

## Circular RNA Sequencing

### 1. Sample Requirements

Sample Type	Amount	Volume	Concentration	*RIN (Agilent 2100™)	Purity (NanoDrop™)
Total RNA	≥ 2 µg	≥ 20 µL	≥ 50 ng/µL	Animal ≥ 7, Plant/Fungus ≥ 6.5, with smooth baseline	OD260/280 ≥ 2.0; no degradation or contamination

\*RIN: RNA Integrity Number

### 2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 150 bp
Recommended sequencing depth	≥ 40 million read pair per sample
Data quality	Guaranteed ≥ 80% bases with Q30 or higher
**Turnaround time	Within 5 weeks from project verification to data releasing without bioinformatic analysis

\*\*Turnaround time varies depending on the project volume.

### 3. Data Analysis Contents

Standard Analysis	
Data Quality Control: Filtering reads containing adapter or uncertain nucleotides or of low quality, and statistic summary of data quality	
Mapping Clean Reads to Reference Genome	
circRNA Identification	Length Distribution of circRNAs
	Sources of circRNAs
	Distribution of circRNA on the Chromosomes
Transcript Expression Quantification	
Correlation Analysis Between Groups (Only for Samples with biological replicates)	
Differential Expression Analysis (Only for Compared Groupings)	
Functional Analysis of Differentially Expressed circRNA Source Genes (Only for Compared Groupings)	Gene Ontology (GO) Enrichment Analysis
	Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis
CircRNA Target Gene Prediction	