Single Molecule, Real-Time (SMRT®) Sequencing **directly detects DNA modifications** by measuring variation in the polymerase kinetics of DNA base incorporation during sequencing. With high throughput, long reads, and the sensitivity to detect epigenetic modification **without amplification or chemical conversions**, the PacBio® Systems offer scalable solutions for assessing DNA modifications in bacterial and eukaryotic genomes.

- Characterize bacterial DNA modifications and methyltransferase recognition motifs
- Identify hyper- and hypo-methylated CpG islands to explore eukaryotic gene expression and regulation
- Observe allele-specific methylation by using long reads to phase SNPs when bisulfite sequencing
- Leverage epigenomic data to cluster contigs and associated plasmids in metagenomic communities
- Detect strand-specific modification such as hemimethylation
- Uncover DNA modifications and methylation events with hypothesis-free analysis

**GENOME-WIDE METHYLATION PATTERNS COMPARED IN 230 DIVERSE PROKARYOTES**

**N^6-ADENINE METHYLATION FOUND IN MAMMALIAN GENOME**

(a) SMRT Sequencing workflow used to identify N^6-adenine methylation in Alkbh1 knockout embryonic stem cells. (b) The levels of 6mA are controlled by Alkbh1, whereby increasing its levels in ES cells led to gene silencing. (c) A downregulation of the dax1 gene, located 30 kb from 6mA-enriched young full-length L1 repeat element, was also observed.

Phylogenetic tree of 230 sequenced prokaryotic organisms. Outer bars indicate the number and types of active MTases detected per genome.

pacb.com/epigenetics
FROM DNA TO EPIGENETIC INFORMATION

Library Preparation
- Prepare long-insert SMRTbell™ library
- For SMRT bisulfite sequencing, generate SMRTbell libraries from long amplicons (1–2 kb) following bisulfite treatment
- Size-selection options enrich for longest inserts
- Use recommended gDNA library inputs
  - > 5 μg for large genome project
  - Low-input options (100 ng)
- Library automation supported

SMRT Sequencing with PacBio Systems
- Take advantage of the Sequel™ System to reduce project costs and generate 7X more reads compared with the PacBio RS II
- Achieve ~10 kb average read lengths, with some reads as long as 60 kb
- Simultaneously capture epigenetic information

Data analysis with SMRT Analysis or PacBio DevNet
- Characterize bacterial methylomes with SMRT Analysis epigenomic tools for modification detection and motif analysis
- Identify hypo- and hyper-methylated CpG regions in eukaryotic genomes with > 20-fold coverage
- Detect CpG methylation across 1.5 kb regions with SMRT bisulfite sequencing

KEY REFERENCES