Type: Report

Title: Allododecaploid yeasts: synthetic hybrids of six species

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Short title: Synthetic six-species hybrids

Field codes: Biotechnology

Abstract

Polyploidy generates diversity by increasing the number of copies of each chromosome. Many plants, animals, fungi, and other eukaryotes are ancient or recent polyploids, including some of the best-known evolutionary radiations, crops, and industrial organisms. Polyploidy facilitates differentiation and adaptation to new environments, but the tools to test its limits are lacking. Here we develop an iterative Hybrid Production (iHyPr) method to produce allododecaploid yeast strains with a base ploidy of 12n. Chromosomal instability increased dramatically as additional copies of the genome were added. These six-species hybrids rapidly improved their fitness during adaptive laboratory evolution. This new method for making synthetic hybrids will enable basic research on polyploidy, cancer, and chromosome biology, as well as more applied research on biofuels, bioproducts, and synthetic biology.

One sentence summary: We constructed six-species synthetic hybrids and showed that they were chromosomally unstable but able to adapt rapidly.

Keywords: *Saccharomyces*, synthetic hybrids, interspecific hybridization, biotechnology, genome instability

Main text

Polyploidy is a widespread phenomenon in biology. Organismal life cycles manipulate ploidy to promote dispersal, environmental robustness, and species diversification (*1*). One of the major drivers of evolutionary innovation is through whole genome duplication (*2, 3*). Polyploidization promotes genome instability and increases mutation rates, both of which can facilitate short-term adaptation to new environments (*4*). The karyotypic diversity generated by polyploidization can lead to tumorigenesis in some cancer types (*5*), but it can also be exploited to develop yeast strains to generate new bioproducts (*6*).

The baker's yeast *Saccharomyces cerevisiae* has been one of the most important model organisms to study polyploidy in the context of evolution (*7*), its effects on mutation rate (*4*), and as a model of how cancer progresses as clonal populations adapt through driver mutations (*8*). Experimental evolution assays in *S. cerevisiae* and comparisons of the genomes of industrial *Saccharomyces* interspecific hybrids have also uncovered many of the same genetic mechanisms that occur during the clonal expansion of tumorigenic cells, such as aneuploidy and loss-of-heterozygosity (*9, 10*).

To experimentally test the limits of chromosome biology and polyploidy in yeasts, we developed an iterative Hybrid Production (iHyPr) method to construct a series of higher-order interspecific hybrids. First, we used two differentially marked HyPr plasmids, which each encode a drug-inducible *HO* gene (*ho*mothallic switching endonuclease) that promotes mating-type switching, to efficiently generate and select for two-species hybrids (*11*). Next, using two additional differentially marked HyPr plasmids, we crossed these two-species hybrids to construct three-species and four-species hybrids. Finally, we

constructed six-species hybrids using three different crossing schemes (Figure 1A, Figure S1).

In all three schemes, diploid genomes were successfully introduced from each of the six parental species, but some species contributed many fewer chromosomes than others to the final strains. Thus, even though the base ploidy of the final strains was allododecaploidy (12n), a ploidy level acquired by a handful of plants and animals in the wild (*12, 13*), none were euploid (Figure S2). During construction, chromosome losses were widespread and outnumbered gains (t-test *p*-value < $1.66*10^{-2}$) (Figure S2). These aneuploidies rose dramatically as the number of species donating genomes increased (linear regression r^2 = 0.78, *p*-value = $3.33*10^{-6}$) (Figure 1B, Figure S2). Complete chromosomal aneuploidies were much more common than aneuploidies caused by unbalanced translocations (97.31% versus 2.69% of total detected chromosomal aberrations). Mitochondrial genome inheritance had a significant impact on the retention of the nuclear genome (ANOVA multifactor *p*-value = $1.21*10^{-3}$), with the mitochondrial genome donor tending to also contribute more nuclear chromosomes (Figure S3-S5).

To determine if the inherent chromosomal instability of these six-species hybrids could be harnessed, we tested how these new six-species hybrids altered their kinetic parameters during adaptive laboratory evolution (ALE) for an estimated 80 generations in a medium containing glucose or in another medium containing xylose, a sugar poorly metabolized by most *Saccharomyces* species (*14*). To provide some growth capability upon which to improve, we chose a *S. cerevisiae* parental strain that had been engineered by inserting xylose utilization genes into Chromosome IV (*15, 16*). Ancestral six-species hybrids grew slowly, and despite differing from each other in chromosomal composition

(Figure S2), single-colony isolates of all 12 ALE replicates outperformed their ancestors in culturing conditions identical to the ALE (Wilcoxon rank sum test, *p*-value = $3.51*10^{-4}$). This improvement in maximum growth rate on xylose occurred regardless of whether they were evolved on xylose or glucose, and many evolved strains outperformed the *S. cerevisiae* reference strain (Figure 1C). In microplate culturing conditions, evolved hybrid populations grew as much as 71% faster on xylose than the reference *S. cerevisiae* strain, and populations evolved on xylose outperformed those evolved on glucose (Wilcoxon rank sum test, *p*-value = $1.29*10^{-2}$) (Table S2, Figure S6), giving further support to the utility of iHyPr and ALE.

Higher-order synthetic hybrids allow investigators to rapidly combine traits from many different parents, such as the xylose consumption of the cellulosic biofuel reference strain of *S. cerevisiae* with traits from other *Saccharomyces* species. Both ALE selection regimes improved performance on xylose, probably partly due to the removal of interspecific incompatibilities through chromosomal loss and partly due to condition-specific effects (Figure 1C, Figure S2, Figure S6). The karyotypic diversity generated allowed for selection from a great deal of phenotypic diversity (Figure S2), a process of great interest for many industrial applications. The fitness improvements observed here also mimic the genetic processes seen in tumor cells escaping antitumorigenic treatments, where polyploidy drives genomic instability and evolution (*8*). In summary, higher-order allopolyploids constructed with iHyPr rapidly acquire genome aberrations that involve multiple species and can rapidly adapt to new environmental conditions. This new technology pushes the yeast cell toward its limits in pursuit of basic research

questions in chromosome biology and evolutionary genetics, as well as potential industrial applications.

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Authors' contributions: Conceived the experiments: DP WGA RLW CTH. Engineered strains: RVM WGA RLW. Data generation: RLW. Data analysis: DP. Wrote the paper: DP CTH.

Competing interests: The Wisconsin Alumni Research Foundation has filed a patent application entitled "Synthetic yeast cells and methods of making and using same" (describing the HyPr method with WGA, DP, and CTH as inventors).

Data and material availability: Raw genome sequencing data has been deposited in NCBI's SRA database, Bioproject PRJNA476226.

Supplementary Materials

Materials and Methods

Supplementary Text

Figures S1-S6

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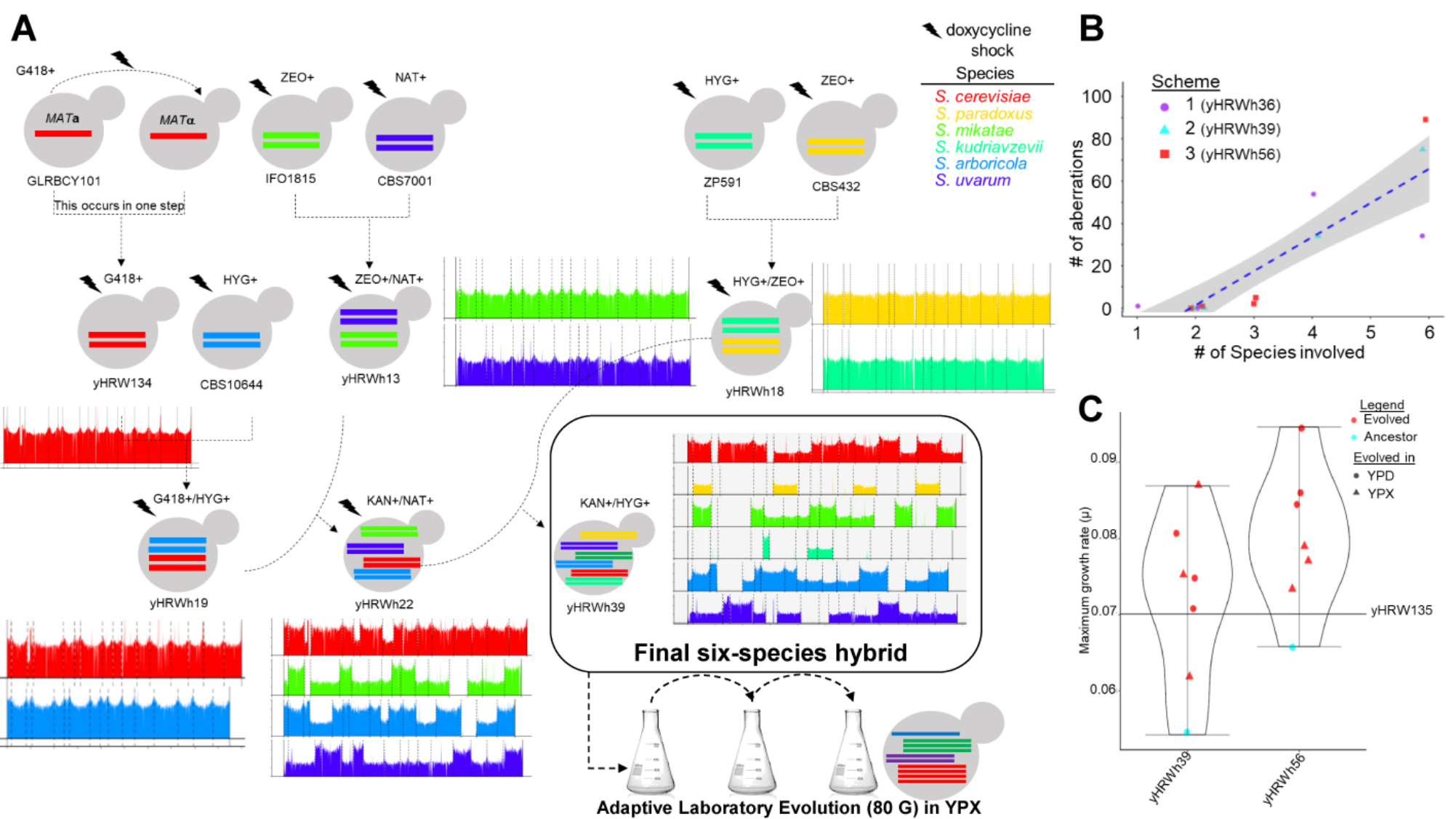
Figure 1. The generation of the six-species hybrids and their use as a tool to study genome instability and improve industrial strains. A) Synthetic hybrid generation scheme using the iHyPr method. The example shown is the six-species hybrid yHRWh39. Chromosomes were colored according to their species designation, with height representing copy number, using the sppIDer pipeline (*17*). For an extended explanation and other schemes, see Figure S1. Arrows mark hybridization steps. For additional intermediary and six-species hybrid nuclear and mitochondrial genomes with higher resolution, see Figure S3, S4. B) Number of chromosomal aberrations inferred versus the number of species contributing genomes (Figure S2, S5). Synthetic hybrids generated from each independent scheme are represented with different shapes and colors. A linear regression line and the 95% confidence interval of the fit are represented with a discontinuous blue color and grey shadow, respectively. C) Box plots for the individual evolved colonies isolated from YPX or YPD plates after ALE and their synthetic hybrid ancestors. Kinetic parameters were tested in 3 mL YPX on a rotating culture wheel, identically to how they were evolved for 80 Generations. The average values (n=6) of

maximum specific growth rates (μ, defined as (ln(OD2)-ln(OD1))/(T2-T1)) for the *S. cerevisiae* reference strain (black line, yHRW135 was derived from yHRW134 by plasmid loss), ancestor (light blue dots), and evolved six-species strains (red dots) are shown (Table S1). Different shapes indicate the media in which the synthetic six-species hybrids were evolved. Color points differentiate the ancestor from the evolved hybrids. Additional kinetic parameters from microplate experiments performed on evolved populations are shown in Figure S6 and Table S2.


A
doxycycline shock
Species
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum
G418+
MATa
MATα
GLRBCY101
This occurs in one step
ZEO+
IFO1815
NAT+
CBS7001
HYG+
ZP591
ZEO+
CBS432
G418+
yHRW134
HYG+
CBS10644
ZEO+/NAT+
yHRWh13
HYG+/ZEO+
yHRWh18
G418+/HYG+
yHRWh19
KAN+/NAT+
yHRWh22
KAN+/HYG+
yHRWh39
Final six-species hybrid
Adaptive Laboratory Evolution (80 G) in YPX
B
Scheme
1 (yHRWh36)
2 (yHRWh39)
3 (yHRWh56)
# of aberrations
# of Species involved
C
Legend
Evolved
Ancestor
Evolved in
YPD
YPX
Maximum growth rate (μ)
yHRW135
yHRWh39
yHRWh56

Supplementary Materials for

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Materials and Methods

Yeast strains and maintenance

The reference strain chosen for improvement was GLBRCY101, a haploid derivative of a *Saccharomyces cerevisiae* strain engineered and aerobically evolved for the consumption of xylose (GLBRCY73) (*16, 15*). Representative strains were selected from five additional *Saccharomyces* species based on published nuclear and mitochondrial genomes (Table S3). These six parent strains were used to generate the six-species hybrids. Yeast strains were stored in cryotubes with YPD (1 % yeast extract, 2 % peptone, and 2 % glucose) and 15 % glycerol at -80 °C. Routine cultures were maintained in YPD plus 2 % agar plates at 24 °C.

Two new *Hy*brid *Pr*oduction (HyPr) plasmids

We previously published two HyPr plasmids with *natMX* (pHCT2) and *hphMX* (pHMK34) resistance cassettes (*11*). Following our previously described methodology, we amplified the *ble* (ZEOcyn resistance) and *nptII* (G418 resistance) coding regions for marker swaps to generate pHRW32 and pHRW40 plasmids, respectively. The new HyPr plasmids enabled complex, iterative crossing schemes without adding extra steps to remove one of the two HyPr plasmids between the hybridization steps.

Saccharomyces transformation with HyPr plasmids

Before transforming GLBRCY101 with a HyPr plasmid, we removed its nuclear *KanMX* cassette by swapping the *KanMX* marker to *TkMX* (*18*). Next, we transformed this strain using a short DNA fragment designed to allow the *TkMX* gene to be removed via homologous recombination and selecting for successful marker loss on synthetic complete (SC) + FUdR medium (0.17 % yeast nitrogen base, 0.5 % ammonium sulfate, 0.2 % complete drop out mix, 2 % glucose, and 50 µg/ml 5-fluorodeoxyuridine). *S. cerevisiae* yHWA85 and representative strains of *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevii*, *Saccharomyces arboricola*, and *Saccharomyces uvarum* were transformed with one of the four HyPr plasmid versions (Table S3). Transformation of yeast strains was done using the lithium acetate/PEG-4000/carrier DNA method, as previously described (*19*), with previously described modifications for particular species (*11*). Cells were suspended in YPD, followed by incubation at each species' optimal temperature (i.e. 30 °C for *S. cerevisiae* and room

temperature for all others, including hybrids) for 3 h before being plated on selective medium. Selective media consisted in YPD plus 2 % agar plates with the appropriate selectable drug(s). *S. cerevisiae* yHWA85 was first diploidized using the HyPr plasmid pHRW40, creating yHRW134 for subsequent crosses. The generation of this diploid strain occurred in one step, which was confirmed by polymerase chain reaction (PCR) amplification of the *MAT* loci (see below). The experimental reference strain yHRW135 was derived from yHRW134 by screening for spontaneous plasmid loss.

iHyPr (*i*terative HyPr) method for sequentially generating higher-order hybrids

Following the HyPr method to facilitate mating-type switch (*11*), we pre-cultured strains with differentially marked HyPr plasmids in the presence of doxycycline to express the endonuclease encoded by *HO*, which is under a Tet-ON promoter; each plasmid also contains the full machinery for inducible expression of the promoter. To generate the six-species hybrids yHRWh36 and yHRWh39, we first forced three separate pairs of species to mate, generating two-species hybrids. In each case, once the three two-species hybrids were generated, we crossed two of those two-species hybrids to create a four-species hybrid, which finally was crossed with the last two-species hybrid to generate the six-species hybrid. To generate the six-species hybrid yHRWh56, two separate two-species hybrids were separately crossed with diploid *Saccharomyces* strains from other species to create two separate three-species hybrids, which were then mated to generate the six-species hybrid (Figure S1). Before each cross, parental strains were transformed with differentially marked HyPr plasmids (Table S3, Figure S1) and treated with doxycycline in YPD at room temperature, except for *S. cerevisiae* where 30 °C was applied. As previously described (*11*), the doxycycline triggers the expression of the Ho endonuclease, which cuts one or more *MAT***a**/*MAT*α idiomorphs and generates mating-compatible strains that behave as either *MAT***a** or *MAT*α. A sample of each culture was combined in a 1-mL Eppendorf tube and patched on a YPD plate. After 2-3 days, a sample was taken with a toothpick and streaked on a YPD plate supplemented with the corresponding drugs to select for successful matings. In contrast to the original HyPr method, we pre-cultured the new hybrid in YPD with one of the two drugs used during the selective medium step, and that hybrid was then crossed with another strain containing one or two of the other HyPr plasmids not used previously. During these subsequent

steps, we expected (and phenotypically verified) the loss of the HyPr plasmid containing the drug-resistance cassette not under selection. This approach and the additional HyPr plasmids made for this study facilitated the iterative crosses required to make six-species hybrids by avoiding the steps of plasmid removal and minimizing the number of generations between crosses (Figure S2).

Mating-type and PCR-RFLP confirmation of strains

Diploidization of the *S. cerevisiae* strain was confirmed by PCR at the mating-type locus. Hybrid statuses were confirmed by restriction fragment length polymorphism (RFLP) analysis. We used the Standard Taq Polymerase (New England Biolabs, Ipswich, MA), and the primers listed in Table S4. Genomic DNA was extracted using the phenol:chloroform method on a strain grown from pre-culture to saturation in YPD. Aliquots of 700 µl of saturated culture were located in 1.5 mL microcentrifuge tubes that contained acid-washed beads. Each tube was centrifuged at maximum speed (15000 rpm) for 5 minutes, and the supernatant was removed. 200 µl of buffer EB (10 mM Tris-Cl, pH 8.0), 200 µl of DNA lysis buffer (10 mM Tris pH 8.0, 1 mM EDTA, 100 mM NaCl, 1 % SDS, 2 % Triton X-100), and 200 µl of phenol:chloroform were added to each tube. Vigorous vortexing was performed for 3-4 minutes, followed by 5 minutes of centrifugation at maximum speed. The top aqueous layer was transferred to 1 mL 100 % EtOH. After an inversion mixture, DNA was precipitated at -80 °C for at least 10-15 minutes. A second centrifugation at maximum speed was performed, and the supernatant was discarded. We washed the pellet with 700 µl of 70 % EtOH, and we centrifuged again to remove any residue or trace of the supernatant. The pellet was dried and resuspended in 100 µl of EB at 50-60 °C for 30 minutes. To remove RNA, we incubated the solution with 0.5 µl of 10 mg/ml RNase A for 30 minutes at 37 °C. DNA was quantified with a Qubit 2.0 Fluorometer (ThermoFisher Scientific). For PCR-RFLP, resulting PCR products were digested with a restriction enzyme or a combination of multiple restriction enzyme assays able to discriminate among *Saccharomyces* species (New England Biolabs, Ipswich, MA). An extended PCR-RFLP pattern, developed in previous publications (*11, 20*) and this study, are detailed in Table S5. Undigested PCR products were visualized on a 1.5 % agarose gel, while digested PCR products were visualized on a 3 % agarose gel.

Adaptive laboratory evolution (ALE) and colony selection

Two of the three six-species hybrids (during construction, the third lost *S. cerevisiae* chromosome IV, where *Scheffersomyces (Pichia) stipitis* xylose utilization genes had been inserted) were evolved in triplicate at room temperature in tubes with two independent media conditions: 3.0 mL YPD or 3.0 mL YPX (1 % yeast extract, 2 % peptone, and 2 % xylose). Three to five days of fermentation were performed to allow cells to consume the sugars, and an aliquot of each replicate was transferred at of 0.1 optical density at 600 nm (OD_{600}) to a fresh medium until it reached approximately 80 generations. A colony from each independent ALE experiment, regardless of whether they were evolved in glucose or xylose, was selected on YPX plates (1 % yeast extract, 2 % peptone, 2 % xylose, and 2 % agar).

Genome sequencing and chromosome composition

Genomic DNA (gDNA) samples from the diploidized *S. cerevisiae* strain and the two-, three-, and six-species hybrids were submitted to the DOE Joint Genome Institute for paired-end Illumina sequencing. Libraries were constructed according to the manufacturer's instructions. Sequencing of the flow cell was performed on an Illumina MiSeq using MiSeq Reagent kits, following a 2x150 nucleotide (nt) indexed run recipe. Curated raw reads were submitted to the SRA database as Bioproject PRJNA476226 (Table S6).

Genomic characterization was performed with `sppIDer` (*17*). Our combined nuclear reference genome was built with the genome assemblies of *S. cerevisiae* GLBRCY22-3 (*21*), which is a close relative of the biofuel reference strain used here; *S. paradoxus* CBS432; *S. arboricola* CBS10644 (*22*); *S. mikatae* IFO1815; *S. kudriavzevii* ZP591; *S. uvarum* CBS7001 (*23*); and *Saccharomyces eubayanus* FM1318 (*24*). Our combined mitochondrial reference genome was built with the mitochondrial assemblies of the aforementioned strains (*22, 24, 25*), except for CBS7001, whose mitochondrial genome is still not completely assembled (*26*). Instead, we used the mitochondrial genome of a close relative, *S. uvarum* CBS395 (*25*). Raw Illumina paired-end reads and the combined reference genomes were the input data of `sppIDer`, which is essentially a wrapper that runs published tools to map the short reads to the combined reference genomes and creates several colorful and visually intuitive outputs (*17*). Here, we show depth of coverage plots from those species contributing genomes.

The percentage of each *Saccharomyces* nuclear genome retained in the hybrid was calculated as follows:

$$Pspp = (Ct * Ws / Gs)*100$$

where Pspp, is the percentage for one of the parental species; Ct, is the number of windows with a coverage mean value above 2; Ws, is the window size; and Gs is the reference genome size for that parental species. This calculation yielded a good approximation of contribution of each species to the genome of the hybrid. For each strain, the number of chromosomes, and the ploidy were estimated from the `sppIDer` plots. The number of chromosomal aberrations was based on the number of gains, losses, or unbalanced translocations detected in the `sppIDer` plots. One chromosomal gain, loss, or unbalanced translocation was counted as one aberration. Aberrations observed in one hybrid and maintained in the offspring of subsequent crosses were not counted again; only new aberrations for each cross were reported in the aberration plot (Figure 1B).

Microtiter plate growth curves

We compared the growth kinetics of the *S. cerevisiae* reference strain yHRW135, the ancestors of the two six-species hybrids retaining the chromosome IV (yHRWh39, yHRWh56), and populations of the evolved hybrids. Growth was tested in YPD and YPX at room temperature. Strains or populations were pre-cultured in 3.0 mL YPD or YPX, depending of the medium tested. After pre-culture, 10 µl of saturated culture was inoculated into a 96-well plate (Nunc, Roskilde, Denmark) containing 240 µl of identical medium as the pre-culture. Spaces between the wells in the plates were filled with sterile H_2O to maintain the humidity of the plates. The reference strain was cross-inoculated in all conditions; for example, yHRW135 pre-cultured in YPX was tested in both YPD and in YPX.

To monitor the growth of strains and populations in the different media conditions, we inoculated 96-well plates and placed them in a BMG FLUOstar Omega at 20 °C. Absorbance at 595 nm was monitored every 15 min for 2 days. Background absorbance was subtracted from the average of three negative controls containing the uninoculated medium being tested. Kinetic parameters for each condition were calculated in `GCAT v6.3` (*27*). Median and standard deviations from three independent biological replicates

were calculated in `R` (*28*) (Table S2). For each medium condition, parameters were normalized against the reference strain, yHRW135, which had been pre-cultured and grown in the medium tested.

Culture wheel growth curves

Strains isolated from single colonies from evolved hybrids, ancestral hybrids, and the reference strain (yHRW135) were pre-cultured in YPX, and inoculated at an initial OD_{600} of 0.1 into 3 mL glass tubes containing YPX. Growth was monitored by measuring OD_{600}. Kinetic parameters were calculated as above. Median and standard deviations from six independent biological replicates were calculated as above. These experimental conditions most closely matched the conditions in which the strains were evolved, and they are reported in Figure 1C and Table S1.

Statistical analysis

Data analysis and plots were performed in `R` (*28*). A linear model of regressions were added to the plot in Figure 1B using the `geom_smooth` option in the R package `ggplot2`, and r^2 and significance of regression were calculated with `summary(lm(y ~ x))`, where x was the number of species, and y was the number of observed aberrations.

The impact of mitochondrial inheritance (Figure S5B) in the retention of the nuclear genome of those hybrids involving *S. cerevisiae* was tested using a multifactor ANOVA in `R`, using `summary(aov(P ~ M * C))`, where P is the percentage retained of the *S. cerevisiae* nuclear genome; M is the mitochondrial genome, which was encoded as a binary character (either as the *S. cerevisiae* mitochondrial genome or that of another species); and C is the type of strain (i.e. classified as parental *S. cerevisiae*; two-, three-, four-, or ancestral six-species hybrid; and evolved six-species hybrid).

t-tests for significant differences between frequency of chromosome gains and losses and Wilcoxon rank sum tests for significant differences in the kinetic parameters shown in Figure 1C and S6, respectively, were performed in `R`.

Supplementary Text

High instability of chromosome III

Industrial conditions offer a niche to interspecific hybrids due to the stressful conditions (*29-35*). The existence of allotriploids and allotetraploids have generated speculation

about rare-mating as one of the mechanisms for the generation of hybrids (*36-38*). For rare-mating, one of the diploid cells must convert the heterozygous *MAT* locus into a homozygous state (*MAT***a**/*MAT***a** or *MAT*α/*MAT*α) or a hemizygous state (*MAT***a/-** or *MAT*α/-), such as after a double-strand break. Such mating-compatible diploids are then able to mate with a compatible cell of the opposite sex (*39*).

HyPr exploits the rare-mating system by heterologously expressing the *HO* gene from differentially marked plasmids (*11*). We expected that Ho would cut one copy of the heterozygous *MAT* locus of the diploid strain and use homology repair to convert the locus from heterozygous to homozygous, presumably over a small gene conversion patch using either a homologous chromosome or a silent mating cassette as a template. Although both this mechanism and larger breakage-induced replication events likely occur some of the time at this locus, 88.9% of the translocations involving chromosome III were unbalanced (Figure S2), suggesting other repair mechanisms are also leading to mating-type locus hemizygosity or homozygosity. For example, the high number of unbalanced translocations targeting chromosome III (40 % or six of fifteen hybrids) might support the occurrence of imperfect Non-Homologous End Joining (NHEJ) events, perhaps promoted by overexpression of the *HO* gene (*39*). Another scenario for the frequent rearrangements in chromosome III might be the high instability observed for that particular chromosome (*40*). Synthetic hybrids between *S. cerevisiae* and *S. kudriavzevii* have demonstrated how easily chromosome III of one of the parents can be lost, rendering the hybrid competent to mate again (*41*). Recent studies of interspecific hybrids from the genus *Zygosaccharomyces* have also shown that inactivation of one of the *MAT* locus copies can also restore sexual competency (*42, 43*). Regardless of the precise mechanisms at work, the iHyPr method clearly facilitated the recovery of the sexual competency of higher-order hybrids by controlling and exploiting these naturally occurring mechanisms to generate interspecific hybrids.

Heteroplasmic state

During interspecific hybridization, hybrids can inherit one of the two parental mitotypes or a recombinant version (*44*). In general, one of the parental mitotypes was quickly fixed during the generation of our hybrids here, except for: the allotetraploid *S. kudriavzevii* x

S. mikatae yHRWh4, the allotetraploid *S. cerevisiae* x *S. uvarum* yHRWh10, and the six-species hybrids yHRWh36, which were all heteroplasmic (Figure S4).

These three exceptions were all part of the same crossing scheme, and we offer a set of related possible explanations. The presence of selfish elements, such as homing endonucleases, could explain why multiple mitotypes were retained in yHRWh4. In this case, a portion of *Smik-COX1*, a gene with a high number of introns invaded by homing endonuclease genes (*45*), seems to have been introduced into the *Skud* mitochondrial genome (mtDNA) (Figure S4A,C). The most intriguing result occurred while generating the yHRWh10 hybrid, which retained most of both parental mtDNAs (*S. cerevisiae* and *S. uvarum*) and remained in a heteroplasmic state (Figure S4A). We recently demonstrated that, during formation of *S. cerevisiae* x *S. uvarum* hybrids, the frequency of petite generation (e.g. strains with incomplete or no mitochondrial genome) was higher when the hybrid inherited a *S. uvarum* mtDNA (*26*). Introgression of the F-*Sce*III homing endonuclease gene into a *Suva*-mtDNA restored normal mitochondrial retention (*26*). In yHRWh10, we did not detect the presence of F-*Sce*III, which might have influenced the loss of mtDNA in its descendants. For example, the six-species hybrid yHRWh36 retained only a small region of *Sarb*-mtDNA and *Suva*-mtDNA (Figure S4C). The role of F-*Sce*III in the stabilization of the mitochondrial genome is still unclear, but it may be playing an important role in our crosses. In another recent study, mtDNA inheritance was also demonstrated to be dominated by one parental strain due to nuclear-mitochondrial interactions, rather than occurring stochastically (*46*).

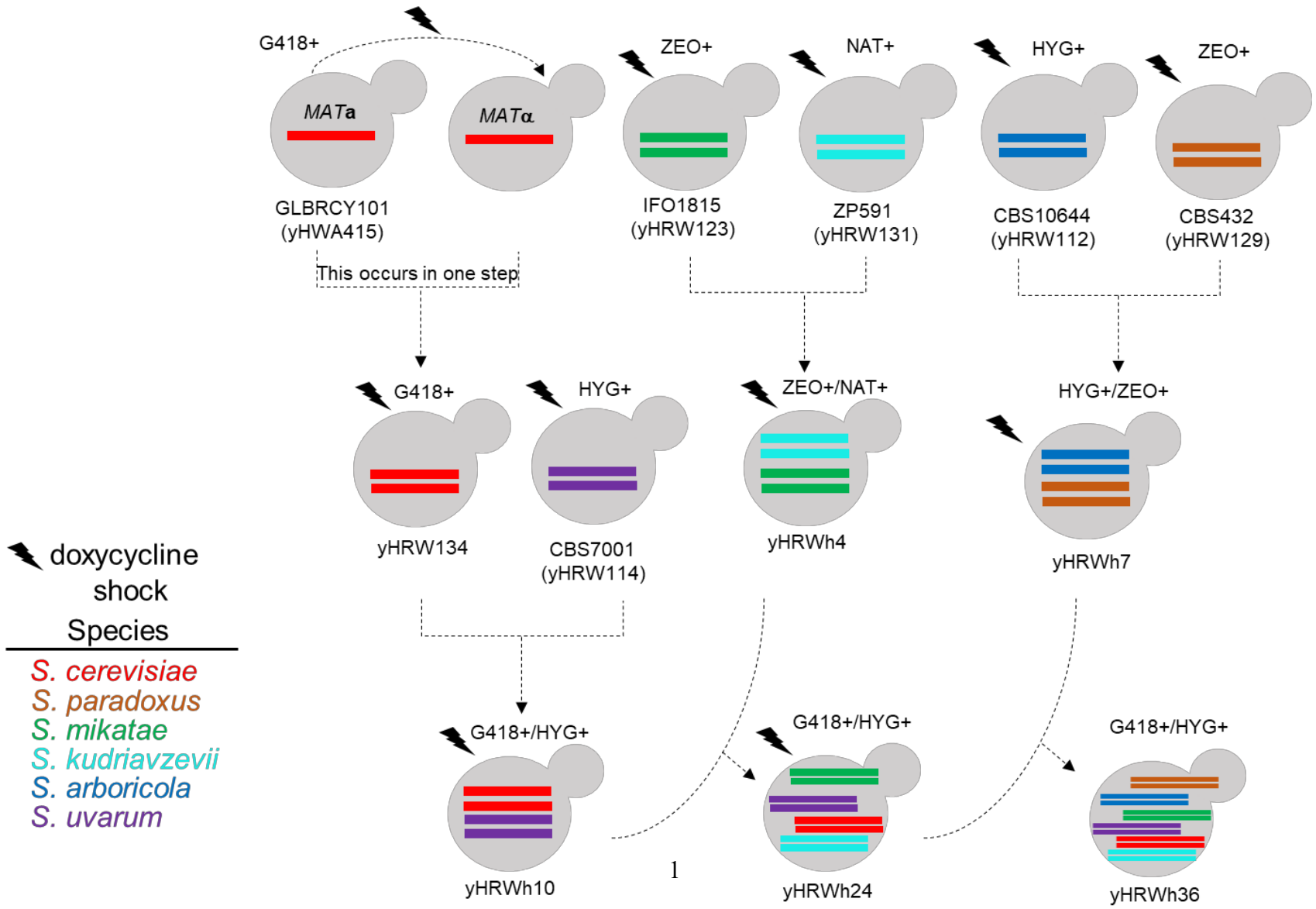
The loss of mitochondrial genomes in particular hybrid combinations, and the unusually high or low coverage (Figure S4) might suggest that interactions between nuclear-encoded mitochondrial proteins with the mtDNA are unbalanced. In such cases, one model proposes that an oligomeric circular mtDNA form precedes ρ^- strain formation (*47*). The Illumina read coverages for some regions of the mtDNAs were surprisingly varied in some hybrids, such as yHRWh8, yHRWh13, and most of the hybrids used for yHRWh36 generation (Figure S4). Formation of mtDNA concatemers are regulated by levels of Din7p and Mhr1p (*47*); when hybrids form, these protein levels might be misregulated due to different cis- and trans-regulation, which could increase or decrease the copy numbers of specific mitochondrial regions. Technical artifacts from Illumina

sequencing also cannot be excluded, and this phenomenon and process merits further study.

Table and Figure captions

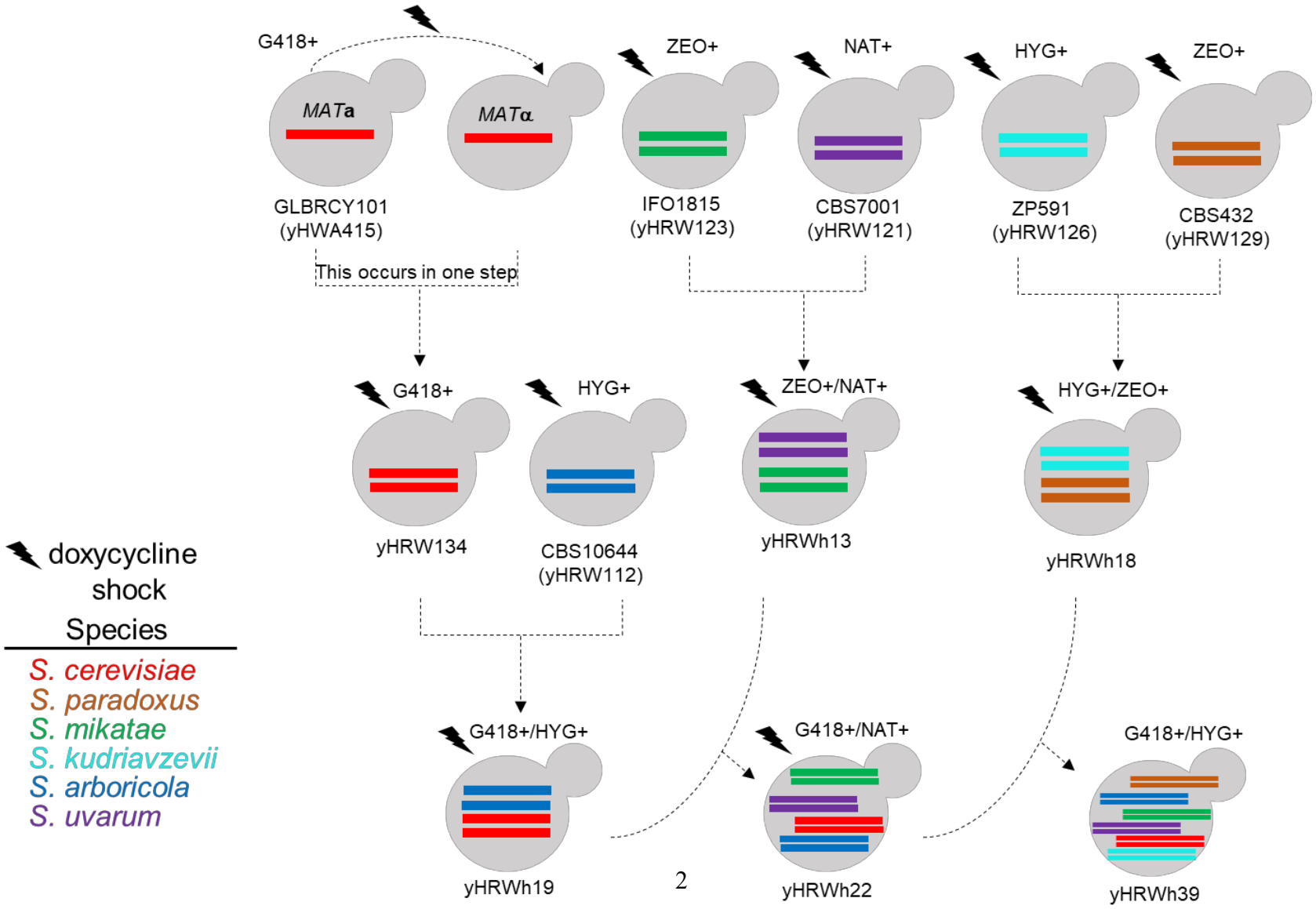
A

Scheme 1: yHRWh36



B

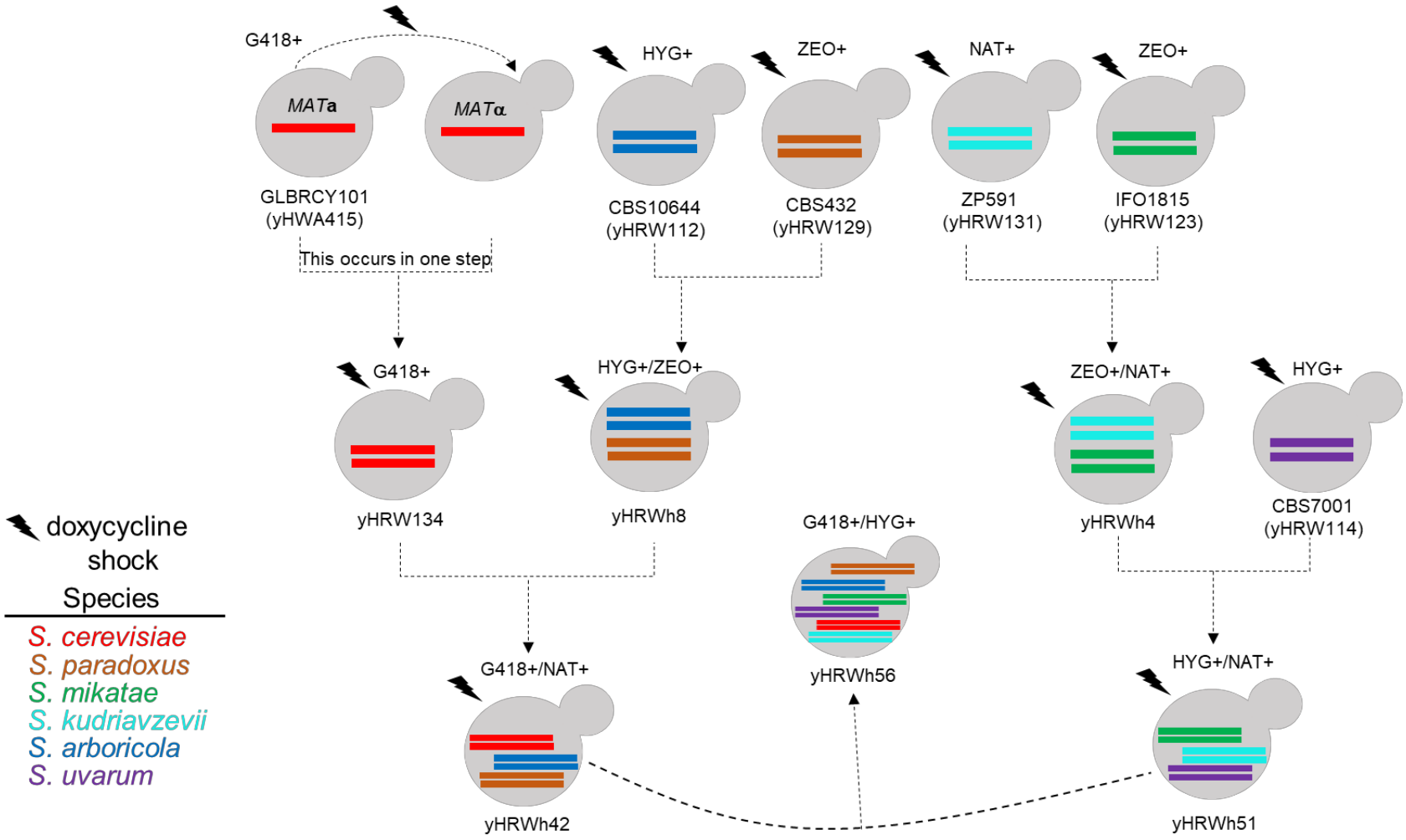
Scheme 2: yHRWh39



2

C

Scheme 3: yHRWh56



3

Figure S1 Schematics for the generation of three six-species *Saccharomyces* hybrids.

The hybridization steps necessary to generate the six-species hybrids yHRWh36, yHRWh39, and yHRWh56 are represented in panels A), B) and C), respectively. Yeast cells are represented in gray, and chromosomes are colored according to the *Saccharomyces* species. The strain names of our lab's copy of some strains (Table S2) are displayed in parentheses below the original culture collection strains. Drug-marker resistance is indicated above yeast cells. Systematic crosses are highlighted with arrows to form a pedigree. The black lightning bolt symbol represents the doxycycline shock to promote *MAT*ing-type switching or loss to facilitate hybridization.

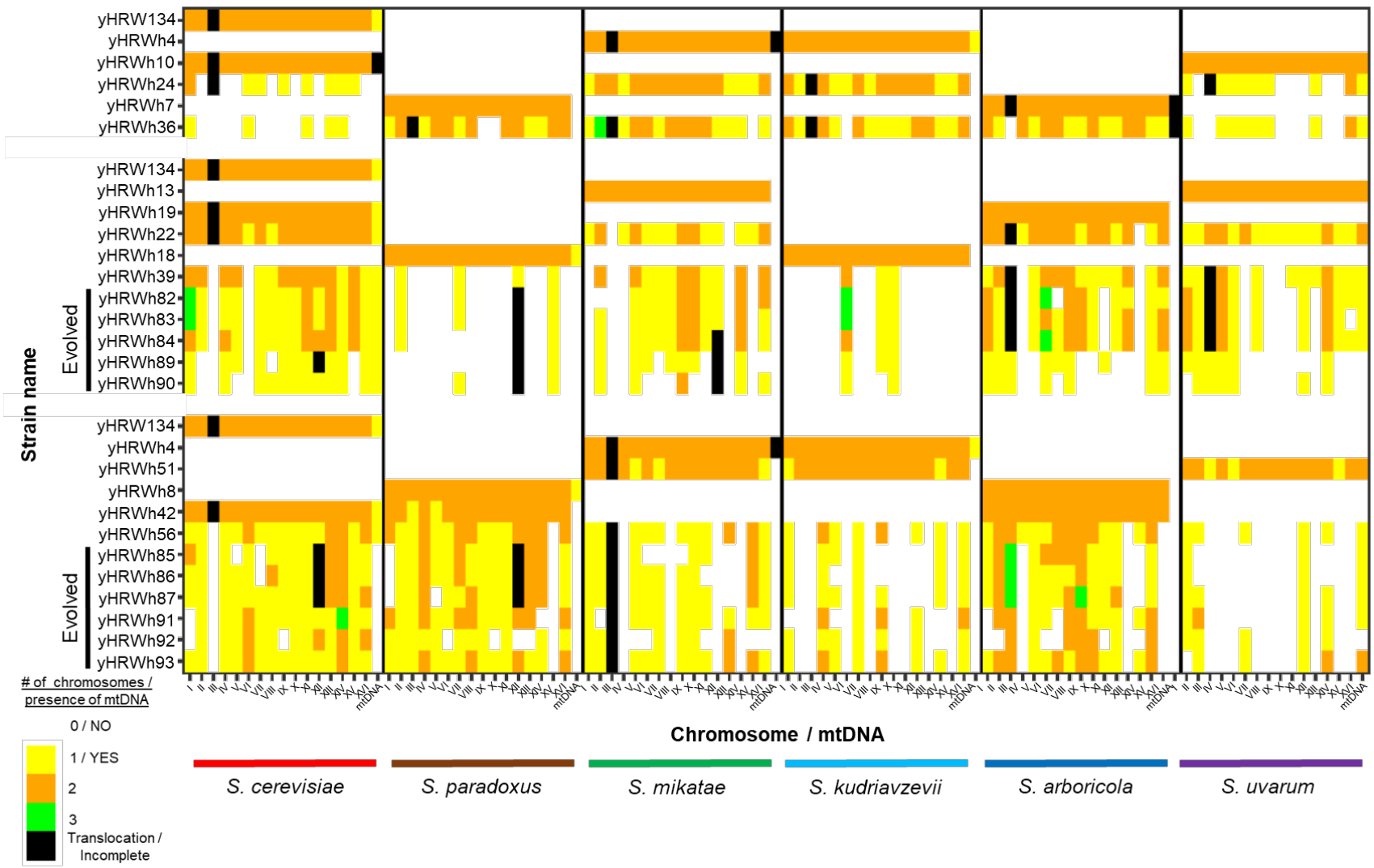
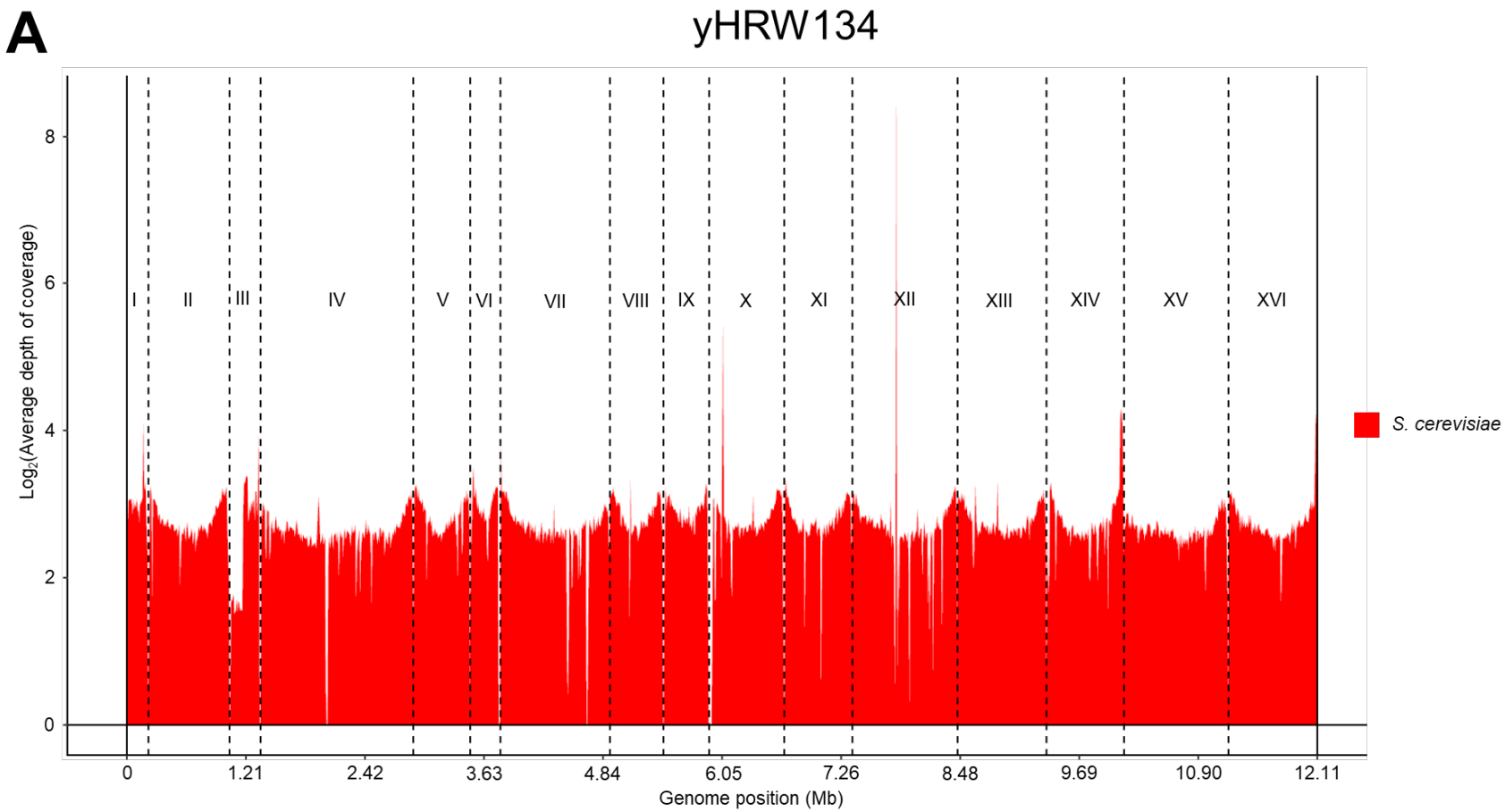


Figure S2 *Saccharomyces* species genome contribution to synthetic hybrids.

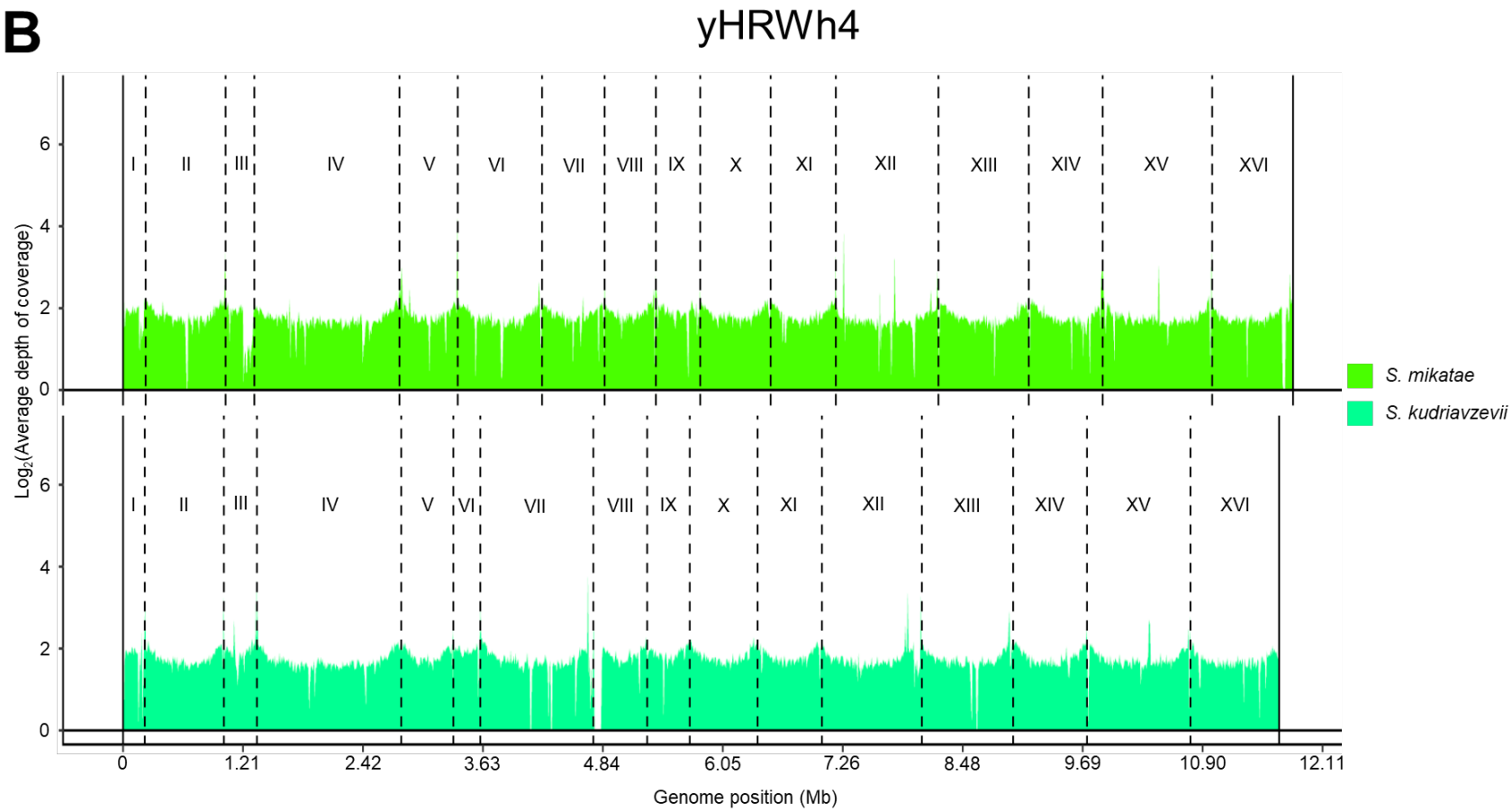
Number of chromosomes for each synthetic hybrid and the *Saccharomyces* species designation were inferred from `sppIDer` plots (Figure S3). The chromosome content was colored according to the species donor. Mitochondrial inheritance

16 was inferred based on the `mitosppIDer` output (Figure S4). Presence, absence, or incomplete mitochondrial genomes
17 are colored in yellow, white, and black, respectively. Synthetic hybrids were ordered as they were used to generate the next
18 hybrid (Figure S1). Diploidized GLRBCY101 (yHRW134) and yHRWh4 are shown multiple times because of their use in
19 multiple crossing schemes. Note that considerable karyotypic diversity continued to be generated during 80 generations of
20 ALE, but each evolved strain is easily recognized as more similar to its ancestral six-species hybrid.
21


A
yHRW134
Log2(Average depth of coverage)
8
6
4
2
0
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. cerevisiae
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)

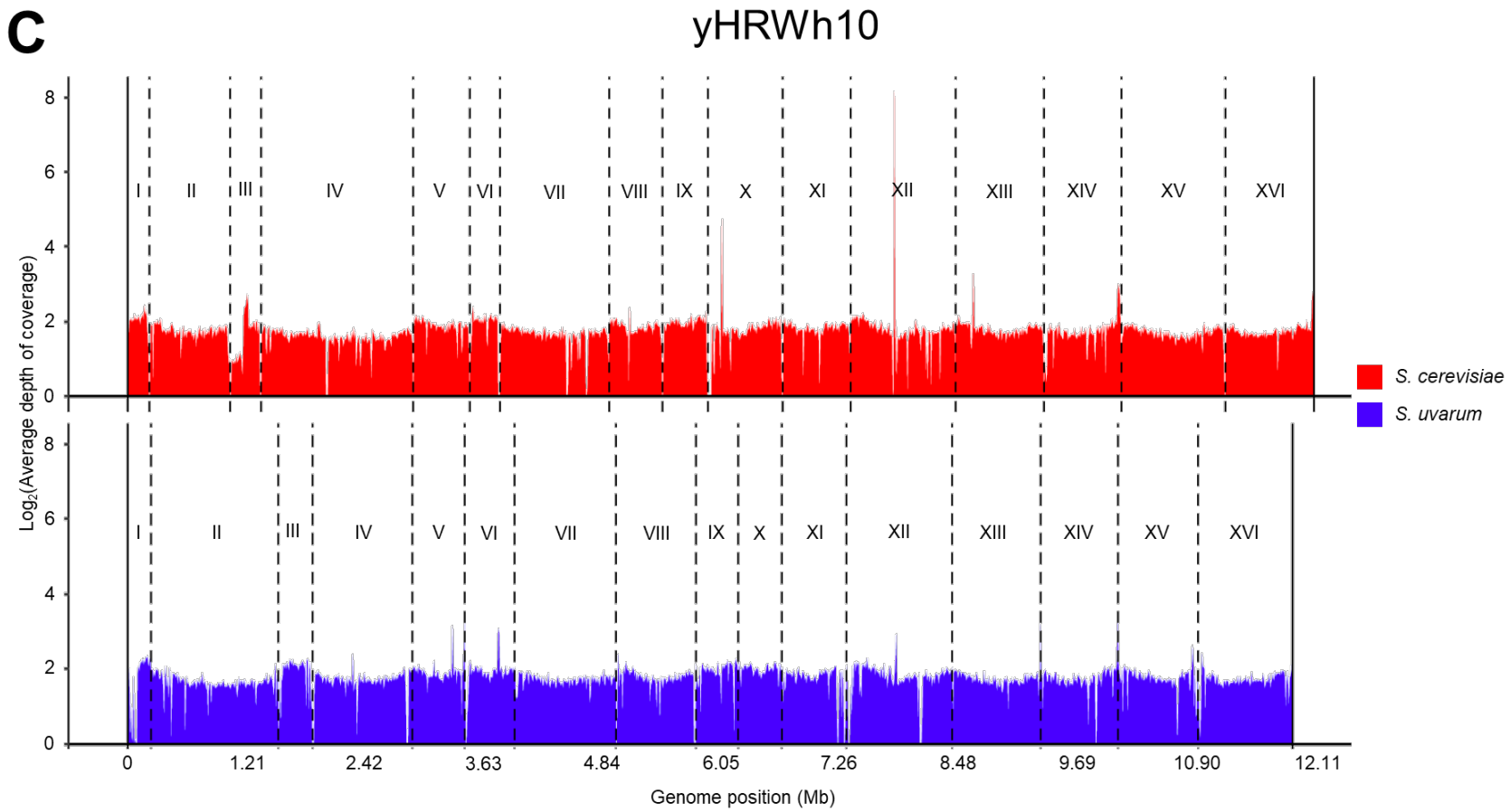
22

23


B
yHRWh4
Log2(Average depth of coverage)
Genome position (Mb)
S. mikatae
S. kudriavzevii
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
0
2
4
6
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11

24

25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yHRWh10
Log2(Average depth of coverage)
Genome position (Mb)
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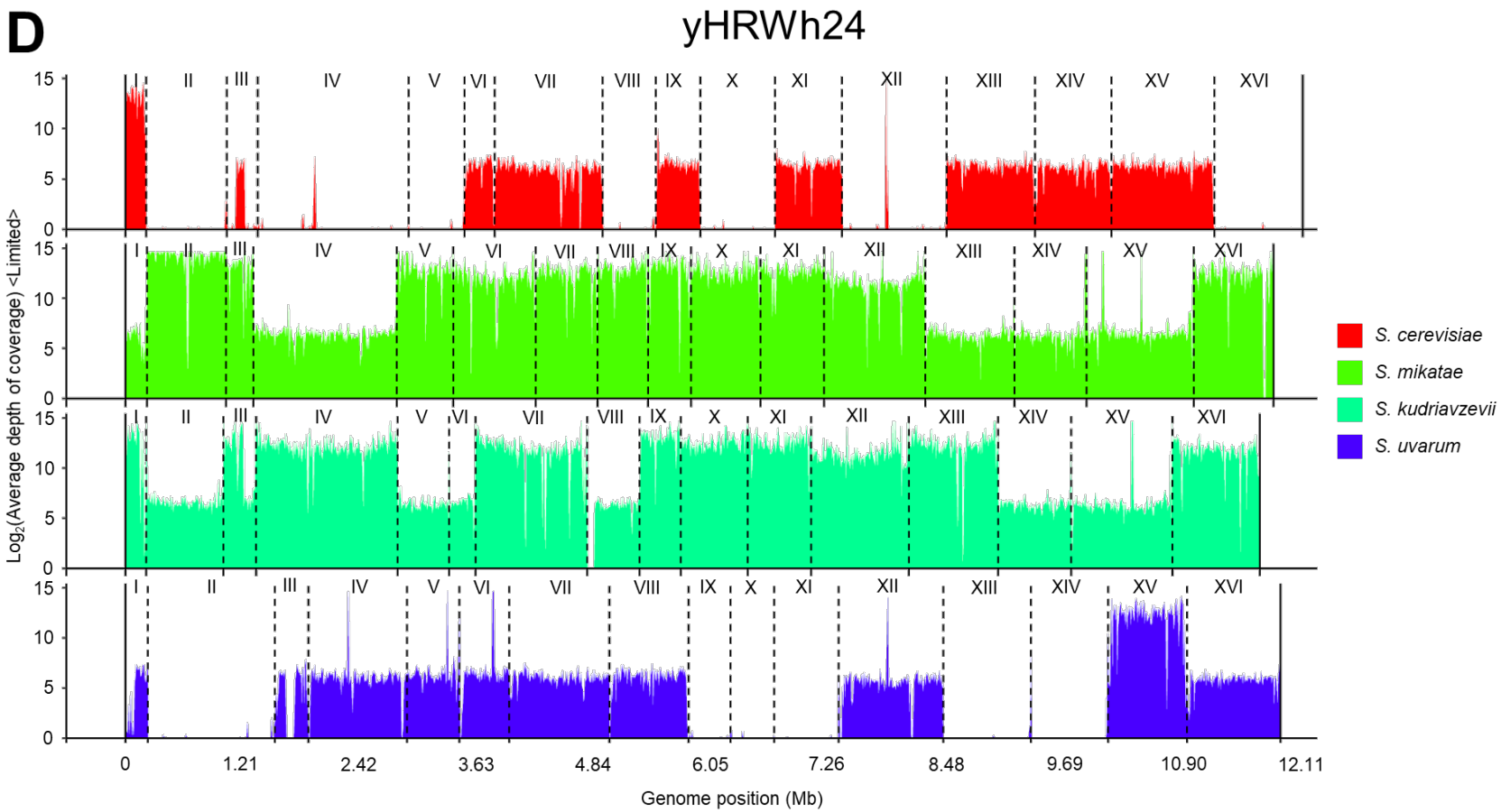
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. cerevisiae
S. uvarum

26

27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yHRWh24
Log2(Average depth of coverage) <Limited>
Genome position (Mb)
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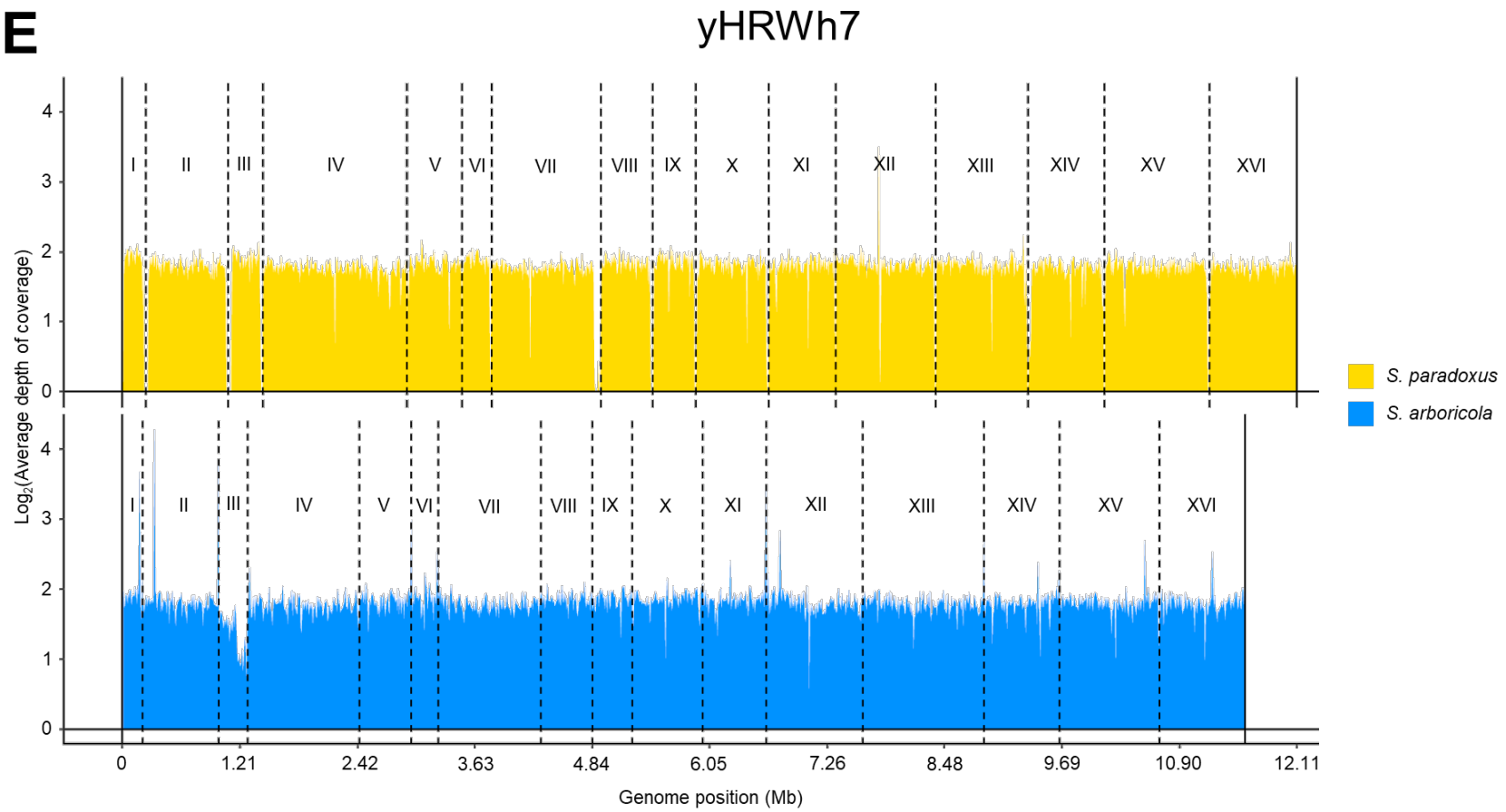
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. cerevisiae
S. mikatae
S. kudriavzevii
S. uvarum

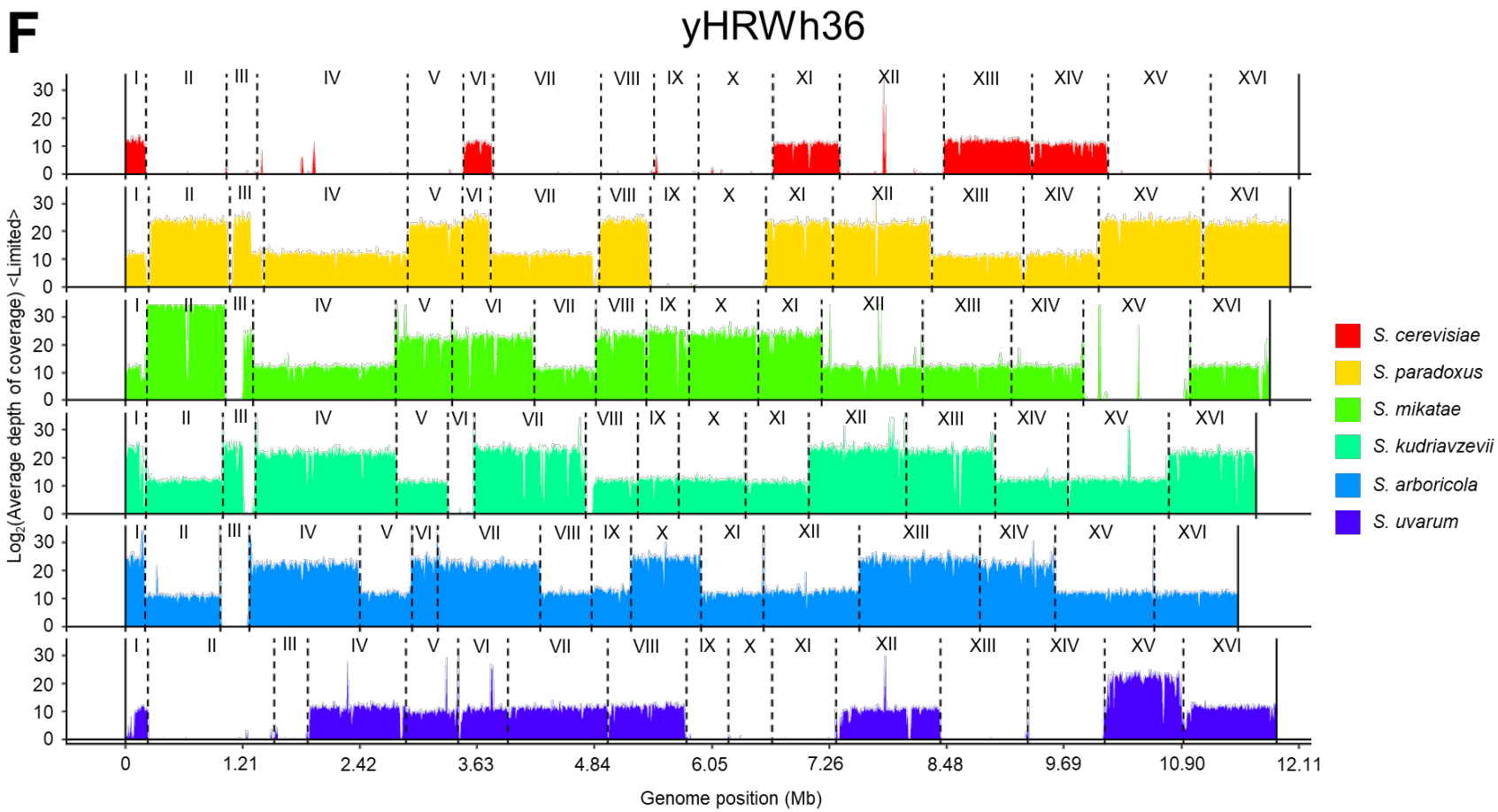
28

29


E
yHRWh7
Log2(Average depth of coverage)
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. paradoxus
S. arboricola
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)

30

31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yHRWh36
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Genome position (Mb)
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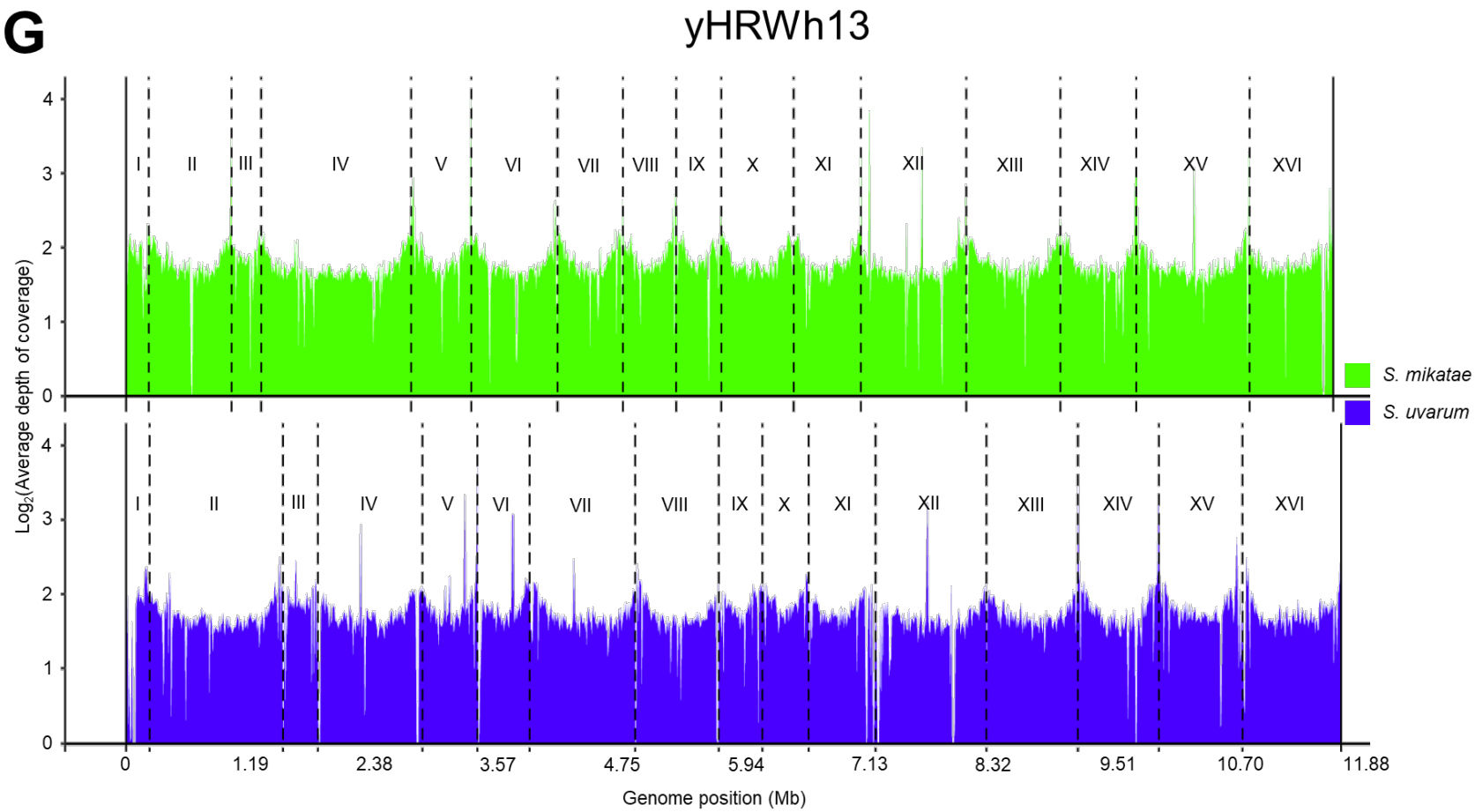
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

32

33


G
yHRWh13
Log2(Average depth of coverage)
Genome position (Mb)
S. mikatae
S. uvarum
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
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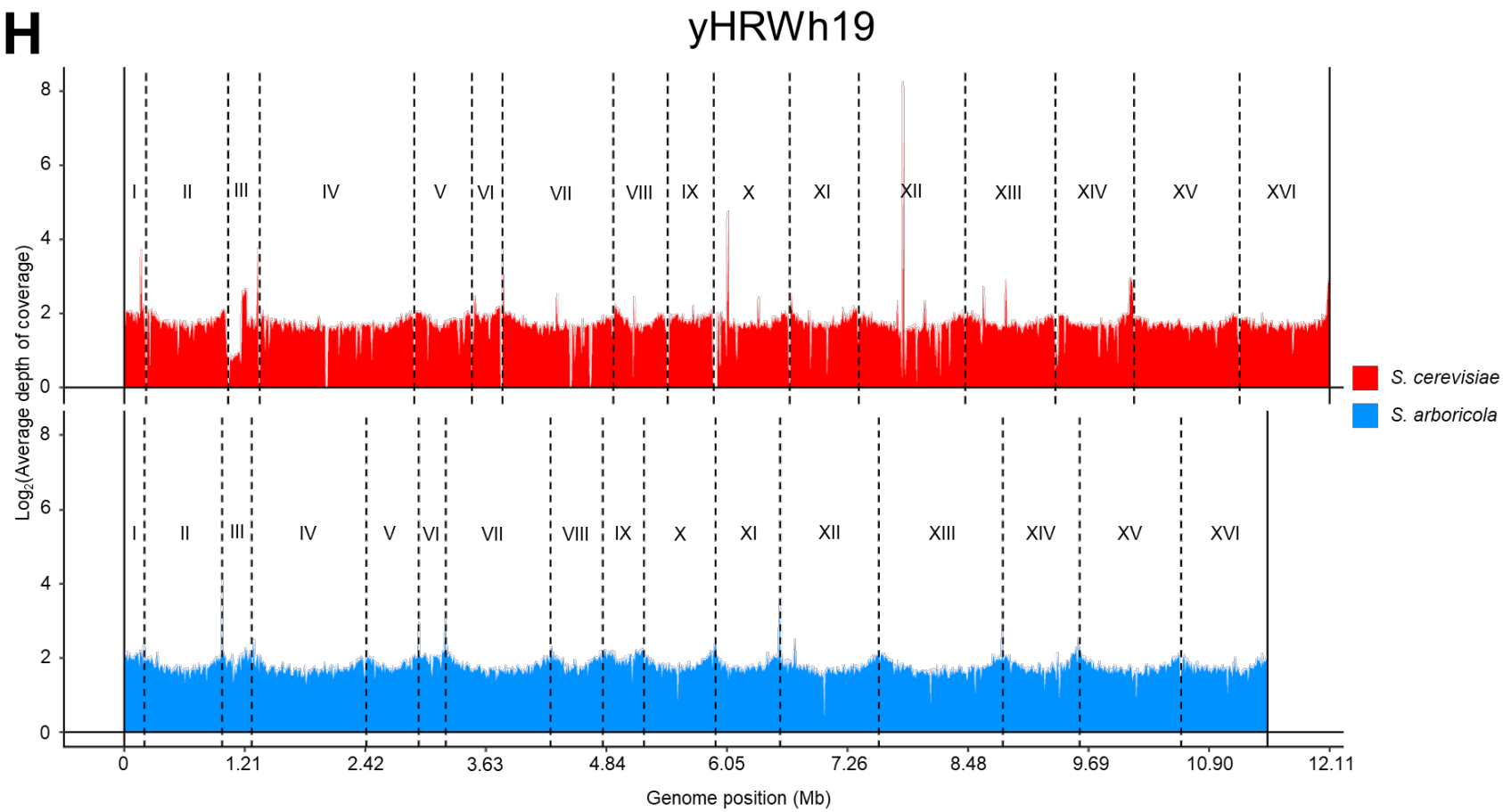
8.32
9.51
10.70
11.88

34

35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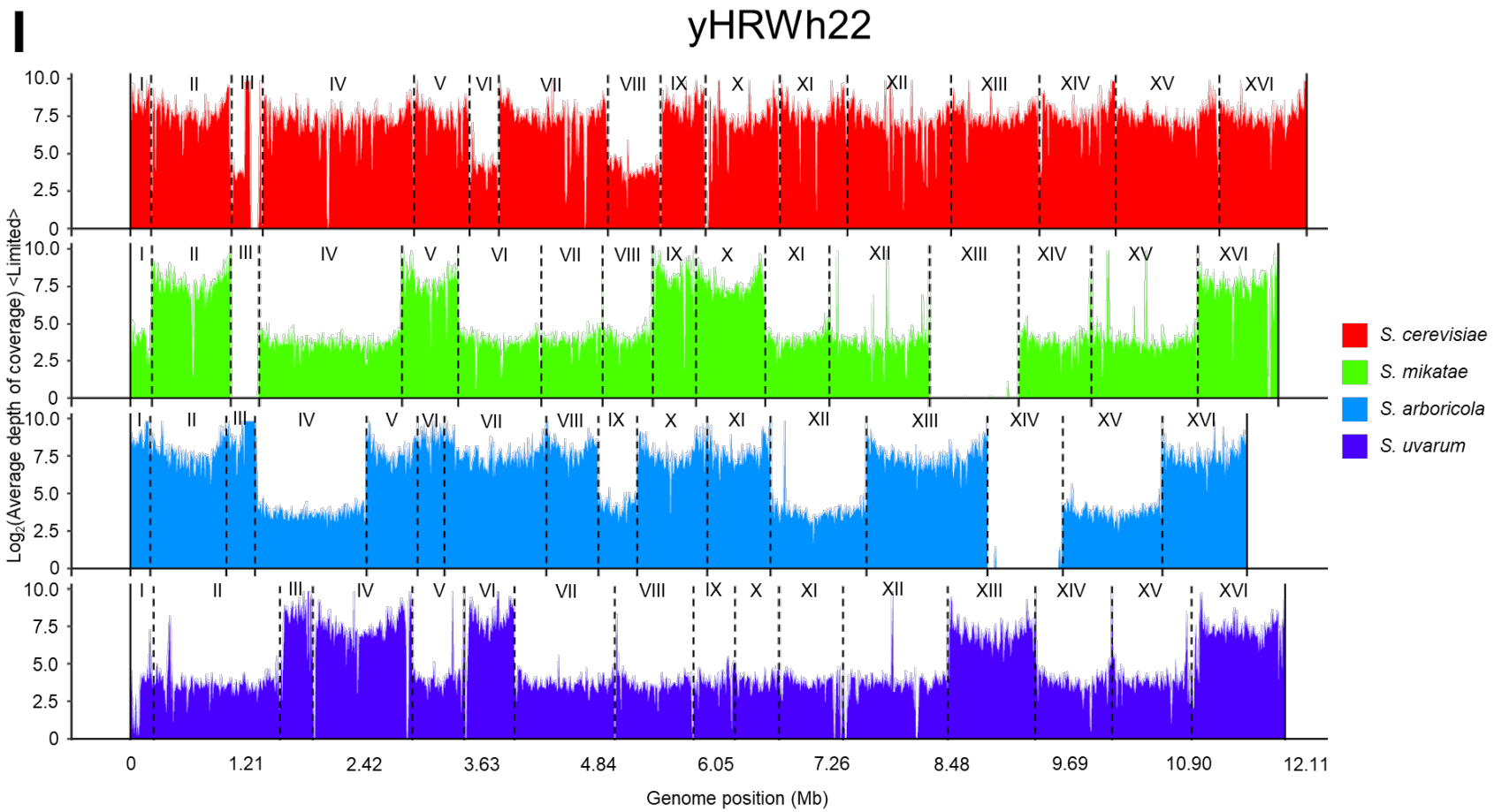
yHRWh19
Log2(Average depth of coverage)
8
6
4
2
0
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. cerevisiae
S. arboricola
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)

36

37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Log2(Average depth of coverage) <Limited>
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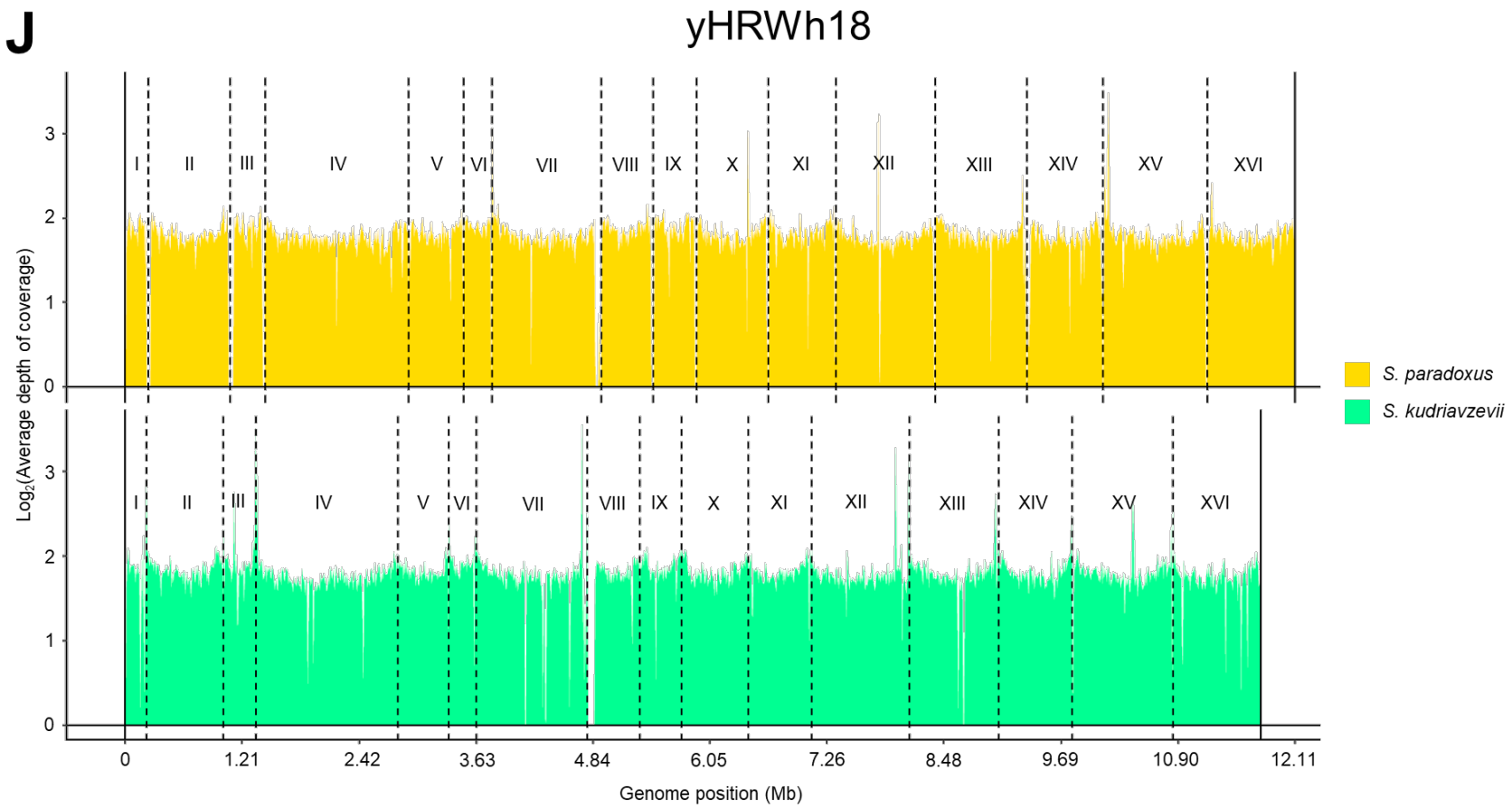
5.0
2.5
0
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)
S. cerevisiae
S. mikatae
S. arboricola
S. uvarum

38

39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yHRWh18
Log2(Average depth of coverage)
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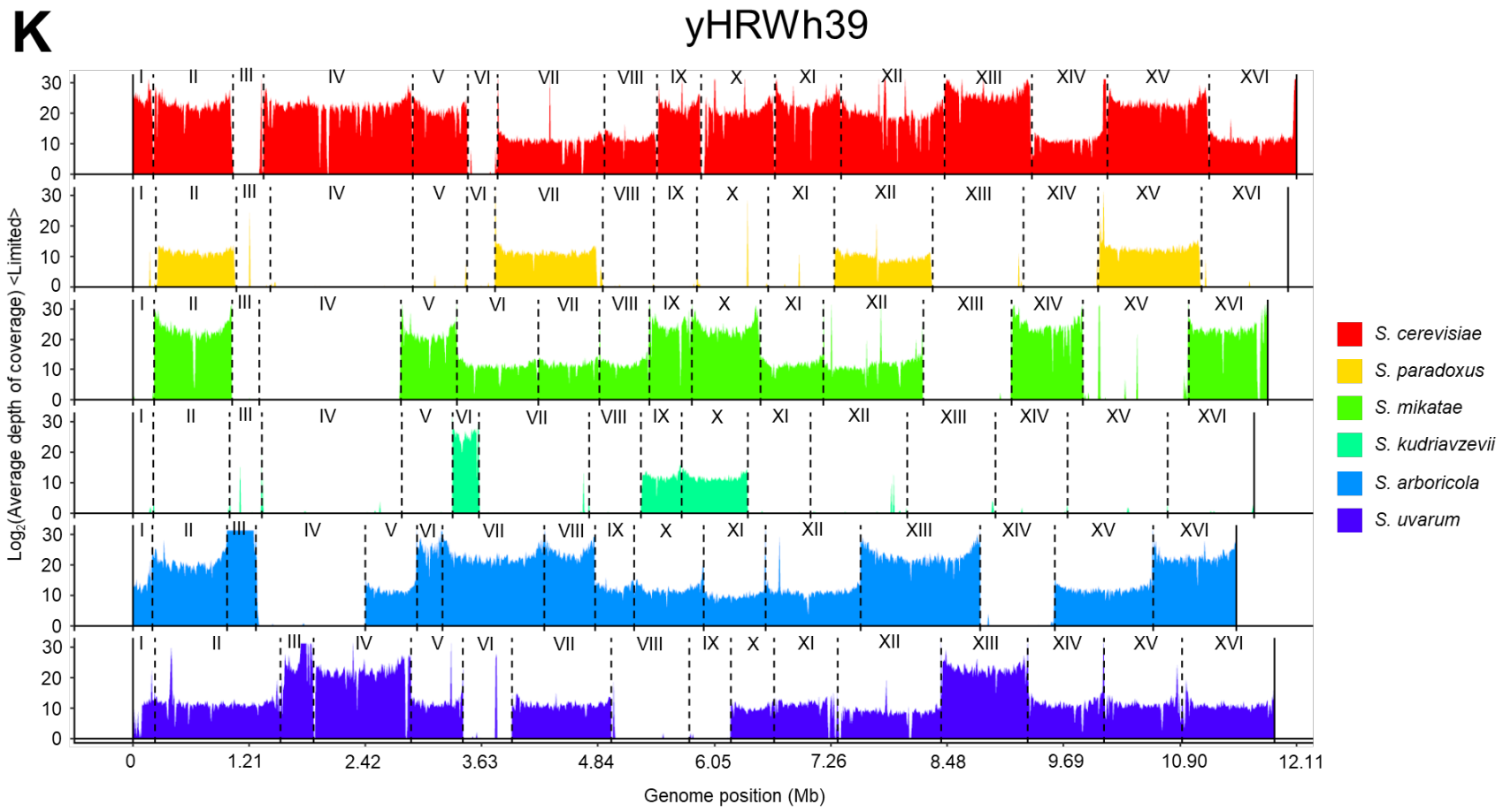
1
2
3
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. paradoxus
S. kudriavzevii
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)

40

41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yHRWh39
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Genome position (Mb)
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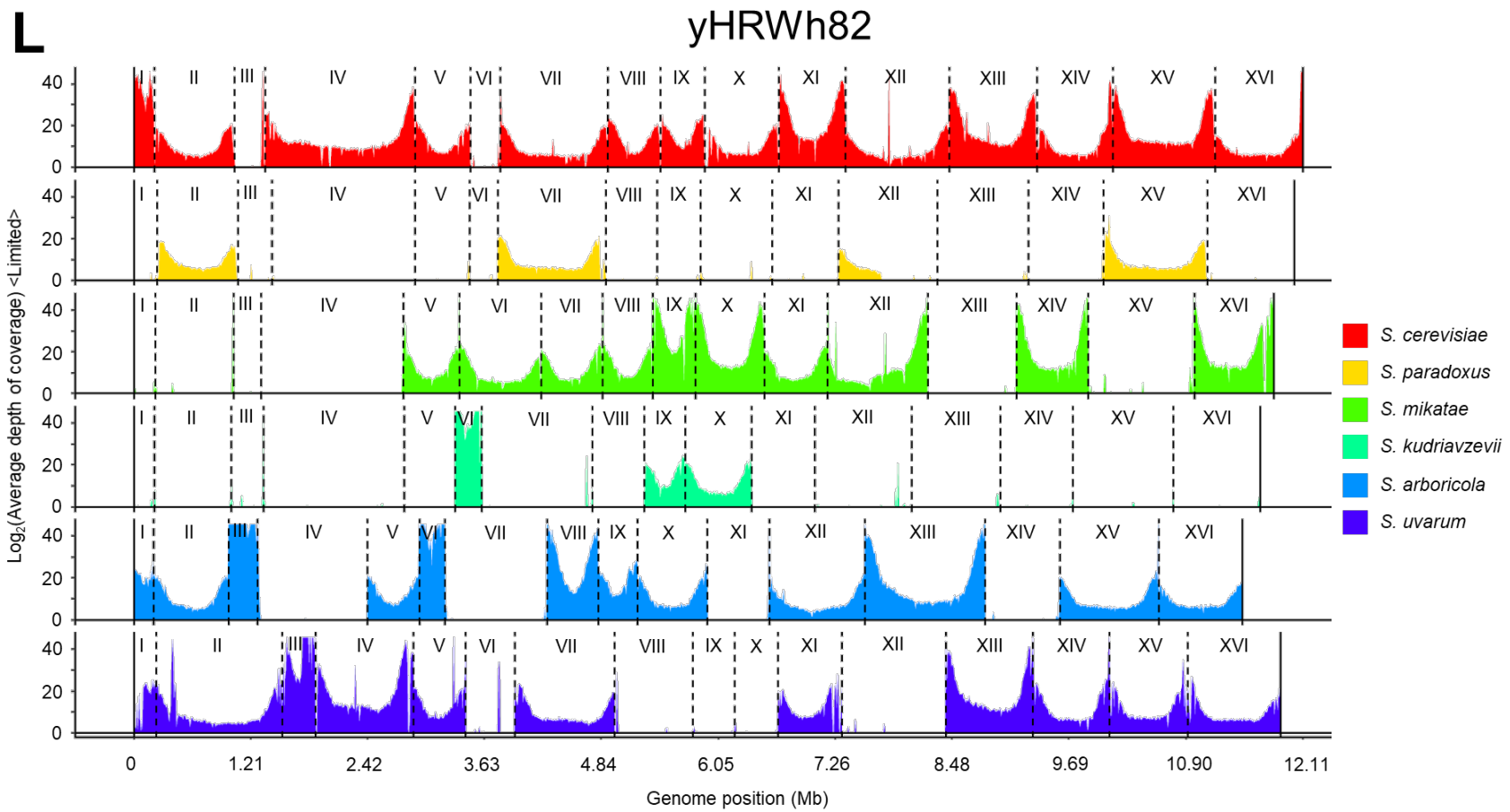
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

42

43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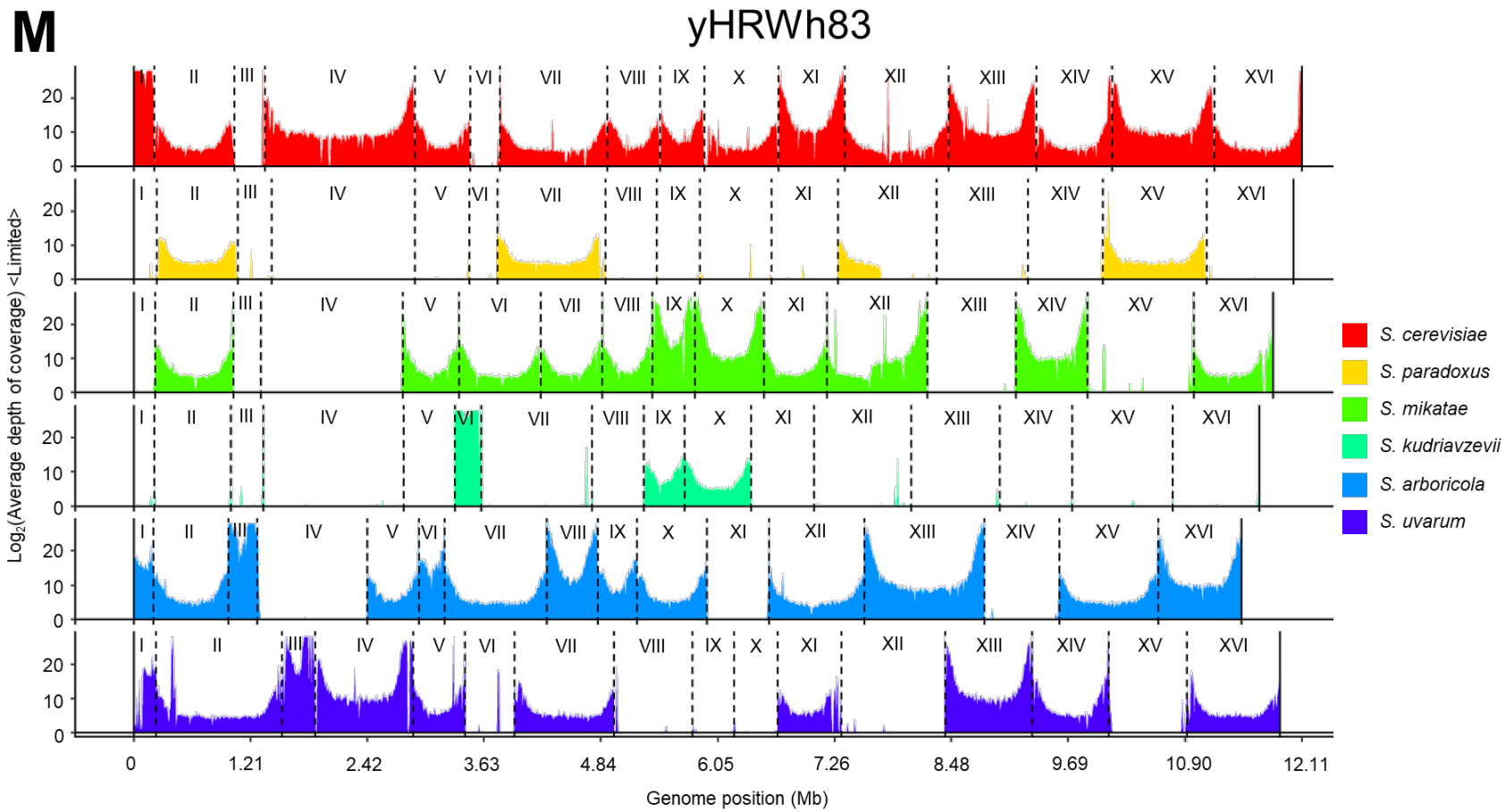
yHRWh82
Log2(Average depth of coverage) <Limited>
Genome position (Mb)
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

44

45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yHRWh83
Log2(Average depth of coverage) <Limited>
Genome position (Mb)
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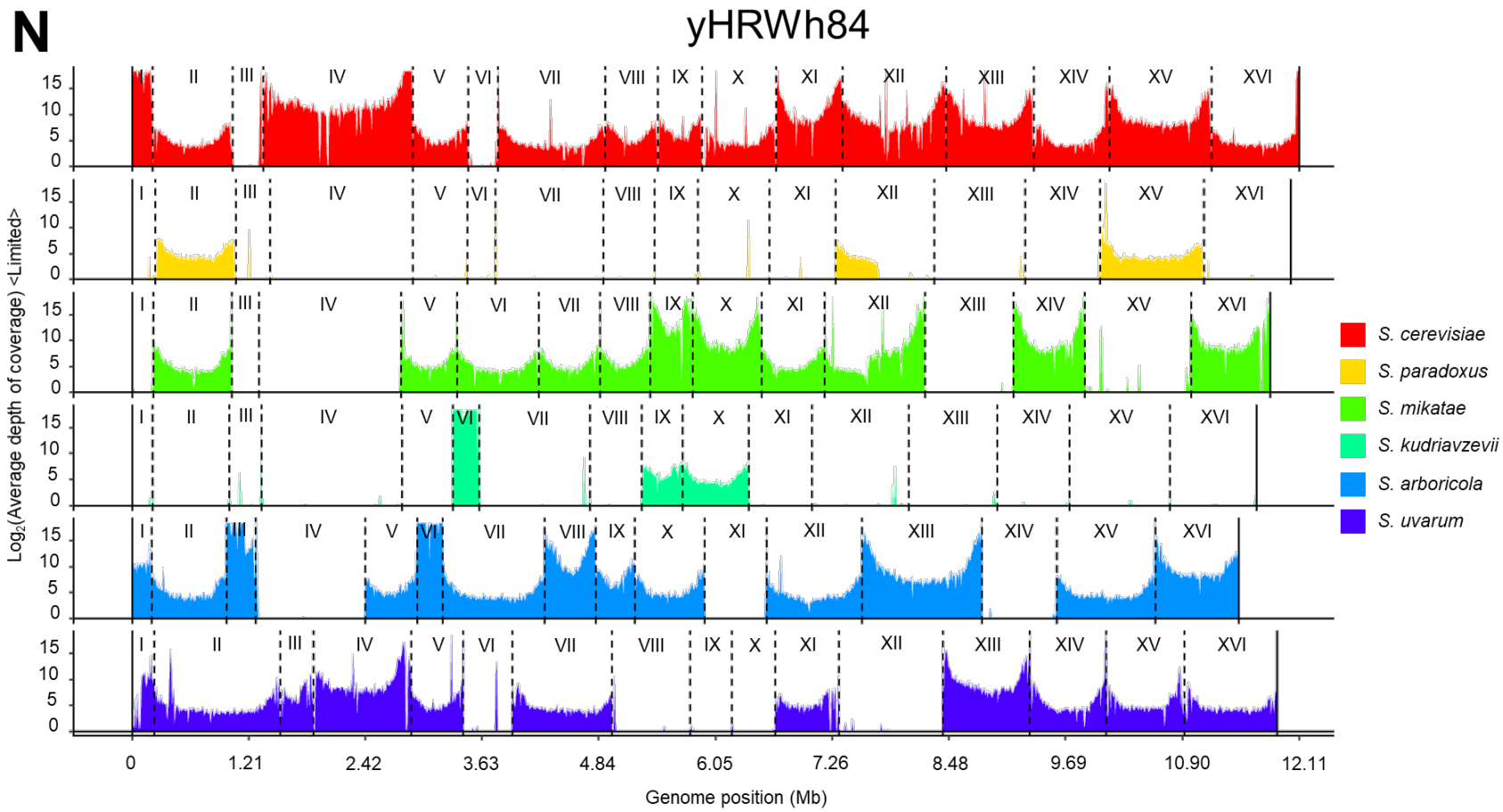
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

46

47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yHRWh84
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Genome position (Mb)
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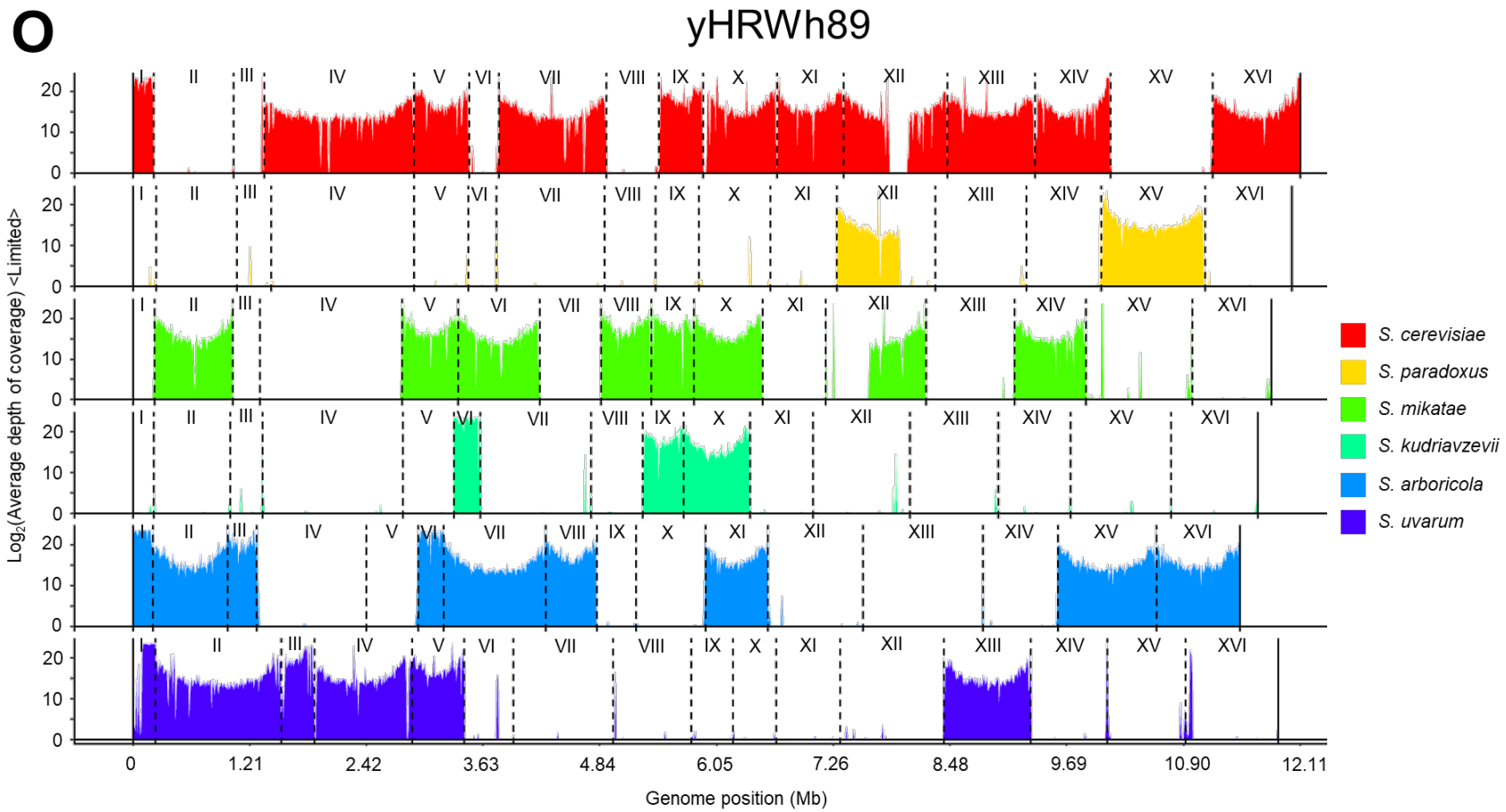
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

48

49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yHRWh89
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Genome position (Mb)
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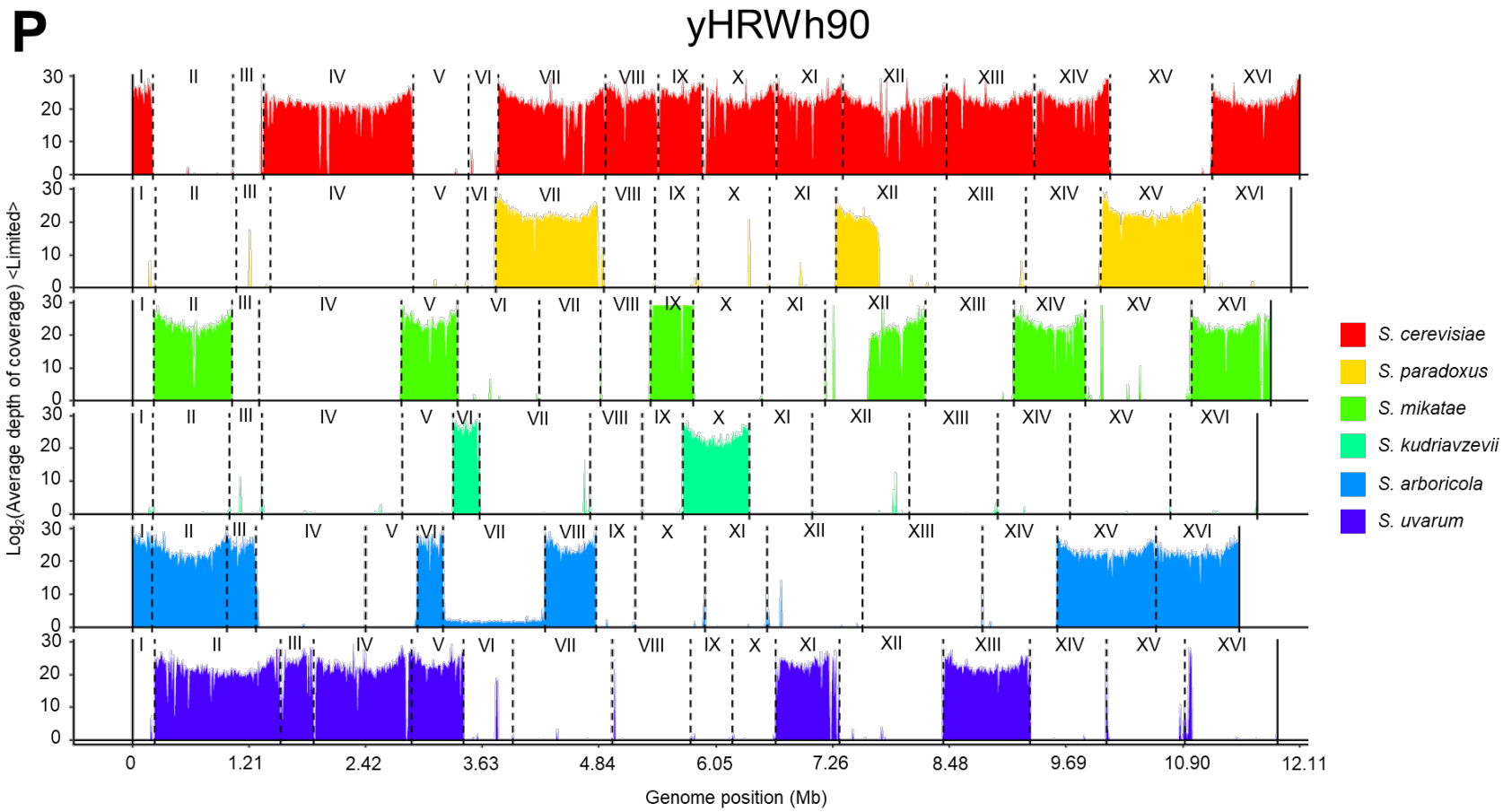
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

50

51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Log2(Average depth of coverage) <Limited>
Genome position (Mb)
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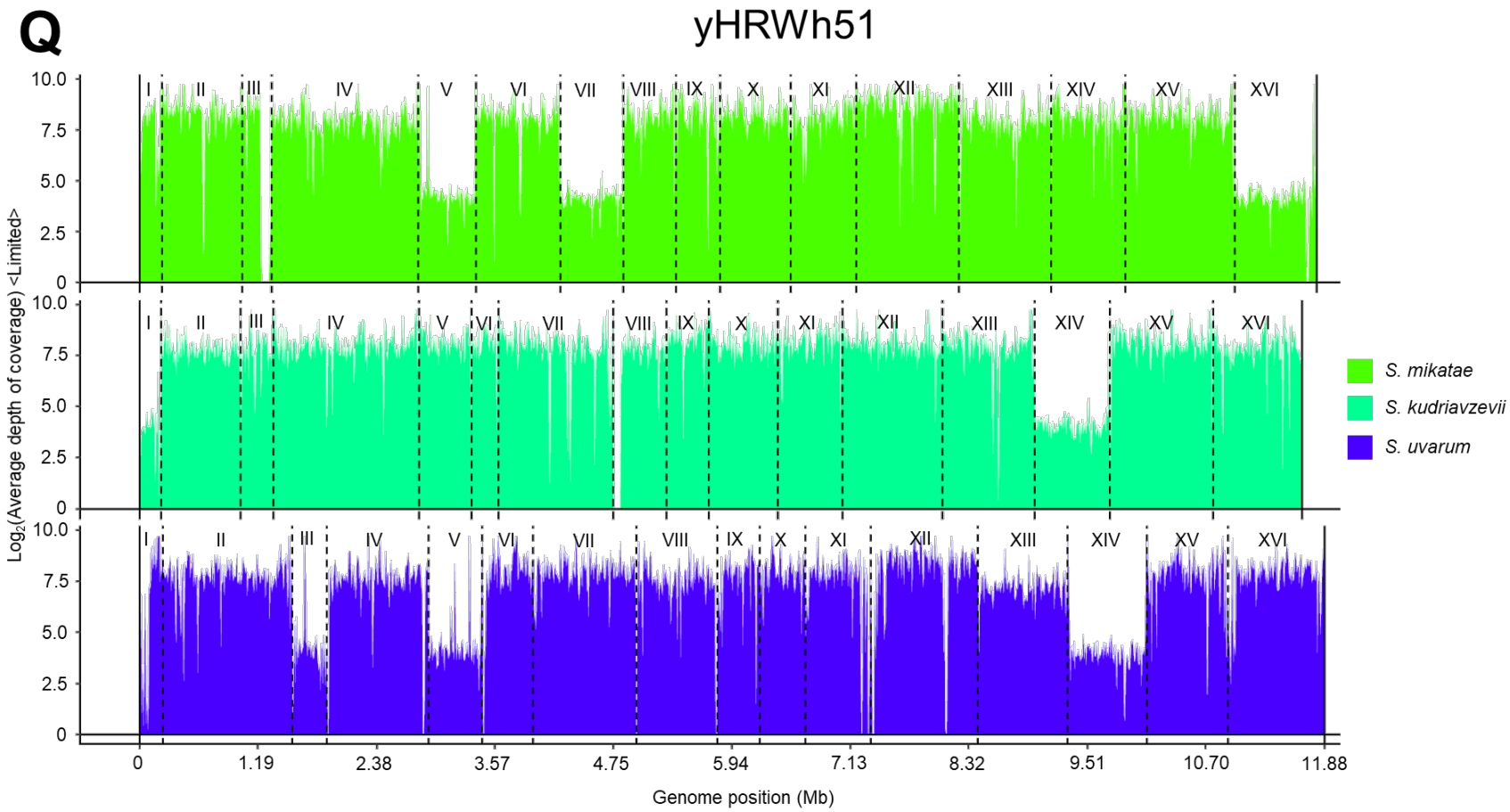
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

52

53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5.0
2.5
0
I
II
III
IV
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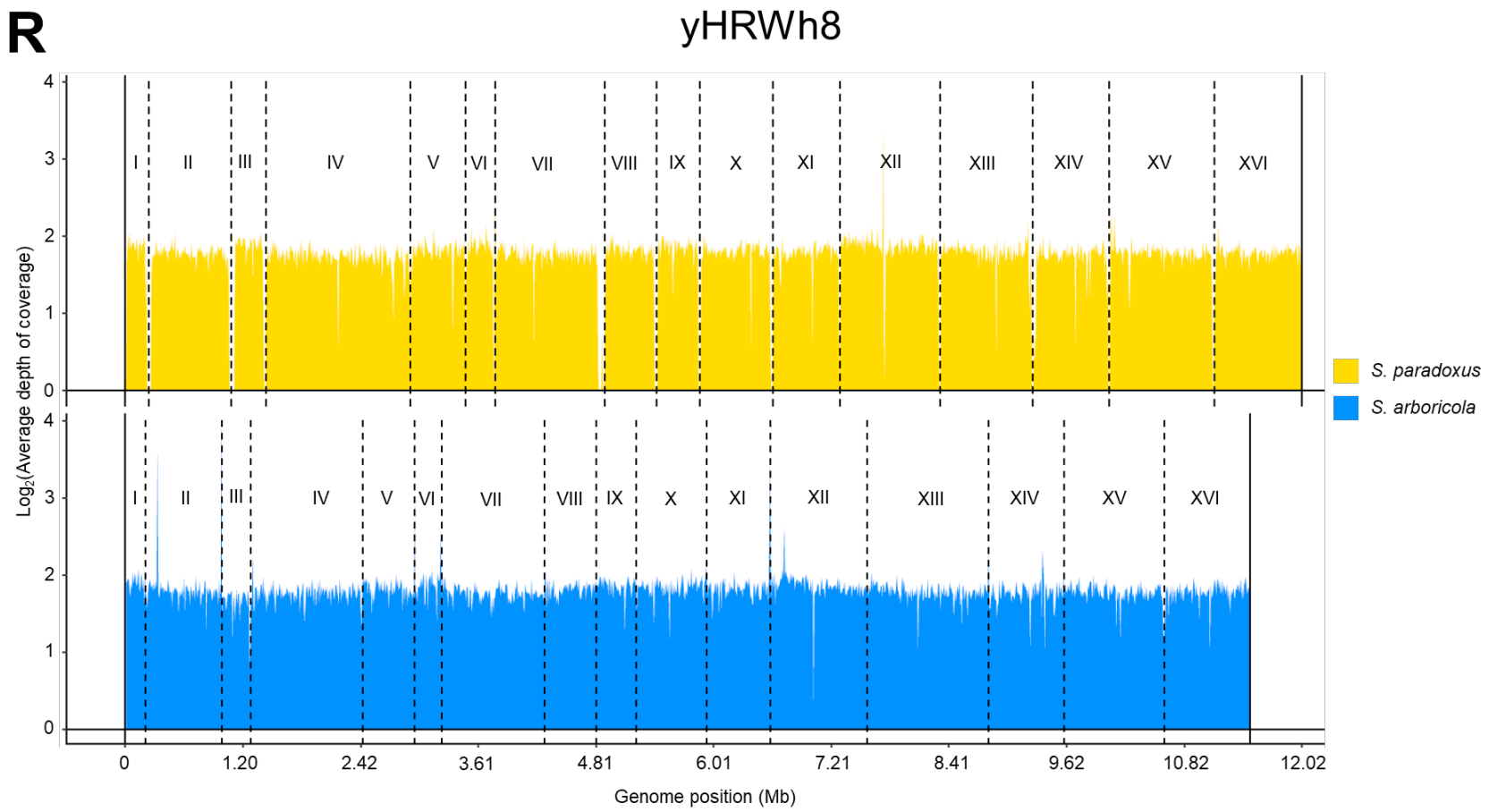
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
S. mikatae
S. kudriavzevii
S. uvarum
0
1.19
2.38
3.57
4.75
5.94
7.13
8.32
9.51
10.70
11.88
Genome position (Mb)

54

55


R
yHRWh8
Log2(Average depth of coverage)
Genome position (Mb)
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II
III
IV
V
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VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
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2.42
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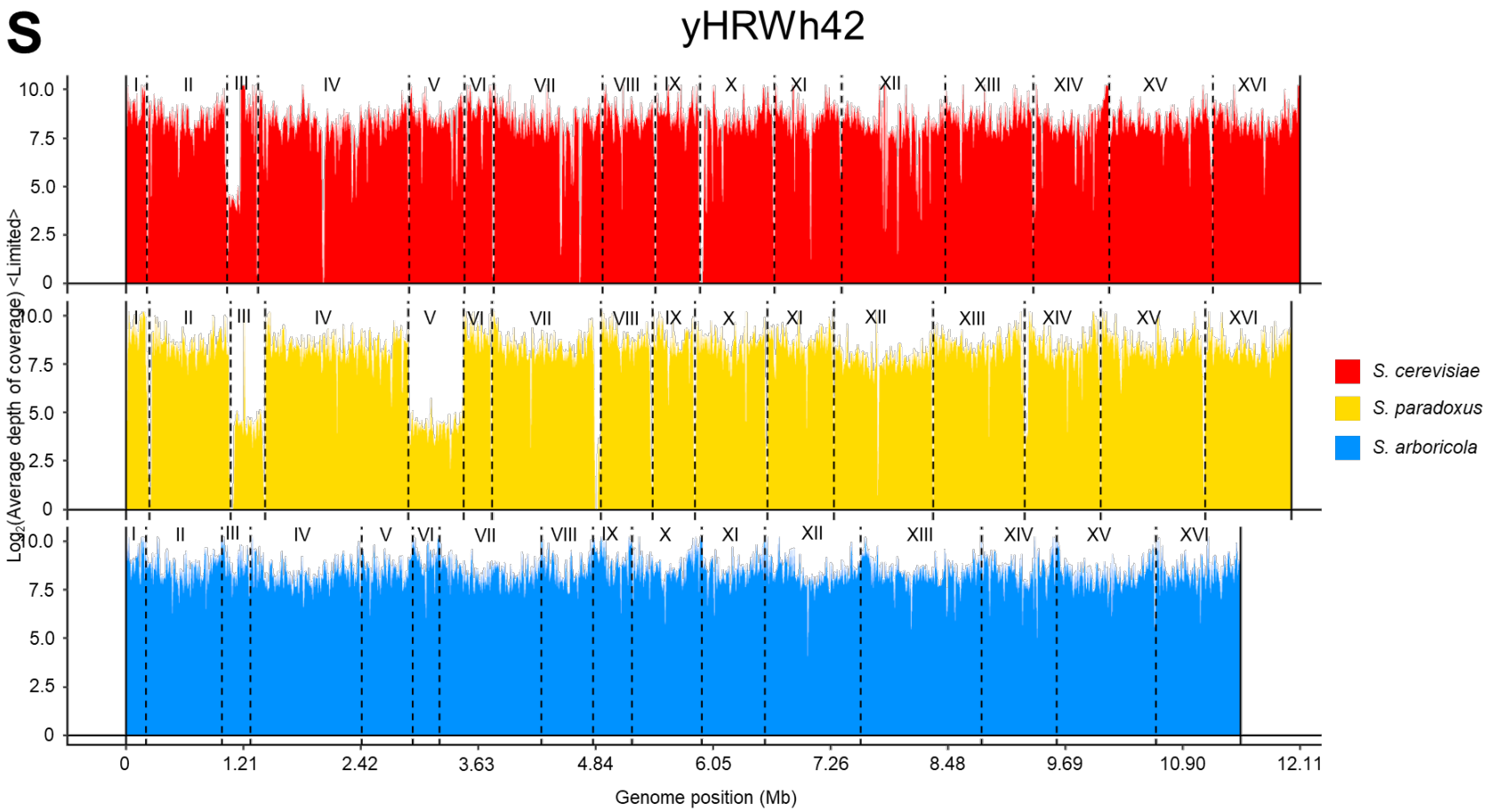
10.82
12.02
S. paradoxus
S. arboricola

56

57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III
IV
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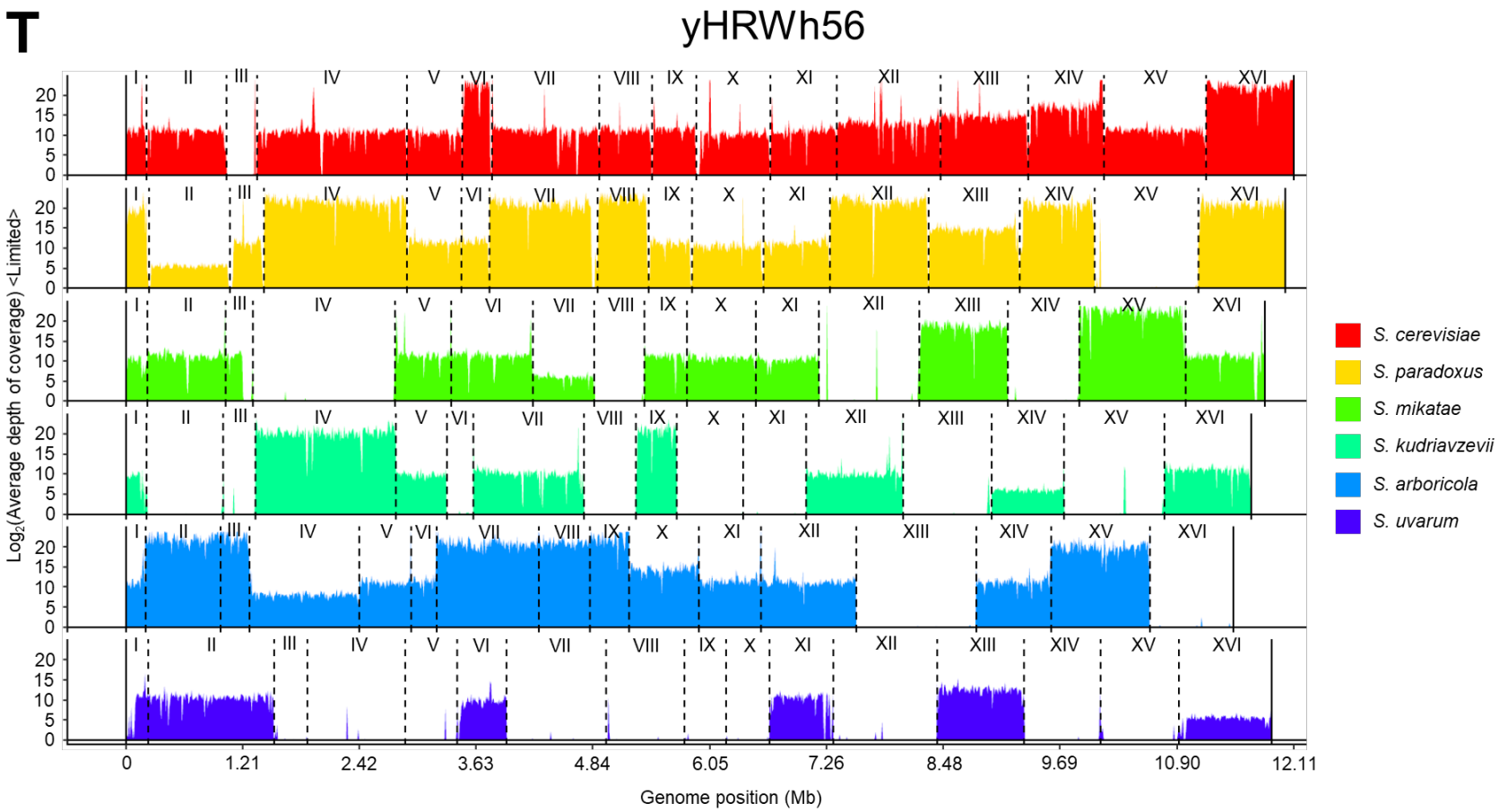
XII
XIII
XIV
XV
XVI
0
1.21
2.42
3.63
4.84
6.05
7.26
8.48
9.69
10.90
12.11
Genome position (Mb)
S. cerevisiae
S. paradoxus
S. arboricola

58

59


T
yHRWh56
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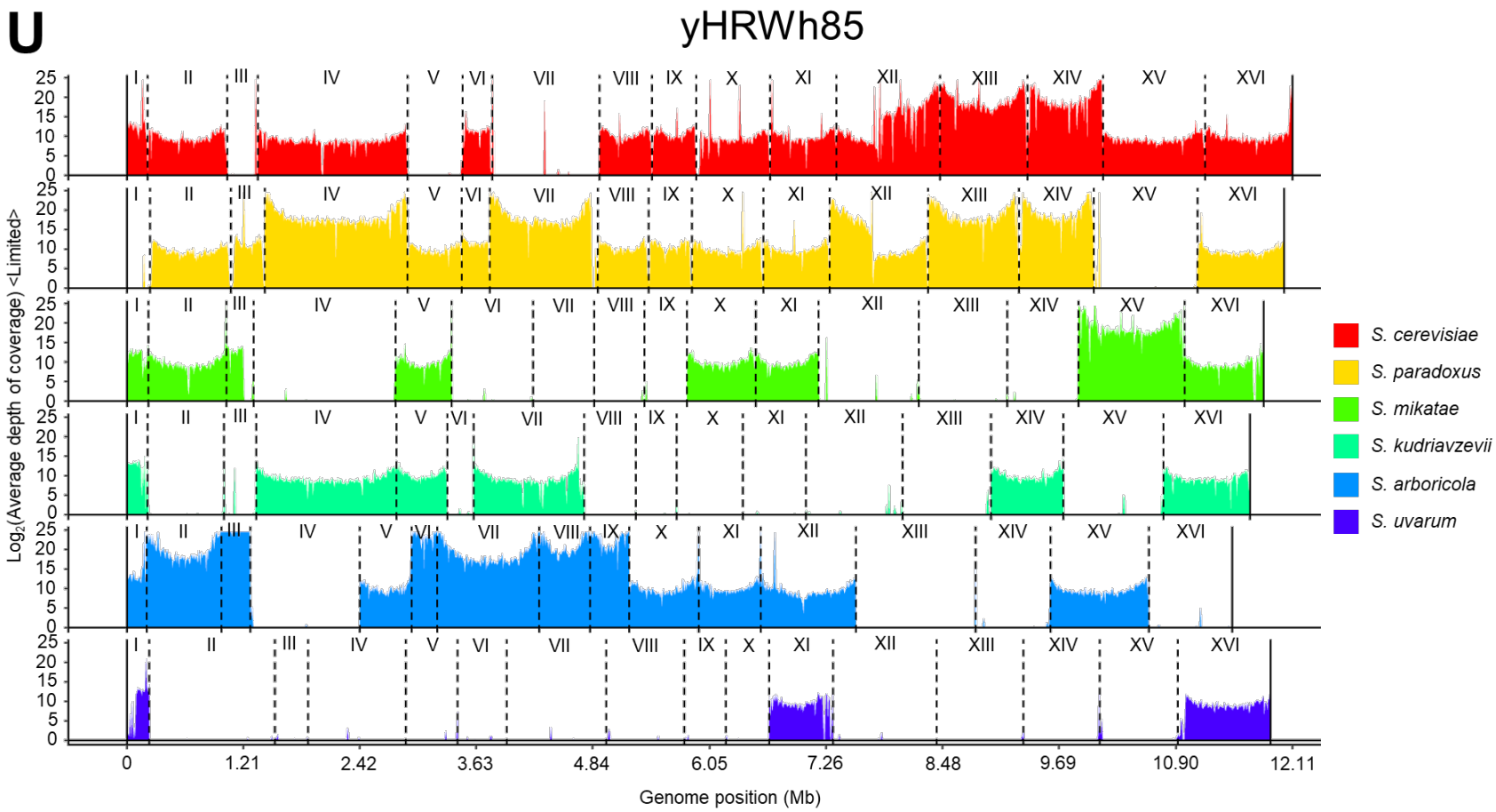
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

60

61


U
yHRWh85
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S. paradoxus
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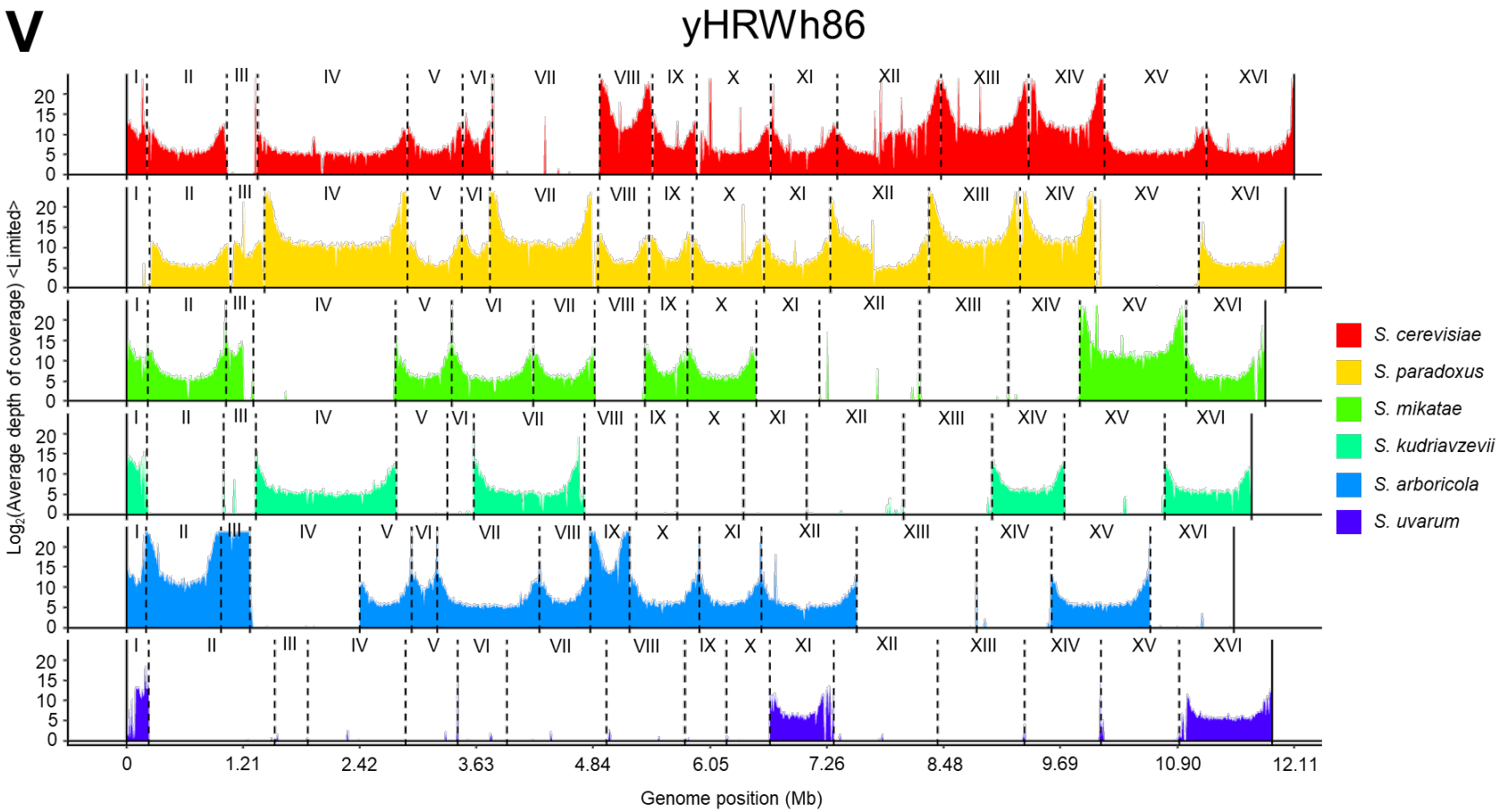
S. kudriavzevii
S. arboricola
S. uvarum

62

63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yHRWh86
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S. paradoxus
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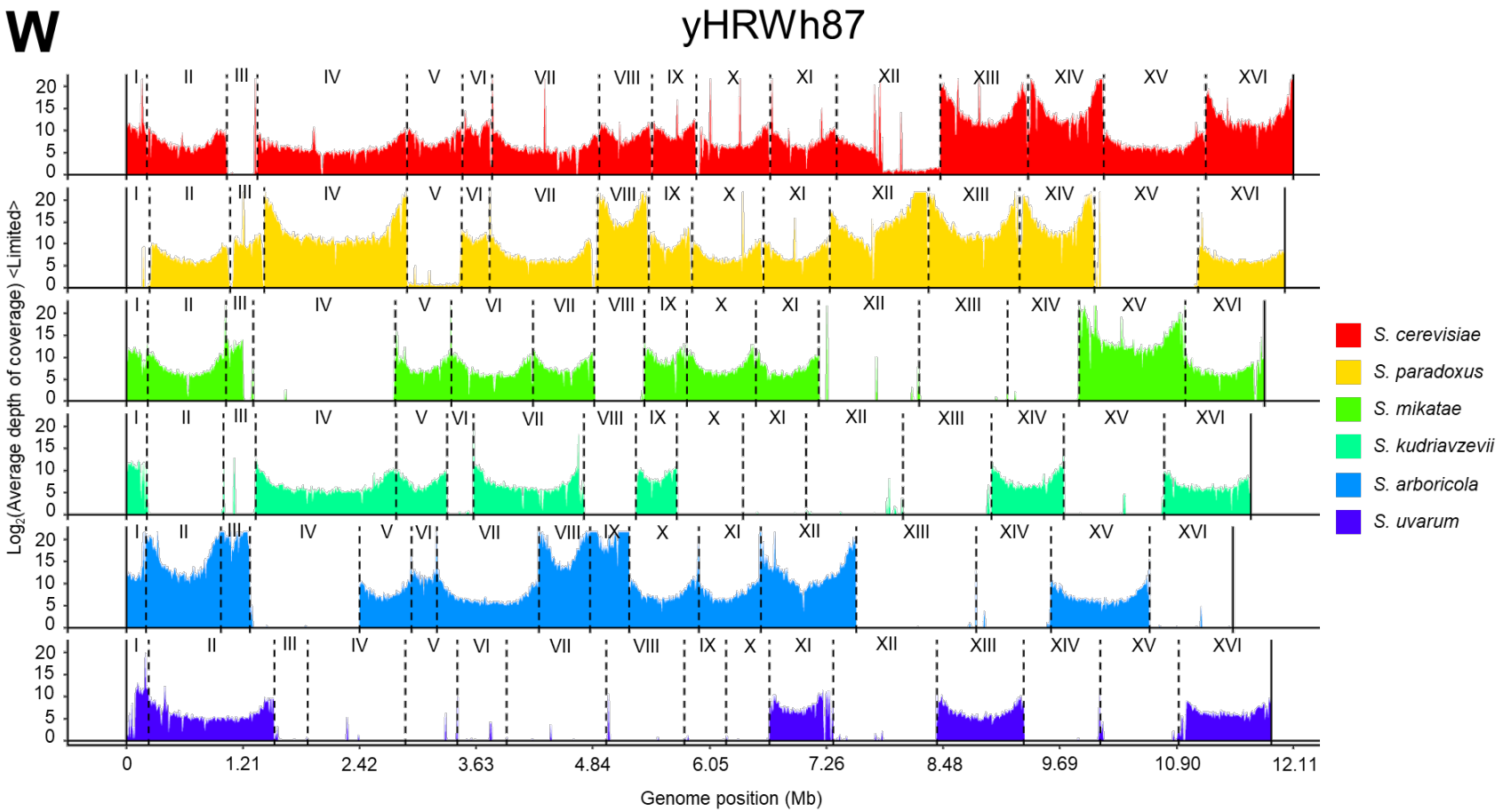
S. kudriavzevii
S. arboricola
S. uvarum

64

65


W
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S. paradoxus
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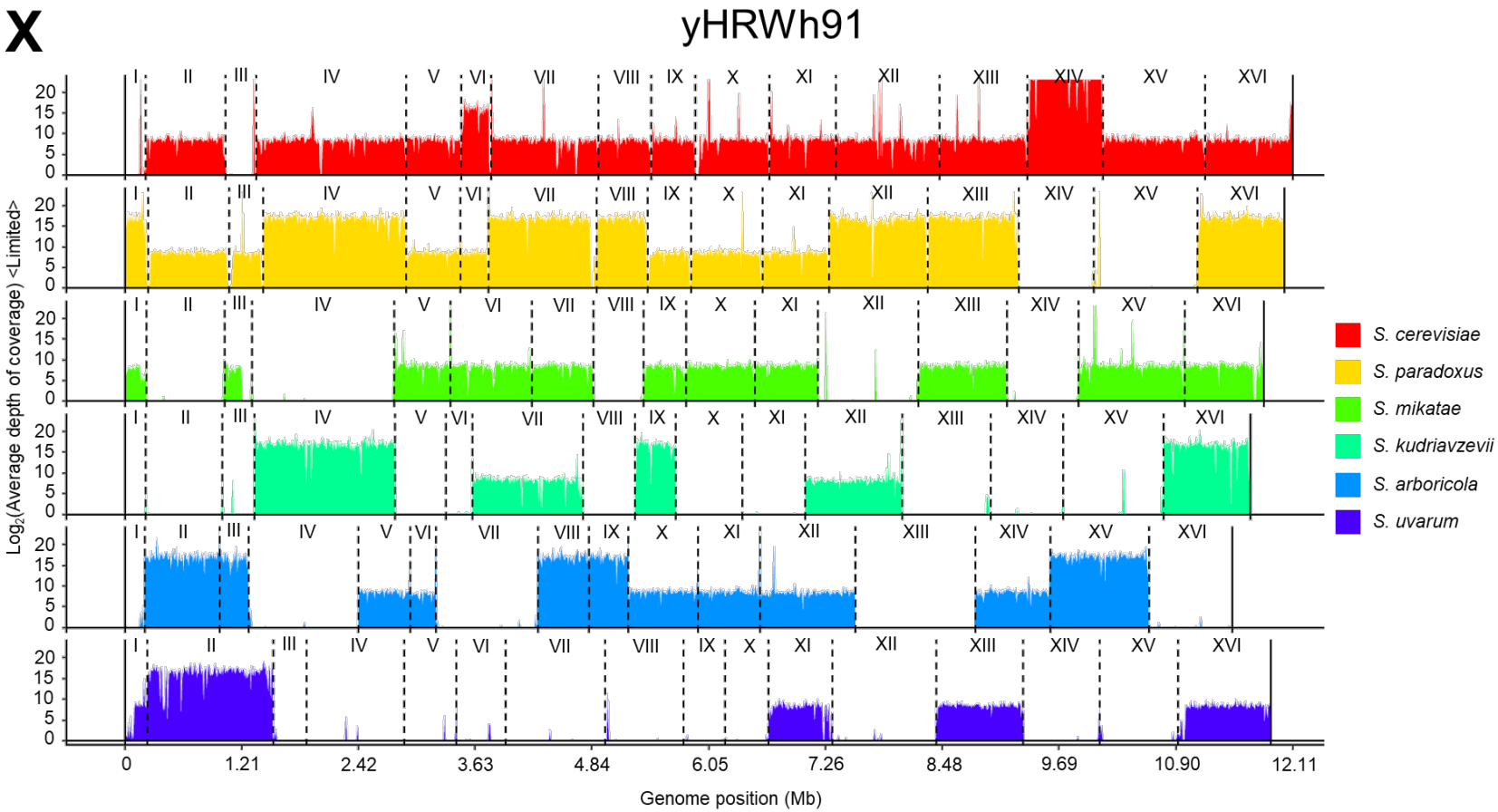
S. kudriavzevii
S. arboricola
S. uvarum

66

67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S. paradoxus
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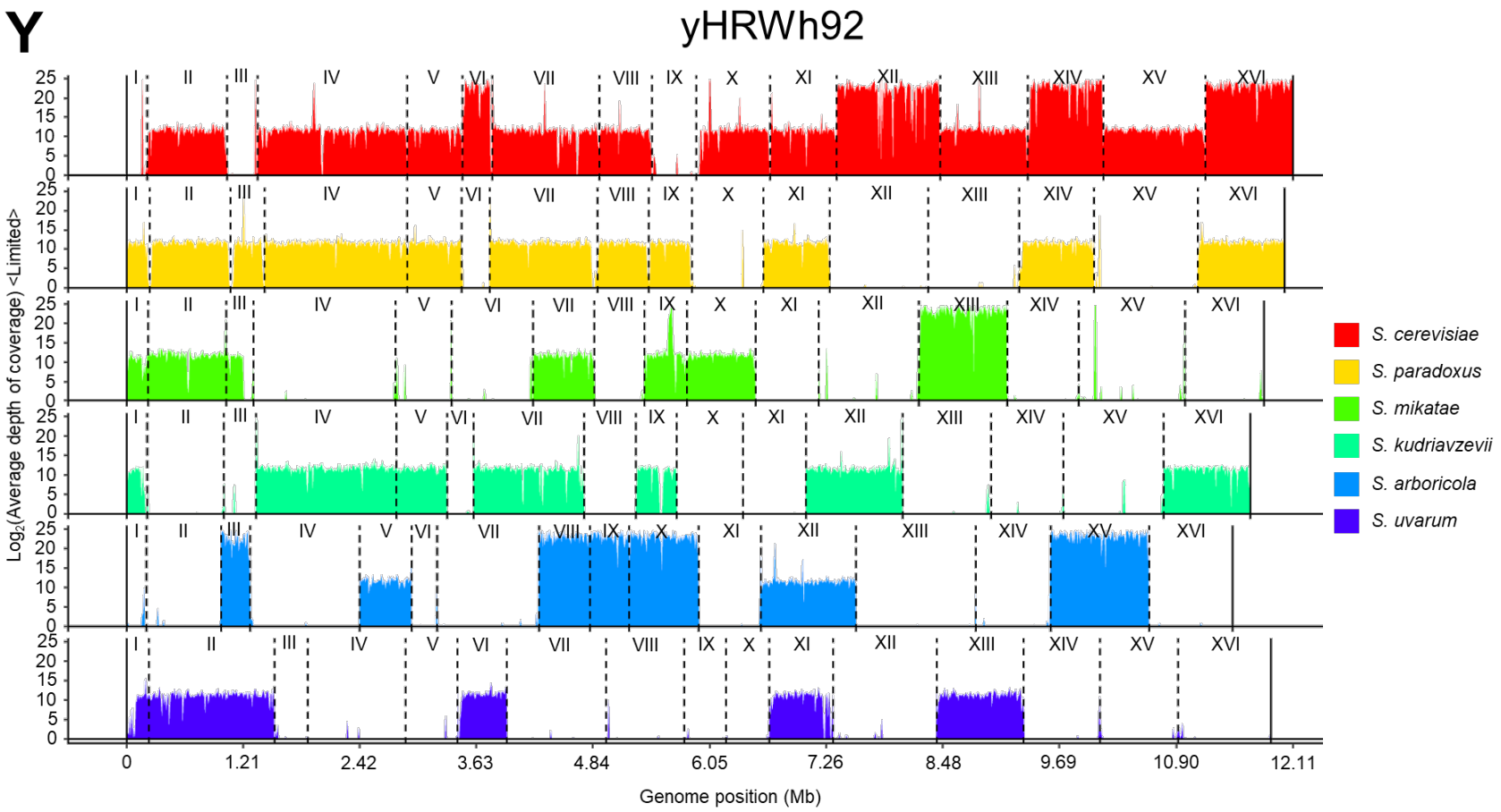
S. kudriavzevii
S. arboricola
S. uvarum

68

69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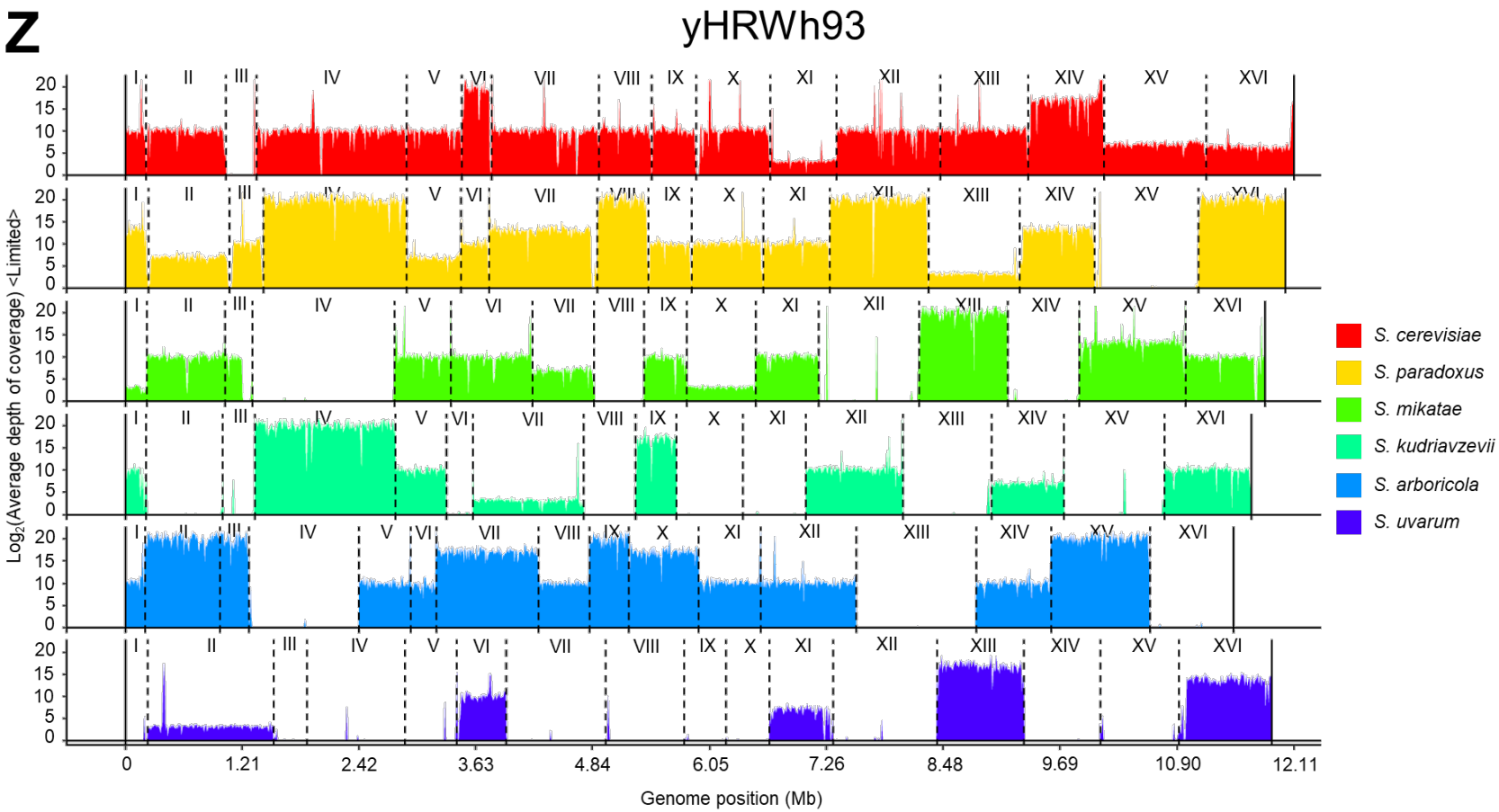
Genome position (Mb)
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

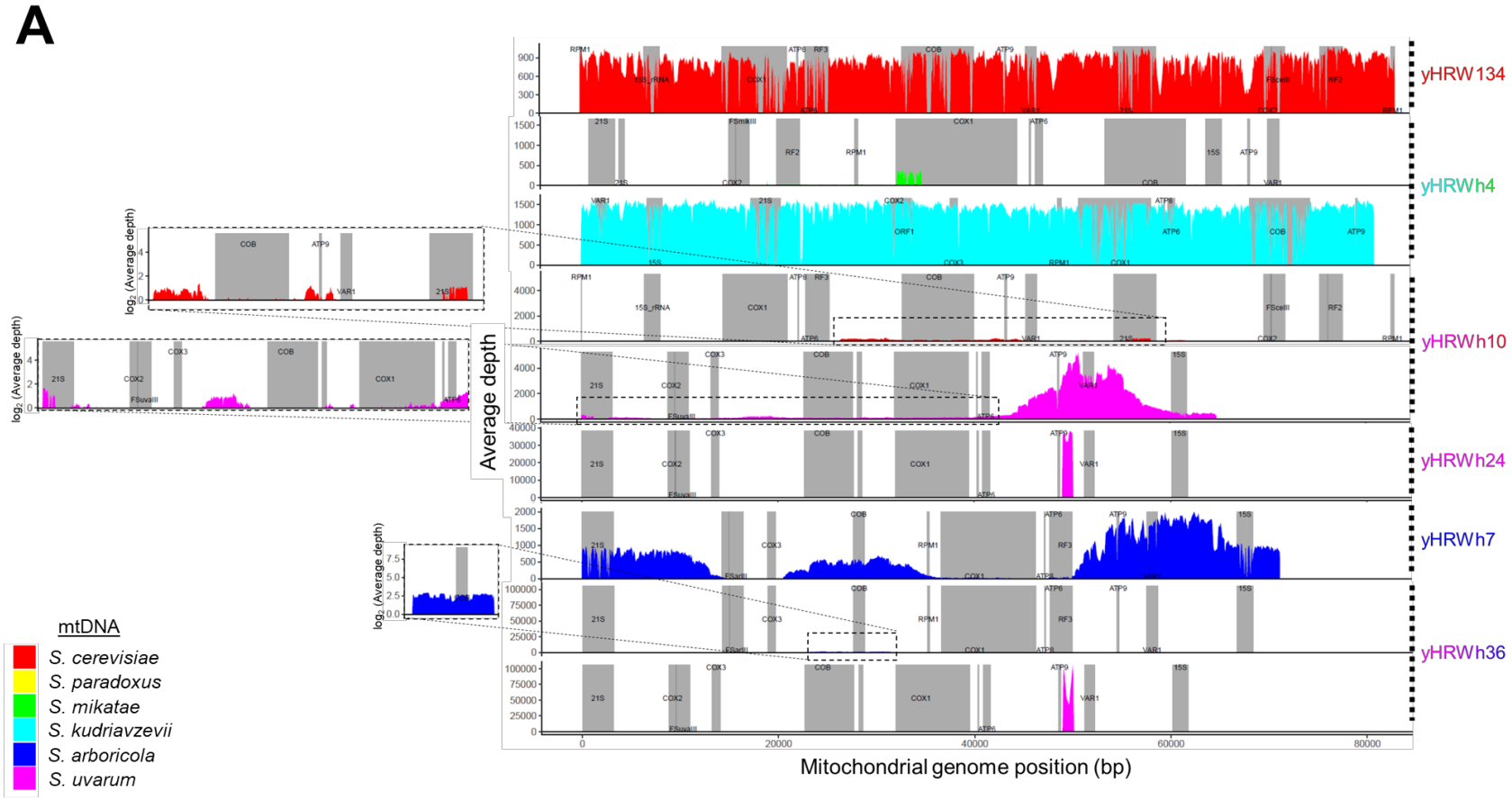


72

73 **Figure S3 Nuclear genome composition of the diploidized *S. cerevisiae* reference strain and the synthetic and**
74 **evolved hybrids.**

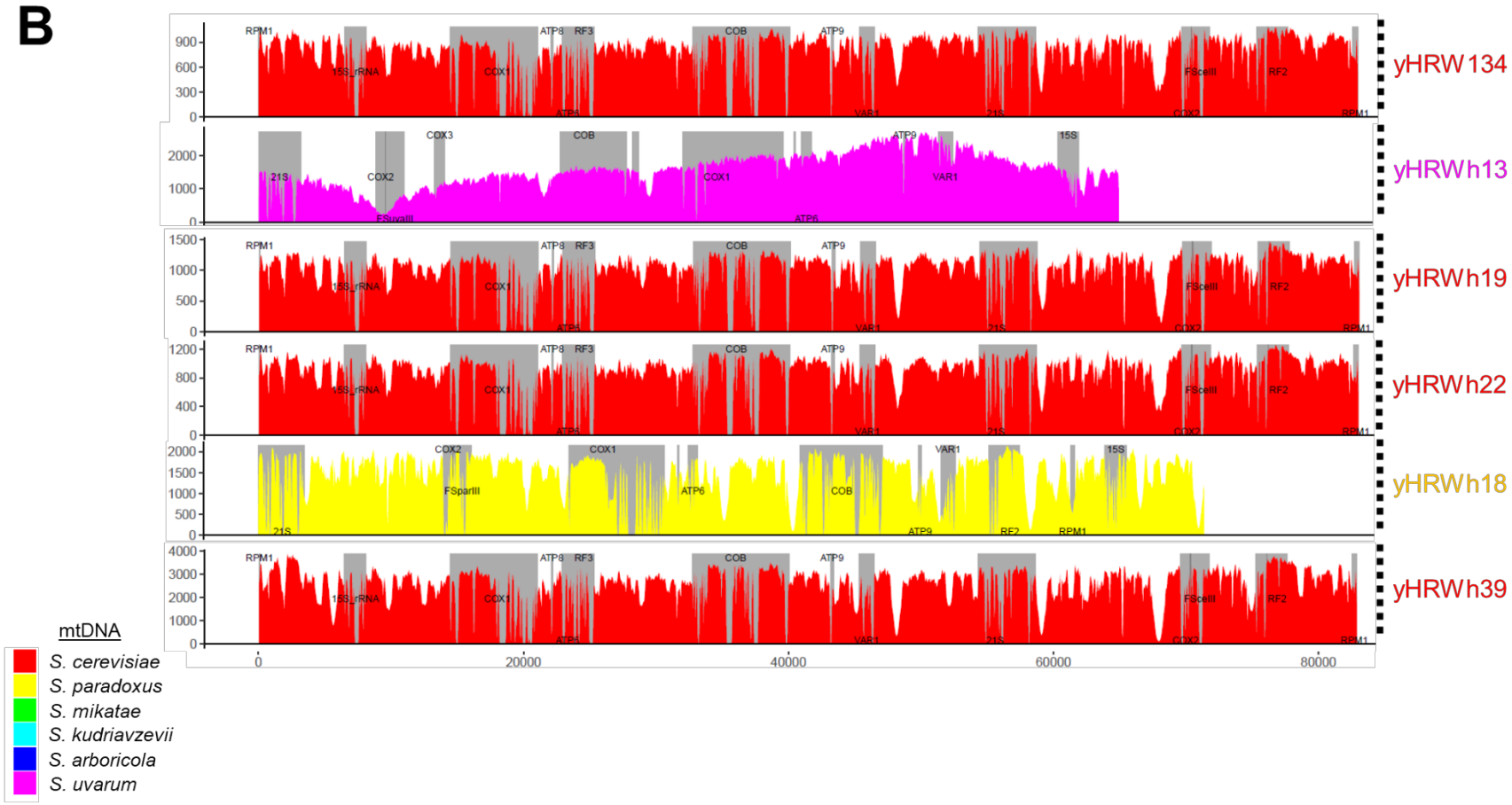
75 Panels A-Z are the `sppIDer` outputs for the diploidized reference strain of *S. cerevisiae* (GLBRCY101) and the synthetic
76 and evolved hybrids. Sequencing coverage values are colored according to each *Saccharomyces* species' contribution in
77 that portion of the genome. Panels were ordered to represent synthetic hybrid data based on the order they were used to

78 generate the next hybrid (Figure S2). `sppIDer` produces multiple plots (*17*), but here we show the $\log_2$ of the average
79 coverage of ~8 Kbp windows normalized to the genome-wide average coverage. To improve the resolution of the three-,
80 four-, and six-species hybrid plots, window coverage values were normalized to the genome-wide average coverage, and
81 values were limited to the 99% percentile and below.
82


A
yHRW134
yHRWh4
yHRWh10
yHRWh24
yHRWh7
yHRWh36
Average depth
log2 (Average depth)
Mitochondrial genome position (bp)
mtDNA
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum

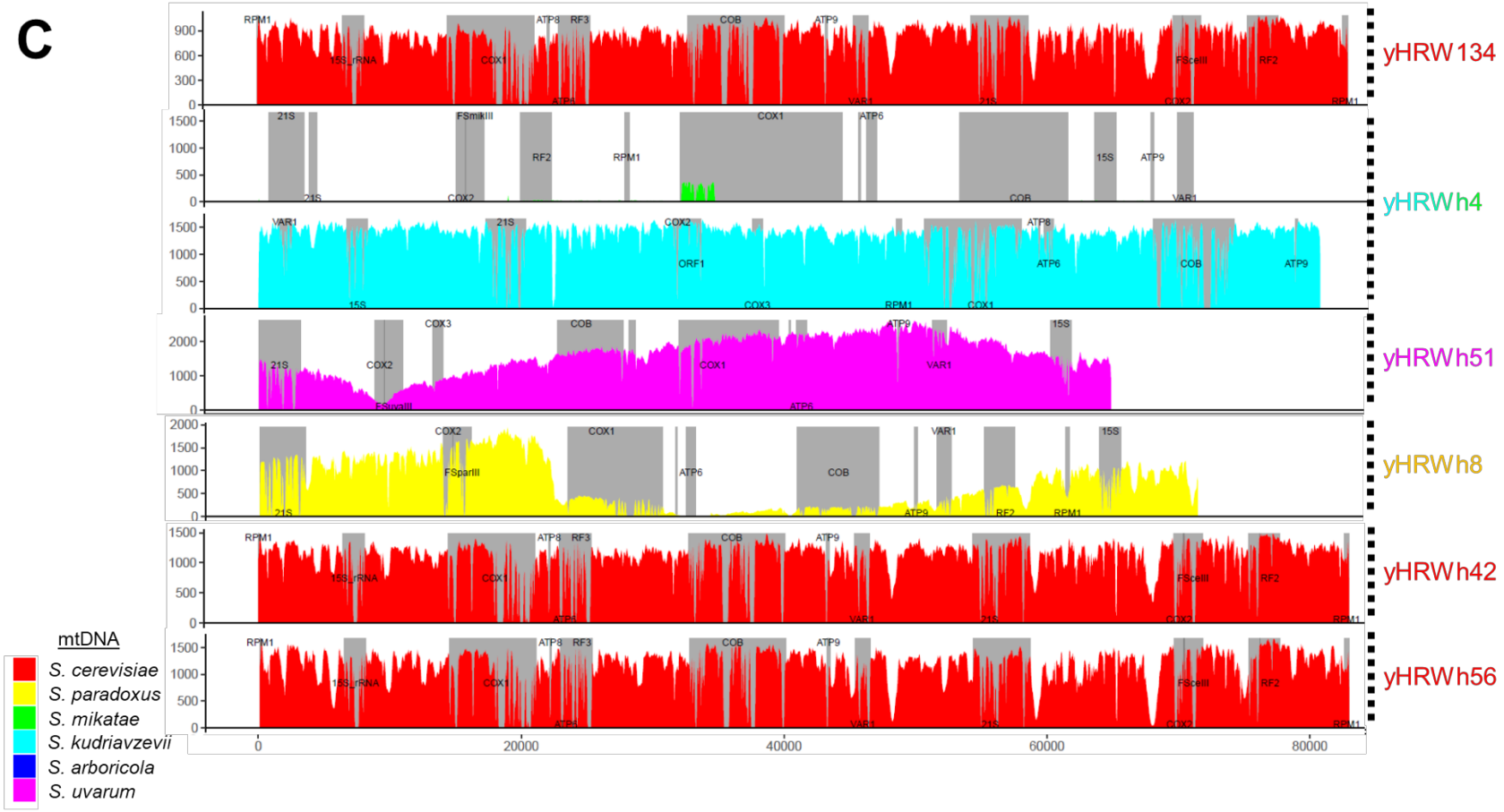
83

84


B
yHRW134
yHRWh13
yHRWh19
yHRWh22
yHRWh18
yHRWh39
mtDNA
S. cerevisiae
S. paradoxus
S. mikatae
S. kudriavzevii
S. arboricola
S. uvarum
0
20000
40000
60000
80000

85

86



87

88 **Figure S4 Mitochondrial genome inheritance of the diploidized *S. cerevisiae* reference strain and the synthetic and**

89 **evolved hybrids.**

90 Panels A-C are the `sppIDer` outputs for the diploidized reference strain of *S. cerevisiae* (GLBRCY101) and the synthetic

91 and evolved hybrids. Sequencing coverage values are colored according to each *Saccharomyces* species' contribution in

92 that portion of the mitochondrial genome. Each panel contains the mitochondrial genome inheritance for the synthetic hybrid

93 used for generating the individual six-species hybrids (Figure S2). `sppIDer` produces multiple plots (*17*), but here we show
94 the $\log_2$ of the average coverage of ~44 bp windows normalized to the mitochondrial genome-wide average coverage. When
95 a synthetic hybrid is formed between ρ^+ parental strains, a heteroplasmic state can be maintained for several generations,
96 but eventually, a parental or recombinant mitochondrial genome is generally fixed (*44*). In some hybrids, this heteroplasmic
97 state persisted, and the names of hybrids are colored in a gradient according to the detected mitochondrial genomes; these
98 colors are also displayed in Figure S5. Due to the unusually high coverage of *ATP9* or *ATP9-VAR1-15S rRNA* of *S. uvarum*
99 in panel A), additional inset plots with limited y-axes are shown.
100

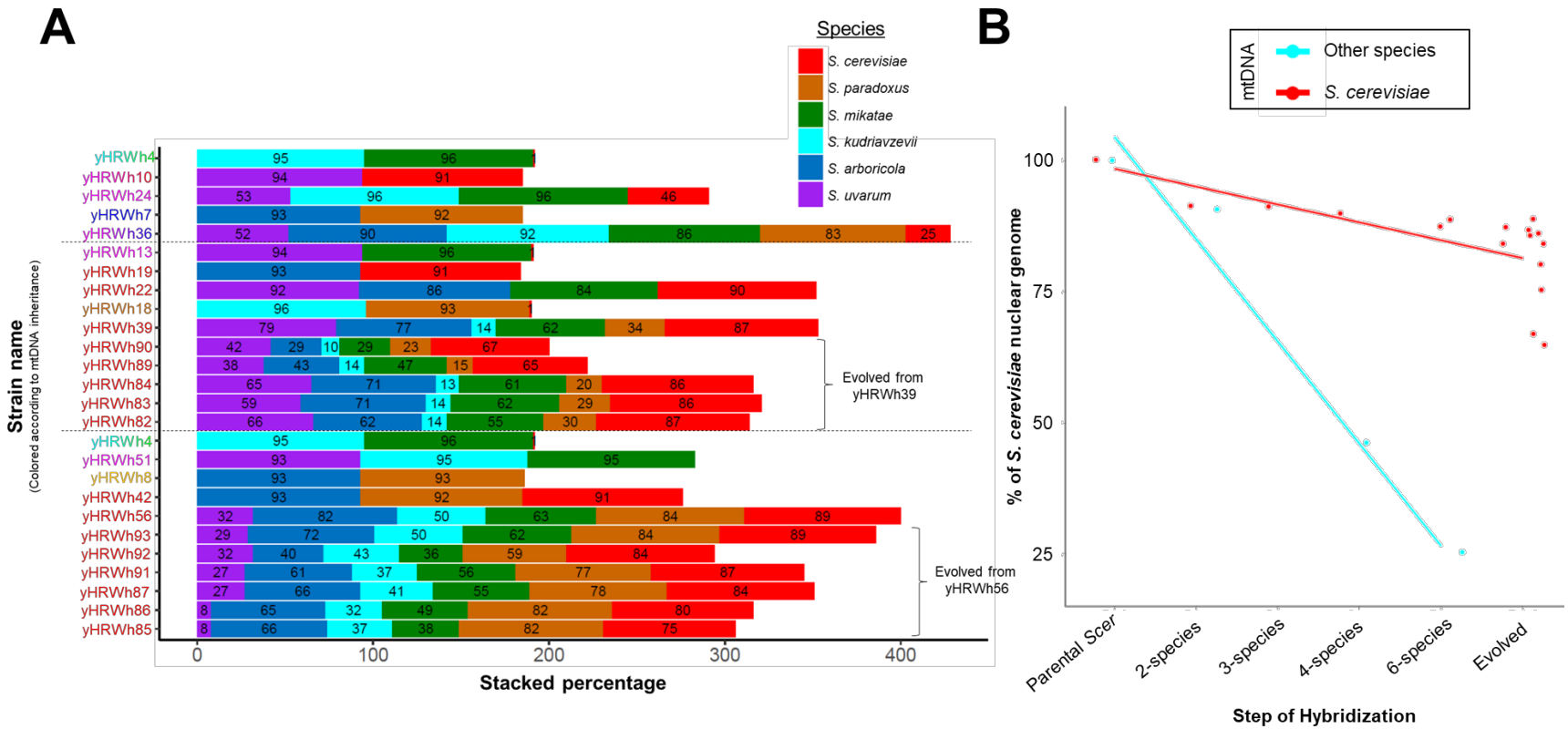


Figure S5 Percentage of retained *Saccharomyces* genomes in synthetic hybrids.

A) The percentage of each *Saccharomyces* species genome is stacked in the bar plot for each synthetic hybrid. Synthetic hybrids are displayed in the order in which they were used (Figure S1). yHRWh4 is shown multiple times because of its use in two crossing schemes. We did not expect 100% genome contribution for each *Saccharomyces* species, even for recently created hybrids, because some genomic regions (e.g. repeats) are not unambiguously detectable with Illumina sequencing data. Percentage bars are colored according to each species' contribution. The strain names are colored based on the mitochondrial inheritance inferred from `mitosppIDer` output (Figure S4). Synthetic hybrids with two or more detected

109 mitochondrial genomes or regions are shown as gradient color based on the species' contributions. B) The nuclear
110 composition of the *S. cerevisiae* parent, synthetic hybrids, and evolved hybrids is plotted according to mitochondrial
111 inheritance. Hybrids with or without the *S. cerevisiae* mitochondrial genome are colored in red and light blue, respectively.
112

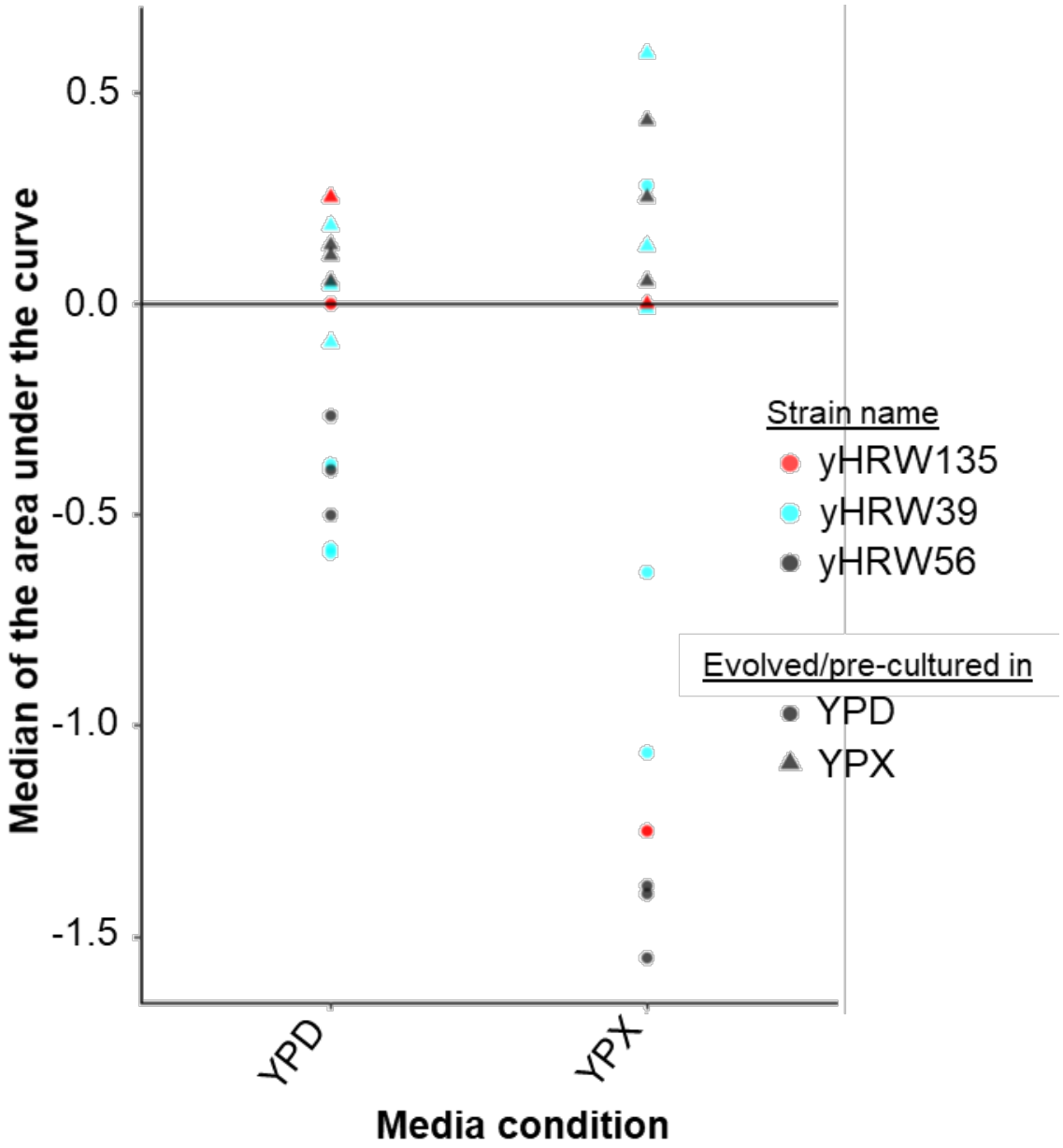


Figure S6 Growth kinetics for ancestral and evolved six-species hybrids in a microplate.

Growth measured as area under the curve (AUC) for the ancestor and evolved six-species hybrids, after normalization to the *S. cerevisiae* reference strain, yHRW135 (full microplate kinetic parameters are reported in Table S2). Different shapes indicate the media in which the synthetic six-species hybrids were evolved. Colors differentiate parent strains, while each data point represents an evolved replicate population.

Table S1 Kinetic parameter information for the *S. cerevisiae* reference strain and ancestral and evolved synthetic hybrids on a culture wheel after colony selection.

Strain	Species	Evolved in	spec.growth.median	spec.growth.sd	ODmax.median	ODmax.sd
yHRW135	*S. cerevisiae*	Not evolved	0.0701	0.0123	3.1023	1.0531
yHRWh39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	Not evolved	0.0543	0.0125	1.2039	0.2819
yHRWh56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	Not evolved	0.0659	0.0156	1.2343	0.1269
yHRWh82	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0747	0.0113	1.3832	0.1477
yHRWh83	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0807	0.0162	1.4838	0.1543
yHRWh84	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0707	0.0207	1.8881	0.2159
yHRWh85	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0844	0.0102	1.2993	0.1865
yHRWh86	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0946	0.0121	1.5119	0.2020
yHRWh87	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	0.0857	0.0146	1.4272	0.1814
yHRWh88	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0619	0.0096	1.4349	1.1908
yHRWh89	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0755	0.0077	1.5429	0.0906
yHRWh90	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0869	0.0179	1.5438	0.1635
yHRWh91	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0790	0.0111	1.5561	0.0182
yHRWh92	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0734	0.0124	1.7413	0.2344
yHRWh93	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	0.0772	0.0161	1.3500	0.2050

spec.growth.median = median (n=6) of the maximum exponential growth rates achieved by the sample in independent cultures
spec.growth.sd = standard deviation (n=6) of the maximum exponential growth rates achieved by the sample in independent cultures
ODmax.median = median (n=6) of the estimated growth plateaus achieved by the sample in independent cultures
ODmax.sd = standard deviation (n=6) of the estimated growth plateaus achieved by the sample in independent cultures

Table S2 Kinetic parameter information for the *S. cerevisiae* reference strain and synthetic hybrids.

Strain	Species	Pre-culture	Medium of kinetic measurement	Evolved culture	mtDNA inheritance	spec.growth.median	spec.growth.sd	spec.growth.norm.median	spec.growth.perc	ODmax.median	ODmax.sd	ODmax.norm.median	ODmax.perc	AUC.median	AUC.sd	AUC.norm.median	AUC.perc
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	1	*S. cerevisiae*	0.1616	0.0130	-1.4790	-64.1256	1.1968	0.1611	-0.3568	-21.9087	28.3000	5.2735	-0.5799	-33.0969
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	1	*S. cerevisiae*	0.1771	0.0072	-1.3461	-60.6659	1.6035	0.1702	0.0652	4.6252	43.6000	7.3276	0.0437	3.0733
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	1	*S. cerevisiae*	0.1810	0.0041	-1.3150	-59.8075	1.2406	0.1828	-0.3050	-19.0563	28.1000	5.9254	-0.5901	-33.5697
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	1	*S. cerevisiae*	0.1639	0.0066	-1.4586	-63.6145	1.4832	0.1861	-0.0473	-3.2259	39.7000	4.2673	-0.0915	-6.1466
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	2	*S. cerevisiae*	0.1653	0.0184	-1.4458	-63.2909	1.3167	0.1192	-0.2191	-14.0888	32.5000	5.0362	-0.3802	-23.1678
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	2	*S. cerevisiae*	0.1787	0.0074	-1.3338	-60.3281	1.6308	0.0521	0.0896	6.4090	48.1000	2.0404	0.1854	13.7116
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	2	*S. cerevisiae*	0.1823	0.0081	-1.3045	-59.5139	1.2197	0.1622	-0.3295	-20.4167	29.9000	5.8960	-0.5005	-29.3144
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	2	*S. cerevisiae*	0.1765	0.0052	-1.3514	-60.8098	1.5115	0.0454	-0.0200	-1.3798	43.9000	1.8009	0.0536	3.7825
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	3	*S. cerevisiae*	0.1897	0.0190	-1.2470	-57.8685	1.4094	0.1302	-0.1209	-8.0387	35.2000	4.1940	-0.2651	-16.7849
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	3	*S. cerevisiae*	0.1733	0.0131	-1.3774	-61.5090	1.4784	0.1272	-0.0519	-3.5365	45.8000	4.6177	0.1147	8.2742
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPD	3	*S. cerevisiae*	0.1796	0.0032	-1.3260	-60.1123	1.3237	0.1460	-0.2115	-13.6333	32.2000	4.6307	-0.3936	-23.8771
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPD	3	*S. cerevisiae*	0.1703	0.0065	-1.4033	-62.1928	1.5874	0.0468	0.0506	3.5728	46.6000	1.8148	0.1397	10.1655
yHRW135	*S. cerevisiae*	YPD	YPD		*S. cerevisiae*	0.4503	0.1683	0.0000	0.0000	1.5326	0.3479	0.0000	0.0000	42.3000	12.5301	0.0000	0.0000
yHRW135	*S. cerevisiae*	YPX	YPD		*S. cerevisiae*	0.2055	0.0071	-1.1318	-54.3644	1.6941	0.1638	0.1445	10.5352	50.4000	6.0494	0.2528	19.1489
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	1	*S. cerevisiae*	0.0775	0.0144	0.4733	38.8321	0.3218	0.0287	0.6694	59.0386	7.3600	1.5627	0.2804	21.4521
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	1	*S. cerevisiae*	0.0692	0.0248	0.3103	23.9928	0.2461	0.1275	0.2827	21.6476	6.6600	4.5517	0.1362	9.9010
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	1	*S. cerevisiae*	0.0599	0.0034	0.1014	7.2836	0.1372	0.0251	-0.5600	-32.1711	3.9000	0.5786	-0.6358	-35.6436
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	1	*S. cerevisiae*	0.0739	0.0175	0.4042	32.3320	0.2954	0.1112	0.5461	46.0134	9.1400	3.4405	0.5929	50.8251
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	2	*S. cerevisiae*	0.0354	0.0062	-0.6572	-36.5903	0.1333	0.0214	-0.6024	-34.1362	2.9000	0.4805	-1.0633	-52.1452
yHRW39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	2	*S. cerevisiae*	0.0525	0.0072	-0.0885	-5.9491	0.2217	0.0338	0.1323	9.6057	6.0100	0.5033	-0.0120	-0.8251
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	2	*S. cerevisiae*	0.0372	0.0085	-0.5862	-33.3898	0.1087	0.0232	-0.8967	-46.2875	2.3300	0.8187	-1.3790	-61.5512
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	2	*S. cerevisiae*	0.0456	0.0136	-0.2924	-18.3478	0.2323	0.0793	0.1994	14.8251	6.2900	2.5603	0.0537	3.7954
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	3	*S. cerevisiae*	0.0421	0.0085	-0.4083	-24.6505	0.1331	0.0242	-0.6042	-34.2157	2.3000	0.7650	-1.3977	-62.0462
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	3	*S. cerevisiae*	0.0606	0.0103	0.1185	8.5581	0.2682	0.0591	0.4066	32.5597	7.2200	1.8231	0.2527	19.1419
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPD	YPX	3	*S. cerevisiae*	0.0355	0.0034	-0.6524	-36.3788	0.1169	0.0097	-0.7909	-42.2021	2.0700	0.1311	-1.5497	-65.8416
yHRW56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*	YPX	YPX	3	*S. cerevisiae*	0.0956	0.0179	0.7766	71.3145	0.2261	0.0111	0.1601	11.7377	8.1900	0.5262	0.4345	35.1485
yHRW135	*S. cerevisiae*	YPD	YPX		*S. cerevisiae*	0.0334	0.0094	-0.7428	-40.2420	0.1396	0.0034	-0.5350	-30.9823	2.5500	0.2606	-1.2488	-57.9208
yHRW135	*S. cerevisiae*	YPX	YPX		*S. cerevisiae*	0.0558	0.0379	0.0000	0.0000	0.2023	0.1131	0.0000	0.0000	6.0600	4.1196	0.0000	0.0000

spec.growth.median = median (n=3) of the maximum exponential growth rates achieved by the sample in independent cultures
spec.growth.sd = standard deviation (n=3) of the maximum exponential growth rates achieved by the sample in independent cultures
spec.growth.norm.median = median (n=3) of the maximum exponential growth rates achieved by the sample in independent cultures, normalized by dividing by the maximum exponential growth rate of the reference strain pre-cultured in the same medium (Pre-culture column) of the growth test medium (Medium of kinetic measurement column)
spec.growth.perc = percentage difference of the maximum exponential growth rates achieved by the sample in independent cultures (n=3) compared to the reference strain pre-cultured in the same medium (Pre-culture column) of the growth test medium (Medium of kinetic measurement column)
ODmax.median = median (n=3) of the estimated growth plateaus achieved by the sample in independent cultures
ODmax.sd = standard deviation (n=3) of the estimated growth plateaus achieved by the sample in independent cultures
ODmax.norm.median = median (n=3) of the estimated growth plateaus achieved by the sample in independent cultures, normalized by dividing by the estimated growth plateau of the reference included in the same experiment (plate run)
ODmax.perc = percentage difference of the estimated growth plateaus achieved by the sample in independent cultures (n=3) compared to the reference strain pre-cultured in the same medium (Pre-culture column) of the growth test medium (Medium of kinetic measurement column)
AUC.median = median (n=3) of the area under the curve achieved by the sample in independent cultures
AUC.sd = standard deviation (n=3) of the area under the curve achieved by the sample in independent cultures
AUC.norm.median = median (n=3) of the area under the curve achieved by the sample in independent cultures, normalized by dividing by the area under the curve of the reference included in the same experiment (plate run)
AUC.perc = percentage difference of the area under the curve achieved by the sample in independent cultures (n=3) compared to the reference strain pre-cultured in the same medium (Pre-culture column) of the growth test medium (Medium of kinetic measurement column)

Table S3 Wild, engineered, and synthetic hybrid strain information.

Genotypic information of wild *Saccharomyces* species and engineered strains for generating synthetic hybrids.

Strain	Synonym 1	Synonym 2	Synonym 3	Descended from	Parent A	Parent B	Species	Population	Genotype	Comment	Publication
GLBRCY73	yHDPN35			GLBRCY22			*Saccharomyces cerevisiae*	Engineered	*MAT***a***/MATα*; *ho*Δ*::XYL123-KanMX*; p^{+Scer} (C2_H13)	Evolved in YPX, aerobically	(15)
GLBRCY101		yHDPN47		GLBRCY73			*Saccharomyces cerevisiae*		*MAT***a** *ho*Δ*::XYL123-KanMX*; p^{+Scer} (C2_H13)	Dissected from GLBRCY73	(14)
yHWA85				GLBRCY101			*Saccharomyces cerevisiae*		*MAT***a** *ho*Δ*::XYL123*; p^{+Scer} (C2_H13)		This study
yHWA415				yHWA85			*Saccharomyces cerevisiae*		*MAT***a** *ho*Δ*::XYL123*; p^{+Scer} (C2_H13); [*pHRW40-KanMX*]		This study
yHRW134					yHWA415	yHWA415	*Saccharomyces cerevisiae*		*ho*Δ*::XYL123/ho*Δ*::XYL123*; p^{+Scer} (C2_H13); [*pHRW40-KanMX*]		This study
yHRW135				yHRW134			*Saccharomyces cerevisiae*		*ho*Δ*::XYL123/ho*Δ*::XYL123*; p^{+Scer} (C2_H13)		This study
yHRW116	FM472	CBS432					*Saccharomyces paradoxus*	EU	*MAT***a***/MATα*; p^{+Spar} (C2_H61)		(49)
yHRW129				yHRW116			*Saccharomyces paradoxus*		*MAT***a***/MATα*; p^{+Spar} (C2_H61); [*pHRW32-ZeoMX*]		This study
yHRW119	yHAB8	IFO1815					*Saccharomyces mikatae*	Asia A	*MAT***a***/MATα*; p^{+Smik} (C2_H74)		(14)
yHRW123				yHRW119			*Saccharomyces mikatae*		*MAT***a***/MATα*; p^{+Smik} (C2_H74); [*pHRW32-ZeoMX*]		This study
yHRW118	FM1009	ZP591					*Saccharomyces kudriavzevii*	EU	*MAT***a***/MATα*; p^{+Skud} (C2_H90)		(50)
yHRW126				yHRW118			*Saccharomyces kudriavzevii*		*MAT***a***/MATα*; p^{+Skud} (C2_H90); [*pHMK34-HygMX*]		This study
yHRW131				yHRW118			*Saccharomyces kudriavzevii*		*MAT***a***/MATα*; p^{+Skud} (C2_H90); [*pHCT2-NatMX*]		This study
yHRW111	FM1198	CBS10644					*Saccharomyces arboricola*	Asia A	*MAT***a***/MATα*; p^{+Sarb} (C2_H76)		(51)
yHRW112				yHRW111			*Saccharomyces arboricola*		*MAT***a***/MATα*; p^{+Sarb} (C2_H76); [*pHMK34-HygMX*]		This study
yHRW110	yHDO562	FM1132	CBS7001				*Saccharomyces uvarum*	HOL-EU	*MAT***a***/MATα*; p^{+Suva} (C2_H77)		(50)
yHRW114				yHRW110			*Saccharomyces uvarum*		*MAT***a***/MATα*; p^{+Suva} (C2_H77); [*pHMK34-HygMX*]		This study
yHRW121				yHRW110			*Saccharomyces uvarum*		*MAT***a***/MATα*; p^{+Suva} (C2_H77); [*pHCT2-NatMX*]		This study
yHRWh4					yHRW123	yHRW131	94% *S. mikatae* x 95% *S. kudriavzevii*		p^{+Skud}; [*pHRW32-ZeoMX*][*pHCT2-NatMX*]		This study
yHRWh7					yHRW112	yHRW129	92% *S. arboricola* x 90% *S. paradoxus*		p^{+Sarb}; [*pHRW32-ZeoMX*][*pHMK34-HygMX*]		This study
yHRWh8					yHRW112	yHRW129	93% *S. arboricola* x 91% *S. paradoxus*		p^{+Spar}; [*pHRW32-ZeoMX*][*pHMK34-HygMX*]		This study
yHRWh10					yHRW134	yHRW114	90% *S. cerevisiae* x 93% *S. uvarum*		$p^{+Scer/Suva/hc-Scer}$; [*pHRW40-KanMX*][*pHMK34-HygMX*]		This study
yHRWh13					yHRW123	yHRW121	95% *S. mikatae* x 92% *S. uvarum*		p^{+Suva}; [*pHRW32-ZeoMX*][*pHCT2-NatMX*]		This study
yHRWh18					yHRW126	yHRW129	95% *S. kudriavzevii* x 91% *S. paradoxus*		p^{+Spar}; [*pHRW32-ZeoMX*][*pHMK34-HygMX*]		This study
yHRWh19					yHRW134	yHRW112	89% *S. cerevisiae* x 93% *S arboricola*		p^{+Scer}; [*pHRW40-KanMX*][*pHMK34-HygMX*]		This study
yHRWh22					yHRWh19	yHRWh13	79% *S. cerevisiae* x 56% *S. arboricola* x 24% *S. mikatae* x 25% *S. uvarum*		p^{+Scer}; [*pHRW40-KanMX*][*pHCT2-NatMX*]		This study
yHRWh24					yHRWh10	yHRWh4	43% *S. cerevisiae* x 94% *S. kudriavzevii* x 93% *S. mikatae* x 49% *S. uvarum*		$p^{+hc-Suva}$; [*pHRW40-KanMX*][*pHCT2-NatMX*]		This study
yHRWh36					yHRWh24	yHRWh7	24% *S. cerevisiae* x 92% *S. kudriavzevii* x 85% *S. mikatae* x 50% S. *uvarum* x 90% *S. arboricola* x 82% *S. paradoxus*		$p^{+hc-Sarb/hc-Suva}$; [*pHRW40-KanMX*][*pHMK34-HygMX*]		This study
yHRWh39					yHRWh22	yHRWh18	86% *S. cerevisiae* x 13% *S. kudriavzevii* x 61% *S. mikatae* x 78% *S. uvarum* x 76% *S. arboricola* x 34% *S. paradoxus*		p^{+Scer}; [*pHRW32-ZeoMX*][*pHCT2-NatMX*]		This study
yHRWh42					yHRW134	yHRWh8	87% *S. cerevisiae* x 93% *S. arboricola* x 83% *S. paradoxus*		p^{+Scer}; [*pHRW32-ZeoMX*][*pHRW40-KanMX*]		This study
yHRWh51					yHRW114	yHRWh4	76% *S. uvarum* x 83% *S. mikatae* x 86% *S. kudriavzevii*		p^{+Suva}; [*pHCT2-NatMX*][*pHMK34-HygMX*]		This study
yHRWh56					yHRWh42	yHRWh51	87% *S. cerevisiae* x 49% *S. kudriavzevii* x 62% *S. mikatae* x 30% *S. uvarum* x 82% *S. arboricola* x 82% *S. paradoxus*		p^{+Scer}; [*pHRW40-KanMX*][*pHCT2-NatMX*]		This study
yHRWh82				yHRWh39			87% *S. cerevisiae* x 14% *S. kudriavzevii* x 55% *S. mikatae* x 66% S. *uvarum* x 62% *S. arboricola* x 30% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh83				yHRWh39			86% *S. cerevisiae* x 14% *S. kudriavzevii* x 62% *S. mikatae* x 59% S. *uvarum* x 71% *S. arboricola* x 29% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh84				yHRWh39			86% *S. cerevisiae* x 13% *S. kudriavzevii* x 61% *S. mikatae* x 65% S. *uvarum* x 71% *S. arboricola* x 20% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh88				yHRWh39					p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study
yHRWh89				yHRWh39			65% *S. cerevisiae* x 14% *S. kudriavzevii* x 47% *S. mikatae* x 38% S. *uvarum* x 43% *S. arboricola* x 15% *S. paradoxus*		p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study
yHRWh90				yHRWh39			67% *S. cerevisiae* x 10% *S. kudriavzevii* x 29% *S. mikatae* x 42% S. *uvarum* x 29% *S. arboricola* x 23% *S. paradoxus*		p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study
yHRWh85				yHRWh56			75% *S. cerevisiae* x 37% *S. kudriavzevii* x 38% *S. mikatae* x 8% *S. uvarum* x 66% *S. arboricola* x 82% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh86				yHRWh56			80% *S. cerevisiae* x 32% *S. kudriavzevii* x 49% *S. mikatae* x 8% *S. uvarum* x 65% *S. arboricola* x 82% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh87				yHRWh56			84% *S. cerevisiae* x 41% *S. kudriavzevii* x 55% *S. mikatae* x 27% *S. uvarum* x 66% *S. arboricola* x 78% *S. paradoxus*		p^{+Scer}	Evolved in YPD, aerobically for 80 generations	This study
yHRWh91				yHRWh56			87% *S. cerevisiae* x 37% *S. kudriavzevii* x 56% *S. mikatae* x 27% *S. uvarum* x 61% *S. arboricola* x 77% *S. paradoxus*		p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study
yHRWh92				yHRWh56			84% *S. cerevisiae* x 43% *S. kudriavzevii* x 36% *S. mikatae* x 32% *S. uvarum* x 40% *S. arboricola* x 59% *S. paradoxus*		p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study
yHRWh93				yHRWh56			89% *S. cerevisiae* x 50% *S. kudriavzevii* x 62% *S. mikatae* x 29% *S. uvarum* x 72% *S. arboricola* x 84% *S. paradoxus*		p^{+Scer}	Evolved in YPX, aerobically for 80 generations	This study

C2_HXXX: Indicates the COX2 haplotype based on (*48*)
XYL123, abbreviation for the *Sch. stipitis XYL1-XYL2-XYL3* cassette.

158 **Table S4 Primers.**

159 PCR primers used to confirm mating-type switches and hybridization.

Primer collection name	Primer name	Species target	Target gene	Strand	Sequence (5'-3')	Annealing T °C	Reference paper
oHRW247	MAT common	All *Saccharomyces* species	*MAT*	Reverse	GAACCGCATGGGCAGTTTACCTTT	48.1	(11)
oHRW248	Mata	All *Saccharomyces* species	*MAT*	Forward	CTCCACTTCAAGTAAGAGTTTGGGT	50.1	(11)
oHRW249	Matalpha	All *Saccharomyces* species	*MAT*	Forward	TTACTCACAGTTTGGCTCCGGTGT	52.3	(11)
oHDP022	BRE5_5.1	*Scer, Sarb, Skud, Smik, Spar*	*BRE5*	Forward	TGATTATAGCCACGGGTGARATGTTYTGG	55.5	(11)
oHDP023	BRE5_5.2	*Scer, Suva, Seub, Spar*	*BRE5*	Forward	TGATTATAGCCACKGGTGARATGTTTTGG	55.5	(11)
oHDP024	BRE5_3.2	All *Saccharomyces* species	*BRE5*	Reverse	TTCAKTCATCAAYTTTGAGGCCCATGTCAT	55.5	(11)
oHDP025	GAL4_5.2	All *Saccharomyces* species	*GAL4*	Forward	TGYGCCAAGTGTCTGAAGAAYAACTGGGA	55.5	This study
oHDP026	GAL4_3.2	All *Saccharomyces* species	*GAL4*	Reverse	GCGATTTCAATCTGRTTATTRTACARCATCAT	55.5	This study
oHDP027	GSY1_5.2	All *Saccharomyces* species	*GSY1*	Forward	ATTGGSAAAAGAATATTCGAGCATRCRATGAG	55.5	This study
oHDP028	GSY1_3.2	All *Saccharomyces* species	*GSY1*	Reverse	AATTTCTTACCACCGGCAAGRGTATYCATATT	55.5	This study
oHDP029	KEL2_5.2	All *Saccharomyces* species	*KEL2*	Forward	TATCTTTTCAATATYAATTCTTATAARTGGAC	55.5	This study
oHDP030	KEL2_3	All *Saccharomyces* species	*KEL2*	Reverse	GCAGTTTCAAGTTCYTTYTGYTG	55.5	(20)
oHDP031	PPR1_5.2	All *Saccharomyces* species	*PPR1*	Forward	CRGGTGTTTGGTATACYATGGG	55.5	This study
oHDP032	PPR1_3	All *Saccharomyces* species	*PPR1*	Reverse	TAAAGATAAGTCATACCTGTCATRAACAT	55.5	(20)

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161 *Sarb*, *S. arboricola*; *Scer*, *Saccharomyces cerevisiae*; *Seub*, *S. eubayanus*; *Skud*, *S. kudriavzevii*; *Smik*, *S. mikatae*; *Spar*, *S. paradoxus*; *Suva*, *S. uvarum.*

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163 **Table S5 PCR-RFLP expected *Saccharomyces* bands.**

	Specie (Strain)	*S. cerevisae* (GLBRCY73) Biofuel	*S. paradoxus* (CECT1939T/CBS432) EU	*S. mikatae* (IFO1815) Asia A	*S. kudriavzevii* (ZP591) EU	*S. arboricola* (CBS10644) Asia A	*S. uvarum* (CBS7001) HOL-EU
Gene	**Restriction enzyme**						
GSY1	PCR SIZE	*767*	*767*	*767*	*767*	*767*	*767*
YFR015C	*Eco* RI	496+275	496+275	767	767	767	767
Chr VI	*Hae* III	638+82+47	720+47	767	661+106	767	515+226+26
	Msp I (*Hpa* II)	597+159+15	430+339	754+15	326+270+162+15	381+339+51	381+326+51+15
KEL2	PCR SIZE	*1202*	*1196*	*1220*	*1214*	*1223*	*1241*
YGR238C	*Hinf* I	742+246+136+87	742+457	742+381+103	594+454+172	742+484	742+502
Chr VII	*Hae* III	1007+121+50+17+7	694+307+121+50+17+7	1025+121+74	822+226+92+74	466+365+197+92+74+29	681+365+121+74
	Msp I (*Hpa* II)	781+229+196	559+222+169+168+86	724+496	657+336+225	996+229	898+345
PPR1	PCR SIZE	*706*	*706*	*706*	*706*	*706*	*706*
YLR014C	*Hae* III	397+309	706	706	541+165 (observed 600)	397+277+32	309+267+90+40
	Hinf I	378+225+109	297+225+131+62	297+225+131+62	425+225+62	244+136+124+110+104	377+124+104+62+51
Chr XII	*Xba* I	706	706	706	706	706	461+249
	ScrF I	227+200+164+115	211+200+118+115+46+16	364+342	364+209+133	342+274+90	364+342
	Taq I	235+184+166+127	184+166+129+112+108+17	166+126+112+108+91+60+40+17	360+184+166	251+166+126+91+60+22	259+184+142+71+58
BRE5	PCR SIZE	*782*	*782*	*782*	*785*	*794*	*779*
YNR051C	*Hae* III	279+270+204+20+9	762+20	782	438+327+20	370+240+170+14	471+288+20
Chr XIV	*Hinf* I	289+228+99+66+50+42+8	290+231+102+69+63+22+20	231++156+141+138+51+41+30+12	171+165+138+114+72+69+42+20+12	228+153+129+93+55+50+44+42	335+301+143
	Msp I (*Hpa* II)	388+195+88+78+41	353+195+164+41+37	314+312+158	724+50+15	383+365+50	779
GAL4	PCR SIZE	725	*740*	*740*	*740*	*749*	*737*
YPL248C	*Scr* FI		749	740	409+183+79+69	670+79	405+184+79+69
Chr XVI	*Taq* I	207+203+181+140	222+214+166+140+6	396+166+94+46+31+13	436+105+90+70+47	445+166+142	306+295+91+46+7
	Xba I	614+115	629+115	740	740	638+115	737
	Msp I (*Hpa* II)	543+184	558+184	558+184	740	749	315+242+184

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165 Recommended restriction enzyme

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167 **Table S6 Summary of whole genome sequencing statistics.**

168 Read length, coverage, and SRA accession numbers for sequencing libraries.

Strain Name	Species	Run Type	Read Length	Average Insert Size	Average Coverage	Kit	Bioproject
yHRW134	*S. cerevisiae*		2x151	251.5	21X	MiSeq Reagent	PRJNA476226
yHRWh4	*S. mikatae* x *S. kudriavzevii*		2x151	250.9	11X	MiSeq Reagent	PRJNA476226
yHRWh7	*S. arboricola* x *S. paradoxus*		2x151	250.6	9X	MiSeq Reagent	PRJNA476226
yHRWh8	*S. arboricola* x *S. paradoxus*		2x151	250.8	13X	MiSeq Reagent	PRJNA476226
yHRWh10	*S. cerevisiae* x *S. uvarum*		2x151	249.8	13X	MiSeq Reagent	PRJNA476226
yHRWh13	*S. mikatae* x *S. uvarum*		2x151	250.9	12X	MiSeq Reagent	PRJNA476226
yHRWh18	*S. kudriavzevii* x *S. paradoxus*		2x151	251.1	12X	MiSeq Reagent	PRJNA476226
yHRWh19	*S. cerevisiae* x *S arboricola*		2x151	250.5	11X	MiSeq Reagent	PRJNA476226
yHRWh22	*S. cerevisiae* x *S. arboricola* x *S. mikatae* x *S. uvarum*		2x151	250.2	6X	MiSeq Reagent	PRJNA476226
yHRWh24	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum*		2x151	252	7X	MiSeq Reagent	PRJNA476226
yHRWh36	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	248	12X	MiSeq Reagent	PRJNA476226
yHRWh39	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	248	11X	MiSeq Reagent	PRJNA476226
yHRWh42	*S. cerevisiae* x *S. arboricola* x *S. paradoxus*		2x151	251	8X	MiSeq Reagent	PRJNA476226
yHRWh51	*S. uvarum* x *S. mikatae* x *S. kudriavzevii*		2x151	249	7X	MiSeq Reagent	PRJNA476226
yHRWh56	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	251	10X	MiSeq Reagent	PRJNA476226
yHRWh82	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	402	7X	MiSeq Reagent	PRJNA476226
yHRWh83	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	374	5X	MiSeq Reagent	PRJNA476226
yHRWh84	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	305	4X	MiSeq Reagent	PRJNA476226
yHRWh85	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	199	6X	MiSeq Reagent	PRJNA476226
yHRWh86	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	219	4X	MiSeq Reagent	PRJNA476226
yHRWh87	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	202	5X	MiSeq Reagent	PRJNA476226
yHRWh88	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*					MiSeq Reagent	
yHRWh89	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	275	5X	MiSeq Reagent	PRJNA476226
yHRWh90	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	250	7X	MiSeq Reagent	PRJNA476226
yHRWh91	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	192	6X	MiSeq Reagent	PRJNA476226
yHRWh92	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	195	6X	MiSeq Reagent	PRJNA476226
yHRWh93	*S. cerevisiae* x *S. kudriavzevii* x *S. mikatae* x *S. uvarum* x *S. arboricola* x *S. paradoxus*		2x151	181	7X	MiSeq Reagent	PRJNA476226

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