

Basic Workflow for *Strongyloides* RNA-seq Browser



1) Pick a Species: Select a *Strongyloides* species using the dropdown menu. Users may choose between *S. stercoralis*, *S. ratti*, *S. papillosus*, or *S. venezuelensis*. Note that comparisons of known homologs across species is available when viewing individual gene expression data.



2) Input Genes/Keywords: Type or upload a file containing gene IDs or other supported search terms.



3) Pick Display: Use the red drop down menu panel to switch between three options for visualizing gene expression: 1) a heatmap plotting all genes (when the search finds multiple genes), 2) a box-and-whiskers plot of single gene expression across life stages, and 3) a data table reporting gene expression values.



4) View or Download Expression Data: Plots/tables will be displayed in, and can be downloaded from, the Gene Expression Across Life Stages panel. Note: these plots DO NOT contain statistical evidence of differential gene expression.



5) Pick Comparisons for Differential Gene Expression Analysis: To test the degree of differential gene expression for your genes of interest, use the Step 2 panel to set up statistical comparisons. These may be between two life stages (e.g. parasitic females vs free-living females) or between a single life stage and a group (iL3s vs parasitic and free-living females).



6) Differential Gene Expression Plots: For each pairwise comparison requested in the previous step, the browser will generate a volcano plot that displays differential expression of selected genes as colored circles, as well as relative expression for all genes as black circles. The x-axis indicates the relative expression of genes across the selected life stage comparison; the y-axis indicates statistical significance. Positive x-axis values indicate a gene is upregulated in the target life stage, negative values indicate downregulation. In general, fold change values between -1 and 1 and/or p -values less than 0.05 should not be considered meaningful differences in expression across life stages.



7) Download Differential Gene Expression Results: Differential gene expression values (log-fold change) and associated p -values, as well as average counts per million for each life stage and gene description data, may be viewed and downloaded using the provided DGE Table panels, which are located at the bottom of the browser page.