



JOHNS HOPKINS
BIOMEDICAL ENGINEERING

Detecting methylation and chromatin accessibility on long DNA sequences

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Timp Lab

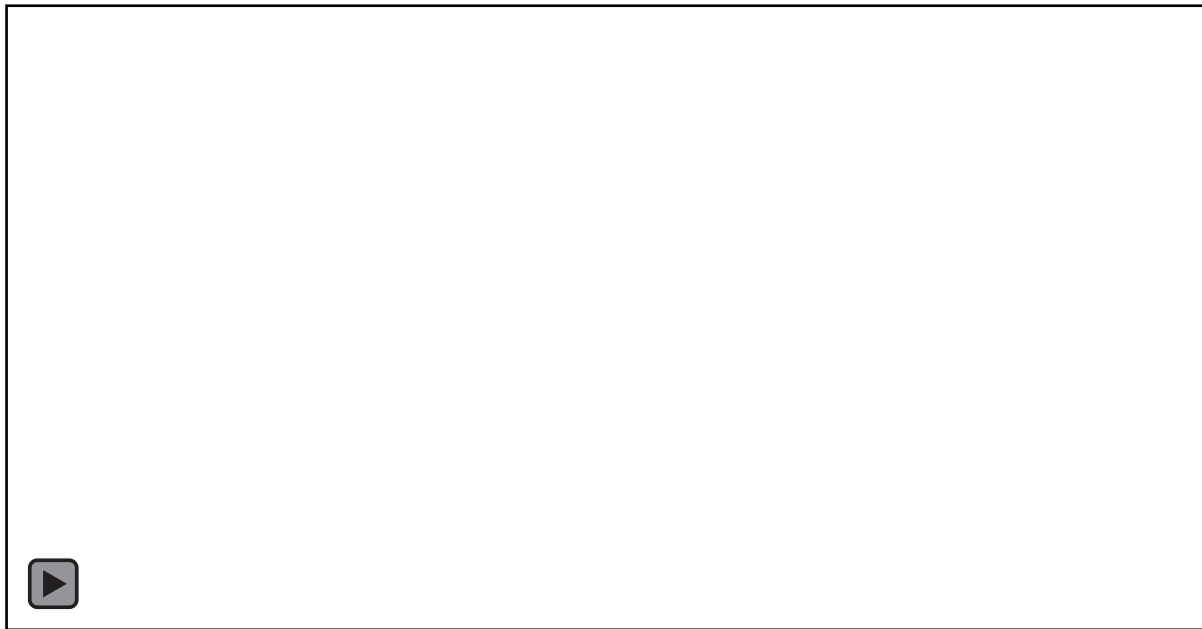
Johns Hopkins University

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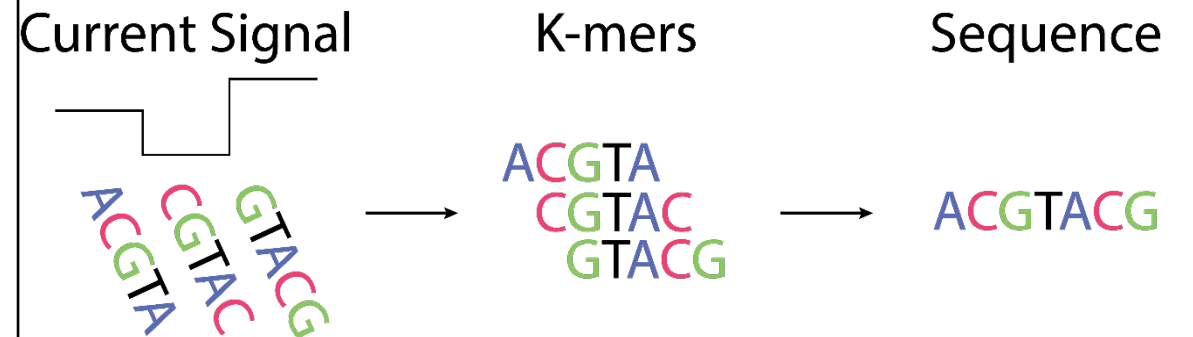
Nanopore Sequencing

DNA sequencing by strands going through a nanopore

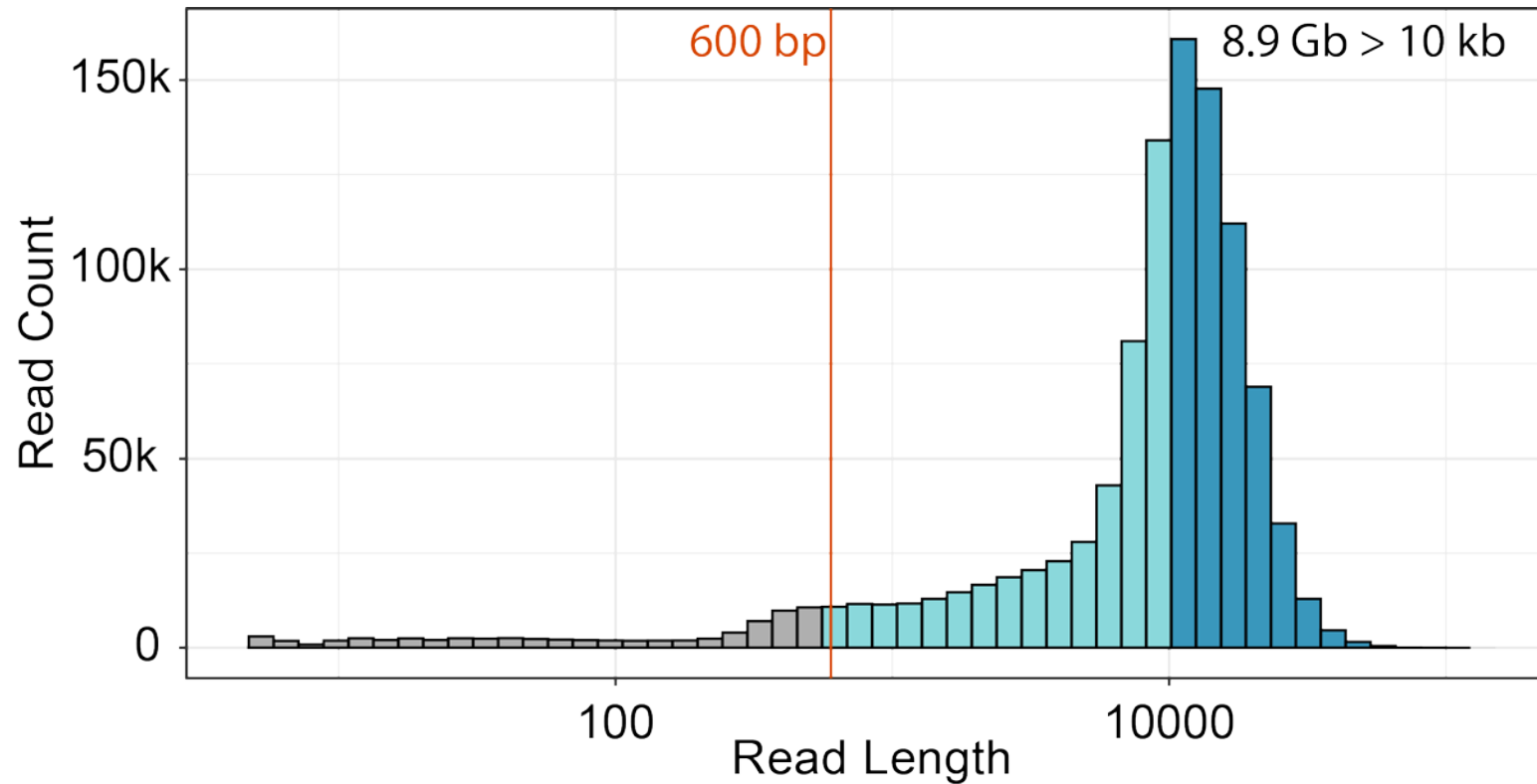
- Current across the nanopore is measured as DNA passes through
- The current modulation is dependent on chemical structure



Oxford Nanopore Technologies



Nanopore Sequencing



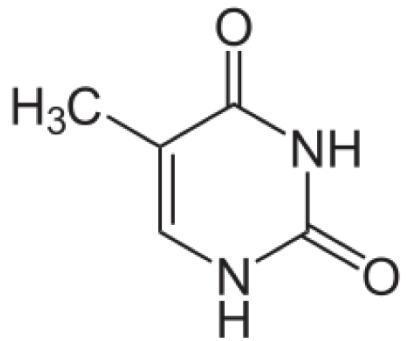
Nanopore sequencing generates long DNA sequences

- Observe genomic and epigenomic patterns on single strands

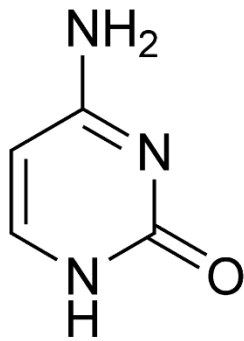
Nanopore Sequencing

Nanopore can detect DNA modifications

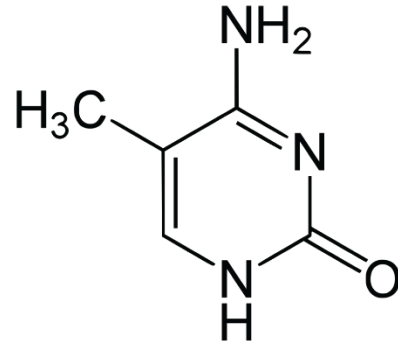
- CpG methylation
- Exogenous Labeling



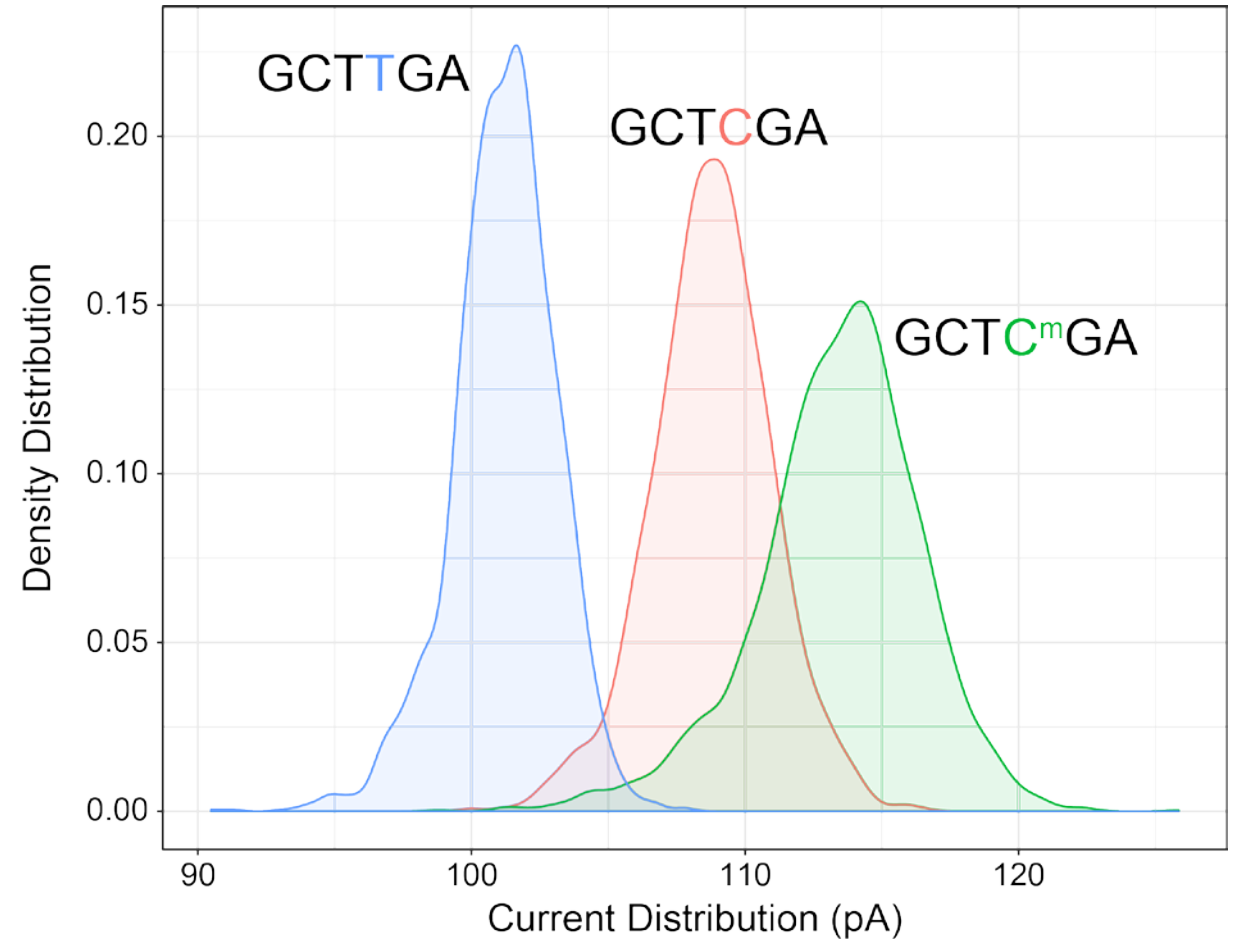
Thymine



Cytosine



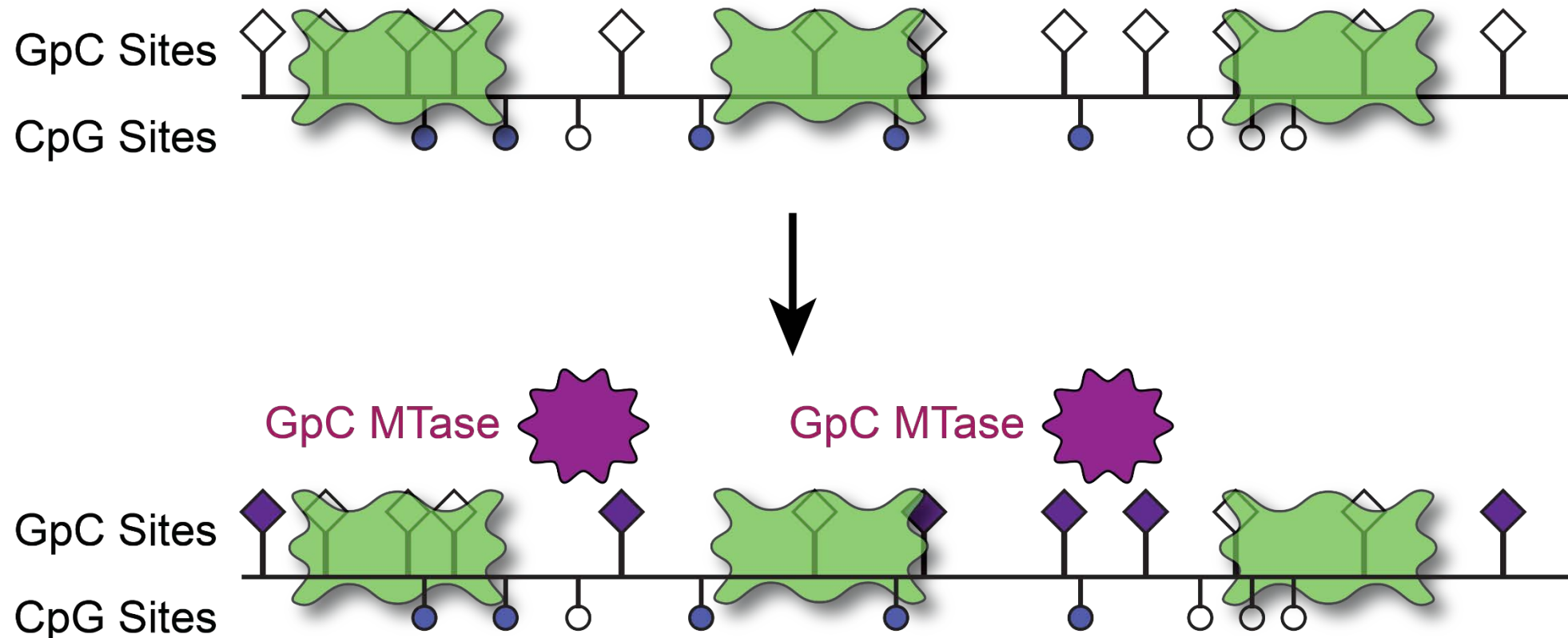
5-methylCytosine



Simpson et. al *Nature Methods* 2017

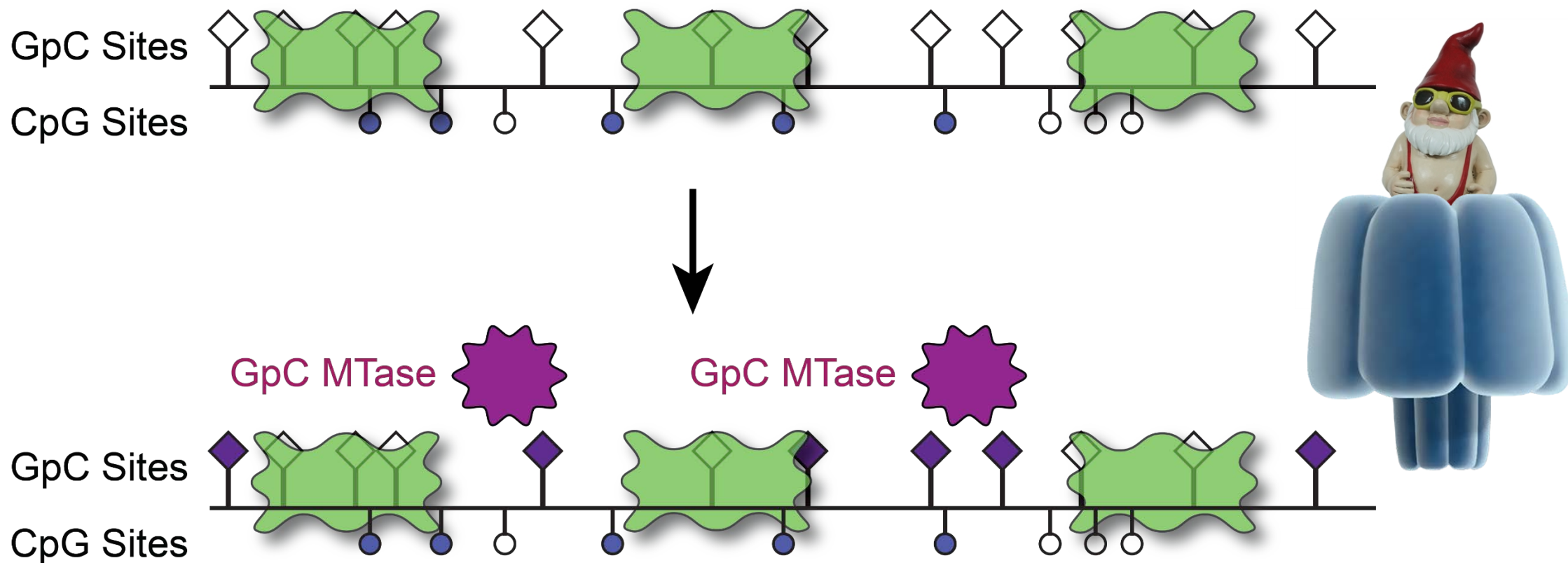
Exogenous Labeling

- NOMe-seq : **N**ucleosome **O**ccupancy and **M**ethylome **s**equencing (Kelly et. al. *Genome Res.* 2012)
Simultaneously measures DNA methylation (CpG) and chromatin accessibility (GpC)



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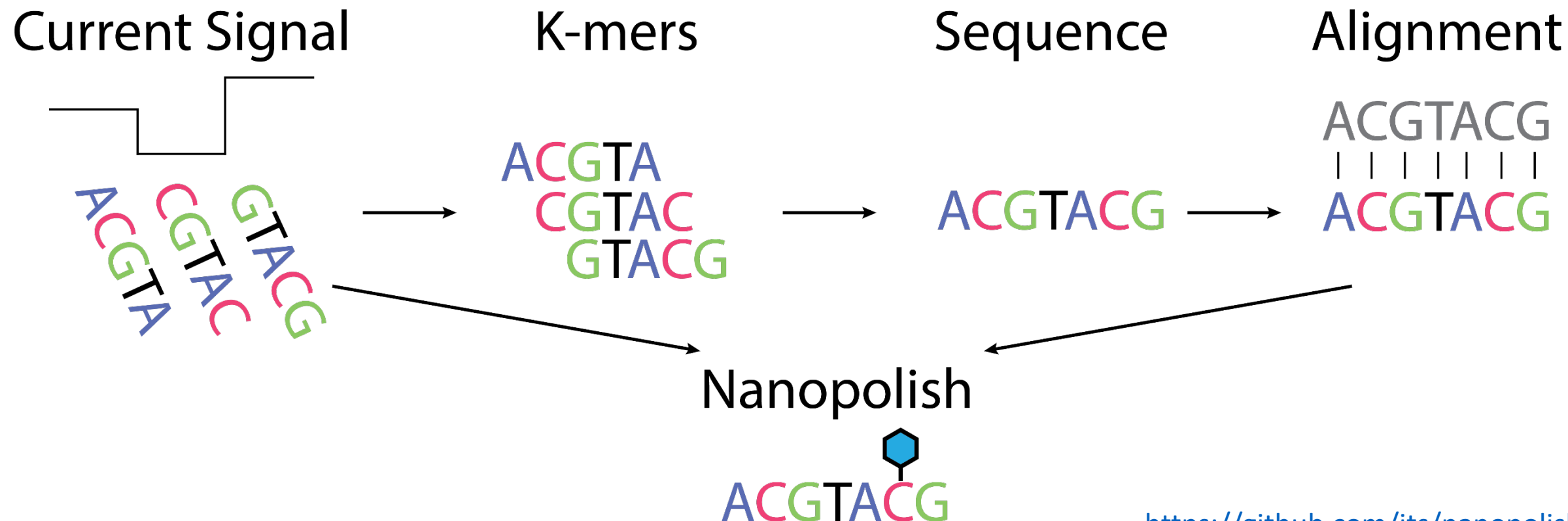


Detecting DNA methylation

Nanopolish : post-alignment methylation caller using a hidden Markov model

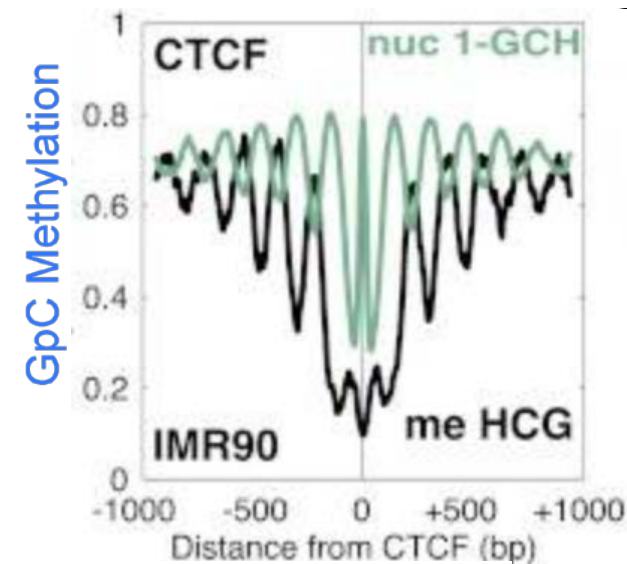
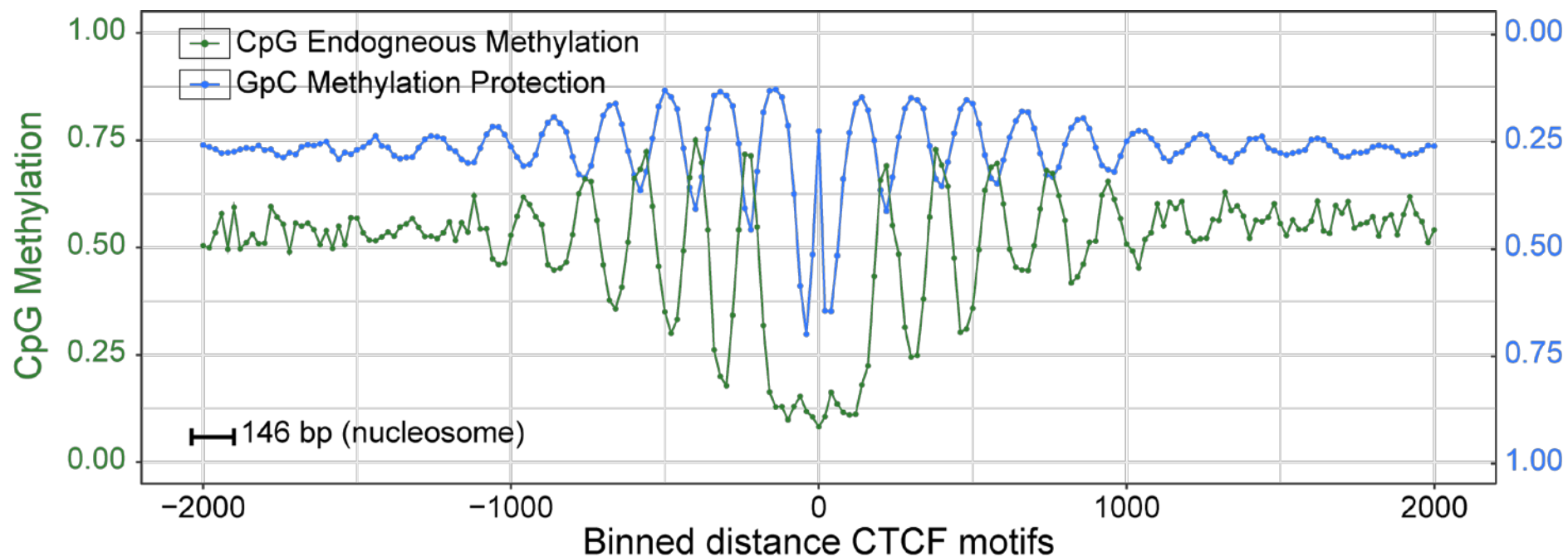
(Simpson, et. al. *Nat. Method* 2017)

- Nanopolish can be trained to detect various DNA modifications



<https://github.com/jts/nanopolish>

NanoNOMe – Validation with GM12878

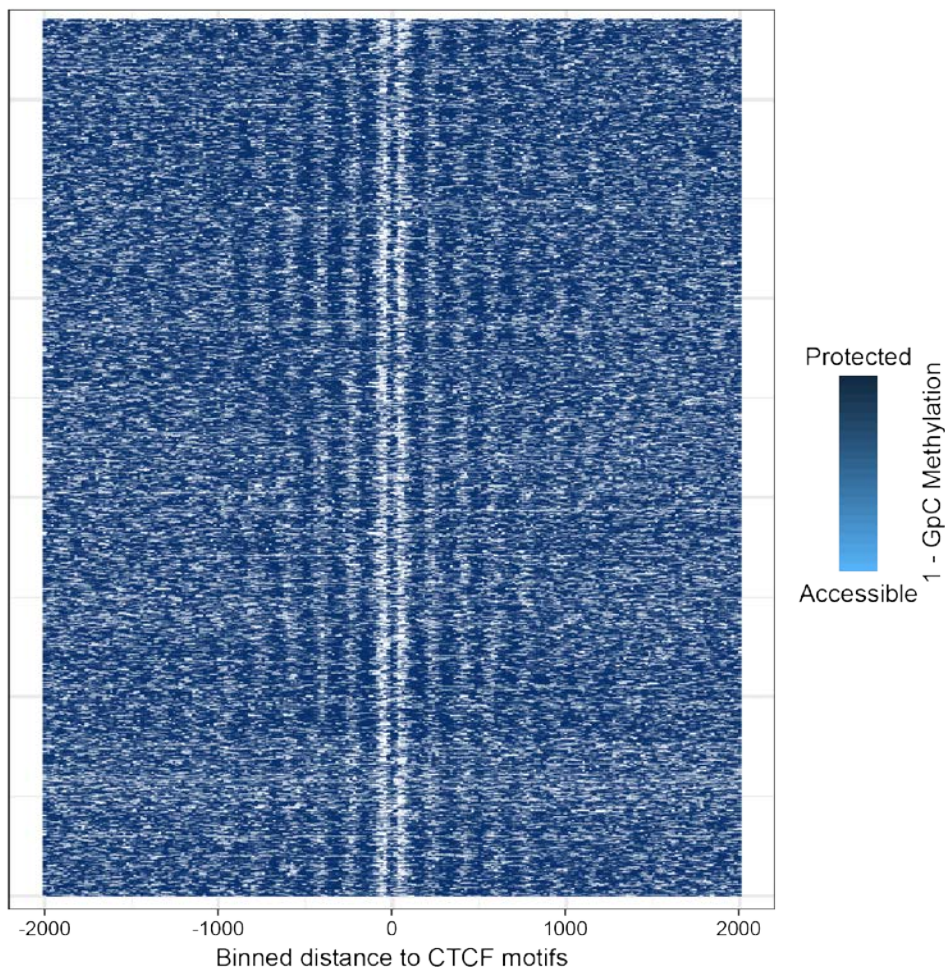


Kelly, et. al. *Genome Res.* 2012

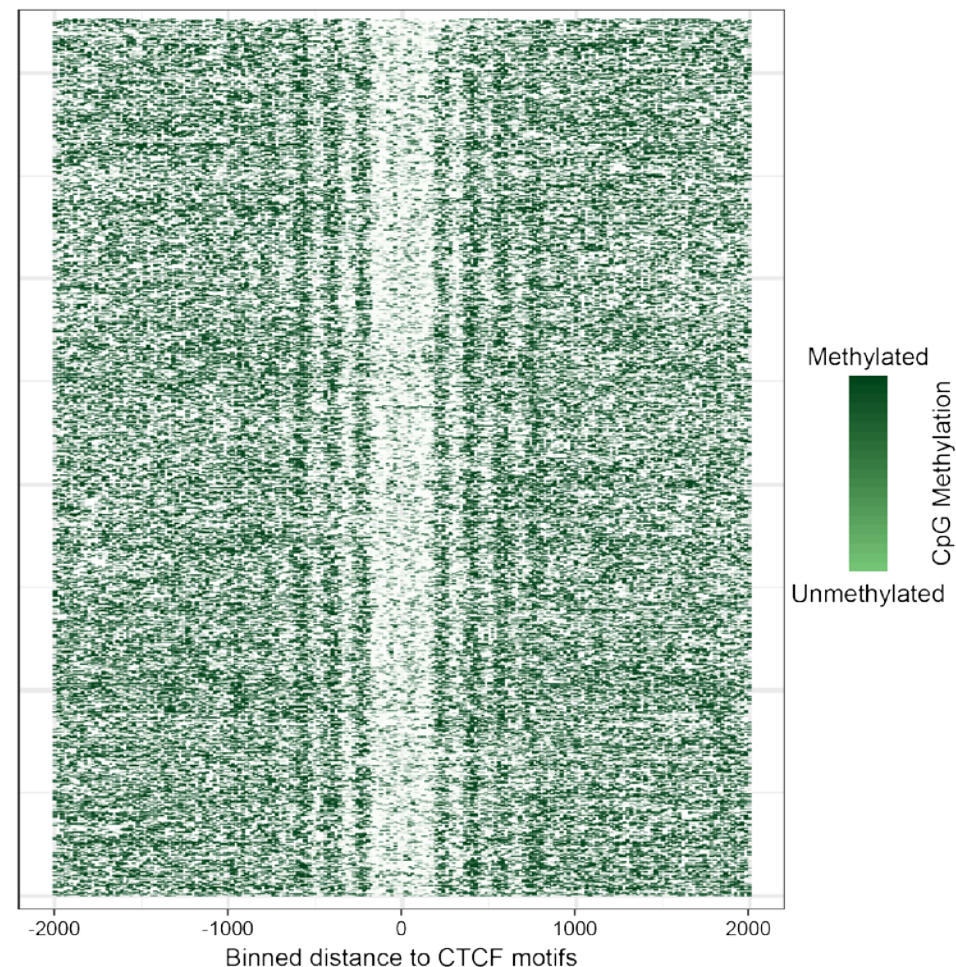
- Anticorrelation of DNA methylation and open chromatin
- Chromatin states around CTCF agrees with NOMe-seq

NanoNOMe – Validation with GM12878

Chromatin Protection (1-GpC)

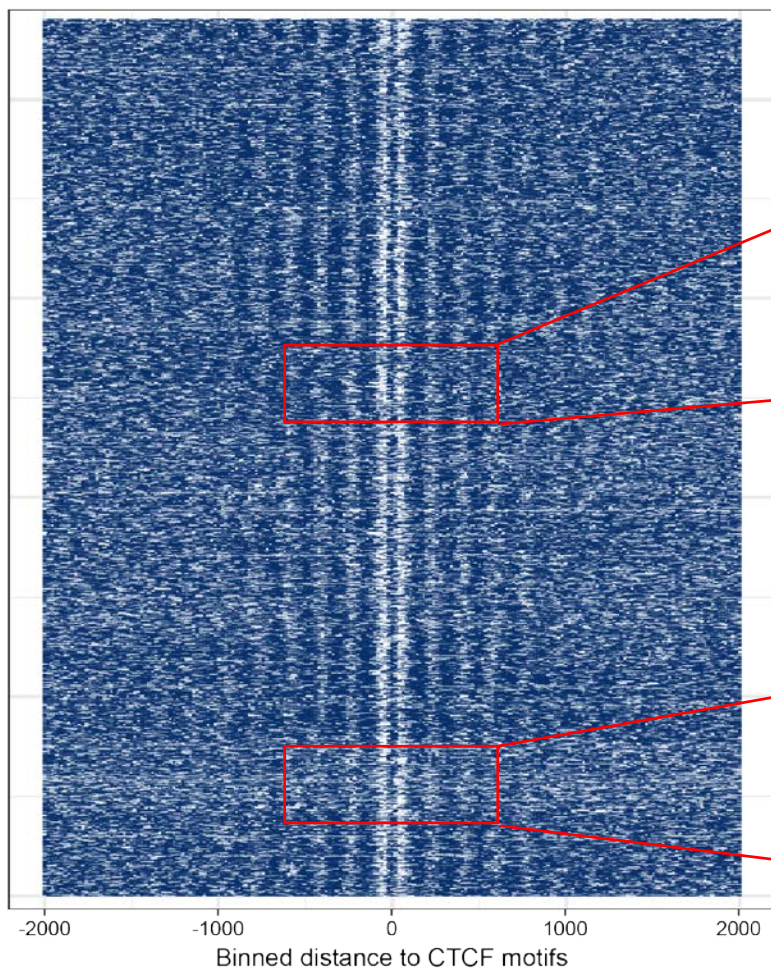


Endogenous Methylation (CpG)

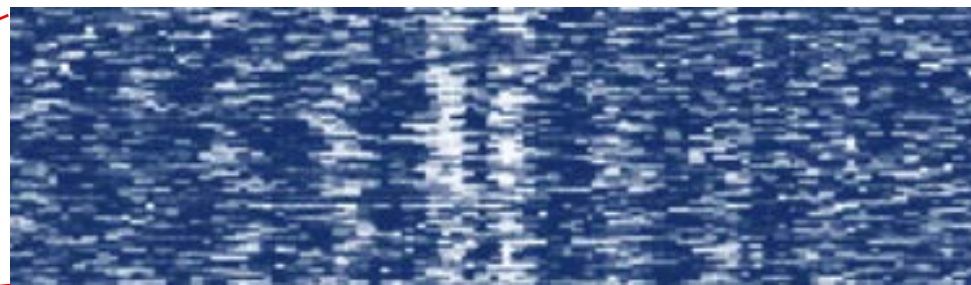


NanoNOMe – Validation with GM12878

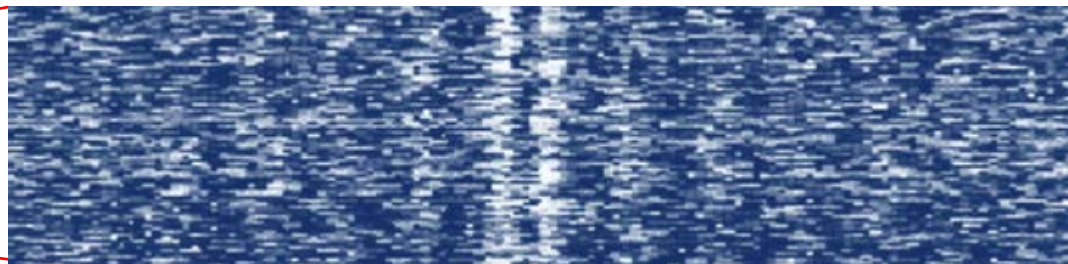
Chromatin Protection (1-GpC)



Shift in nucleosome positioning



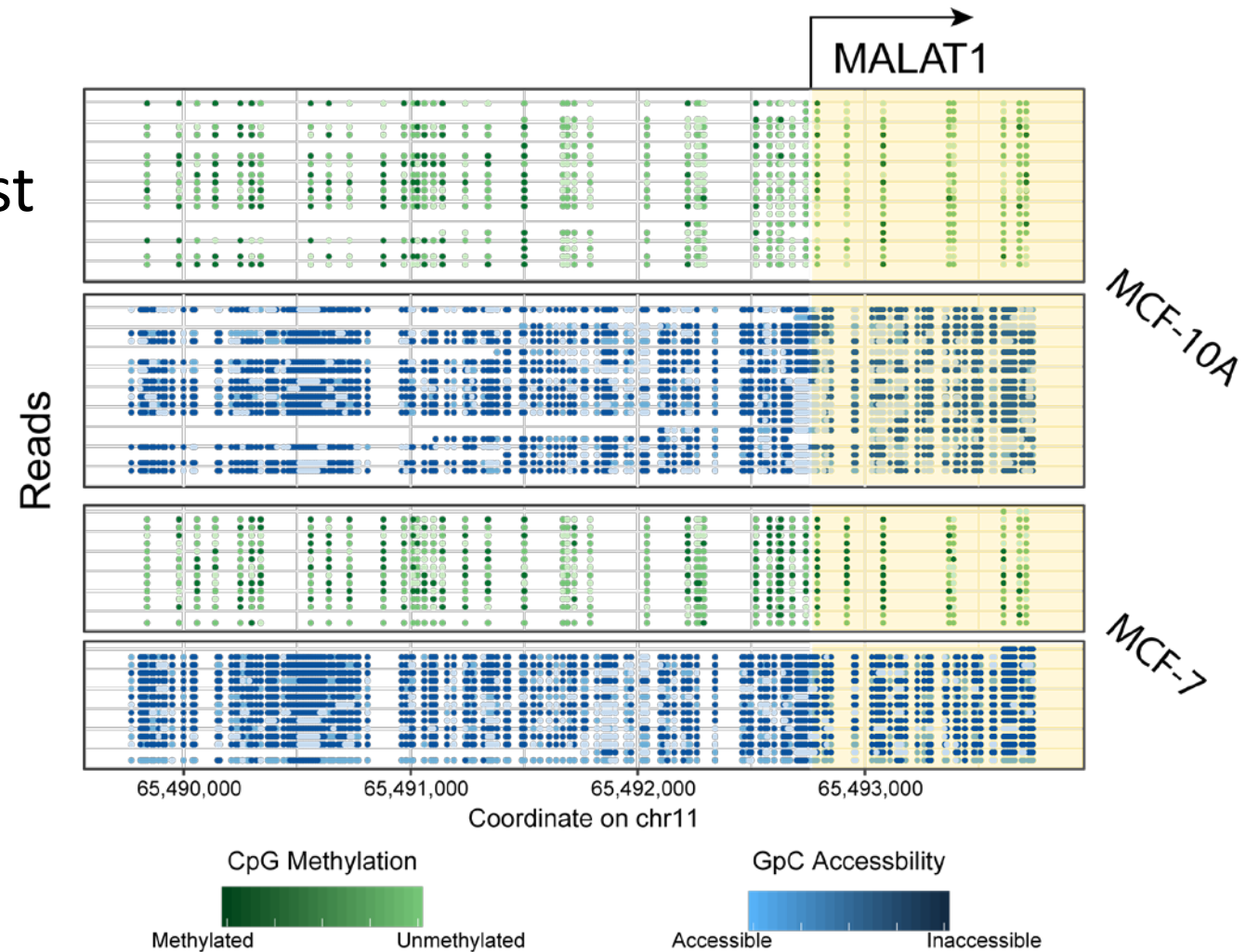
Loss of homogeneity



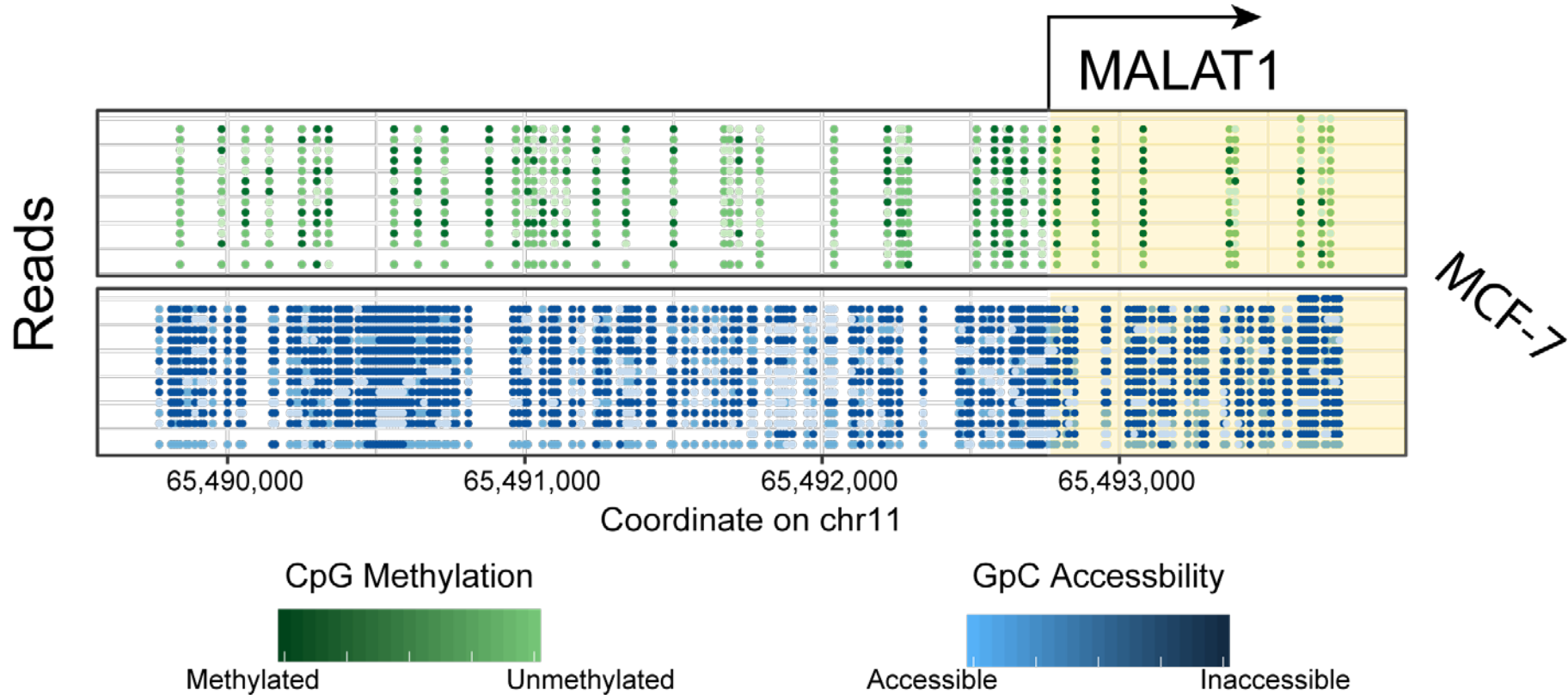
NanoNOMe on Breast Cancer Cell Lines

MCF-10A vs MCF-7

- MALAT1 : downregulated in breast cancer

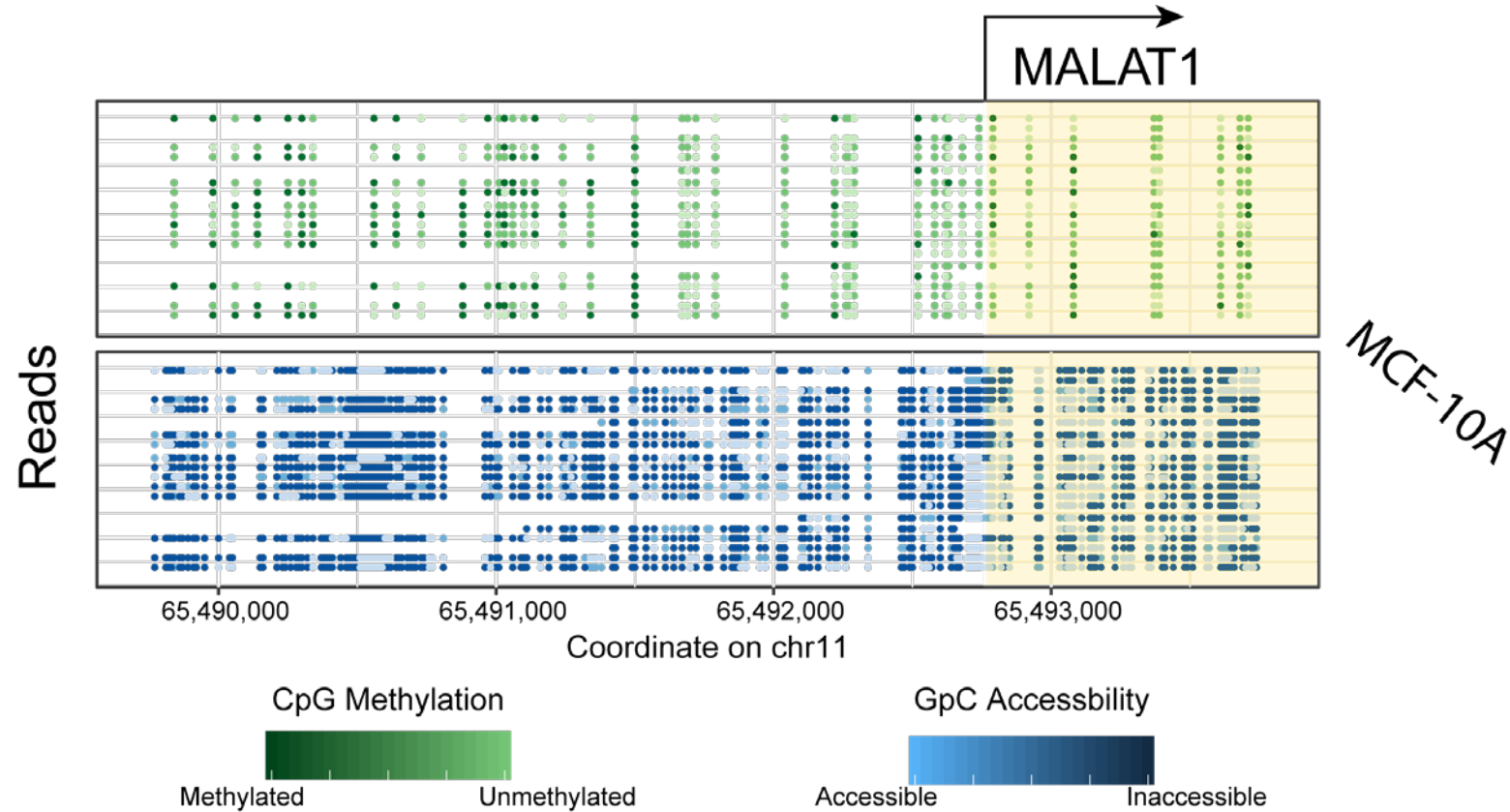


NanoNOMe on Breast Cancer Cell Lines



- Promoter region is methylated
- Promoter is inaccessible

NanoNOMe on Breast Cancer Cell Lines

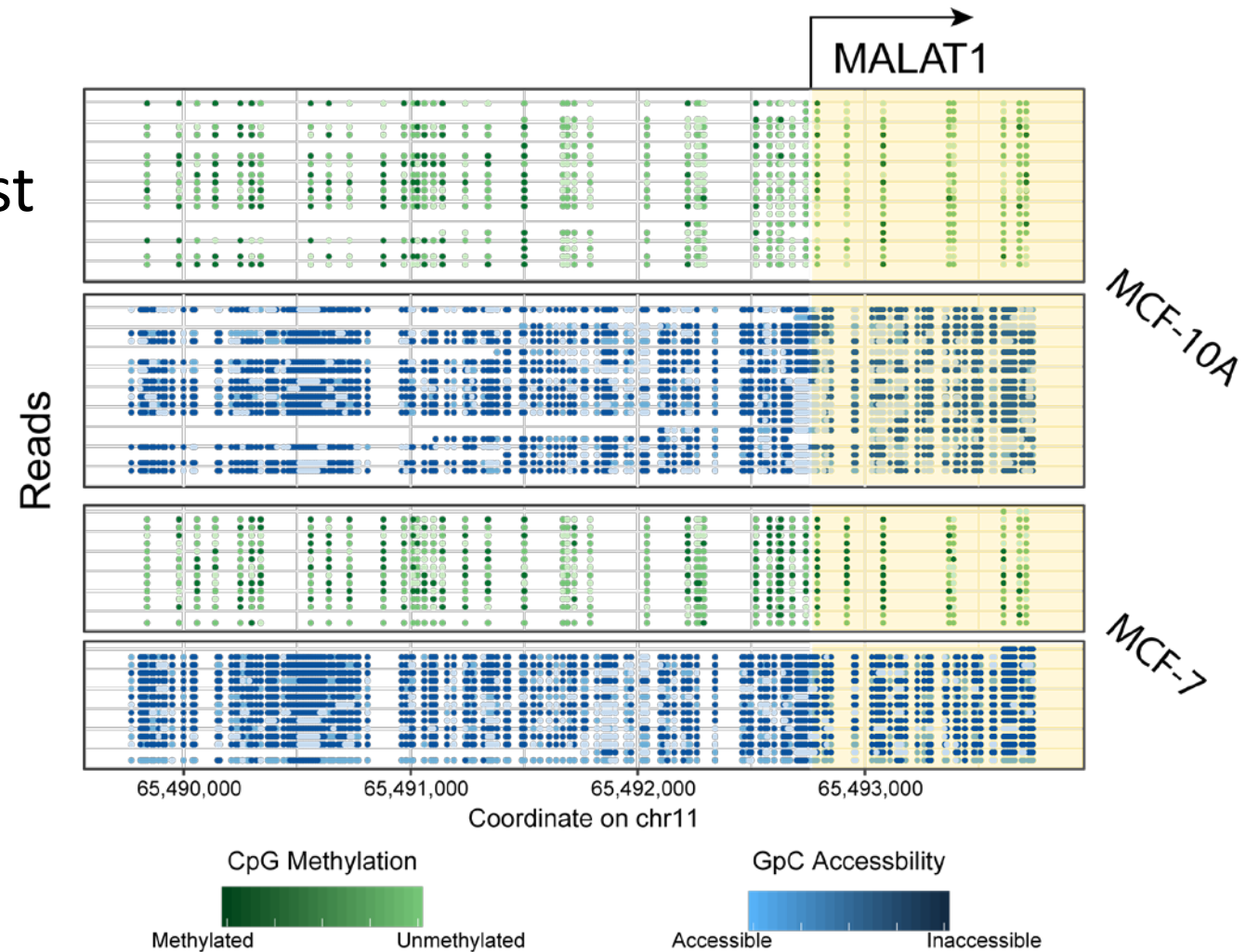


- Promoter region is unmethylated
- Promoter is accessible in an allele-specific manner

NanoNOMe on Breast Cancer Cell Lines

MCF-10A vs MCF-7

- MALAT1 : downregulated in breast cancer
- Expression in MCF-10A :
 - Unmethylated promoter
 - Allele-specific accessible promoter
- Silencing in MCF-7 :
 - Methylated promoter
 - Inaccessible promoter



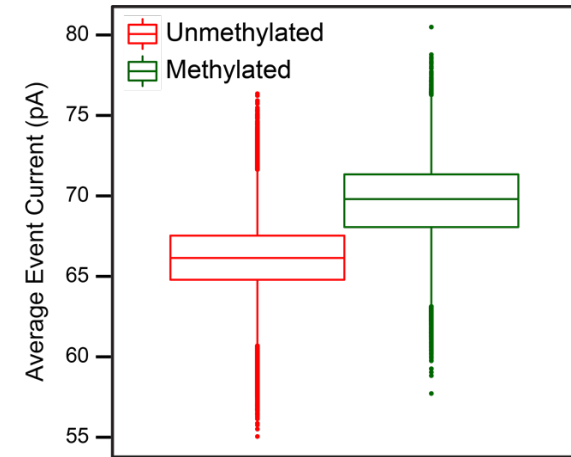
Conclusion and Future Work

Using nanoNOMe, we can observe :

- CpG methylation and chromatin accessibility simultaneously on long reads
- Heterogeneity of chromatin states
- Allele-specific chromatin states

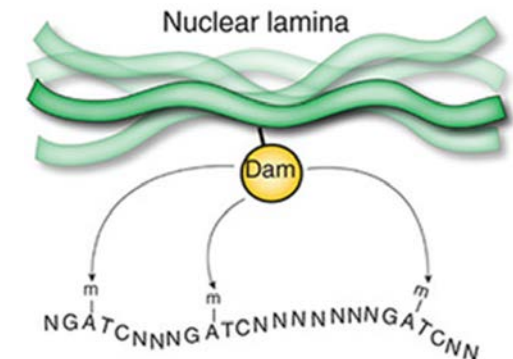
Future Work

- Phase chromatin states based on SNPs
- Different methylation motifs
 - Dam (GATC)
 - EcoGII (A)
- Other exogenous labeling techniques
 - damID – with Reddy lab at JHMI



AGATCG

Nuclear
environment



Acknowledgements



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