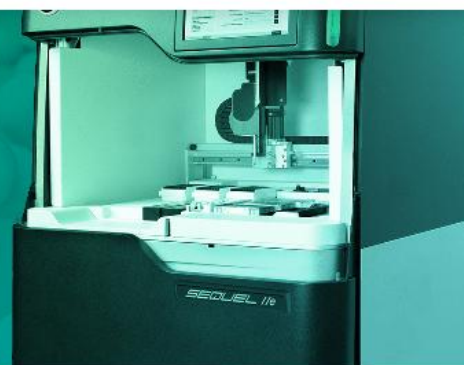


EXHIBIT F

EPIGENETICS

See a new dimension to genomes with the most accurate sequencing reads and DNA methylation in a single experiment

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SEQUENCING METHODS

[Whole genome](#)
[Targeted](#)
[RNA](#)
[Epigenetics](#)
[Metagenomics](#)

EPIGENETICS SOLUTIONS

Access the epigenome to explore DNA modifications without any special library preparation needed



HUMAN AND EUKARYOTE EPIGENETICS

Use 5-base HiFi sequencing to study epigenetics of humans and other eukaryotes. Measure 5mC methylation at CpG sites directly from the sequencing instrument without the need for bisulfite treatment or other special library preparation methods.

[Download example data](#)



MICROBIAL EPIGENETICS

Build a more comprehensive picture of how microbes use epigenetic modification for immune evasion by characterizing DNA modifications and methyltransferase recognition motifs for 4mC and 6mA.

[Learn more](#)

INTRODUCTION TO EPIGENETICS WITH LONG-READ SEQUENCING

The unique chemistry of long-read HiFi sequencing technology enables researchers to directly reveal the epigenetic landscape of samples.

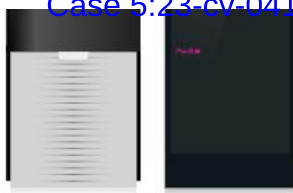
HiFi sequencing provides the most accurate genome-wide calls for SNVs, indels, and SVs. With 5-base HiFi sequencing, the same sequencing library identifies genome-wide methylation patterns associated with gene activity and other biological functions. Further, long HiFi reads enable phasing of the genetic and epigenetic variants into parental haplotypes.



HiFi SEQUENCING

MEASURE THE GENOME AND EPIGENOME IN A SINGLE SEQUENCING RUN





IN A SINGLE SEQUENCING RUN

On-instrument 5-base HiFi sequencing detects 5mC methylation in standard sequencing runs without any changes to library prep or sequencing workflows required.

EPIGENETIC SEQUENCING - HOW PACBIO COMPARES

HiFi sequencing is the only technology that provides accurate genetic and epigenetic information from a single sequencing library.

	Methylation microarrays	Short-read sequencing	Nanopore sequencing	HiFi 5-base sequencing
SNVs	×	✓	✓	✓
Indels	×	✓	×	✓
SVs	×	×	✓	✓
Haplotype phasing	×	×	Limited	✓
Genome-wide	×	✓	✓	✓
5mC in CpG contexts	Limited	Requires special library preparation	Requires special data processing. Conflated with basecalling	✓

ADVANTAGES OF EPIGENETIC ANALYSIS WITH 5-BASE HIFI SEQUENCING

With the power of long-read sequencing, you can achieve:

Epigenetics in every run — no bisulfite treatment required

Unlike methods that require chemical conversion of DNA, HiFi sequencing detects modifications in native DNA through impacts on the kinetics of base incorporation.

High accuracy of sequence and methylation

Methylation detection with HiFi sequencing is highly concordant to bisulfite sequencing.

Access the full genome

Access difficult regions of the genome like repeats and centromeres that are beyond the reach of short-read sequencing.

Phasing

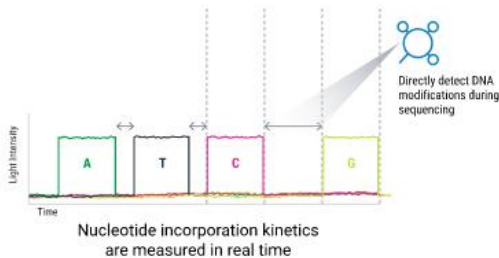
Identify allele-specific methylation, whether due to parental imprinting, genetic variation, or repeat expansions.

“SMRT sequencing is opening up new diagnostic avenues, such as the ability to determine tandem repeat lengths, interruptions, and even epigenetics in a single test at base pair resolution.”¹

— Ardui, et al., 2018¹

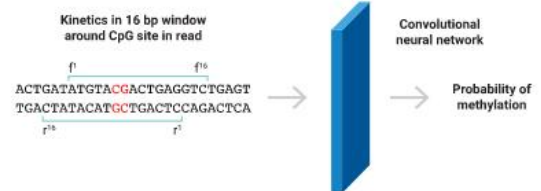
HOW 5-BASE HIFI SEQUENCING CALLS METHYLATION STATUS

HiFi sequencing provides two channels of information: fluorescence and kinetics. Utilizing both enables highly accurate reads (fluorescence) plus methylation status (kinetics) from a single library.



HiFi sequencing observes a polymerase incorporating fluorescently labeled nucleotides complementary to a native DNA strand. The label identifies the base (A, C, G, T). Epigenetic modifications like 5mC impact polymerase kinetics — how fast bases are incorporated. No special library prep is required.

- A convolutional neural network model processes polymerase kinetics to determine the methylation status of each CpG site in a HiFi read.
- The model runs directly on the Sequel IIe system and is also available in SMRT Link.
- Methylation status is output using the [BAM standard MM and ML tags](#).



APPLICATION BRIEF

MEASURING DNA METHYLATION WITH 5-BASE HIFI SEQUENCING

Genome-wide detection and phasing of genetic and epigenetic variants from a single library prep. HiFi sequencing of a single sample detects methylation patterns across the genome, such as hypomethylation at transcription start sites. Sequencing multiple samples identifies differential methylation.

[Learn more](#)



PB-CPG-TOOLS

The pb-CpG-tools collection provides tools for secondary analysis of CpG methylation data from PacBio HiFi reads. Starting from a pileup of HiFi reads with methylation tags, the tools calculate the percent of reads methylated at every CpG site in the genome.

[Access tools](#)



DIRECT DETECTION OF DNA METHYLATION

See how scientists use PacBio sequencing to detect methylation with basepair resolution.

[Read publication](#)

GENOME-WIDE DETECTION OF CYTOSINE METHYLATION

Read how researchers use the kinetics in HiFi reads to determine methylation status at CpG sites.

[Access manuscript](#)

DNA 5MC DETECTION AND METHYLATION PHASING

Read how circular consensus sequencing enables genome-wide detection of cytosine methylation by single molecule real-time sequencing.

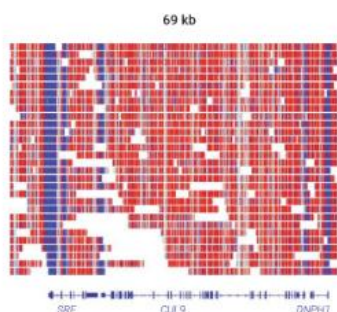
[Access manuscript](#)

EXPLORE

Did you know we have over 10,000 articles, reports, papers, and videos related to epigenetic research?

[Explore resources](#)

WHAT 5-BASE SEQUENCING REVEALS



REGIONAL METHYLATION PATTERNS

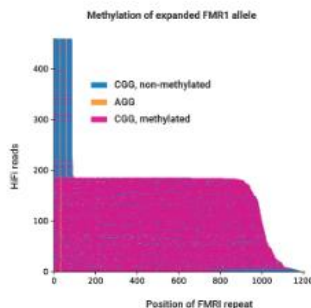
Methylation levels vary across the genome in many species. In vertebrates like human most CpG sites are methylated. Active gene transcription start sites are often hypomethylated.

In this example genomic region, 5-base HiFi sequencing of the human HG002 sample shows overall hypermethylation (red) with hypomethylation (blue) specifically at transcription start sites.

PHASED GENETICS AND EPIGENETICS

HiFi sequencing enables simultaneous phasing of reads into maternal and paternal haplotypes and detection of methylation. This reveals allele-specific methylation patterns, which can be due to genetic variation (where epigenetic status is affected by a difference in sequence) or parental imprinting (where epigenetic status is affected by whether a chromosome was inherited from the mother or father).

In this example, the HG002/3/4 trio from *Genome in a Bottle*, HiFi reads show the expected maternal imprinting at the gene *PEG3*. HiFi sequencing allows phasing of the haplotypes per sample, the trio identifies which allele is transmitted from which parent, and 5-base sequencing shows allele-specific methylation.



HiFi sequencing phases and identifies hypermethylation of expanded *FMR1* repeats in NA07537.

METHYLATION AND DISEASE

Atypical methylation patterns contribute to rare diseases like Prader-Willi syndrome and are important factors in pathogenic repeat expansion, such as the CGG expansion at the *FMR1* locus that cause Fragile X syndrome. With high accuracy, long reads, and methylation detection, HiFi sequencing is ideal for characterizing these repeat expansions.

APPLICATIONS FOR EPIGENETIC ANALYSIS

5mC at CpG sites is the predominant epigenetic mark in vertebrates like humans. It is also useful — though not comprehensive — in other eukaryotic species such as plants, which employ 5mC methylation in CpG contexts alongside other motifs. For microbes, which employ a wider variety of modifications, [alternative analysis tools are recommended](#).

Methylation	Species	HiFi 5-base sequencing
5mC at CpG sites	Human + other vertebrates	✓
5mC at various motifs	Other eukaryotes, including plants	✓ Useful though partial view
4mC and 6mA	Microbes	Enabled through SMRT Link microbial genome analysis

EPIGENETIC SEQUENCING WORKFLOW AT A GLANCE



STANDARD LIBRARY PREP

- No bisulfite or other enzymatic treatment

[Library prep kits](#)



STANDARD SEQUENCING RUN

- Simultaneously detect accurate base sequence and accurate epigenetic modifications

[SMRT sequencing](#)



SIMPLE ANALYSIS

- Detect microbial base modifications and motifs with the microbial genome analysis application in SMRT Link
- Call 5mC at CpG sites directly from the sequencing instrument or in SMRT Link
- Visualize 5mC annotation directly in IGV

COMMON QUESTIONS ABOUT PACBIO EPIGENETIC SEQUENCING

WHICH EPIGENETIC MARKS DOES HIFI SEQUENCING IDENTIFY?



DOES 5-BASE HIFI SEQUENCING DISTINGUISH 5HMC FROM 5MC?



FEATURED LONG-READ SEQUENCING SYSTEMS

5-base genome sequencing is now possible. With PacBio long-read sequencers you can gain immediate access to the epigenome with no special workflow or data processing steps required.

[Explore products](#)

TALK WITH AN EXPERT

If you have a question, need to check the status of an order, or are interested in purchasing an instrument, we're here to help.

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PacBio

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