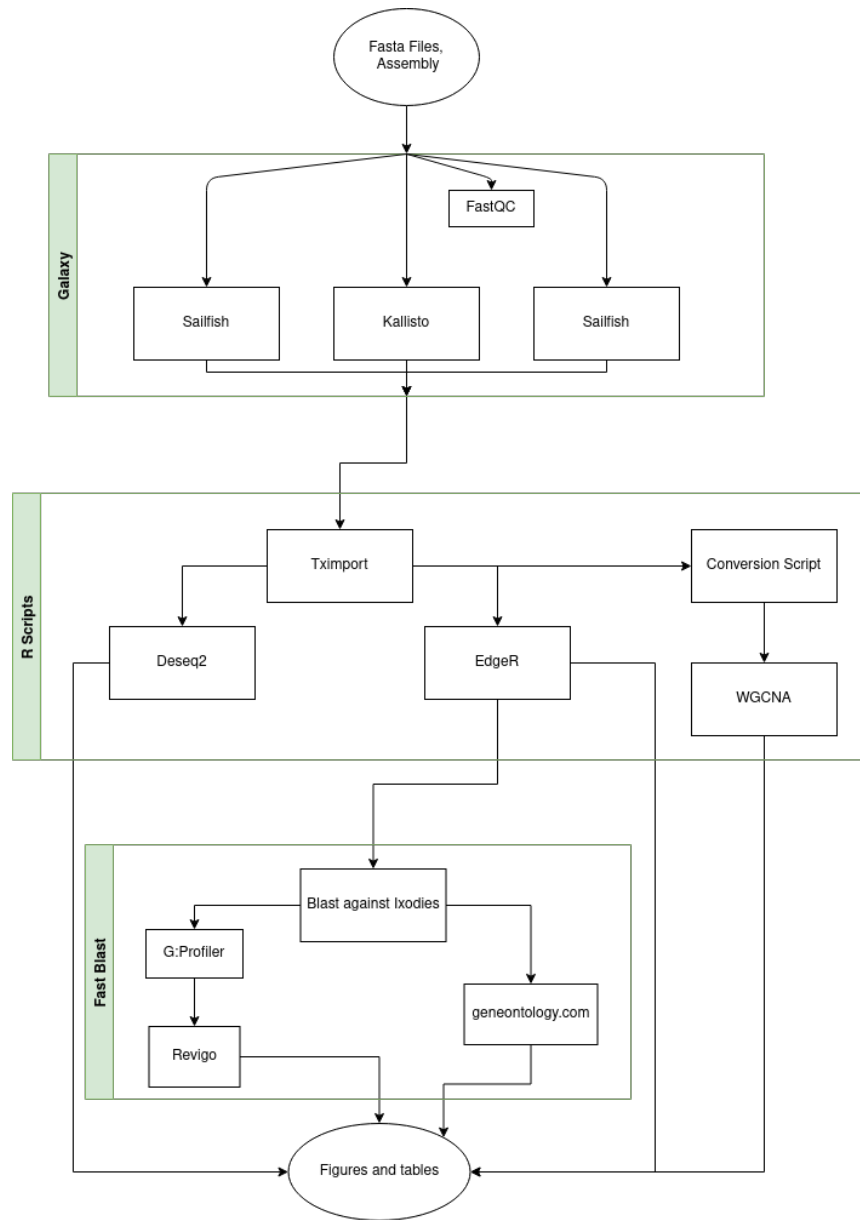


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3.1 Transcriptomic Workflow Overview



3.1.1 Quantification

The Raw high-throughput RNA-sequencing data from each trial were saved as individual FASTQ files. The FASTQ files were uploaded to the Galaxy

platform (usegalaxy.org) where the quantification occurred. First, Galaxy's 'FastQC' tool was used to determine the quality of the raw data and to mitigate the effects of amplification bias ([insert settings here](#)). The Kmer test within 'FastQC' was enabled and set at a length of 7bp. Following this initial quality-check, the raw files were trimmed with 'Trimmomatic' using its default settings ([description](#)). FastQC was run on the trimmed files to confirm its quality.

After the data was trimmed, transcript abundance quantification was performed by the Galaxy's 'Sailfish quant' tool using the default settings ([description](#)).

3.2 Differential expression analysis

DESeq2([Reference](#)) and EdgeR([Reference](#)) were used to test for differential expression between the legs, body, and pesticide treatment (Deet and Perm). The transcript-level counts were quantified using the TPM (transcripts per million) generated from the 'Sailfish quant' tool. The differentially expressed genes were estimated using an adjusted p value cutoff of less than 0.05 for multiple testing using a false discover rate FDR) (Benjamini and Hochberg, 1995), and a log 2 -fold change ≥ 2 . The differential analysis becomes more reliable when the results of the similar tools are compared.

3.3 WGCNA

3.4 GO analysis

3.4.1 Blast

The significantly differentially expressed contigs were searched (BLASTx) against the NCBI *Ixodites scapularis* SwissProt protein database with an expectation value (e-value) of 0.001. The highest scoring blast hits were assigned a gene ID for each contig. For each search, a positive BLAST hit was functionally profiled using g:Profiler and the PANTHER Classification System.

3.4.2 GProfiler

Functional profiling of overrepresented gene sets were statistically listed with enriched terms in gProfiler. g:GOST was the g-profiler tool used for functional enrichment analysis. The GProfiler output is sent to Revigo.

3.4.3 Revigo

The R script output of REVIGO (Rudjer Boskovic Institute) generates tree-maps of the genes. These maps show the function of the enriched genes.

3.4.4 Geneontology.org

The output of blast is sent to Geneontology.org (not sure if this section is needed (didn't use the data at all))

4 Results

4.1 Figure 1: methods comparison between EdgeR and Deseq2

This figure compares the differential expression data from both EdgeR and Deseq2. The P value is small, indicating that EdgeR and Deseq2 have similar results.

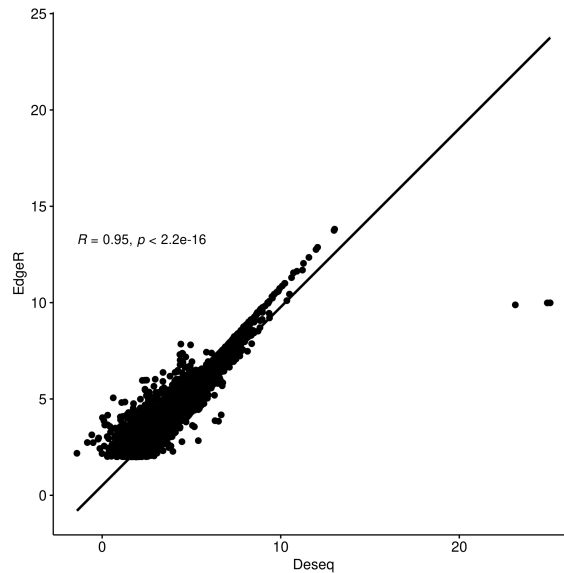


Figure 1: The quantification files were run through both EdgeR and Deseq2. Each axis plots the expression values as given by its respective title.

4.2 Figure 2: Perm and Deet repellency and survival

4.3 Figure 3: Leg vs Body general (control)

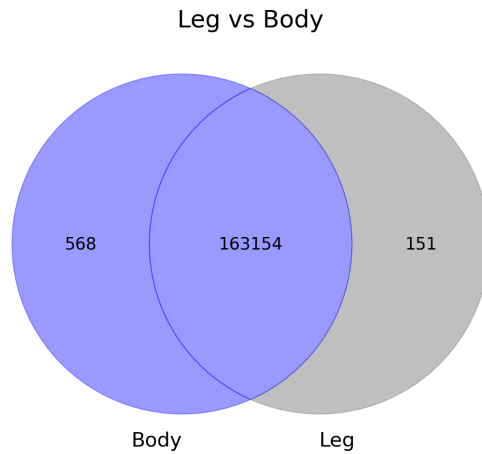


Figure 2: Comparison of up-regulated genes in the body and leg compared to the total number of genes from the sample.

4.3.1 TODO supplemental tables

4.4 Figure 4: Leg vs Body Specifics overlap with Ixodies

Refer to paper in google drive. Discussion.

4.5 Figure 5: Response to DEET In Leg and Body

4.5.1 Body

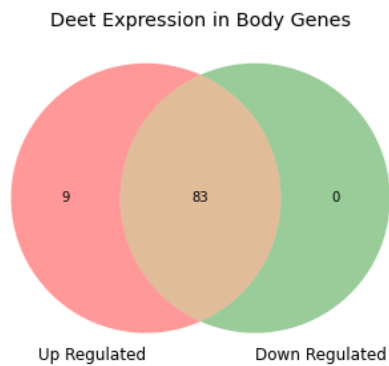


Figure 3: Differentially expressed genes with respect to the body are further compared based on differential expression with respect to the Deet comparison.

4.5.2 Leg

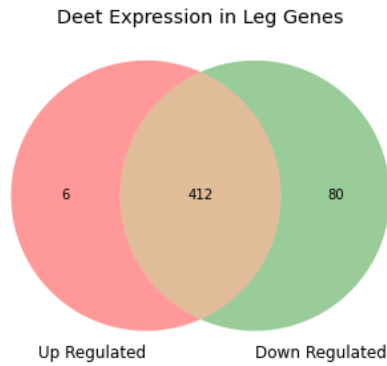


Figure 4: Differentially expressed genes with respect to the leg are further compared based on differential expression with respect to the Deet comparison.

4.5.3 TODO supplemental tables

4.6 Figure 6: Response to PERM in Leg and Body

4.6.1 Body

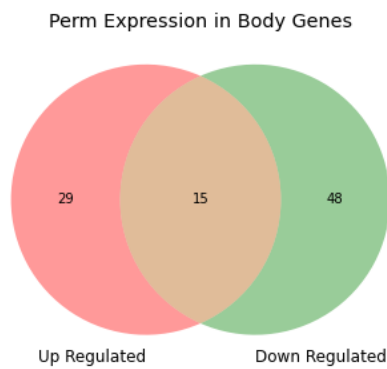


Figure 5: Differentially expressed genes with respect to the body are further compared based on differential expression with respect to the Perm comparison.

ison.

4.6.2 Leg

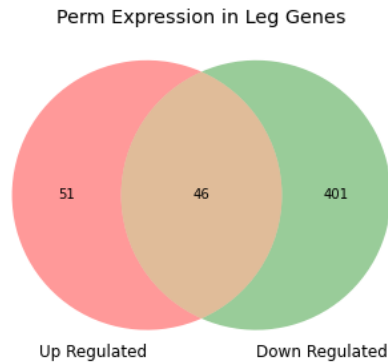


Figure 6: Differentially expressed genes with respect to the leg are further compared based on differential expression with respect to the Perm comparison.

4.6.3 TODO supplemental tables

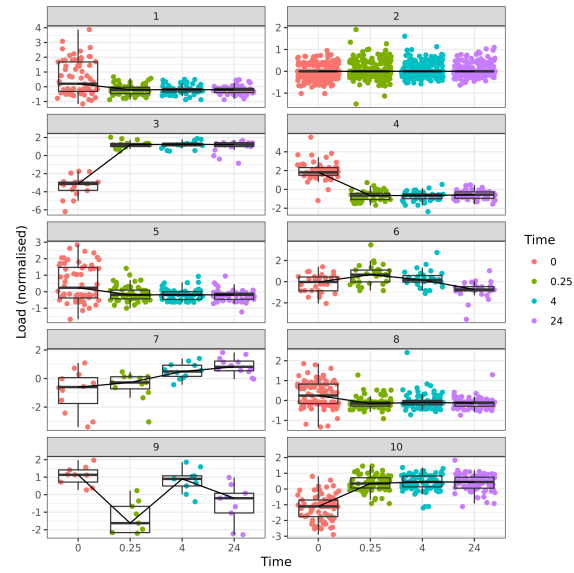
4.7 Figure 7: WGCNA

4.8 Figure 8: Overlap Between the response, body and leg

Don't think we need it now.

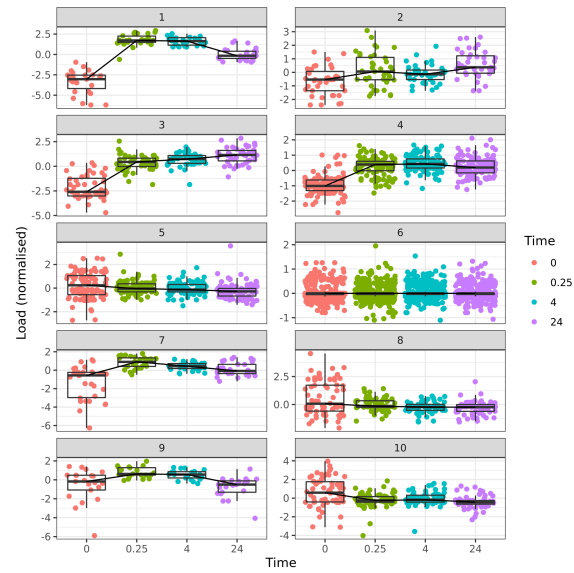
4.9 Figure 9: Time Course Legs

4.9.1 DEET



1. Supplemental Tables figure9/DEET/Legbox

4.9.2 PERM



1. Supplemental Tables figure9/PERM/Legbox

4.10 Figure 10: Time Course Body

4.10.1 DEET

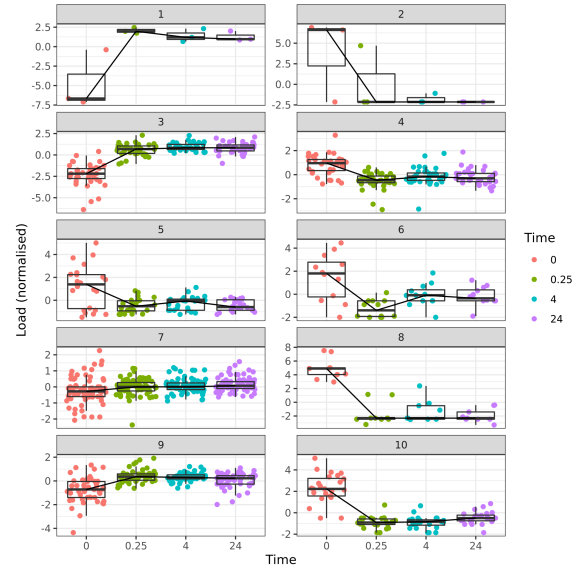
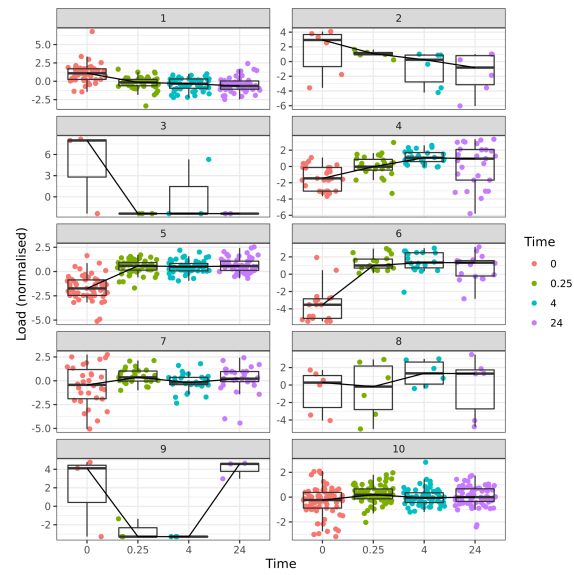


Figure 7: K-mean clusters of differential expression data

1. Supplemental Tables [figure10/DEET/Bodybox/](#)

4.10.2 PERM



1. Supplemental Tables [figure10/PERM/Bodybox/](#)

4.11 Figure 11: GO analysis (treemaps)

5 Discussion