

## **Package Version**

```
openxlsx 4.2.8  
gridExtra 2.3  
pheatmap 1.0.12  
RcolorBrewer 1.1-3  
lubridate 1.9.4  
forcats 1.0.0  
stringr 1.5.1  
dplyr 1.1.4  
purrr 1.0.4  
readr 2.1.5  
tidy 1.3.1  
tibble 3.2.1  
ggplot2 3.5.1  
tidyverse 2.0.0
```

## **09d**

We developed a customized R-based analytical pipeline to visualize differentially expressed genes (DEGs) across multiple experimental conditions using heatmaps. Gene expression data were first filtered based on log2 fold-change (log2FC) and false discovery rate (FDR) thresholds, followed by the removal of genes with TPM values below a specified cutoff across all biological groups. The resulting expression matrix was normalized using either max-based scaling or Z-score transformation, followed by final rescaling to ensure comparability across samples. Hierarchical clustering was applied to both genes and samples, with customizable cluster numbers. Heatmaps were generated using the **pheatmap(1.0.12)**. The pipeline also quantifies upregulated and downregulated genes, assigns cluster labels, and outputs structured summary tables for downstream interpretation.

## **Segex**

We implemented a dedicated R-based visualization module to generate heatmaps from transcriptomic signal intensity data without applying differential expression filtering. Raw expression values were extracted from SEGEX-formatted result files. The resulting expression matrix was normalized using either max-based scaling or Z-score scaling followed by rescaling, depending on the selected mode. Hierarchical clustering was applied to both rows (genes/probes) and columns (samples), with customizable cluster numbers. Heatmaps were generated using the **pheatmap(1.0.12)** under both clustered and non-clustered configurations. For each scaling method, the sample order, clustering results, and normalized expression values were saved into summary Excel files to facilitate downstream interpretation.

### **ChIP-seq**

We implemented an R-based pipeline to visualize signal intensities from ChIP-seq or similar genomic region-based data using hierarchical clustering heatmaps. Input matrices were derived from multiple sheets of an Excel file, where rows represented genomic regions and columns corresponded to distinct experimental conditions. Optionally, log2-to-linear or linear-to-log2 transformations were performed prior to normalization.

Each matrix was normalized using either max-based scaling or Z-score transformation, followed by further rescaling to ensure comparability across features. Sample-level annotations were automatically inferred and color-coded to reflect group structures. Hierarchical clustering was applied to both rows and columns with customizable cluster numbers. Heatmaps were generated using the **pheatmap(1.0.12)**, and both clustered and non-clustered views were exported in PDF format. In addition, the sample order, clustering assignments, and normalized signal matrices were saved in structured Excel workbooks to facilitate downstream interpretation and reproducibility.