**SUPPLEMENTARY INFORMATION**

**Table S1. Mitonuclear Strain Table**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **mtDNA→**  **nuclear↓** | **YJM975** | **273614N** | **YPS606** | **Y55** | **BC187** | **SK1** | **L-1528** | **YIIc17\_E5** | **Y12** |
| **YJM975** | NCYC 35941 | SP15a1 𝜌62 | SP15a1 𝜌272O | SP15a1 𝜌92O | SP15a1 𝜌122∆ | SP15a1 𝜌113 | SP15a1 𝜌203 | SP15a1 𝜌72 | SP15a1 𝜌262O |
| **273614N** | SP6a1 𝜌152 | NCYC  3585 1 | SP6a1 𝜌272 | SP6a1 𝜌92 | SP6a1 𝜌122 | SP6a1 𝜌113 | SP6a1 𝜌203 | SP6a1 𝜌72 | SP6a1 𝜌262 Δ |
| **YPS606** | SP27a1 𝜌152 | SP27a1 𝜌62 | NCYC  3606 1 | SP27a1 𝜌92 | SP27a1 𝜌122 | SP27a1 𝜌113 | SP27a1 𝜌203 | SP27a1 𝜌72 | SP27a1 𝜌262 |
| **Y55** | SP9a1 𝜌152 | SP9a1 𝜌62 | SP9a1 𝜌272 | NCYC  3588 1 | SP9a1 𝜌122 | SP9a1 𝜌113 | SP9a1 𝜌203 | SP9a1 𝜌72 | SP9a1 𝜌262 |
| **BC187** | SP12a1 𝜌152 | SP12a1 𝜌62 | SP12a1 𝜌272 | SP12a1 𝜌92 | NCYC  3599 1 | SP12a1 𝜌113 | SP12a1 𝜌203 | SP12a1 𝜌72 | SP12a1 𝜌262 |
| **SK1** | SP11a1 𝜌153∆ | SP11a1 𝜌63∆ | SP11a1 𝜌273∆ | SP11a1 𝜌93∆ | SP11a1 𝜌123∆ | NCYC 35861∆ | SP11a1 𝜌203∆ | SP11a1 𝜌73∆ | SP11a1 𝜌263∆ |
| **L-1528** | SP20a1 𝜌152 | SP20a1 𝜌62 | SP20a1 𝜌273 | SP20a1 𝜌93 | SP20a1 𝜌123 | SP20a1 𝜌113 | NCYC 35911 | SP20a1 𝜌73 | SP20a1 𝜌263 |
| **YIIc17\_E5** | SP7a1 𝜌152 | SP7a1 𝜌62 | SP7a1 𝜌272 | SP7a1 𝜌92 | SP7a1 𝜌122 | SP7a1 𝜌113 | SP7a1 𝜌203 | NCYC  35901 | SP7a1 𝜌262 |
| **Y12** | SP26a1 𝜌152 | SP26a1 𝜌62 | SP26a1 𝜌272 | SP26a1 𝜌92 | SP26a1 𝜌122 | SP26a1 𝜌113 | SP26a1 𝜌203 | SP26a1 𝜌72 | NCYC  36051 |

Individual strain names for each mitonuclear genome combination are provided. All strains are *MAT***a** *ura3::KanMX ho::HygMX*.

1. Cubillos et al., 2009; 2. Paliwal et al., 2014; 3. S. Paliwal and H. Fiumera

O. Strains used for matings to generate recombinant diploids and controls.

Δ. Strains excluded from analysis due to flocculation or contamination

**Table S3: Media recipes**

|  |  |  |
| --- | --- | --- |
| Media | Metabolic Pathway | Recipe |
| SD | Fermentation | 0.67% yeast nitrogen base without amino acids, 2% dextrose |
| CSM | Fermentation | 0.08% Complete Supplement Mixture (Sunrise Science Products), 0.67% yeast nitrogen base without amino acids, 2% dextrose |
| YPD | Fermentation | 2% dextrose, 2% peptone, 1% yeast extract |
| SOE | Fermentation | 1% sucrose, 0.5% fructose, 0.15% peptone, 0.1% yeast extract, 0.5% dextrose |
| CSMEG | Mitochondrial respiration | 0.08% Complete Supplement Mixture (Sunrise Science Products), 0.67% yeast nitrogen base without amino acids, 3% ethanol, 3% glycerol |
| YPEG | Mitochondrial respiration | 1% yeast extract, 2% peptone, 3% ethanol, 3% glycerol |
| YPEGM | Mitochondrial respiration | 1% yeast extract, 2% peptone, 3% ethanol, 3% glycerol, 20 µM menadione |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Forward primer  (5’ -> 3’) | Reverse primer  (5’ -> 3’) | PCR conditions | Reference |
| *ATP6* | GATCAATTTGAAATTAGACTATTATTTGG | TGCATCTTTTAAATATGATGCTG | 95° -1 min, (95°-30s, 49°-30s 68°-2 min)30, 72°-5 min | This work |
| *COX2* | GGTATTTTAGAATTACATGA | ATTTATTGTTCATTTAATCA | 95° -1 min, (94°-30s, 49°-45s, 68°-2 min, )30, 68°-5 min | (Belloch *et al.* 2000) |
| *COX3* | TATGCCTTCACCATGACC | TCCAACATGATGTCCAGC | 95° -1 min, (95°-30s, 49°-30s 68°-2 min)30, 72°-5 min | (Hsu and Chou 2017) |

**Table S4: PCR primers and conditions**

**Table S5. Non-parental Phenotypes**

|  |  |  |
| --- | --- | --- |
| Media | Temperature  (°C) | Phenotypically distinct strains |
| CSM | 30 | 0 |
| 35 | 2 |
| 37 | 2 |
| CSMEG | 30 | 0 |
| 35 | 0 |
| 37 | 2 |
| SOE | 30 | 0 |
| 35 | 0 |
| 37 | 28 (15) |
| YPD | 30 | 0 |
| 35 | 2 |
| 37 | 1 |
| YPEG | 30 | 0 |
| 35 | 3 |
| 37 | 7 (3) |
| YPEGM | 30 | 0 |
| 35 | 5 (1) |
| 37 | 3 |

Strains with recombinant mtDNAs whose growth phenotypes were significantly different from strains with either parental mitotype are indicated. A total of 41 strains were tested for each condition. Parentheses indicate number of strains significantly different after a Bonferroni correction.

**Table S9. Variance Component Analyses**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Media | Temperature  (°C) | Nuclear | Mitotype | Nuclear x  Mitotype | Residual | Variance | Normalized  Variance |
| CSM | 30 | 84.2% | 0.0% | 6.8% | 8.9% | 1404042493 | 10058.70 |
| 35 | 96.0% | 0.0% | 1.4% | 2.5% | 4070752463 | 27062.25 |
| 37 | 96.0% | 0.0% | 2.1% | 1.9% | 4551915599 | 36743.87 |
| CSMEG | 30 | 28.7% | 0.7% | 12.5% | 58.1% | 262066091 | 2695.22 |
| 35 | 72.8% | 3.3% | 8.8% | 15.1% | 370539419 | 4480.51 |
| 37 | 67.9% | 4.1% | 17.4% | 10.5% | 648653887 | 9908.97 |
| SOE | 30 | 56.4% | 0.9% | 17.1% | 25.6% | 354953640 | 6534.77 |
| 35 | 55.7% | 0.0% | 27.2% | 17.1% | 706973944 | 14388.74 |
| 37 | 76.4% | 0.1% | 15.9% | 7.6% | 889286999 | 24513.76 |
| YPD | 30 | 72.7% | 0.0% | 12.7% | 14.6% | 9845740451 | 43153.03 |
| 35 | 70.2% | 1.2% | 6.7% | 22.0% | 4678801380 | 22438.39 |
| 37 | 80.6% | 0.0% | 6.0% | 13.4% | 6918640032 | 32629.14 |
| YPEG | 30 | 72.7% | 0.0% | 16.5% | 10.8% | 4354175900 | 38822.03 |
| 35 | 39.0% | 1.4% | 41.3% | 18.4% | 2188542030 | 16552.17 |
| 37 | 64.1% | 6.1% | 12.4% | 17.4% | 1969776569 | 16247.35 |
| YPEGM | 30 | 77.8% | 0.3% | 14.0% | 7.8% | 5149706874 | 51793.93 |
| 35 | 37.3% | 10.9% | 27.3% | 24.5% | 1488587396 | 11755.24 |
| 37 | 33.6% | 18.3% | 16.0% | 32.1% | 1481953868 | 13745.37 |

Variance components were estimated from the models used for ANOVAs and are expressed as percentages of total phenotypic variance. Normalized variance was determined by dividing total variance by the grand mean for that condition. Variance and normalized variance are expressed in the arbitrary units used to designate colony size.

**Table S10: Genotyping of Putative mtDNA Recombinants.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genotype Pattern | | | Number of strains | Novel banding patterns from total mtDNA RFLPs |
| *ATP6* | *COX2* | *COX3* |  |  |
| YJM975 | YJM975 | YJM975 | 24 | 6 (of 8 tested) |
| Y12 | Y12 | Y12 | 12 | 4 (8) |
| YJM975 | Y12 | YJM975 | 2 | 1 (1) |
| YJM975 | YJM975 | Y12 | 1 | 0 (0) |
| YJM975 | Y12 | Y12 | 0 | 0 (0) |
| Y12 | YJM975 | Y12 | 1 | 0 (1) |
| Y12 | Y12 | YJM975 | 0 | 0 (0) |
| Y12 | YJM975 | YJM975 | 3 | 3 (3) |

Mitochondrial recombination was assessed based on Restriction digests of complete mtDNAs or individual genes. The number of strains showing each possible recombination pattern are indicated. Strains that showed recombination in complete mtDNAs are indicated with the total number tested in parentheses.

**Table S11: Petite Frequency in Crosses**

|  |  |  |  |
| --- | --- | --- | --- |
| mtDNA Cross | Number of *petite* strains | Number of strains tested | *petite* frequency |
| YJM975 x Y12 | 0 | 41 | 0% |
| YJM975 x YPS606 | 2 | 89 | 2.3% |
| YJM975 x Y55 | 9 | 64 | 14.1% |
| YPS606 x Y55 | 2 | 88 | 2.3% |

Reported are the numbers of diploids with putatively recombinant mtDNAs that could not support respiration (*petites)* produced from each cross. These *petite* strains were excluded from subsequent analyses.