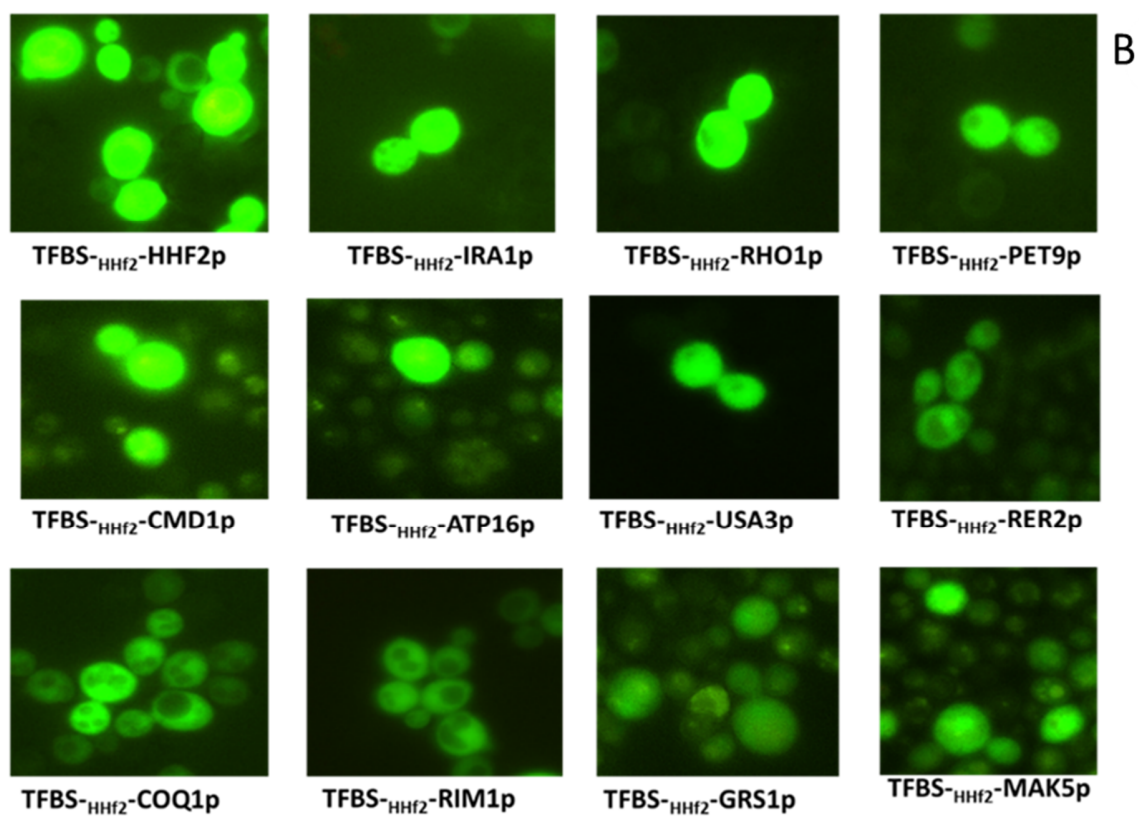
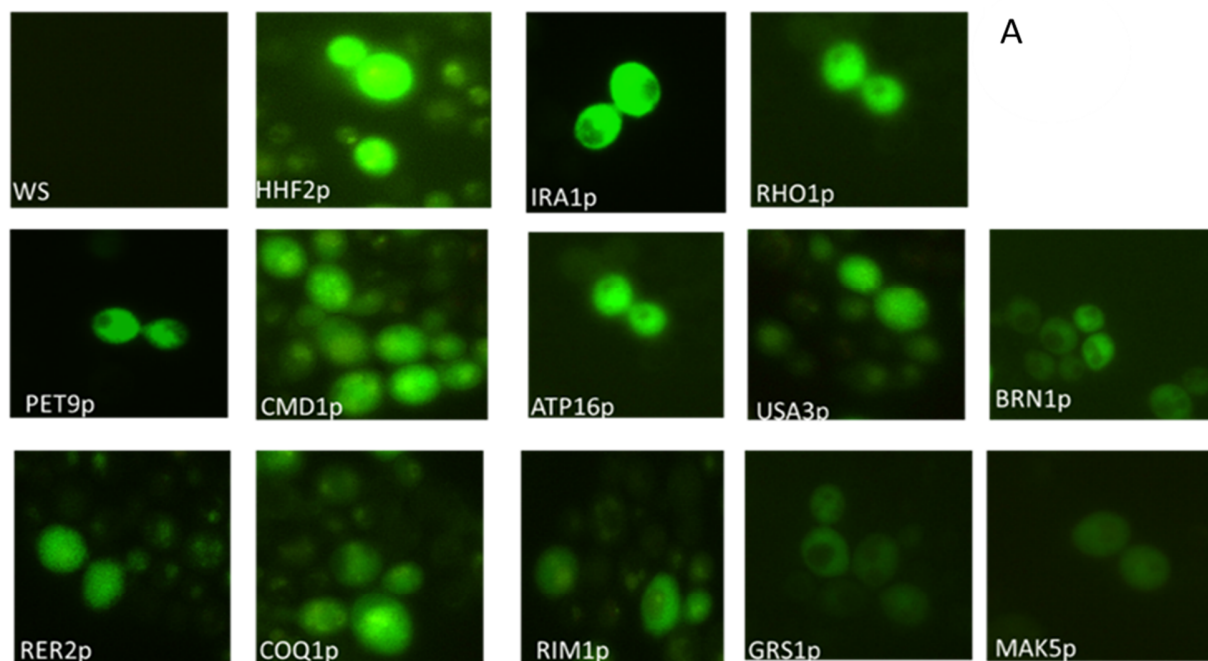


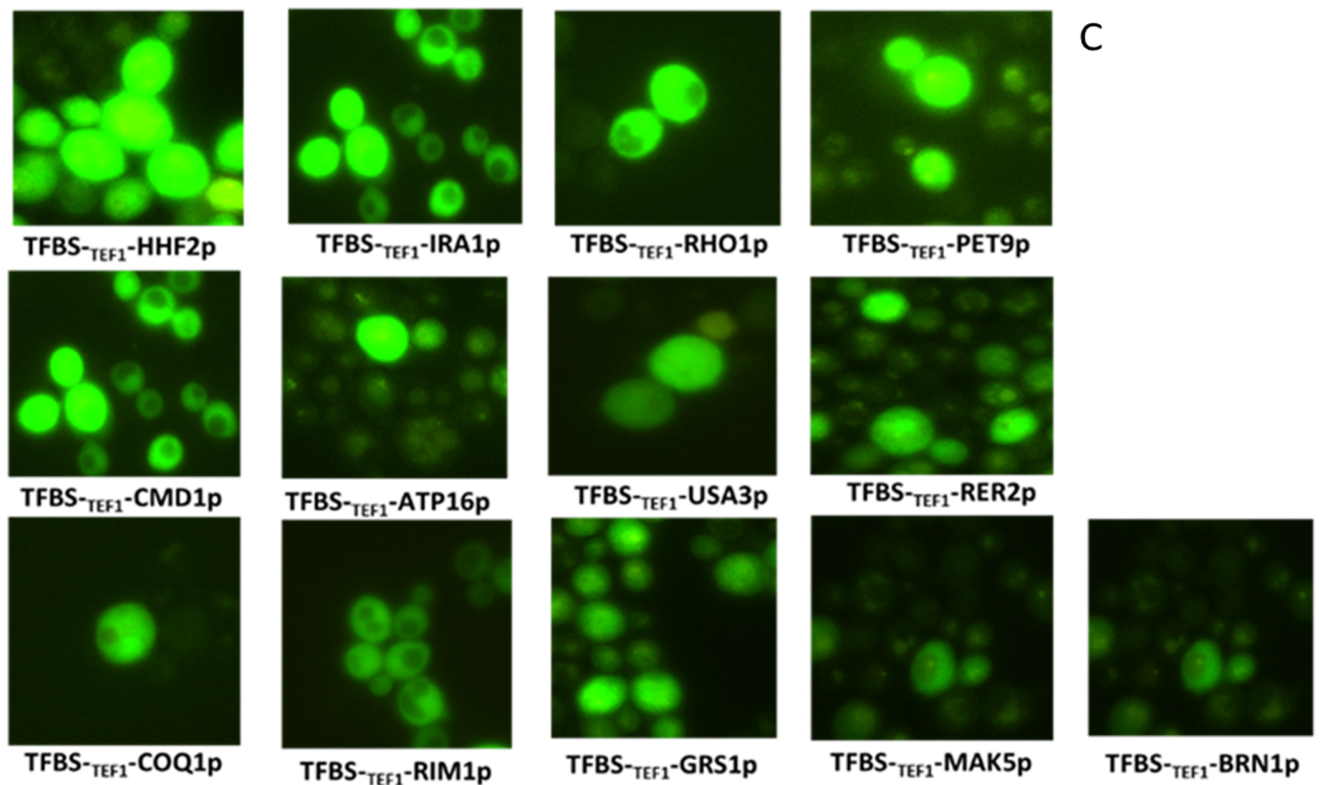
WS



FES strain

Supplementary figure.4. Nile red staining dye was used to visualize the lipid droplets biogenesis and accumulation of squalene in engineered strain FSE compared with wild strain.





Supplementary figure.5. (a) Confocal microscopic analysis of wild type yeast promoters. (b) Confocal microscopic analysis of engineered promoters containing TFBS of HHF2p. The attachment of TFBS of HHF2p enhanced the strength of all promoters. (c) Confocal microscopic analysis of engineered promoters containing TFBS of TEF12p. The attachment of TFBS of TEF1p enhanced the strength of all promoters.

