Small 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 ⩾ 7.5, Plant/Fungus ⩾ 7, with smooth baseline	OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation or contamination
Exosome RNA	⩾ 20 ng	⩾ 10 µL	-	Peak between 25-200nt, FU> 10, with no peak > 2000nt	

*RIN: RNA Integrity Number

2.Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Single-end 50 bp
Recommended sequencing depth	⩾ 10 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 (for miRNA)	
Data Quality Control: Filtering reads containing adapter or uncertain nucleotides or of low quality, and statistic summary of data quality	
Summary of Length Distribution	
Common and Specific Sequence Summary	
Mapping Clean Reads to Reference Genome	
Functional Classification and Annotation of small RNA	Identification Known miRNA
	Non-coding Transcripts Annotation
	Repeat Sequence Annotation (Annotation of the reference genome should be provided)
	Exon and Intron Annotation
	Novel miRNA Prediction
miRNA Base Edit	
miRNA Family Analysis	
miRNA Expression Quantification	

Correlation Analysis Between Groups **(Only for Samples with biological replicates)**	
Differential Expression Analysis **(Only for Compared Groupings)**	
Target Gene Prediction of Known and Novel miRNA	
Functional Analysis of **Differentially Expressed Genes (Only for Compared Groupings)**	Gene Ontology (GO) Enrichment Analysis
	Kyoto Encyclopedia of Genes and Genomes **(KEGG)** Pathway Enrichment Analysis

Advanced Analysis (for association of mRNA and miRNA) (mRNA-seq data for corresponding samples is needed simultaneously.)	
Conjunction Analysis of Differentially Expressed mRNA and Differentially Expressed miRNA Downstream Targeting genes	Cluster Analysis
	GO Enrichment Analysis
	KEGG Enrichment Analysis
	Protein Protein Interaction Analysis

Advanced Analysis (for association of mRNA and miRNA) (mRNA-seq data for corresponding samples is needed simultaneously.)	
Data Quality Control: Filtering reads containing adapter or uncertain nucleotides or of low quality, and statistic summary of data quality	
Summary of Length Distribution	
Mapping Clean Reads to Reference Genome	
piRNA Analysis	Identification of piRNA
	piRNA Base Bias Analysis
	Quantification Analysis
	Chromosome Distribution Analysis
	Source Gene Analysis
	Functional Enrichment Analysis
piRNA Cluster Analysis	Quantification Analysis
	Chromosome Distribution Analysis
	Differential Expression Analysis
	Adjacent Gene Analysis

Standard Analysis (for siRNA) (only for samples with virus infection)
Data Quality Control: Filtering reads containing adapter or uncertain nucleotides or of low quality, and statistic summary of data quality
Summary of Length Distribution
Sequence Assembly
Contig Classification and Annotation
Mapping Clean Reads to Reference (Host Genome, Nr Database, Nt Database, Virus ReSeq Database)
Summary of Virus Species Candidate