# Impact of histone-modifying factors and carbon sources on chromatin accessibility in *S. cerevisiae*

Gabriel O. Amador*, Alyssa M. Benjamin†, Danny Cotter†, Jason K.W. Cheng†, Pagé C. Goddard†, Emily Greenwald†, Kathryn J. Hanson†, Mervenaz Koska*, Rosa Ma†, Dylan G. Maghini†, Keri J. Ngo*, Roshni Patel†, Hannah D. Rosenblatt*, Jarod E. Rutledge†, Kelly A. Tomins†, Rachel A. Ungar†, Yiu-Cheung E. Wong*, Georgi Marinov†, Amr Mohamed†, Anna Shcherbina†, Avanti Shrikumar†, Laksshman Sundaram†, Anshul Kundaje†, and William J. Greenleaf†

†Stanford University, Department of Genetics *Stanford University, Department of Developmental Biology

## INTRODUCTION


**Figure 1**. Strain and media conditions tested. YPD: yeast extract peptone-dextrose, YPGE: yeast extract glycerol-ethanol.

Gene expression is affected by a myriad of genetic and environmental factors. Chromatin accessibility influences the ability of transcription factors and transcriptional machinery to bind DNA.

Chromatin accessibility is partially regulated by histone-modifying proteins, such as histone chaperone ASF1 and acetyltransferase RTT109, which are involved in chromatin assembly and disassembly[1,2]. Additionally, environmental factors such as the availability of various carbon sources impact metabolic processes[3] and, in turn, global gene expression.

## OBJECTIVES

Evaluate how chromatin accessibility in *S. cerevisiae* is impacted by:

- media culture conditions
- deletion of chromatin-modifying factors, RTT109 and ASF1

with assay for transposable-accessible chromatin using sequencing (ATAC-seq)[4].

## EXPERIMENTAL DESIGN

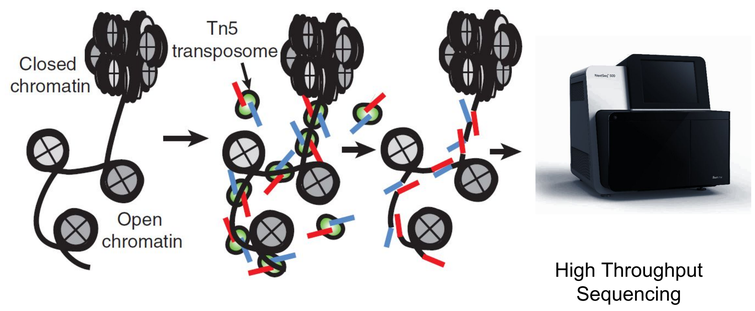



**Figure 2**. Schematic for transposase mediated adapter ligation in ATAC-seq (modified from Buenrostro et al. (2013)).

- Three strains of *S. cerevisiae* (wild-type, *Δrtt109,* and *Δasf1*) were grown in either glucose media (YPD) or glycerol/ethanol media (YPGE)
- Experimental design was optimized by distributing strain and media conditions amongst researchers, but some confounding factors were allowed to occur so that we could explore batch effect analysis
- ATAC-seq libraries were prepared according to the protocol established by Buenrostro et al. 2013.
- Libraries were quantified using Qubit and Screentape and standardized before paired-end sequencing on NextSeq
- QC and expression analysis was performed as depicted in **Fig. 3**.

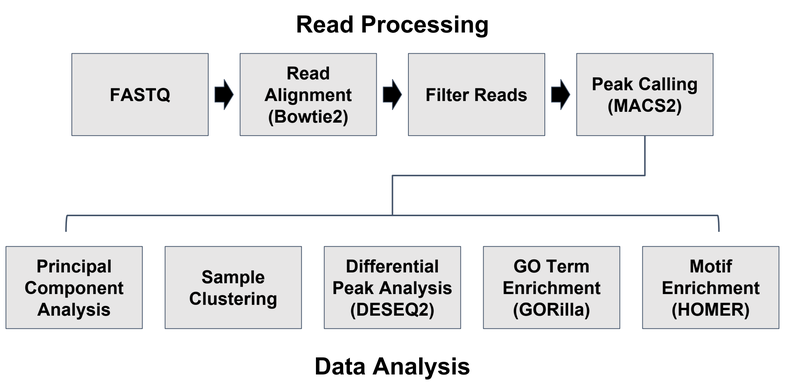



**Figure 3**. Schematic of data analysis pipeline.

## FRAGMENT LENGTH DISTRIBUTION

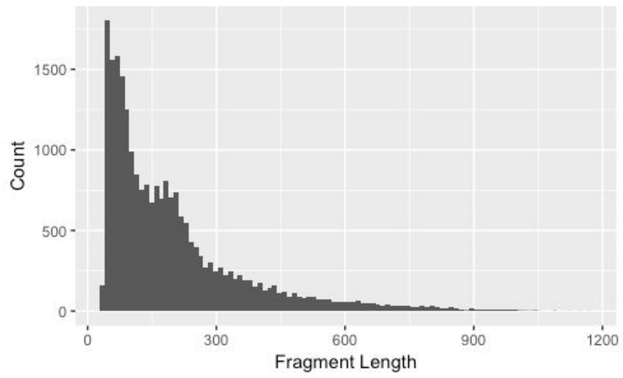



**Figure 4.** Distribution of fragment length aggregated between all samples. Median fragment length was 150 bp with a minimum length of 40 bp and maximum length of 1175 bp.

## PRINCIPAL COMPONENT ANALYSIS

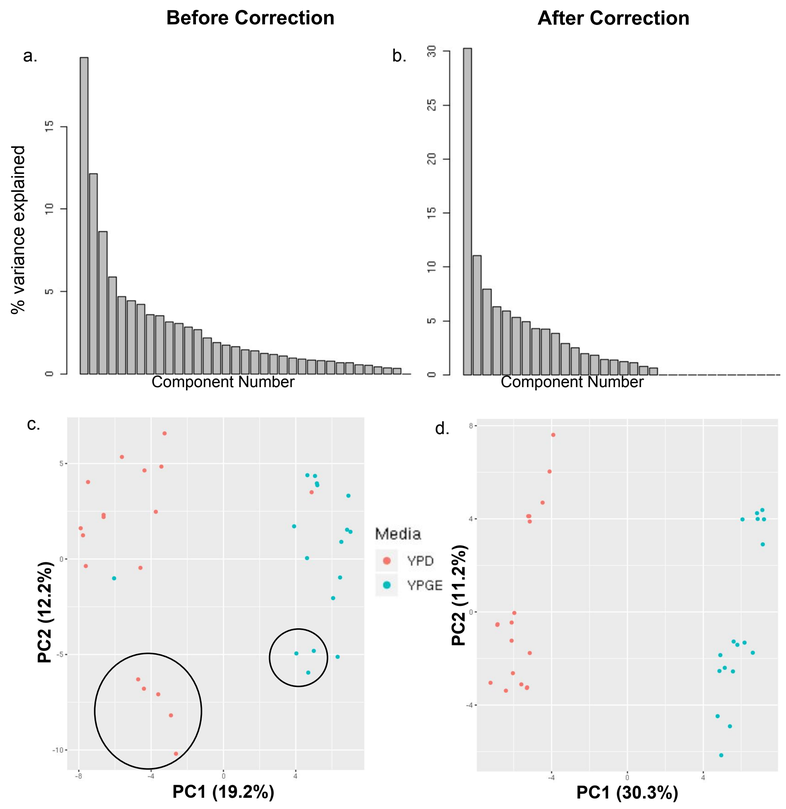



**Figure 5.** Skree plots (a,b) and PCA plots (c,d) demonstrating percentage of variance explained by each principal component before (a,c) and after (b,d) correcting for batch effects (circled) and a sample swap.

Initial principal component analysis (PCA) revealed both batch effects and a sample swap. As shown in the original PCA plot (Fig. 5), the circled samples run by a single experimenter are systematically different. The effect of the experimenter was removed through using a linear mixed effects model with the media, strain, and experimenter being the explanatory effects. After subtracting out the contribution from the experimenter, the residual values were used for further data analysis. By removing the batch effect by experimenter and re-labeling the swapped samples, the first principal component explained 30.3% of the variation of the data.

## DIFFERENTIAL PEAK ANALYSIS

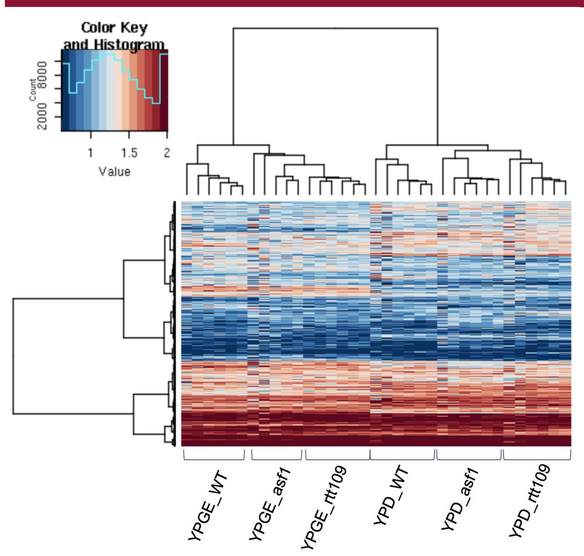
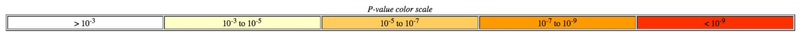



**Figure 6.** Heat map demonstrating differential peaks between experimental groups. Clustering analysis demonstrated distinct peak patterns between medium and strains

## GO ENRICHMENT




GO term enrichment analysis was performed for the genes nearest the peaks identified by ATAC-Seq. Relative to the YPD-cultured control group, YPGE-treated groups were enriched for GO terms relating to thiamine synthesis and metabolism. Thiamine, and its phosphate derivatives, are necessary for the first step of alcoholic fermentation in yeast[5]. Additionally, compared to the two mutant strains, the WT has upregulation in genes relating to cellular response to abiotic stimulus.

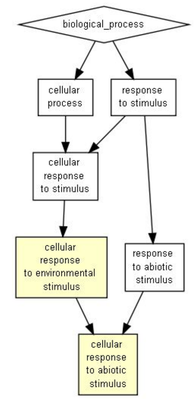



**Figure 7:** GO term enrichment for biological processes.

## HOMER Motif Analysis

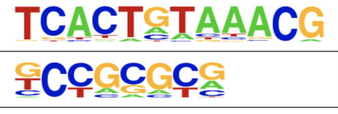
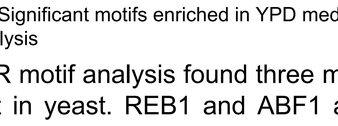
| Rank | Motif | P-value | Best Match/Details |
|---|---|---|---|
| 1 |  | 1e-32 | REB1/MA0363.1/Jaspar(0.948) More Information \| Similar Motifs Found |
| 2 |  | 1e-16 | hb/dmmpmm(Noyes)/fly(0.848) More Information \| Similar Motifs Found |
| 3 |  | 1e-14 | ABF1/ABF1_YPD/62-ABF1(Harbison)/Yeast(0.869) More Information \| Similar Motifs Found |
| 4 |  | 1e-14 | SUT1/MA0399.1/Jaspar(0.764) More Information \| Similar Motifs Found |
| 5 |  | 1e-13 | sd/dmmpmm(Bergman)/fly(0.725) More Information \| Similar Motifs Found |

**Table 1.** Significant motifs enriched in YPD media versus YPGE media from HOMER motif analysis

HOMER motif analysis found three motifs enriched in YPD samples present in yeast. REB1 and ABF1 are known to be DNA binding proteins involved in transcriptional activation, and SUT1 is a transcription factor.

## V-Plot

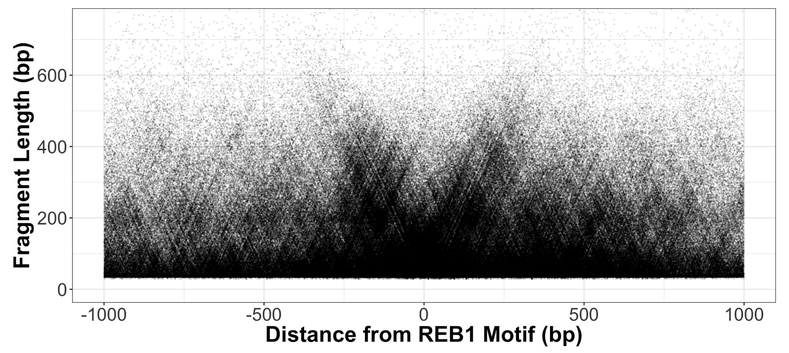



**Figure 8.** V-plot demonstrating transcription factor binding enrichment at the REB1 motif in YPD-cultured yeast relative to YPGE-cultured yeast.

To validate our HOMER motif analysis, we more closely examined the top hits by looking for characteristic “V” fingerprints left behind by transcription factors (using a position-aware 2D fragment length distribution). We looked at REB1, since it was the highest enriched motif in YPD samples (Table 1).

## FUTURE DIRECTIONS

In order to further elucidate the role of histone-modifying factors, more in-depth gene expression analyses will be performed to compare the conditions of fermentable versus non-fermentable media. We will investigate the specific protein-protein or protein-chromatin interactions of the genes that were differentially expressed in this study. Deletion of other histone-modifying genes can also help us further understand chromatin accessibility and transcriptional regulation in yeast.

## REFERENCES

[1] Le, S., Davis, C., Konopka, J.B., and Sternglanz, R. (1997). Two new S-phase specific genes from *Saccharomyces cerevisiae*. *Yeast,* **13**(11): 1029-1042. doi: 10.1002/(SICI)1097-0061(19970915)13:11<1029::AID-YEA160>3.0.CO;2-1

[2] Schneider, J., Bajwa, P., Johnson, F.C., Bhaumik, S.R., and Shilatifard, A. (2006). Rtt109 is required for proper H3K56 acetylation. *Journal of Biological Chemisstry,* 281: 37270-37274. doi: 10.1074/jbc.C600265200

[3] Jiang, F., Rizavi, H.S., and Greenberg, M.L. (1997). Cardiolipin is not essential for the growth of *Saccharomyces cerevisiae* on fermentable or non-fermentable carbon sources. *Molecular Biology,* **26**(3): 481-491. PMID: 9402019

[4] Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature Methods*, **10**(12): 1213–8. http://doi.org/10.1038/nmeth.2688

[5] Marobbio CMT, Vozza A, Harding M, Bisaccia F, Palmieri F, Walker JE. Identification and reconstitution of the yeast mitochondrial transporter for thiamine pyrophosphate. The EMBO Journal. 2002;21(21):5653-5661. doi:10.1093/emboj/cdf583.