Single-molecule simultaneous profiling of DNA methylation and DNA-protein interactions with Nanopore-DamID.

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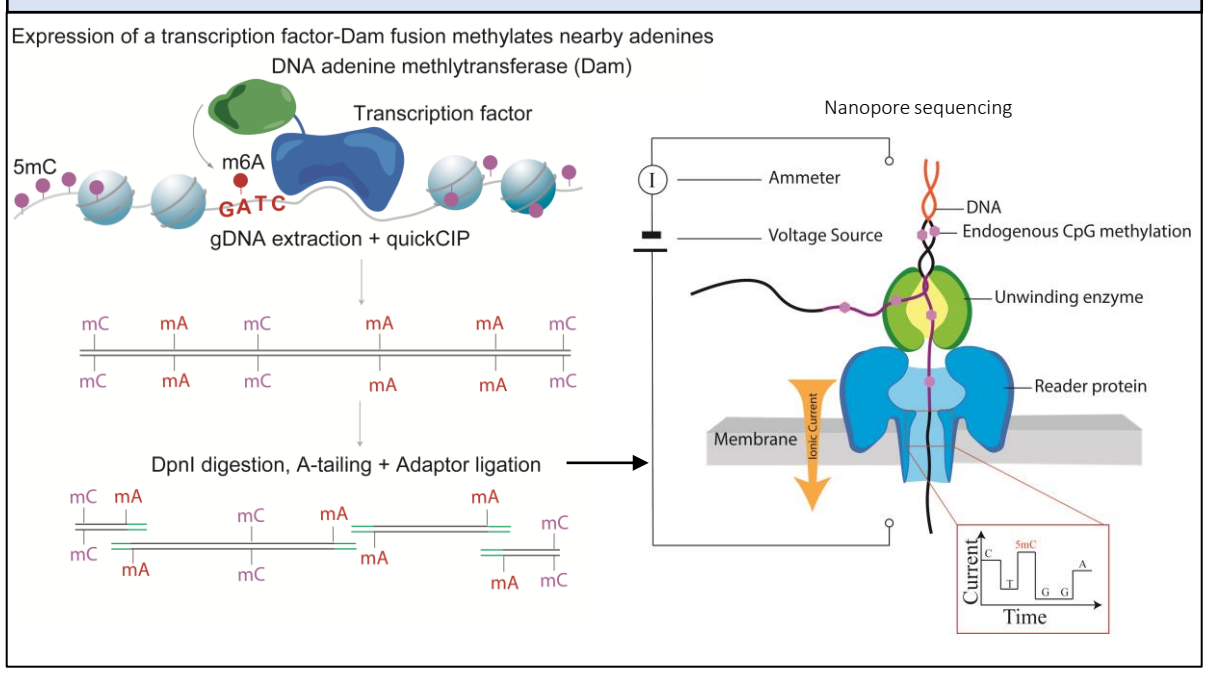
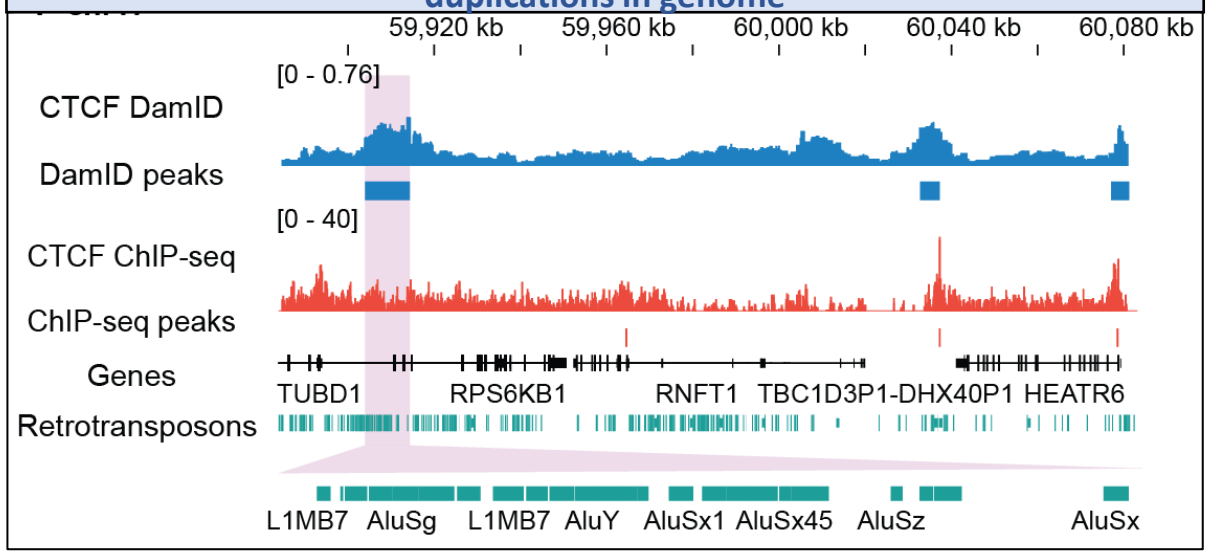
ABSTRACT

Protein-DNA interactions and DNA methylation control mammalian gene expression. Here we present Nanopore-DamID to simultaneously detect cytosine methylation and DNA-protein interactions from single molecules, via selective sequencing of adenine-labelled DNA. Assaying LaminB1 and CTCF binding with Nanopore-DamID, we identify strict CpG methylation maintenance at transcriptional start sites amidst generalised hypomethylation of LaminB1-associated heterochromatin. Promoter methylation is required for escape of a small subset of genes from Lamin-associated repression. We detect novel CTCF binding sites in highly repetitive regions and allele-specific binding to imprinted genes and the active X chromosome, highlighting the importance of DNA methylation to transcription factor activity.

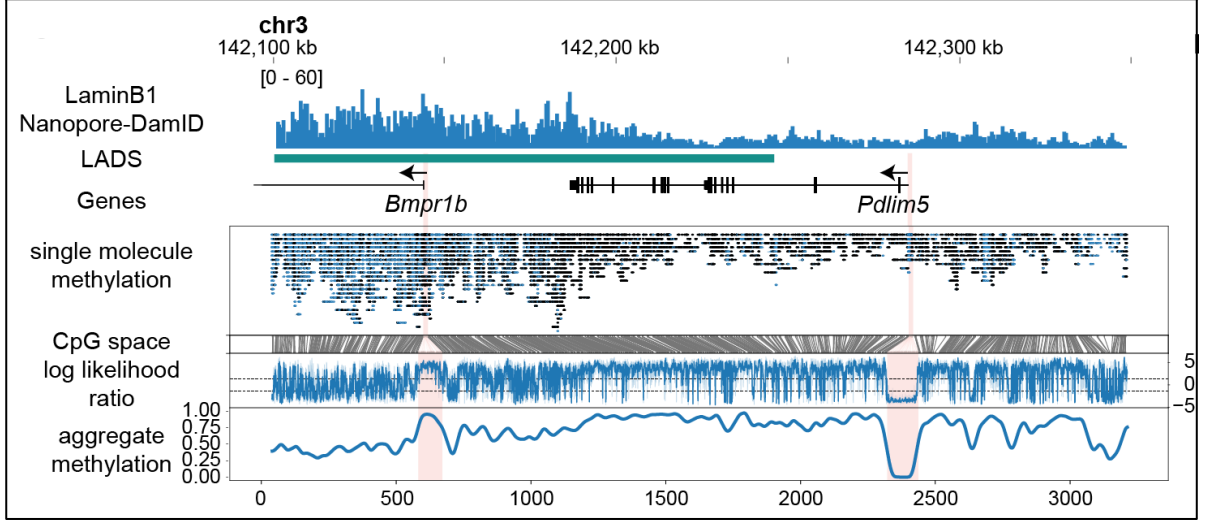
Nanopore-DamID Methodology



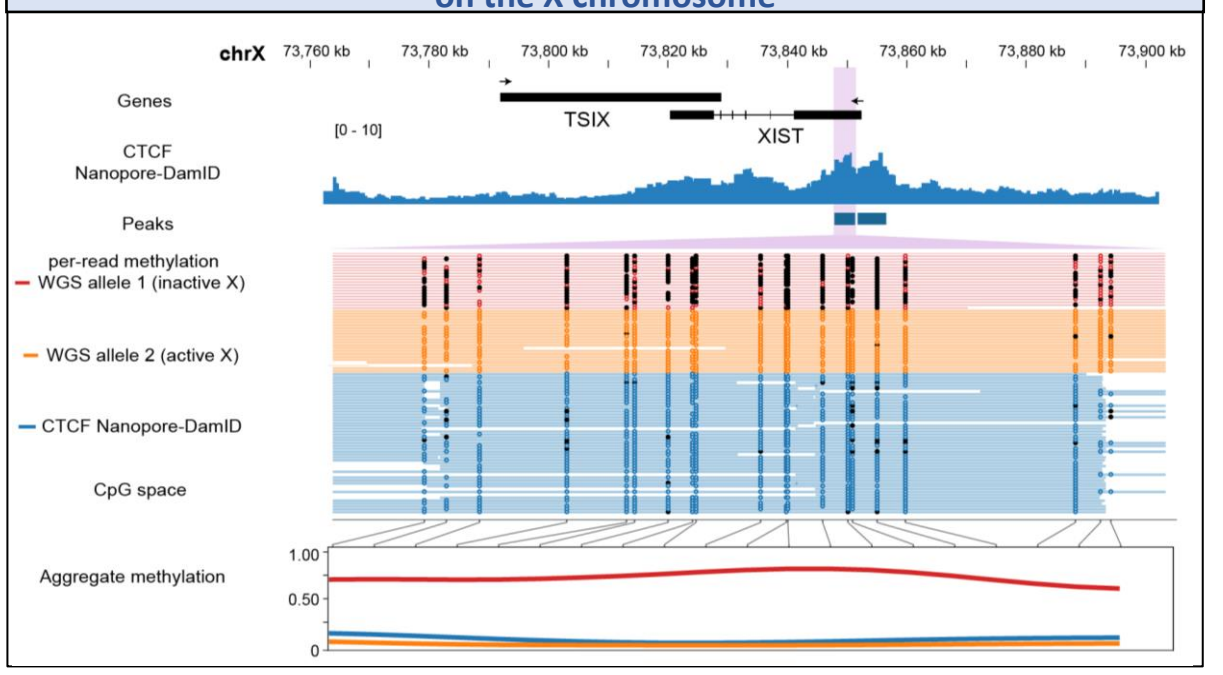
Nanopore-DamID can identify CTCF at TE-rich regions and segmental duplications in genome



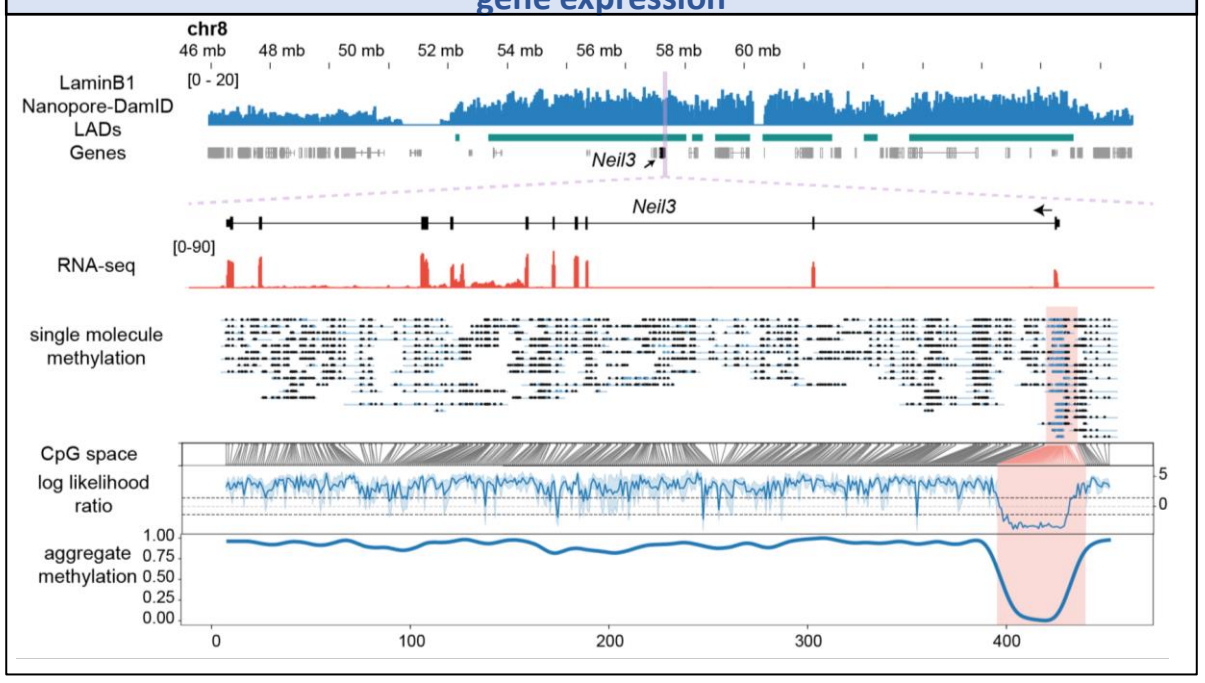
Nanopore-DamID simultaneously reveals Lamin-DNA interaction and associated endogenous CpG methylation patterns.



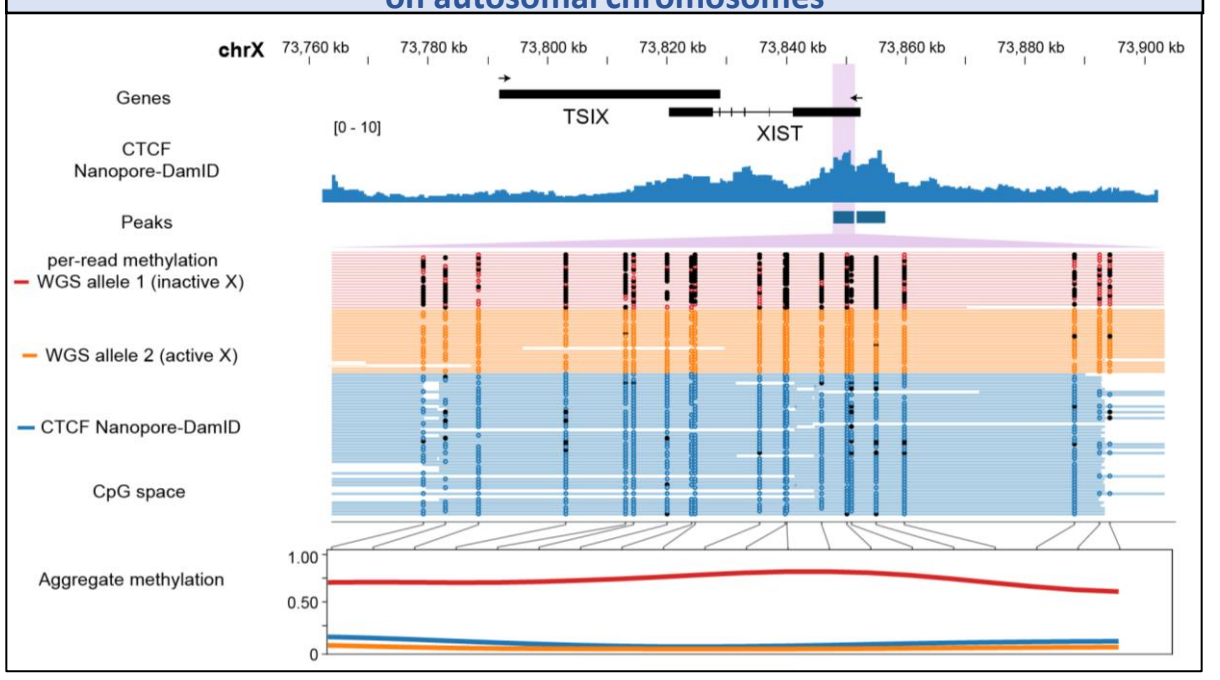
Nanopore-DamID identifies allele specific transcription factor interaction on the X chromosome



Lamin-DNA interactions along with endogenous CpG methylation regulate gene expression



Nanopore-DamID identifies allele specific transcription factor interaction on autosomal chromosomes



Conclusion

Nanopore-DamID revealed that genes in heterochromatic LADs (Lamin Associated Domains) were hypomethylated, however their promotors maintained high CpG methylation. Inversely, genes in iLADs (inter Lamin Associated Domains) were hypermethylated but their promotor regions were hypomethylated. A small subset of genes in LADs which were expressed had hypomethylated promotors, indicating that demethylation is necessary but not sufficient for genes to escape LAD associated gene repression[3]. Additionally, Nanopore-DamID identified CTCF-binding sites at TE-rich region and segmental duplications not detected by ChIP seq from ENCODE. The results of CTCF Nanopore-DamID revealed that CTCF binding on the XIST locus occurs exclusively at its active hypomethylated allele. Similarly, CTCF bound alleles are largely demthylated at the autosomal paternal locus KCNQ1OT1. These examples demonstrate the power of Nanopore-DamID to detect single-molecule allele specific transcription factor-DNA methylation interactions. Overall, Nanopore-DamID enables novel insights into the regulation of gene expression by DNA-protein interactions and DNA methylation

REFERENCES

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ACKNOWLEDGEMENTS

Australian Government
National Health and Medical Research Council
NHMRC
CSL
The University Of Queensland
AUSTRALIA
AIBN
Australian Institute for Bioengineering and Nanotechnology
mater research