**Additional Information for Systematic Improvement of Isobutanol Production from D-xylose in Engineered *Saccharomyces cerevisiae***

Peerada Promdonkoy1, Wiparat Siripong1, Joe James Downes2,3, Sutipa Tanapongpipat1, and Weerawat Runguphan1

1National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

2University of Kent, Canterbury, Kent, UK

3 Present address: Syngenta, Jealott’s Hill International Research Station, Bracknell, Berkshire RG42 6EY, UK

Corresponding Author

Tel: +66 02-564-6700, Fax: +66 02-564-6701

Address: National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

E-mail: weerawat.run@biotec.or.th

**1. Primers used in this study**

**1.1 Primers for gene deletions**

**Primer for *GRE3* deletion**

Forward

5’ TAATATAAATCGTAAAGGAAAATTGGAAATTTTTTAAAGATGCATAGGCCACTAGTGGAT 3’

Reverse

5’ TGTTCATATCGTCGTTGAGTATGGATTTTACTGGCTGGATCACAGCTGAAGCTTCGTACG 3’

**Primer for *PHO13* deletion**

Forward

5’ TTATAGCTTGCCCTGACAAAGAATATACAACTCGGGAAAATGCATAGGCCACTAGTGGAT 3’

Reverse

5’ TCAAAAAGTAATTCTACCCCTAGATTTTGCATTGCTCCTCTACAGCTGAAGCTTCGTACG 3’

**1.2 Primers for plasmid construction**

Bold nucleotides designate the Kozak sequence; underlined nucleotides are restriction sites

**pRS416Tef1-SsXKS1**

S1. Forward

5’ ATAGGATCC**AAAACA**ATGACCACTACCCCATTTG 3’

S2. Reverse

5’ ATAGAATTCTTAGTGTTTCAATTCACTTTCC 3’

**pRS416Tef1-HXT7\_F79S**

S3. Forward

5’ GCAATCTAATCTAAGTTTTCTAGAACTAGTGGATCC**AAAACA**ATGTCACAAGACGCTGCT 3’

S4. Reverse

5’ CCAGTATCCCAACCGGAAACGAAACCACCAAAGG 3’

S5 Forward

5’ TTGGTGGTTTCGTTTCCGGTTGGGATACTGGTAC 3’

S6 Reverse 2

5’ GAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTTATTTGGTGCTGAACATTCT 3’

**pRS416Tef1-CtXYL1**

S7 Forward

5’ ATAGGATCC**AAAACA**ATGTTTAAATTTTTCACTTCTC

S8 Reverse

ATAGAATTCTTAAACAAAGATTGGAATGTTG

**pRS416Tef1-SsXYL1**

S9 Forward

5’ ATAGGA TCC**AAAACA**ATGCCTTCTATTAAGTTGAAC 3’

S10 Reverse

5’ ATACCCGGGTTAGACGAAGATAGGAATCTT 3’

**pRS416Tef1-CtXYL2**

S11 Forward

5’ ATATATCCGCGG**AAAACA**ATGACTGCAAACCCATCA 3’

S12 Reverse

5’ ATATATGAATTCCTATTCTGGACCATCAATTAAAC 3’

**pRS416Tef1-SsXYL2**

S13 Forward

5’ ATATATCCGCGG**AAAACA**ATGACTGCTAACCCTTCC 3’

S14 Reverse

5’ ATATATGAATTCTTACTCAGGGCCGTCAAT 3’

**pUG72-TDH3-LlkivDmit-2A-ScADH7mit**

S15 Forward

5’ ATACGACTCACTATAGGGAGACCGGCAGATCCGCGGGAGTTTATCATT 3’

S16 Reverse

5’ AGTGTACATTGTTTTCCGCGGTTTGTTTGTTTA 3’

S17 Forward

5’ GGTTCTTTGTTGACTTGTGGAGATGTTGAGGAGAATCCAGGACCAATGCTTTACCCAGAAAAATTT 3’

S18 Reverse

5’ GACGGTATCGATAAGCTTGATATCGAATTCCTATTTATGGAATTTCTTATCATA 3’

S19 Forward

5’ ACAAACAAACCGCGGAAAACAATGTACACTGTCGGA 3’

S20 Reverse

5’ CTCAACATCTCCACAAGTCAACAAAGAACCCCTTCCTTCTGCTCTGGATTTGTTCTGTTCTGCGAA 3’

S21 Reverse

5’ AAGTTCTTGTGGCTGGCTTGAAAAATCTTATAGATTGACGTAGTGAAAGCATTGTTTTCCGCGGTTT 3’

S22 Forward

5’ ATTTTTCAAGCCAGCCACAAGAACTTTGTGTAGCTCTAGATATCTGCTTTACACTGTCGGAGATTAC 3’

S23 Reverse

5’ aAGTTCTAGTAGCAGGCTTAAAGAAtCTTATAGATTGACGTAGTGAAAGCATTGGTCCTGGATTCTC 3’

S24 Forward

5’ ATTCTTTAAGCCTGCTACTAGAACTtTGTGTAGCTCTAGATATCTGCTTCTTTACCCAGAAAAATTTCAG 3’

**pRS416Tef1-ScIlv2-2A-ScIlv5-2A-ScIlv3-Ura3**

S25 Forward

5’ CTTGCTCATTAGAAAGAAAGCATAGCAATCTAATCTAAGTTTTCTAGAACTAGTGGATCCAAAACAATGATCA GACAATCTACGCTA 3’

S26 Reverse

5’ TGGTCCTGGATTCTCCTCAACATCTCCACAAGTCAACAAAGAACCCCTTCCTTCTGCTCTGTGCTTACCGCCTGTACG 3’

S27 Forward

5’ AGAGCAGAAGGAAGGGGTTCTTTGTTGACTTGTGGAGATGTTGAGGAGAATCCAGGACCATTGAGAACTCAAGCCGCC 3’

S28 Reverse

5’ AGGACCAGGGTTTTCTTCTACGTCACCGCATGTTAGTAGACTTCCTCTACCCTCAGCTCTTTGGTTTTCTGGTCTCAACTT 3’

S29 Forward

5’ AGAGCTGAGGGTAGAGGAAGTCTACTAACATGCGGTGACGTAGAAGAAAACCCTGGTCCTGGCTTGTTAACGAAAGTTGCT 3’

S30 Reverse

5’ GTGACATAACTAATTACATGACTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTCAAGCATCTAAAACACAACC 3’

**Plasmid pRSII416-TDH3-LlkivDmit-2A-ScADH7mit**

S31 Forward

5’ ATATGGGCCCGAGTTTATCATTATCAATACTGCC 3’

S32 Reverse

5’ ATATCCCGGGGGCCGCAAATTAAAGCCT 3’

**Plasmids pRSII426Tef1-ScHXT7\_F79S, pRSII426Tef1-SsXYL1, pRSII426Tef1-SsXYL2 and pRSII426Tef1-ScIlv2-2A-ScIlv5-2A-ScIlv3-Ura3**

S33 Forward

5’ ATATATGCGGCCGCATAGCTTCAAAATGTTTCTACTC 3’

S34 Reverse

5’ ATATATGAGCTCGGCCGCAAATTAAAGCCT 3’

**pRSII426Tef1-SsXKS**

S35 Forward

5’ ATATATCTGCAGATAGCTTCAAAATGTTTCTACTC 3’

S36 Reverse

5’ ATATATGCGGCCGCGGCCGCAAATTAAAGCCT 3’

**Plasmid pRSII426-TDH3-LlkivDmit-2A-ScADH7mit**

S37 Forward

5’ ATATATGCGGCCGCGAGTTTATCATTATCAATACTGCC 3’

**1.3 Primers for chromosomal integration**

**Integration into *ARS208* site**

Forward

5’ AGGGACCATACACACGTCCGCTAAACAAAAGATCTTGGGCATCAGCTGAAGCTTCGTACG 3’

Reverse

5’ CCCTCTTTTCCACCCCGTGAAAAACAGAATATCATTAACCGGACGACTCACTATAGGGCG 3’

**Integration into *YMRWΔ15* site**

Forward

5’ GATGACTGTTTCTCAAACTTTATGTCATTTTCTTACACCGCACAGCTGAAGCTTCGTACG 3’

Reverse

5’ ACCGCGAAGATTTATAATGGTTTATCGGTTGCATTTTCCATGACGACTCACTATAGGGCG 3’

**Integration into *ARS308* site**

Forward

5’ GAAATTTCAACATTAACTTCGAATTTTTTTCTTTTTATCTAACAGCTGAAGCTTCGTACG 3’

Reverse

5’ TAGAAGTGGTAGCAATATGTAGCAAAGAAGACAAGTAATCCTACGACTCACTATAGGGCG 3’

**Integration into *ARS720* site**

Forward

5’ GTTACTGTTGATTGTTCGTTTATTTGTATAATTGAGTTTACACAGCTGAAGCTTCGTACG 3’

Reverse

5’ ATTTATAAGTTTGCTTTTTGTCACTCTCTTGGCCCTAATTACACGACTCACTATAGGGCG 3’

**Integration into *ARS1309* site**

Forward

5’ ATTCTAGTATCAAAGAAACTTACTATGACGCAGTTTAGGATCCAGCTGAAGCTTCGTACG 3’

Reverse

5’ ACACTGAATAAACAAGGGGCTTTACGATGGAGTAGTAGACCTACGACTCACTATAGGGCG 3’

**Integration into *YORWΔ22* site**

Forward

5’ CACCGGAGCTTGGATATGATAAACGAAATATTCTTGAATCGTCCGCGGGAGTTTATCATT 3’

Reverse

5’ CGTGATAAACGATCGCCATAACTAACAGGTATAAATGGCAGCAAGCTTCGTACGCTGCAG 3’

**Table S1.** Plasmids generated in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Plasmid name | Overexpressed gene(s) | Promoter used for overexpression | Plasmid origin of replication | Selectable marker | Reference |
| pRSII416-SsXKS | *XKS* from *S. stipitis* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII416-SsXR | *XYL1* from *S. stipitis* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII416-SsXDH | *XYL2* from *S. stipitis* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII416-ScHXT7mut | *HXT7F79S* from *S. cerevisiae* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII416-LlkivD-T2A-ScADH7 | *LlkivD*\_mito from *Lactococcus lactis*, *ADH7* from *S. cerevisiae* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII416-ScIlv2-T2A-ScIlv5-T2A-ScIlv3 | *Ilv2*, *Ilv5* and *Ilv3* from *S. cerevisiae* | *TEF1* | CEN6/ARS4 | Ura3 | This study |
| pRSII426-SsXKS | *XKS* from *S. stipitis* | *TEF1* | 2μm | Ura3 | This study |
| pRSII426-SsXR | *XYL1* from *S. stipitis* | *TEF1* | 2μm | Ura3 | This study |
| pRSII426-SsXDH | *XYL2* from *S. stipitis* | *TEF1* | 2μm | Ura3 | This study |
| pRSII426-ScHXT7mut | *HXT7F79S* from *S. cerevisiae* | *TEF1* | 2μm | Ura3 | This study |
| pRSII426-LlkivD-T2A-ScADH7 | *LlkivD*\_mito from *Lactococcus lactis*, *ADH7* from *S. cerevisiae* | *TEF1* | 2μm | Ura3 | This study |
| pRSII426-ScIlv2-T2A-ScIlv5-T2A-ScIlv3 | *Ilv2*, *Ilv5* and *Ilv3* from *S. cerevisiae* | *TEF1* | 2μm | Ura3 | This study |