Electronic Supplementary Material

Inter-chromosomal insertions into wild-type chromosomes induced by SCRaMbLE

Sijie Zhou[1,2]*, Junyanrui Li[1,2]*, Xichen Cui[1,2], Ying Wang[1,2], Ying-Jin Yuan (✉)[1,2]

1 Frontiers Science Center for Synthetic Biology and Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

2 Frontiers Research Institute for Synthetic Biology, Tianjin University, Tianjin 300072, China

E-mail: yjyuan@tju.edu.cn

*These authors contributed equally to this work.

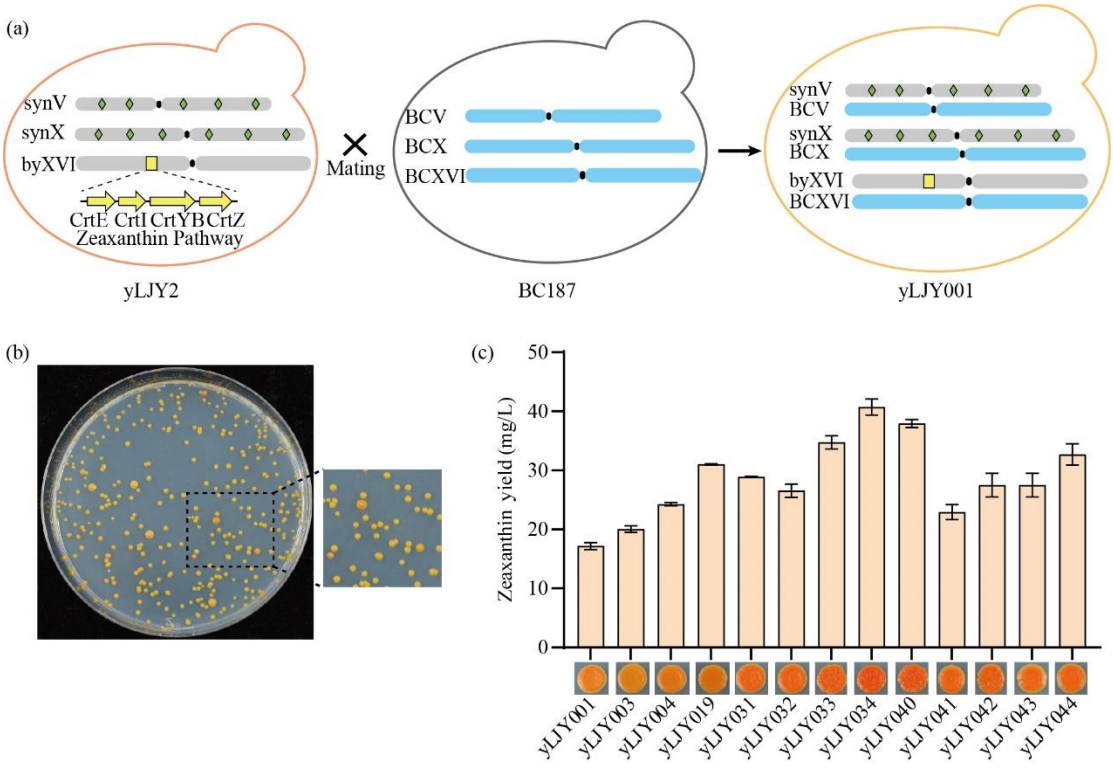

Fig. S1 SCRaMbLE of heterozygous diploid yeast with two synthetic chromosomes for improving the biosynthesis of zeaxanthin. (a) Schematic diagram illustrating the formation of heterozygous diploid strain yLJY001. The zeaxanthin pathway was integrated at the *YPL062W* gene locus on chromosome

XVI of the haploid synV&X strain. Previous study has reported that knocking out of the *YPL062W* gene is beneficial to produce lycopene, a precursor of zeaxanthin [1]. (b) The SCRaMbLEd library of heterozygous diploid yeast was plated on SC-Leu agar plates and incubated at 30 °C for 72 h. (c) Production verification of SCRaMbLEd strains was conducted using HPLC method, with yLJY001 serving as the control strain.

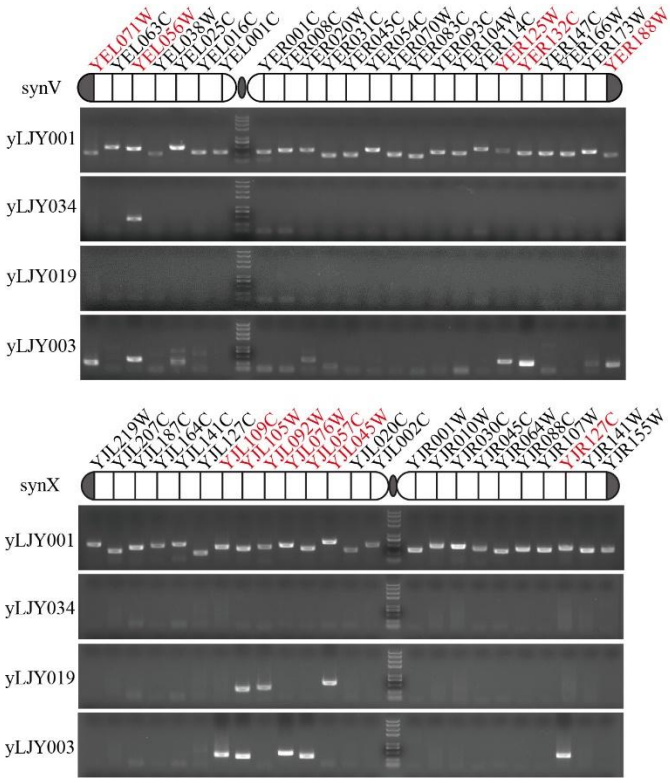

Fig. S2 PCRTag analysis. PCRTags of genes *YEL071W*, *YEL056W*, *YER125W*, *YER132C*, *YER188W*, *YJL109C*, *YJL105W*, *YJL092W*, *YJL076W*, *YJL057C*, *YJL045W*, and *YJR127C* are marked in red, indicating that these regions are still present in the strains yLJY034, yLJY019, and yLJY003 respectively.

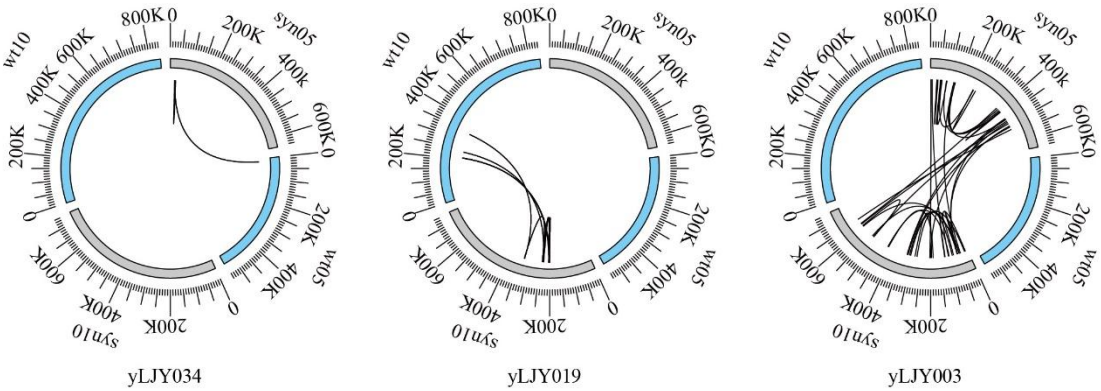

Fig. S3 CIRCOS diagrams for SVs in SCRaMbLEd strains yLJY034, yLJY019, and yLJY003. Each line in the diagram represents an interaction between partners.

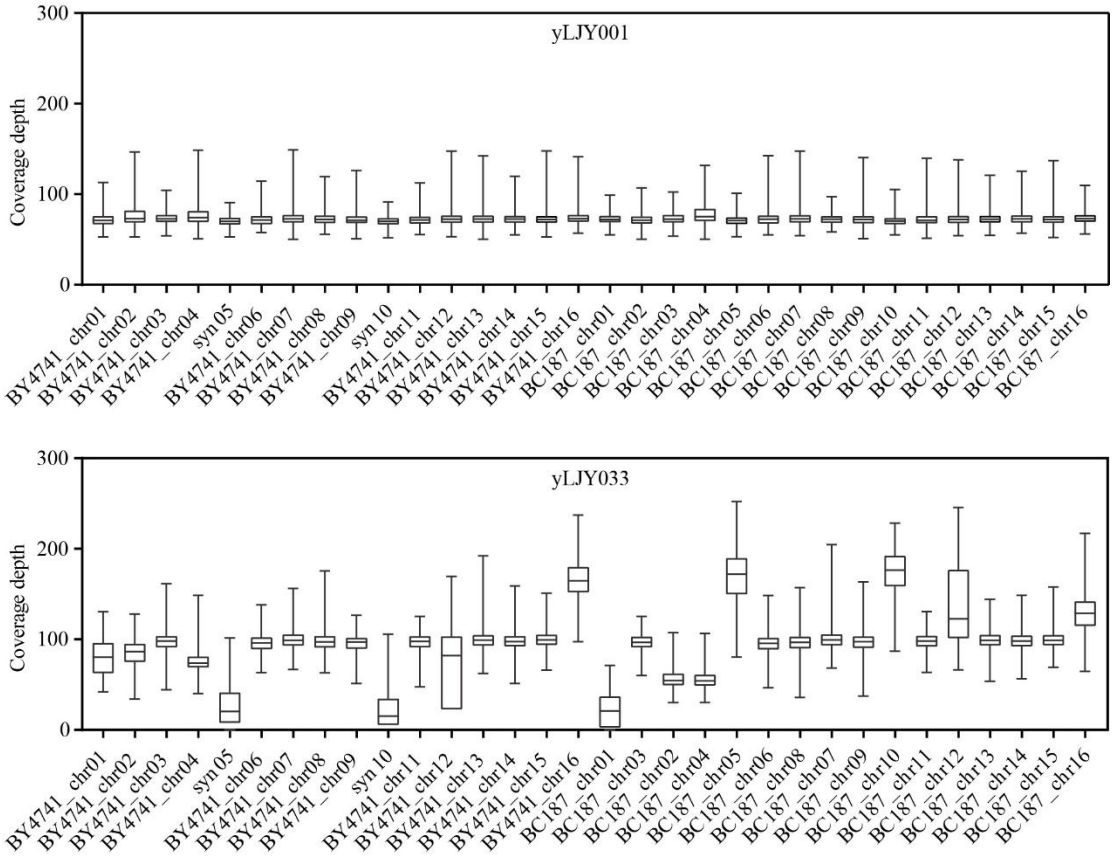

Fig. S4 Boxplots of whole genome sequencing read coverage depth. Loss of heterozygosity was observed in chromosomes V, X, and XII of SCRaMbLEd strain yLJY033. Aneuploidy of chromosomes I and XVI was also detected in SCRaMbLEd strain yLJY033. Strain yLJY001 is control strain. The coverage depth represents the chromosome copy number determined from WGS results. The center line in each box indicates the median read coverage depth, the upper bound of the box indicates the upper quartile, the lower bound indicates the lower quartile, the upper whisker indicates the maximum read coverage depth and the lower whisker indicates the minimum read coverage depth.

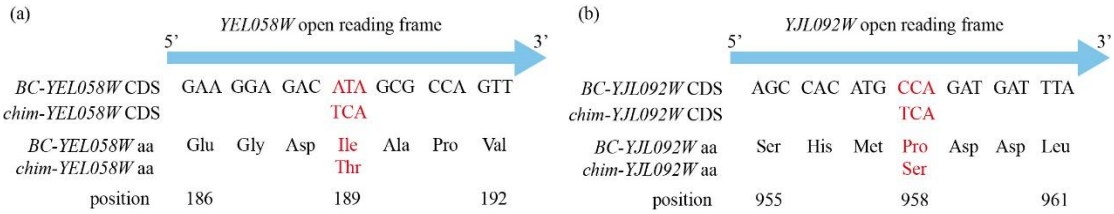

Fig. S5 Missense mutations in the chimeric gene. (a) Sequence alignment revealed that *chim-YEL058W* had a missense mutation Ile189Thr compared to *BC-YEL058*. (b) Sequence alignment revealed that *chim-YJL092W* had a missense mutation Pro958Ser compared to *BC-YJL092W*.

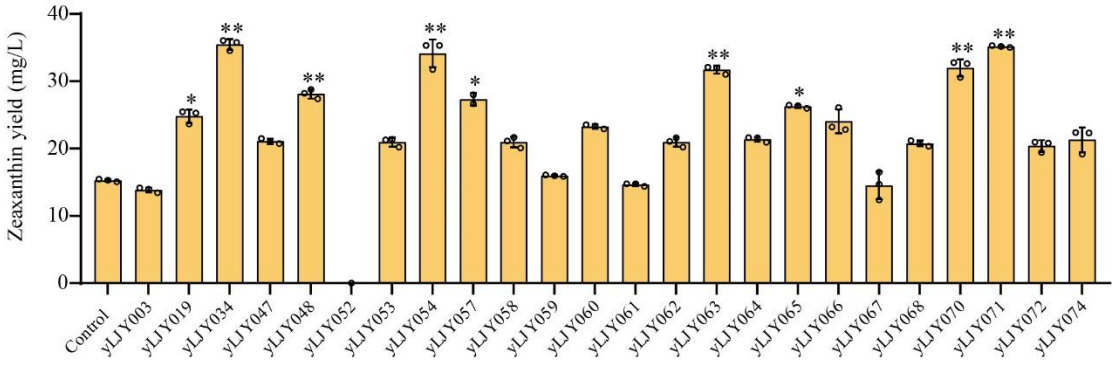

Fig. S6 HPLC was utilized to measure zeaxanthin production in SCRaMbLEd strains with insertion events. A total of 24 SCRaMbLEd strains (as indicated in Fig. 5) were evaluated for zeaxanthin production. Quantification was conducted in biological triplicates for each strain, as depicted. The error bars denote the standard deviation from three replicates. Unpaired test, **P* < 0.01, ***P* <0.001.

Table S1 Yeast strains used in this study.

Strains	Description	Sources
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[2]
synV&X	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[3]
BC187	*MATα ura3*::*KanMX*	[4]
yLJY1	synV&X, *YPL062W*::zeaxanthin pathway with *Leu2* marker	This study
yLJY001	Diploid of BC187 and yLJY1	This study
yLJY002	Introducing plasmid pLJY01 into strain yLJY001	This study
yLJY003	SCRaMbLEd strain from the yLJY002	This study
yLJY004	SCRaMbLEd strain from the yLJY002	This study
yLJY019	SCRaMbLEd strain from the yLJY002	This study
yLJY031	SCRaMbLEd strain from the yLJY002	This study
yLJY032	SCRaMbLEd strain from the yLJY002	This study
yLJY033	SCRaMbLEd strain from the yLJY002	This study
yLJY034	SCRaMbLEd strain from the yLJY002	This study
yLJY038	yLJY001 synV centromere with *Ura3* marker strain	This study
yLJY039	Introducing plasmid pLJY02 into strain yLJY038	This study
yLJY040	SCRaMbLEd strain from the yLJY002	This study
yLJY041	SCRaMbLEd strain from the yLJY002	This study
yLJY042	SCRaMbLEd strain from the yLJY002	This study
yLJY043	SCRaMbLEd strain from the yLJY002	This study
yLJY044	SCRaMbLEd strain from the yLJY002	This study
yLJY045	SCRaMbLEd strain from the yLJY038	This study
yLJY046	SCRaMbLEd strain from the yLJY038	This study
yLJY047	SCRaMbLEd strain from the yLJY038	This study
yLJY048	SCRaMbLEd strain from the yLJY038	This study
yLJY049	SCRaMbLEd strain from the yLJY038	This study
yLJY050	SCRaMbLEd strain from the yLJY038	This study
yLJY051	SCRaMbLEd strain from the yLJY038	This study
yLJY052	SCRaMbLEd strain from the yLJY038	This study
yLJY053	SCRaMbLEd strain from the yLJY038	This study
yLJY054	SCRaMbLEd strain from the yLJY038	This study
yLJY055	SCRaMbLEd strain from the yLJY038	This study
yLJY056	SCRaMbLEd strain from the yLJY038	This study
yLJY057	SCRaMbLEd strain from the yLJY038	This study
yLJY058	SCRaMbLEd strain from the yLJY038	This study
yLJY059	SCRaMbLEd strain from the yLJY038	This study
yLJY060	SCRaMbLEd strain from the yLJY038	This study
yLJY061	SCRaMbLEd strain from the yLJY038	This study
yLJY062	SCRaMbLEd strain from the yLJY038	This study
yLJY063	SCRaMbLEd strain from the yLJY038	This study
yLJY064	SCRaMbLEd strain from the yLJY038	This study
yLJY065	SCRaMbLEd strain from the yLJY038	This study
yLJY066	SCRaMbLEd strain from the yLJY038	This study

yLJY067	SCRaMbLEd strain from the yLJY038	This study
yLJY068	SCRaMbLEd strain from the yLJY038	This study
yLJY069	SCRaMbLEd strain from the yLJY038	This study
yLJY070	SCRaMbLEd strain from the yLJY038	This study
yLJY071	SCRaMbLEd strain from the yLJY038	This study
yLJY072	SCRaMbLEd strain from the yLJY038	This study
yLJY073	SCRaMbLEd strain from the yLJY038	This study
yLJY074	SCRaMbLEd strain from the yLJY038	This study

Table S2 Plasmids used in this study.

Plasmid	Description	Sources
pZea	pRS416, pFBA1-crtE-tFBA1-pTEF1-crtI-tTEF1-leu-pGPD-crtYB-tGPD-pTDH1-crtZ-tGPD	This study
pLJY01	pRS416, pGAL1-CreEBD-tTDH2	[5]
pLJY02	pRS413, pGAL1-CreEBD-tTDH2-hygB	This study

Table S3 48 pairs of synV and synX PCRTags used in this study.

PCRTag	syn-F (5'-3')	syn-R (5'-3')
YEL071W1	CAGCGGTAGTAACAAACGTCATGATGAC	ATTCAAACGCTCGGTAACGGCAGCGCTA
YEL063C2	ATCACGACGGTCACTGTCAATATCAACG	TACTAAGGGCGGCGTCCCTTATATCGCT
YEL056W1	CTCACACCACAGTAGCAATTTGTTGGCT	GCCACCAACAATTGGCAATGAGTGACTG
YEL038W1	TCCAGCTCACGACAGCTTAGACTTAAAC	AACTGGAGCGTTGCCTGGTCTACTG
YEL025C4	CAAAGCAGGAAAACTGTAAGCCTCCAAG	CGTCCCTAACAATTTGAACCAGGCTGTT
YEL016C1	TGAATGACCATAGCTGTCGACGTTTGGG	TAATACCGCCGATCCAATCTGGCAATTG
YEL001C1	AAACAAAGTAGGAGGAGCAGCGATACGA	TGAGGACACTGCCACTTTGGCTTACAAC
YER001W1	GGTTGCTGGCAGAGGCATCGTTTTATCA	CTTGTATGAGCCAAAATCGCCTCTCCAT
YER008C4	GCCGGTTTTGTGATCTGGACGACTGAAA	TTCAGCCCATCACAGCCGTAATGTTTCA
YER020W1	TCAACCTAGCTTAAGCGATGCCAGCTCA	GCTTGGTAAAGCCCATAAGGTGCTGATA
YER031C1	TGAGATGGTAGGACCGTTTGGGGCACTA	TCCAACCGAGGAGAGTAAAACCTTCGCT
YER045C1	ACTGTCACCGCTAGGAATGCTTGGG	CCCTTCAGCTGCTATCTATCCTTCATTC
YER054C2	GCTCTTGCTAGGAGGCATATCAGTTCTA	CAGATCAGGTAACGGCGTTCAAGCTCGT
YER070W3	GCGTCCAGGCGCTTTCGCTTTGTATTTA	CTTCTCATATCTAGTGTACAAGGCCTCG
YER083C1	TGACAACAAGCTCAACAAGTCTGGGGTT	TGCTGAAAGCCCATTGGATGCCCCATCA
YER093C1	TGAACTGGTGGTCAAGCTACGTTGTCTC	AAGCCCAACTTCACCATTGATGGCTCGT
YER104W1	TATCGATACCAGTCCAAGCGTTGTCAGC	GTATTCGGCCATATCGCTTGGCAAGGCA
YER114C3	TGAGGCACGGTTTAAACTTCTACCGTTG	TGATAGCATCGGCCCTCCTGACAGATTT
YER125W1	CAGTTCAGCCACTCGTCAATATAGCAGC	ACCTTCAGGGGTGAATCTCTGCTCCCAA
YER132C5	GATCTTGCTGCTACCTTTGCTGGCAGGA	TATCTTTGGCGGTTTGATGGTTAGCCCA
YER147C1	CTTATCACCGCTGCATCTTGAAACACGA	CGTCAGCATGAACGAGTTAGATAGCAGA

YER166W3	CAGACAGTCAGGCTCAAACTCAGAAGCC	AGCCTCAGCCAATAACTCGATGCAGTCA
YER173W2	GGTCGAGAATGGTATTGATGTCGTTGAC	GCTCAAACTCTCTGAAATGACAGGAGCC
YER188W1	TCGTAGAAGCGACGCTTTGGGTGTTACC	GTTCCAACACAACATCTGACCAGGTCTG
YJL219W11	TTTAGACGCTACCGAACCTCCAATCGAT	TAAAGTACCACGAATTTGCTTTGGGGCG
YJL207C3	GACATCGCTGTCTAAGGTACAACCCAAA	CTTAGACGCTTGGGCCGAGGTTGTT
YJL186W2	ATTGCGTTTACCAGTCTTGACCAGAAGC	GTAAGCCAAAACGCTGTATTTACCGCCA
YJL164C1	TCTAAACAAGTCGGCGTAAGGATCTTCG	TTATATCGCTCCAGAGGTTGTCTCAACC
YJL141C1	TGACTTGGTAACTGAAACCAATGGGCTG	CCCAGCCTATACTTCAAGCAACACCTCA
YJL127C1	CAAAGGGGTTGACTGGATGCTACTCAAG	ACACGACAACGCTTTGTTAGGTCAGGCT
YJL109C4	GCCAACGGTGTCATAGCTGCTTTCAACA	CCCAAGCCCATACGCTAAAACCGTTTTA
YJL105W1	CGGCTGTATTTGTGGCAGCTCAGATAGT	GGTAGGAGCAGTGTAGTGCAATTGTCTC
YJL092W2	TAGTAACAAGAGCGGTCAGAGCTTGACC	AGCAGCATCGGCTAAGAATTTGGCGTTT
YJL076W4	CCCACCTCCAAGCAGCAAAATTGTTAGT	AGGCTCTGGAATCTTTAACATACCGCTG
YJL057C2	TAAGCTCATGTAGCTCAACCAGCTACCG	AACCGCTAAAACCGTTGAGGAGACCTTG
YJL045W1	TGGCCGTGCCTATTTTAGCTGTACCAGC	GGTAGGTAAAACTGGGATAGGCTCCTGA
YJL020C2	TAAGTTTGAGATGGCTGGTGGAGCTGAC	ACCACCTCCTCACCAAAACGTTACCGCT
YJL002C1	GACAAACTTCTCATCGCTACCGCTACTA	TAGCTTCGAGTTCGGTCCATGGGAAGAC
YJR0012	CAGCTTAAGACGTGCTAACAGCTTCAGA	ACCAGCACCGCCTGACATCATTTCG
YJR010W2	CGGTTTCAGCCCATTAACCGGTTTCTTA	TAACTCTCTATGAGCACGGTGCATAGGG
YJR030C1	AGTGCTTGGGCTCAAACTGCTGGTCTGA	CCAACAAAGCGTCACTCCAGTTAGCGCT
YJR045C2	ATTTTCACTTAAGCCGCTGCTGCCAGCG	TTTAAGTGGCGAGGTCACCGATGTTTTG
YJR064W2	CAGTACCAATGGCAGAATCGTCCCTCGT	TCTTTGTTTGTCGGCTTCCTCGCTAACG

YJR088C1	AGCGTACCACCATAATTCAGCGTCCAAA	TGGTGATAGCGCTTTGACCGAAGGTAAT
YJR107W1	ATTAGGTGCTGCTTTGGCTTCATTAGCC	TAAGCCTGACATGCCCTCTCTGAAACCG
YJR127C4	GCTTGGGATGGTGTTTGGAAATGAGCTA	TGCCCCACACCAAGTTGGTTTCAGC
YJR141W1	TTCAAGCATTATGAGCTTGCCTGACGGT	GGTAACTTGACCCAAAACAGCGCTACAT
YJR155W1	CATGGGTGGTGGTCGTTTCCAGTCAAAG	GCTTGGCTTTTTAGTGACGGCAGGATCG

Table S4 192 pairs of synV and synX PCRTags used in this study.

PCRTag	syn-F (5'-3')	syn-R (5'-3')
YEL071W1	CAGCGGTAGTAACAAACGTCATGATGAC	ATTCAAACGCTCGGTAACGGCAGCGCTA
YEL067C1	GACGGCGCTAACGAAGATAGTGCTACAT	TTGCAGCAGAAGATTCCCACCAGACAAT
YEL064C1	TGAAAAGATGGCACGCAAAACATGTGGG	TGCTTTGGGTTTCATCGTCGACTGGACC
YEL061C1	ACCATCGAAACCGGTGTTTGAGCTACCA	CGAAGTTGCTGGTCCTTTGTTTCAGGAC
YEL058W2	TGTCGGTCGTGATAGCAGAGAATCATCA	CTTTAACTGAGGGCCGCCAATGCCGTTA
YEL055C2	ATCAGCCAAAGGTCTGTCCAAGGTTTCG	CAAGGAGGCTACCTCAGCTTTGGTTAAA
YEL052W1	CGGCTTGAAGAGTGTTTTCTCAAGAGGT	AGCAACATCGGTAACTTGGAACTCATCG
YEL050C1	TAACAAAGCAATGTGGCTACTACGGCCA	TAGCCCAGGCTTACGTTGGTATAGAAGT
YEL047C1	CTTCCATGAGCTTGATCTATCGTTAGGG	CCAATTAGGCGGTCATAGCGTTGCTCGT
YEL044W1	CGGTTTCCCAAGCCGTTTTAAAAGCGCT	AGCACCACGTAACTTCAAATACTCCTGG
YEL042W1	CAGTGTCGGCGCTGCCAATAGTTTG	ACCCATAATACTGACGCCGTCGCCTTCA
YEL040W1	AAGCACCGCTACTAGCAGTAGTAAGACC	GCCAGCATTGTTGCCTGACATGCTTGAG
YEL036C2	GAAACCATGTCTGTCGCTGAAACTCTGG	CGGCTGGCAACAGGGCGATAGA
YEL032W3	CAGCAGTGGCGTTGGCTTAACTGCT	AACGTCATACTGACCGAAGACTGGGTTA
YEL030W1	TGCTCGTATCACTGCTAGCGACATCAGC	AACCAACTCTCTCTCGCCTTGAAAGACC
YEL025C4	CAAAGCAGGAAAACTGTAAGCC	CGTCCCTAACAATTTGAACCAGG

YEL022W1	TCCAAGCCACAGCGAGAAATCAACCAACGGTGCT	CTGTTAGGATACAACTGTAATCTGTCGCCGCTG
YEL020C2	ACTCAAATCGGCGCCTGGGCTAACGTTA	CTTGCCAACTCCTATGGCCAAAGGTATC
YEL016C1	TGAATGACCATAGCTGTCGACGTTTGGG	TAATACCGCCGATCCAATCTGGCAATTG
YEL015W1	AGTCTTGACCGTCGCTAGCCAAAGTGGT	GTCTTGTTGTGGCTGGCTTTGATGGGTA
YEL011W1	CTACGAAGCTCATGTTGGCATCAGCTCA	TAAACCGTCCTCAACATTCTTGCTGGCG
YEL007W2	CAGCAGCGCCAGCGCTTATAAAAGAGCT	TGGTAATGGGTGTTGATGCTGATGTTGG
YER011W1	TCCTTCAAGCAGCGCCGCTCCT	GCCGTCAGTGATTTGGCTAATAGCGCTA
YER013W2	CGGTCACGATGTCGGTTACACTATCCGT	GGCAGCCTCGATATAGTCCATTTGTGGG
YER016W1	CAGCAGCTTAGGTATCAATGGCTCACGT	GCCGTTAACCTCACCGTTTGAATTAGCG
YER019W1	GGCTCCTCACAACAGTAAATCATTGGGT	ACCGTCCTCATGGTTGATTTTAGCTTCG
YER023W1	CAGTTTAGCCGCCGGTTGGACC	AACACATAAACCAGCGATGGTGGTGCCG
YER025W1	TAGACATGTCAGCTTCGTCGATTGCCCA	CTTTAAATCCTCAATTTCGGCGCCAGGT
YER027C1	TGAGCTTGATGAGGCGTCAATGTCGTCG	CAACGAGGCCAGTTTAGCTTATACCTTC
YER032W1	ACGTAGCCCATTGAGATTTACCAGCAGC	CAATGGCTTAAAAGGCTCGATGCTAGGG
YER033C1	GTTTTTACTACCACCTCTCAATGGGCTC	ACACAGCCAGCAGCCACATTACGCT
YER038C1	ACCTTGGCTACGTGATGAGCCGAATAAA	CACCATTACCACCACCGTTGCTAAATTG
YER041W1	CGGTCCTTCAAGCATCACCAGTCATTCA	ATGCATAATAGCAACGGTGCTAGGCCAA
YER043C1	GCCATCAACCAAACTTTCACGGCAACCA	ATTAAAGGGTGCTAGAATCGCCGGCTGC
YER045C1	ACTGTCACCGCTAGGAATGCTTGGG	CCCTTCAGCTGCTATCTATCCTTCATTC
YER047C1	TGAACTTAAACTGCTCCACTGGACCAAG	TATGTTGTTGTTCGGTCCTCCTGGCACC
YER049W2	CGGCGATTTAGCTAACTTAAGCGGCTTA	CTTCCACTTACGATCTGGGTCAGGCAAA
YER051W1	CTCAGGTGTTTACGTTCCAAACG	GTTCTCCTCAGCTTTCTCACCGTT

	TTGGT	AACC
YER053C1	ACGCTTGCTAGCAACGCTCATAC TCTCA	TAAGCAGCAGACCACCATGCCAC CATTC
YER054C1	GCTCTTGCTAGGAGGCATATCAG TTCTA	CAGATCAGGTAACGGCGTTCAAG CTCGT
YER056C2	AGTGCCGCCGATGATAATTAAGC AGCCA	TTTCAGCGTTTTCGGCGCTGAATT AGGT
YER059W1	GATCTTGGATGGCGATACCTCAA ATAGC	TGGGGTGGTGGTAACTGAGTTAC AACGT
YER060W2	TTTCAGCGTCTTTGGCGCTGAGT TAGGT	AGTGTAATCGGCAGCGTAGGTGG TCCAA
YER060W-A1	CTTCTTCAGTGTTGTTGCTGGCTT GGCT	GGCAACGGTATACATACCTGGAA CGTTG
YER061C1	ACTACGACCAGGCAACAAAGCG CTAGCA	AACCGCTTGTGCTACCGGCAACA ATAGT
YER063W1	TAGCGAACCAGCTGCTATCGAAG AACCT	GCTATCGATGTCAGCTTGGTCTTG GCCA
YER064C1	GTCAGCGCTGGTTGAGCTTCTGG TA	CGGTTTCGACGCTAGCTTAGCTC CTATT
YER065C1	TCTGCTAAAATTGTGAACGGCCA AGGCA	TATGAGAGCTAGAGCTTTCGCCC CTTAC
YER066W1	CCATCGTGCTTTGGTTGGCTTGTT AGGT	GGTGTCGCTTCTAACCAATAAAC CTGAG
YER069W1	CGATACCGCCAGCACTTTGAATA GCAGC	TGGCAAAGCCATGACCCAGAAAT CAACG
YER070W1	GCGTCCAGGCGCTTTCGCTTTGT ATTTA	CTTCTCATATCTAGTGTACAAGGC CTCG
YER073W1	CGGTGGTGCTCGTCACGGTTCA	GATGCCGCTCTGACCAAAACCGC CAAAT
YER075C1	ACTGTCGCTTTCCAATGACAACA TGCTC	TAGTGTCATCCCAGAATGGTTTC AGCAC
YER078C1	TAAGCTGTTGCTGCCGGTGGCAA CAACT	AGACGCTTTCGCCGAAAAATGGG AAGGT
YER081W1	CGATTTGGATTATGCCACTTCACG TGGC	GTCTTTCATGGCGGCGAATTGAG GAGCT
YER082C1	GCTCCACAAACTAACGGTGCCAT TGCTG	CCACTTGTTAATCACCGGTAGAA AGGGC
YER086W1	TAGCCAAGGCGTTGGCTTAAGCA GTAGA	TCTCTCTTCGGCTAACTTAGCGCA TTCG
YER087W1	TCAATGGCTACCTTTGGGTTTGC GTAGC	GTCAGCCCAAGCTGAGACGAATG GAATC
YER089C1	ATCAGCGAAGCTTCTAACGCTGG TTCTA	CCCAGATATCTTGGAGCACAGCT TGGAC
YER091C1	GATAGGAGGACGAACGTAACGA	TCCTACCACCACCATCGGCAGTT

	CTGCCA	TTCCA
YER093C3	AAAGCTGTGGCTGTGACGACCTC TAGGT	TAGAGGCATGCGTGTTATTGAGG GTTTC
YER096W1	CAACCCTTCAGGTAGCGGTAGCA GCAAT	ATAAGCATCGCCCAACAAATACT GAGCG
YER098W1	TGATCCAAGTATCGCTAAGAGCC CTTCA	AGCTCTGTCTTCGTAGGTGGTGC TACTG
YER099C1	GACGTCGCCAACCAATAACATTC TGCTG	CGCTAACTTGTTAGAAACCGCTG GCTGC
YER101C1	GCTATGTGATAAAATGTCGTAGCC GCCA	CGGCGCTGCTGTTTTAAGCGAAC ACTTT
YER103W1	CGAAAGAGCTAAACGTACCTTAA GCAGC	GTAGGCGACAGCCTCGTCTGGAT TGATA
YER105C1	TGGAACTGAACCTGATTCACGGC TTGAC	CCCACAAGGCTATGCTAACGTTT TCGCT
YER109C1	AGCGATTGACAAAGGACTGCTAG CGCTG	AGCCCCACCAACCAAGACCGCT
YER110C1	ATCGTCAATATGACCGCTCAAGC TGCTA	TTGGAATGCTATCGACGAAAGTA CCCGT
YER111C1	GCTGCTGGTGTTACCAGCTGAAA ATGGG	TATCGTCCCTGATGGTCCAATGCA ATCA
YER113C1	ACCAACGCTGTTAGCCAATGACA TCCAG	CCACTGCCCAGGTGCTAGCAAAA ATTAT
YER116C1	CTTTGAACGGCACAAGGCACAG TGACCA	CATGCACACTGAAGAGCCTGAAG CTTCA
YER119C1	AAAGCTGATGCTGGTACTACCGG TAGCA	CGCCACTGAATCAAGCCCATTAA TCAGA
YER122C1	ACGTGAGGCGGTCAAAATACTTG AACGG	AAGCGATAGCCCATTAGATACTG ACAGC
YER125W1	CAGTTCAGCCACTCGTCAATATA GCAGC	ACCTTCAGGGGTGAATCTCTGCT CCCAA
YER129W1	ATTGGCCGCCTCATCAACCAACT CAAGT	TGGAACATGATCGAAGTCCCAGC TGACG
YER132C1	TGATGAACGACGGCTTGGAGCCT TAGGA	CAGACGTGCTAGCCATCCATTGC AAAGC
YER136W1	TCAATCAGTCGCTAGATATGGCA AGAGC	ATGGCCTTTACTACAGACATTGTG AGCG
YER141W1	TAGACCTTTCAGCTTGAGTAGTC CAGTC	ACCAATGGCTCTGCCCCATAAAC GG
YER144C1	AGTGCTTGGGGTGTTCAACTTGC TCAAG	CAGCGTTATCAAGCCATTATCAGG TACC
YER148W1	TGGCGCTAAGTCAGAGGACGATA GCAAA	GCCTGGGAATAATTCAGGCTCGT AACTT
YER151C1	GCAACTGCTGGTACCAACACGGC	CATCGAGCCTTTAGGCAGCATCG

	TGTTA	CTTTG
YER153C1	CAAAACGCCGGCTAACAACAATGGCAAA	CGCTTTGGCTCACGCTGATACCATC
YER155C1	ACTGAAACTAGCTCTGCTGTCACGACGG	TATCAGCGAGCGTTTCCAAGAGCAGGGT
YER158C1	GGTGGTAGTGCTCAAGCTGCTAGGAACT	TGTCAATAACCATGAAACCGCCGTTCCA
YER161C1	TGGGCCGTTTGAATGCTTGCTCTTTGAA	AAGAAGCATCGGTGCTTCACACGCTCCT
YER163C1	GCTGGTCAACAAAACTCTCTTGCCGCTC	AGCTAATCCTGGTCGTGTTGCTACCTTG
YER166W1	CAGACAGTCAGGCTCAAACTCAGAAGCC	AGCCTCAGCCAATAACTCGATGCAGTCA
YER170W1	TCGTTACGTCCATGTCCCATCAGGTCGT	GGTTTCACCGCTAACAGTGCCGAAGATA
YER174C1	GTCGTCACTGCTACCTGATGATTCCTCG	CCCATGTAAGACCATGTCACAGGTTTTG
YER176W1	CGAGAGACCTAGCACCTCATATAGCTTG	AGCAGCGACAACAGGGTCTGATTCAACA
YER178W1	TGCCAGCCGTAGTAGTGCTATGACC	ATCATAGGCCTTAACTTCGGCTTCGGTA
YER180C1	TCTTTCAAAGACTGGGTAGGTACCGGCG	TTTGGATTACATCCCAGACAGTCCTAGC
YER182W1	CGGTGGTTCATTCTTGGGTGGTTGGTAT	AACACCGCCAGGGGTGTTCAAAGCTCTA
YER184C1	GTGTGAGGCGCTTGAGGCATCCAAGAAT	TGCTAGCTTGCAGAAGGGTTTGGCTAAT
YER188W1	TCGTAGAAGCGACGCTTTGGGTGTTACC	GTTCCAACACAACATCTGACCAGGTCTG
YJL219W1	TTTAGACGCTACCGAACCTCCAATCGAT	TAAAGTACCACGAATTTGCTTTGGGGCG
YJL217W1	CAGCGACGGTCATGCTATGTTGTCATCA	GACCTTTAAACCACCTCTCTCTGGGGTA
YJL214W1	CAGTATCTCAGTCTTGGCTCCAATGTTG	AACTAAGAATCTTGGGCTTTCTGGGACG
YJL212C1	GGCATAAGCGGTGCTACTGGTCAAAGCA	TGAGGATGTTAACAACTTGACCGCTACC
YJL209W1	TGTTAGTCAAGTCAGCAGTGTTCGTCAC	AACCAAGTGGACGTGGTCGTGTAAAGGA
YJL207C1	GCTAAACAAAGCGTCGTTGCTGCTGGTA	CAGAGGTAGCACCGTTAGAAACGAAGGT
YJL204C1	ATTTGAAGCTGAGCTGGCGTCGGTTTGG	TGCTGGTGCTAACTTTAGAAAGGTCGTC
YJL198W1	TAGTAGCGGCTTATTGAGCACTAT	GCTAGTAGCATTGCCTTTAACGA

	CGCT	CGCTA
YJL192C1	GATGACACCAGCTAACTTCCATC TGCTA	TGCCAACTTGCCATTGAATGATG GCGTC
YJL185C1	GCTGGTATTTTCTAAGCTGCTGCT AGCA	TGATGGCGTTAACCACGGTTTCC CAGTC
YJL181W1	TAATGGTACCTATAGCTTGGTCGC TAGC	TGAACGACCTAATTCCAAAACCT GGTGG
YJL178C1	AGCAGTTGAACGACCCAAAATAC GGCTG	GAACGGTGATGGTGACAACGGC AAAGAC
YJL174W1	CAACGGTGCTTACCCAAGCTTGT CAAAA	GGTAGCTTGAGCATTATTGGTGGT AGTG
YJL165C1	AGGAGGGCTTCTAGGGGTACTTA ATGAA	GTTGAGCTTAAGCCCATCAAGAG AACCT
YJL163C1	GCCACGACCAATGCCTAAGATGC TGATA	CGCTACCATGGGTGTTGGTCCTAT GTTC
YJL161W1	CTTATACGGTACCGGCTTAGCTTG TTTG	TGGAGGAAAACCCAAGACTGAG ACAACG
YJL158C1	ATCTGAATCGCCTAAGGCTAAAT AGCCG	TGACTCACAAGCTCAAGCCACCA CCACT
YJL156C1	GCTTCTGCTGTTGCTGAAAGCAT CACTG	CCATTCATCAGGTCACTCAGGTA GCAAA
YJL154C1	AGCTGGTGGACCTGAACGGCTG	TACCGCTCCTCCTAATAGCCCTGT C
YJL148W1	CGAGAGTAGCTTGACCCAGGAC AATTTG	GACTTCCTCGGCAACGTGAAAGT CTTCA
YJL145W1	CGCTAATGGTGATGCTAACAAGA AAGCC	GACCCAACCAAAGACAGTTGGA ACGTTG
YJL139C1	ACTAACGCCGATGCTGTGAACTG GAGCA	CGCTTACGCTTTCTTAACTGACA GCGGT
YJL134W1	TTCAAGTCACACCGCTAACGCTA CCGGT	GACACCCATAAAAGCGACTGAGT CTTGA
YJL132W1	CAGTAGTCCTGGTTCATTTGACG CTCGT	AATACGGCCAGTCAACTGCATAT GGTCG
YJL130C1	GGCCAAGGCGCTACTTCTGCTTA ATCTA	CGCCAACAGCGTTGATGAGGCTT TAGCT
YJL128C1	AGGACCTTCAGTTGAGCTGCTAG TACTG	CAAAGCTCCAGCCATCATTAATAC CCCT
YJL125C1	AACAACGTTAGCGTTTAAGCTGG CAGCA	CTTACAACCTACCCCAGAATTATG GACC
YJL123C1	ACTACCGCTACTACTCCACCAATT GCTG	TGTCAAAGGTGGCAGCAACAGC GTTGCT
YJL121C1	TGGATCGGCAGCGGTAAAGACTG AAGTG	TGAGGCTACCCAAGATCCATTAC ACTTG
YJL116C1	GGTTCTGCACAAGGCGATACTAT	CGTCAGTTTAGATTGGTTGGGTTT

YJL111W1	CGCTTCGTCGATGCTGTTTTGAGTTTGGACCGT	CGGCAGCGCCGGTTTCTTCAACTTGACGCAAT
YJL108C1	GTCACTAGTTGAGGTGGTGATAGTTTCG	TAACAGCACCGAGTTCACCGCTGCTTTG
YJL103C1	GTCAGCGTCACTATGTGAGCTTGAATCG	CCCAAACCCATCAGCTAGCACCAGA
YJL099W1	TAGAAGCTTAAACTGTGGTTGGGACAGC	AGCAAACAAGGCTTTTTCGTACTCCTCC
YJL097W1	TAGCAGCCGTTTGTTGGTCGTTTTGGGT	GATGATTCTCAATTCTGAAGCGACGCCG
YJL094C1	GCTCAAACTCTTTCTGACGCCGTCTCTA	TTTGTTCTGGCGTGAAGCTACCGCTGCT
YJL091C1	GCTGCTTTTTTTGCTAGCAGCGACAACA	CCCAGTTACCCGTATGGTCCCTAGA
YJL087C1	GCTACCAGTAGCGCTACAATTGTCCAAA	TCACAACGTTACTGCCGTTGCTGAATAC
YJL082W1	CGAAAGAAGCAAGAACAGCTCAAGCCCA	AACGGCGCCAATAGCTGGAGGCAACAAT
YJL080C1	AGCGACTGATGATTCACTGGCCAACAAG	AGTCGGCGAAGCCATTACTTACGCTCGT
YJL078C1	GGTACGAGCGCCCAATGAGGTTGAACTT	TCAAACTTCAGAGTTGACTACCAGCCCA
YJL074C3	AACCAAGGCGTCCTCAGGTAACAAACCG	TGATGCTTTGGAAGGCATCACCACCACT
YJL071W1	GGGTGCTAAGCCAAGCTCAAACTCACCT	TAAGTCCAAAACGGCGACTGATCTTTGG
YJL068C1	AGCAAAAGCGCTGCAGCTCTTATAACGT	CGCTATCGTTTTCCCAGACACCAGTCCT
YJL062W1	CAGTAATGACTTGAGCGAGCATGACAGT	GTGTGGACCGTCCTTGTGACCAATGTGA
YJL058C2	ATGCAACATGCTAACTGGGTTTGGGCTA	AGGTAGATACATGAAGGGTAAAGCCAGC
YJL057C1	ATAGGTATGTGAGCTACGGCTTGGTAAG	CGGTTTGAGCACCGAACAGTTGGTTAGT
YJL052W1	TGCCAGTGGCAATATCATTCCTAGTAGC	GACGACACGAGCACTATAGCCATATTCA
YJL049W1	ATGGGTCCGTTCATGGAAAGAGAGCACC	TGAGGTGATATCTTCAACTTCGGCAGCG
YJL047C1	GCCACCGGTATTTGATGATTGCAAGCTA	CGTTTTGCCACAAGAGATGGACGACACC
YJL044C1	GCCTTGTGAAACCAATTCATGCAACTCG	CTTCTATCAACCTCCTAAGACCGGTTCA
YJL039C1	ACGTTTGCTTAAGCTAGCGACAC	AGCTGCCGCTAACTTAGACGCCT

	TACCG	TAGTT
YJL036W1	TGGTAGCAGCAGCGGTTATGTTT CATAC	ACGTTGTGAGAATCTGTCACCAG CGATA
YJL033W1	CGCTATTGTTTCAACCTTAAGCCC TAGC	GTCAACTTGGACGACCCAGTCAA CAGCT
YJL029C1	AGCTGAGCTTGGCAAAACCAAG CTTTCG	CTTCAGAGTTGATGACGAAGCTG GTAGT
YJL023C1	AAAACCGTCCCAGTCTAAGTTAG CGACT	CGGCGACAAAGCTATCAGAGAGT ATGTC
YJL019W2	CAGCAATGTTCCTCAGCAGTACA GCTCA	GCAACCTGGTTCATTCTCGCATTG CCAA
YJL012C1	CAAACTAATTCTGACACGAGCGT CGCCT	CGCTGAAGCTCATCGTTTACGTT GGTAC
YJL006C1	TGGGTCAGCGTTTAATAAATGGT CAGGG	CCCACAACACAGTATCGCTTTAG CTATC
YJR010W1	TATCAGCGGCACCGAGTTACGTA GAAGA	CAACTGGATGTCAGCGCTTGAGC TAGTG
YJR015W1	AAAGGCTTTCCCACCAAGCTCAG GTAGC	GGCGTAGCTGATACTTGGAACAA CCATT
YJR019C1	TGGGTGGTGTTTATGCAAGCTGC TCTCA	CGAAGAGGCTACCAGCTTGTTTC AGAAG
YJR025C1	AACGGTGTCGGCGAATCTGACTG GTGAA	TATGATCGTTGGCGGTCCAAACG AACGT
YJR031C1	GAAAACGCTTGACAATGGGTGTG AGGCT	GGCTGTTGAGTTGGACGTCGATT CAATC
YJR035W1	CGTTAACAGCCATGCTGGTTCAA GCAGC	ACTACGAGCAAAAGTGATGCCGT CATCA
YJR039W1	CAGTAGCCCAATCGTTAGTATCG ACGCT	AACTTGGCTAACGGTAGCTTCCT CAACG
YJR041C1	GTAACTGTCTGGACCCTTACGAC TGCAA	CGCTAGTGAAATCACTGATGACA TGGCC
YJR045C1	GCTGATATCGCTGGTGCTTAAGC CAGCA	AAGTACCAATGGCGATACCCACT TAGGC
YJR051W1	TAGCGGCTTAGCTGGTTTGACTA CCTCA	GTGAGTTCTAGGGACGCTATGGC CA
YJR053W1	CAGTAACTCAACTTCAGCTTTCT GGAGC	AGCTAAGATGGTATCCTGGGTGT CATTG
YJR056C1	GTTCAAATTACGGGTATCACCAC GACCA	CGCTTTAGATAGCAACGTTACCG CCGAT
YJR059W1	TACCAGCCCAAGTATCTCAGGTT CAGGC	GTTGCTTCTTTGTGGCAAACCAG CATGG
YJR062C1	TGAGGTTGAATCATCGCTTGGAA CACGT	CCCACAGGGTGAGCAGATTTTCA ACTAT
YJR068W1	CCGTATCATCGATCCATTGGCTAG	GTGTGGAACAACGCCGGCCAATT

	TAGA	CTTCA
YJR070C1	TAATGAAGGAACGGCGGCTGGTGAG	CCAAGAACCTATGGTTCGTCATGAAGCT
YJR074W1	TGTTCCTCCAGGTTTCATCGATGCCAGC	AGCTTGGCTAGCCTCCTCTTTGGTTAAT
YJR076C1	ATTACGTCTGGTGGTAGTACTGCTGCTA	TGAAATGGGTGGTGACGTCGGTACCATC
YJR083C1	GCTGGTAGGGGTGCTTTCGCTTGATCTA	TTCAGATCATGCTTCACCAATCAGCACC
YJR089W1	GTCAAGCTCAGTCATCAGCACCCCAGTT	GCTAGCAATTGGAGTACCGGTCTCG
YJR091C1	GCTGCTCCAGATACCTGAGCTGGTTCTT	TAAAAATGGCGCCGAGTTCAGTAGCTCA
YJR094C1	GCTTGATGAAAAACTATAGGCAGCGGCG	TGCTAGTGACAACGTTTACGAGGCTGAC
YJR096W1	CAAGAGCAAGGGCTTGGTTGTTGAAGCT	ATCGGTACATTCCCAGTCAGTTGGCTCG
YJR100C1	GTAACCGCCACCGAAGCTCAAAAAGCCA	CGATAGTACCCATTACCATGATGGCAAG
YJR103W1	ACATGTTATCAGTCGTGAAAGACGTGGC	AGTTGGCTTGGTTTTCTGTTCGCCGTGG
YJR106W1	CATCAGCGACCCAAATTCATTGAGAAGC	AACCTCGCCGATGCTCAAGCTAACGCTA
YJR109C1	GTCATCGGTTGACTCGGCACGTTTACTA	TCACGTTCCTTTGCCACCTTCAGGCATC
YJR122W1	CCCTCAAAGTTTTCGTGTCGTTACCAGT	GTAGGTTCTAGCGGTTAACTCTTGACCA
YJR124C1	GCTGCTAGGAATAGTACTGCTAGCCATG	TGCTTTAGTCGGTACCATCGGTTCAGCC
YJR126C1	TGAGTTAGCGTCGCCAGCTGATGAGCTA	TAGCGTTCCAATGTCAGCCCGTGACGTT
YJR129C1	ACGGATGGTAGCACTCAACAAGCATAAC	CCACAAACCATTGCAAGAGTTAGCTCCA
YJR132W1	TTCAATCGTCACCCAGTTGGCCAGCAGA	ACCGGTCAAAGGATCCTCTTCCAAATCG
YJR134C1	ACCATTGCTGTTGCTCAACTGAACTGAG	GTTGGGTGAATTGGAATCAGACCCAAGC
YJR136C1	GGTCTGCAACAATAAGACGCCTCTCAAC	TTGCGCTATCACCGTTAGTGACGAGGTT
YJR138W1	TAGTACCGGCTCAAGCAATGACCCTTCA	ATCTGGATTGTGGACACGATCGTAGTGA
YJR140C1	GCTGACATCATTGACAGCGCTTGATTCG	CATGAAAGGTGCTTTAACCAGCCCAGTT
YJR147W1	TTTGAGCTTAGTCAAGGCCCACC	ATGTTGCAAGCAGCTATCTGGAC

Table S5 Summary of insertion sites in the nanopore sequencing strains.

Strain name	Length of insertion (bp)	Gene involved	Distance from the wt centromere (bp)
yLJY003	183,046	YJR138W,YER186C-YER188W, YJR075W,YJL197W-YJL193W,YER159C-YER158C,YJL049W,YJL050W, YER176W-YER175C,YJL191W,YJL192C,YJL197W-YJL193W,YJL055W-YJL057C, YJL213W,YJL159W,YJL160C,YJR014W-YJR011C,YJL110C,YJL109C,YJL073W-YJL077W-A,YJL171C-YJL174W,YJL148W,YEL071W,YEL072W,YJL105W,YER167W-YER169W,YJR127C-YJR128W,YER124C-YER128W,YEL009C-YEL006W,YER162C-YER164W,YJR068W,YJR067C,YJR121W-YJR119C,YJL198W-YJL200C,YEL009C-YEL006W,YER132C,YER135C-YER133W-A,YEL030C-A,YEL030WYEL049W,YEL044W-YEL046C,YER048C,YER053C-A-YER052C	236,094
yLJY019	9,229	YJL045W,YJL092W,YJL093C,YJL104W	200,017
	6,187	YJL092W,YJL105W,YJL104W	86,544
yLJY034	5,985	YEL059C-A-YEL056W	108,058
yLJY047	108,307	YER038C-YER089C	75,797
	187,522	YJL209W-YJL102W	202,846
	10,100	YJR155W-YJR158W	279,290
yLJY048	34,996	YEL072W-YEL057C	106,974
	90,146	YER008C-YER053C	16,006
	74,641	YER158C-YER188W	333,310
	41,478	YJR141W-YJR158W	247,664
yLJY052	261,140	YER188W-YER066W	138,118
	172,316	YJL167W-YJL082W	153,118
yLJY053	57,674	YER166W-YER188W	355,000
	85,569	YJL222W-YJL174W	334,165
	73,094	YJL079C-YJL042W	73,149
	4,442	YJR110W-YJR113C	185,554
	41,469	YJR141W-YJR158W	247,679
yLJY054	155,077	YER118C-YER188W	245,758
	55,094	YJL102W-YJL080C	149,214
	25,191	YJR151C-YJR158W	263,816

yLJY057	3,944	YEL055C	99,609
yLJY058	101,714	YEL070W-YEL018W	28,852
	286,115	YER055C-YER188W	112,791
	149,966	YJL098W-YJL026W	43,448
	17,517	YJR152W-YJR158W	271,187
yLJY059	50,366	YEL070W-YEL044W	82,072
	305,492	YER047C-YER188W	92,725
	129,922	YJL221C-YJL145W	284,763
	56,007	YJL101C-YJL079C	145,175
yLJY060	145,081	YER077C-YER150W	162,867
yLJY061	2,563	YEL059C-A-YEL058W	108,010
	22,687	YER087C-B-YER096W	178,081
	3,689	YER152W-A-YER154W	317,986
	30,812	YJL204C-YJL184W	344,962
	15,044	YJL129C-YJL120W	236,646
	2,357	YJL092W,YJL091C	172,983
	2,699	YJL049W,YJL048C	86,396
yLJY062	44,069	YEL070W-YEL049W	88,277
	21,064	YER006W-YER014W	10,660
	196,635	YER051W-YER188W	102,222
	40,823	YJL100W-YJL084C	158,644
	52,433	YJR061W-YJR092W	101,882
	128,914	YJR095W-YJR158W	161,641
yLJY063	29,456	YEL048C-YEL031W	58,415
	57,944	YJL222W-YJL196C	361,838
	2,113	YJL093C	179,685
	97,674	YJR064W-YJR123W	107,536
yLJY064	33,741	YEL068C-YEL051W	93,185
	33,420	YER103W-YER117W	210,491
	15,855	YER144C-YER152W-A	295,689
	4,611	YER179W-YER183C	379,786
	69,131	YJL215C-YJL174W	335,180
	59,875	YJL171C-YJL135W	270,432
	94,105	YJL132W-YJL090C	172,019
	42,057	YJL087C-YJL069C	47,118
	67,228	YJR095W-YJR134C	160,197
yLJY065	76,787	YEL070W-YEL029C	54,651
	98,127	YER147C-YER188W	310,085
	75,917	YJR127C-YJR158W	210,541
yLJY066	117,938	YER133W-A-YER188W	287,756
	70,590	YJL079C-YJL043W	75,597
	77,679	YJR126C-YJR158W	208,807
yLJY067	57,840	YEL071W-YEL042W	77,007
	156,476	YER008C-YER082C	15,641

	18,560	YJL222W-YJL214W	401,591
	32,687	YJL213W-YJL197W	365,123
	54,986	YJL098W-YJL076W	138,003
yLJY068	59,646	YER086W-YER113C	176,586
	10,596	YER139C-YER145C	296,001
	11,014	YER184C-YER188W	397,892
	1,261	YJL078C	143,650
	25,171	YJR150C-YJR158W	260,674
yLJY070	46,167	YEL044W-YEL022W	34,597
	43,417	YJR066W-YJR092W	110,825
	125,537	YJR095W-YJR158W	161,873
yLJY071	114,541	YER077C-YER132C	162,866
	18,557	YJL222W-YJL214W	401,575
yLJY072	78,359	YER093C-YER130C	191,056
	90,826	YJL222W-YJL171C	329,293
	7,819	YJR154W-YJR158W	277,138
yLJY074	122,718	YER086W-YER145C	176,580
	88,120	YJL222W-YJL172W	330,775

References:

1. Chen Y, Wang Y, Liu M, Qu J, Yao M, Li B, Ding M, Liu H, Xiao W, and Yuan Y. Primary and secondary metabolic effects of a key gene deletion (Δ*YPL062W*) in metabolically engineered terpenoid-producing *Saccharomyces cerevisiae*. Applied and Environmental Microbiology, 2019, 85(7): e01990
2. Brachmann C B, Davies A, Cost G J, Caputo E, Li J, Hieter P, and Boeke J D. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast, 1998, 14(2): 115-132
3. Xie Z X, Mitchell L A, Liu H M, Li B Z, Liu D, Agmon N, Wu Y, Li X, Zhou X, Li B, et al. Rapid and efficient CRISPR/Cas9-based mating-type switching of *Saccharomyces cerevisiae*. G3 (Bethesda), 2018, 8(1): 173-183
4. Liti G, Carter D M, Moses A M, Warringer J, Parts L, James S A, Davey R P, Roberts I N, Burt A, Koufopanou V, et al. Population genomics of domestic and wild yeasts. Nature, 2009, 458(7236): 337-341
5. Jia B, Wu Y, Li B Z, Mitchell L A, Liu H, Pan S, Wang J, Zhang H R, Jia N, Li B, et al. Precise control of SCRaMbLE in synthetic haploid and diploid yeast. Nature Communications, 2018, 9(1): 1933