Normalization Methods

RPKM/FPKM Issues

Reads/Fragments Per Kilobase Million - biased by composition

TPM Calculation

Transcripts Per Million - better for comparison

DESeq2 Normalization

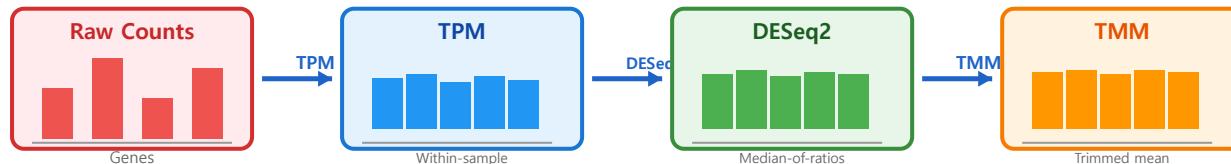Median-of-ratios method for differential expression

TMM Method

Trimmed Mean of M-values - robust to outliers

Batch Correction

ComBat, limma removeBatchEffect for technical variation

💡 **Choose normalization method based on your downstream analysis goals**

Raw Counts

Genes

→ TPM →

TPM

Within-sample

→ DESeq →

DESeq2

Median-of-ratios

→ TMM →

TMM

Trimmed mean

Method Selection

TPM: Sample comparison
DESeq2: DE analysis (count)
TMM: edgeR pipeline
RPKM: Avoid for DE

Pick based on analysis goal