

Recombinant *Saccharomyces cerevisiae* strain development for rapid glucose and xylose co-fermentation

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Table S1. Primers used in this study

| Primer | Description | Sequence (5'→3') ^{a,b} |
|----------------------------|---|---|
| Episomal plasmid | | |
| pPYXp.pu | <i>PGK1</i> promoter (pPY1) | AGC <u>ACTAGTTATTTAGATTCCCTGACTTCAAC</u> |
| pPYXp.pd | <i>Orpinomyces</i> XI (pPYXo) | CCC <u>GAATTCTGTTTTATATTGTTGTAAAAAG</u> |
| XIou | <i>Piromyces</i> XI (pPYXp) | TTAG <u>AATTCAAAATGACAAAGGAATACTT</u> CCCA |
| XIod | <i>Piromyces</i> XI (pPYXpXp) | AAT <u>CTCGAGTTATTGGTACATAGCAACAA</u> TTG |
| pPYXp.xu | <i>Orpnomyces</i> XI (pPYXoXo) | CCCG <u>AATTCAAAATGGCTAAGGAATATT</u> CCCAC |
| pPYXp.xd | URA3 | ACGT <u>TCTAGATTATTGGTACATGGCAACAA</u> TAGCT |
| PGK | NTS2-2 upstream partial(pNTS-1) | AAT <u>ACTAGTTATTTAGATTCCCTGACTTCA</u> AC |
| Cyc1TT | NTS2-2 downstream partial(pNTS-2) | TTAA <u>ACTAGTGCAAATTAAAGCCTTCGAGC</u> GTCCA |
| pPYXpXo.pgku NaeI | pNTS | AAGCCGGCTATTTAGATTCCCTGAC |
| pPYXpXo.cyc1tt d | LoxP-KanMX- LoxP (XoNK) | AA <u>ACTAGTCCGCAAATTAAAGCCTT</u> |
| Integrating plasmid | | |
| xpxpura3un | Orpinomyces XI (XoNK) | TTT <u>GGATCCAGCTTTCAATTCATCTTT</u> |
| xxura3dn | URA3-pNTS (XXUN) | ccttgaaactacctc <u>GGGTAATAACTGATATAATT</u> |
| xpxpnts2un | <i>Piromyces</i> XI | gaggtagttcaagg <u>TGACA</u> |

| | | |
|-------------------|-----------------------|--|
| Xpxpnts2-21d | <i>Orpinomyces XI</i> | ttaaatgtaaaatgGCCTATCGG |
| XpXpnts2-22u | <i>ACT1</i> | cattttacattaaa <u>TGAAGGGAGGATAGTAGTAAA</u> G |
| XpXpnts2dn | | ATAGTCGACTTTCCAAAGTGACAGGTGC |
| XoUNNTS2-2u | | AACATATGGAGGTAGTTCAAGGTGACAG |
| XoUNNTS2-2d | | tttccaaagtgacagGTGCC |
| XoUNKanMXu | | ctgtcacttgaaaa <u>CAGCTGAAGCTCGTACGCT</u> |
| XoUNKanMXd | | AAGAATTGCATAGGCCACTAGTGGATCT G |
| XoUN pXIqcu | | AAGGATCCTATTTAGATTCCCTGACT |
| XoUN pXIqcd | | AAC <u>CTGCAGGCCGCAAATTAAAGCCTTCG</u> A |
| xpxpura3un | | TTT <u>GCCGGCAGCTTTCAATTCATCTTT</u> |
| xpxpnts2dn | | ATAGAT <u>ATCTTTCCAAAGTGACAGGTGC</u> |
| XI qRT-PCR | | |
| qpcrXIpirou | | ATGGATGGTCACTTACCATACT |
| qpcrXIpirod | | CACATAGCGAACCGTAACCAAT |
| qpcrXIoriu | | ATGACAAAGGAATACTTCCAAC |
| qpcrXIorid | | ACCATGCCATAGCGAATCTAAC |
| qpcrACT1u | | ATGGATTCTGGTATGTTCTAGC |
| qpcrACT1d | | TTACAGATCAGTCAATATAGGAG |

^a Restriction endonuclease sites in primer overhangs (if any) were underlined.

^b The bases written in lowercase are the recombinant sequences involved in the construction of integrating plasmid

36a₂XpXpUN



39a₂XoNK



Figure S1. Drop-plate experiments on YP agar plates containing 20 g L⁻¹ xylose. Cells were incubated at 30°C for 3 days.