Description of Additional Supplementary Files

File Name: Supplementary Data 1
Description: rDNA variants with their frequencies across isolates after filtering annotation descriptions for POSITION, VF(AF in LoFreq), INDEL, HRUN, QUAL_SCORE, SB, DP and DP4 are as in LoFreq NAME - Isolate name (from Peter et al., 2018 10.1038/s41586-018-0030-5) ACCESSION - SRA accession id (from Peter et al., 2018 10.1038/s41586-018-0030-5) POSITION - Nucleotide position in the rDNA prototype (S288c reference) REFERENCE - Nucleotide in the S288C reference at corresponding POSITION ISOLATE - Variant sequence in analyzed isolate VF - Intragenomic Variant frequency (iVF) INDEL - indicates if the variant is an indel HRUN - Homopolymer length to the right of reported indel position QUAL_SCORE - Quality score SB - Strand bias DP - Raw depth DP4 - Counts for ref-forward bases, ref-reverse, alt-forward and altreverse bases one_copy_VF - calculated iVF corresponded to a variant in one rDNA copy based on total rDNA copy number in this isolate

File Name: Supplementary Data 2
Description: Calculated Shannon's entropy at each position in rDNA across all rDNA copies from all isolates pooled together POSITION - position in the rDNA prototype A, T, G, C, INDEL - number of sequences with A, T, G, C, and indels at a given position (indels of all sizes are lumped together in INDEL) H - Shannon's entropy

File Name: Supplementary Data 3
Description: Variant pairs with coherent frequencies across all isolates NAME - Isolate name (from Peter et al., 2018 10.1038/s41586-018-0030-5) ACCESSION - SRA accession id (from Peter et al., 2018 10.1038/s41586-018-0030-5) POSITION_1 - Nucleotide position in the rDNA prototype for variant 1 in a pair VARIANT_1 - Sequence of variant 1 in a pair VF_1 - Intragenomic variant frequency (iVF) of variant 1 POSITION_2 - Nucleotide position in the rDNA prototype for variant 2 in the pair VARIANT_2 - Sequence of variant 2 in the pair VF_2 - Intragenomic variant frequency (iVF) of variant 2

File Name: Supplementary Data 4
Description: Variant pairs with coherent frequencies analyzed by ecological niche NAME - Isolate name (from Peter et al., 2018 10.1038/s41586-018-0030-5) ACCESSION - SRA accession id (from Peter et al., 2018 10.1038/s41586-018-0030-5) NICHE - ecological origin (from Peter et al., 2018 10.1038/s41586-018-0030-5) POSITION_1 - Nucleotide position in the rDNA prototype for variant 1 in a pair VARIANT_1 - Sequence of variant 1 in a pair VF_1 - Intragenomic variant frequency (iVF) of variant 1 POSITION_2 - Nucleotide position in the rDNA prototype for variant 2 in the pair VARIANT_2 - Sequence of variant 2 in the pair VF_2 - Intragenomic variant frequency (iVF) of variant 2

File Name: Supplementary Data 5
Description: rRNA variant annotation SUBUNIT - Ribosomal subunit (SSU - small, LSU - large) RRNA - rRNA DNA_POSITION - Nucleotide position in the rDNA prototype (S288c reference); rDNA coordinates DNA_REFERENCE - Nucleotide in the S288C reference at corresponding POSITION; rDNA coordinates DNA_ISOLATE - Variant sequence in analyzed isolate; rDNA coordinates RNA_POSITION - Nucleotide position in the prototype (S288c reference); rRNA coordinates RNA_REFERENCE - Nucleotide in the S288C reference at corresponding POSITION; rRNA coordinates RNA_ISOLATE - Variant sequence in analyzed isolate; rRNA coordinates VF - Intragenomic variant frequency (iVF) SHELL - Subunit shells (from Bernier et al. 2014 10.1039/c3fd00126a) ES - Expansion segments (from Ben-Shem et al. 2011 10.1126/science.1212642) RP - Ribosomal proteins (from Ben-Shem et al. 2011 10.1126/science.1212642) CNE - Presence in eukaryotic conserved nucleotide elements (from Doris et
al. 2015 10.1261/rna.051144.115); defined only for 25S #BRIDGE_WITHIN_5A - Presence within 5 Angstrom of intersubunit bridges (Bridges are from Ben-Shem et al. 2011 10.1126/science.1212642)
#INDEL - Indel variants #NAME - Isolate name (from Peter et al., 2018 10.1038/s41586-018-0030-5)
#ACCESSION - SRA accession id (from Peter et al., 2018 10.1038/s41586-018-0030-5)

File Name: Supplementary Data 6
Description:
1) raw_rDNA_var_calls - raw rDNA .vcf files prior to additional filtering
2) Sequencing_Sultanov_etal – raw rDNA .vcf files (for DNA- and total RNA sequencing) generated in this study and the rDNA coverage for each sample
3) rDNA_S288c.fsa - the S288c rDNA copy prototype sequence used in this study
4) rDNA_S288c.bed - annotations associated with the rDNA prototype
5) rDNA_S288c_benchmark.bed - annotations for benchmarking
6) positions_in_homopolymers.txt – nucleotide positions in the S288c rDNA prototype that are embedded in the 10-nt poly(A/T/G/C) sequences.