

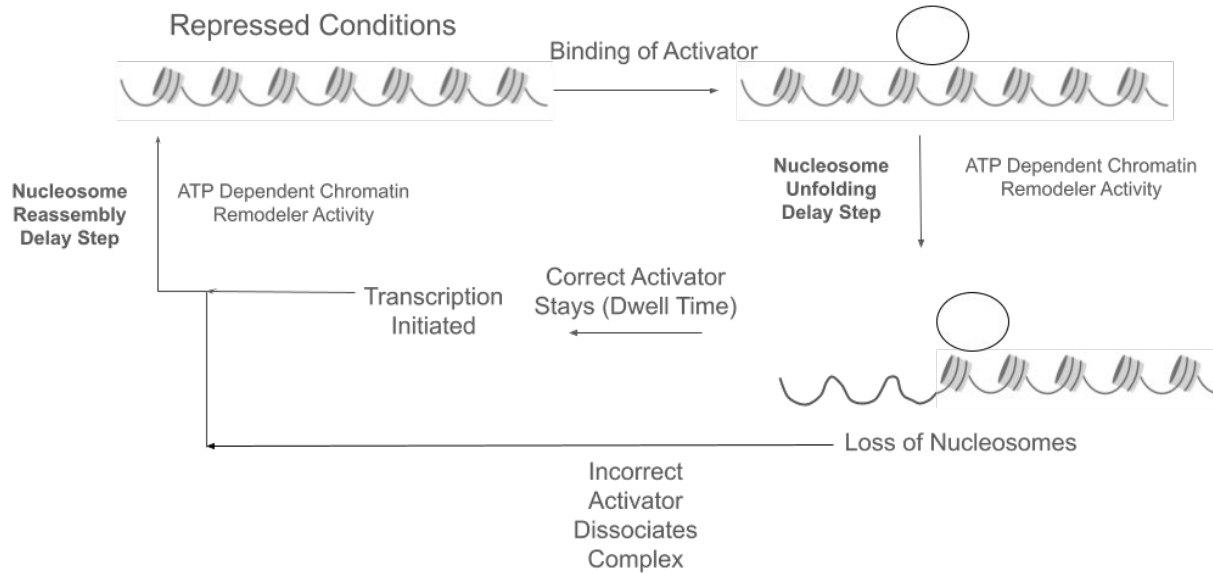
# The dynamics of chromatin structure in the context of kinetic proofreading

How might cells enhance transcriptional specificity?

By Nathaniel Wolff

In conjunction with H.Boeger, Ph.D and Brett Meisner

# Potential Kinetic Proofreading of Transcriptional Activation



- Proposed Kinetic Proofreading Step involves subsequent disassembly and reassembly of nucleosomes
- Reassembly might occur in 2 steps like NCP assembly

->Would result in dynamic chromatin structure

How do we determine **if** chromatin structure becomes dynamic during transcription? Verify **how** it becomes dynamic?

# Main Implications to test

Q1: Does the activated distribution of nucleosome DNA lengths (bubble sizes) tend to be smaller when transcriptionally active?

May tell us whether or not the chromatin is becoming dynamic in the first place.

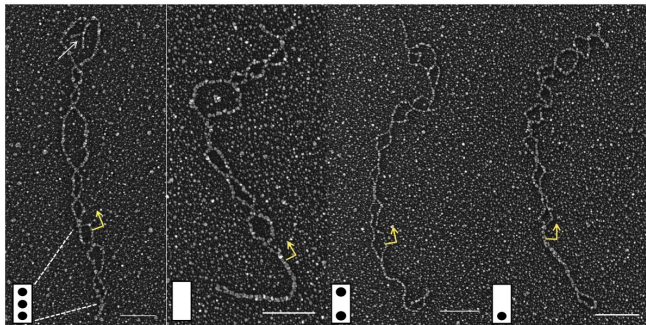
-> Assess whether repressed distribution is stochastically greater than activated using Mann-Whitney U Test

Q2: Do the modalities of the distributions differ?

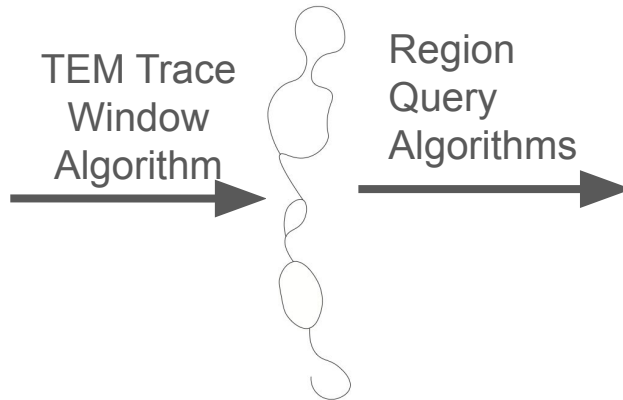
Verifies the mechanism of Kinetic Proofreading, 2 step nucleosome reassembly following disassembly

-> Statistical test to assess modality of both distributions, Ashman's D on fitted Gaussian Mixture Models

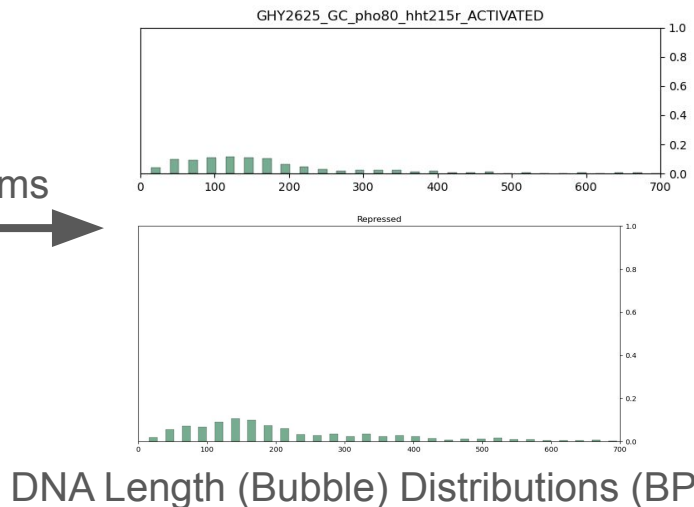
# Approach: TEM Trace Repo ([GeneRing](#))



TE Micrograph of Chromatin Fibers



Chromatin Traces

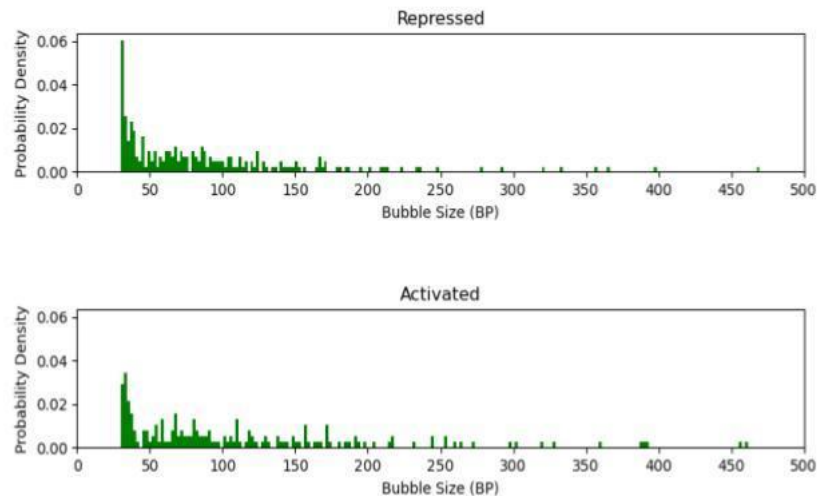
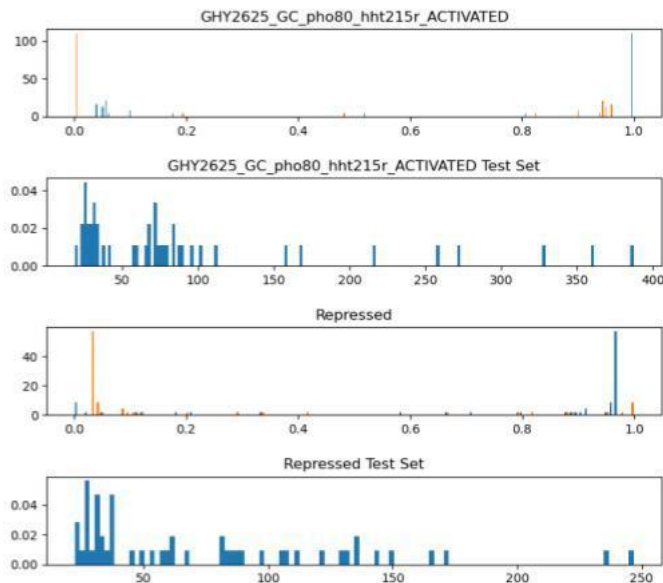


DNA Length (Bubble) Distributions (BP)

Overarching Goal: Write region query algorithms/scripts to:

1. Determine the distributions of nucleosomal DNA lengths (bubble sizes) over PHO5 promote
2. Fit bimodal Gaussian Mixture Models (SciKit learn) to distributions and compare the Ashman's D of each
3. Parse distributions into format compatible with Mann-Whitney U Test and evaluate

# Results: Bubble Size Distributions and GMM fits



Left - Gaussian Mixture Models fit to Repressed and Active distributions

Right - Figure 5: Bubble Size Distributions for Strain 3 (Transcriptionally Activated) and Strain 1 (Repressed)

# Results: Statistical Results

|                                       |   |
|---------------------------------------|---|
| Mann Whitney U                        | Repressed and Active: 0.06 (Not Significant*)<br><br>*Alpha value of .05              |
| Ashman's D of Gaussian Mixture Models | Repressed: 1.0 (Non-Bimodal*)<br>Active: 1.3 (Non-Bimodal*)<br><br>*Cutoff of $D > 2$ |

# Conclusions

## **Mann Whitney U:**

We cannot conclude that either repressed distributions is stochastically greater than the activated distribution

->No solid evidence in favor of existence of proposed kinetic proofreading step at all

## **Ashman's D:**

We cannot conclude that the distributions have different modalities or that either is bimodal

->Distributions might be the same modality (appears so by inspection)

->Likely unimodal, or “sawtooth” shape

->Kinetic proofreading step might occur by a less intuitive mechanism than a 2 step reassembly + disassembly

# Overall discussion

We have not found evidence in favor of our proposed kinetic proofreading step

->The mechanism might not be as intuitive as we expected

-> The closeness of the MWU P value indicates that the data might just be too small to assess stochastic difference

->Still, interrogating nucleosome occupancies with this dataset could help us verify that unfolding of nucleosomes is occurring, with a potentially more significant p value



# Sources and Acknowledgements

1. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD. Nucleosomes unfold completely at a transcriptionally active promoter. *Mol Cell*. 2003 Jun;11(6):1587-98. doi: 10.1016/s1097-2765(03)00231-4. PMID: 12820971.
2. Shelansky. Robert. GeneRing, (2015), GitHub repository, <https://github.com/rshelans/GeneRing>
3. *Nucleosome Assembly during DNA Replication*, [www.cell.com/current-biology/pdf/S0960-9822\(95\)00245-4.pdf](http://www.cell.com/current-biology/pdf/S0960-9822(95)00245-4.pdf). Accessed 11 Mar. 2024.
4. Gnedin, Oleg Y. “Quantifying Bimodality.” University of Michigan, 19 Feb. 2010.
5. Brown CR, Mao C, Falkovskaia E, Jurica MS, Boeger H (2013) Linking Stochastic Fluctuations in Chromatin Structure and Gene Expression. *PLoS Biol* 11(8): e1001621. <https://doi.org/10.1371/journal.pbio.1001621>
6. Roger D. Kornberg, Chromatin Structure: A Repeating Unit of Histones and DNA. *Science* 184, 868-871(1974). DOI:10.1126/science.184.4139.868
7. Carter-Orsquo;Connell, Ian, et al. “Genome-Wide Characterization of the Phosphate Starvation Response in Schizosaccharomyces Pombe - BMC Genomics.” *BioMed Central*, BioMed Central, 12 Dec. 2012, [bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-13-697](http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-13-697)

Many thanks to Ben Ticknor, my housemate, rising Ph.D in Statistics, for his help

Questions?