

## Long Non-coding RNA Sequencing

### 1. Sample Requirements

Sample Type	Amount	Volume	Concentration	*RIN (Agilent 2100™)	Purity (NanoDrop™)
Total RNA	≥ 500 ng	≥ 15 μL	≥ 50 ng/μL	≥ 5.5, with smooth baseline	OD260/280 >2.0 ; OD260/230 ≥ 2.0; no degradation or contamination
Exosome RNA	≥ 5 ng	≥ 15 μL	-	Freagments between 25-200 nt, FU> 10	

\*RIN: RNA Integrity Number  
FU: Fluorescent unit

### 2. Sequencing Parameters

Platform	illumina Novaseq 6000
Read length	Paired-end 150
Recommended sequencing depth	≥ 12G raw data per sample
Data quality	Guaranteed ≥ 85% bases with Q30 or higher
Turnaround time	Within 3 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 and Transcript Prediction	
Structural Analysis	Alternative Splicing (AS) Quantification and Differential Expression Analysis (Only for Compared Groupings)
	SNP/InDel Detection and Annotation Analysis
lncRNA Prediction	Transcript Assembly
	Filtering of Candidate lncRNA
Transcript Expression Quantification	
Correlation Analysis Between Groups (Only for Samples with biological replicates)	
Differential Expression Analysis (Only for Compared Groupings)	
lncRNA Target Gene Prediction	Co-location Prediction of lncRNA and mRNA
	Co-expression Prediction of lncRNA and mRNA
Functional Analysis of <b>Differentially Expressed mRNA and Differentially Expressed lncRNA Targeting Genes (Only for Compared Groupings)</b>	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)	