

SUPPLEMENTARY MATERIAL

TABLE OF CONTENTS

Supplemental Figures:

Supplemental Figure 1: Whole genome alignment of the JHU_Cniv_v1 mitochondrial contig and the *C. nivariensis* mitochondrial genome.

Supplemental Figure 2: Coverage histograms

Supplemental Figure 3: Telomere positions reference based scaffolds

Supplemental Figure 4: Whole genome alignments between related yeasts

Supplemental Figure 5: Whole genome alignment of JHU_Cniv_v1 and the *C. nivariensis* reference genome

Supplemental Tables:

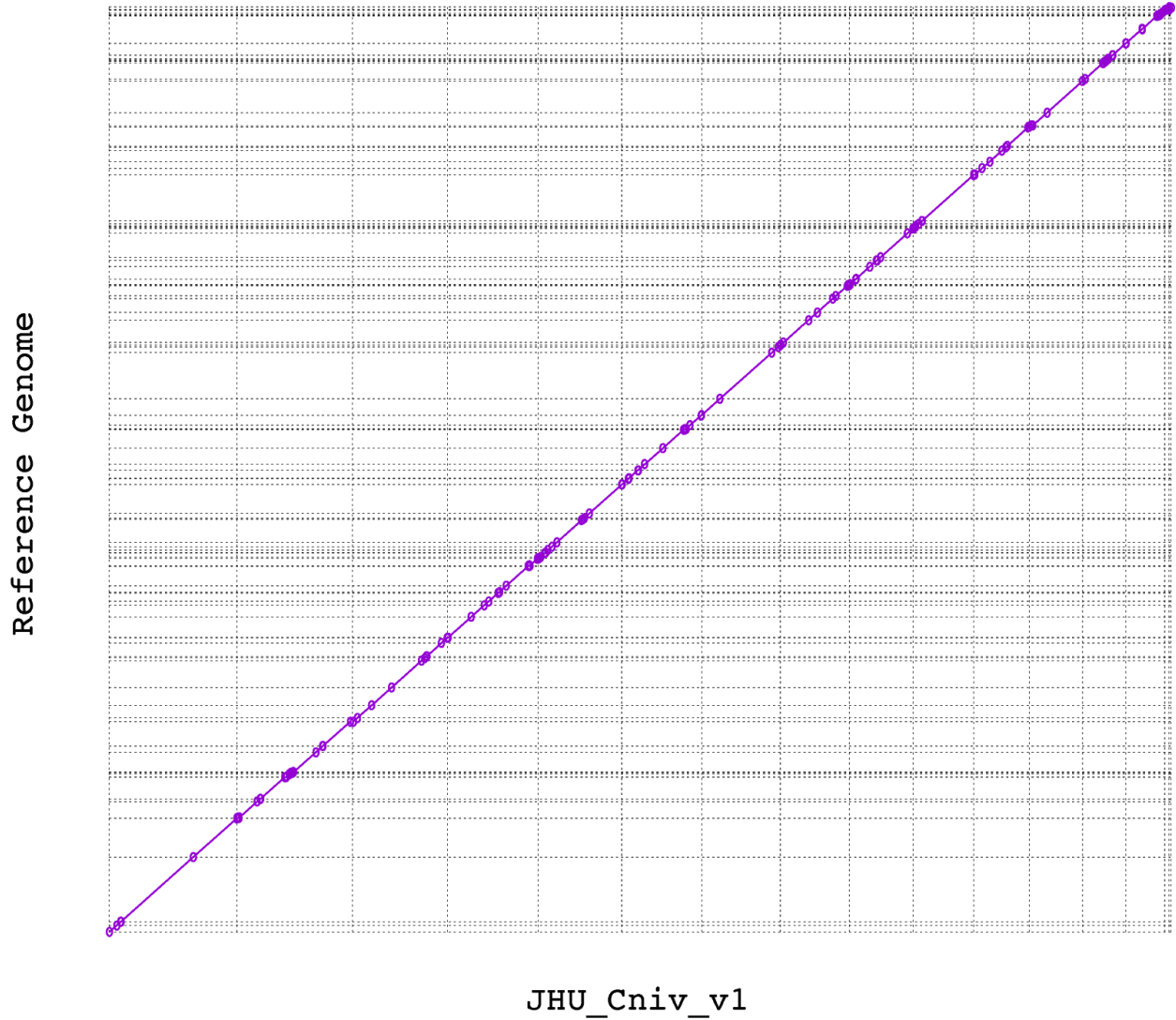
Supplemental Table 1: Contig lengths and contig telomere counts

Supplemental Table 2: Contributions from each annotation software

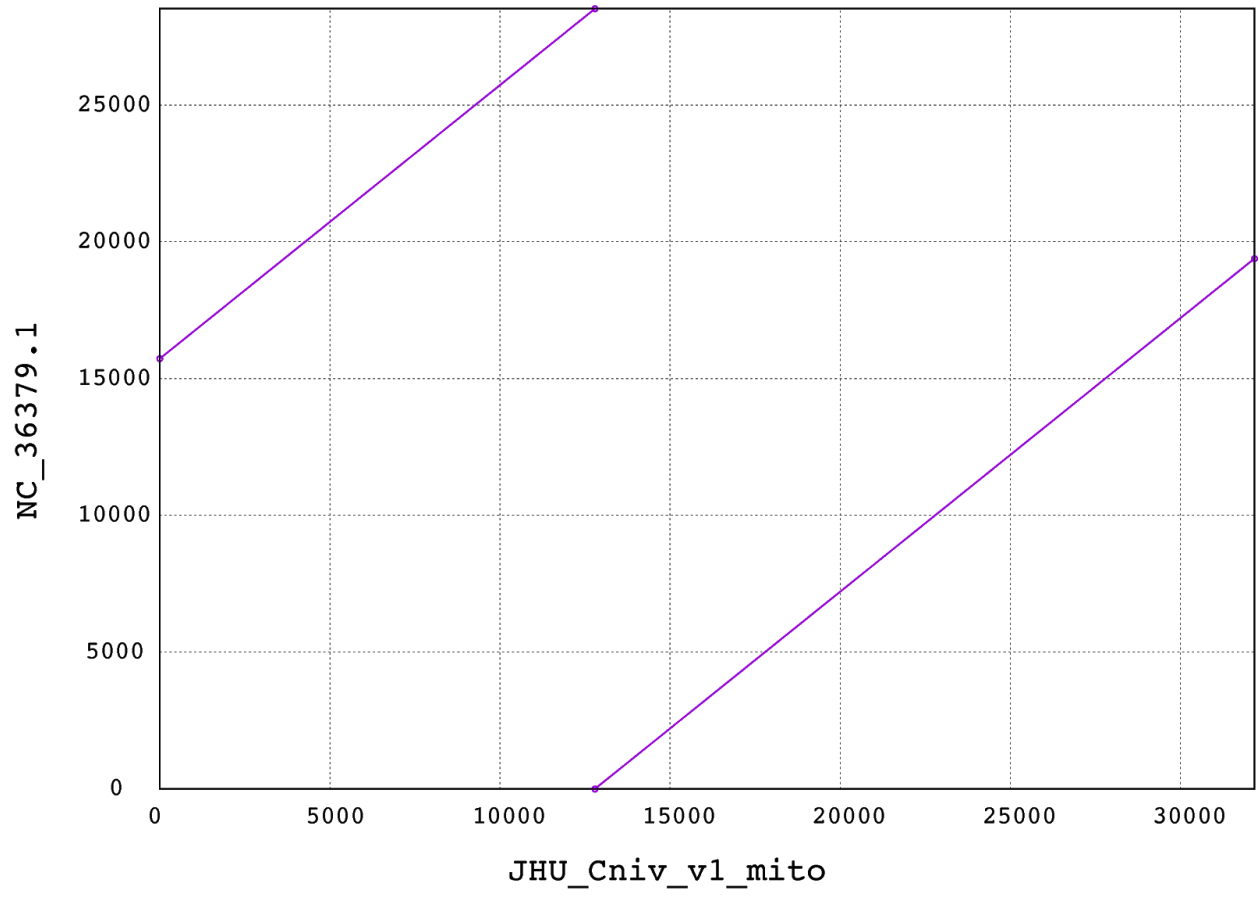
Supplemental Table 3: Gene and exon counts of JHU_Cniv_v1 and related yeasts

Supplemental Data:

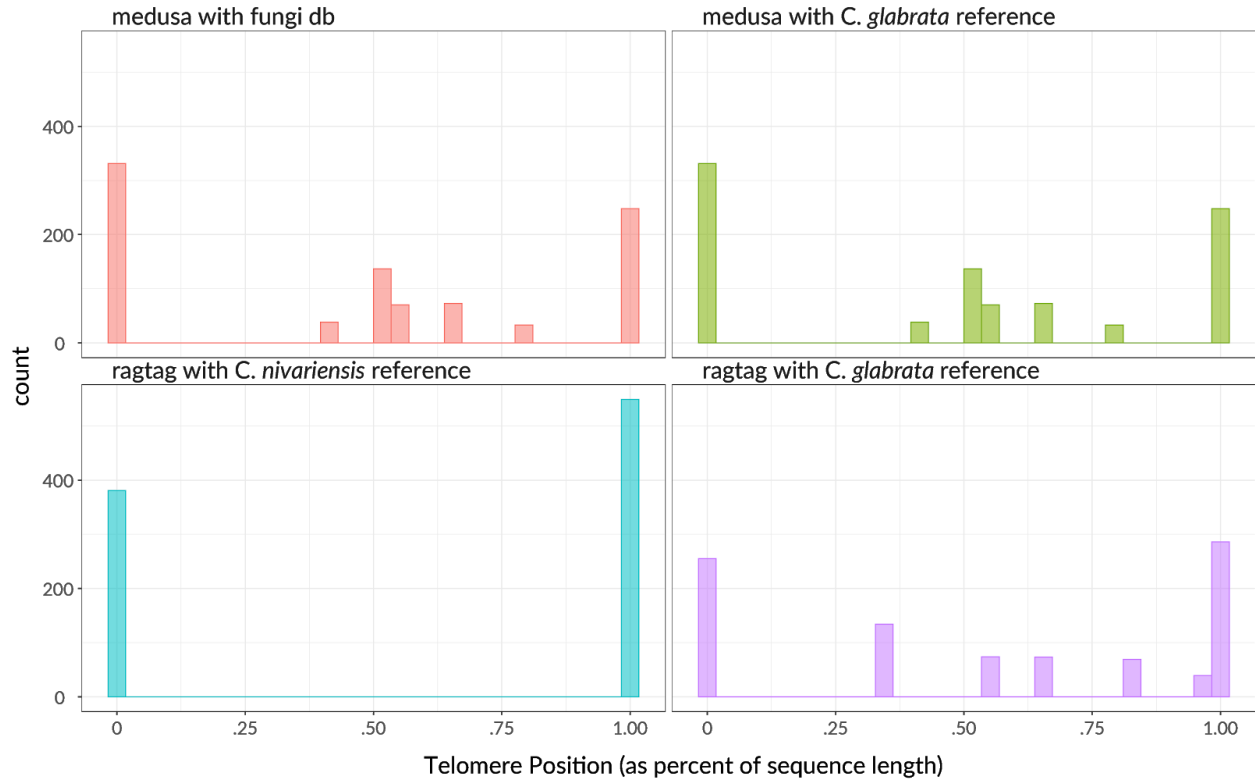
Supplemental Data 1: Copy numbers of subtelomeric homologues



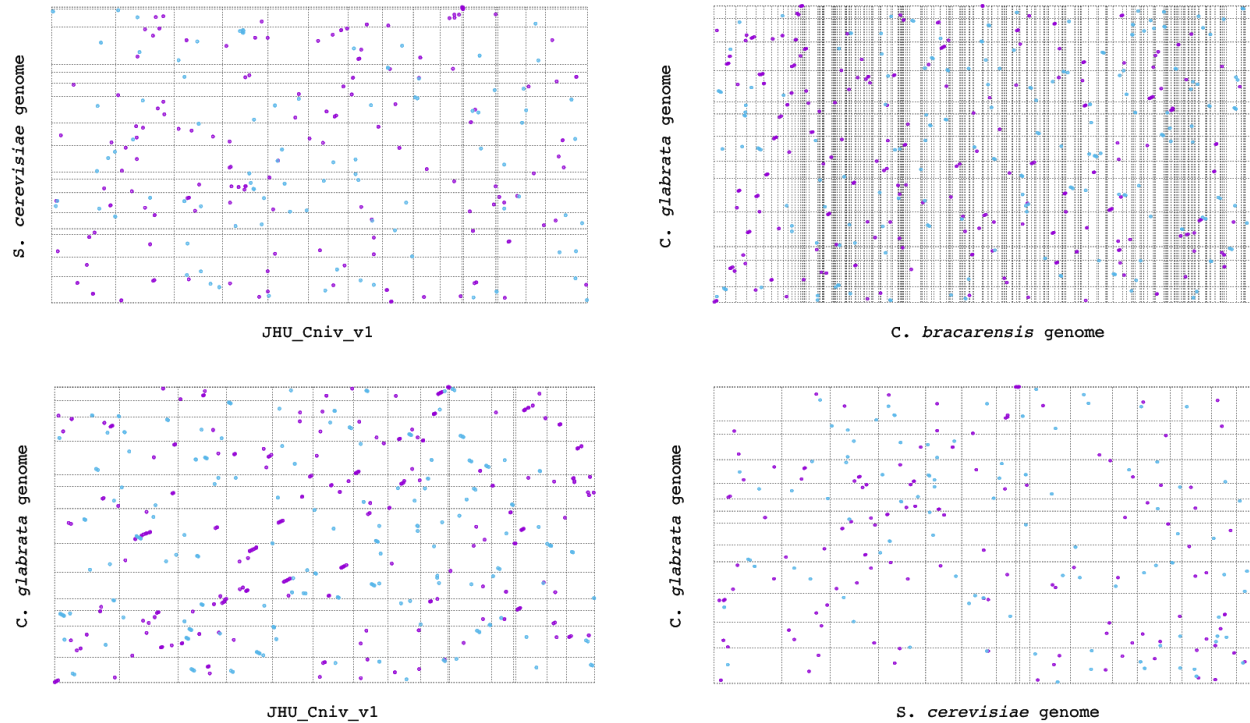
Supplemental Figure 1: Whole genome alignment of the current reference genome (y axis) compared and our new assembly (x axis). Alignments match with no notable structural variants, and very little missing or duplicated sequence.



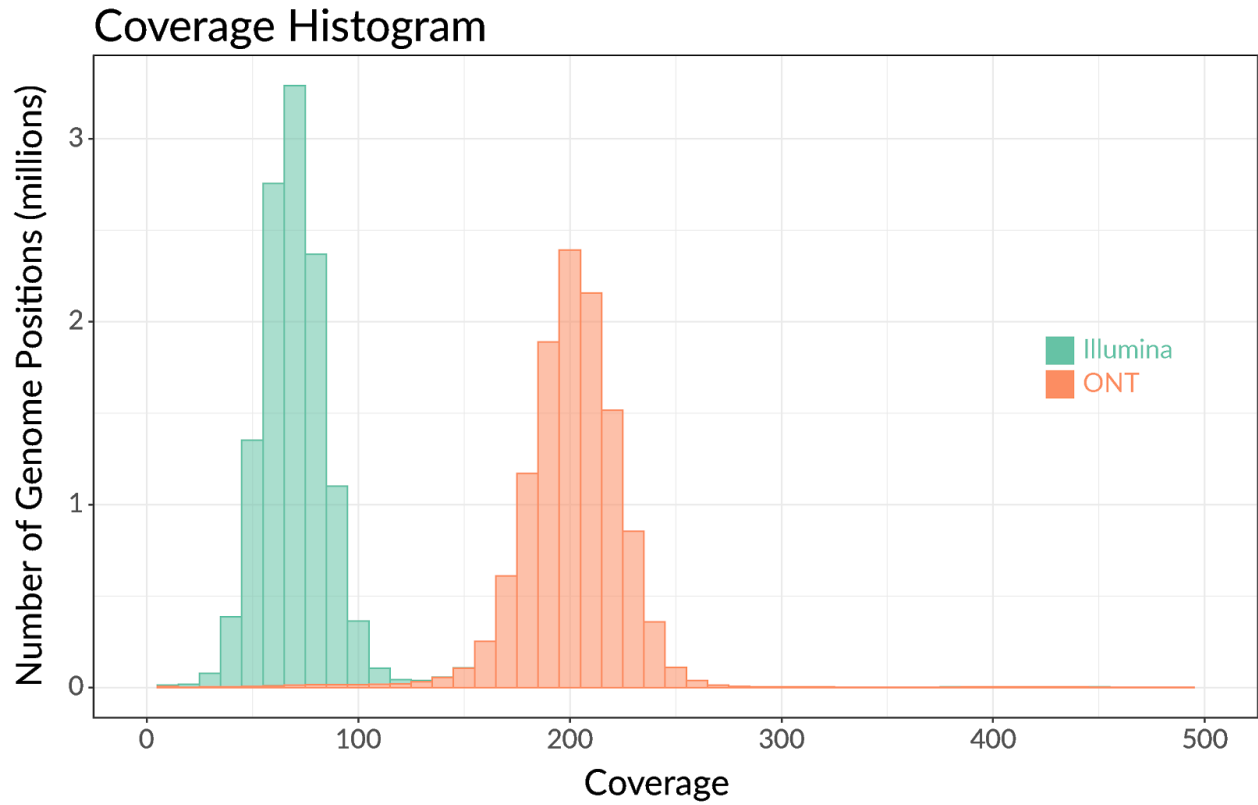
Supplemental Figure 2: Alignment of our 32Kb circular contig (x axis) with the completed mitochondrial genome of the *C. nivariensis* reference genome (y axis). The final 3662bp of this contig appears twice in the reference genome.



Supplemental Figure 3: Histogram of telomere repeat positions in our assembly, and in scaffolds produced by RagTag and MeDuSa. When MeDuSa is used with a database including the reference genomes of *C. nivariensis*, *C. glabrata*, *C. bracarensis*, and *N. delphensis*, telomeres are placed in the middle of contigs. The same result is produced when only the *C. glabrata* genome is used for scaffolding with MeDuSa, and MeDuSa fails to run when only the *C. nivariensis* reference is used. When the *C. nivariensis* reference genome is used for scaffolding with RagTag, no changes are made. When the more contiguous *C. glabrata* genome is used with RagTag, telomere sequences are again placed in the middle of sequences, suggesting a scaffolding error.



Supplemental Figure 4: Whole genome alignment of our new assembly against the *S. cerevisiae* (top left), and *C. glabrata* (bottom left) reference genomes. For both, there are no long alignments, suggesting that there is little similarity in genome structure between these species and *C. nivariensis*. *C. bracarensis*, a close relative to both *C. glabrata* and *C. nivariensis*, also shares little genome similarity to *C. glabrata* (top right), suggesting that yeast genomes within the glabrata clade are not generally similar enough to support inter-species reference based scaffolding. We also compared *C. glabrata* to the highly contiguous and complete *S. cerevisiae* genome (bottom right) to check that genome contiguity alone did not bias the genome similarity detected.



Supplemental Figure 5: Histogram of coverage per base in our assembly by filtered (>3kb) ONT reads and trimmed Illumina reads.

Contig	Length (bp)	Forward Telomeres	Reverse Telomeres
tig01	1423475	35	38
tig02	1283968	0	39
tig03	1060011	35	39
tig04	933062	36	26
tig05	1010854	0	36
tig06	885783	35	38
tig07	879540	39	35
tig08	763992	34	33
tig09	714796	35	47
tig10	675194	36	36
tig11	594828	32	26
tig12	617546	36	0
tig13	481613	38	41
tig14	434809	33	33
tig24	44616	0	39
JHU_Cniv_v1_mito	28512	0	0

Supplemental Table 1: Contig lengths and the number of times the forward and reverse telomere sequence appears in each.

	Total	Gene	Exon
Augustus (BRAKER)	23,497	5,028	6,109
Genemark.hmm (BRAKER)	36	6	12
Liftoff glabrata	263	130	2
Liftoff cerevisiae	42	21	0
Liftoff albicans	0	0	0
StringTie	2,141	824	1,175

Supplemental Table 2: Annotation contributions from each software

	Total Exons	Total Genes
JHU_Cniv_v1	7,298	5,859
<i>C. glabrata</i>	5,629	5,448
<i>S. cerevisiae</i>	6,760	6,420
<i>C. albicans</i>	6,732	6,263

Supplemental Table 3: Gene and exon counts of our annotation and currently available reference annotations.