**Methylome Profiling Using Third-Generation Sequencing: A Comparison of PacBio and ONT in a PHA-Producing Bacterium**

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There are two major third-generation sequencing technologies that allow DNA methylation detection on a genome-wide scale: Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT). Since methylation detection is performed directly from raw sequencing signals, specialized tools for particular sequencing platforms need to be used. Here, we used kineticsTools and Modkit to detect methylation in PacBio and ONT, respectively. In this case study, we used *Caldimonas thermodepolymerans*, a thermophilic bacterium that produces polyhydroxyalkanoate (PHA), a naturally occurring biodegradable polymer potentially usable to design bioplastics. Understanding its epigenetic regulation, particularly DNA methylation, will play a crucial role in optimising its biotechnological and industrial potential. Initial comparison of all the reported methylation positions revealed that ONT identified significantly more sites (~4.4 million) than PacBio (~300,000), with all the sites detected by PacBio being identified by ONT, suggesting a difference in their baseline sensitivity or signal reporting between platforms. To get more confident methylation sites, we applied stringent filters (methylation percentage ≥95%) and found that PacBio retained ~29000 sites while ONT retained only ~11000 sites, with ~8000 high-confidence sites concordantly detected by both. This indicated that ~74% of ONT high-confidence calls were supported by PacBio. However, PacBio identified a larger set of filtered sites meeting its criteria; this likely arises primarily due to distinct algorithmic approaches used to assign confidence scores, thus reflecting how each platform processes and thresholds methylation signals. Motif analysis showed stronger agreement for major shared methylation motifs (e.g., CTGCAG 6mA, GAGCTC 4mC, GAYAN...GTG 6mA) with more than 99.6% site-level concordance. However, notable platform-specific differences were observed with ONT showing several uniquely identified motifs with a high fraction likely involving 5mC modification, while PacBio reported only a few low-fraction motifs. Our study shows that while both platforms reliably capture methylation patterns, algorithm sensitivity and filtering threshold differences notably influence detection outcome, especially for non-canonical motifs. Understanding these platform-specific differences is essential for studying epigenetic regulation of genes involved in PHA biosynthesis. It guides future efforts to optimise industrial microbes through methylome engineering and synthetic biology approaches.

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