



Whole Genome Bisulfite Sequencing (WGBS)



DNA methylation at the C5 position of cytosine plays a crucial role in gene expression and chromatin remodelling, and perturbations in methylation patterns are implicated in the development of cancer, neurodegenerative diseases, and neurological disorders. Therefore, the mapping of methylated bases (the methylome) is critical to understanding gene expression and other processes subject to epigenetic regulation.

Medikonia provides whole genome sequencing of bisulfite-converted DNA, as an effective method to identify individually methylated cytosines on a genome-wide scale. Methylome analysis is an increasingly valuable research tool with a range of applications, including studies on gene regulation, stem cell differentiation, embryogenesis, aging, cancer and other diseases, and phenotypic diversity and evolution in plants and animals.

Image by Christoph Bock (Max Planck Institute for Informatics) (Own work)
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The Advantage

- Extensive experience: over 400 samples successfully sequenced and a bioinformatics analysis team composed entirely of Ph.D. scientists.
- Unsurpassed data quality: We guarantee that $\geq 80\%$ of bases have a sequencing quality score $\geq Q30$, exceeding Illumina's official guarantee of $\geq 75\%$.
- Comprehensive data analysis: We use widely-accepted mainstream software such as Bismark and a mature in-house pipeline for mapping, DMR analysis, functional analysis and data visualization.

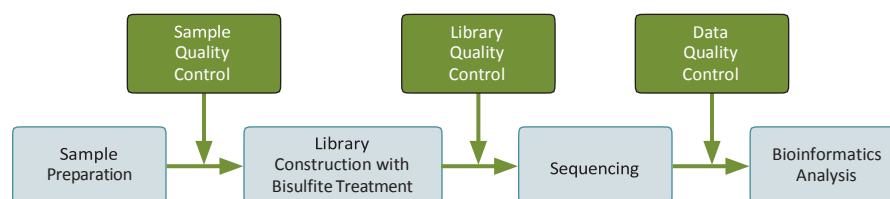
Project Workflow

SEQUENCING STRATEGY

- 200 bp ~ 400 bp insert DNA library
- HiSeq platform, paired-end 150 bp

DATA QUALITY GUARANTEE

- $Q30 \geq 80\%$



SAMPLE REQUIREMENTS

- DNA amount: $\geq 5 \mu\text{g}$
- DNA concentration: $\geq 50 \text{ ng}/\mu\text{l}$
- Purity: $\text{OD}_{260/280} = 1.8 - 2.0$ without degradation or RNA contamination

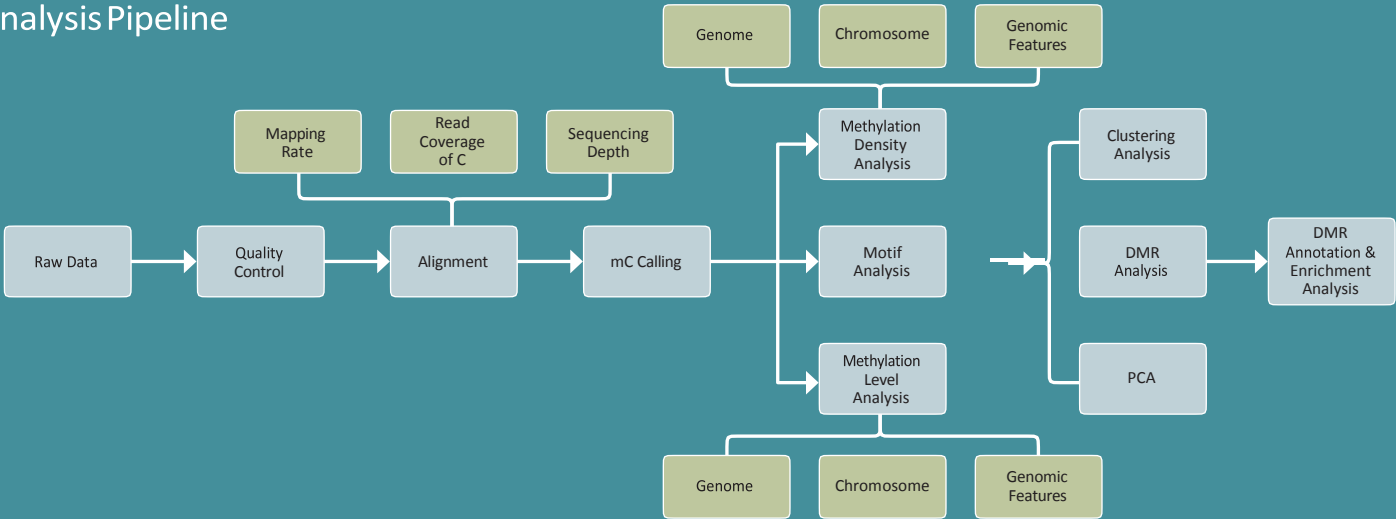
TURNAROUND TIME

- 15 working days from verification of sample quality (without data analysis)
- The data analysis turnaround is project-dependent.

RECOMMENDED SEQUENCING DEPTH

- $\geq 30\times$

Analysis Pipeline



Project Example

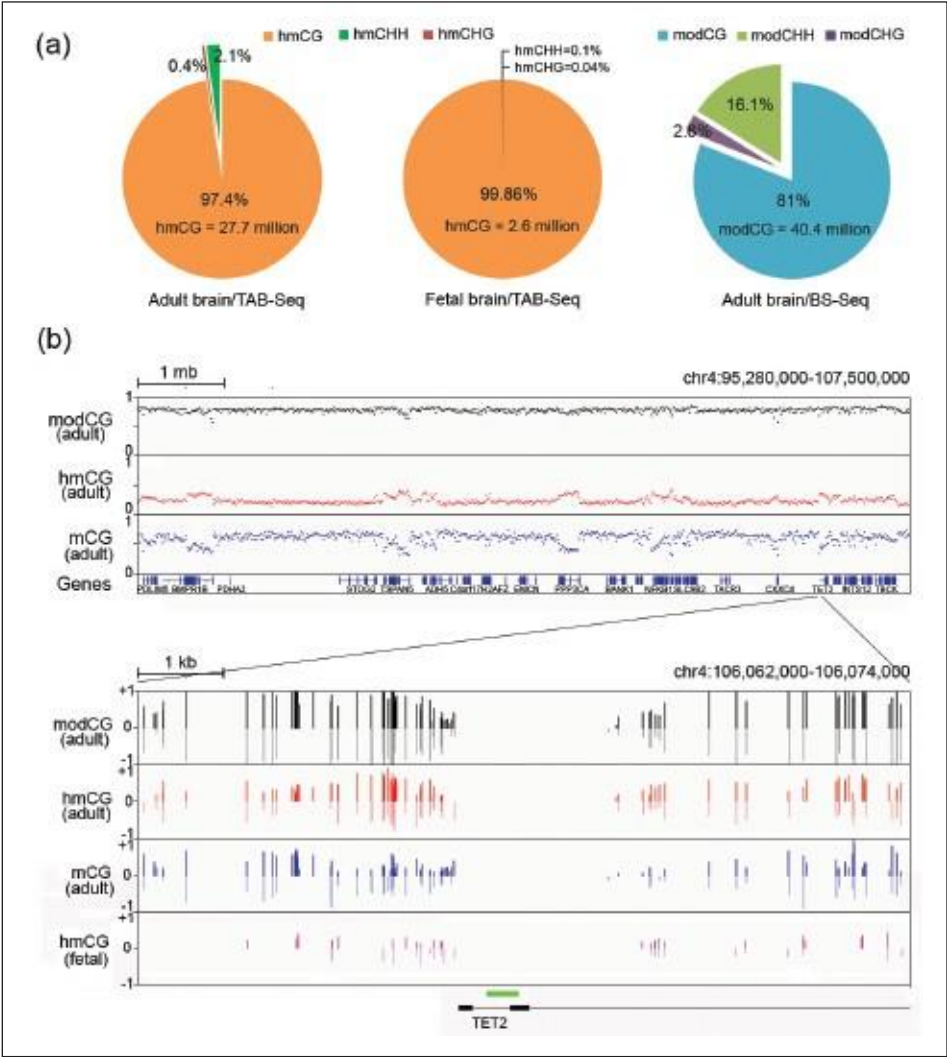
The following study utilized Medikonia’s expertise in WGBS.

Whole-Genome Analysis of 5-Hydroxymethylcytosine and 5-Methylcytosine at Base Resolution in the Human Brain

Genome Biology, 15: R49 (2014).

Different forms of DNA methylation may differentially modulate gene expression and chromatin structure, with consequences for organ development and function. There is particular interest in the role of 5-hydroxymethylcytosine (hmC), which is abundant in some types of mammalian cells. In this study, genome-wide sites of hmC and 5-methylcytosine (mC) in the human brain were mapped to single-base resolution using, respectively, bisulfite sequencing and Tet-assisted bisulfite sequencing. Strand biases were observed with higher levels of hmC and mC associated, respectively, with the sense and antisense strand of gene bodies. Levels of hmC increased during developmental stages, with highest levels present in adult brains, and hydroxymethylation-enriched sites included actively transcribed regions and 5’ splicing sites at exon-intron boundaries, indicating roles for hmC in the regulation of gene expression and splicing.

Base-resolution hydroxymethylome and methylome in the human brain. (a) The percentages of hmCs or modCs in the adult or the fetal brain in the contexts of CG, CHH, and CHG. (b) Examples of the hmC, mC, and total modification (hmC+ mC) profiles are shown for a genomic region of 12 Mb on chromosome 4 as a scatterplot (upper panel) and for a 12 kb region surrounding the TSS of the TET2 gene as a bar chart. The green box indicates the CpG island located in the TET2 promoter.



Medikonia Data

REPRESENTATIVE SAMPLES SHOWING DATA QUALITY OF MEDIKONIA’S WGBS:

Sample Name	Raw Reads	Raw Bases	Clean Reads	Clean Bases	Error Rate	Q20	Q30	GC Content	BS Conversion Rate
Sample1	750864918	112.63G	686418034	102.96G	0.03%	94.62%	86.52%	23.01%	99.29%
Sample2	762188782	114.14G	618495260	92.61G	0.04%	94.35%	86.41%	21.08%	99.66%
Sample3	789026912	118.35G	721080112	108.16G	0.02%	96.54%	90.63%	21.12%	99.66%
Sample4	784535120	117.68G	760489944	114.07G	0.03%	92.81%	85.49%	20.70%	99.63%

EXAMPLES OF PUBLICATIONS USING MEDIKONIA’S EXPERTISE

Journal	Title
Genome Biology, 15: R49 (2014)	Whole-genome analysis of 5-hydroxymethylcytosine and 5-methylcytosine at base resolution in the human brain.
Nature Communications, 3:850 (2012)	An atlas of DNA methylomes in porcine adipose and muscle tissues.