



DATA NOTE

Identifying genes required for respiratory growth of fission yeast [version 1; referees: 4 approved]

Michal Malecki^{1,2}, Jürg Bähler ¹

¹Research Department of Genetics, Evolution & Environment and Institute of Healthy Ageing, University College London, London, UK

²Department of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

v1 First published: 15 Nov 2016, 1:12 (doi: [10.12688/wellcomeopenres.9992.1](https://doi.org/10.12688/wellcomeopenres.9992.1))
 Latest published: 15 Nov 2016, 1:12 (doi: [10.12688/wellcomeopenres.9992.1](https://doi.org/10.12688/wellcomeopenres.9992.1))

Abstract

We have used both auxotroph and prototroph versions of the latest deletion-mutant library to identify genes required for respiratory growth on solid glycerol medium in fission yeast. This data set complements and enhances our recent study on functional and regulatory aspects of energy metabolism by providing additional proteins that are involved in respiration. Most proteins identified in this mutant screen have not been implicated in respiration in budding yeast. We also provide a protocol to generate a prototrophic mutant library, and data on technical and biological reproducibility of colony-based high-throughput screens.

Open Peer Review

Referee Status: 

	Invited Referees			
	1	2	3	4
version 1				
published 15 Nov 2016	report	report	report	report

- Christopher Herbert**, CEA, CNRS, University Paris-Sud, University Paris-Saclay France
- Luis A. Rokeach**, Université de Montréal Canada
- Jim Karagiannis**, University of Western Ontario Canada
- Makoto Kawamukai**, Shimane University Japan

Discuss this article

[Comments](#) (0)

Corresponding author: Jürg Bähler (j.bahler@ucl.ac.uk)

How to cite this article: Malecki M and Bähler J. **Identifying genes required for respiratory growth of fission yeast [version 1; referees: 4 approved]** Wellcome Open Research 2016, 1:12 (doi: [10.12688/wellcomeopenres.9992.1](https://doi.org/10.12688/wellcomeopenres.9992.1))

Copyright: © 2016 Malecki M and Bähler J. This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Grant information: This work was supported by the Wellcome Trust [095598].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The author(s) declare no competing interests.

First published: 15 Nov 2016, 1:12 (doi: [10.12688/wellcomeopenres.9992.1](https://doi.org/10.12688/wellcomeopenres.9992.1))

Introduction

Energy metabolism is fundamental for cell growth and function, and cells need to tune metabolic pathways to optimize their physiology in response to different nutrient or physiological conditions¹. Metabolism in yeast can be manipulated by the growth medium: on glucose yeast cells proliferate by fermentation, while on glycerol they shift towards respiration. Much of our fundamental knowledge on energy metabolism stems from research with *Saccharomyces cerevisiae*, including systematic screens for genes involved in cellular respiration^{2,3}, while *Schizosaccharomyces pombe* provides a potent complementary model system⁴. We have recently applied large-scale functional and regulatory analyses on energy metabolism in fission yeast⁵. The data presented here provide additional, complementary insights into genes that are required for respiration.

Here, we screened the *S. pombe* deletion collection⁶ for mutants affecting growth on solid media containing glycerol as a main carbon source. Glycerol is a non-fermentable carbon source, which forces cells to gain energy by respiration rather than fermentation. We compared respiratory growth on glycerol to fermentative growth on glucose (control) to identify mutants that show defects in respiration. This screen differed in three respects from screens we previously reported⁵: 1) we used the latest version 5 of the gene-deletion library, which features over 400 more mutants (3420 vs 3003) and corrections of erroneous deletions; 2) we generated a prototrophic library of this latest version using an improved protocol that minimizes contamination by wild-type cells and optimizes library quality; 3) we determined colony sizes at densities of only 384 colonies per plate (instead of 1536 colonies), since larger colonies provide a higher dynamic range and sensitivity that outperforms data from higher density screens despite the reduced number of technical repeats; and 4) we applied a different data analysis and only used glycerol as a non-fermentable carbon source, while the previous study also measured respiratory growth on galactose⁵.

Materials and methods

Yeast media

YES: 5g/L yeast extract, 30g/L glucose, 0.25g/L adenine, histidine, leucine, lysine, uracil (Formedium PMCUCL1000)

EMM agar (Formedium PMD0100)

ME agar (Formedium PCM 0810)

YE glucose: 5g/L yeast extract (Bacto 212750), 30g/L glucose (Fisher G/0500/53)

YE glycerol: 5g/L yeast extract (Bacto 212750), 30ml/L glycerol Fisher BP229-1), 1g/L glucose (Fisher G/0500/53)

For solid media, 2% agar (VWR 20767.298) was added.

Auxotroph deletion library

The auxotroph haploid deletion library version 5.0 was obtained from Bioneer (<http://www.bioneer.com/>). The basic genotype is *h+* *ade6-M210* (or *ade6-M216*) *ura4-D18 leu1-32*. Each strain contains a different non-essential gene deleted with the kanamycin cassette⁶. This library contains 3420 strains.

Prototroph deletion library

The prototroph deletion library was prepared by backcrossing the auxotroph deletion library to a *h* 972 strain with its H1 box deleted with a hygromycin cassette (*h* 972 *H1::hyg^r*; strain created in this work). The cassette was amplified on the template of the pFA6a family plasmid⁷ using the following primers (Life Technologies) (Left: AAAGTAATACATGGATTTTACTGCCCTGATTCTATCGAAATATGCTGTTTTTTTTTATTTCGTTTTTATT-TATTTTCAATAACGGATCCCCGGGTTAATTAA; Right: GGGAAGGGGAAGGTAGAAGGGCGCACACAAAAAGGGAAAATTGGAGGGAGAATGAATGACACGAACAGCAT-AATTGGAAAGAATTCGAGCTCGTTTAAAC). Replacing the H1 box with hygromycin allows to select stable *h* mating types in the progeny; this mating-type selection prevents mating during the screen, and enables the use of this prototroph collection for synthetic genetic array assays.

Prototroph deletion library preparation

The auxotroph deletion library was arrayed onto 9 PlusPlates (Singer Instruments) with solid YES medium, at 384 colonies per plate using the RoToR HDA robot (Singer Instruments), and grown for 2 days at 32°C. The prototroph strain (*h* 972 *H1::hyg^r*) was grown in liquid YES medium overnight and subsequently arrayed onto 3 PlusPlates with YES medium at 384 colonies per plate and grown for 3 days at 32°C. The freshly grown prototroph strain was then mixed with the freshly grown auxotroph deletion collection on PlusPlates with solid ME medium, using the RoToR HDA robot mate protocol. Each plate of wild-type strains was used to mix with 3 plates of the auxotroph collection. Subsequently, the 9 plates obtained were wrapped in cling film and stored at 25°C for 3 days to facilitate mating and sporulation.

After incubation, the plates were moved into a well humidified 45°C incubator for another 3 days. This step is designed to kill parental strains and diploids, because only spores are resistant to this high temperature. Next, using the RoToR HDA robot, spores were copied onto PlusPlates with YES medium and grown for 2 days at 32°C to allow spore germination before the selection steps. To improve efficiency, the selection was performed first in liquid medium. Cells were transferred from PlusPlates with solid YES medium to 384-well plates filled with 60µl/well of YES containing hygromycin and kanamycin (0.1mg/ml each) and kept overnight at 32°C. Subsequently, cells were transferred onto agar PlusPlates with YES supplemented with hygromycin and kanamycin (0.1 mg/ml each) and grown for two days at 32°C. For the second selection step, cells were transferred from YES plus hygromycin and kanamycin plates onto a 384-well plate filled with 60µl/well of EMM medium and grown overnight at 32°C. Cells were then copied onto PlusPlates with solid EMM medium and grown for 2 days at 32°C. Subsequently, cells were transferred onto PlusPlates with YES supplemented with hygromycin and kanamycin and grown for two days. The resulting prototroph library was then transferred onto 384-well plates with 60µl of YES with 20% glycerol and stored at -80°C.

Screening procedure

For each biological repeat, the deletion library (prototroph or auxotroph) was freshly plated from -80°C glycerol stocks onto

PlusPlates with solid YES medium at 384 colonies per plate. After 3 days incubation at 32°C, libraries were copied onto YES plates with kanamycin, and incubated again for 2 days, then were copied again onto two sets of PlusPlates with solid YES medium and incubated for two more days. These plates were used as an initial template for the screen and treated as technical repeats. The library from each set of YES plates was copied onto PlusPlates with YE glucose or YE glycerol medium and grown for at least 2 days at 32°C. Cells grew more slowly on YE glycerol and were left for up to four days. After incubation, pictures of the plates were taken using a Canon camera (PC1305) and MultiDoc-It imaging system (UVP). In total, two technical repeats of the screen were obtained for each of the two biological repeats carried out, with both the auxotroph and the prototroph libraries, resulting in four sets of colonies grown on glycerol medium and four sets of colonies grown on glucose medium to be recorded for each library (Figure 1A and B).

Data treatment

Quantitation of colony sizes was performed with the *gitter* R package (v. 1.1.1) (<http://omarwagih.github.io/gitter/>)⁸. For normalisation, we used an in-house tool (Townsend StJ., Rallis C., Bähler J., unpublished data). In brief, absent colonies were identified and excluded from the normalisation process; for each plate, colony sizes were normalised to the plate median, corrected for row- or column-specific artefacts (i.e. edge effects). A median filter was applied to identify and correct for local spatial variations. Initial data analysis revealed some noise, although correlations between data sets were all positive, with the strongest correlations being obtained for the technical repeats and with weaker correlations between biological repeats grown on the same carbon source (Figure 1A). Similarly, clustering analysis showed that data are most similar among technical repeats (Figure 1B). Due to the high variability between biological repeats, we decided not to average values from the 4 repeats for each carbon source. First, we only averaged data from the corresponding technical repeats, and filtered out colonies showing high variability between repeats (standard error >0.4), being absent in one of the repeats, or with very small colony sizes on glucose (normalised median size <0.12). Second, we separately calculated for each biological repeat the colony size ratios between glycerol and glucose.

Dataset validation

We obtained 16 sets of colony size data from the screen: 8 sets each for the auxotroph and prototroph mutant library backgrounds, consisting of 2 independent biological repeats each with colonies grown on either YE glucose or YE glycerol media, with each biological repeat consisting of two technical repeats. The data from technical repeats strongly correlated, while the data from biological repeats showed much weaker correlations (Figure 1A and B). There was also a substantial difference between most data based on the auxotroph library and those based on the prototroph library (Figure 1B). The distributions of colony-size ratios (growth on glycerol/growth on glucose) also differed substantially between the biological repeats (Figure 1C, upper graphs).

We analysed the top-100 screen hits showing the lowest colony-size ratios for each of the 4 biological repeats for functional enrichments

within the Gene Ontology (GO; <http://geneontology.org/>) ‘Cellular Component’ category, using the AnGeLi tool (www.bahlerlab.info/AnGeLi)⁹ with the deletion library genes as a background list. Since the screen was designed to detect respiratory-deficient mutants, we expected an enrichment in genes encoding mitochondrially localised proteins. This analysis revealed the expected enrichments in the GO term ‘mitochondrion’ (GO: 0005739 PomBase, www.pombase.org)¹⁰, which reached 50% for biological repeat 1 ($p < 1.1E-15$) and 40% for biological repeat 2 ($p < 1.2E-8$) for the auxotroph library, and 33% for biological repeat 1 ($p < 5E-4$) and 37% for biological repeat 2 ($p < 5.5E-6$) for the prototroph library. These strong enrichments in the key expected GO term indicate that the screen produced biologically relevant results. We then analysed the overlap of genes obtained from the 2 biological repeats for both the auxotroph and prototroph libraries using a threshold value. To choose the optimal threshold for each library, we plotted the enrichments in genes with the GO term ‘mitochondrion’ as a function of increasing thresholds for the biological repeats and overlap (Figure 1C, lower graphs). The thresholds were set such that the overlap between the biological repeats showed $\geq 40\%$ enrichment for the GO term ‘mitochondrion’. For the auxotroph library, a threshold of <0.86 for the colony-size ratio (growth on glycerol/growth on glucose) gave an overlap of 166 genes between the biological repeats, which showed 44% enrichment in genes associated with the GO term ‘mitochondrion’ (Figure 1D and E). For the prototroph library, a threshold of <0.75 for the colony-size ratio gave an overlap of 99 genes, which showed 40% enrichment in genes associated with the GO term ‘mitochondrion’ (Figure 1D and E).

We then merged the results obtained from both genetic backgrounds (auxotroph and prototroph). The GO term ‘mitochondrion’ showed an enrichment of 37% in the merged list (Figure 1E). Substantial overlaps were also evident with independently identified genes affecting *S. pombe* growth on glycerol (FYPO: 0001934 PomBase¹¹), as well as with genes resulting from our previous screen using an older version of the Bioneer deletion library (version 2.0; <http://www.bioneer.com/>) and a different prototroph library⁵ (Figure 1F). We also compared our screen hits with orthologous genes that affect respiratory growth in budding yeast (*Saccharomyces* genome database; www.yeastgenome.org), where the genes resulting from the new screen showed a stronger overlap than the genes from our previous screen (Figure 1F).

Conclusions

As we have shown before⁵, the genetic background of the deletion library can strongly affect the screen results. The commonly used auxotroph mutant background has shortcomings for investigating energy metabolism in particular, yet also provides complementary insights. We find that the mutant library in the prototroph background shows less variation in growth as measured by colony sizes, which likely reflects the absence of genetic interactions from the three auxotrophic mutants. We also report quite large variations among independently repeated screens, which highlights the importance to perform biological repeats. The genes implicated in respiration in the previous screen⁵ and in the new screen reported here compare as follows: 149 genes have only been identified in the previous screen, 55 genes have been identified in both screens, and 166 genes have only been identified in the new screen. These substantial

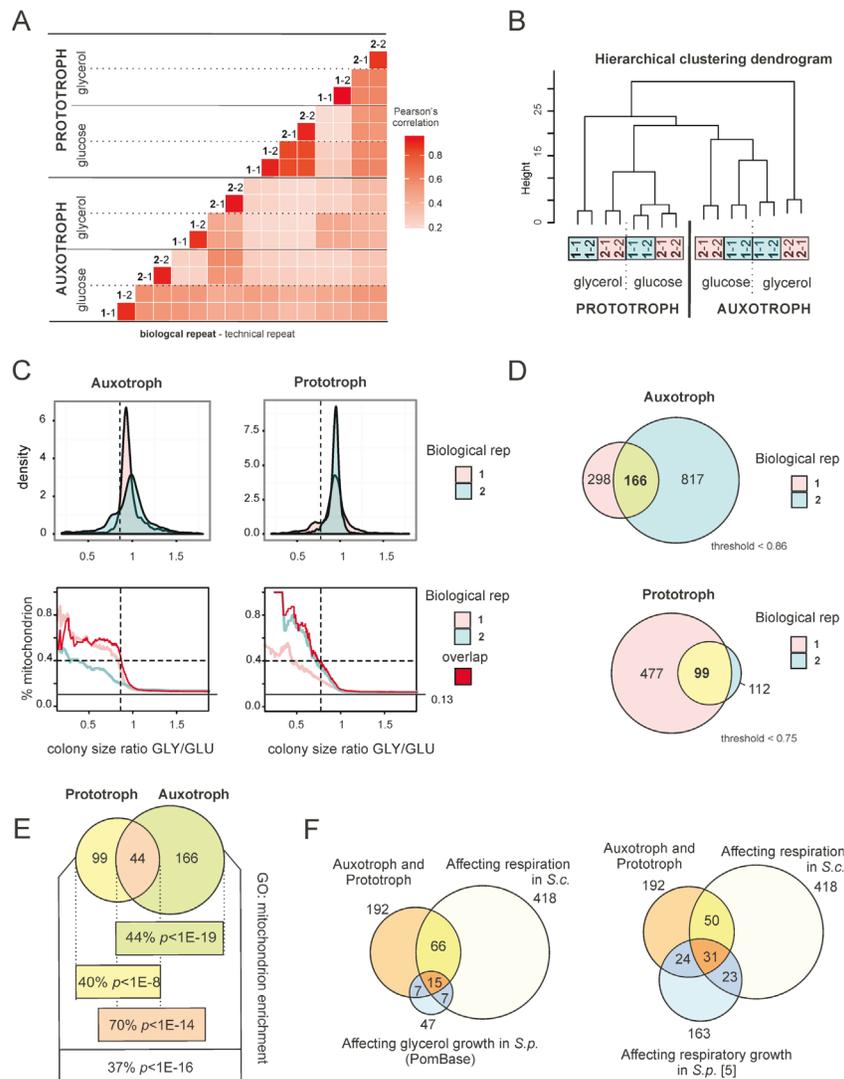


Figure 1. Validation of screen results. (A) Relationships between all 16 data sets, consisting of biological and technical repeats on fermentative (glucose) and respiratory (glycerol) media for both auxotroph and prototroph libraries as indicated. Pearson's correlations between data sets are visualised on the heat map using the *ggplot2*R package v.2.1.0 (<http://ggplot2.org/>; *ggcorr* function, method "pairwise", "pearson"). The biological repeats are indicated in bold numbers, followed by numbers for technical repeats. (B) Hierarchical clustering of all 16 data sets using the *dist* (method "Euclidean") and *hclust* (method "ward.D") R functions. Data sets are labelled as in (A), with biological repeats colour-coded as in (C) and (D). (C) Upper graphs: Distributions of colony size ratios (growth on glycerol/growth on glucose) and prototroph and auxotroph libraries. The vertical dashed lines indicate the chosen thresholds for gene lists in the lower graphs. Lower graphs: Functional enrichments between biological repeats. The percentages of genes with the Gene Ontology (GO) term 'mitochondrion' (for each biological repeat and the overlap list, see colour legend) are plotted as a function of different colony-size ratios for both auxotroph (left) and prototroph (right) libraries. The vertical dashed lines indicate the thresholds chosen to generate gene lists that show $\geq 40\%$ enrichment for the GO term 'mitochondrion' (horizontal dashed lines). The solid horizontal line indicates the proportion of genes associated with the GO term 'mitochondrion' among all genes ($\sim 13\%$). (D) Overlaps between biological repeats. The chosen colony-size ratio thresholds (C) were used to generate gene lists for both biological repeats and genetic backgrounds. Venn diagrams with numbers of genes whose mutants showed colony-size ratios values lower than the thresholds. The overlapping genes were used for further analyses in (E) and (F). (E) Gene lists obtained from the chosen colony-size ratio thresholds (C) for auxotroph and prototroph libraries and their overlap were tested for enrichment of genes associated with the GO 'mitochondrion' term using AnGeLi⁹. The percentages of mitochondrial genes and the corrected *p* values for the enrichment of mitochondrial genes is provided for each gene list, colour-coded as in the Venn diagram. (F) Gene lists obtained from the chosen colony size ratio thresholds (C) for auxotroph and prototroph libraries were combined (Auxotroph and Prototroph), and compared with three other relevant gene lists: 1) genes which have previously been annotated in PomBase to affect *S. pombe* (*S.p*) growth on glycerol (left Venn diagram); 2) genes which we have independently identified⁵ to affect respiratory growth (right Venn diagram); and 3) genes affecting respiratory growth in *S. cerevisiae* (*S.c.*), obtained from 3 phenotype categories in the *Saccharomyces* genome database (www.yeastgenome.org): respiratory growth absent, decreased or decreased rate. All the lists were limited to genes present in the *S. pombe* deletion library (some respiratory genes are essential in *S. pombe*, but not in *S. cerevisiae*) and showing orthologues in both yeasts, based on the manually curated list in PomBase¹⁰.

differences not only reflect the differences in the mutant library and methods (see above), but also the large variations in respiratory-deficient phenotypes, as also observed in *S. cerevisiae*². This variation and experimental noise may be even higher in *S. pombe* for the following reasons: 1) *S. pombe* depends on respiration to a greater extent than *S. cerevisiae*¹², and respiratory mutants may therefore affect growth also in fermentative media, thus diminishing growth differences between respiratory and fermentative conditions; and 2) *S. pombe* generally grows very poorly on glycerol, which reduces the sensitivity to record differences in colony sizes. The data reported here provide an additional resource of respiratory genes, which complements and enhances data from the previous screens.

Taken together, the partial overlaps between data obtained in different screens, as well as the variability of the data from different biological repeats and strain backgrounds, suggests a high plasticity of the respiratory phenotype. A similar phenomenon has been noticed in budding yeast². Thus, merging data from different high-throughput screens provide complementary information and together increase the sensitivity to systematically identified genes relevant for respiratory growth.

Data availability

Raw data are deposited in the Open Science Framework (DOI: <http://dx.doi.org/10.17605/OSF.IO/VCHE3>¹³; <https://osf.io/vche3/>).

Data from this and the previous screen⁵ will be also be available on our website (www.bahlerlab.info/resources).

Author contributions

JB and MM planned the experiments. MM performed the work and analysis and prepared the first draft of the manuscript. JB prepared final version of the manuscript.

Competing interests

The author(s) declare no competing interests.

Grant information

This work was supported by the Wellcome Trust [095598].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank Maria Rodriguez-Lopez for designing the primers for H1 box deletion, for the fruitful discussion and support. We thank Stjohn Townsend for help, advice and discussion regarding data normalisation, and Mimosa Hoti for help with handling of the libraries.

References

- Lunt SY, Vander Heiden MG: **Aerobic glycolysis: meeting the metabolic requirements of cell proliferation.** *Annu Rev Cell Dev Biol.* 2011; **27**: 441–464. [PubMed Abstract](#) | [Publisher Full Text](#)
- Merz S, Westermann B: **Genome-wide deletion mutant analysis reveals genes required for respiratory growth, mitochondrial genome maintenance and mitochondrial protein synthesis in *Saccharomyces cerevisiae*.** *Genome Biol.* 2009; **10**(9): R95. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Steinmetz LM, Scharfe C, Deutschbauer AM, *et al.*: **Systematic screen for human disease genes in yeast.** *Nat Genet.* 2002; **31**(4): 400–404. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hoffman CS, Wood V, Fantes PA: **An Ancient Yeast for Young Geneticists: A Primer on the *Schizosaccharomyces pombe* Model System.** *Genetics.* 2015; **201**(2): 403–423. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Malecki M, Bitton DA, Rodriguez-Lopez M, *et al.*: **Functional and Regulatory Profiling of Energy Metabolism in Fission Yeast.** *Preprints.* 2016: 2016100054. [Publisher Full Text](#)
- Kim DU, Hayles J, Kim D, *et al.*: **Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*.** *Nat Biotechnol.* 2010; **28**(6): 617–623. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hentges P, Van Driessche B, Tafforeau L, *et al.*: **Three novel antibiotic marker cassettes for gene disruption and marker switching in *Schizosaccharomyces pombe*.** *Yeast.* 2005; **22**(13): 1013–1019. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wagih O, Parts L: **gitter: a robust and accurate method for quantification of colony sizes from plate images.** *G3 (Bethesda).* 2014; **4**(3): 547–552. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bitton DA, Schubert F, Dey S, *et al.*: **AnGeLi: A Tool for the Analysis of Gene Lists from Fission Yeast.** *Front Genet.* 2015; **6**: 330. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wood V, Harris MA, McDowall MD, *et al.*: **PomBase: a comprehensive online resource for fission yeast.** *Nucleic Acids Res.* 2012; **40**(Database issue): D695–699. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Harris MA, Lock A, Bahler J, *et al.*: **FYPO: the fission yeast phenotype ontology.** *Bioinformatics.* 2013; **29**(13): 1671–1678. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chiron S, Gaisne M, Guillou E, *et al.*: **Studying mitochondria in an attractive model: *Schizosaccharomyces pombe*.** *Methods Mol Biol.* 2007; **372**: 91–105. [PubMed Abstract](#) | [Publisher Full Text](#)
- Malecki Michal: **"Respiratory Mutants S.pombe."** *Open Science Framework.* 2016. [Data Source](#)

Open Peer Review

Current Referee Status:    

Version 1

Referee Report 28 November 2016

doi:[10.21956/wellcomeopenres.10768.r17616](https://doi.org/10.21956/wellcomeopenres.10768.r17616)



Makoto Kawamukai

Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, Matsue, Japan

The work done by Malecki and Baehler is the screening of the *S. pombe* mutants that did not grow well on the yeast extract medium containing mainly glycerol comparing with glucose. They used auxotrophic and prototrophic mutants and repeated experiments twice. Statistical analysis was conducted. The data presented here is sound and is useful. A couple things I am concerned.

1. Variation of two independent experiments looks high. It is partly because they judged by only plate works. To be truly respiration defective, the authors are better to measure oxygen consumption of the mutants. That is the definition of respiration deficiency.
2. Did the authors find out the mutants that are required for glycerol metabolism but not relevant to respiration? Did the authors omit these mutants from the list of respiration defective mutants. I could not reach the list of the mutants the authors selected.
3. We are also using a deletion mutant library of Bioneer Corp. It still contains the strains that are not deleted properly or apparently possess extragenic unknown mutation(s). Several must-be-respiration defective mutants such as coenzyme Q less mutants we are studying¹ are not included in the Bioneer library. Without re-checking by own-self, it still needs to be careful for interpretation using this library.

References

1. Hayashi K, Ogiyama Y, Yokomi K, Nakagawa T, Kaino T, Kawamukai M: Functional conservation of coenzyme Q biosynthetic genes among yeasts, plants, and humans. *PLoS One*. 2014; **9** (6): e99038 [PubMed Abstract](#) | [Publisher Full Text](#)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Reader Comment 05 Jan 2017

Michal Malecki, University College London, UK

We thank the reviewer for the helpful and constructive review. Our response to the issues raised is given below.

The issue of variation between experiments has been addressed in detail in our answer to Christopher Herbert's comments.

Regarding the second comment, we did not filter out the mutants responsible for glycerol metabolism but not for respiration. All the lists described in the text are available in the Open Research Framework depository as a Supplementary Table. We will soon also provide an integrated data set, combining the results of the screen described in this report and results from previous screen (Fig 1F, data from Malecki et al. 2016). This integrated dataset will be more useful than the data from the single screens, as detailed in our response to Christopher Herbert.

Regarding the last comment, we are aware that some of the library mutants are miss annotated (although this is minimal; in the latest version of the Bioneer library). Moreover, some phenotypes may also originate from deletion of regulatory sequences of neighbouring genes. As stressed by the reviewer, it is important to check any phenotypes of interest obtained by high-throughput studies, preferably by preparing independent deletion mutants. Our screen delivers a conservative list of genes that are involved with high probability in respiratory metabolism. This resource can be used for deeper follow-up studies, but any mutant of interest should be independently verified first.

Reference:

Malecki M, Bitton D, Rodríguez-López M, Rallis C, Calavia NG, Smith GC, Bähler J; Functional and regulatory profiling of energy metabolism in fission yeast. *Genome Biol.* 2016 Nov 25;17(1):240

Competing Interests: No competing interests were disclosed.

Referee Report 25 November 2016

doi:[10.21956/wellcomeopenres.10768.r17842](https://doi.org/10.21956/wellcomeopenres.10768.r17842)



Jim Karagiannis

Department of Biology, University of Western Ontario, London, ON, Canada

The authors describe a genome-wide screen for fission yeast genes required for respiratory growth. While the study is similar to a previously reported screen by the same group¹ it differs in several important aspects:

1. A more up-to-date version of the gene deletion set is used.
2. An improved method for generating a prototrophic gene deletion library is employed.
3. Colonies are screened at lower densities allowing for more accurate measurements.

Taken together, these changes both improve the quality of the data set and provide novel information.

The methods used for screening and data analysis are rigorous and clearly described. All data have been deposited to the Open Science Foundation website and are freely available. The quality of the data set is supported by the fact that the identified genes are indeed enriched for those encoding mitochondrially

localised proteins (as one would expect in a screen for respiratory deficient mutants). It should also be noted that many of the fission yeast hits in the screen have not been identified in budding yeast. I thus feel that the data represent an important, novel, and useful foundational resource for those with an interest in energy metabolism.

The data also provide useful information with respect to various technical aspects of performing genome-wide screens. First, the authors - perhaps not surprisingly - show that the results of the screen are affected by the presence/absence of auxotrophic markers. Second, the study reveals large variability between biological repeats. While these results are somewhat troubling - in the sense that they make interpretation of this and other genome-wide screens more difficult - it is nevertheless critically important that researchers be made aware of:

1. Issues related to the confounding affects of auxotrophic markers.
2. The importance of performing multiple biological repeats when conducting genome-wide screens.

References

1. Malecki M, Bitton D, Rodriguez-Lopez M, Rallis C, Garcia Cavalia N, Smith G, Bahler J: Functional and Regulatory Profiling of Energy Metabolism in Fission Yeast. *Preprints*. 2016.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Reader Comment 05 Jan 2017

Michal Malecki, University College London, UK

We thank the referee for his positive comments about our work.

Competing Interests: No competing interests were disclosed.

Referee Report 24 November 2016

doi:[10.21956/wellcomeopenres.10768.r17944](https://doi.org/10.21956/wellcomeopenres.10768.r17944)



Luis A. Rokeach

Department of Biochemistry and Molecular Medicine, Université de Montréal, Montreal, QC, Canada

The title and abstract are appropriate.

Article content: the results and design are adequately explained.

The results presented by Malecki and Bähler constitute an interesting and needed contribution to the field energy metabolism. The techniques developed in this paper will be useful to other researchers wishing to carry out similar gene screenings.

The data significantly broaden the knowledge acquired in a previous study. The wide variation in the

biological datasets obtained with different repeats is intriguing. Some other potential explanations to these observations would have been useful. Certainly, these variations require further experimental scrutiny.

It would have been very convenient that the genes identified in these screens be presented by their name (instead of number) on a lists appended to the article. As presented now, the data are less useful to the reader.

As yeast mutants affected in respiration show also aging phenotypes, in future efforts it would be very interesting to investigate how the mutation identified would impact chronological life span.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Reader Comment 05 Jan 2017

Michal Malecki, University College London, UK

We thank the reviewer for the helpful, constructive review. Our response to the issues raised is given below.

We agree that the discrepancy between biological repeats and between different screens is surprising. Some more explanations and views of this phenomenon are provided in our answer to Christopher Herbert's comments. Answering referee request we will introduce gene names into Supplementary Table 1.

The suggestion to check the ageing phenotypes of identified mutants is a good one. This is on our list of future experiments and should be interesting, especially in the light of the fact that some mutants affecting respiration seem to dramatically shorten lifespan, while others actually increase the lifespan (1).

1. Rallis C, López-Maury L, Georgescu T, Pancaldi V, Bähler J; Systematic screen for mutants resistant to TORC1 inhibition in fission yeast reveals genes involved in cellular ageing and growth. *Biology Open* 2014 Feb 15;3(2):161-71

Competing Interests: No competing interests were disclosed.

Referee Report 21 November 2016

doi:[10.21956/wellcomeopenres.10768.r17607](https://doi.org/10.21956/wellcomeopenres.10768.r17607)



Christopher Herbert

Institute of Integrative Biology of the Cell, CEA, CNRS, University Paris-Sud, University Paris-Saclay, Gif-sur-Yvette Cedex, France

The data note by Malecki and Bähler describes a screen to identify genes required for respiration in *S. pombe*. It differs from their previous study, in that an improved and more complete version of the

deletion library was used, the method for transferring the deletions into a prototrophic background has been improved, colony sizes were determined at a much lower density and galactose medium was no longer used. Taken together, these changes should give a simpler and more robust dataset.

The methods used to produce and analyze the dataset are clearly and succinctly explained and the treated and raw data are available in the Supplementary Tables.

The authors find that, as they have previously shown, that approximately twice as many genes are identified in the auxotroph compared to the prototroph screen. This is no surprise, auxotrophic background is known to modify phenotypes, in particular *ura4* mutations often exacerbate respiratory phenotypes. Indeed, it could be argued that in future, resources would be better used if more biological repetitions were performed to produce a more robust dataset, rather than comparing auxotrophs with prototrophs.

To my mind the most surprising, and perhaps worrying, result is the large differences seen between the biological repeats (Figure 1D) for both the prototroph and auxotroph strains. This is surprising as the “biological repeat” was essentially taking the strains out of the freezer, and they underwent two rounds of glucose growth before being tested for respiratory growth. This is an observation that requires further investigation; yeast biology would not be where it is today if strains had a 50% chance of changing their phenotype every time they came out of the freezer.

The analysis in Figure 1D-F is interesting, I realize that this is a data note, not an article, but I have not been able to find the corresponding Tables it in the supplementary data. I recommend that the data Tables corresponding to Figure 1D-F be included in the supplementary data, and all the Tables should include standard gene names not just gene IDs, for most people interested in respiratory functions in *S. pombe* Cbp3 means something, SPCC4B3.17 does not.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Reader Comment 05 Jan 2017

Michal Malecki, University College London, UK

We thank the reviewer for the helpful and constructive review. Our response to the issues raised is given below.

As requested, we have now introduced standard gene names in Supplementary Table 1. Most of the categories mentioned in Figure 1D-F are indicated in Supplementary Table 1, and genes from this categories can be filter out using indications.

About the differences between biological repeats. We have provided possible explanations for these differences in the Conclusion. These differences are quite striking, and our screens seem not to be saturated. Together with the results from the previous screen, it is also evident that some mutants previously described as respiratory deficient exhibit such a phenotype only in one or the other of the two distinct screens. Although this discrepancy between screens obtained using different versions of the deletion collection might reflect annotation mistakes, this cannot be the reason if differences are observed between biological repeats of screens using the same deletion

collection. As mentioned in the paper, the respiratory phenotype appears to show particularly high plasticity. Another possible reason is that different amounts of biomass for given mutant were printed in different biological repeats. It is known that the amount of starting biomass placed on solid media with stressor will impact growth. Although the phenotyping method is largely automated, minimizing differences in biomass transferred between plates, the biomass amount will depend on initial colony size and state (for example age). Waking up a deletion collection on agar plates leads to some differences in colony sizes which does not only depend on genetic background. Genome-wide screens are by necessity somewhat noisy, producing both false positives and negatives, and our data highlight this feature and that the integration of multiple screens can provide rich complementary insights.

We point out, however, that both screens and the biological repeats in the second screen produced reliable and biologically coherent results, as indicated by enrichment of genes encoding mitochondrial proteins. These insights were supported by a conservative analysis of the data.

It is evident, given that the screen reported here and a complementary previous screen (Malecki et al. 2016) are not saturated and apply distinct methodology, integration of the screen results provides insights greatly exceeding those from the single screens. We will soon provide an updated version of the Data Note to highlight such joint results, and provide the data on our website and the Open Science Framework depository.

Reference:

Malecki M, Bitton D, Rodríguez-López M, Rallis C, Calavia NG, Smith GC, Bähler J; Functional and regulatory profiling of energy metabolism in fission yeast. *Genome Biol.* 17:240 (2016)

Competing Interests: No competing interests were disclosed.
