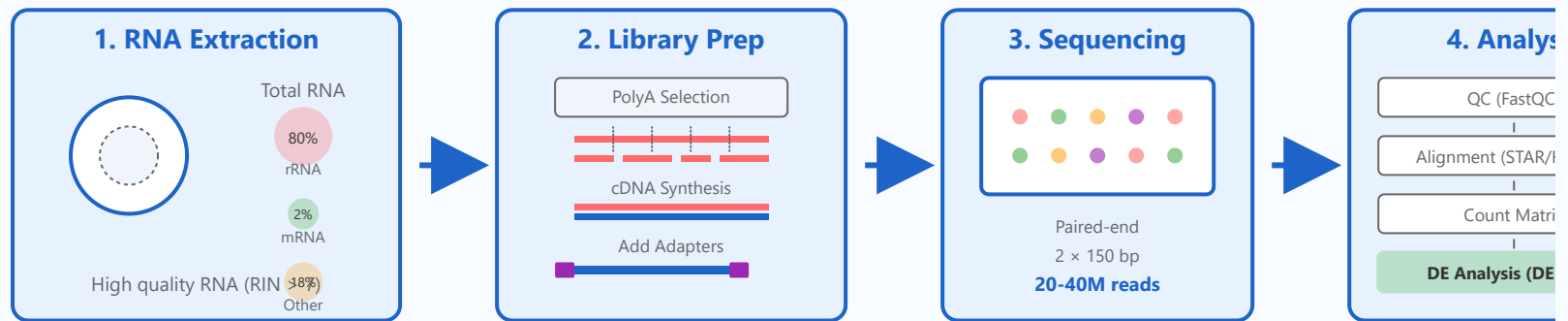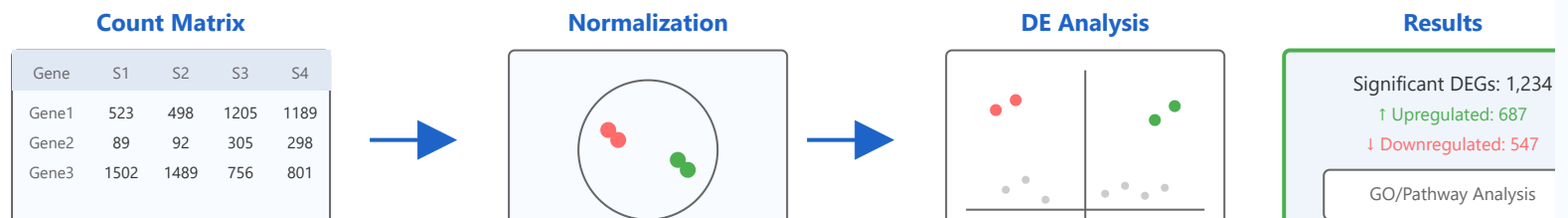# RNA-seq Overview

RNA-seq Workflow & Mechanism

Complete RNA-seq Pipeline: From Cells to Expression Profiles

1. RNA Extraction

Total RNA

80% rRNA

2% mRNA

18% Other

High quality RNA (RIN 8)

2. Library Prep

PolyA Selection

cDNA Synthesis

Add Adapters

3. Sequencing

Paired-end
2 × 150 bp
20-40M reads

4. Analys

QC (FastQC)

Alignment (STAR/

Count Matri

DE Analysis (DE

Expression Analysis & Results

Count Matrix

Gene	S1	S2	S3	S4
Gene1	523	498	1205	1189
Gene2	89	92	305	298
Gene3	1502	1489	756	801

Normalization

DE Analysis

Results

Significant DEGs: 1,234

↑ Upregulated: 687

↓ Downregulated: 547

GO/Pathway Analysis

Common RNA-seq Tools

Alignment:
STAR, HISAT2, TopHat2

Quantification:
featureCounts, HTSeq, Salmon

DE Analysis:
DESeq2, edgeR, limma

Visualization:
IGV, R/ggplot2

Applications

- Differential gene expression
- Alternative splicing analysis
- Novel transcript discovery
- Allele-specific expression

Key Considerations

- Biological replicates (≥3)
- Read depth (20-40M reads)
- Strand-specific protocols
- Batch effect correction