

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

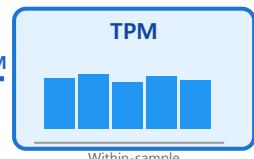
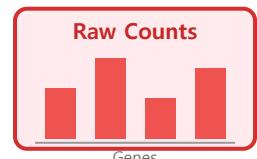
Trimmed Mean of M-values - robust to outliers

Batch Correction

ComBat, limma removeBatchEffect for technical variation



Choose normalization method based on your downstream analysis goals



Method Selection

TPM: Sample comparison

DESeq2: DE analysis (count)

TMM: edgeR pipeline

RPKM: Avoid for DE

Pick based on analysis goal