# 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; OD260/230 ≥ 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 ≥ 85% 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 | |