Identifying EOR-1 Binding Sites and Accessible Regions in Relation to NHR-25 in *C. elegans*

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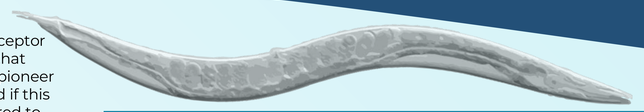
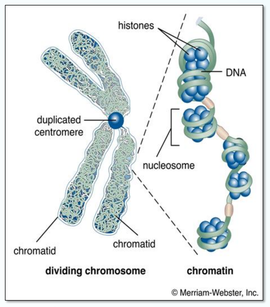
Abstract

Transcription factors (TFs) are proteins that bind to DNA sequences and regulate gene expression. NHR-25 is a TF in *C. elegans* of the nuclear hormone receptor family that is highly conserved across species. EOR-1 is another TF encoded by GAGA motif. Previous studies showed that GAGA motif is enriched at sites that change chromatin accessibility in development and serves as pioneer-like factor in *Drosophila*. These suggested that EOR-1 may have pioneer activity. A pioneer factor (PF) works via binding to condensed chromatin regions, rearranging nucleosomes, and opening free regions. To determine if EOR-1 acts as a PF and if this activity is essential for NHR-25 binding, we performed Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) in *eor-1* mutants compared to wild type. Initial results identified regions of accessibility in the two conditions. To compare changes in accessibility to EOR-1 binding we will perform Cleavage Under Target and Release Under Nuclease (CUT&RUN) on EOR-1 and on NHR-25 in an *eor-1* mutant. We expect to see similar GAGA motif in sequences where EOR-1 binds and the differential NHR-25 association when *eor-1* is mutated if *eor-1* is necessary for NHR-25 biding. This study will give us insight into the relationship between the two proteins to increase the understanding of regulatory roles between TFs that is essential to organismal development.

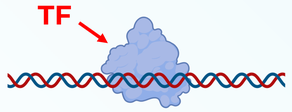


Background



Chromosomes contain **chromatin**, composed of **nucleosomes** that packages the **DNA double helix** carrying genetic information.

Transcription factors (TFs) are proteins that bind to DNA sequences & regulate gene expression.

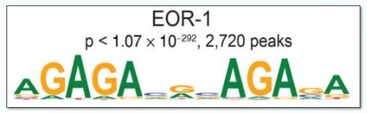


Nuclear Hormone Receptor-25 (NHR-25):

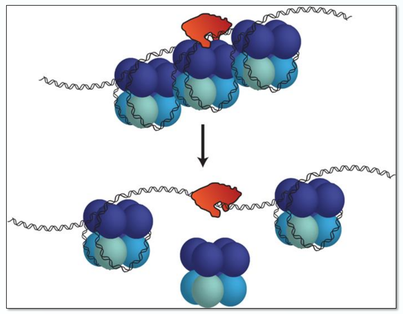
- Essential for *C. elegans* development
- Abundant in larvae stage 1 (L1)
- Expressed in hypodermal & vulval precursor cells[1]

EOR-1:

- GAGA motif[2]
- Enriched at where NHR-25 binds to
- Associated with longer fragments, suggesting nucleosome periodicity related to EOR-1 binding



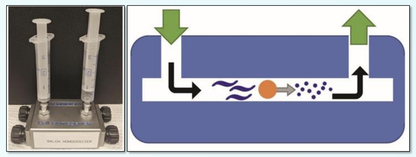
Pioneer Factor Model[3]



Is EOR-1 a pioneer factor of NHR-25?

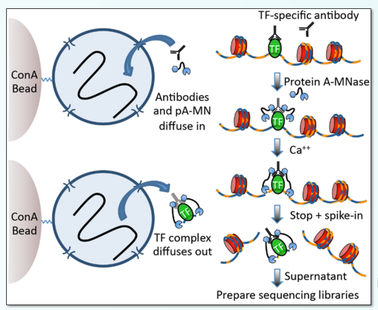
1. What are the specific binding sites of EOR-1? → CUT&RUN
2. Does EOR-1 contribute to increased chromatin accessibility? → ATAC-seq

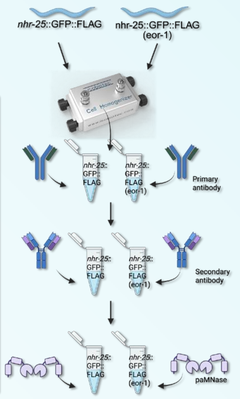
Methodology



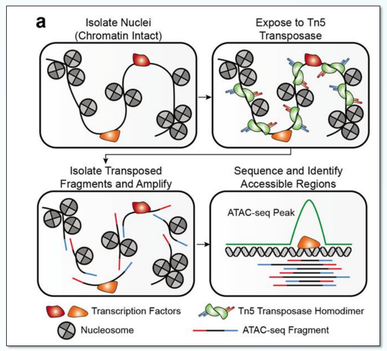
Nuclei Homogenization using the Balch Homogenizer[3]

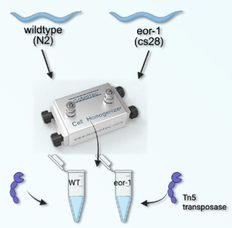
CUT&RUN[4,5]



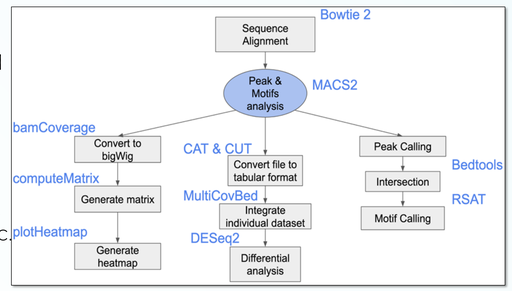


ATAC-seq[5,6]

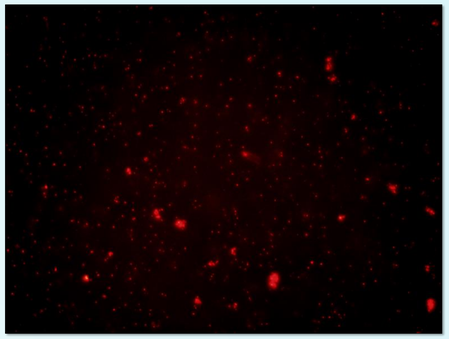




ATAC-seq data analysis pipeline using Galaxy[7], DESeq2[8], RSAT[9], etc.

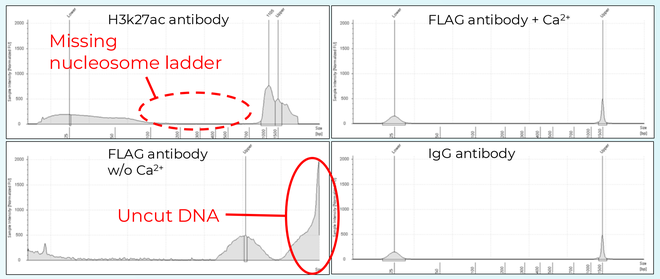


Results

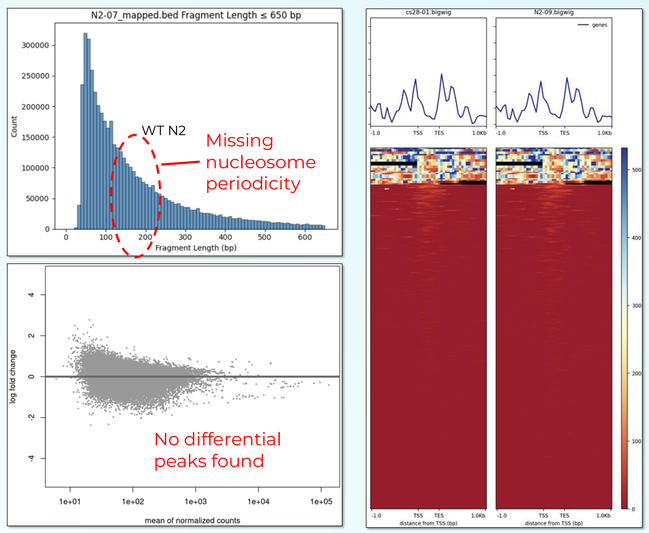


Optimized Nuclei Homogenization

Fluorescent signals are successfully homogenized nuclei from L1 *C. elegans* stained by propidium iodide. Every 1,000 worms correspond to 1 million cells.

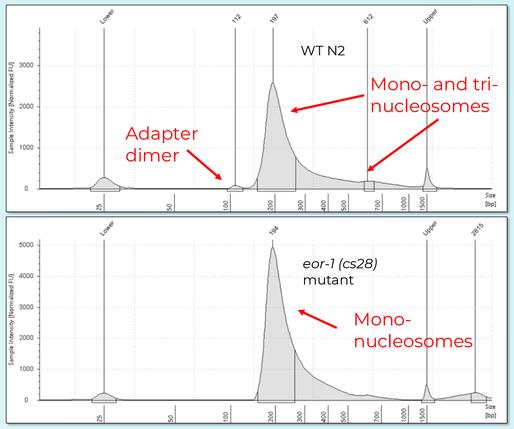


CUT&RUN TapeStation QC
Without Ca^{2+}, no periodic fragments of DNA was found.



Previous ATAC-seq Data Analysis

- Quality control analysis showed unpromising data from precious ATAC-seq prep (top-left & right)
- No significance found for differential peaks (bottom-left)



ATAC-seq TapeStation QC after Optimization
Mono- and tri-nucleosome fragments found indicates promising tagmentation using the Illumina Tn5

Conclusions

We optimized the protocol for nuclei homogenization and its quantification.

CUT&RUN:

- Ca^{2+} is essential for pa-MNase enzymic reaction.

ATAC-seq:

- Promising nucleosome ladder for library QC.

Future Directions:

- Repeat replicates of CUT&RUN and ATAC-seq and send out for sequencing.
- Analyze sequencing data using the analysis pipeline.

Acknowledgements

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