## Supplementary material

# Endogenous 2μ plasmid editing for pathway engineering in *Saccharomyces cerevisiae.*

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Figure S1. Structure of wild type endogenous 2μ plasmid (pE2μ), two sites were chosen for editing to insert target DNA element.



Figure S2 Structure of CRISPR/cas9 plasmid.



Figure S3. Fluorescence for strain harboring different plasmid in cultivation of 30 generation.



Figure S4. Comparation of Sc594 and Sc530. **(A)** Diluted culture of Sc530 and Sc594 were plated on YPD plate at 90th generation. **(B)** Fluorescence for Sc530 and Sc594 in cultivation of 90 generation.



Figure S5. Average PCN of Sc366, Sc343, Sc584 after 120h fermentation.



Figure S6. Comparation of relative mRNA level of Sc343 and Sc584 to strain Sc366 for gene ADS, CYP71AV1, DBR2





Figure S7. Tool box of the pE2μ multi-copy system for increasing the copy number of the target DNA element in different host.

Table S1. Primer used in this study

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| --- | --- |
| Primer | Sequence |
| 18Q2a-pgi1t-R | GTTCGGATGTGATGTGAGAACTGgtagtttagtgtttttcttccagtgcgag |
| 18Q2a-pgi1t-F | aaacgcggatcCAAATCGCTCTTAAATATATACCTAAAGAAC |
| 18Q2a-homodown-F | ttacgcggatccttattatacaggttcaaatatactatctgtttcagggaaaac |
| 18Q2a-homodown-R | atctgtgcttcattttgtaggtttaaactagctagaccgagaaagagactagaaatg |
| 18Q2a-homoup-F | ttctcggtctagctagtttaaacctacaaaatgaagcacagatgcttcgttaacaaag |
| 18Q2a-homoup-R | tttaggactagtcctggagaaactattgcatctattgcatag |
| 18Q4-pTDH3-F | ttaccggaattcggatccgagaccAGTTTATCATTATCAATACTGCCATTTCAAAGAAT |
| 18Q4-adh1t-R | acatacgtctcagtagctcgaggagaccggtagaggtgtggtcaataagag |
| 2μori-test-F | gcgttgcatttttgttctacaaaatg |
| biobrick-R | attaccgcctttgagtgagc |
| 18Q-test-5 | atgccttataaaacagctatagattgc |
| 18Q-test-15 | GATTGCGCCTGAGCGAGACGAAATAC |
| 18Q3a-cas9-F | taacgcggtaccAGCTCATAGCTTCAAAATGTTTCTACTCCTTTTTTACTC |
| 18Q3-cas9-R | gagctcgcatgcCCGCAAATTAAAGCCTTCGAGCGTCCCAAAAC |
| 18Q3-pSNR52-F | taatcgggcatgcgagctcTCTTTGAAAAGATAATGTATGATTATGCTTTC |
| 18Q0b-pSNR52-R | aaacaaatacatacattgtcttccGATCATTTATCTTTCACTGCGGAGAAGTTTCG |
| 18Q0b-gRNA-F | Cggaagacaatgtatgtatttgttttagagctagaaatagcaagttaaaataaggc |
| 18Q3-cyc1t-R | ggataagaatgcggccgcaaagccttcgagcgtcccaaaac |
| 20dTPI-leu2-F1 | CCTTTTCTGGCATCCAGTTTTgattcaagaaatatcttgaccgcag |
| 20dTPI1-leu2-F2 | CAGCTTCCTCTATTGATGTTACACCTGGACACCCCTTTTCTGGCATCCAGTTTTga |
| 20TPI1-leu2-F3 | TTGGTGGAAGATTACCCGTTCTAAGACTTTTCAGCTTCCTCTATTGATGTTACACC |
| 20dTPI1-leu2-R1 | AAAAAGCGCCTTGCTTTTTGTTtgcaccatatcgactacgtcgtaag |
| 20dTPI1-leu2-R2 | tTCAATTGTTAAATGCTTTTCTTCTTTTTATTAGAAAAAGCGCCTTGCTTTTTGTTtg |
| 20dTPI1-leu2-R3 | GTTGATATAGAGGTGTTCAATTGTTAAATGCTTTTCTTCTTTTTATTAGAAAAAGC |
| 20QRcT-F1 | acgtgcccgatcaactcgagtgccacctATCTTCAGTGGCATGTGAGATTCTCC |
| 20QRcT-F2 | cattatggtgaaagttggaacctcttacgtgcccgatcaactcgagtgcc |
| 20QRcT-F3 | caaaaaatacgcccggtagtgatcttatttcattatggtgaaagttggaacctc |
| 20QRcT-R1 | ttaatgtcatgataataatggtttcttGTAAATCTACCGTCCCTTACAAGAAC |
| 20QRcT-R2 | gatacgcctatttttataggttaatgtcatgataataatggtttcttGTAAATCtac |
| 20QRcF-R3 | ttttatctgaaattctgcctcgtgatacgcctatttttataggttaatgtcatg |
| 18Q0d-gRNA-F | GATCgaaaatcacgtaatacttctgttttagagctagaaatagcaagttaaaataaggc |
| 18Q0d-pSNR52-R | gctctaaaacagaagtattacgtgattttcGATCATTTATCTTTCACTGCGGAGAAG |
| 18Q4f0-D-R | CAGATCTCTAGACCATTTGACACTTGATTTGACACTTCTTTTTTTTTTTATTTATG |
| 18Q4f0-D-F | TCAATAGGATCCGTTTAAACGGAAGAAGATGTTATGAAGCTCG |
| 18Q4f0b-2μ-F | GTTTAAACGGATCCCGGATGAAAGGTAGTCTAGTACCTCCTGTG |
| 18Q4f0b-2μ-R | TTATAGCGGCCGCACTAGTTATGATCCAATATCAAAGGAAATGATAGCA |
| qALG9-F | CCAATTGTTTAATCCGGGCTG |
| qALG9-R | CAGTGGACAGATAGCGTAGAG |
| qRFP-F | GTTCATATGGAAGGTTCAGTTAATGGTC |
| qRFP-R | CAAGCAAATGGTAATGGACCACCT |
| qADS-F | GCAATCTTTGGCTAACGACGTTG |
| qADS-R | CAAAGCTGGGTTAGTAGAGAAAGCG |
| qCYP71AV1-F | AATTGCCACTATACTATCCCGTGC |
| qCYP71AV1-R | AGTGATGCAAGAACTTTTTAGATGGG |
| qDBR2-F | TGTGGTGGTTACACCAGAGAATTGG |
| qDBR2-R | GTGGGTGTAGAAGGTAGCTCTGTC |
| 18Q0l-gRNA-F | TCataatggtttcttagacgtcgttttagagctagaaatagcaagttaaaataagg |
| 18Q0l-pSNR52-R | gctctaaaacgacgtctaagaaaccattatGATCATTTATCTTTCACTGCGGAGAAG |