

RIP-Seq

1. Sample Requirements

Sample Type	Required Amount	Volume	Concentration	Peaks Distribution	Purity
Enriched RNA Sample	≥ 30 ng Recommend ≥ 50 ng	≥ 20 μ L	≥ 1.5 ng/ μ L	Fragments should be longer than 1000 bp.	OD _{260/280} >2.0 No degradation or contamination

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 150
Recommended Sequencing Depth	≥ 20 million read pair per sample for the species with reference genome;
Data quality	Guaranteed $\geq 85\%$ bases with Q30 or higher
Turnaround time	31 days for fewer than 48 samples from project verification to data releasing

3. Data Analysis Contents

Standard analysis
Data quality control (get rid of reads containing adapter or with low quality; Q20, Q30, error rate distribution, GC distribution, total bases)
Mapping onto reference genome (mapping rate, reads distribution, rRNA content)
Peak calling
Motif prediction
Peak annotation (downstream or overlapping gene, peak distribution in functional region of gene and transcript)
Functional analysis of peak-associated genes (Gene Ontology, pathway)
Visualization of RIP-seq data