Novel detection method for 5mCpG on DNA Viruses: Hepatitis B Virus

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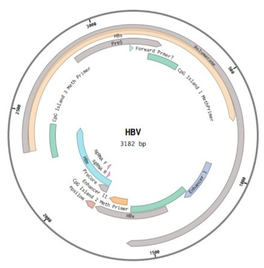
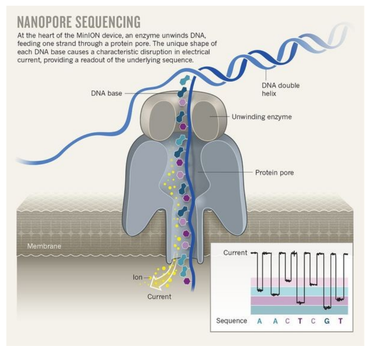
INTRODUCTION

Methylation sequencing can provide insights into transmission events and disease progression. Hepatitis B is a relaxed circular DNA virus and is one of the causative agents of viral hepatitis. HBV infection is a major health problem worldwide and can cause both acute and chronic disease. According to the World Health Organization (WHO), more than 240million people exhibit chronic HBV infections and about 600,000 people die every year due to the consequences of HBV. The methylation patterns of HBV can provide valuable information regarding chronicity and disease activity.

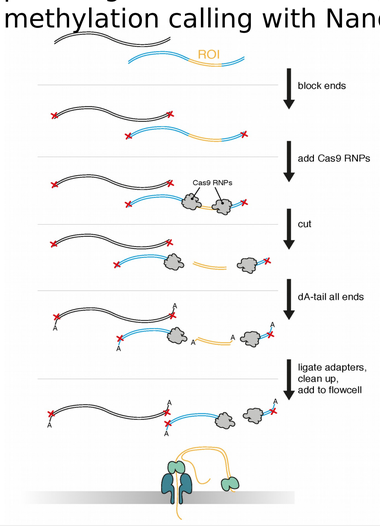
Previously, the analysis of DNA base modifications on native DNA is not possible without bisulfite conversion steps and PCR which degrade DNA and add additional biases. 'Third generation' sequencing approaches such as Oxford Nanopore Technologies (ONT) can potentially overcome these limitations. **Here we have developed a novel technique for detecting modified bases on HBV that is not dependent on bisulfite conversion or PCR**.



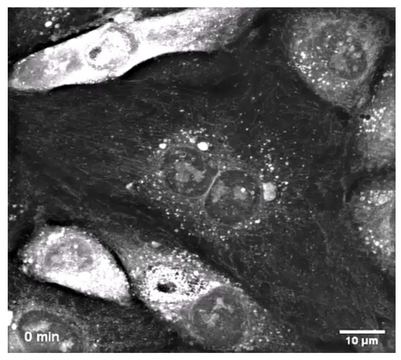


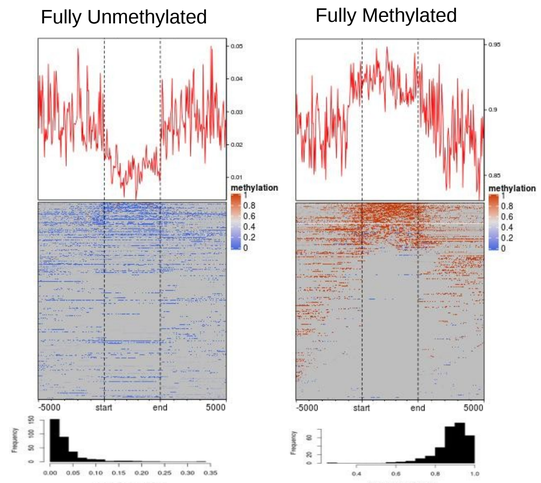
METHODS

Primary Human Hepatocytes (PHH) or HepaRG cells were naturally infected with Hepatitis B Virus (Genotyoe D) for 2 weeks before total DNA extraction. HBV Fully unmetylated control (FU) was prepared by nested PCR amplification and nuclear DNA FU control by whole genome amplification of HepaRG DNA. Fully Methylated controls (FM) were prepared by M.SssI treatment to methylate CpG dinucleotides from the amplified DNA controls. The Cas9 targeted sequencing protocol employing single cutting for each strand (2x sgRNAs in total) was used to enrich in HBV DNA prior to sequencing with an ONT MinION devise and R9.4.1 Flow cell. De novo assembly was performed with Canu followed by assembly polishing with MEDAKA, alignment with minimap2 and methylation calling with Nanopolish.



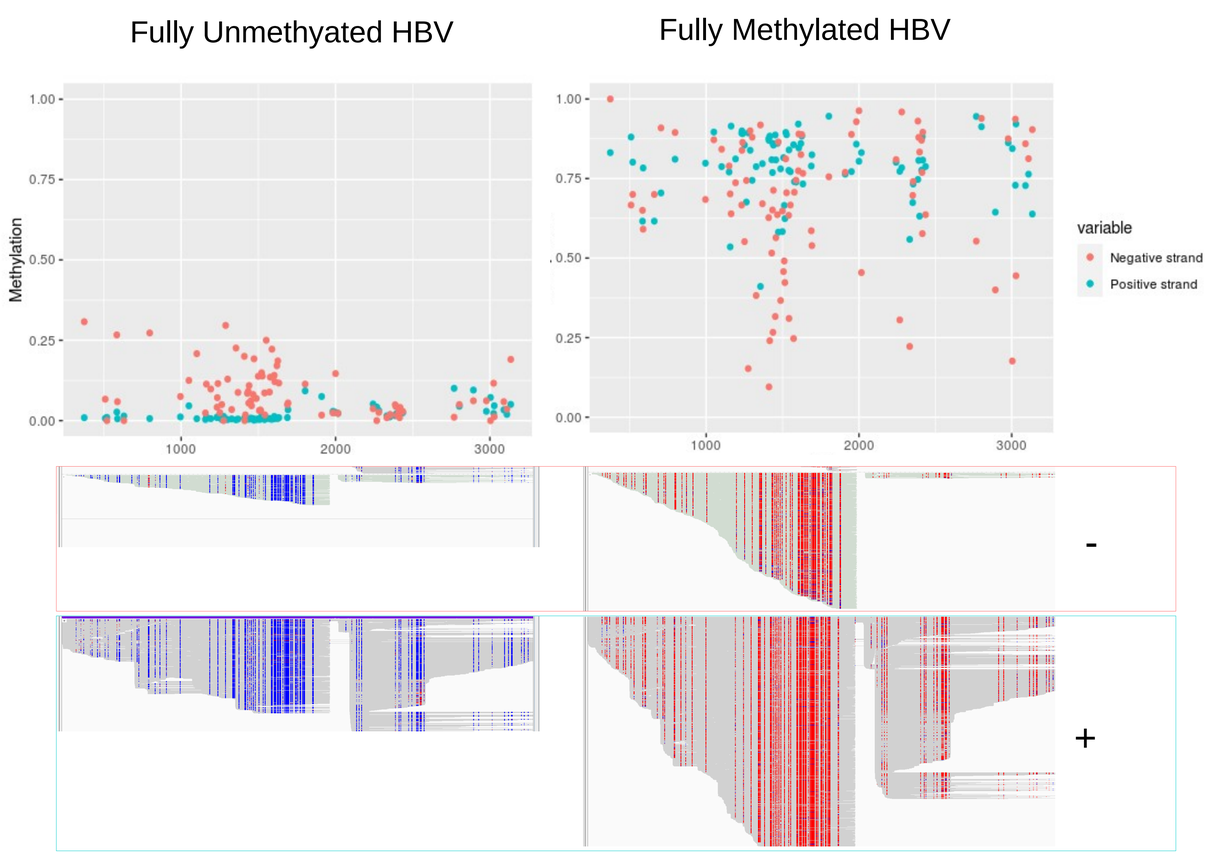
RESULTS: NUCLEAR DNA



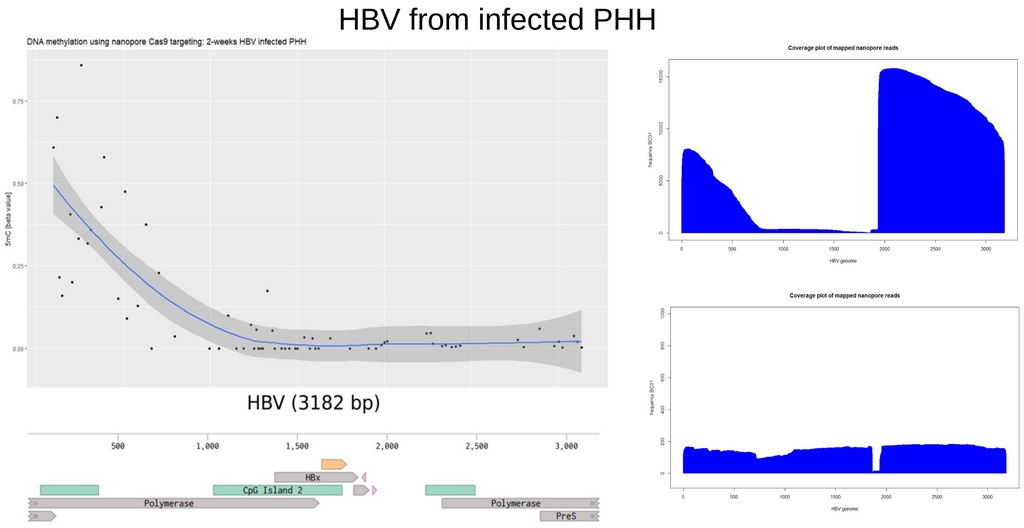


Nuclear DNA extracted from HepaRG cells was used to recapitulate expected patterns of DNA methylation in Fully unmethylated and fully methylated controls. We observed a clear absence of DNA methylation in the FU controls and increased methylation in the FM controls. As such, we were confidant that Nanopore sequencing was able to detect the modified base 5mC in nuclear DNA. However, since the models used were trained on human DNA, we also further validated these tools with HBV FM and FU controls.

RESULTS: HBV DNA



We observed a clear absence of DNA methylation in the FU HBV control. Some residual reads were methylated, however, after visualising the single molecules we could see that the low levels of 5mC observed were likely due to contamination of the original HBV DNA. For the FM controls we also observed the expected result of increased frequency of methylated CpG sites. We also identified several reads that were unmethylated, however, we attributed this to the efficiency of the M.SssI DNA methyltransferase enzyme.



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HBV sequenced from infected PHH exhibited high levels of DNA methylation at CGI 1. This follows expected patterns of HBV genotype D methylation. In addition, a huge disparity in strand coverage was observed; however, this was due to the increased presence of viral replication intermediates, but an important consideration nonetheless.

ACKNOWLEDGEMENTS

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CONCLUSIONS & DIRECTIONS

- We are able to reproduce expected patterns of DNA methylation in hepatitis virus using ONT based sequencing techniques
- It is important to consider strand specific coverage for non-nuclear DNA
- **This approach displays potential for the sensitive detection of viral methylation in cellular infection models as well as large tissue samples**
- More work is needed to detect non CpG methylation using ONT sequencing data as well as continue to develop this tool to be useful for smaller quantities of DNA

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