# Streamlined workflow for bacterial methylation analysis using nanopore data

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Recent advantages in sequencing technologies enable direct detection of DNA methylation such as N4-methylcytosine, 5-methylcytosine or N6-methyladenine without further chemical treatment or specific protocol usage. Therefore, efficiently studying bacterial epigenetics at the whole-genome level is possible. Although obtaining methylation data is straightforward, their further postprocessing remains challenging as there is a lack of comprehensive tools that connect the methylation information with the functional genomic context. Therefore, we proposed a comprehensive computational pipeline for genome-wide methylation profiling using nanopore sequencing data. The proposed approach consists of basecalling and methylation detection using Dorado, followed by quality filtering. Then, *de novo* assembly of genomes is performed, and reads with detected methylation are mapped. Methylation calls are exported as bedMethyl files and further processed using our designed tool MethylomeMiner to link them with genomic features such as genes and intergenic regions. Additionally, it is possible to assign functional categories to methylated genes via COG classification. The pipeline was tested on 10 *Klebsiella pneumoniae* genomes sequenced using the ONT P2S platform. Over 60,000 methylated positions were detected per genome, with N6-methyladenine being the most abundant type. Approximately 47% of methylation sites were located in coding regions, and many affected genes were involved in key cellular functions such as transcription and metabolism. Our solution provides a practical framework for routine exploration of bacterial methylomes, enabling insights into epigenetic regulation and its potential impact on gene expression, adaptation, and pathogenicity.

Acknowledgement: This work was supported by GA23-05845S.