Viewing DESeq2 results and creating a gene list

Once we have performed DESeq2 to identify differentially expressed genes, we can create a list of significantly differentially expressed genes using cutoff thresholds.

- Double click the **Feature list** data node to open the task report

The task report spreadsheet will open showing genes on rows and the results of the DESeq2 on columns (Figure 1).

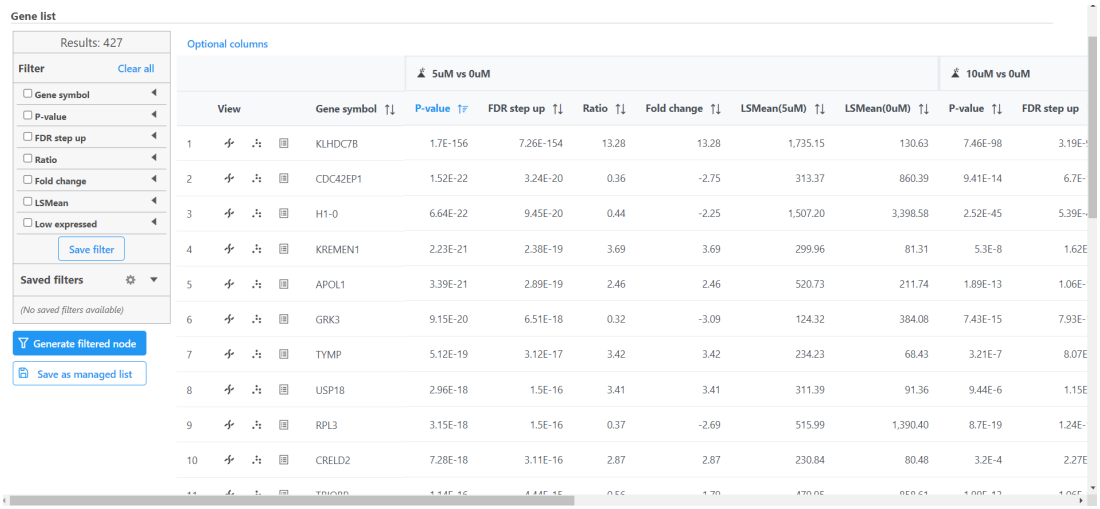


Figure 5. Viewing the DESeq2 task report spreadsheet

To get a sense of what filtering thresholds to set, we can view a volcano plot for a comparison.

- Click  next to the *5uM vs. 0uM* comparison

A volcano plot will open showing p-value on the y-axis and fold-change on the x-axis (Figure 2). If the gene labels are on (not shown), click on the plot to turn them off.

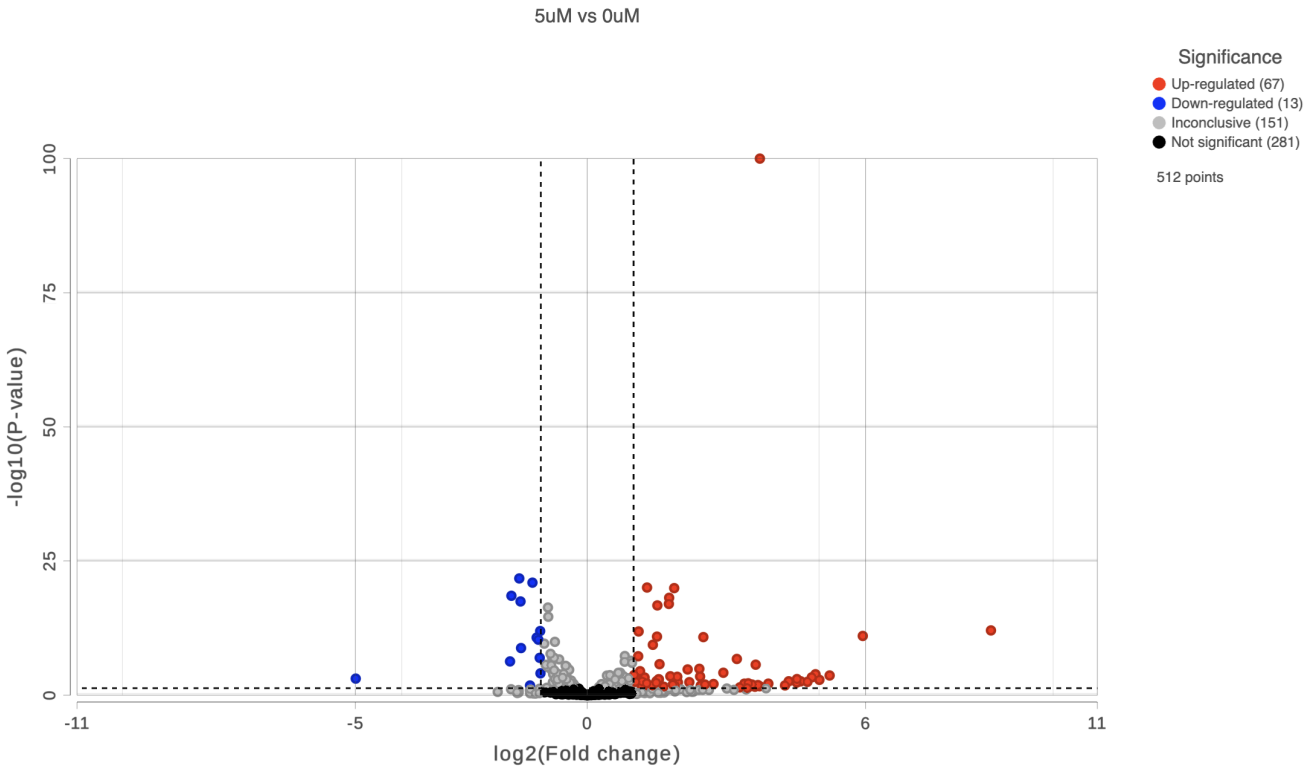


Figure 6. Viewing DESeq2 results with a volcano plot

Thresholds for the cutoff lines are set using the *Statistics* card (*Configuration panel > Configure > Statistics*). The default thresholds are *|2|* for the X axis and *0.05* for the Y axis.

- Switch to the browser tab showing the *DESeq2 report*
- Click **FDR step up**
- Click the triangle next to *FDR step up* to open the *FDR step up* options
- Leave **All contrasts** selected
- Set the cutoff value to **0.05.** Hit **Enter**.

This will include genes that have a FDR step up value of less than or equal to 0.05 for all three contrasts, 5M vs. 0M, 10M vs. 0M and 5M:10M vs. 0M. FDR step up is the false discovery rate adjusted p-value used by convention in microarray and next generation sequencing data sets in place of unadjusted p-value.

- Click **Fold-change**
- Click the triangle next to Fold-change to open the *Fold-change* options
- Leave **All contrasts** selected
- Set to *From* **-2** to **2** with **Exclude range** selected. Hit **Enter**.

Note that the number of genes that pass the filter is listed at the top of the filter menu next to *Results:* and will update to reflect any changes to the filter. Here, 27 genes pass the filter (Figure 3). Depending on your settings, the number may be slightly different.

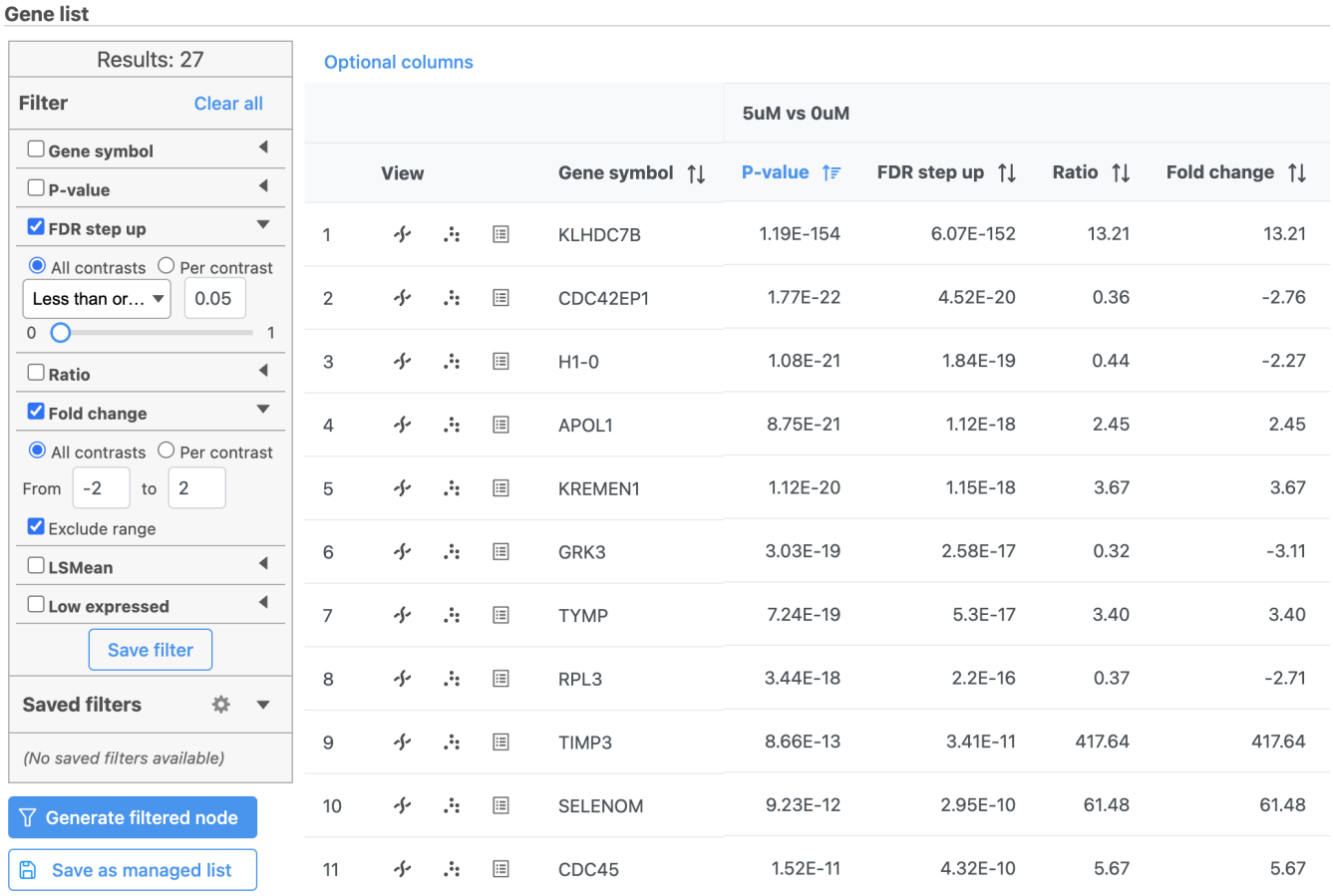
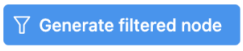


Figure 7. Applying filters to the DESeq2 results spreadsheet

- Click  to create a data node with only the genes that pass the filter

This creates a *Filter list* task node and a *Filtered feature list* data node (Figure 4).

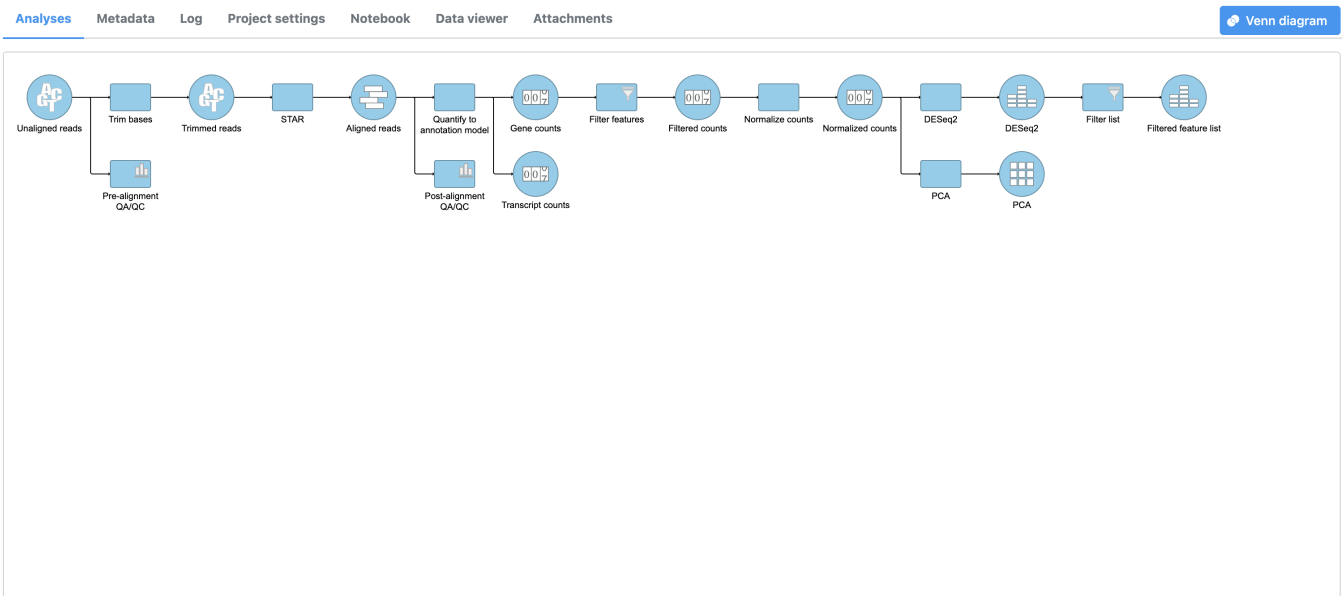


Figure 8. Filter list and a new Feature list node are added to the pipeline

« Performing differential expression analysis with DESeq2 Viewing a dot plot for a gene »

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