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Genome-Wide Analysis of Methylome in the Mouse Brain using Long-Read Sequencing Technology

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Abstract

DNA methylation is an epigenetic modification that transfers a methyl group onto the C-5 position of the cytosine to form 5-methylcytosine. DNA methylation regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factor(s) to DNA, especially in regulation of Allele Specific Expression (ASE). In this study, we used Oxford Nanopore long-read sequencing technology to profile methylome in the two inbred mouse strains, C57BL/6J (B6) and DBA/2J (D2). Compared with bisulfite conversion followed by Illumina Sequencing, long-read sequencing technology allows us to achieve much longer read length of 4,653.675 base pairs on average while maintaining an average percent identity of 90.775%. We detected millions of methylation events and 1,465 differentially methylated regions (DMRs) between B6 and D2. Understanding more about how DNA methylation patterns of these mice affect neurological phenotype will further research into drug development for neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD).

Introduction

- Oxford Nanopore long-read direct DNA sequencing allows for a much more accurate analysis of DNA methylation patterns.
- Using Hidden Markov Modeling (HMM), DNA methylation may be predicted from signal-level scores without the need of Bisulfite conversion.
- Gnas is a complex, well-known imprinted gene that displays both maternal and paternal imprinting in an isoform dependent manner.[1]
- H19 is one of the most well documented imprinted genes. The H19 gene is transcriptionally silenced through 5-mC modifications on the paternal allele.[2]

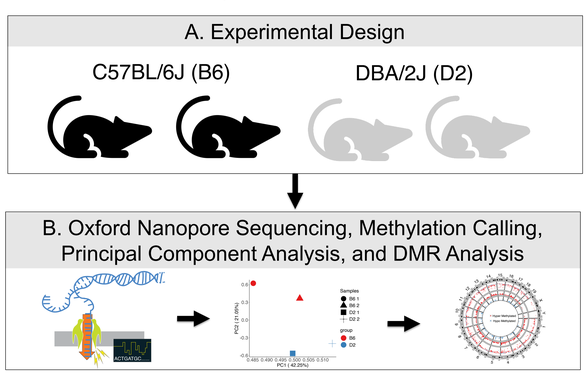

Figure1: Experimental design and brief graphical workflow.

Methods

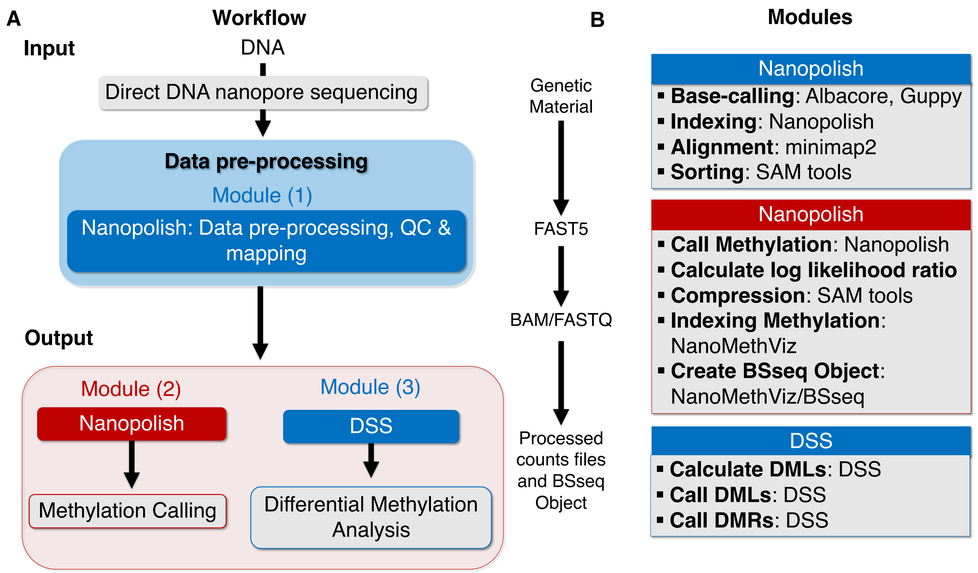

Figure 2: The standard workflow followed to produce the differentially methylated regions from the signal-level data produced by the Oxford Nanopore MinION sequencer.

Results

- We found through the DMR analysis that the C57BL/6J mouse strain had 1259 methylation regions hyper methylated in reference to the DBA/2J strain.
- There were just 206 regions of the DBA/2J strain having higher mean methylation level compared to C57BL/6J.

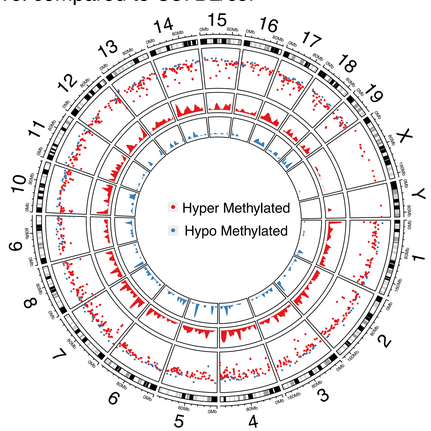

Figure 3: Plot of genomic density and rainfall plot showing hyper and hypo differentially methylated regions in C57BL/6J compared to DBA/2J.

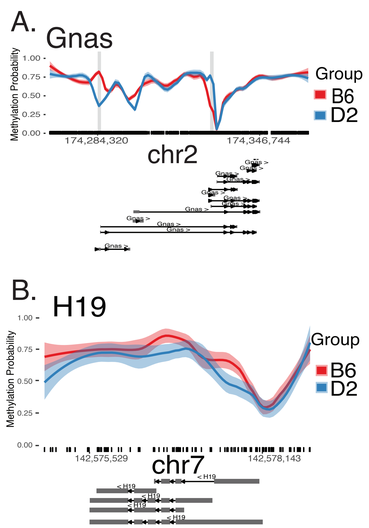

Figure 4: Graphical representation of the methylation probability by genomic position for known imprinted genes.

Conclusions

- We found the C57BL/6J inbred mouse strain to be hyper methylated in comparison to the DBA/2J inbred strain.
- DNA methylation patterns of bulk brain tissue in these two classically studied inbred strains may contribute to the large neurological phenotypic differences between them.
- Our future directions with this project will be to integrate the detected DMRs with previously found genes displaying allele specific expression in the same mouse strains to elucidate allele specific methylation patterns.
- Our goal for this project is to be able to detect allele specific methylation patterns that correlate to the distinct phenotypic differences between the two mouse strains.

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References

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