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RNA-Seq Workflow Guide

A one-page reference from raw sequencing reads to differential expression and biological interpretation.

Workflow

Step	Purpose	Typical outputs
1. Experimental design	Define conditions, controls, replicates and metadata.	Sample sheet
2. Obtain FASTQ	Generate or download raw sequencing reads.	FASTQ / FASTQ.gz
3. Quality control	Assess base quality, adapters and read composition.	FastQC / MultiQC reports
4. Preprocess if needed	Trim adapters or low-quality bases when justified.	Clean FASTQ
5. Align / quantify	Map reads or estimate transcript abundance.	BAM or quantification files
6. Count genes	Summarize reads by annotated genes.	Count matrix
7. Differential expression	Normalize and test condition effects.	DEG table
8. Visualize	Assess samples and results.	PCA, heatmap, volcano plot
9. Functional analysis	Interpret affected pathways and processes.	GO / pathway results

Key principle: Good experimental design and sample metadata matter as much as the software. Keep raw data, metadata, scripts and outputs organized from the start.