Analysis of Micrococcal Nuclease Digest Bias Relative to Chromatin Profiling Assays in Maize and Human

Zehta Glover[1,2], Ayush Singh[1], Jane Benoit[1], Hank W Bass[1]

(1) Department of Biological Science, Florida State University, Tallahassee, FL, USA

(2) IDEA Grant Recipient, Florida State University, Tallahassee, FL, USA

Background

Understanding gene regulation remains a major goal in biology. In order to understand gene regulation, it requires knowledge of chromatin structure and the occupancy of transcription factors on chromatin. (reviewed by Tsompana and Buck, 2014).

In the Bass lab at FSU we have been adapting the use of micrococcal nuclease (MNase, E.C. 3.1.31.1) as a biochemical probe of fixed chromatin structure (Cole and Dennis, 2020, Parvathaneni et al., 2020, Rodgers-Melnick et al., 2016, Turpin et al., 2018, Vera et al., 2014, Wheeler et al., 2020). MNase is referred to as a relatively non-specific endo-exonuclease, but early reports indicate an AT-rich preference (Horz and Altneburger, 1981; Dingwall et al., 1981). MNase bias remains a critique of MNase-based assays although it has been reported to be negligible in mapping nucleosomes (Allan et al., 2012). However, for analysis of small DNA particles in nucleosome-free DNA, this bias has not been carefully characterized.

My research aims to define MNase sequence bias on maize and human genomic data. This will be achieved through the following aims:

AIM 1 - Produce and sequence NGS libraries of small fragments from light partial digests of total DNA from human and maize
AIM 2 - Develop gain of footprint assay using YY1 DNA-binding protein
AIM 3 - Characterize MNase digestion patterns through bioinformatic analysis

Methods

In order to achieve AIM 1, human DNA will be obtained through collaboration with the J.H. Dennis lab (Biological Science Department, FSU). The DNA will remain frozen until it is time for MNase digestion. For maize, I will isolate maize total DNA from frozen earshoot tissue using the Qiagen Plant Maxi DNA kit (Cat no. 68163), following manufacturer's instructions as follows. Once total naked DNA is collected (for maize or human) MNase digestion will be carried out. In order to stimulate a partial digest similar to chromatin digests, I will carry out the protocol described as MOA-seq by Savadel et al., (2021) using lower MNase concentrations. After MNase titration, I will make libraries, sequence, and align them to the genome following the methods and procedures for MOA-seq (Savadel et al., 2021).

For the next aim, I will attempt a novel gain of footprint assay (GOF). I will carry out the same MNase digestion, but in the presence of DNA binding proteins, to recover fragmented DNA and map the genomic libraries. For this experiment, I will use the YY1 DNA binding protein for both human and maize DNA.

For my third and final Aim, I will characterize the MNase digestion patterns of naked human and maize DNA both with and without the addition of the YY1 binding protein. This entails a comparison of the control Naked DNA to past MOA-seq projects, as well as, a base composition analysis of the fragments of MNase cut DNA.

Name	Digest	Total DNA	DNA	Read
ZSG01	rep1	~ 1 µg	maize	80M
ZSG02	rep2	~ 1 µg	maize	80M
ZSG03	rep3	~ 1 µg	maize	80M
ZSG04	rep4	~ 1 µg	maize	80M
ZSG06	rep1	~ 1 µg	human	80M
ZSG07	rep2	~ 1 µg	human	80M
ZSG08	rep3	~ 1 µg	human	80M
ZSG09	rep4	~ 1 µg	human	80M

Figure 1. Table of pooled Bioreplicates for Library sequencing. ZSG01 – ZSG04 are partially digested Maize DNA. ZSG06 – ZSG10 are partially digested Human DNA.

MNase Digestion of Maize and Human DNA

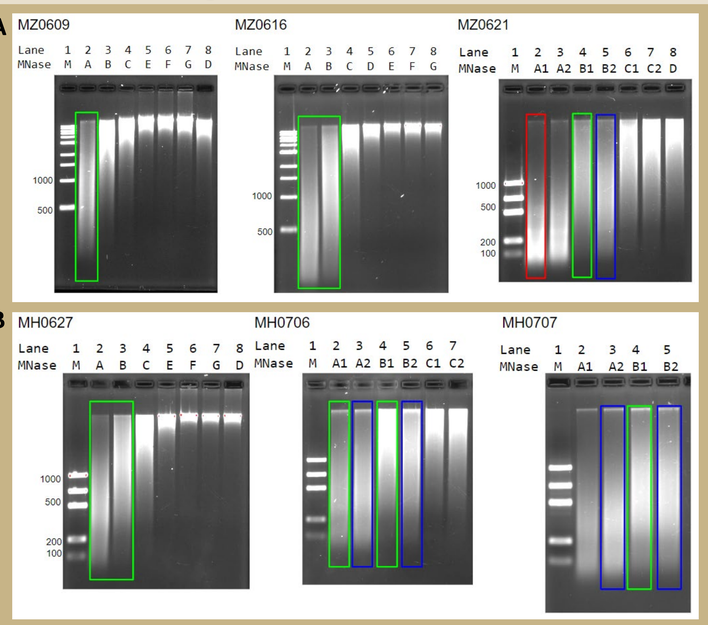

Figure 2. DNA samples subjected to MNase at a series of concentrations (Lane A is highest concentration, Lane 3 is the lowest concentration) from a dilution series. Partial digests similar to MOA-seq were selected (boxed lanes) for DNA library preparation to sequence the small fragments. Panel A is digested Maize DNA and Panel B is digested Human DNA.

Analysis: Control DNA vs MOA-seq

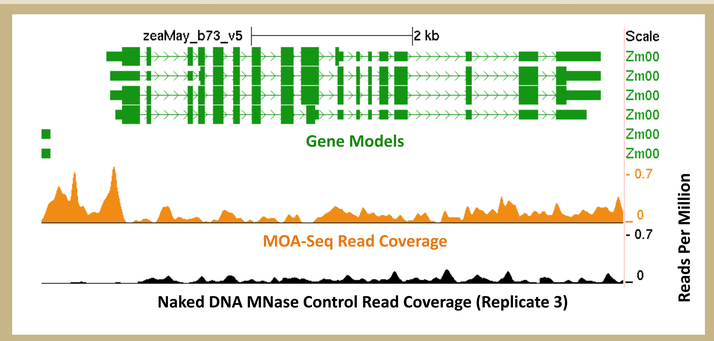

Figure 3. Control Read Coverage Compared to MOA-seq profiling. Genome Browser screenshot showing data around one gene comparing MOA (orange, from Savadel et al., 2021) to replicate 3 control (Black, my data unpublished).

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Analysis: Base Composition around Left Side of DNA Fragments

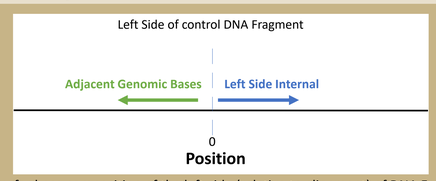

Figure 4. Key for base composition of the left side (relative to alignment) of DNA Fragments.

Analysis: Base Composition around Maize and Human DNA Fragments

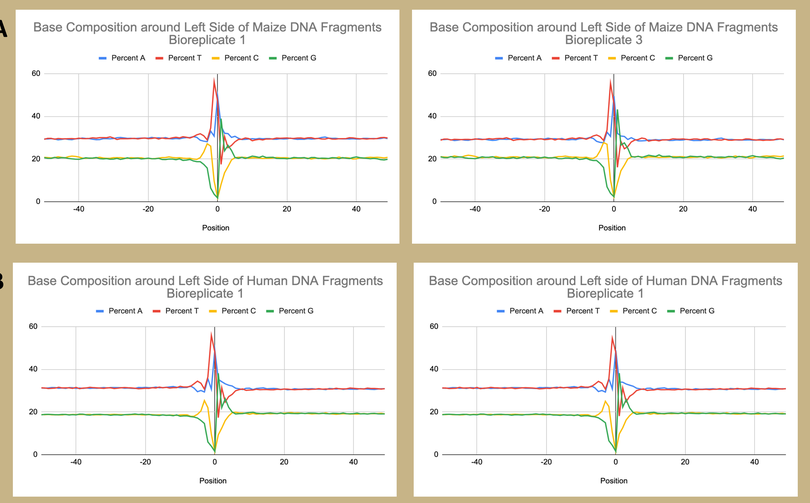

Figure 5. Base composition around the Left side (relative to alignment) of DNA Fragments. (A) Bio-replicates 1 and 3 of partially digested maize DNA. (B) Bio-replicates 1 and 3 of partially Digested human DNA. Position 0 marks the first base of the left side of the DNA fragment.

Summary

Early Analysis reveals possible MNase AT-bias during digestion.

- By mapping the centers of the MNase digested DNA fragments and comparing them to MOA-seq data some false positive peaks are observed.
- The left flanks of the MNase digested DNA displays peak of AT-richness at the left cut.
- The extent of the bias and how it may affect data will be further investigated.

Future Directions

- I will proceed with a novel gain of footprint assay by pre-incubating the isolated DNA with a protein (YY1) and then carrying out the MNase digestion.
- YY1 has a known DNA binding sequence,and should define a true DNA-protein footprint.

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