Whole Genome Bisulfite Sequencing
(Gene Methylation)

1. Sample Requirements

Sample Type	Required Amount	Volume	Concentration	Purity
Genomic DNA	⩾ 100 ng	⩾ 20 µL	⩾ 5 ng/µL	OD260/280=1.8-2.0; 0 < OD260/230 < 3; No degradation or contamination

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000/NovaSeq X Plus
Read length	Paired-end 150
Recommended Sequencing Depth	⩾ 30× coverage for the species with reference genome;
Data quality	Guaranteed ⩾ 85 % bases with Q30 or higher
Turnaround time	Typical 24 working days for fewer than 24 samples from project verification to data releasing without bioinformatic analysis

3. Data Analysis Contents

Standard analysis
Data quality control (filtering reads containing adapter or with low quality; Q20, Q30, error rate distribution, GC distribution, total bases)
Mapping onto reference genome (mapping rate, duplication rate, sequencing depth, reads coverage)
mCs detection, methylation level calculation
(1) Methylation level and frequency distribution in different sequence context (CG, CHG, CHH)   (2) Methylation level and frequency distribution in different chromosomes   (3) Methylation level and frequency distribution in different functional elements (promoter, 5'UTR, exon, intron, 3'UTR)
Correlation and clustering
Differentially methylated regions (DMRs), Differentially Methylated Promoter (DMPs) detection and annotation
Function enrichment (Gene Ontology and KEGG Pathway) of DMR-associated genes and DMP-associated genes
Visualization of BS seq data