

1. Sample Requirements

| Sample Type | Remarks | Amount | RIN | Volume | Concentration | Purity |
|--------------|----------------------|---------|---|---------|---------------|-------------------------------------|
| | | | (Agilent 2100TM) | | | (NanoDropTM/Agarose gel) |
| Total RNA | Strongly Recommended | ≥ 4 µg | Animal ≥ 6.5, Plant ≥ 6, with smooth baseline | ≥ 20 µL | ≥ 50 ng/µL | OD260/280>2.0 ; |
| | Required | ≥ 2 µg | | | | No degradation or DNA contamination |
| Exosomal RNA | Strongly Recommended | ≥ 60ng | Peak between 25-200nt, FU> 10, no peak > 2000nt | ≥ 10 µL | - | OD260/280>2.0; |
| | Required | ≥ 40 ng | | | | No degradation or DNA contamination |

2. Sequencing Parameters

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|------------------------------|---|
| Platform | Illumina Novaseq 6000 |
| Read length | Paired-end 150 |
| Recommended Sequencing Depth | ≥15G~20G raw data per sample |
| Data quality | Guaranteed ≥80% bases with Q30 or higher |
| Turnaround time | Within 18 working days from project verification to data releasing without bioinformatic analysis |

3. Data analysis

| Standard Analysis (Species with Reference) | |
|--|---|
| 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 and Transcript Prediction | |
| Structural Analysis | Alternative Splicing (AS) Quantification and Differential Expression Analysis (Only for Grouping with control samples) |
| | SNP/InDel Detection and Annotation Analysis |
| lncRNA prediction | Transcript Assembly |

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|---|---|
| | Filtering of Candidate lncRNA |
| Transcript Expression Quantification | |
| Correlation Analysis Between Groups (Only for Samples with biological replicates) | |
| Differential Expression Analysis (Only for Grouping with control samples) | |
| lncRNA Target Gene Prediction | Co-location Prediction of lncRNA and mRNA |
| | Co-expression Prediction of lncRNA and mRNA |
| Functional Analysis of Differentially Expressed mRNA and Differentially Expressed lncRNA Targeting Genes (Only for Grouping with control samples) | Gene Ontology (GO) Enrichment Analysis |
| | Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis |
| | Transcription Factors Functional Annotation analysis |
| | Protein Protein Interaction Analysis |
| Fusion Gene Analysis (Only for Grouping with TUMOR or CANCER tissue/cell line of human & mouse) | |