DESeq2 Normalization for Aneuploidy

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First, download files from the AWS server. The files are located in: /scratch/Shares/public/sread2022/data\_files/include/deseq2\_normalization/

1. Load in your files. Change the path to wherever you stored your files:

Graphical user interface, text

Description automatically generated

1. Load in and clean up your counts file to be DESeq2-compatible. Run DESeq2 with a batch correction:

Text

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1. Generate your results table (shrinking log2FoldChange is optional). Also calculate a per-chromosome median fold change:

Graphical user interface, text, application, email

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1. Generate some plots for visualization:

Graphical user interface, text, application

Description automatically generated

1. You’ll notice that for chr21, most genes are at 1.5x expression (as expected), but a subset are closer to a fold-change of 1.0, and are not considered significant. What can we say about these genes?

**Normalization Method 1: Adjusting Counts By Ploidy**

1. Our metadata table lists the ploidy status for each sample. We can define a function which will print the copy number of the gene based on the sample’s ploidy status. We use this to generate a matrix corresponding to the copy number for each gene in each sample:

Graphical user interface, text

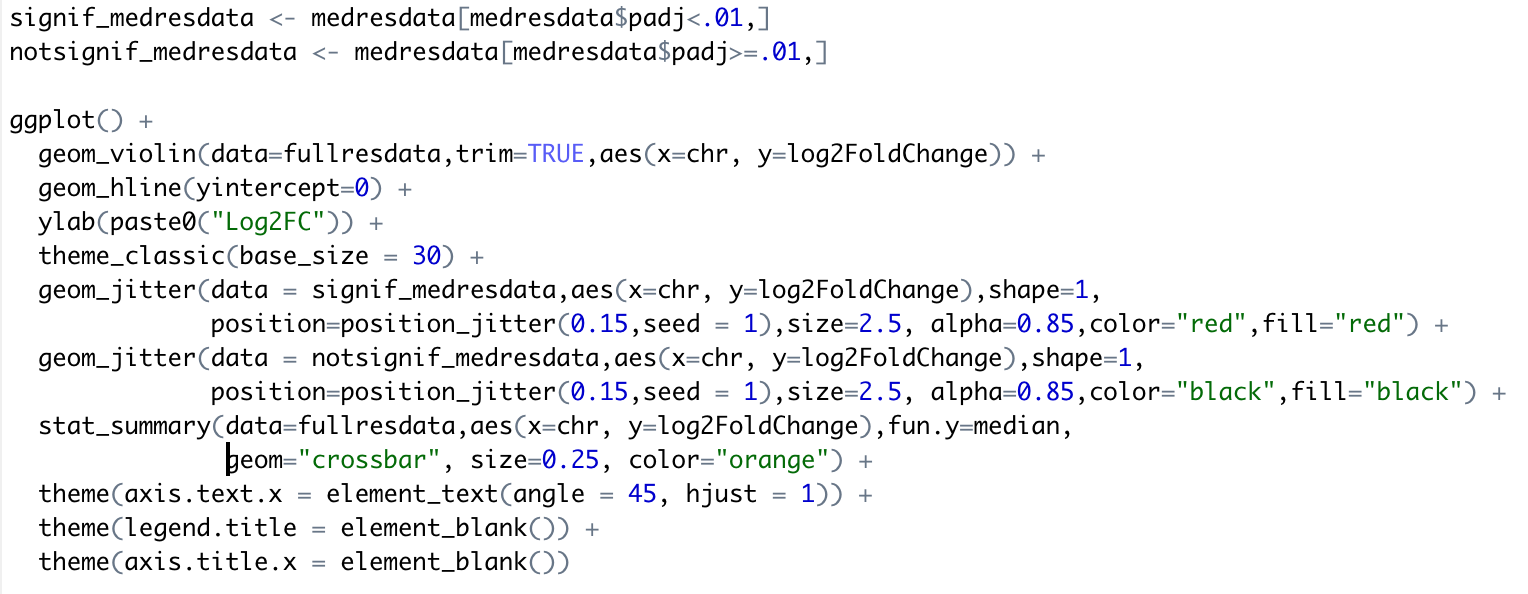
Description automatically generated

1. Re-run DESeq2 with the new normalization matrix:

Graphical user interface, text, application

Description automatically generated

1. Visualize our results



**Method 2: Change the alternative hypothesis**

1. Re-run DESeq2, using an adjusted alternative hypothesis

Graphical user interface, text, application, email

Description automatically generated

1. Visualize the results:

Graphical user interface, text, application

Description automatically generated