www.sciencemag.org/content/355/6329/eaaf4706/suppl/DC1

Supplementary Materials for

Bug mapping and fitness testing of chemically synthesized chromosome X

Yi Wu,* Bing-Zhi Li,* Meng Zhao, Leslie A. Mitchell, Ze-Xiong Xie, Qiu-Hui Lin, Xia Wang, Wen-Hai Xiao, Ying Wang, Xiao Zhou, Hong Liu, Xia Li, Ming-Zhu Ding, Duo Liu, Lu Zhang, Bao-Li Liu, Xiao-Le Wu, Fei-Fei Li, Xiu-Tao Dong, Bin Jia, Wen-Zheng Zhang, Guo-Zhen Jiang, Yue Liu, Xue Bai, Tian-Qing Song, Yan Chen, Si-Jie Zhou, Rui-Ying Zhu, Feng Gao, Zheng Kuang, Xuya Wang, Michael Shen, Kun Yang, Giovanni Stracquadanio, Sarah M. Richardson, Yicong Lin, Lihui Wang, Roy Walker, Yisha Luo, Ping-Sheng Ma, Huanming Yang, Yizhi Cai, Junbiao Dai, Joel S. Bader, Jef D. Boeke, Ying-Jin Yuan†

*These authors contributed equally to this work.
†Corresponding author. E-mail: yjyuan@tju.edu.cn

Published 10 March 2017, *Science* **355**, eaaf4706 (2017)
DOI: 10.1126/science.aaf4706

This PDF file includes:

Additional Supplementary Material:

The following information related to *synX* can be accessed on the www.syntheticyeast.org website: synX design diagram, PCRTag sequences, Feature summary table (wild type X, designed synX, physical strain yYW0115; yeast_chr10_9_01), Variants in physical strain (yeast_chr10_9_01), Minichunk plasmids, PCR primers used in this paper.

Supporting Online Material

Table of Contents

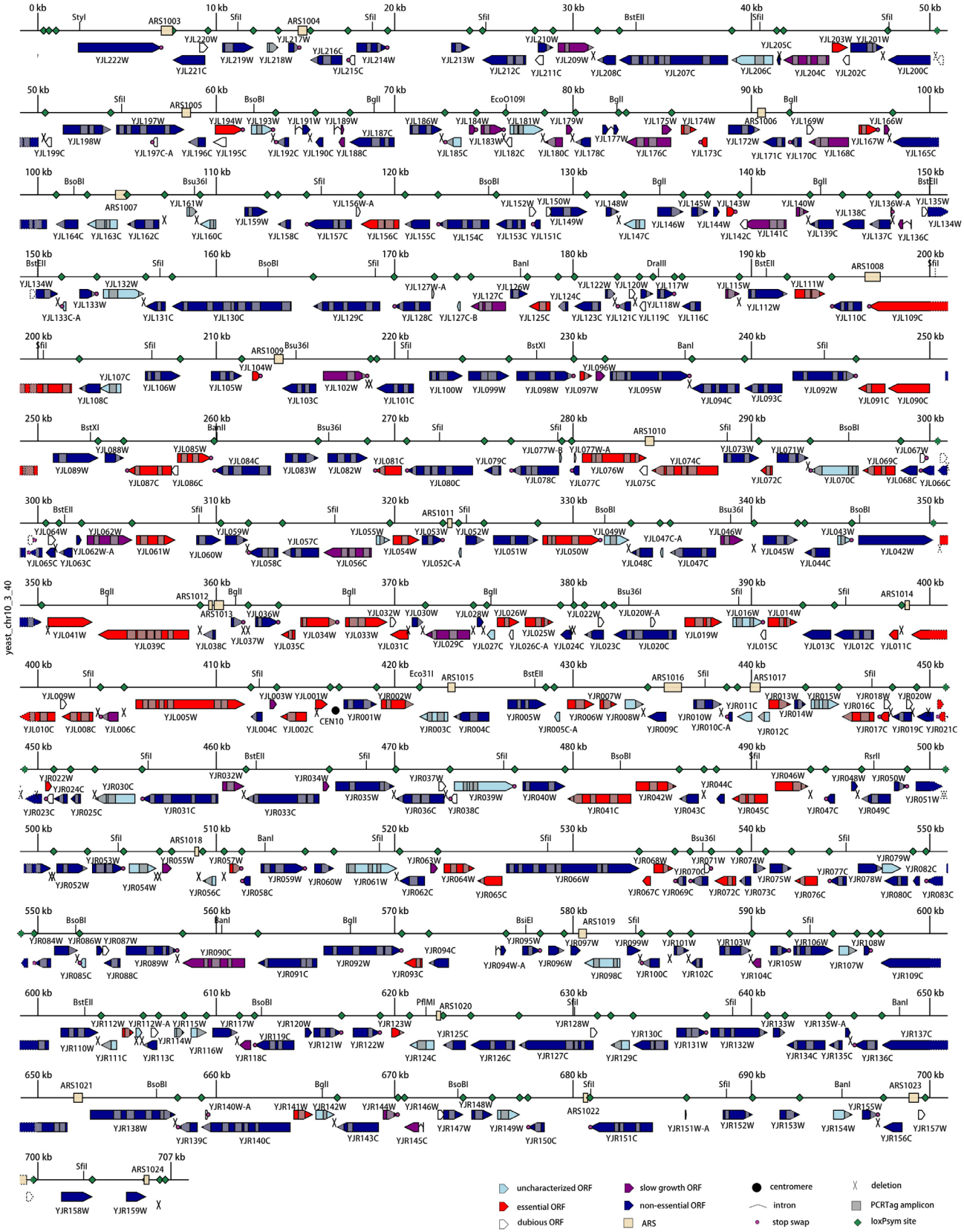
yeast_chr10_3_40
uncharacterized ORF
essential ORF
dubious ORF
slow growth ORF
non-essential ORF
ARS
centromere
intron
stop swap
deletion
PCRTag amplicon
loxPsym site

Fig. S1. Designed map of *synX* (chromosome version yeast_chr10_3_40). Open reading frames (ORFs) - red, essential; dark blue, non-essential; purple, null mutation confers a slow growth phenotype; light blue, uncharacterized; white, dubious/pseudogene. Autonomously replicating sequences (ARSs) are labeled in pale yellow. Locations marked "×" are present in the native chromosome and deleted in *synX*. Green diamonds represent loxPsym sites embedded in the 3′UTR of non-essential genes and at several other landmarks. Gray bars in ORFs symbolize PCRTag pairs. Fuchsia circles indicate stop codons swap (TAG recoded to TAA). Rare-cutting restriction enzyme sites bordering ~10 kb chunks are also shown. In most cases, the appearance of a deletion symbol in an overlap region with portions of genes was caused by deletion of excess loxPsym sites inserted during the design process. All the versions of *synX* can be accessed from BioStudio (http://54.160.105.26/gbrowse2/) with permission.

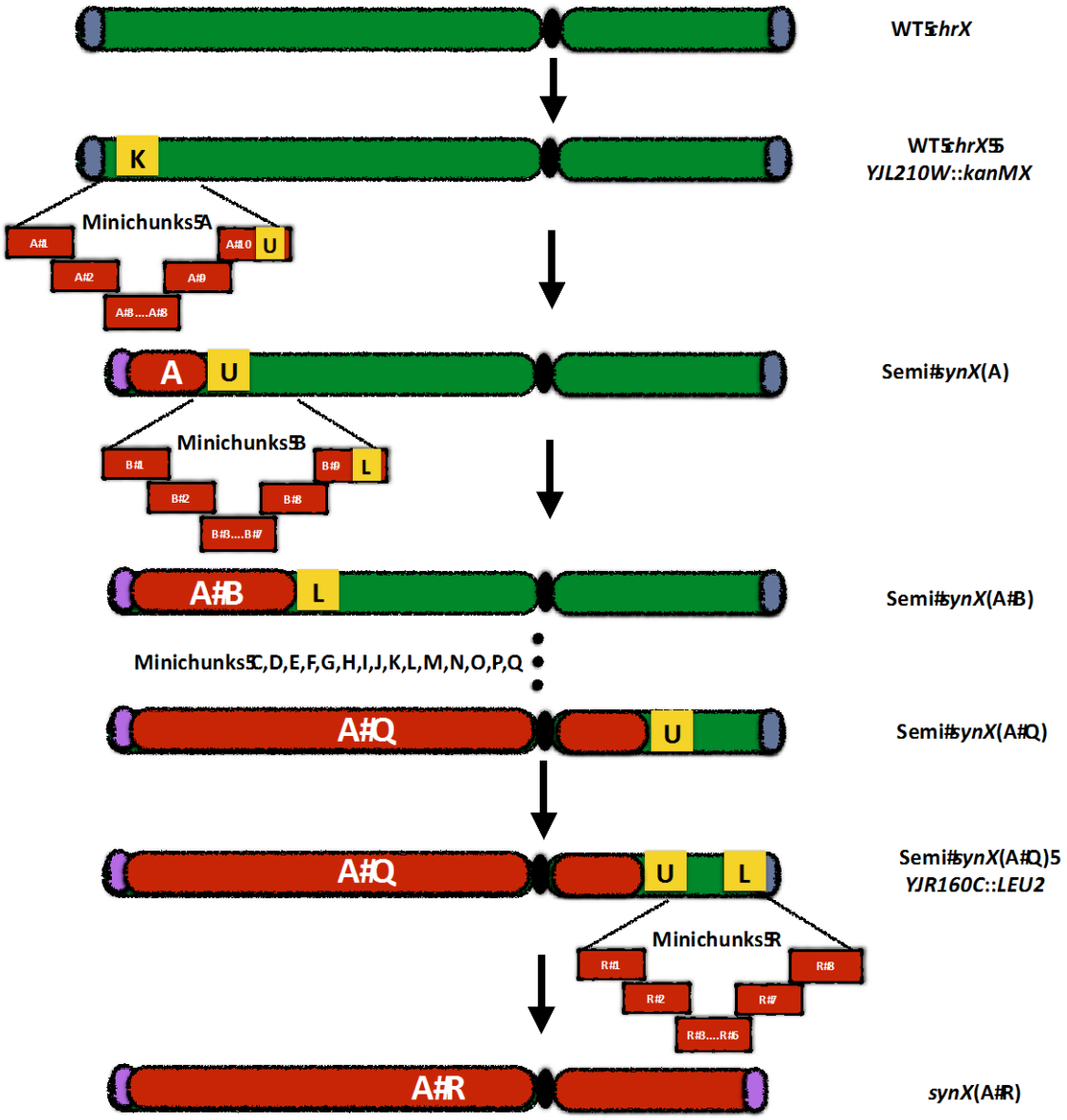

Fig. S2. *SynX* construction. Replacement of native yeast chromosome *X* with 5-kb synthetic minichunks. 18 iterative one-step assemblies and replacements of native segments of yeast chromosome *X* were carried out by using pools of overlapping synthetic DNA minichunks (table S1). Genetic markers (*KanMX4*, *LEU2* or *URA3*) were introduced in both boundary of replaced region in every incorporation step, which enabled high-efficiency screen of complete replacement of chromosome *X* in yeast. K, *KanMX4*; U, *URA3*; L, *LEU2*.

YJL219W1 YJL218W1 YJL217W1 YJL216C2 YJL216C1 YJL214W1 YJL214W2 YJL213W1 YJL212C2 YJL212C1 YJL210W1 YJL209W1 YJL209W2 YJL208C1 YJL207C6 YJL207C3 YJL207C1 YJL207C4 YJL207C2 YJL207C5 YJL206C1 YJL206C2 YJL204C2 YJL204C1 YJL204C3 YJL201W2 YJL201W1* YJL200C2 YJL200C1 YJL198W2 YJL198W3 YJL198W1 YJL197W2 YJL197W3 YJL197W4 YJL197W1 YJL196C1 YJL193W1 YJL192C1 YJL187C1 YJL187C2 YJL186W2 YJL186W1* YJL185C1 YJL183W1 YJL181W2* YJL181W1 YJL180C1
SYN
WT
YJL178C1 YJL176C2 YJL176C1 YJL174W1 YJL172W2 YJL172W1 YJL171C1 YJL170C1 YJL168C2 YJL168C1* YJL167W1 YJL165C2 YJL165C3 YJL165C1 YJL164C1 YJL163C1 YJL163C2 YJL162C2 YJL162C1 YJL161W1 YJL160C1 YJL159W1 YJL158C1* YJL157C2 YJL157C1 YJL156C2 YJL156C1 YJL155C1 YJL154C2 YJL154C1 YJL154C3 YJL153C1 YJL153C2 YJL149W1 YJL148W1 YJL147C1 YJL146W1 YJL145W1 YJL141C1 YJL141C2* YJL140W1 YJL139C1 YJL137C1 YJL134W1 YJL133W1 YJL132W2 YJL132W1 YJL131C1
SYN
WT
YJL130C7 YJL130C4 YJL130C3 YJL130C5 YJL130C6 YJL130C1 YJL130C2 YJL129C2 YJL129C4 YJL129C3 YJL129C1 YJL128C2 YJL128C1 YJL127C1 YJL127C2 YJL126W1 YJL125C1 YJL124C1 YJL123C1 YJL122W1 YJL121C1 YJL118W1 YJL117W1 YJL116C1 YJL115W1 YJL112W1 YJL112W2 YJL111W1 YJL111W2 YJL110C1 YJL110C2 YJL109C1 YJL109C2 YJL109C4 YJL109C3* YJL109C5 YJL108C1 YJL107C1 YJL106W2 YJL106W1 YJL105W1 YJL105W2 YJL103C1 YJL103C2 YJL102W2 YJL102W1 YJL101C1 YJL101C2
SYN
WT
YJL100W1 YJL100W2 YJL099W1 YJL099W2 YJL098W2 YJL098W3 YJL098W1 YJL097W1 YJL095W1 YJL095W2 YJL095W3 YJL095W4 YJL094C3 YJL094C1 YJL094C2 YJL093C1* YJL093C2 YJL092W4 YJL092W3 YJL092W1 YJL092W2 YJL091C1 YJL090C1 YJL090C2 YJL089W1 YJL088W1 YJL087C2 YJL087C1 YJL085W1 YJL085W2 YJL084C3 YJL084C1* YJL084C2 YJL083W1 YJL083W2 YJL082W1 YJL082W2 YJL081C1 YJL080C3 YJL080C4 YJL080C2 YJL080C1 YJL079C1 YJL078C1 YJL078C2 YJL078C3 YJL076W1 YJL076W4
SYN
WT
YJL076W3 YJL076W2 YJL074C2 YJL074C3 YJL074C4 YJL074C1 YJL073W2* YJL073W1 YJL072C1 YJL071W2 YJL071W1 YJL070C2 YJL070C3* YJL070C1 YJL069C2 YJL069C1 YJL068C1 YJL066C1 YJL063C1 YJL062W1 YJL062W2 YJL061W1 YJL061W2 YJL060W1* YJL059W1 YJL058C2 YJL058C1 YJL057C2 YJL057C1 YJL056C1 YJL056C2 YJL056C3 YJL055W1 YJL054W1 YJL053W1 YJL052W1 YJL051W2 YJL051W1 YJL050W2 YJL050W1 YJL050W3 YJL049W1 YJL048C1 YJL047C3 YJL047C1 YJL047C2 YJL046W1 YJL045W1
SYN
WT
YJL045W2 YJL044C1 YJL043W1* YJL042W3 YJL042W1 YJL042W2 YJL042W4 YJL039C4 YJL039C1 YJL039C5 YJL039C2 YJL039C3 YJL038C1 YJL037W1 YJL036W1 YJL035C1 YJL034W1 YJL034W2 YJL033W2* YJL033W1 YJL030W1 YJL029C2 YJL029C1 YJL026W1 YJL025W2 YJL025W1 YJL023C1 YJL020C2 YJL020C1 YJL020C3 YJL019W1 YJL019W2 YJL016W2 YJL016W1 YJL014W1 YJL014W2 YJL012C1 YJL012C2 YJL010C2 YJL010C1 YJL008C2 YJL008C1 YJL006C1 YJL005W6 YJL005W1 YJL005W4 YJL005W2 YJL005W3
SYN
WT

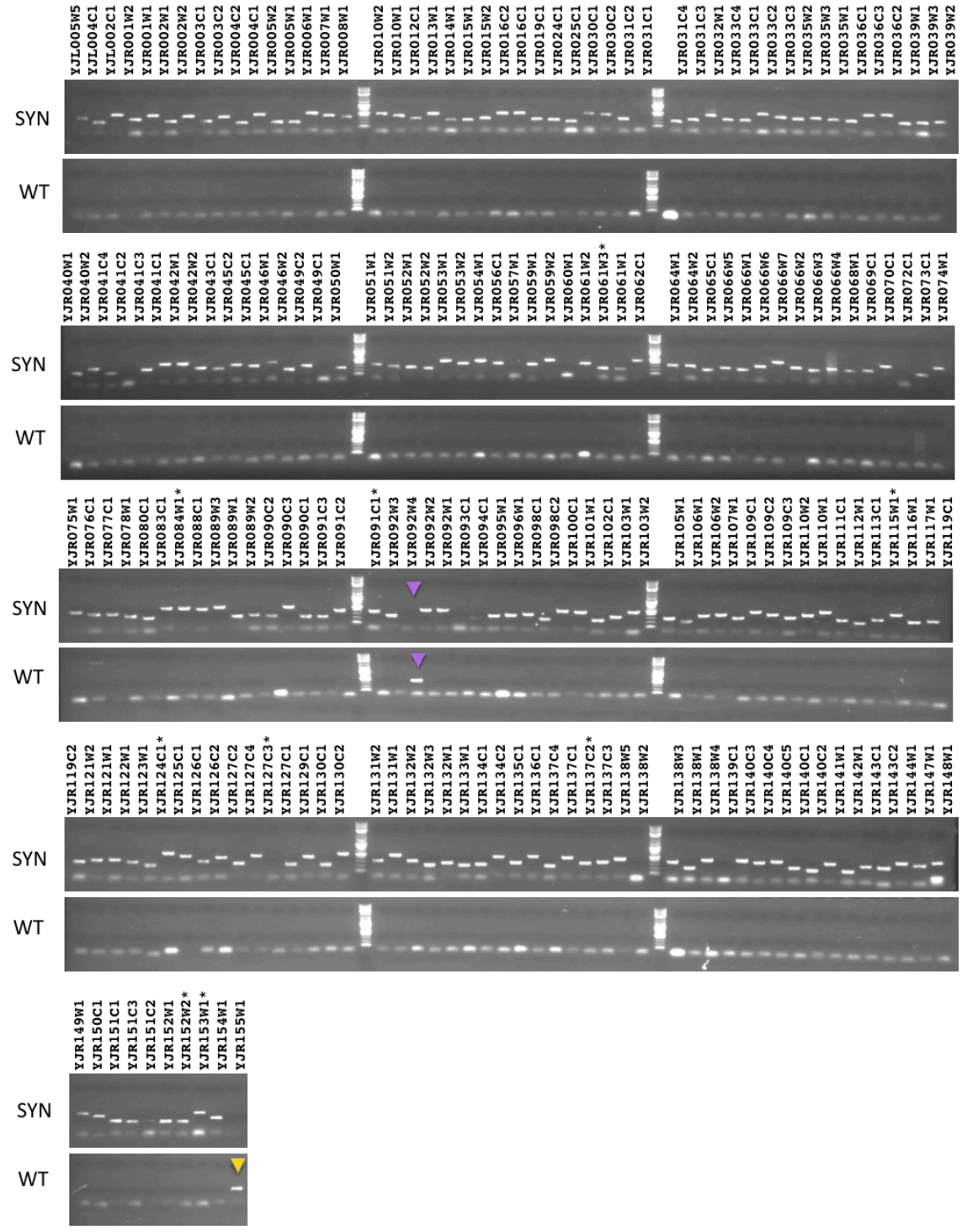
YJL005W5
YJL004C1
YJL002C1
YJR001W2
YJR001W1
YJR002W1
YJR002W2
YJR003C1
YJR003C2
YJR004C2
YJR004C1
YJR005W2
YJR005W1
YJR006W1
YJR007W1
YJR008W1
YJR010W2
YJR010W1
YJR012C1
YJR013W1
YJR014W1
YJR015W1
YJR015W2
YJR016C2
YJR016C1
YJR019C1
YJR024C1
YJR025C1
YJR030C1
YJR030C2
YJR031C2
YJR031C1
YJR031C4
YJR031C3
YJR032W1
YJR033C4
YJR033C1
YJR033C2
YJR033C3
YJR035W2
YJR035W3
YJR035W1
YJR036C1
YJR036C3
YJR036C2
YJR039W1
YJR039W3
YJR039W2
SYN
WT
YJR040W1
YJR040W2
YJR041C4
YJR041C2
YJR041C3
YJR041C1
YJR042W1
YJR042W2
YJR043C1
YJR045C2
YJR045C1
YJR046W1
YJR046W2
YJR049C2
YJR049C1
YJR050W1
YJR051W1
YJR051W2
YJR052W1
YJR052W2
YJR053W1
YJR053W2
YJR054W1
YJR056C1
YJR057W1
YJR059W1
YJR059W2
YJR060W1
YJR061W2
YJR061W3*
YJR061W1
YJR062C1
YJR064W1
YJR064W2
YJR065C1
YJR066W5
YJR066W1
YJR066W6
YJR066W7
YJR066W2
YJR066W3
YJR066W4
YJR068W1
YJR069C1
YJR070C1
YJR072C1
YJR073C1
YJR074W1
SYN
WT
YJR075W1
YJR076C1
YJR077C1
YJR078W1
YJR080C1
YJR083C1
YJR084W1*
YJR088C1
YJR089W3
YJR089W1
YJR089W2
YJR090C2
YJR090C3
YJR090C1
YJR091C3
YJR091C2
YJR091C1*
YJR092W3
YJR092W4
YJR092W2
YJR092W1
YJR093C1
YJR094C1
YJR095W1
YJR096W1
YJR098C1
YJR098C2
YJR100C1
YJR101W1
YJR102C1
YJR103W1
YJR103W2
YJR105W1
YJR106W1
YJR106W2
YJR107W1
YJR109C1
YJR109C2
YJR109C3
YJR110W2
YJR110W1
YJR111C1
YJR112W1
YJR113C1
YJR115W1*
YJR116W1
YJR117W1
YJR119C1
SYN
WT
YJR119C2
YJR121W2
YJR121W1
YJR122W1
YJR123W1
YJR124C1*
YJR125C1
YJR126C1
YJR126C2
YJR127C2
YJR127C4
YJR127C3*
YJR127C1
YJR129C1
YJR130C1
YJR130C2
YJR131W2
YJR131W1
YJR132W2
YJR132W3
YJR132W1
YJR133W1
YJR134C1
YJR134C2
YJR135C1
YJR136C1
YJR137C4
YJR137C1
YJR137C2*
YJR137C3
YJR138W5
YJR138W2
YJR138W3
YJR138W1
YJR138W4
YJR139C1
YJR140C3
YJR140C4
YJR140C5
YJR140C1
YJR140C2
YJR141W1
YJR142W1
YJR143C1
YJR143C2
YJR144W1
YJR147W1
YJR148W1
SYN
WT
YJR149W1
YJR150C1
YJR151C1
YJR151C3
YJR151C2
YJR152W1
YJR152W2*
YJR153W1*
YJR154W1
YJR155W1
SYN
WT

Fig. S3. PCRTag analysis of *synX*. The presence of *synX* and absence of native chromosome *X* was verified by amplification of synthetic PCRTags (SYN) compared to wild type PCRTags (WT). PCRTag analysis of *synX* strain (yYW0115) revealed the presence of SYN PCRTags and absence of WT PCRTags. The amplification of WT PCRTags showed in yellow triangle were amplified in homologous region in other chromosomes. YJL219W1 region is homologous with *YOL156W* in chromosome *XV*; YJL052W1 region is homologous with *YGR192C* in chromosome *VII*; YJR155W1 region is homologous with *YFL057C* in chromosome *VI*. PCRTag YJR092W4 region showed in purple triangle was subsequently incorporated by co-transformation with tR(CCU)J-URA3 integration at *HO* (Fig. S4). All the presence of SYN PCRTags that did not yield amplicons under the used PCR conditions were confirmed by whole genome nucleotide sequencing of *synX* strain (yYW0115).

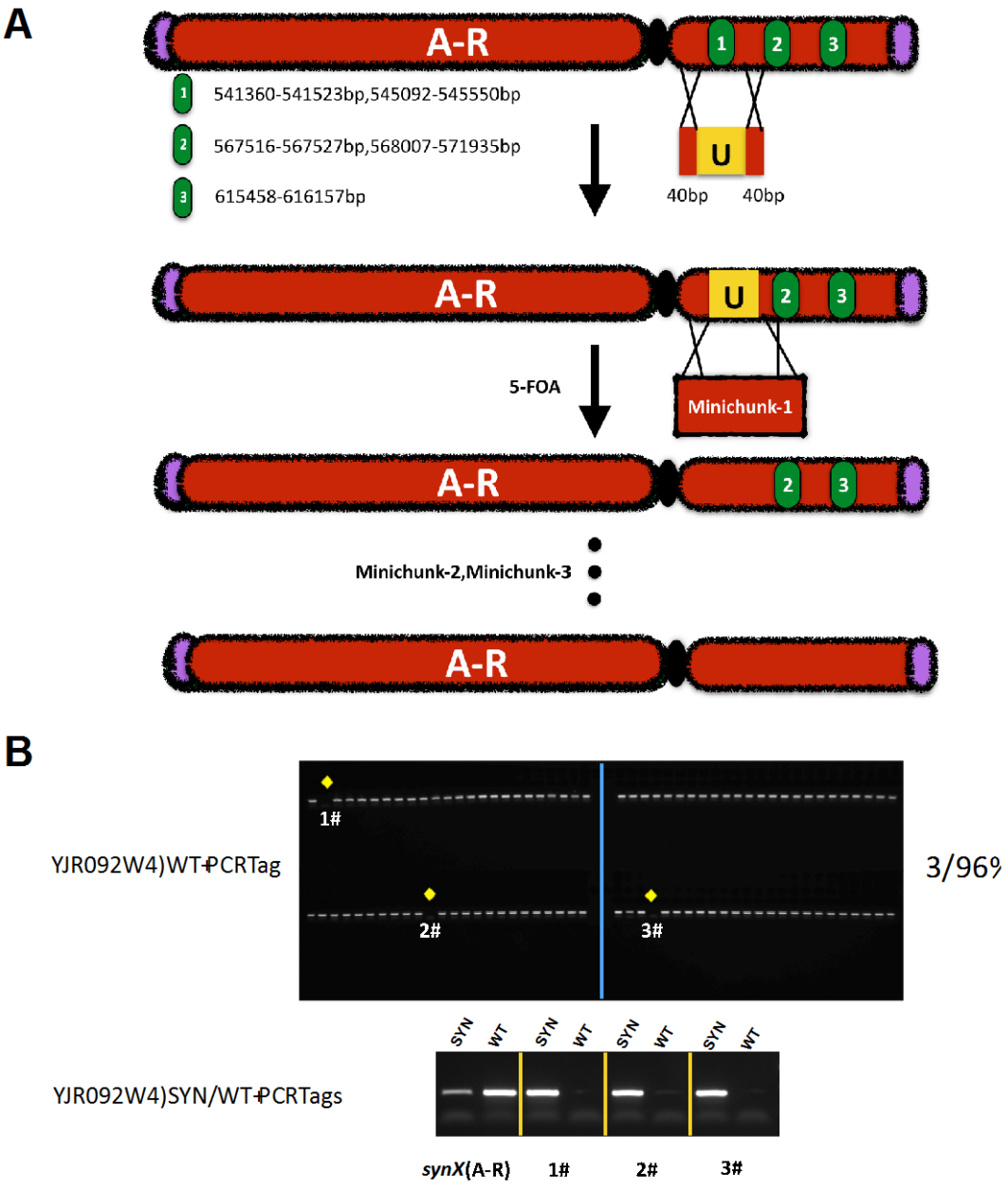

Fig. S4. Replacement of residual wild type sequence with synthetic counterpart. (**A**) Replacement of wild type chromosome regions by two step transformation with synthetic DNA minichunks. Three wild type regions were replaced successively. (**B**) Incorporation of the synthetic YJR092W4 into *synX* by co-transformation. 3 colonies displayed incorporation of synthetic YJR092W4 after 96 colonies with *URA3* marker were screened. U, *URA3*.

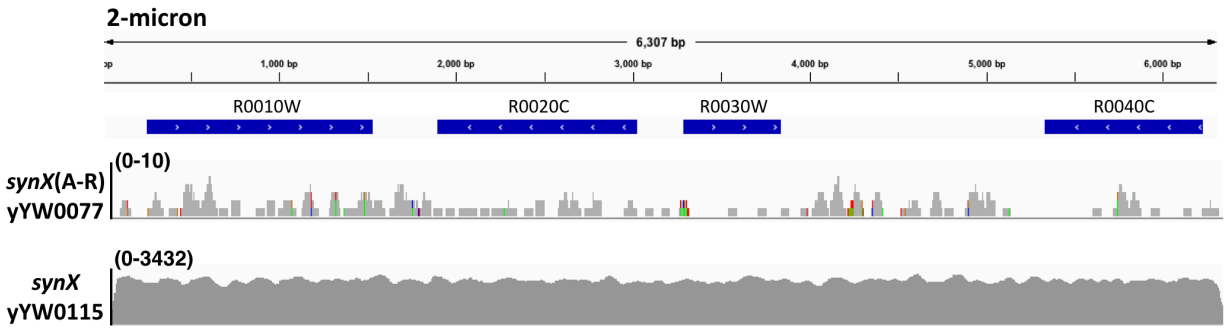

Fig. S5. Plots of read depth for 2-micron in *synX*. Read depth analysis of *synX* (yYW0077) reveal absence of native 2-micron plasmid. 2-micron plasmid were recovered in *synX* strain (yYW0115) after a backcross to semi-*synX(*A-F) strain (yYW0098). The copy number of the 2-micron plasmid in yYW0115 is about 30, relative to the average read depth for other chromosomes.

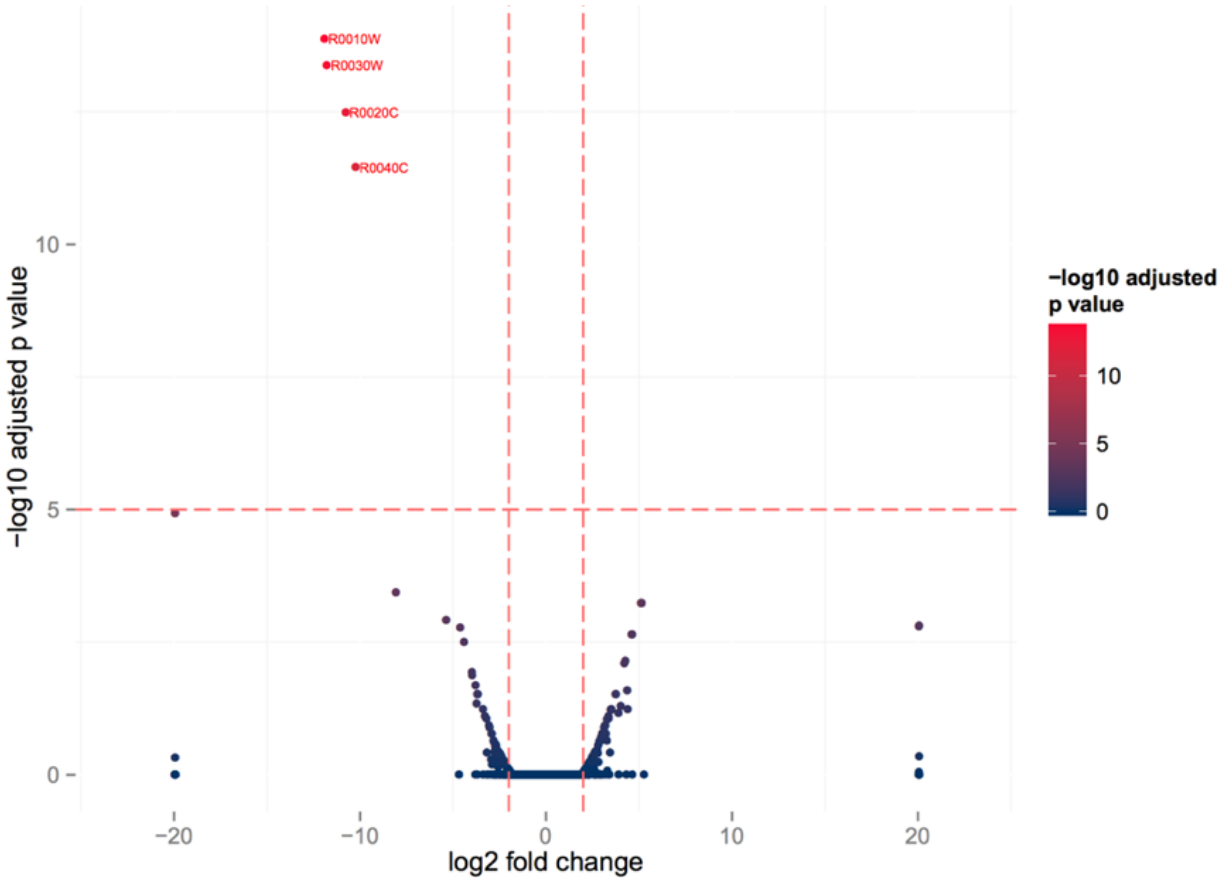

Fig. S6. Transcript profiling of *synX* strain. RNA-Seq analysis of *synX* strain (yYW0077) as compared to wild type (BY4741) is shown in a volcano plot. Genes with significantly altered (shown in red) are all in 2-micron native plasmid. Lack of transcripts reflects 2-micron plasmid loss which was verified by whole genome sequencing for yYW0077 (Fig. S5). The dashed line identifies the False Discovery Rate (FDR) threshold at 5 for -log10 p adjusted value and 2 for absolute value of log2FoldChange.

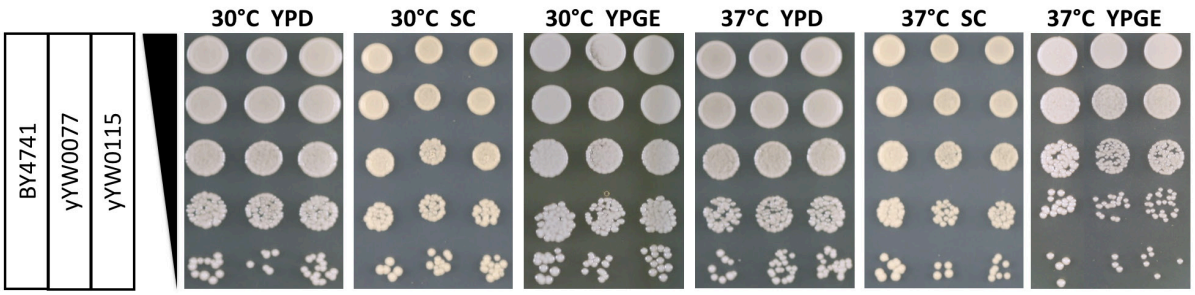

Fig. S7. Growth fitness of *synX* strains (yYW0077 and yYW0115). Ten-fold serial dilutions of saturated cultures of wild type (BY4741), yYW0077 and yYW0115 strains were plated on the indicated media and incubated at noted temperatures. YPD, yeast extract peptone dextrose; SC, synthetic complete medium; YPGE, yeast extract peptone glycerol ethanol.

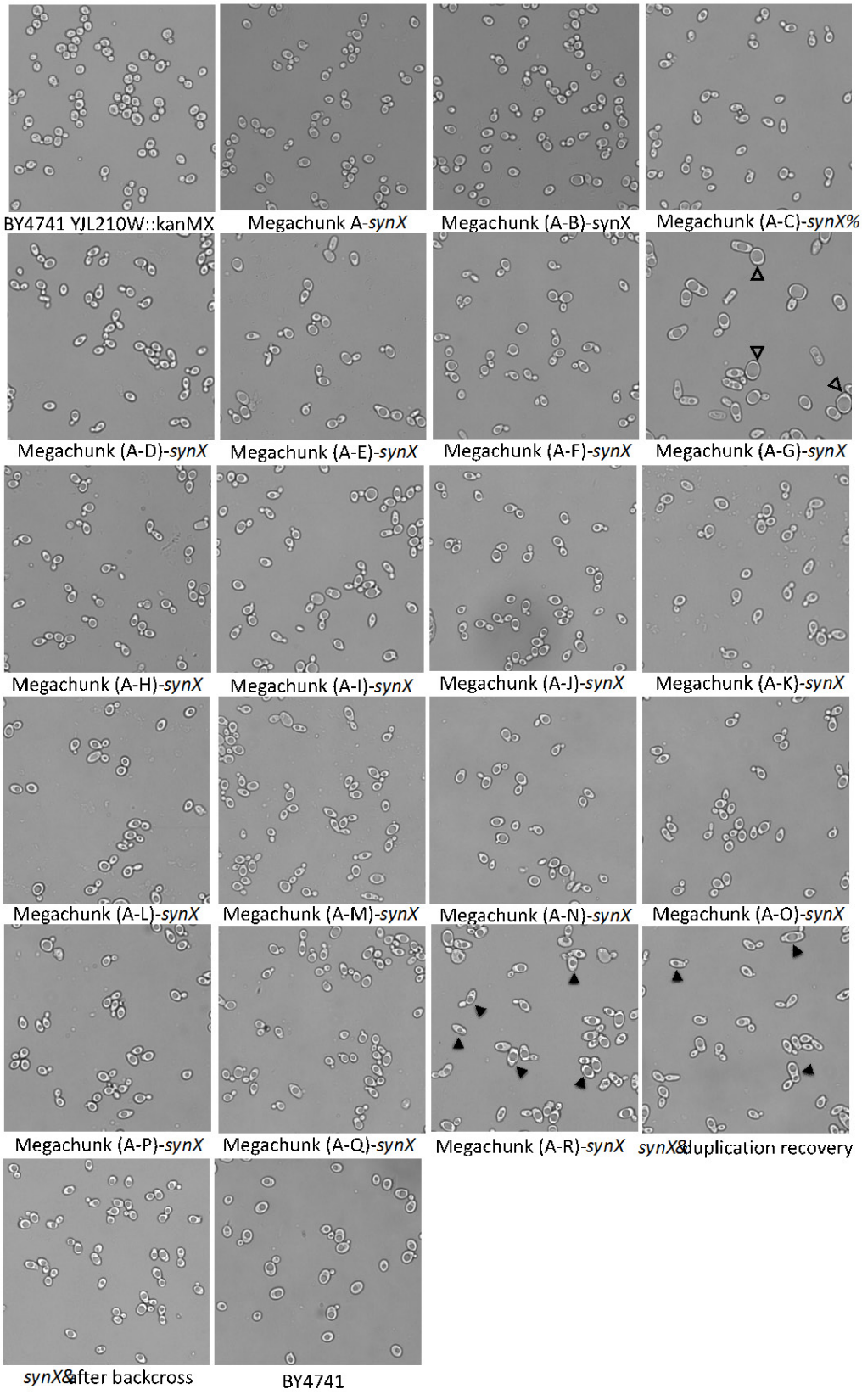
BY4741 YJL210W::kanMX
Megachunk A-synX
Megachunk (A-B)-synX
Megachunk (A-C)-synX%
Megachunk (A-D)-synX
Megachunk (A-E)-synX
Megachunk (A-F)-synX
Megachunk (A-G)-synX
Megachunk (A-H)-synX
Megachunk (A-I)-synX
Megachunk (A-J)-synX
Megachunk (A-K)-synX
Megachunk (A-L)-synX
Megachunk (A-M)-synX
Megachunk (A-N)-synX
Megachunk (A-O)-synX
Megachunk (A-P)-synX
Megachunk (A-Q)-synX
Megachunk (A-R)-synX
synX&duplication recovery
synX&after backcross
BY4741

Fig. S8. *SynX* strain cell morphology. *SynX* (yYW0115) has an elongated and varied size cell morphology after the megachunk R integration. After backcross to BY4742, the morphology of *synX* almost recovered to wild type although it is still subtley elongated. Abnormal cell morphology in megachunk (A-G)-*synX* was caused by genetic marker *URA3* insert in *YJL080C* which was subsequently reversed upon megachunk H incorporation. Arrowheads indicate cells with morphological defect. Cells were grown to mid-log phase in YPD medium at 30°C. Images were collected using an Olympus CX41 microscope.

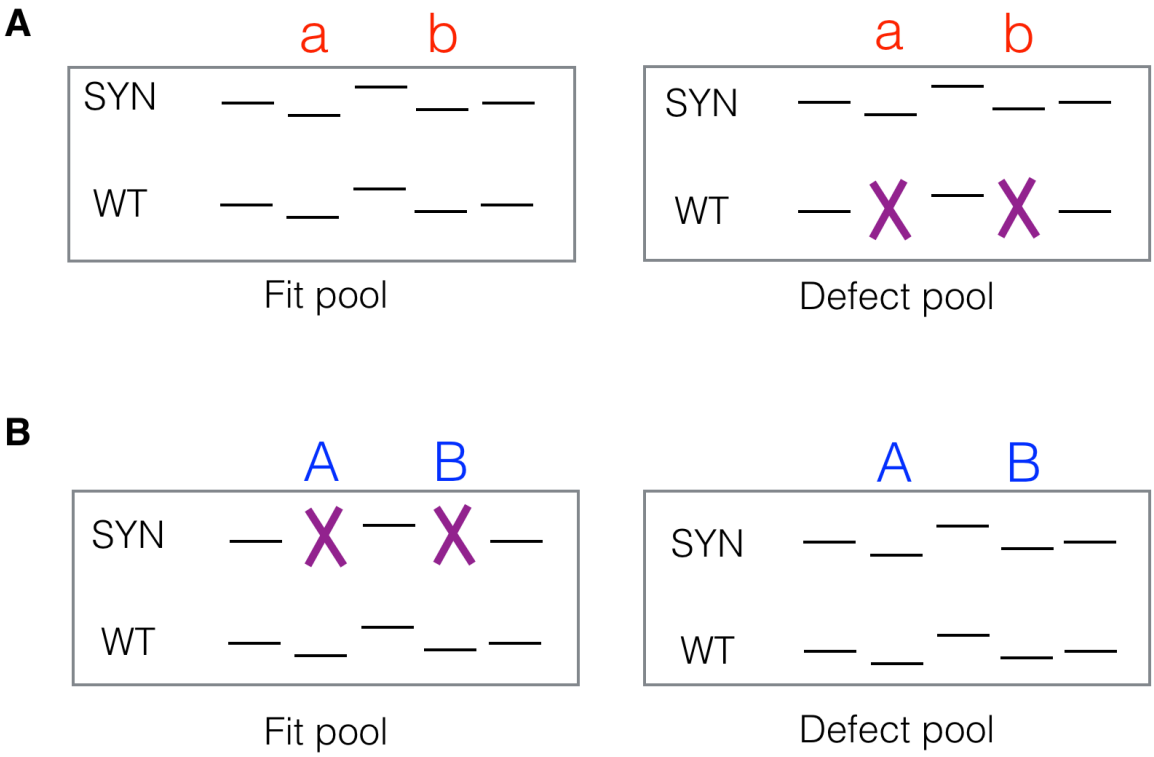

Fig. S9. PoPM can be used in theory to map synthetic defects and multiple defects in synthetic strains. (**A**) Assuming gene "a" and gene "b" are synthetic detrimental interactions, then both SYN and WT PCRTags will be amplified in the fit pool. However, neither of the WT PCRTags will be amplified in the defect pool since the defect will only show up when synthetic gene "a" and synthetic gene "b" both present in a strain. (**B**) Assuming there are bug "A" and bug "B" showing up at the same time, both SYN and WT PCRTags will show up in the defect pool. However, since either bug "A" or bug "B" can cause a defect, none of the SYN PCRTags of bug "A" or bug "B" will be amplified in the fit pool.

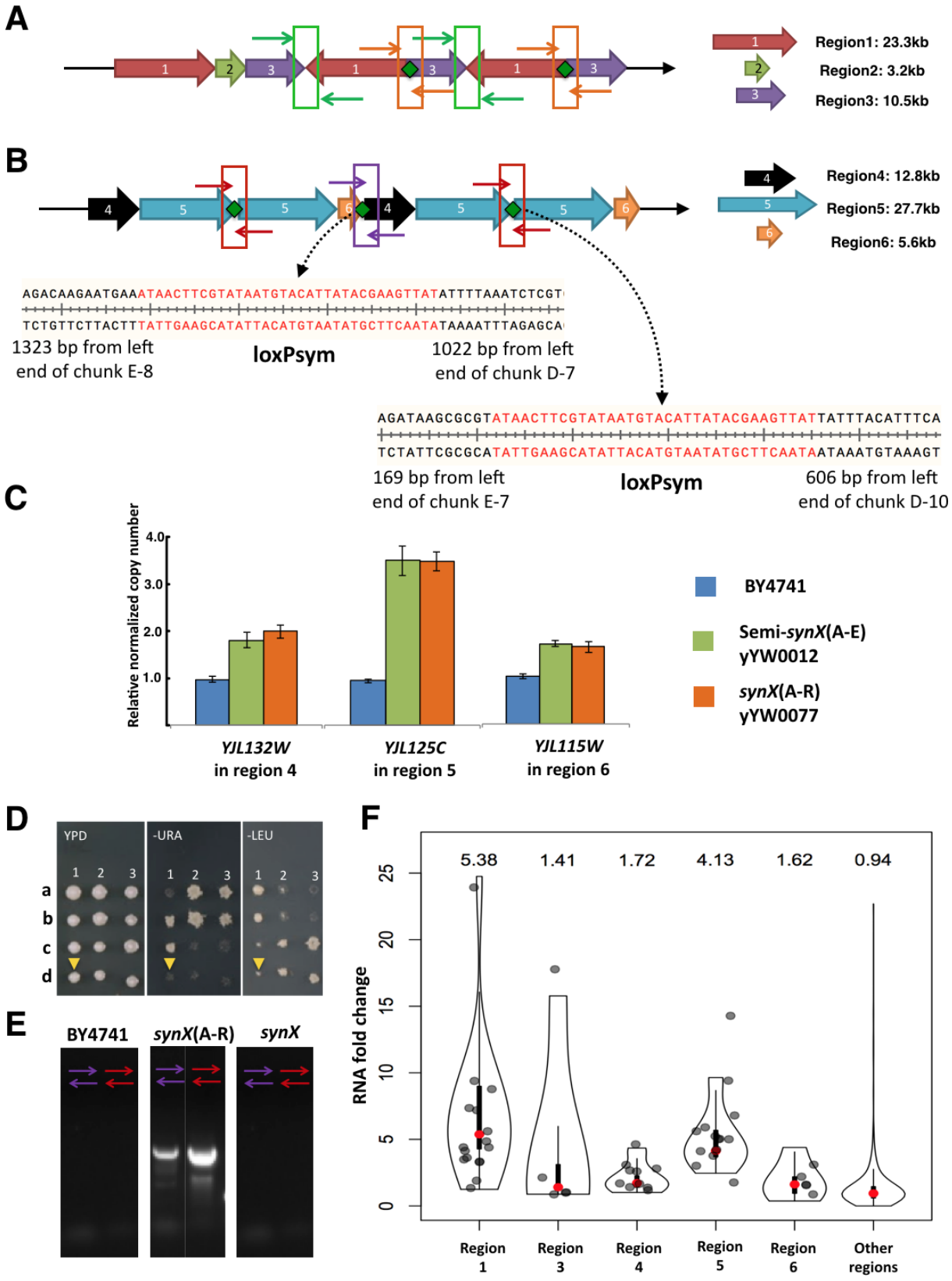
A
Region1: 23.3kb
Region2: 3.2kb
Region3: 10.5kb
B
Region4: 12.8kb
Region5: 27.7kb
Region6: 5.6kb
AGACAAGAATGAAATAACTTCGTATAATGTACATTATACGAAGTTATATTTTAAATCTCGT
TCTGTTCTTACTTTATTGAAGCATATTACATGTAATATGCTTCAATATAAAATTTAGAGCA
1323 bp from left end of chunk E-8
loxPsym
1022 bp from left end of chunk D-7
AGATAAGCGCGTATAACTTCGTATAATGTACATTATACGAAGTTATTATTTACATTTCA
TCTATTCGCGCATATTGAAGCATATTACATGTAATATGCTTCAATAATAAATGTAAAGT
169 bp from left end of chunk E-7
loxPsym
606 bp from left end of chunk D-10
C
Relative normalized copy number
4.0
3.0
2.0
1.0
YJL132W in region 4
YJL125C in region 5
YJL115W in region 6
BY4741
Semi-synX(A-E) yYW0012
synX(A-R) yYW0077
D
YPD
-URA
-LEU
1 2 3
a
b
c
d
E
BY4741
synX(A-R)
synX
F
RNA fold change
0
5
10
15
20
25
5.38
1.41
1.72
4.13
1.62
0.94
Region 1
Region 3
Region 4
Region 5
Region 6
Other regions

Fig. S10. Large duplications and rearrangements in *synX*. (**A**) Predicted duplication and rearrangement structure in region 1-2-3 in megachunk C (Fig. 4) deduced from sequencing depth and junction sequence analysis. (**B**) Duplicated fragments in megachunks D and E were joined via loxPsym sites to form the duplication and rearrangement structure in region 4-5-6. (**C**) Massive duplications and rearrangements occurred during integrative transformation steps E, not subsequently. Intermediate assembly strain semi-*synX*(A-E) showed the same copy numbers in duplication region 4, 5, 6 compared with final chunk integration strain *synX*(A-R). (**D**) Phenotype of dissected tetrads from diploid strain (yYW0111). (**E**) Duplication structure in strain *synX*(A-R) and recovered structure in strain *synX* (yYW0115*)* were verified by PCR using junction primers. (**F**) RNASeq analysis of *synX*(A-R) strain as compared to the wild type in duplication regions. RNA fold change in region 4-5-6 was consistent with DNA fragment copy number in each duplication region. In duplication region 1-2-3, the RNA fold change data shows higher transcription level in region 1 and lower transcription level in region 3. The black dots signify individual genes in the duplication region. The red dot shows mean RNA fold change value in the below region, and the value is given on the top.

Table S1. Deletion of tRNA genes in *synX*.

Anticodon	AAC	ACG	AGA	AGC	AGT	CAT	CCA	CCT *	CTT	GCC	GTA	GTC	TAA	TAG	TCT	TTC
Isotype	Val	Arg	Ser	Ala	Thr	Met	Trp	Arg	Lys	Gly	Tyr	Asp	Leu	Leu	Arg	Glu
Copy number in WT chr10	1	1	1	1	1	3	1	1	1	2	2	4	1	1	2	1
Copy number in genome	14	6	11	11	11	10	6	1	14	16	8	16	7	3	11	14
Copy number in *synX* chromosome	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Deletion rate	0.07	0.17	0.09	0.09	0.09	0.30	0.17	1.00	0.07	0.13	0.25	0.25	0.14	0.33	0.18	0.07

*The single copy tRNA gene, *tR(CCU)J* was relocated to the *HO* locus.

Table S2. Yeast strains used in this study.

Strain name	Description	Genotype
BY4741		*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
BY4742		*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
yYW0001	Starting strain	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YJL210W::kanMX*
yYW0003	semi-*synX* A	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-41382bp-URA3*
yYW0006	semi-*synX*(A-B)	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-77157bp-LEU2*
yYW0007	semi-*synX* (A-C)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-116793bp-URA3*
yYW0010	semi-*synX* (A-D)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-157749bp-LEU2*
yYW0012	semi-*synX* (A-E)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-191739bp-URA3*
yYW0013	semi-*synX* (A-F)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-237184bp-LEU2*
yYW0017	semi-*synX* (A-G)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-273426bp-URA3*
yYW0019	semi-*synX* (A-H)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-309934bp-LEU2*
yYW0036	semi-*synX* (A-I)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-346968bp-URA3*
yYW0037	semi-*synX* (A-J)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-390191bp-LEU2*
yYW0039	semi-*synX* (A-K)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-428760bp-URA3*
yYW0042	semi-*synX* (A-L)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-469319bp-LEU2*
yYW0044	semi-*synX* (A-M)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-505408bp-URA3*
yYW0050	semi-*synX* (A-N)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-546918bp-LEU2 pRS413(HIS3)- tR(CCU)J*
yYW0052	semi-*synX* (A-O) with wild type minichunk O-6*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-584422bp-URA3 pRS413(HIS3)- tR(CCU)J*
yYW0055	semi-*synX* (A-O)*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-584422bp-URA3 pRS413(HIS3)- tR(CCU)J*
yYW0132	semi-*synX* (A-O) with WT YJR093C PCRTag*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-584422bp-URA3 pRS413(HIS3)- tR(CCU)J*
yYW0133	semi-*synX* (A-O) with WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-*

	YJR093C-R PCRTag*	*584422bp-URA3 pRS413(HIS3)- tR(CCU)J*
yYW0062	semi-*synX* (A-P) with wild type minichunk O-6*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-631028bp-LEU2 pRS413(HIS3)- tR(CCU)J*
yYW0072	semi-*synX* (A-P) with wild type minichunk O-6 and loxPsym inserted after YJR120W removed*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-630994bp-LEU2 pRS413(HIS3)- tR(CCU)J*
yYW0074	semi-*synX* (A-Q) with wild type minichunk O-6 and loxPsym inserted after YJR120W removed*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-674448bp-URA3 pRS413(HIS3)- tR(CCU)J*
yYW0077	*synX* (A-R) with wild type minichunk O-6 and loxPsym inserted after YJR120W removed*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-707459bp pRS413(HIS3)- tR(CCU)J*
yYW0082	semi-117kb-*synX* (A-C)	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-116793bp-URA3*
yYW0088	semi-158kb-*synX* (A-D)	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-157749bp-LEU2*
yYW0091	semi-192kb-*synX* (A-E)	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-191739bp-URA3*
yYW0094	semi-237kb-*synX* (A-F)	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-237184bp-LEU2*
yYW0098	semi-237kb-*synX* (A-F) mating type changed	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10-237184bp-LEU2*
yYW0100	yYW0077 615458-616157bp region replaced by SYN fragment*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10*
yYW0101	yYW0100 567516-567527bp, 568007-571935bp region replaced by SYN fragment*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10*
yYW0103	yYW0101 541360-541523bp, 545092-545550bp region replaced by SYN fragment*	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10*
yYW0111	Diploid yYW0098 X yYW0103	*MAT**a**/α his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10*
yYW0113	*synX* spore dissected from yYW0111	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10*
yYW0115	*synX* with wild type tR(CCU)J integrated at *HO* locus	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10 HO::tR(CCU)J-URA3*
yYW0117	yYW0115 with *URA3* knock out	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10 HO::tR(CCU)J*
yYW0134	BY4741 with GFP integrated at	*MAT**a** leu2Δ0 met15Δ0 ura3Δ0 his3Δ1::NAT-*

	HIS3 locus	*GFP*
yYW0153	yYW0115 with RFP integrated at *HIS3* locus	*MAT**a** leu2Δ0 met15Δ0 ura3Δ0 SYN10 HO::tR(CCU)J-URA3 his3Δ1::NAT-dTomato*
yYW0138	BY4742 with Gal-CEN10-URA integrated at CEN10	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 CEN10::pGal1-CEN10-URA3*
yYW0155	Diploid yYW0117 X yYW0138	*MAT**a**/α his3Δ1 leu2Δ0 ura3Δ0 WT CEN10::pGal1-CEN10-URA3 SYN10*
yYW0156	Diploid yYW0155 WT chr10 lost	*MAT**a**/α his3Δ1 leu2Δ0 ura3Δ0 SYN10*
yYW0159	*synX* spore dissected from yYW0156	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SYN10 HO::tR(CCU)J-URA3*
yYW0120	BY4741 FIP1-SYN-YJR093C-R	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R*
yYW0122	BY4741 FIP1-SYN-YJR093C-R 1-Asp codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 1*
yYW0123	BY4741 FIP1-SYN-YJR093C-R 2-Ala codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 2*
yYW0124	BY4741 FIP1-SYN-YJR093C-R 3-Gly codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 3*
yYW0125	BY4741 FIP1-SYN-YJR093C-R 4-Ala codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 4*
yYW0126	BY4741 FIP1-SYN-YJR093C-R 5-Ser codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 5*
yYW0127	BY4741 FIP1-SYN-YJR093C-R 6-Ser codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 6*
yYW0128	BY4741 FIP1-SYN-YJR093C-R 7-Asn codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 7*
yYW0129	BY4741 FIP1-SYN-YJR093C-R 8-Pro codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 8*
yYW0130	BY4741 FIP1-SYN-YJR093C-R 9-Asp codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 9*
yYW0131	BY4741 FIP1-SYN-YJR093C-R 10-Ile codon replaced by WT	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FIP1-SYN-YJR093C-R SYN-WT codon 10*

* Strains with massive duplications and rearrangements.

Table S3. *SynX* version table.

Version name	Strain number	Comment	Details
yeast_chr 10_3_37	NA	Designed version in biostudio	
yeast_chr 10_3_38	NA	Restore *YJR079W* intron (*YJR079W* has overlap region with *YJR080C*)	705bp YJR079W intron restored; evidence the YJR079W is a real gene is very limited.
yeast_chr 10_3_39	NA	Restore PCRTag YJR093C1-Revert to wild type sequence (synthetic YJR093C1-Revert sequence causing grow defect)	571414-571441, "GATGTCTGGGTTTGAACTAGCGCCAGCA" replaced by "TATATCAGGATTACTGGACGCACCTGCG"
yeast_chr 10_3_40	NA	Remove loxPsym site after *YJR120W* (dubious ORF; may disrupt promoter of *ATP2* which is an important gene)	34bp loxPsym site "ATAACTTCGTATAATGTACATTATACGAAGTTAT" inserted after YJR120W was removed
yeast_chr 10_3_41	NA	GFF annotations updated ; sequence unchanged from prior version	
yeast_chr 10_9_01	yYW0115	Genome sequenced, 11 variant nucleotides arose and 2 loxPsym sites were absent	Mutations at 204034 "T" to "C", 225233 "C" to "T", 227311 "T" to "C", 272048 "A" to "G", 311658 "C" to "A", 387191 "T" to "C", 408449 "T" to "C", 511671 "C" to "T", 564352 "C" to "G", 580754 "G" to "T", 705687 "G" to "A"; Two loxPsym sites at position of 392866(*YJL013C*) and 507487(*YJR055W*) were also absent.

Table S4. Sequence variants in the *synX* chromosome.

Position in yeast_chr10_3_40	Reference yeast_chr10_3_40	Variant *synX* yeast_chr10_9_01	ORF	Amino acid Substitution
204034	T	C	YJL107C	Asp-Gly(207)
225233	C	T	YJL099W	Synonymous
227311	T	C	YJL098W	Synonymous
272048	A	G	YJL080C	Leu-Pro (774)
311658	C	A	YJL059W	Ala-Asp (382)
387191	T	C	YJL019W	Ser-Pro (307)
408449	T	C	YJL005W	Tyr-His (986)
511671	C	T	YJR058C	Asp-Asn (77)
564352	C	G	YJR091C	Met-Ile (429)
580754	G	T	YJR098C	Ser-Arg (625)
705687	G	A	YJR159W	Gly-Ser(242)

Two loxPsym sites at position of 392866(*YJL013C*) and 507487(*YJR055W*) were also absent.

Table S5. *SynX* restriction site "landmarks".

Restriction Enzyme	Synthetic coordinates		WT Sequence	New Sequence	Pre-existing/Introduced (P/I)
	Start (bp)	Stop (bp)			
StyI	2341	2349	CCCAAGGTT	CCCAAGGTT	P
SfiI	11202	11216	CCGGCCCTTTTGGCC	CCAGCCTTGTTAGCA	I
SfiI	18746	18760	CCGGCCGTAGTGGCC	CCTGCTGTGGTTGCA	I
SfiI	25103	25117	GGCCACTCCGGCCTC	TGCCACACCTGCTTC	I
BstEII	33528	33536	TGGTAACCT	TGGCAACCT	I
SfiI	40468	40482	GGCCCCCACGGCCAG	GGCTCCGACTGCTAA	I
SfiI	47706	47720	GGCCGAACCGGCCTT	TGCTGAACCAGCTTT	I
SfiI	54687	54701	GGCCAGGATGGCCGA	GGTCAAGATGGAAGA	I
BsoBI	62110	62115	CTCGGG	TTGGGT	I
BglI	68843	68857	TCGCCTAGTTGGCGT	TCGTCTTGTTGGAGT	I
EcoO109I	76251	76257	GGTCCCA	GGTCCCA	P
BglI	82455	82466	GCCAAGGCGGCT	GCCAAGGCTGCT	I
BglI	92227	92238	GCCCAAAAGGCT	GCCAAGCAAAGA	I
BsoBI	102014	102022	AACTCGGGC	AACTCTCGC	I
Bsu36I	108774	108782	ACCTTAGGC	ACCTTAGGC	P
SfiI	115879	115893	GGCCCCCAGGGCCCC	CGCTCCTAAAGCGCC	I
BsoBI	125265	125270	CTCGGG	TTCTGG	I
BglI	134861	134872	AGCCCGATAGGC	TCTCCCATAGGT	I
BglI	143874	143885	GCCCTTAAGGCG	GCCCTTCAATCT	I
BstEII	149977	149985	GGTAACCAT	GGTAATCAT	I
SfiI	156835	156849	GGCCTCCTTGGCCCT	TGCCTCTTTAGCTCG	I
BsoBI	162892	162900	TCTCGGGTG	TCTAGGGTG	I
SfiI	168909	168923	GGCCCCCGCGGCCAG	TGCGCCCGCTGCTAA	I
BanI	177052	177057	GGTGCC	GGGGCT	I
DraIII	184785	184796	GCACCCAGTGTC	GCCCCATCGGTC	I
BstEII	190833	190841	GGGGTAACC	GGTGTCACC	I
SfiI	200299	200313	GGCCGTGGGGGCCGC	AGCAGTAGGGGCAGC	I
SfiI	206381	206395	CTGGCCATCCCGGCC	CTGGCAATCCCAGCG	I
Bsu36I	214468	214476	GTCCTGAGG	GTCTTGAGG	I
SfiI	220736	220750	GGCCGGCGTGGCCTC	AGCTGGTGTTGCCTC	I
BstXI	227954	227968	ACCAGACAGCTGGTT	ACAAGGCAATTAGTT	I
BanI	236279	236287	TCGGCACCA	TCGGCACCA	P
SfiI	244082	244096	GAGGCCATAAAGGCC	GAAGCCATTAAAGCA	I

BstXI	252943	252957	TCCAATTTCCTGGAT	TCCAATTTCCTAGAT	I
BanII	260037	260045	TGAGCCCTG	TGAGCCCTG	P
Bsu36I	266301	266309	TCCTCAGGT	TCTTCTGGT	I
SfiI	272512	272526	GGCCTTAGCGGCCTT	AGCTTTGGCAGCTTT	I
SfiI	279138	279152	GGCCTTCACGGCCAC	TGCCTTGACGGCTAC	I
SfiI	288639	288653	ACGGCCGACCCGGCC	ACCGCTGACCCTGCT	I
BsoBI	295450	295458	GCTCGGGTC	GCTTGGGTC	I
BstEII	301495	301503	CAGGTTACC	TAAATTGCC	I
SfiI	309022	309036	GCGGCCGCCAAGGCC	GCTGCCGCTAAGGCC	I
SfiI	316640	316654	GGCCCCGTCGGCCTT	TGCTCCATCTGCTTT	I
SfiI	324018	324032	GGCCGGATCGGCCGA	GGTAGAATCGGTAGA	I
BsoBI	331776	331781	CCCGAG	CCAGAA	I
Bsu36I	338828	338836	CTCCTTAGG	CTACTTAGA	I
BsoBI	346063	346071	CGCCCGAGT	AGGCCCAGT	I
BglI	353898	353909	GGCCTTGAAGGC	GGCTTTAAAAGC	I
BglI	361082	361093	GGCCTTAATGGC	GGTTTAAATGGT	I
BglI	367471	367482	AGCCTGCAAGGC	AGTTTACAAGGC	I
BglI	375394	375405	GCCACAAAGGCC	GCCACAGAGACC	I
Bsu36I	383126	383134	CACCTTAGG	CACCTTCGG	I
SfiI	389279	389293	GTGGCCGATGTGGCC	GTCGCAGACGTGGCC	I
SfiI	395281	395295	GGCCACTAAGGCCGC	AGCAACCAAAGCAGC	I
SfiI	402928	402942	TCGGCCACAAGGGCC	TCTACCACAGGGTCC	I
SfiI	412404	412418	GGCCAACTTGGCCCA	CGCTAGCTTCGCCCA	I
Eco31I	421476	421487	GGTCTCTAACGC	TGTTTCTAACGC	I
BstEII	427854	427862	AGGTCACCT	AGAAGTCCT	I
SfiI	437396	437410	GCGGCCCGCGAGGCC	GCCGCCAGAGAAGCT	I
SfiI	446749	446763	GGCCTGGCTGGCCCC	GGCCTGGGACGCACC	I
SfiI	456172	456186	GGCCTGGAGGGCCTG	TGCTTGCAATGCTTG	I
BstEII	462235	462243	GGTTACCCT	AGTAACTCT	I
SfiI	468407	468421	AAGGCCGACGTGGCC	AAAGCTGATGTTGCA	I
SfiI	476109	476123	AAGGCCAAGATGGCC	AAGGCAAAGATGGCT	I
BsoBI	482651	482659	ACTCGGGAG	ACTTGGTAG	I
SfiI	490281	490295	GGCCAGTGCGGCCGC	AGCTAAGGCAGCGGC	I
RsrII	496863	496871	CGGACCGCG	TGGACCACG	I
SfiI	504496	504510	ACGGCCGCGCAGGCC	ACGGCTGCTCAAGCA	I
BanI	512733	512738	GGTGCC	GGGGCA	I
SfiI	519139	519153	GGCCGCGAAGGCCTG	GGTAGAGAAGGTTTA	I
SfiI	528574	528588	GTGGCCTCCACGGCC	GTTGCATCAACTGCA	I
Bsu36I	537267	537275	CCTAAGGGT	TCTTAAAGT	I

SfiI	545299	545313	GGCCGGTGCGGCCGA	AGCAGGAGCAGCAGA	I
BsoBI	552118	552126	GCCTCGGGT	GCTTCAGGT	I
BanI	560303	560311	TGGCACCGA	TGGAACACT	I
BglI	567516	567527	AGCCTCTATGGC	AGCCTGTATGGA	I
BsiEI	577348	577356	ACCGGTCGT	ACTGGGCGT	I
SfiI	583510	583524	GAGGCCCCAGAGGCC	GAAGCACCAGAAGCA	I
SfiI	593279	593293	TCGGCCACGGTGGCC	TCTGCAACAGTTGCA	I
BstEII	602624	602632	GGTCACCTG	GGTCATTTA	I
BsoBI	612530	612538	TCTCGGGAC	CCGTGGTAC	I
PflMI	621754	621768	GACCAGCGCATGGAT	GACTAAAGCATGAAT	I
SfiI	630080	630094	GGCCGATGAGGCCGA	AGCTGAACTAGCGCT	I
SfiI	638943	638957	CTGGCCGCGTTGGCC	CTTGCCGCACTGGCT	I
BanI	648302	648307	GGTGCC	GGTACC	I
BsoBI	656648	656656	ACTCGGGAA	ACTCGAGAA	I
BglI	665939	665950	AGCCTGTCGGGC	TCCTTGAGTGGT	I
BsoBI	673543	673548	CCCGAG	CCCGAG	P
SfiI	680986	681000	GGCCACCACGGCCAC	AGCAACTACTGCGAC	I
SfiI	688593	688607	TTGGCCGAGGAGGCC	TTGGCTGAAGAAGCC	I
BanI	695076	695081	GGTGCC	GGCGCT	I
SfiI	702559	702573	GGCCAAGATGGCCCA	GGCCAGGATGGTCCA	I

Table S6. Replication origins in *synX*.

Name	Coordinates on WT Chromosome	Modifications
ARS1001	65-577	Deleted
ARS1002	7445-7943	Deleted
ARS1003	16124-16762	
ARS1004	23662-24158	Insertion of loxPsym site
ARS1005	67469-67950	
ARS1006	99367-99803	
ARS1007	113233-113834	
ARS1008	204029-204917	
ARS1009	228552-229043	
ARS1010	298776-299256	
ARS1011	337281-337529	
ARS1012	374880-375122	
ARS1013	375706-376227	
ARS1014	417195-417440	
ARS1015	442556-442965	
ARS1016	454584-455555	
ARS1017	459337-459895	
ARS1018	540546-540780	
ARS1019	612846-613278	
ARS1020	654375-654614	
ARS1021	683634-684122	
ARS1022	711896-712142	
ARS1023	729983-730513	
ARS1024	737034-737275	Insertion of loxPsym site
ARS1025	744408-745008	Deleted