

Reduced Representation Bisulfite Sequencing (RRBS-Genome Methylation)

1. Sample Requirements

Sample Type	Required Amount	Volume	Concentration	Purity
Genomic DNA	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	OD _{260/280} =1.8-2.0; 0 < OD _{260/230} < 3; No degradation or contamination

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 150
Recommended Sequencing Depth	10 Gb raw data per sample
Data quality	Guaranteed ≥ 85% bases with Q30 or higher
Turnaround time	Typical 7~8 weeks for fewer than 20 samples from project verification to data releasing

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)
Alignment to reference genome (mapping rate, sequencing depth, reads coverage)
Quality controls for 5-mC identification (MspI digestion efficiency analysis and BS conversion rate)
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 functional elements (promoter, 5' UTR, exon, intron, 3' UTR)
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