Supplementary Materials for

Cristae-dependent quality control of the mitochondrial genome

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Figure S1

(A) Quantitative PCR analysis of mtDNA levels of WT or Δcob::ARG8 strains, which were used in the pedigree analysis (Fig. 1C). No difference in mtDNA levels between both strains are apparent. The average value is derived from three biological replicates. The value for each biological replicate was derived from three technical replicates; Error bars indicate SD. n. s. - non significant, t-test.

(B) Carry-over cell material does not support growth upon restreaking. Replica plat- ing during the pedigree analysis results in carry-over of cell material. While such cell material is easily distinguished from growing cells by direct visual inspection of plates, it is difficult to discern on photos. For this experiment, cell material was restreaked on selective plate to demonstrate the difference between carry-over cell material and cell growth. Asterisks indicate carry-over cell material.

(C) PCR analysis corroborates results from the growth-based pedigree assay. Genomic DNA was extracted directly from YPD grown colonies without further incubation from three pedigree lineages and remaining cell material was replica plated onto YPG or SC-ARG (lacking arginine) plates. Presence of the COB gene (present in WT mtDNA, which supports growth on YPG) or the ARG8 gene (present in Δcob::ARG8 mtDNA, which supports growth on SC-Arg) was tested by PCR using specific oligos for the respective genes. PCR results correlate perfectly with the growth based analysis.

(D) Schematic illustration of the pedigree analysis, explaining the skipping of generations on a respective medium. YPG - rich medium containing the non-fermentable carbon source glycerol, SC-Arg - synthetic defined medium lacking arginine.
Figure S2

(A) Pedigree analysis of WT cells. The experiment is virtually identical to the experiment presented in Fig. 1C with the difference that the starting strains for the pedigree analysis had switched mating types. Specifically, in this experiment the strain containing WT mtDNA had mating type \textit{alpha}, whereas the strain containing \textit{\Delta cob::ARG8} had mating type \textit{a}. Striped bars indicate percentage of heteroplasmic cells containing WT and \textit{\Delta cob::ARG8} mtDNA. Grey or red bars indicate percentage of homoplasmic cells containing WT or \textit{\Delta cob::ARG8} mtDNA, respectively.

(B) Pedigree analysis of cells containing \textit{ARG8} inserted neutrally into mtDNA upstream of the \textit{COX2} gene. We cannot distinguish between cells that are heteroplasmic for both mtDNA species and cells where \textit{ARG8} had recombined into WT mtDNA. The shown result, however, indicates that \textit{ARG8} does not confer a strong disadvantage on mtDNA, which could lead to its removal.
Figure S3

(A) Quantitative PCR analysis of mtDNA levels of WT or Δcob::ARG8-TetO-TetR-3xmRuby3 strains, which were used for the microscopy experiments presented in Fig. 1D. A slight increase in the mtDNA levels of the Δcob::ARG8-TetO-TetR-3xmRuby3 is apparent. The average value is derived from three biological replicates. The value for each biological replicate was derived from three technical replicates; Error bars indicate SD. n. s. - non significant, t-test.

(B) Additional microscopic images of the inheritance of either LacO-marked intact or TetO-marked Δcob::ARG8-TetO mtDNA. Images complement data shown in Fig. 1D.

Scale bar: D 10 μm.
**Figure S4**

**(A and B)** Time-lapse microscopy of mating events between WT cells expressing either matrix-targeted NG or matrix-targeted mKate2. Mating events are shown, where either both cells contained WT mtDNA (A) or one cell contained WT mtDNA and the other contained $\Delta cob::ARG8$ mtDNA (B). Selected time-frames for (B) are shown in Fig. 1E.

**(C and D)** Similar to (A), except that cells with deletions of the nuclear-encoded $\Delta dnm1$ (C) or $\Delta atg32$ (D) were used.

Scale bars: A-D 10 $\mu$m.
Figure S5

(A) Schematic illustration of the NG-tagged ATP6 within mtDNA.

(B) Quantitative PCR analysis of mtDNA levels of WT or strains expressing the Atp6-NG protein. The average value is derived from three biological replicates. The value for each biological replicate was derived from three technical replicates; Error bars indicate SD. n. s. - non significant, t-test.

(C) Petite frequency of the Atp6-NG strain in comparison to WT and the Δatp21 strains.

(D) Westernblot analysis of strains harbouring either WT or ATP6-NG mtDNA. Aconitase (Aco1) was used as a loading control.

(E) Widefield fluorescence microscopy images of diploid cells harbouring ATP6-NG mtDNA and expressing nuclear-encoded matrix-targeted mScarlet. Scale bar: E 10 µm.
Figure S6

(A) Time-lapse microscopy of mating events between WT cells harbouring ATP6-NG mtDNA and WT cells harbouring WT mtDNA and expressing nuclear-encoded matrix-targeted Kate2.

(B) Time-lapse microscopy of mating events between cells expressing NG-tagged Cox4 and cells expressing nuclear-encoded matrix-targeted mKate2.

(C) Growth analysis of strains expressing NG fused to Pam16 or the transmembrane domain of Fis1 in comparison to WT. Strains were used in the time-lapse experiment presented in Fig. 2, E and F and Fig. S4D and E.

(D) Westernblot analysis of strains harbouring the NG-tagged version of Pam16. Aconitase was used as a loading control. Note that no signal for free NG is detectable indicating that NG is not cleaved off.

(E) Time-lapse microscopy of mating events between WT cells expressing NG fused to the transmembrane domain of Fis1 and WT cells expressing nuclear-encoded matrix-targeted mKate2.

(F) Time-lapse microscopy of mating events between cells expressing NG-tagged Pam16 and cells expressing nuclear-encoded matrix-targeted mKate2.

Scale bars: A and D-E 10 µm.
Figure S7

(A) Quantification of the equilibration of soluble Su9-mKate2 protein in mating experiments shown in Fig. 3A and B; ** P<0.01, t-test.

(B) Quantification of the Atp6-NG in the daughter cells of zygotes from mating experiments shown in Fig. 3A and B; * P<0.05, t-test.

(C) Spot test of strains expressing Atp6-NG or Atp6-mKate2 compared to the WT and a strain harbouring Δatp6 mtDNA.

(D) Quantification of the co-localization of Atp6-NG and Atp6-mKate2 in daughter cells derived from matings between WT, Δatp20 or Δatp20 cells, in which parental cells contained either ATP6-NG or ATP6-mKate2 mtDNA. The Pearson (PCC) and Manders (MCC) correlation coefficients between Atp6-NG and Atp6-mKate2 signals along the mitochondrial network of daughter cells were determined for multiple cells in three independent experiments; * P<0.05, ** P<0.01, t-test.
Figure S8

(A) Electron micrographs of mitochondria from WT, Δatp20, Δatp21, and Δatp20 Δatp21 cells grown in rich medium containing glucose.

(B) Quantification of cristae shape from cells grown in rich medium containing glucose. For each strain, mitochondria from 50 cells were scored for mitochondrial ultrastructure and grouped into the indicated categories. Shown is the mean ± standard deviation from three independent experiments. Examples of mitochondria with altered cristae shape are depicted on the right.

(C) Electron micrographs of mitochondria from Δatp20 Δatp21 cells grown in rich medium containing galactose. Shown are two representative cells. For each cell, the same mitochondrion was imaged in consecutive 70 nm ultrathin sections.

Scale bars: A and C 500 nm; B 200 nm.
Figure S9

(A) Quantitative PCR analysis of mtDNA levels of WT, Δatp20, Δatp21, Δatp20Δatp21, Δmic10, or Δmic60 strains containing WT or Δcob mtDNA. Strains were used for the pedigree analysis presented in Figure 4A-F. The average value is derived from three biological replicates. The value for each biological replicate was derived from three technical replicates; Error bars indicate SD. n. s. - non significant, t-test.

(B) Petite frequency of the indicated deletion strains.
Movie S1

Mitochondrial morphology during mating events of WT cells. Mating events between two cells containing either WT or $\Delta$cob::ARG8 mtDNA. Cells expressed either matrix-targeted mKate2 (WT mtDNA, cyan) or NG ($\Delta$cob::ARG8 mtDNA, magenta). Mating events were monitored by live-cell microscopy. Brightfield, single fluorescent channels and a merge from both fluorescent channels are shown.

Movie S2

Mitochondrial morphology during mating events of $\Delta$dnm1 cells. Mating events between two $\Delta$dnm1 cells containing either WT or $\Delta$cob::ARG8 mtDNA. Cells expressed either matrix-targeted mKate2 (WT mtDNA, cyan) or NG ($\Delta$cob::ARG8 mtDNA, magenta). Mating events were monitored by live-cell microscopy. Brightfield, single fluorescent channels and a merge from both fluorescent channels are shown. Images were taken in 7 min intervals.

Movie S3

Mitochondrial morphology during mating events of $\Delta$atg32 cells. Mating events between two $\Delta$atg32 cells containing either WT or $\Delta$cob::ARG8 mtDNA. Cells expressed either matrix-targeted mKate2 (WT mtDNA, cyan) or NG ($\Delta$cob::ARG8 mtDNA, magenta). Mating events were monitored by live-cell microscopy. Brightfield, single fluorescent channels and a merge from both fluorescent channels are shown. Images were taken
in 7 min intervals.

**Movie S4**

Diffusion of mitochondrial-encoded Atp6-NG throughout the mitochondrial network of zygotes. Cells expressing matrix-targeted mKate2 (cyan) were mated with cells expressing mtDNA-encoded Atp6-NG (magenta) and were monitored by live cell imaging. Brightfield, single fluorescent channels and a merge from both fluorescent channels are shown. Images were taken in 7 min intervals.

**Movie S5**

Diffusion of Fis-NG throughout the mitochondrial network of zygotes. Cells expressing matrix-targeted mKate2 (cyan) were mated with cells expressing nuclear-encoded Fis1-NG (magenta) and were monitored by live cell imaging. Brightfield, single fluorescent channels and a merge from both fluorescent channels are shown. Images were taken in 7 min intervals.

**Movie S6**

Diffusion of Pam16-NG throughout the mitochondrial network of zygotes. Cells expressing matrix-targeted mKate2 (cyan) were mated with cells expressing nuclear-encoded Pam16-NG (magenta) and were monitored by live cell imaging. Brightfield, single
fluorescent channels and a merge from both fluorescent channels are shown. Images were taken in 7 min intervals.

Table S1

Yeast strains used in this study.

Table S2

Primers used in this study.

Table S3

Plasmids used in this study.
Supplemental Figure 1

A

mtDNA levels
(fold change)

WT  ∆cob

0.0  0.5  1.0  1.5

B

Dissection YPD plate

Homopl. for WT mtDNA

Homopl. for ∆cob mtDNA
Supplemental Figure 1

**c**

**YPD**  
Dissection YPD plate  
Lineage 1  
Lineage 2  
Lineage 3

**YPG**  
Homopl. for WT mtDNA

**SC-Arg**  
Homopl. for ∆cob mtDNA

| Control | Lineage 1 | Lineage 2 | Lineage 3 |
|---------|-----------|-----------|-----------|
| WT ∆arg | WT ∆arg   | WT ∆arg   | WT ∆arg   |
| Zygote  | Gen. 1    | Gen. 2    | Gen. 1    |
| Gen. 4   | Gen. 5    | Gen. 3    | Gen. 4    |
| Gen. 5   | Gen. 5    | Gen. 4    | Gen. 5    |

**COB amplicon**

**ARG8 amplicon**

**D Pedigree analysis, most often observed pattern**

1. **Mating**  
   - Δcob mtDNA  
   - WT mtDNA

2. **Microdissection**
   - Heteroplasmic colony
   - Growth on YPG and SC-Arg

3. **Pedigree analysis, generation skipping**
   - Microdissection
   - Heteroplasmic colony
   - Growth on YPG and SC-Arg
   - Homoplasmic colony
   - Growth only on YPG

- Heteroplasmic colony
- Growth only on YPG
Supplemental Figure 2

A  Wildtype - switched MAT types (N=55)

B  Neutral ARG8 (N=16)
Supplemental Figure 3

A

mtdNA levels [fold change]

WT TetR  
∆cob TetR

n.s.

B

Brightfield  
LacI-3xGFP  
∆cob TetR-3xmRuby3  
Matrix  
TagBFP  
Merge

mtDNA levels [fold change]
Supplemental Figure 5

A

B

C

D

E
Supplemental Figure 8

**A**

Images showing mitochondrial structures in different strains:
- WT
- Δatp20
- Δatp21
- Δatp20Δatp21

**B**

Graph showing the percentage of mitochondria with different characteristics:
- WT
- Δatp20
- Δatp21
- Δatp20Δatp21

**C**

Images of cell 1 and cell 2 in Δatp20Δatp21 strain:
- No cristae
- Onion-like
- Septae

Scale bars are included for each image.
Supplemental Figure 9

A

**mtDNA levels [fold change]**

| mtDNA Genotype | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
|----------------|------|------|------|------|------|------|
| WT             |      |      |      |      |      |      |
| ∆cob          |      |      |      |      |      |      |
| WT-Δatp20     |      |      |      |      |      |      |
| WT-Δatp21     |      |      |      |      |      |      |
| WT-Δmic10     |      |      |      |      |      |      |
| WT-Δmic60     |      |      |      |      |      |      |
| WT-Δatp20Δatp21 |      |      |      |      |      |      |
| WT-Δatp21     |      |      |      |      |      |      |
| WT-Δmic10     |      |      |      |      |      |      |
| WT-Δmic60     |      |      |      |      |      |      |

B

**Pelle frequency [%]**

| Genotype | Pelle frequency [%] |
|----------|---------------------|
| WT       | 0                   |
| Δatp20   | 5                   |
| Δatp21   | 10                  |
| Δmic10   | 15                  |
| Δmic60   | 20                  |
| Δatp20Δatp21 | 25              |
| Δmic10Δmic60 | 20              |
| Name / Alias | Short description | Mating type | Genotype | Source |
|-------------|-------------------|-------------|----------|--------|
| yCO380     | WT                | Mat a       | leu2-3, 112 can1-100 ura3-1 his3-11, 15 mt-LacO | 1 |
| yCO381     | WT                | Mat alpha   | leu2-3, 112 can1-100 ura3-1 his3-11, 15 mt-LacO | 1 |
| yCO391     | ∆arg8             | Mat a       | ade2-1 his3-11, 15 trp1-1 leu2-3, 112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA | 2 |
| yCO354     | ∆arg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11, 15 trp1-1 leu2-3, 112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA | 2 |
| yCJ048     | Neutral ARG8      | Mat alpha   | leu2-3, 112 can1-100 ura3-1 his3-11, 15 ∆arg8::hphNT1 ARG8::mtDNA | This study |
| yCO392     | ∆arg8             | Mat alpha   | ade2-1 his3-11, 15 trp1-1 leu2-3, 112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA | This study |
| yCO969     | ∆arg8::HIS3 Δcob::ARG8M | Mat a     | ade2-1 his3-11, 15 trp1-1 leu2-3, 112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA | This study |
| yCO460     | Δcob::ARG8-TetO-TetR-3xRuby | Mat a    | leu2-3, 112 trp1-1 can1-100 ura3-1 his3-11, 15 ∆arg8::hphNT pvt100u-mtTagBFP HO-Su9-TetR-3xRuby3--KanMX4-HO Δcob::ARG8-TetO | This study |
| yCJ033     | LacO- LacI-3xGFP | Mat alpha   | leu2-3, 112 can1-100 ura3-1 his3-11, 15 mt-LacO HO-Su9-TagBFP-Pcup-Su9-3xGFP-LacI::kanMX4 | This study |
| yCJ009     | HO::Su9-mKate2    | Mat a       | leu2-3, 112 can1-100 ura3-1 his3-11, 15 mt-LacO HO::Su9-mKate2::kanMX6 | This study |
| Strain     | Chromosome | Genotype          | Notes                      |
|-----------|------------|-------------------|----------------------------|
| yCJ010    | HO::Su9-NG | Mat alpha         | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO HO::Su9-NG::kanMX6 | This study |
| yCJ078    | Δarg8::HIS3 Δcob::ARG8M HO::Su9-NG | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M HO::Su9-NG-kanMX6 intronless mtDNA | This study |
| yCJ081    | Δarg8::HIS3 HO::Su9-mKate2 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA HO::Su9-mKate2::kanMX6 | This study |
| yCJ002    | Δdnm1 Δarg8::HIS3 Δcob::ARG8M HO::Su9-NG | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δdnm1::hpNT1 | This study |
| yCJ004    | Δdnm1 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δdnm1::hpNT1 | This study |
| yCJ085    | Δdnm1 Δarg8::HIS3 Δcob::ARG8M HO::Su9-NG | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δdnm1::hpNT1 HO::Su9-NG-kanMX6 | This study |
| yCJ086    | Δdnm1 Δarg8 HO::Su9-mKate2 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δdnm1::hpNT1 HO::Su9-mKate2-kanMX6 | This study |
| yCJ074    | Δatg32 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δatg32::hpNT1 | This study |
| yCJ075    | Δatg32 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δatg32::hpNT1 | This study |
| yCJ076    | Δatg32 Δarg8::HIS3 Δcob::ARG8M HO::Su9-NG | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δatg32::hpNT1 HO::Su9-NG-kanMX6 | This study |
| Strain   | Type            | Gene(s)                           | Markers/Replacements                                      | Notes                        |
|----------|-----------------|-----------------------------------|-----------------------------------------------------------|------------------------------|
| yCJ077   | Δatg32 Δarg8 HO::Su9-mKate2 | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δatg32::hphNT1 HO::Su9-mKate2-kanMX6 | This study                   |
| yCO114   | rho0             | Mat alpha                         | leu2 ura3-52 ade2-101 arg8::URA3 kar1-1 rho0               | 3                            |
| yCO115   | rho+ Δcox2       | Mat a                             | lys2 leu2-3,112 ura3-52 his3HinDIII arg8::hisG rho+ cox2-62 | 3                            |
| yCJ025   | MR6 WT           | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 | 4                            |
| yCJ026   | MR10 Δatp6       | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 Δatp6 | 4                            |
| yCJ043   | ATP6-NG          | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG | This study                   |
| yCJ126   | HO::Su9-mScarlet ATP6-NG | Mat a/alpha                      | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG HO::Su9-mScarlet-URA3 | This study                   |
| yCJ045   | HO::Su9-mKate2   | Mat alpha                         | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO HO::Su9-mKate2-kanMX6 | This study                   |
| yCJ019   | COX4-NeonGreen   | Mat alpha                         | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Cox4-NeonGreen | This study                   |
| yCJ072   | Fis1-NG          | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA FIS1-NG::kanMX6 | This study                   |
| yCJ114   | Pam16-NG         | Mata                              | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO PAM16-NG::kanMX | This study                   |
| yCJ051   | Δatp21 ATP6-NG   | Mat a                             | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG Δatp21::hphNT1 | This study                   |
| yCJ052   | Δatp21 HO::Su9-mKate2 | Mat alpha                        | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Mito Δatp21::hphNT1 HO::Su9-mKate2-kanMX6 | This study                   |
| Strain   | Description                                                                 | Genotype                                                                 | Notes                  |
|----------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|------------------------|
| yCJ053   | Δatp20 ATP6-NG                                                             | Mat a ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG | This study             |
| yCJ054   | Δatp20 HO::Su9-mKate2                                                     | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Mito Δatp20::hphNT2 HO::Su9-mKate2-kanMX6 | This study             |
| yCJ057   | Δatp20 Δatp21 ATP6-NG                                                    | Mat a ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG | This study             |
| yCJ058   | Δatp20 Δatp21 HO::Su9-mKate2                                              | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Mito Δatp21::hphNT1 Δatp20::hphNT2 HO::Su9-mKate2-kanMX6 | This study             |
| yCO754   | Δmic10 ATP6-NG                                                            | Mat a ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG | This study             |
| yCO755   | Δmic10 HO::Su9-mKate2                                                     | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Mito Δmic10::hphNT1 HO::Su9-mKate2-kanMX6 | This study             |
| yCJ066   | Δmic60 ATP6-NG                                                            | Mat a ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-NG | This study             |
| yCJ067   | Δmic60 HO::Su9-mKate2                                                     | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Mito Δmic60::hphNT1 HO::Su9-mKate2-kanMX6 | This study             |
| yCJ120   | ATP6-mKate2                                                               | Mat a ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-mKate2 | This study             |
| yCJ084   | ATP6-NG                                                                   | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 ATP6-NG                  | This study             |
| yCJ123   | ATP6-NG pvt100u-TagBFP                                                    | Mat alpha leu2-3,112 can1-100 ura3-1 his3-11,15 ATP6-NG pvt100u-TagBFP  | This study             |
| yCJ124 | ATP6-mKate2 pvt100u-TagBFP | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-mKate2 pvt100u-TagBFP | This study |
| yCJ127 | ATP6-NG Δatp20 pvt100u-TagBFP | Mat alpha | leu2-3,112 can1-100 ura3-1 his3-11,15 ATP6-NG Δatp20::NatNT2 pvt100u-TagBFP | This study |
| yCJ128 | ATP6-mKate2 Δatp20 pvt100u-TagBFP | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-mKate2 Δatp20::NatNT2 pvt100u-TagBFP | This study |
| yCJ130 | ATP6-NG Δatp21 pvt100u-TagBFP | Mat alpha | leu2-3,112 can1-100 ura3-1 his3-11,15 ATP6-NG Δatp21::hphNT1 pvt100u-TagBFP | This study |
| yCJ132 | ATP6-mKate2 Δatp21 pvt100u-TagBFP | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 ATP6-mKate2 Δatp21::hphNT1 pvt100u-TagBFP | This study |
| yCJ103 | WT LacO-LacI | Mat alpha | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Su9-3xGFP-LacI::kanMX6 | This study |
| yCJ104 | WT matrix-mKate2 | Mat a | leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 HO-Su9-mKate2 | This study |
| yCJ105 | LacO-Lacl Δatp21 | Mat alpha | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Su9-3xGFP-LacI::kanMX6 Δatp21::NatNT2 | This study |
| yCJ106 | matrix-mKate2 Δatp21 | Mat a | leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 HO-Su9-mKate2 Δatp21::NatNT2 | This study |
| yCJ134 | LacO-Lacl Δmic60 | Mat alpha | leu2-3,112 can1-100 ura3-1 his3-11,15 mt-LacO Su9-3xGFP-LacI::kanMX6 Δmic60::hphNT1 | This study |
| yCJ135 | matrix-mKate2 Δmic60 | Mat a | leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 HO-Su9-mKate2 Δmic60::hphNT1 | This study |
| yCJ020 | Δatp20 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δatp20::hphNT1 | This study |
| YCJ022 | Δatp20 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δatp20::hphNT1 | This study |
|------|--------------|-------|-------------------------------------------------|------------|
| YCJ046 | Δatp20Δatp21 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δatp21::hphNT1 Δatp20::kanMX6 | This study |
| YCJ047 | Δatp20Δatp21 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δatp21::hphNT1 Δatp20::kanMX6 | This study |
| YCJ055 | Δatp21 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δatp21::hphNT1 | This study |
| YCJ007 | Δatp21 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δatp21::hphNT1 | This study |
| YCO756 | Δmic10 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δmic10::hphNT1 | This study |
| YCO757 | Δmic10 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δmic10::hphNT1 | This study |
| YCJ070 | Δmic60 Δarg8::HIS3 Δcob::ARG8M | Mat alpha | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 cob::ARG8M intronless mtDNA Δmic60::hphNT1 | This study |
| YCJ071 | Δmic60 Δarg8 | Mat a | ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 CAN1 arg8::HIS3 intronless mtDNA Δmic60::hphNT1 | This study |

1 Osman et al. 2015  
2 Gruschke et al. 2011  
3 Steele et al. 1996  
4 Rak et al. 2007
| Name / Alias   | Sequence                                                                 |
|---------------|--------------------------------------------------------------------------|
| CO356 ∆dnm1 S1| CATTAAGTAGCTACCAGCAGTCTAAATACGGACTAAAGAATG||
|               | CGTACGCTGGAGCTGCAGAC                                                     |
| CO357 ∆dnm1 S2| CGCAATGTTGAAGTAAGATCAAATGAGTAATTATGCAATTA||
|               | ATCGATGAATTCGAGGTGAGCTGCAGAC                                             |
| CO573 ∆atg32 S1| TCACAAAAACGGAAAAATCTGCCAGGAACAGGTAAAAACATGCGTACGCTGCAGGTCGAGCAGC         |
| CO574 ∆atg32 S2| GTGAGTAGGAACGTGTATGTTGTTGATATATGGAAAAAGGTATTATCGAGTAATTTCGAGGTCGAGC     |
| CO888 ∆mic10 S1| TGCTAGGAGGAGAAGGAAACGGAAAAAGACAAAAATATACCAGGCGTACGCTGCAGGTCGAGCAGC   |
| CO889 ∆mic10 S2| TATTTTTTTTTTGAATATATATATAAAGCAGCATCGTGCGCTTAAGACTAAATGCGTACGCTGCAGGTCR  |
| CO986 ∆atp20 S1| ACCTGGCGATAAACATTTCCAGAACGTATACGCTATCAATTCATCTGATCAGATCGTGCTGAGGTCGAC |
| CO987 ∆atp20 S2| ACGAATACAGGTTGTTGGAATGAGATAGGTAATAAGAACAGGATGATGAGTTGAAAGTGCGTACGCTGCAGGTCGAC |
| CO1104 ∆atp21 S1| CGGAACATAACGTATATAGGAACTAGCTGAGTGAGTTAAAGGATCGTACGCTGCAGGTCGAC |
| CO1105 ∆atp21 S2| TAATGATGATATCCATTGCTATCTATTTATGGTGAAGCAGTGCTATTTAATGCGTACGCTGCAGGTCGAC |
| CO1114 qPCR Cox1 fv| CTACAGTATACAGCATTTCAGGA |
| CO1115 qPCR Cox1 rv| GTGCCCTGAATAGATGATAATGGT  |
| CO1116 qPCR Act1 fv| CACCCCTGTTTCTTTTGAAGTG |
| CO1117 qPCR Act1 rv| CGTAGAAGGCTGGAAAGCTGTG |
| CO1196 ∆mic60 S1| GGCATAAGAAGCGATTGAAAGTCTACTAAAAAAGCTAATTCGATGATGATGATGAGTTGAAAGATCGTACGCTGAGGTCGAC |
| CO1197  | AGGTGTAATGACGTACATCTCTTTTCTCTTTGTATTATTCTTTTC AATCGATGAATTGAGCTCG |
|---------|-------------------------------------------------------------------|
| ∆mic60 S2 | GATAATCTACTTTTTTACAACAAATATAAAACAATGGCTCAA AAGGGAAGG |
| CO1268 tagging of Fis1 fw | CCAAGCTTCTTATATAATTCATCCATCCATGGACA |
| CO1269 tagging of Fis1 rv | GGATGAATTATAAGAAGCTTGGTCATGGTACTGA |
| CO1270 backbone for Fis1-NG fw | GCAAGCTAAACAGATCTCTACCTTTCTTGTATTCTAAGAAAGAAAC |
| CO1271 backbone for Fis1-NG rv | CGAATTCAAACAGATCTCTATCTGTG ACTGATGCAAGC AGCAATCGATCGTACGCTGCAGGTCGAC |
| CO1677 tagging of Pam16 fw | GCTGCATGCTTTGCATAACACTTTGTGACGTATTAGGAGGCTT CTTGAATCGATGAAATTGAGGTCG |
| CO1678 tagging of Pam16 rv | GCTGCATGCTTTCGATAACACTTTGTGACGTATTAGGAGGCTT CTTGAATCGATGAAATTGAGGTCG |
| CO562 Amplification of COB for | AATCAAATGTGTATTTAAGTTAGTG |
| CO562 Amplification of COB rev | TTATTATTAACATCTACCGATATAGAAT |
| CO891 Amplification of ARG8 for | TCAAGACCTGAAGATTATGTATCACAAGAGG |
| CO601 Amplification of ARG8 rev | TTAAGCATATACAGCTTGCAGACTAG |
| CO982 S3 tagging of Cox4 | TACAAACTAAAACCTTGTGTTGTTCAATATGATGACCAGCACCATCAA CGGTGACGCTGCTGGTTTA |
| CO982 S2 tagging of Cox4 | AAAAAGTAAAGAGAAGAAGGCAACTTGAATGATAAGATT AATCGATGAATTGAGGTCG |
| Name / Alias | Needed for | Source |
|-------------|------------|--------|
| pCO021      | Janke deletion cassette G418 resistance | 1 |
| pCO059      | Janke deletion cassette NAT resistance | 1 |
| pCO074      | Janke deletion cassette hygromycin resistance | 1 |
| pCO151      | pvt100u-TagBFP | This study |
| pCO282      | pCOB-ST5 | 2 |
| pCO441      | HO-\text{P}_{PGK1}-Su9-mKate2-kanMX6-HO | This study |
| pCO442      | HO-\text{P}_{PGK1}-Su9-NG-kanMX6-HO | This study |
| pCO307      | pCOB/ST5 Arg8+Term+synth-TetO | This study |
| pCO408      | HO-\text{P}_{CUP}-Su9-3xGFP-LacI—\text{P}_{PGK}-Su9-TagBFP-KanMX | This study |
| pCO407      | HO-\text{P}_{CUP}-Su9-TetR-3xRuby3—\text{P}_{PGK1}-Su9-TagBFP-KanMX4-HO | This study |
| pCO444      | Plasmid for biolistic transformation and generation of \textit{ATP6}-mtNeonGreen | This study |
| pCO494      | C-Terminal tagging of proteins with NeonGreen | This study |
| pCOJ103     | Plasmid for biolistic transformation and generation of \textit{ATP6}-mtNeonGreen | This study |

\(^1\) Janke et al. 2004
\(^2\) Gruschke et al. 2011