

PHOSPHATE-LIMITED CHEMOSTATS

0.05 h⁻¹ dilution rate: 13 h doubling time

	CEN.PK rho ⁺	CEN.PK rho ⁰
Klett	140	97 (69%)
Coulter counter		
cells/ml	3.7 x 10 ⁷	2.3 x 10 ⁷ (62%)
mean size (fL)	31	38
Hemocytometer		
cells/ml	3.7 x 10 ⁷	2.0 x 10 ⁷ (54%)
budded cells	32%	21%
Residual glucose	1.80 g/L	0.60 g/L
Residual ethanol	0.24 g/L	0.89 g/L

**Mitochondrial Abundance and
Function is Regulated by
Multiple Genes in Yeast**

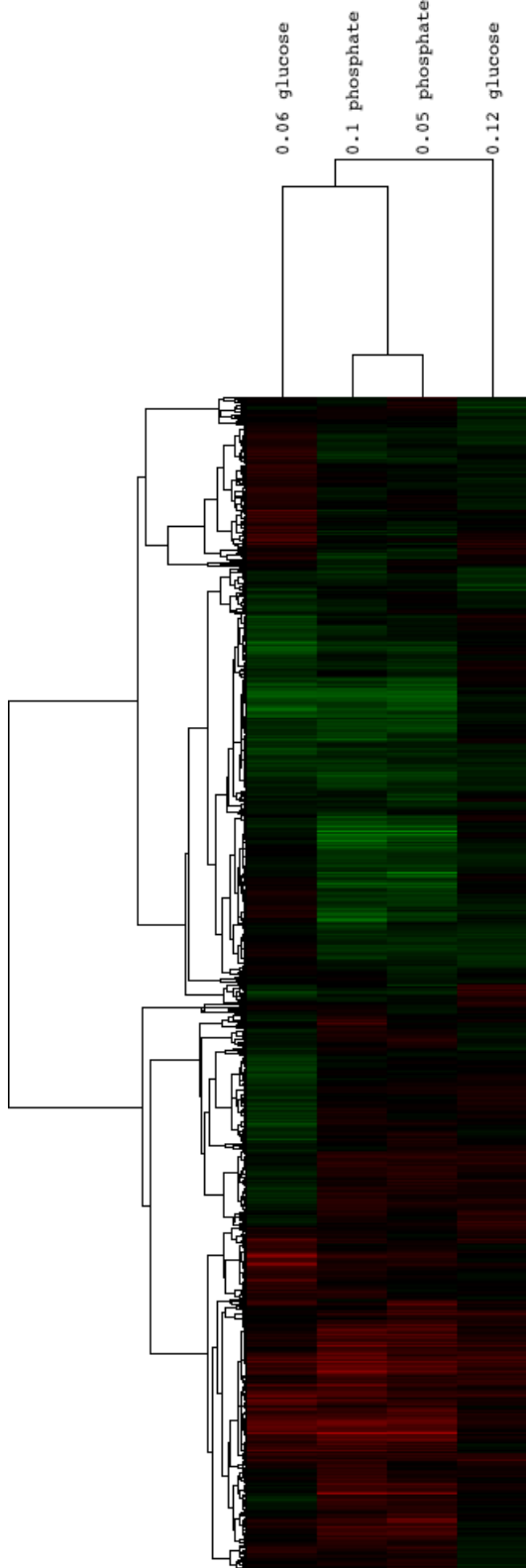
Amy Caudy, Princeton

KITP Evo Cell, Jan 28, 2010

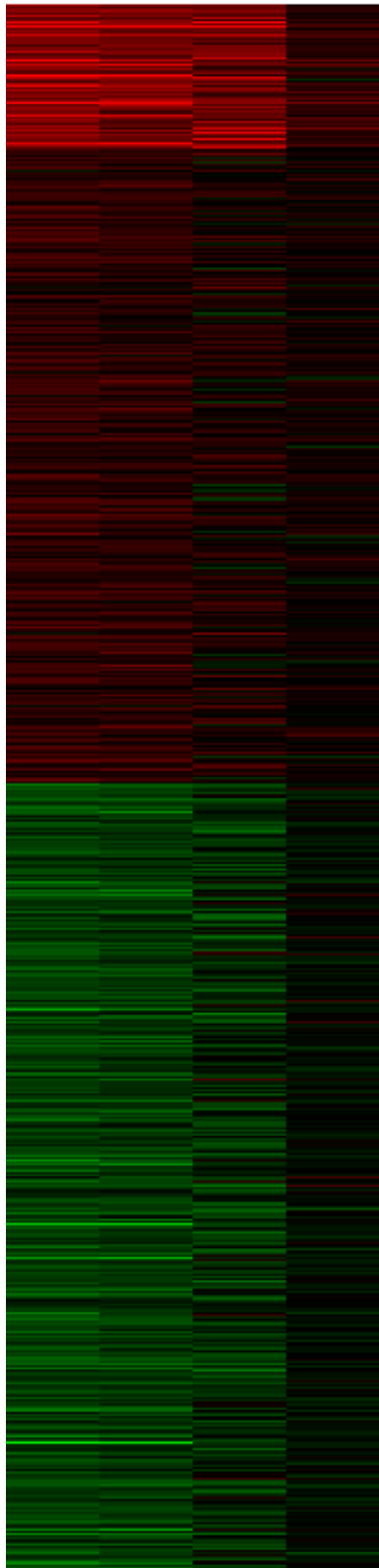
PHOSPHATE-LIMITED CHEMOSTATS

0.1 h⁻¹ dilution rate: 7 h doubling time

	CEN.PK rho+	CEN.PK rho0
Klett PUT IN	102	69 (68%)
Coulter counter cells/ml mean size (fL)	3.2 x 10 ⁷ 29	1.8 x 10 ⁷ (56%) 33
Residual glucose	2.63 g/L	1.83 g/L
Residual ethanol	0.23 g/L	0.66 g/L



Cluster all data from growth rate matched **petite** compared to **wild type**

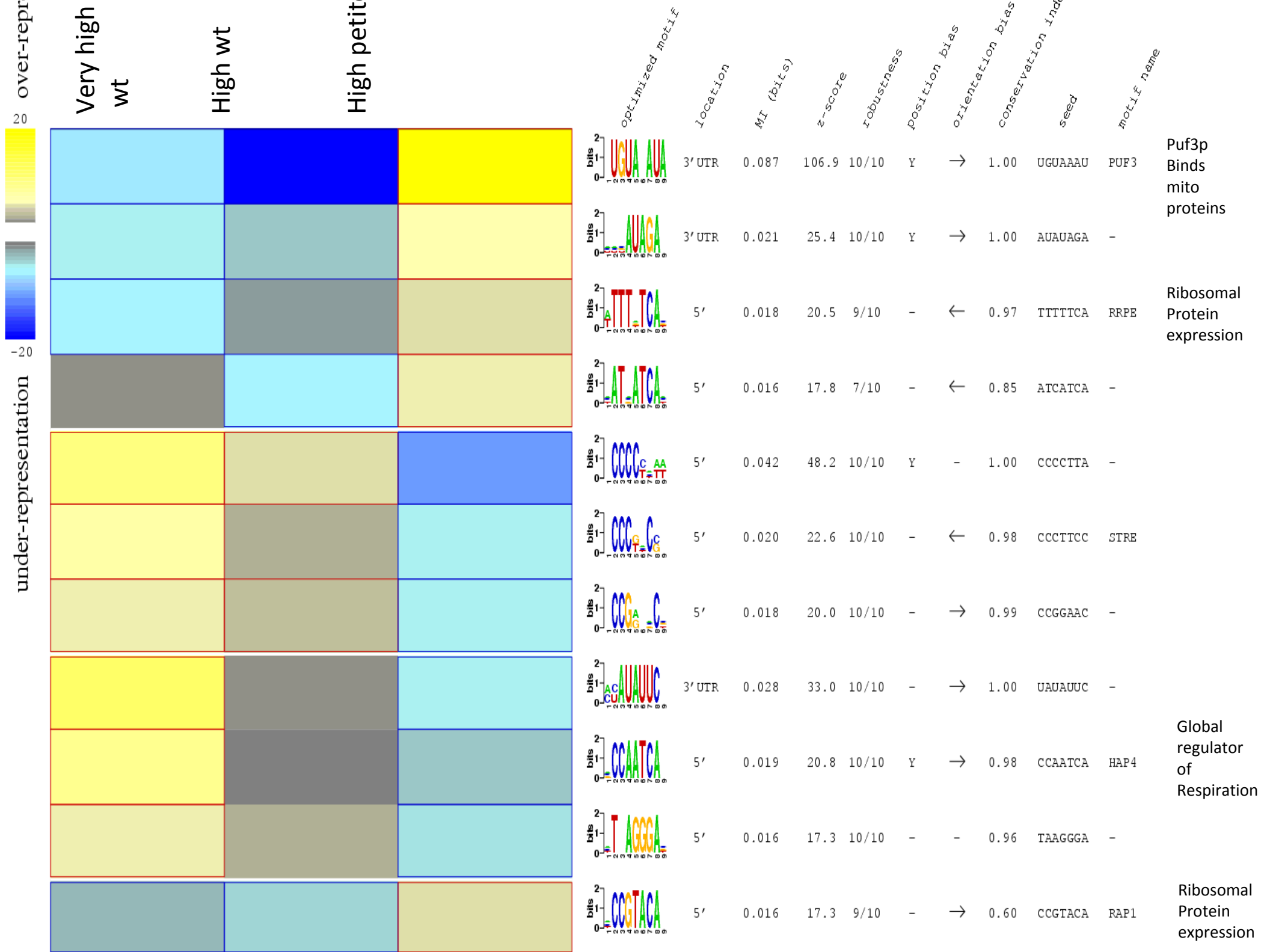


K-means clustering of genes changed 1.5 fold in growth-rate matched **petite** vs **wild type**.

Process: Oxidative phosphorylation, respiratory chain
Component: Mitochondrion

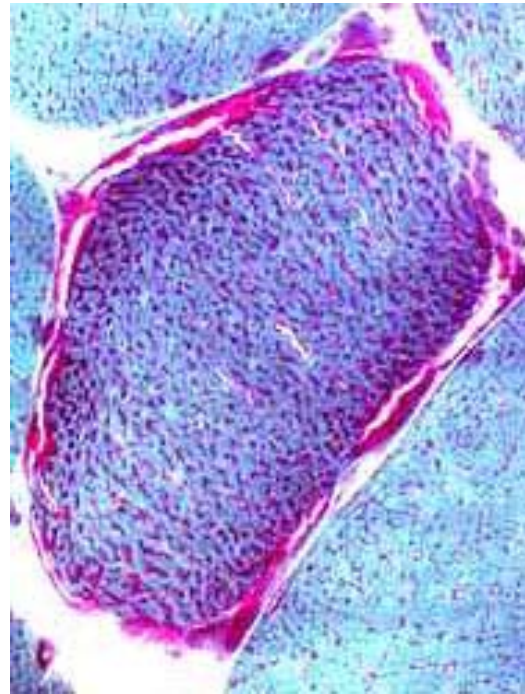
Process: No functional enrichment.
Component: Cell wall, vacuole

Process: Mitochondrial ribosome, mitochondrial protein transport, amino acid metabolism, tRNA acylation, (mito chaperones)
Component: cytosolic and mitochondrial ribosomes, mito envelope



Myopathies Suggest Mitochondrial Copy Numbers are Plastic

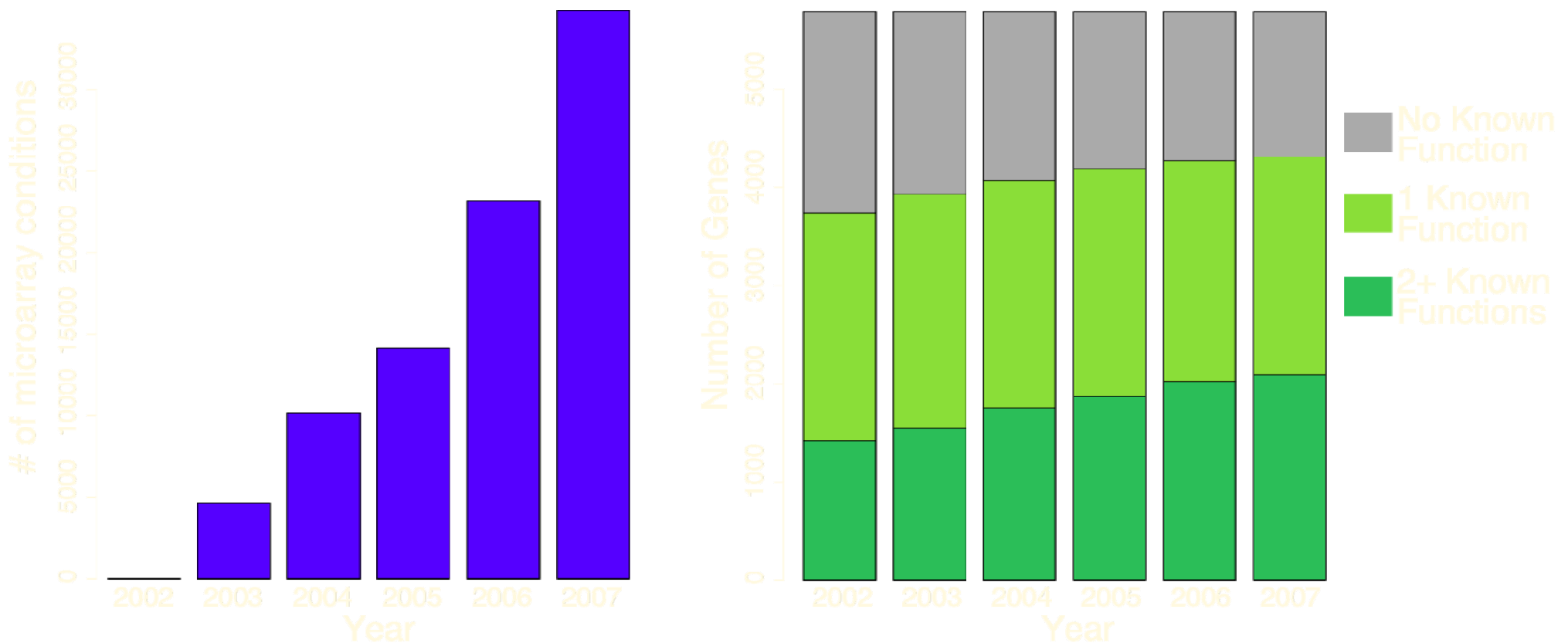
Approximately 50 different nuclear and mitochondrial DNA mutations that compromise mitochondrial function are associated with ragged red muscle fibers.



Washington University Pathology Image Archive.

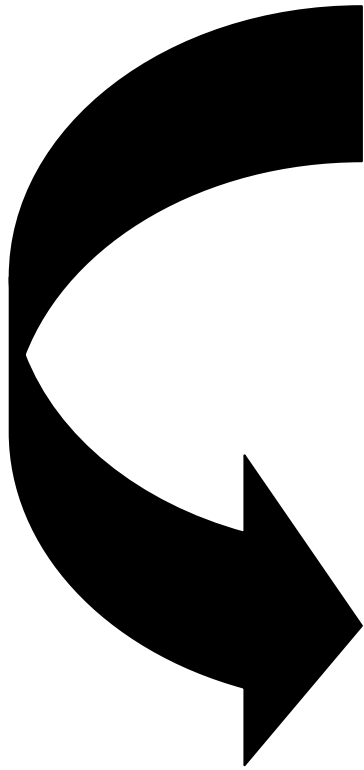
The red staining is the consequence of high numbers of mitochondria.

Huge repositories of biological data...



...are not directly translating into knowledge

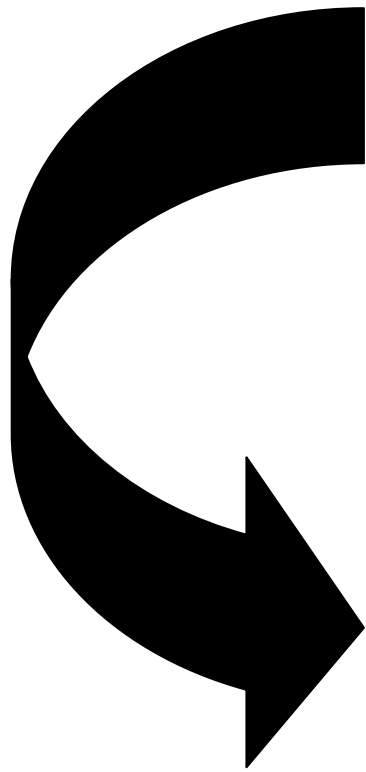
The promise of computational biology



experiments

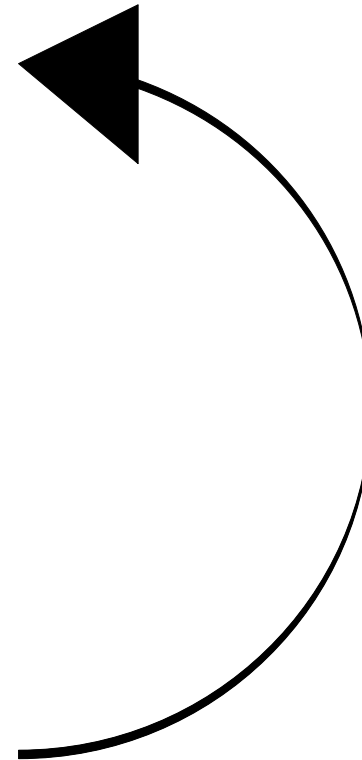
**computational
predictions**

The promise of computational biology

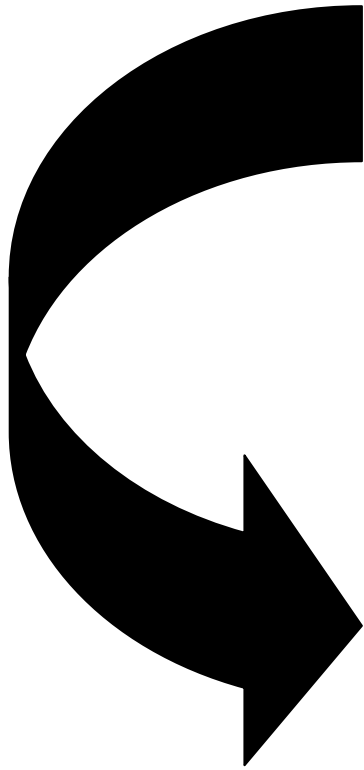


experiments

**computational
predictions**

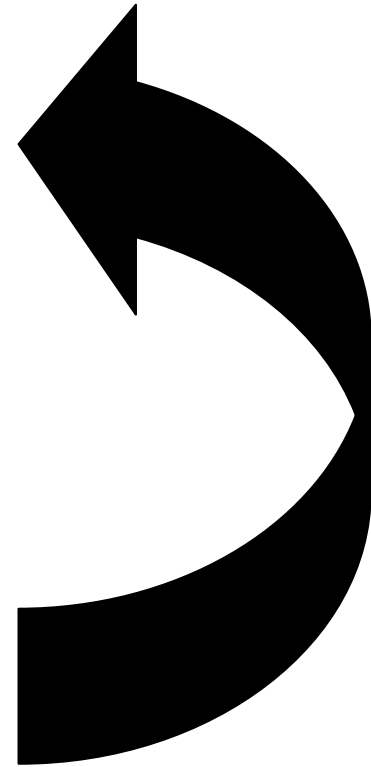


The promise of computational biology

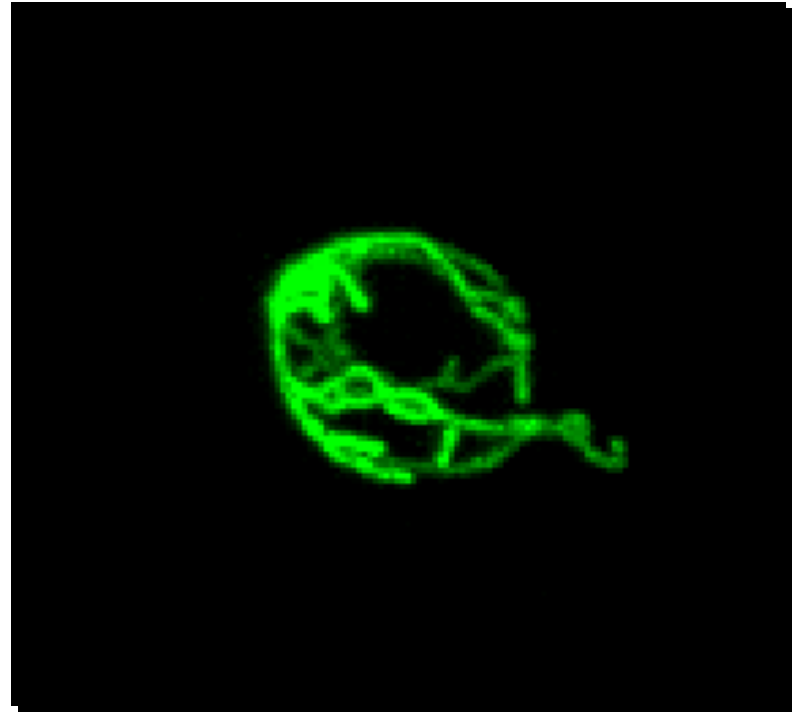
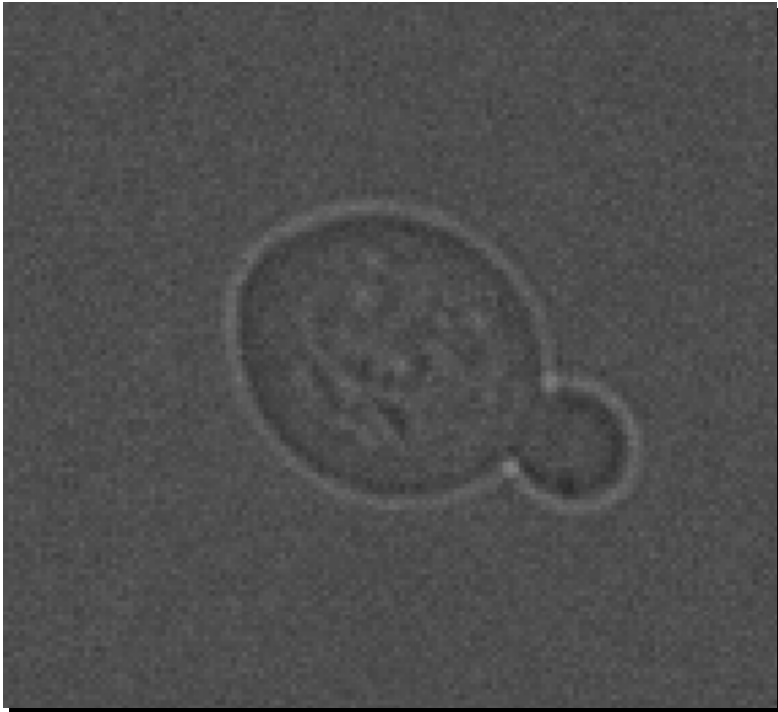


experiments

**computational
predictions**



**Mitochondrial biology is a complex interaction
between the endosymbiont and host**

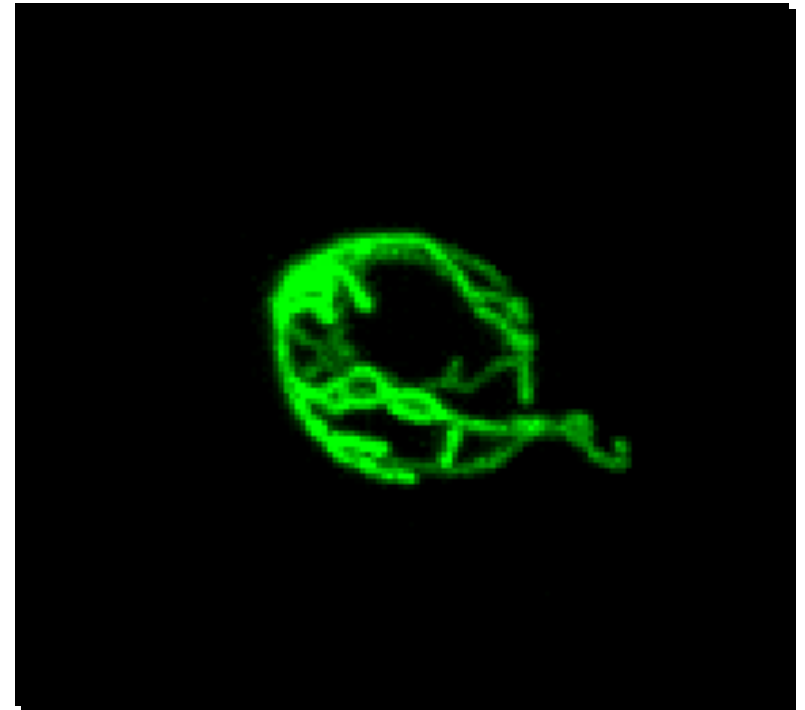


Why mitochondria?

- Implicated in a variety of human diseases
- Complex system of interacting proteins that carry out a range of functions

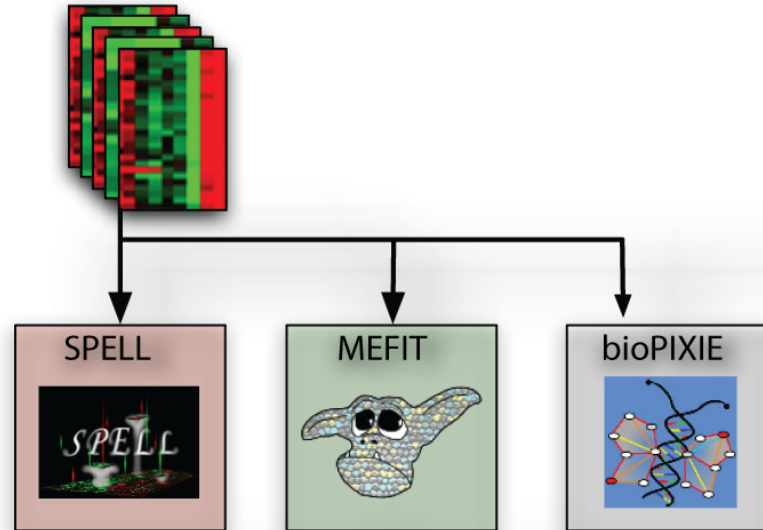
Why yeast?

- Large body of existing experimental data
- Functional annotations for $\sim 3/4$ of genome



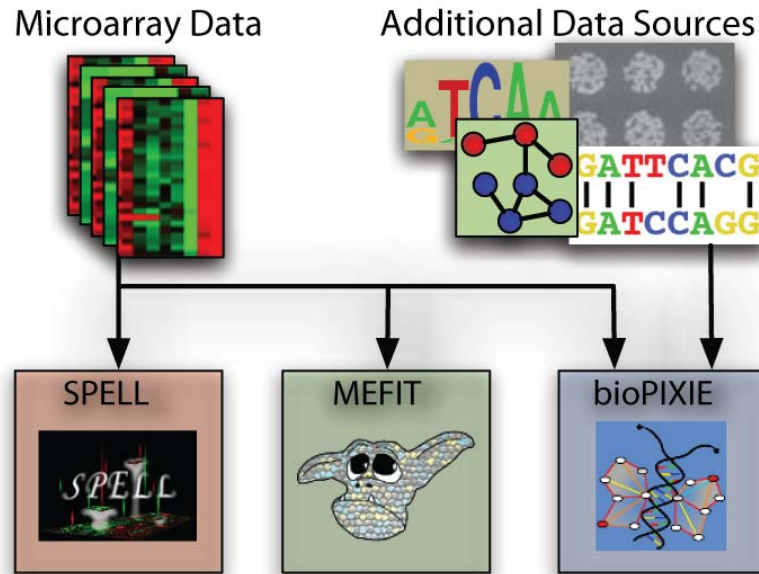
Genomic datasets can be used to computationally predict gene function

Microarray Data



**~2400 microarray
conditions**

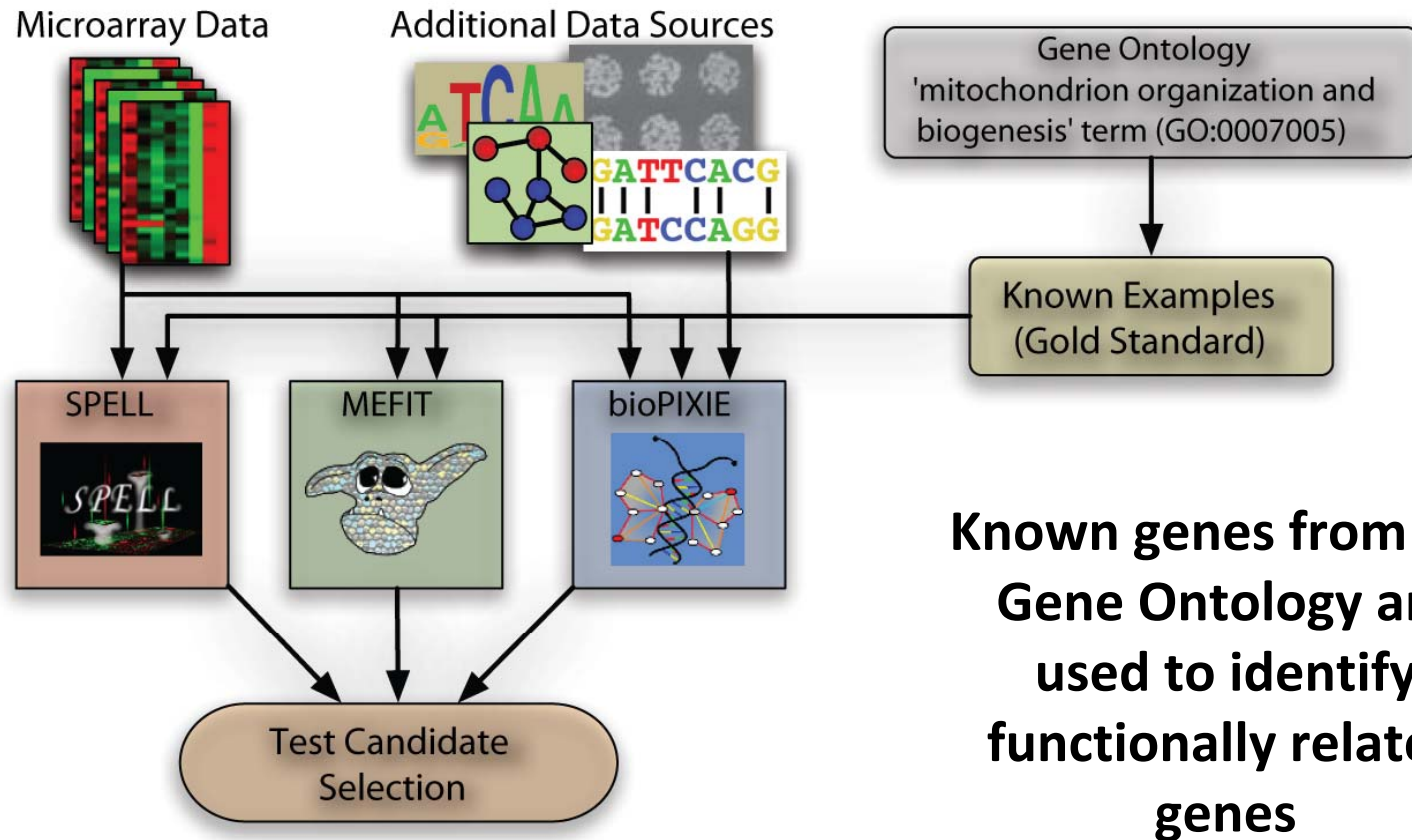
Genomic datasets can be used to computationally predict gene function



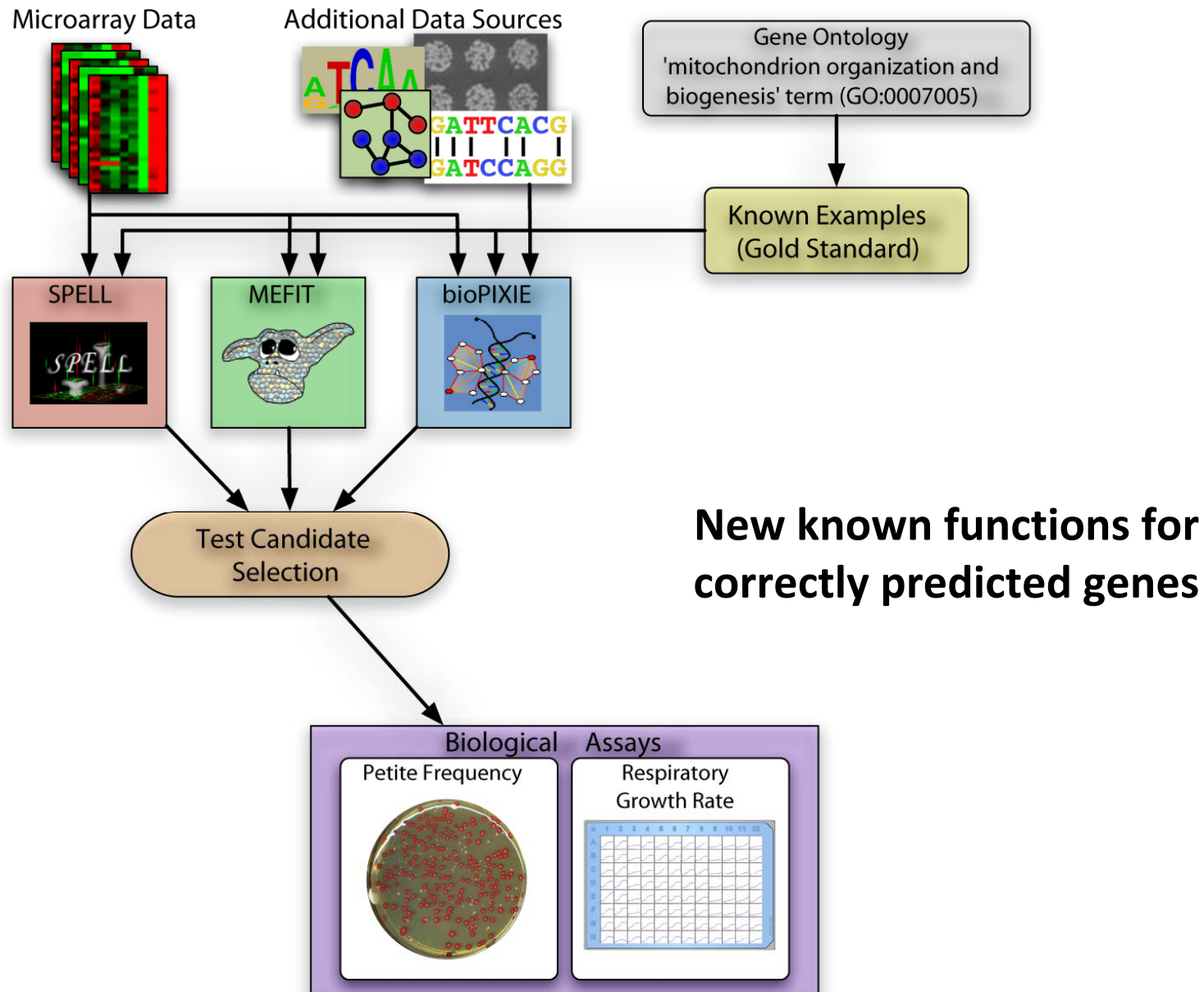
Genetic and physical interaction datasets, primary sequence information

“Guilt by association” identifies functionally related genes

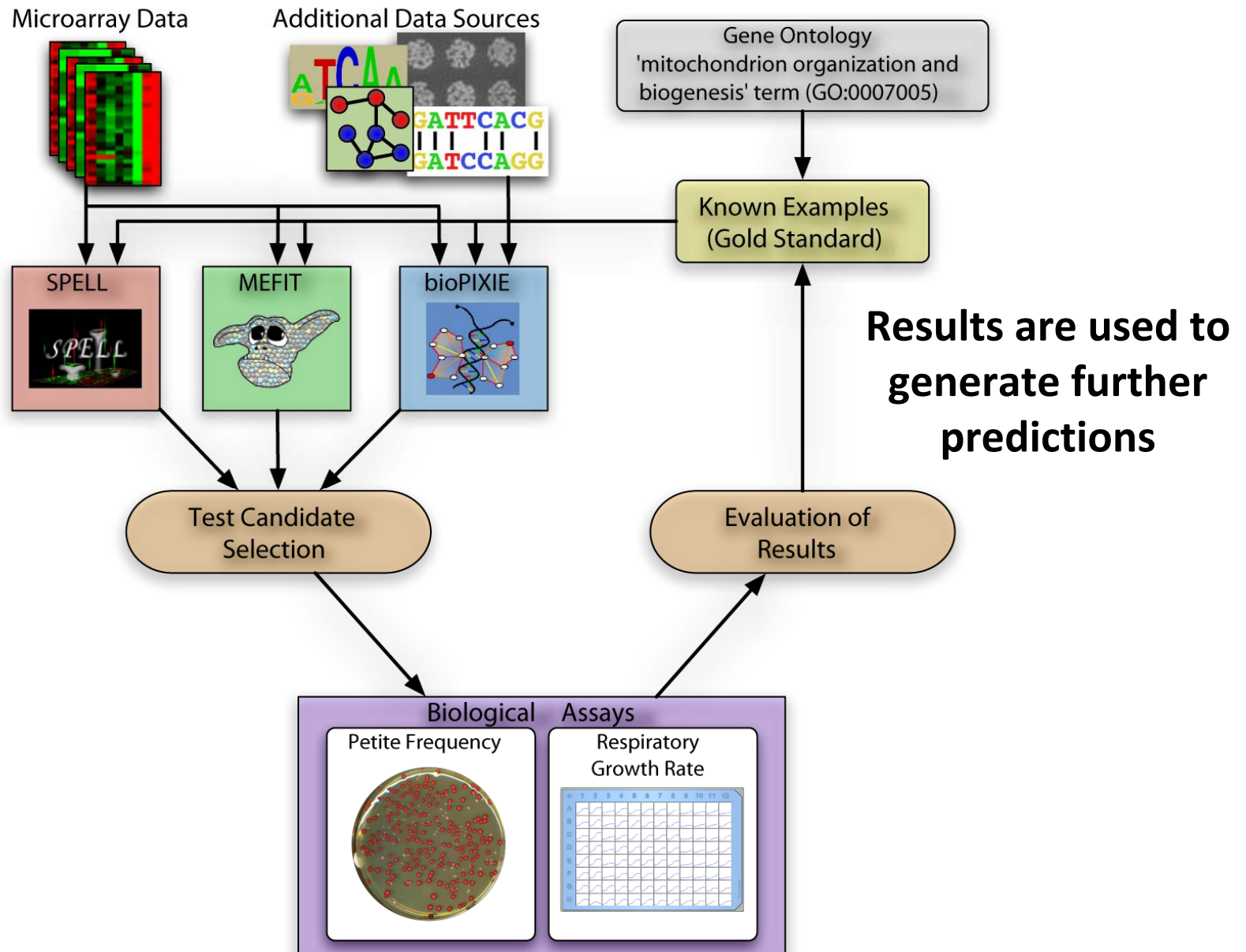
Genomic datasets can be used to computationally predict gene function



Genomic datasets can be used to computationally predict gene function



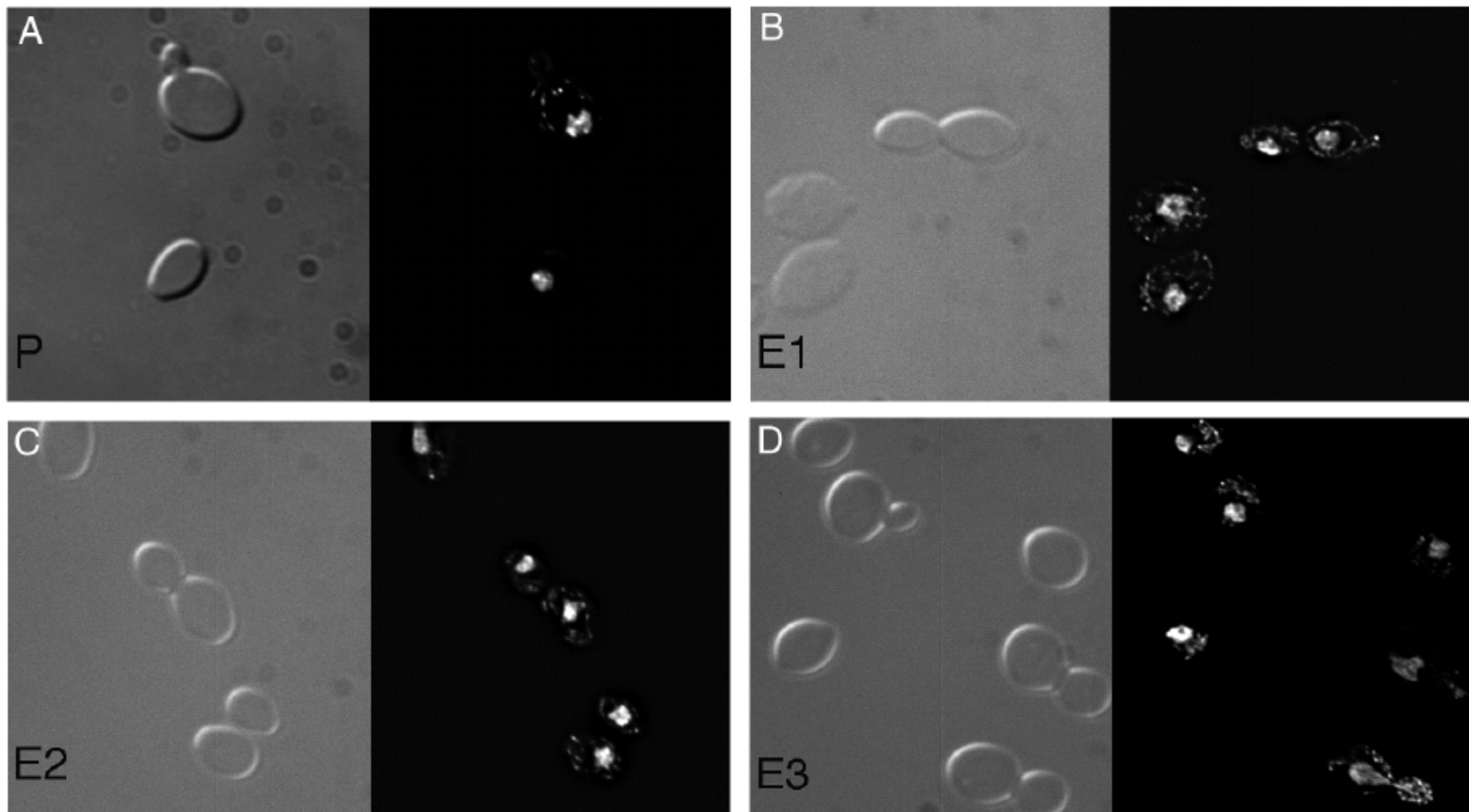
Genomic datasets can be used to computationally predict gene function



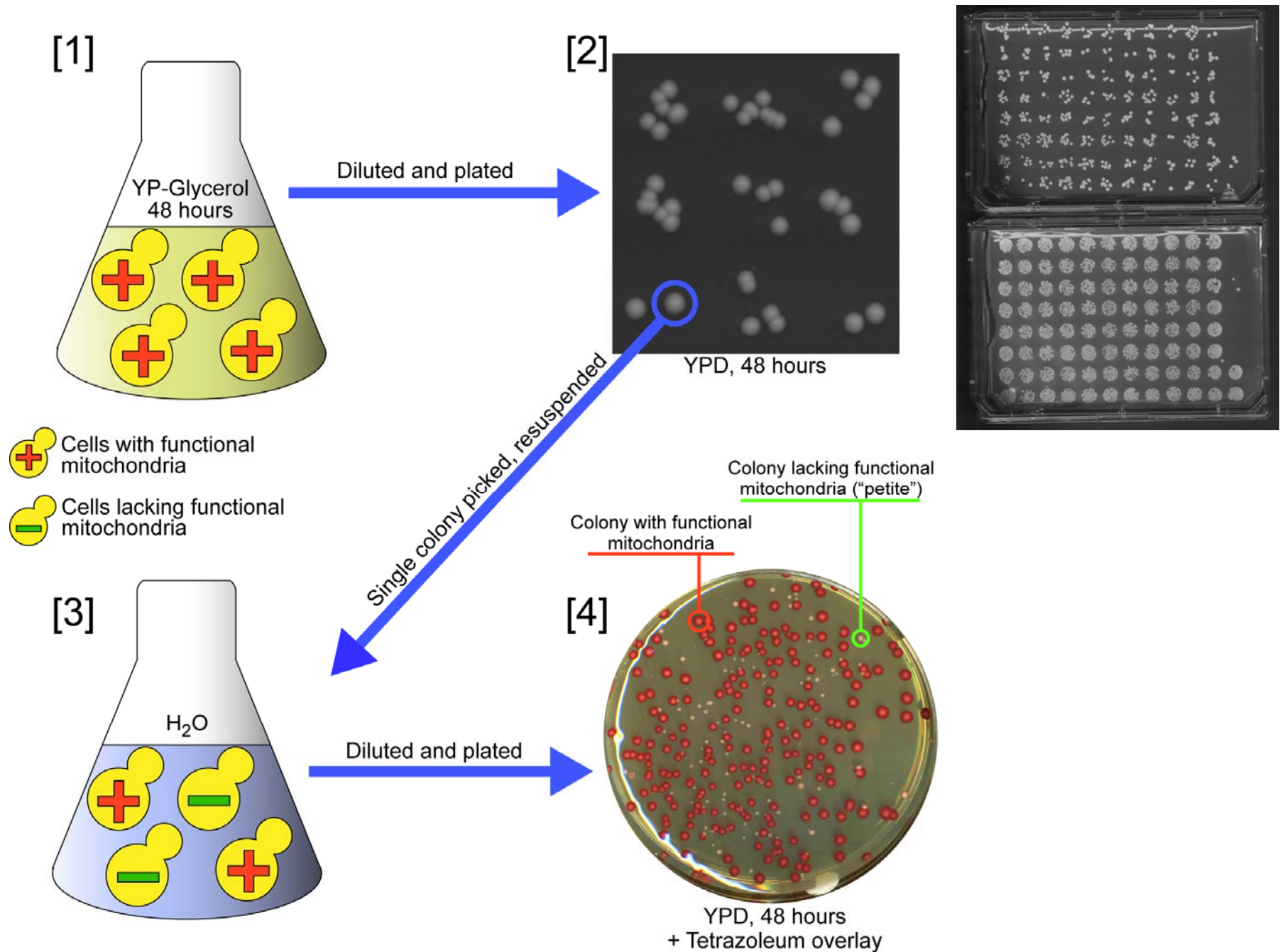
Mitochondrial Copy Number in Yeast Increases After Evolution in Low Glucose

Phase Contrast

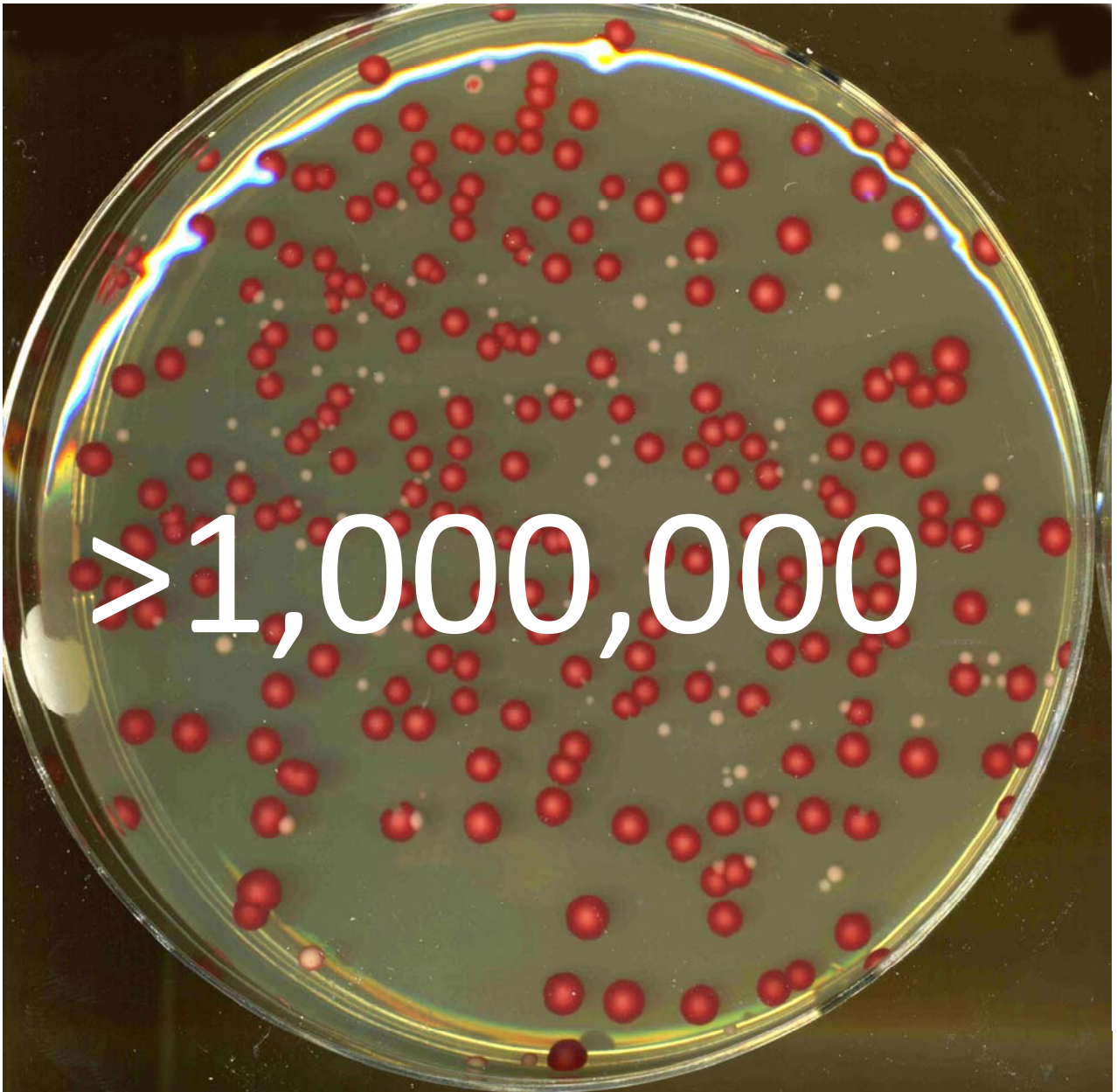
DAPI (DNA)



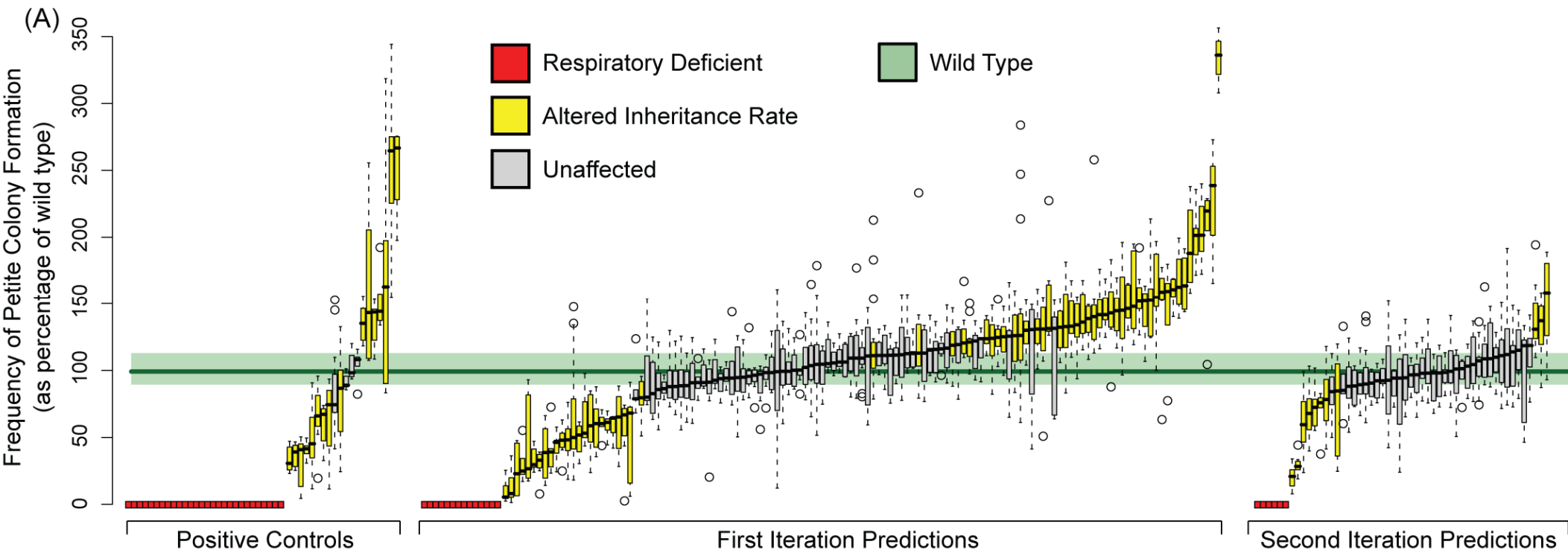
Petite frequency assay: A quantitative measure of mitochondrial inheritance and biogenesis



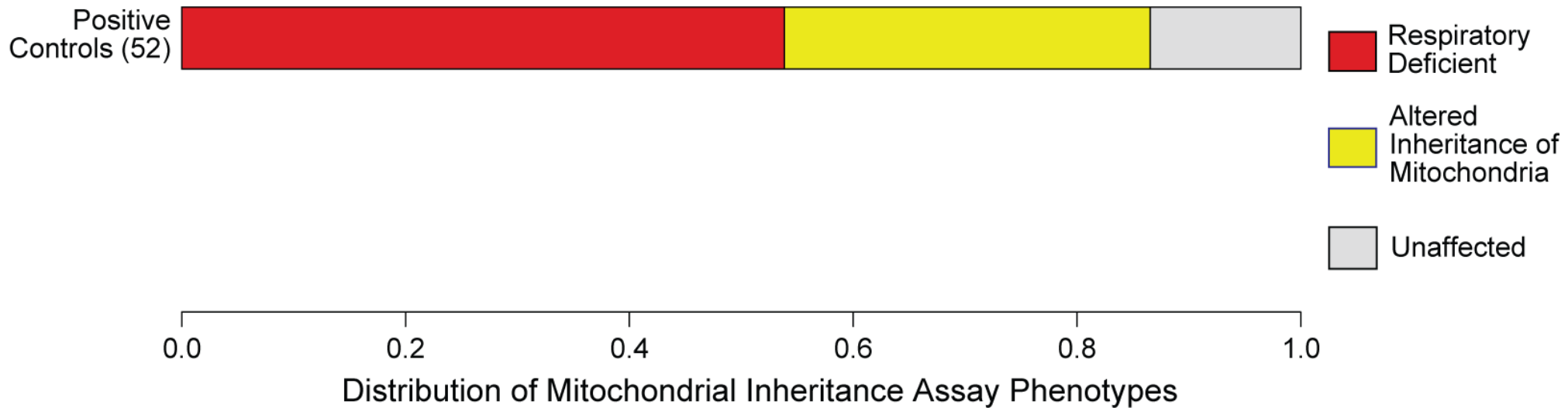
Tetrazolium plate to identify respiratory competent colonies



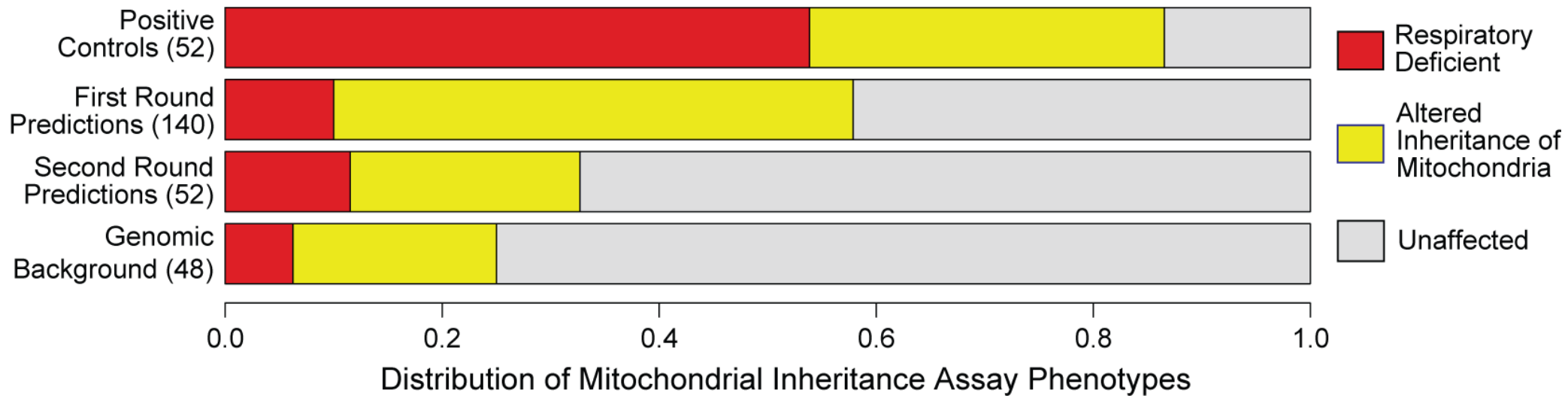
Computational predictions identify 99 mutations that alter mitochondrial inheritance



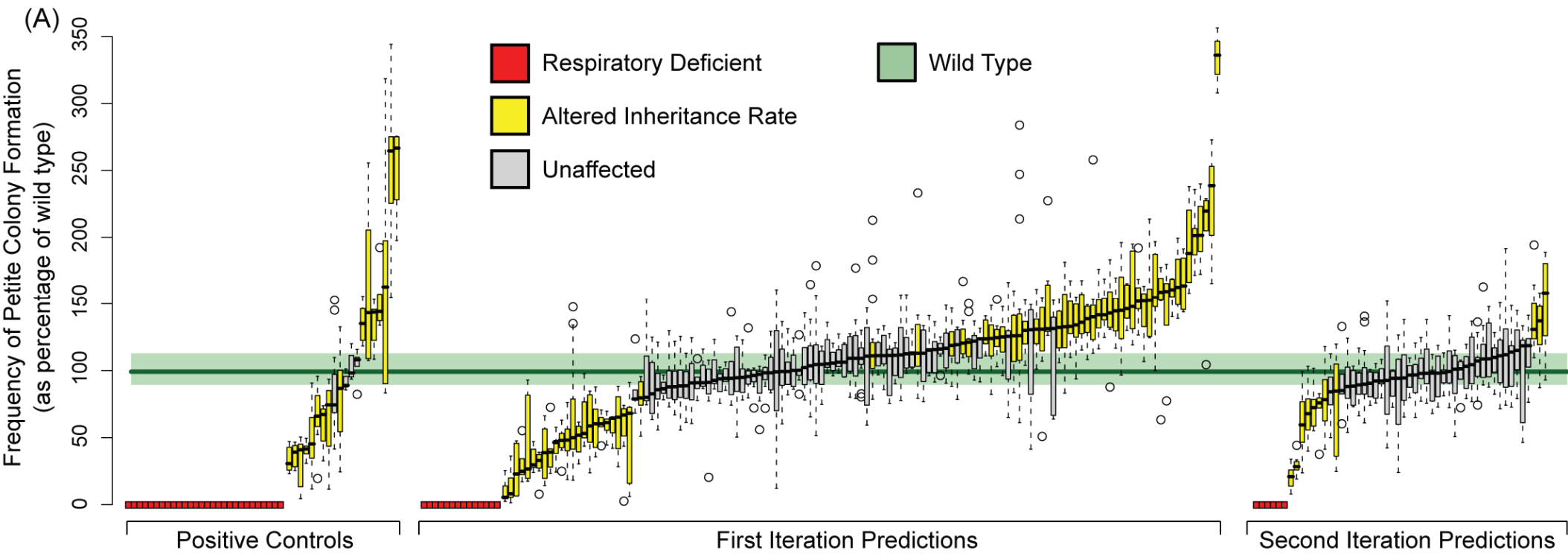
Quantitative Assay Detects Defects in Mitochondrial Function in the Majority of Positive Controls



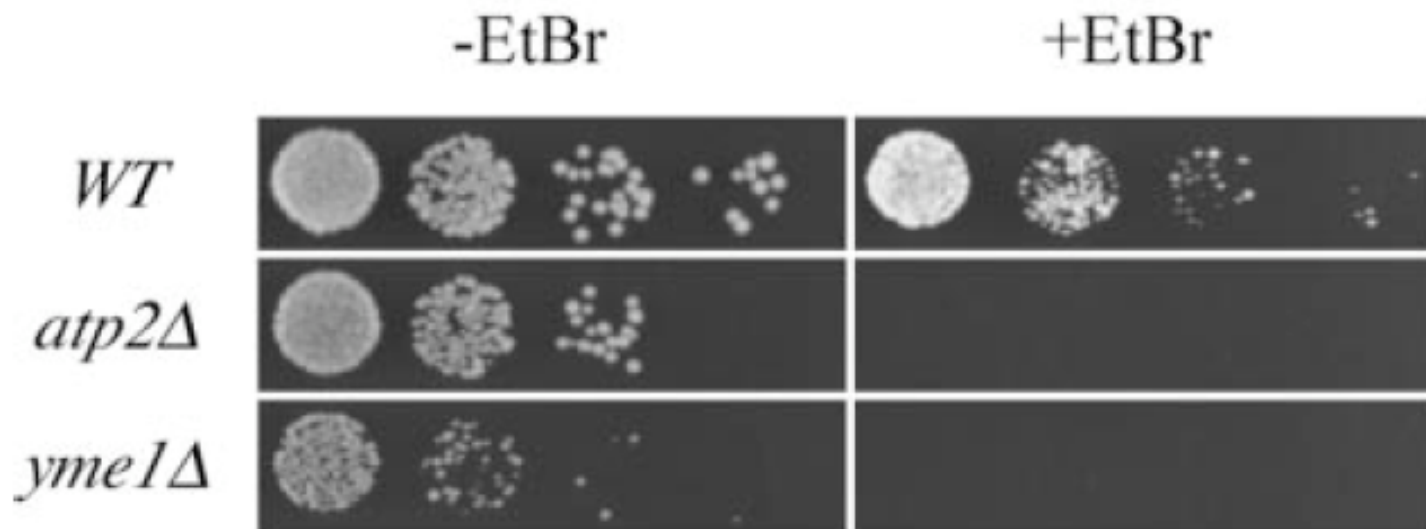
Mutants of predicted genes frequently show modest phenotypes detectable in a quantitative assay



Many mutations decrease petite frequency

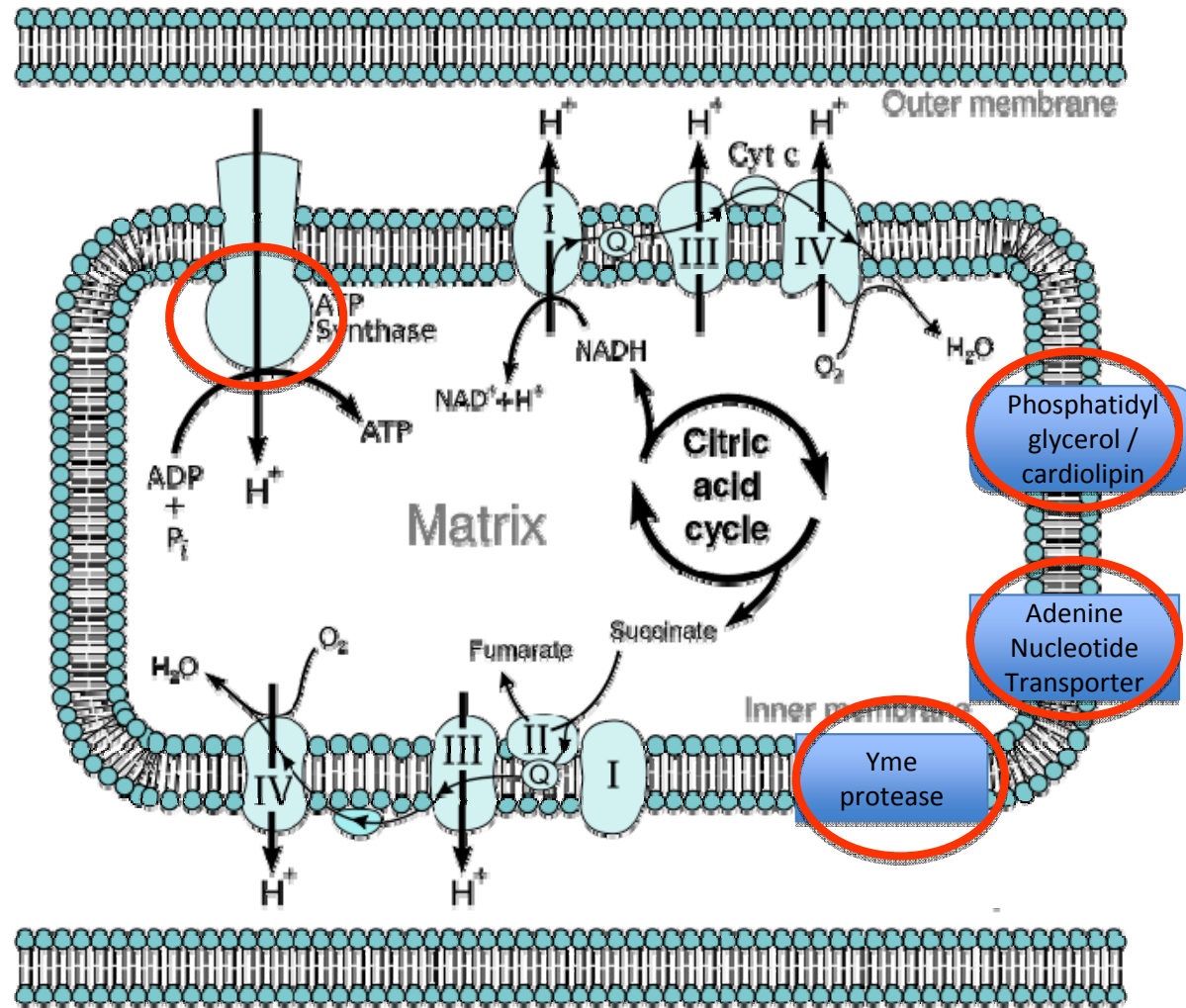


Petite Negative Mutants Produce no Petites

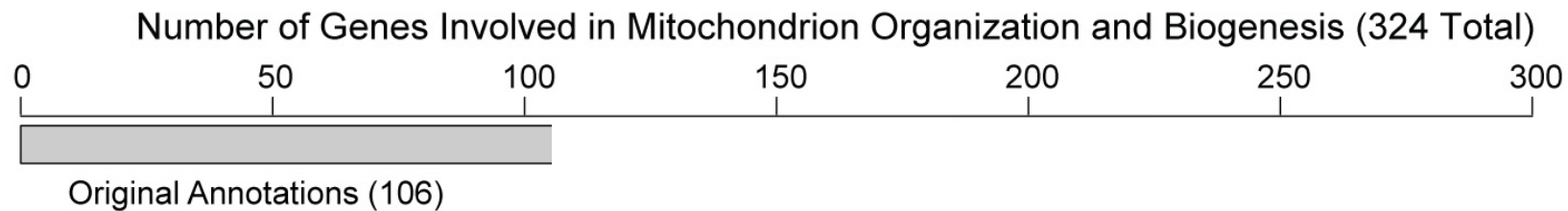


Ethidium bromide causes high frequency of mtDNA loss

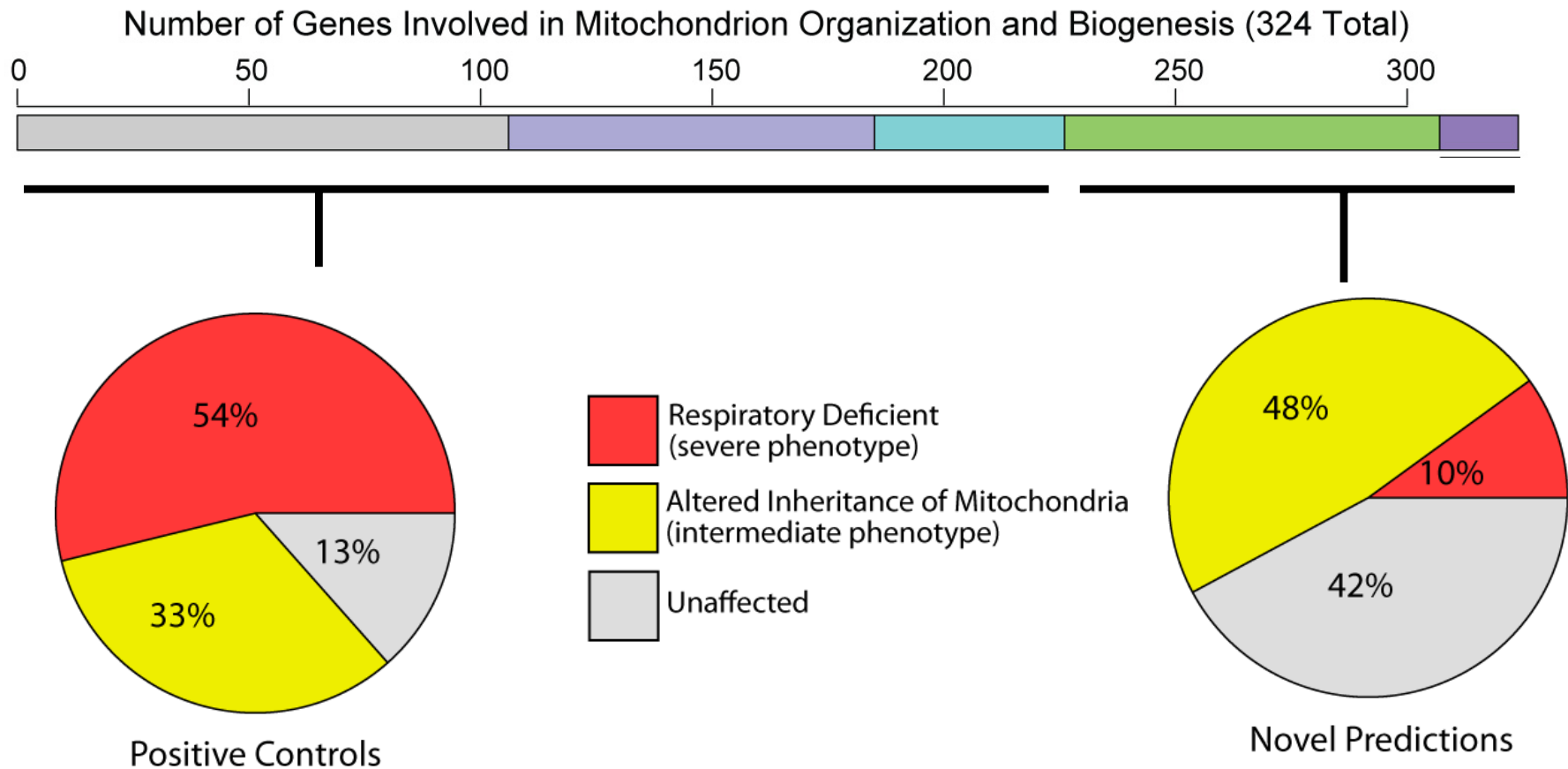
Many petite negative mutations affect ATP turnover and inner membrane properties



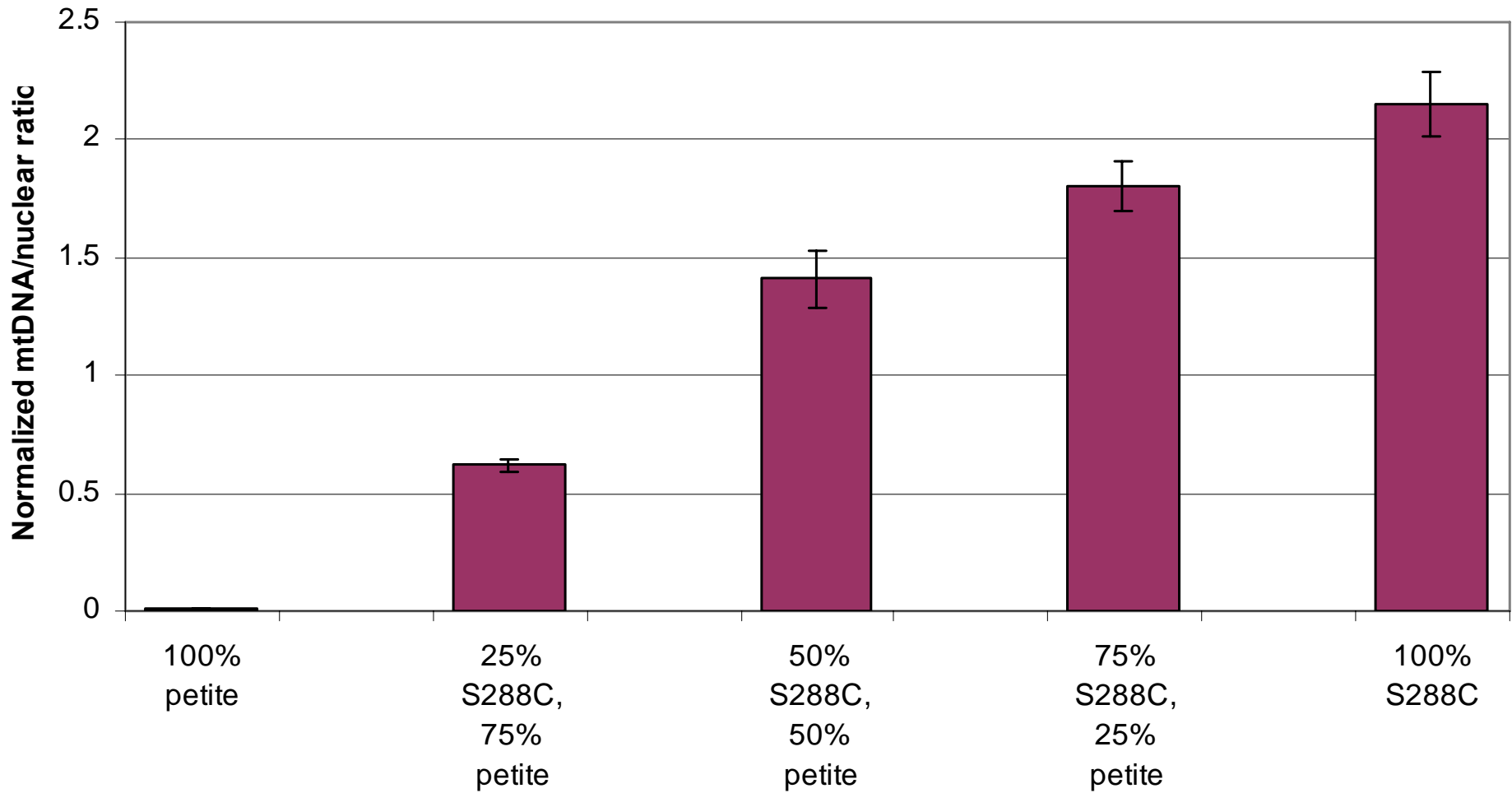
Predictive Framework Increases Number of Genes involved in Mitochondrial Biogenesis and Inheritance from 106 to 324



Predictive Framework Increases Number of Genes involved in Mitochondrial Biogenesis and Inheritance from 106 to 324



Validation of Real Time PCR for Determination of mtDNA to Nuclear DNA Ratio



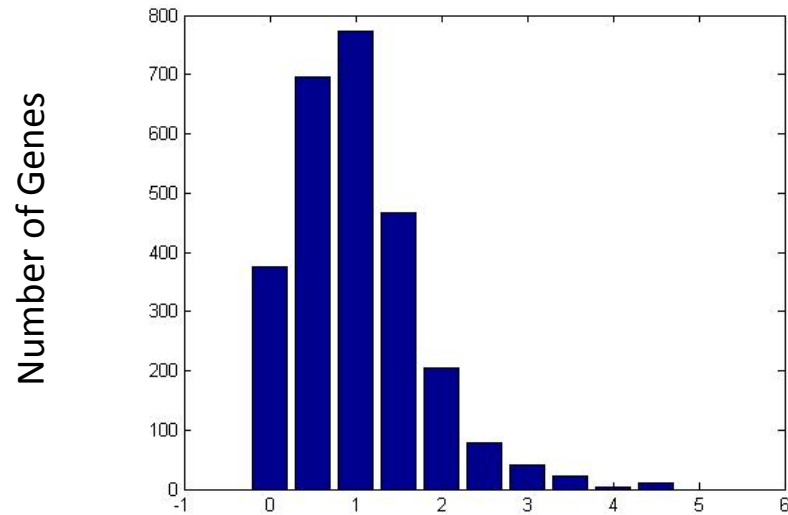
Distribution of mtDNA/Nuclear DNA Ratios in the haploid deletion set

Glucose

Mean Ratio $1.18 \pm .9$

Median Ratio 1.03

3225 informative strains

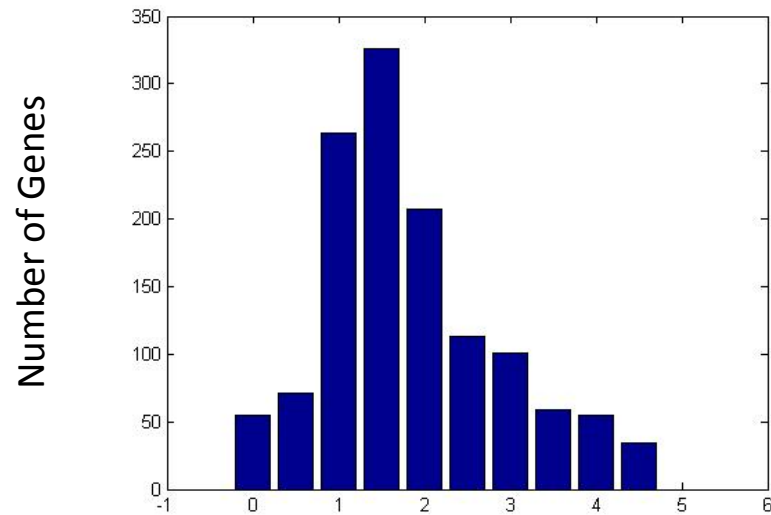


Glycerol

Mean Ratio 2.3 ± 2.7

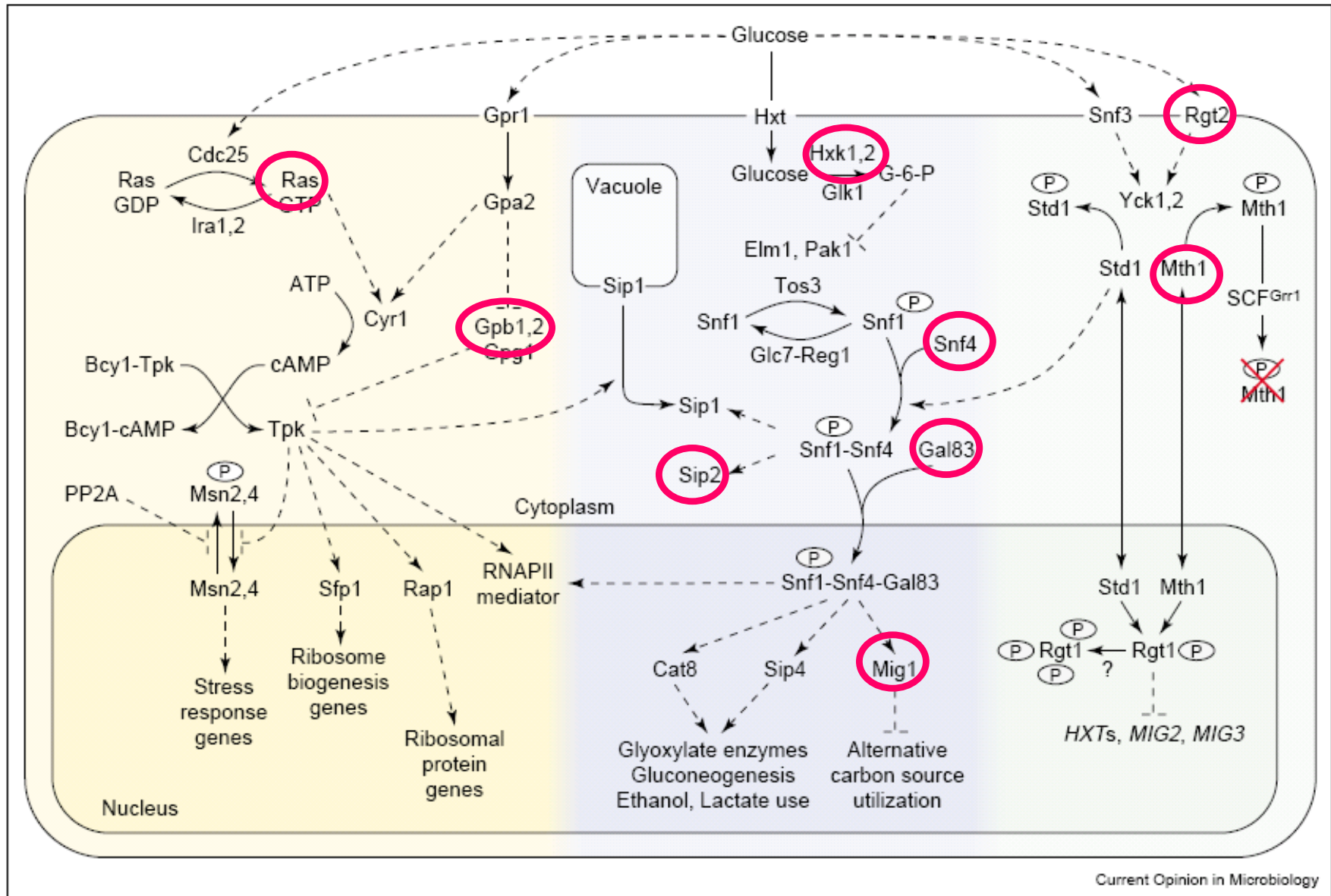
Median Ratio 1.91

2005 informative strains



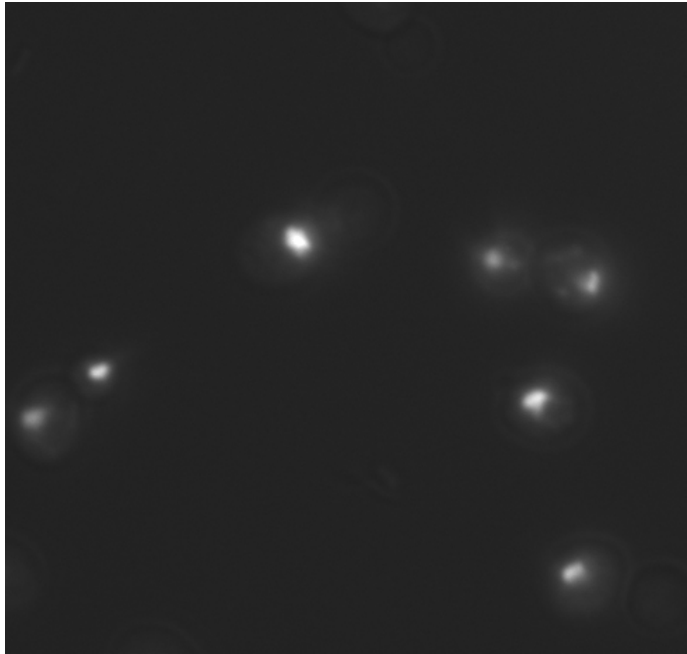
Normalized mtDNA/Nuclear DNA Ratio

Deletion Mutants of Many Members of the Glucose Sensing Pathway Have High mtDNA Copy Number

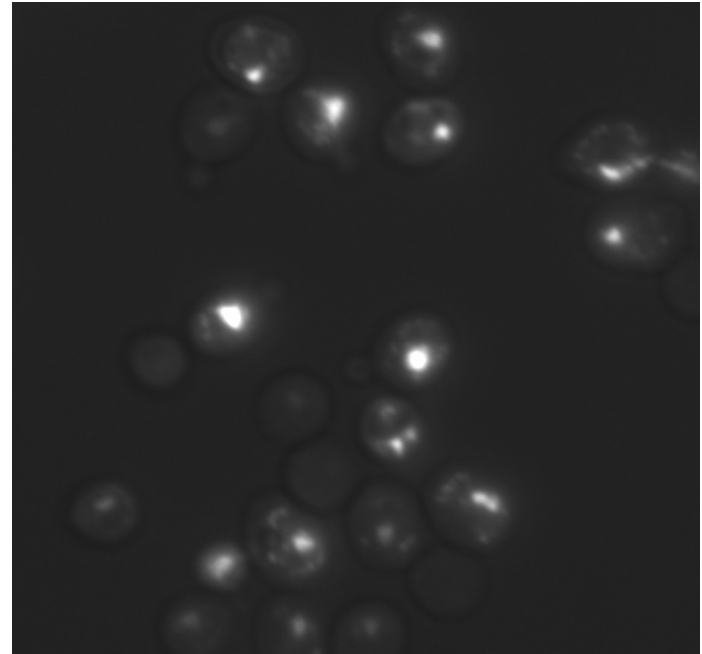


Current Opinion in Microbiology

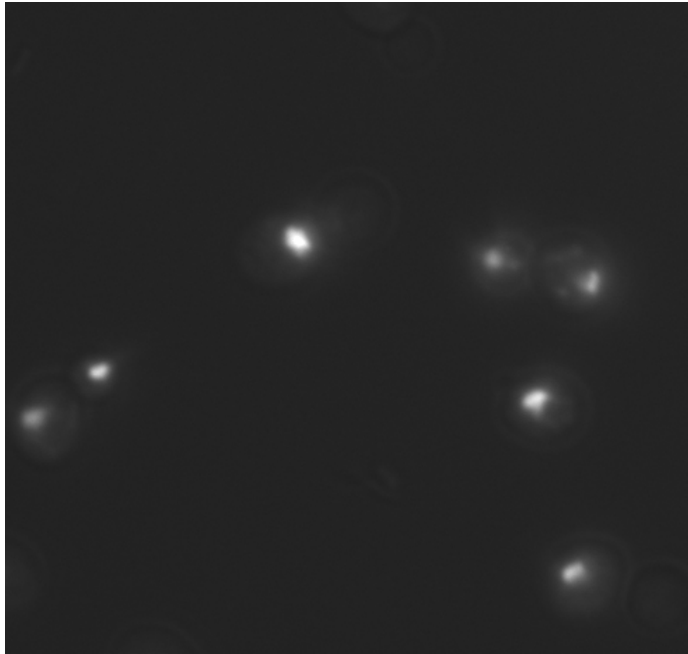
Figure adapted from Schneper, Duvel, and Broach 2004



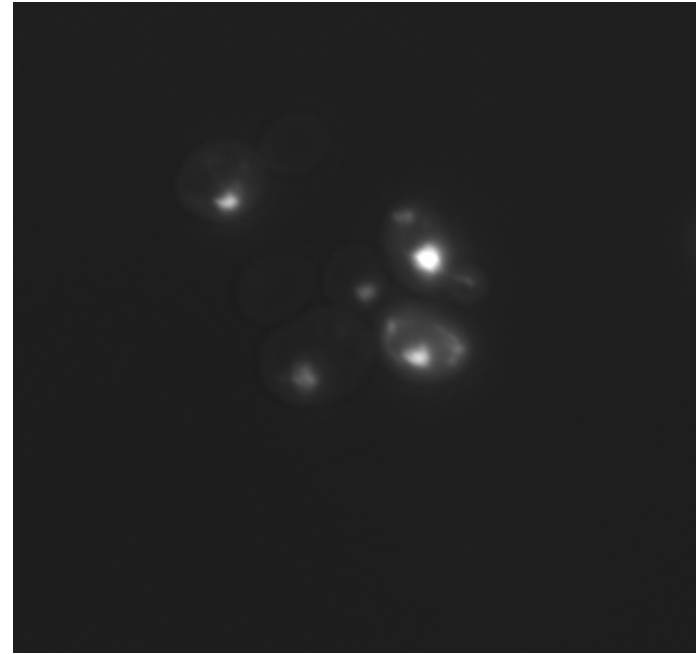
Control Strain



PRX1: Mitochondrial peroxiredoxin (1-Cys Prx) with thioredoxin peroxidase activity, has a role in reduction of hydroperoxides; induced during respiratory growth and under conditions of oxidative stress
3 fold elevated mtDNA on glucose
Growth defect on glycerol

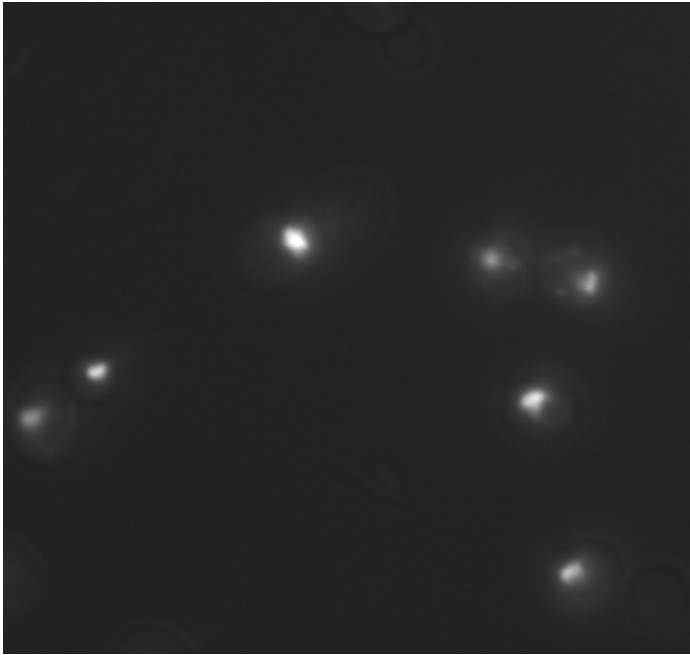


Control strain

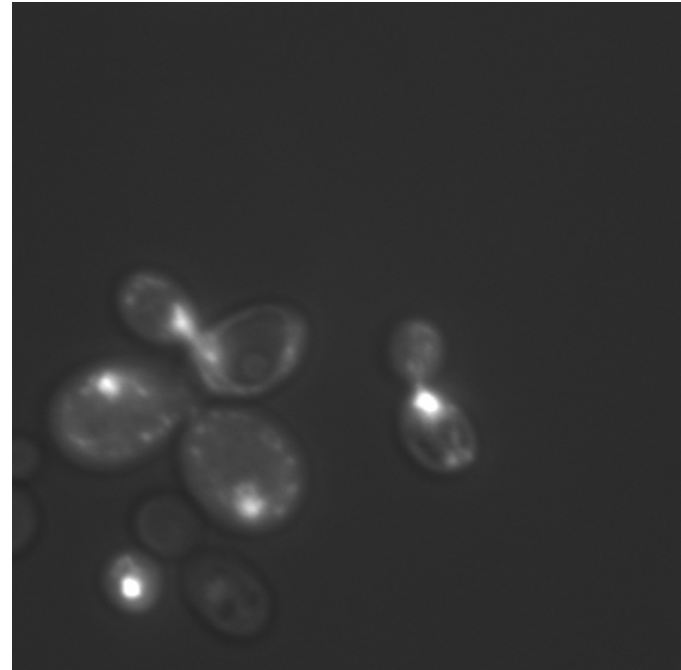


OM14: major outer membrane protein of yeast mitochondria 1

4 fold elevated mtDNA on glucose
Growth defect on glycerol

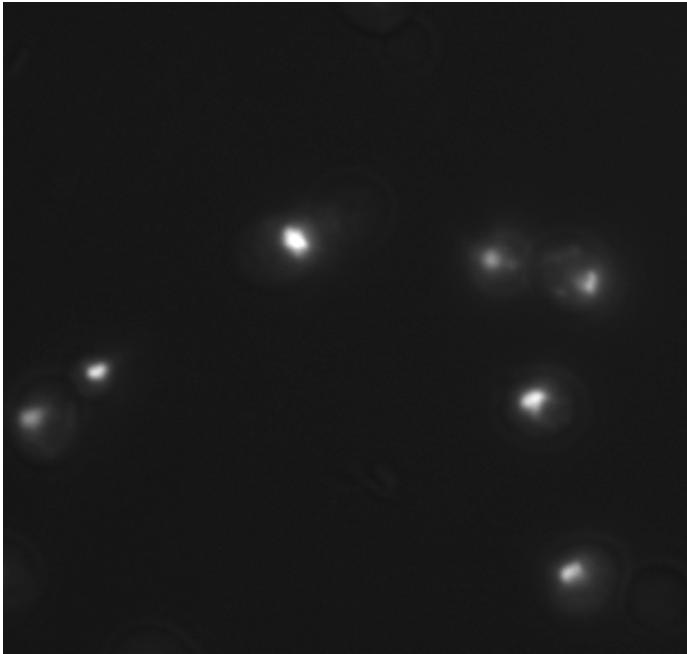


Control Strain



RAD6: Ubiquitin-conjugating enzyme (E2), involved in postreplication repair (with Rad18p), sporulation, telomere silencing, and ubiquitin-mediated N-end rule protein degradation (with Ubr1p)

4-fold elevated mtDNA on glucose
10-fold elevated mtDNA on glycerol

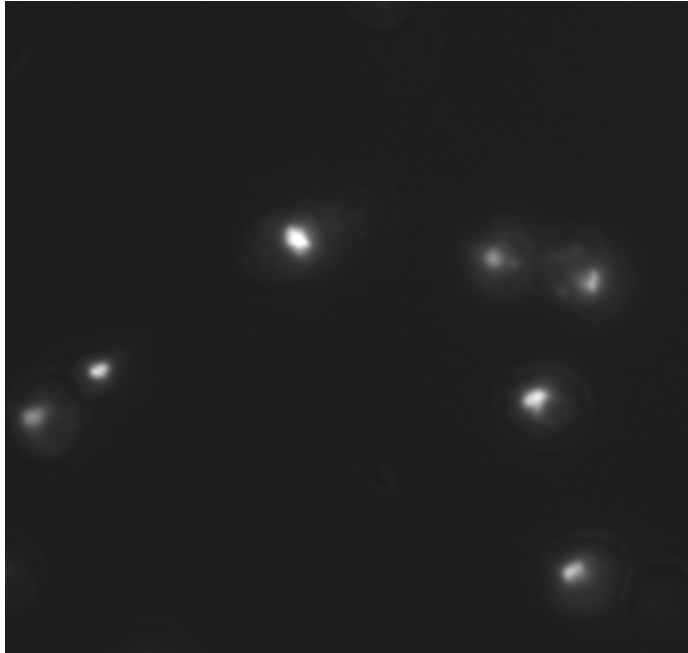


Control Strain

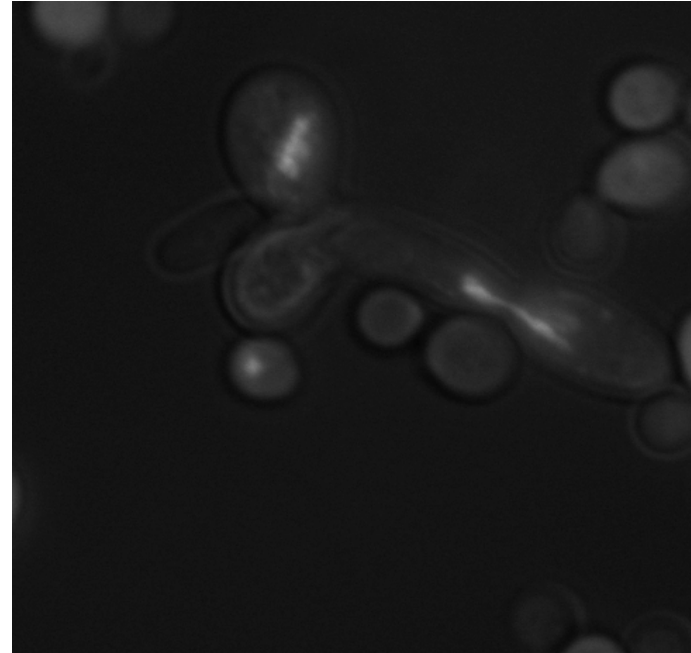


ATG4: Cysteine protease required for autophagy; cleaves Atg8p to a form required for autophagosome and Cvt vesicle generation; mediates attachment of autophagosomes to microtubules through interactions with Tub1p and Tub2p

5-fold elevated mtDNA on glucose
3-fold elevated mtDNA on glycerol



Control Strain



URA8: Minor CTP synthase isozyme (see also URA7), catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to UTP, forming CTP, the final step in de novo biosynthesis of pyrimidines; involved in phospholipid biosynthesis
5-fold elevated mtDNA on glucose
Growth defect on glycerol

Predicted genes are Localized to Cytoplasm as well as Mitochondria

<i>bioPIXIE</i>	<i>SPELL</i>	<i>MEFIT</i>
mitochondrial part (GO:0044429)	mitochondrial part (GO:0044429)	mitochondrial part (GO:0044429)
actin cytoskeleton (GO:0015629)	--	--
mitochondrial distribution (GO:0048311)	--	--
--	mitochondrial ribosome (GO:0005761)	mitochondrial ribosome (GO:0005761)
--	translation (GO:0006412)	translation (GO:0006412)

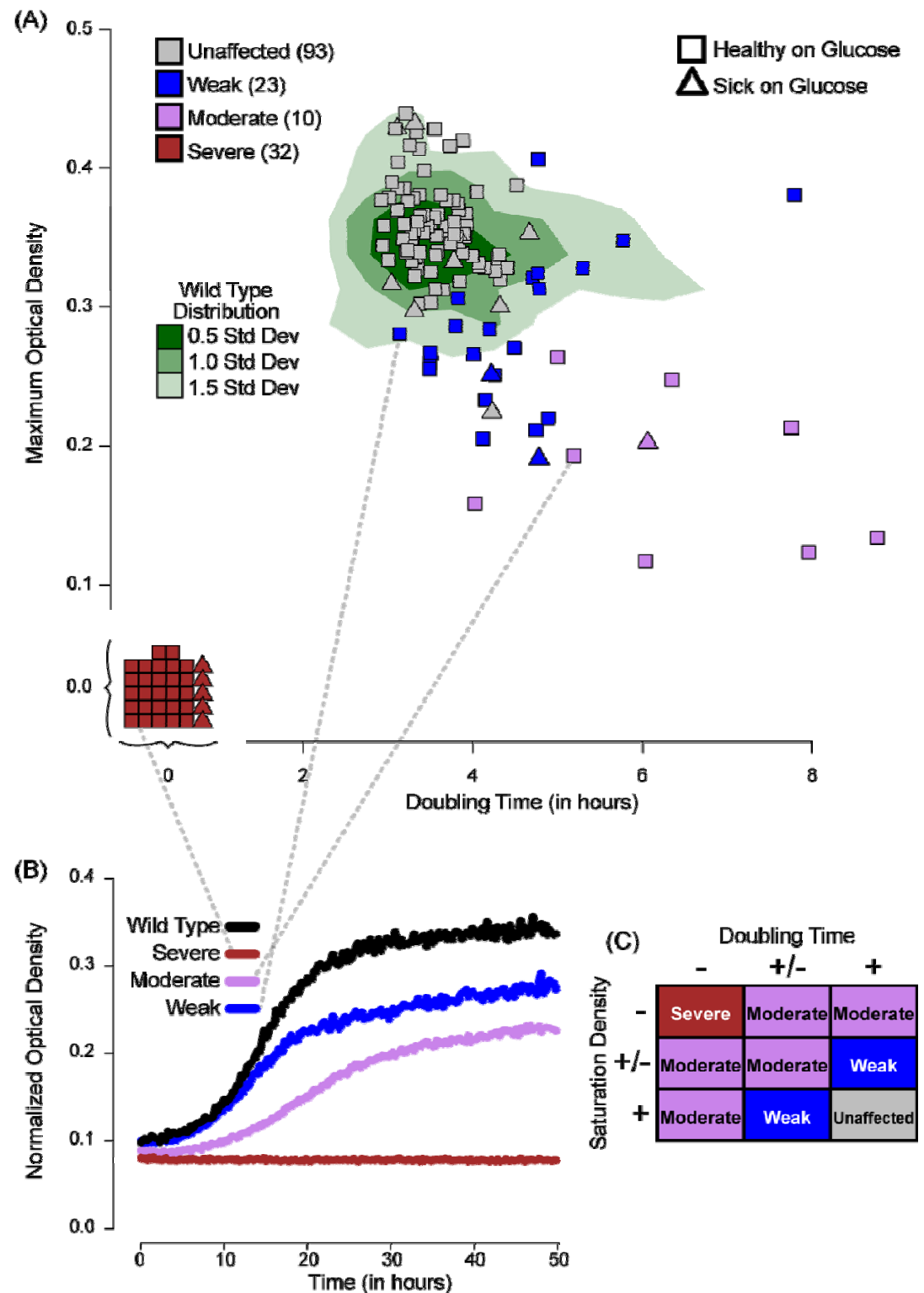
AIM21: A Cytoplasmic Gene Required for Mitochondrial Motility

Actin
Porin (mito)

Petite 150% of wt

Respiratory function is defective in mutants of some predicted genes

- 6 mutants and two paired wild type per plate



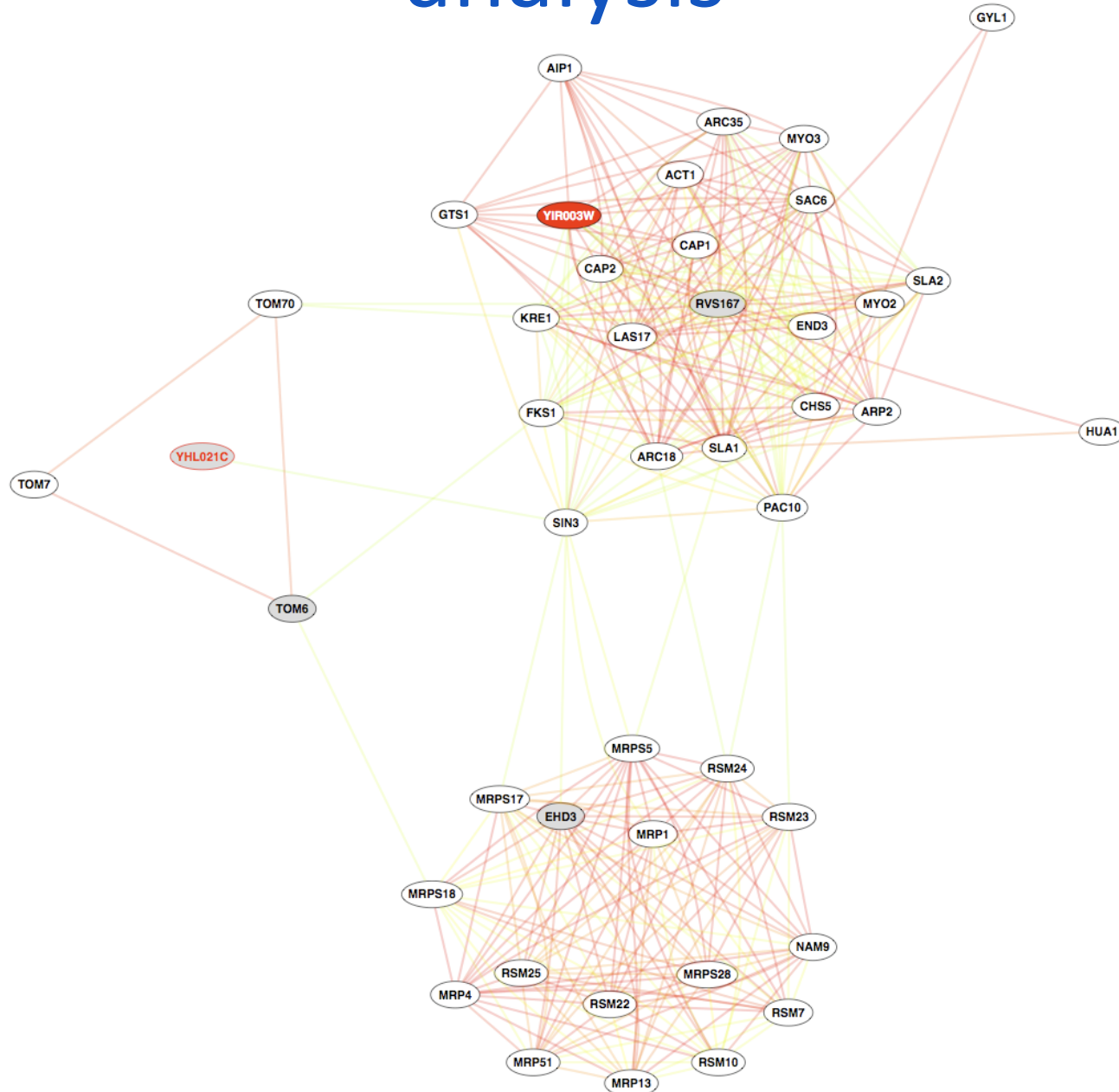
Highly replicated experiments discover new genes with specific roles in respiration

- Seven mutations that cause strong respiratory defects had been missed in previous high throughput screens
- 55 of the 67 mutants tested had weak or no respiratory phenotype
- 12 genes with altered mitochondrial inheritance and respiratory defects include:
 - COX14, a respiratory assembly factor
 - FMC1, an ATP synthase chaperone
 - MRP49, a translation assembly subunit
 - CIT1, citrate synthase

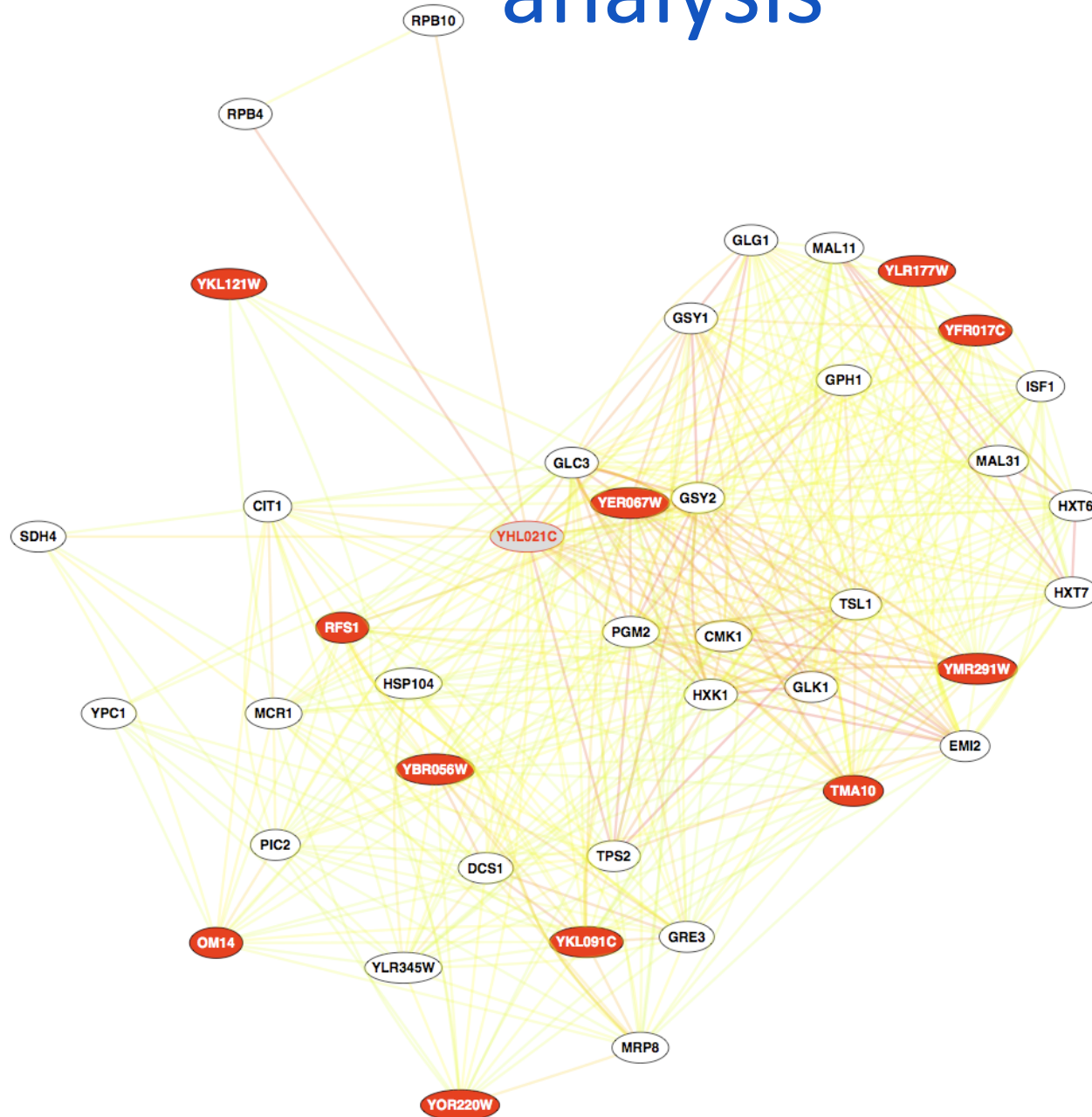
What about those predictions that didn't exhibit a phenotype?

- Of the 60 candidates showing normal mitochondrial inheritance in the first round, 26 retained high confidence scores in the second round of prediction
- 23 of these 26 have known mitochondrial localization
- To identify potential genetic interactions, these 26 mutants were crossed to 4 mutants that have minor alterations in mitochondrial inheritance.

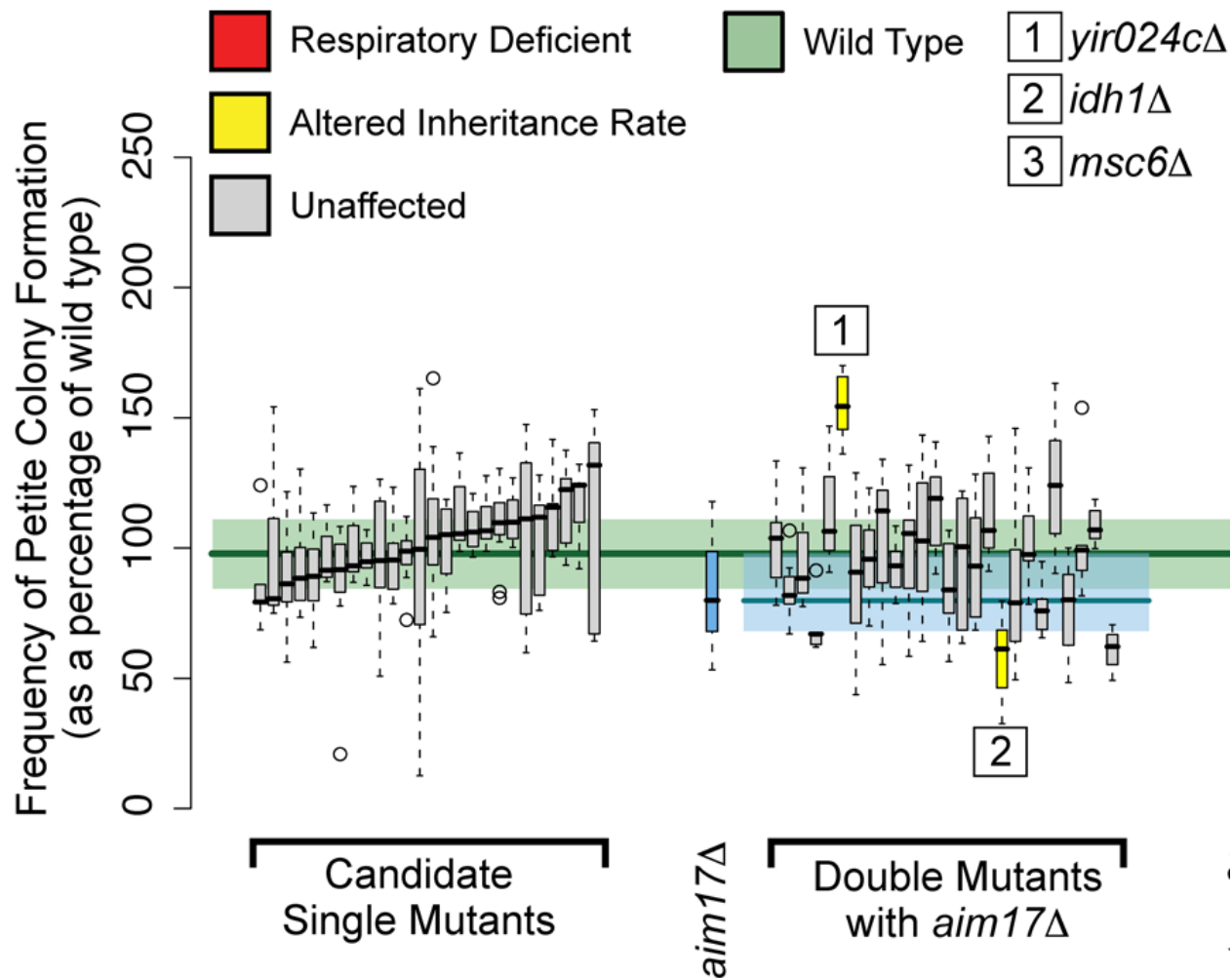
Selecting genes for double mutant analysis



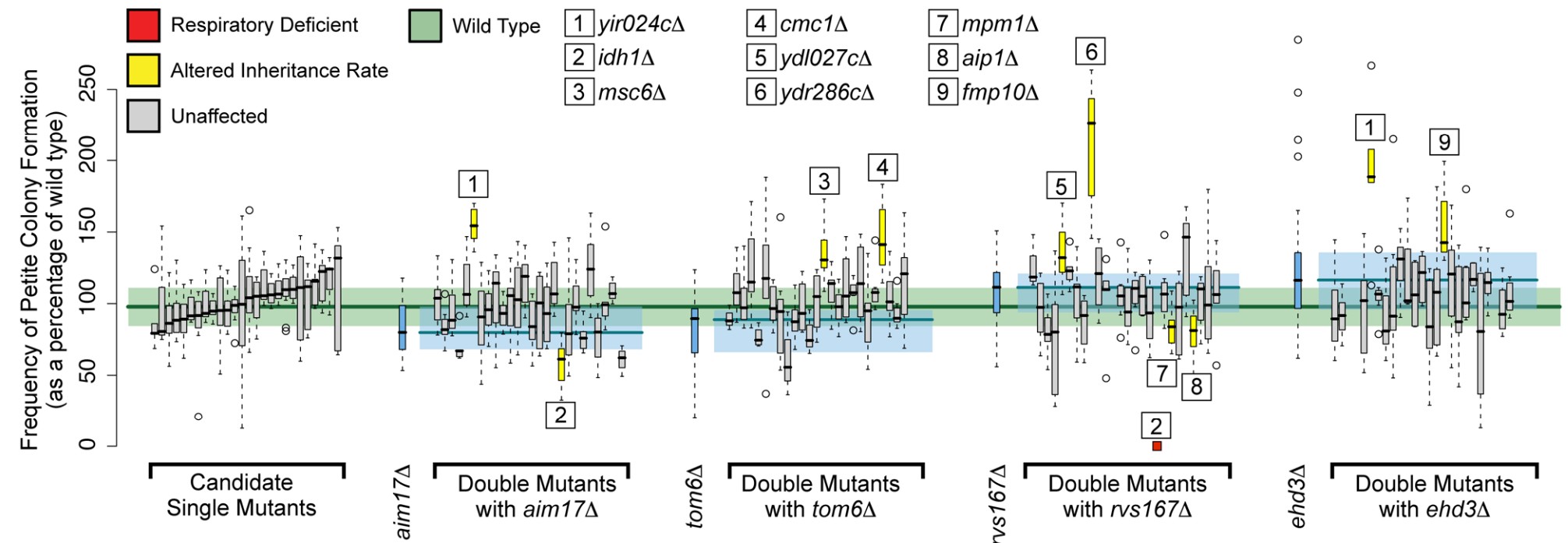
Selecting genes for double mutant analysis



Testing Double Mutants for Synthetic Effects on Petite Frequency

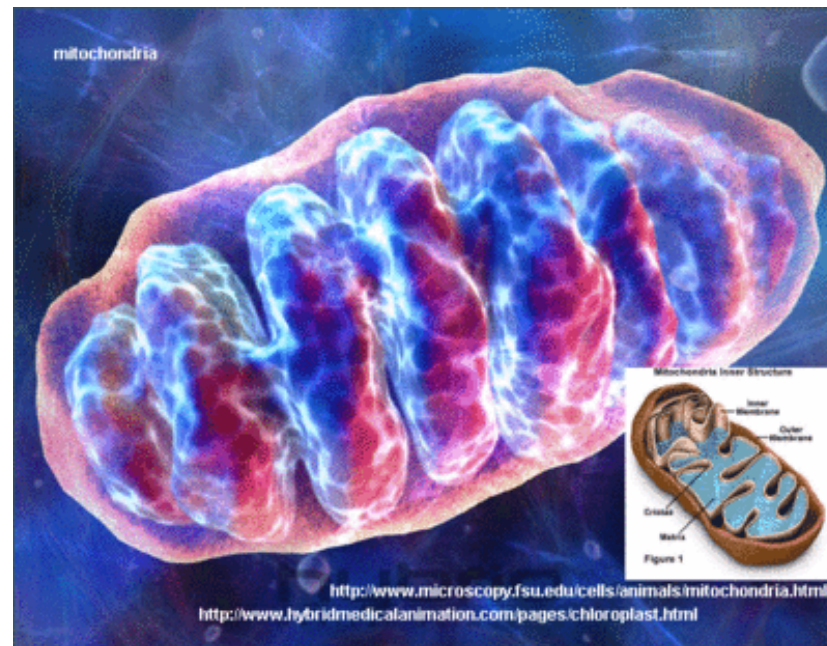


10 of 26 unaffected single mutants show synthetic genetic defects



Mitochondria and human disease

- Mitochondria are a hub of metabolic processes in the cell
- 1 in 5 known human mitochondrial genes is associated with disease
- Over half of the ~100 newly discovered mitochondrial genes have human orthologs
- Several of these human orthologs are known disease genes ranging from cancer (thymoma) to metabolic disorders (Glutricaciduria)



Acknowledgements

- Caudy Lab
 - Alicia Hayes
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 - Dave Cappel
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 - David Hess
 - Chad Myers
 - Matt Hibbs
 - Curtis Huttenhower
- Boone Lab,
University of Toronto
 - Michael Costanzo
 - Jadine Paw



MEFIT	http://function.princeton.edu/mefit
SPELL	http://function.princeton.edu/spell
bioPIXIE	http://function.princeton.edu/pixie
MousePIXIE	http://mousenet.princeton.edu



acaudy@princeton.edu