DNA methylation from bacteria and microbiome using nanopore technology

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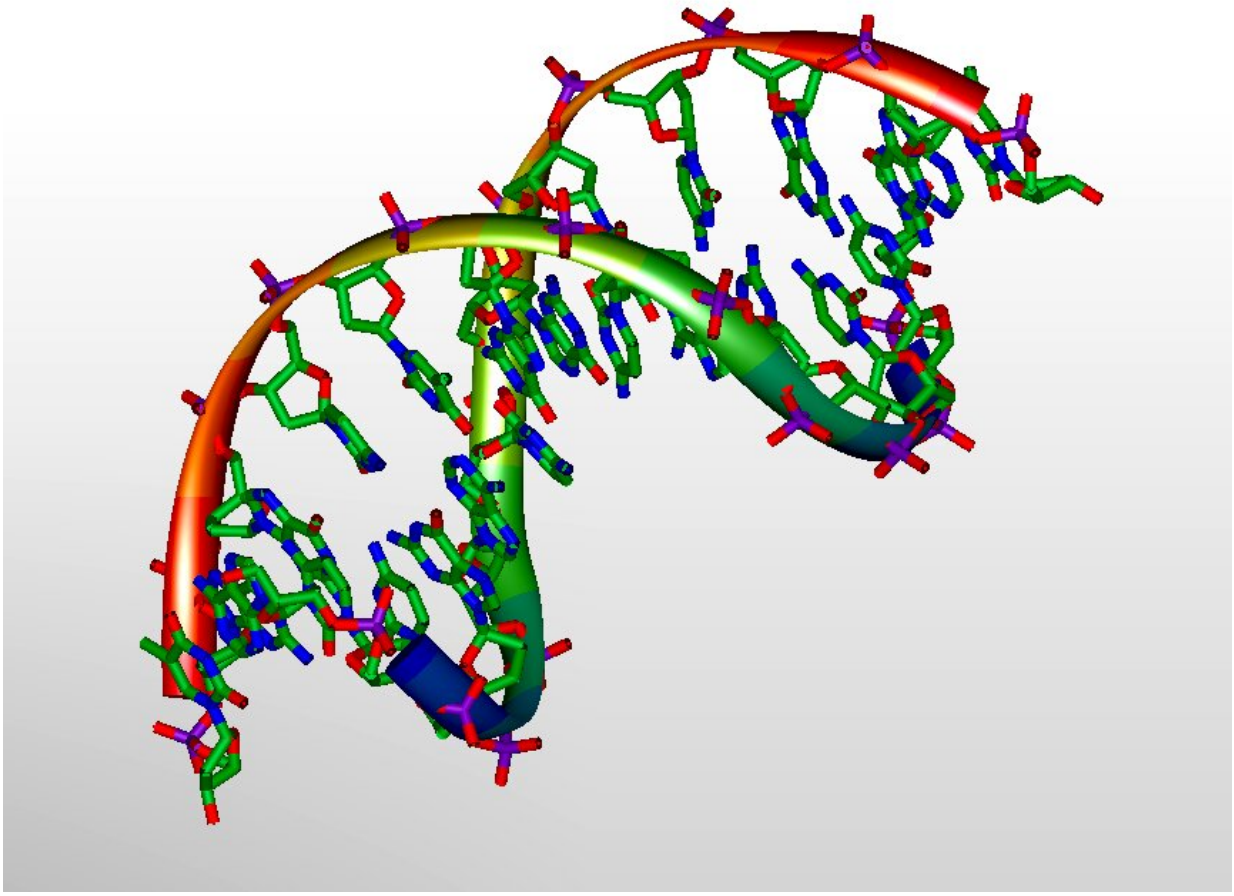

3D-model of DNA. Credit: Michael Ströck/Wikimedia/ GNU Free Documentation License

Bacterial DNA methylation occurs in diverse sequence contexts and plays important functional roles in cellular defense and gene regulation. An increasing number of studies have reported that bacterial DNA methylation has important roles affecting clinically relevant phenotypes such as virulence, host colonization, sporulation, biofilm formation, among others. Bacterial methylomes contain three primary forms of DNA methylation: N6-methyladenine (6 mA), N4-methylcytosine (4mC) and 5-methylcytosine (5mC). The widely used bisulfite sequencing for DNA methylation mapping in mammalian genomes are not effective at resolving bacterial methylomes. Single molecule real-time (SMRT) can effectively map 6mA and 4mC events, and have empowered the study of >4,000 bacterial methylomes in the past ten years. However, SMRT sequencing cannot effectively detect 5mC methylation.

In a new study, researchers developed a method that enables nanopore sequencing for broadly applicable methylation discovery and applied it to individual bacteria in the gut microbiome. In addition, they demonstrated the use of DNA methylation for high-resolution microbiome analysis, mapping mobile genetic elements with their host genomes directly from microbiome samples.

Antibiotics resistance poses great risk to public health. To best combat bacterial pathogens, it is important to discover novel drug targets. Increasing evidence suggests that bacterial DNA methylation plays important roles in regulating bacterial physiology such as virulence, sporulation, biofilm formation, pathogen-host interaction, etc. The new method in this work allows researchers to more effectively discover novel DNA methylation from bacterial pathogens, opening new opportunities to discovery of targets for new inhibitors.

Despite growing appreciation for the role of microbiome in human health, comprehensive characterization of microbiomes remains

difficult. To effectively harness the therapeutic power of microbiome, it is important to understand the specific bacteria species and particular strains within the human microbiome. This new method combines the power of long read sequencing and bacterial DNA methylation to resolve complex microbiome samples into individual species and strains. So it will also empower higher-resolution characterization of the human microbiome for medical applications. The power of methylation-based mapping of mobile genetic elements (often encoding antibiotics resistance genes) to their host genomes also helps track the transmission of genes that confer antibiotic resistance.

By examining three types of DNA methylation in a large diversity of sequence contexts, we observed that nanopore sequencing signal displays complex heterogeneity across methylation events of the same type. To capture this complexity and enable nanopore sequencing for broadly applicable methylation discovery, the researchers generated a training dataset from an assortment of bacterial species and developed a novel method that couples the identification and fine mapping of the three forms of DNA methylation into a multi-label classification design. They evaluated the method and then applied it to individual bacteria and the mouse gut microbiome for reliable methylation discovery. In addition, they demonstrated in the microbiome analysis the use of DNA methylation for binning metagenomic contigs, associating mobile genetic elements with their host genomes, and for the first time, identifying misassembled metagenomic contigs.

Mount Sinai's Gang Fang says, "DNA methylation plays important roles in the human genome, and is widely studied in health and various diseases. DNA methylation is also prevalent in bacteria, but our current understanding is still at a relatively early stage."

An increasing number of studies have reported that bacterial DNA methylation plays important roles in regulating medically relevant

phenotypes of pathogenic bacteria, such as virulence, biofilm formation, virulence, sporulation, among others.

Broader and deeper study of bacterial DNA methylation requires reliable technologies, and the new method fills an important gap in that it now enables the use of Nanopore sequencing to make new discoveries from bacterial genomes. This novel method has broad utility for discovering different forms of DNA methylation from bacteria, assisting functional studies of epigenetic regulation in bacteria, and exploiting bacterial epigenomes for more effective metagenomic analyses.

The study is published in *Nature Methods*.

More information: Discovering multiple types of DNA methylation from bacteria and microbiome using nanopore sequencing, *Nature Methods* (2021). DOI: 10.1038/s41592-021-01109-3

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