

Generating a hierarchical clustering heatmap

To check how well our list of differentially expressed genes distinguishes one treatment group from another, we can perform hierarchical clustering based on the gene list. Clustering can also be used to discover novel groups within your data set, identify gene expression signatures distinguishing groups of samples, and to identify genes with similar patterns of gene expression across samples.

- Click the **Feature list** data node
- Click **Exploratory analysis** in the task menu
- Click **Hierarchical clustering** (Figure 1)

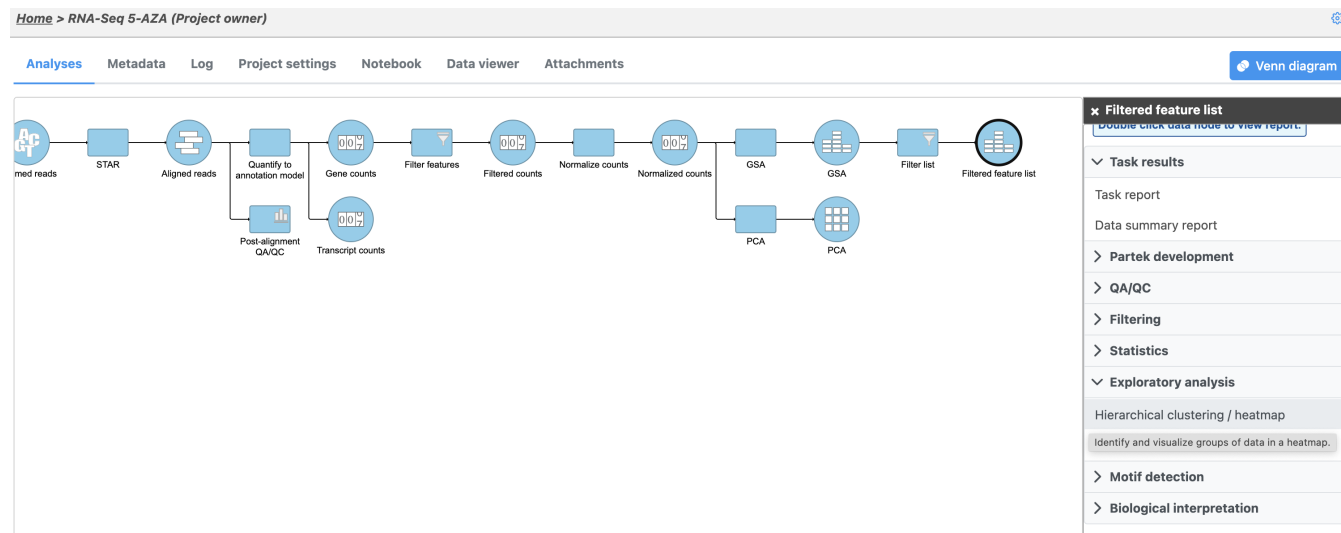


Figure 7. Invoking Hierarchical clustering

The *Hierarchical clustering* menu will open (Figure 2). Hierarchical clustering can be performed with a heatmap or bubble map plot. **Cluster** must be selected under *Ordering* for both *Feature order* and *Cell order* if both the features (columns) and cells (rows) are to be clustered.

Heatmap Bubble map

Ordering

Feature order

☒ Cluster

Cluster by distance metrics to sort based on similarity. Requires at least 3 features.

☐ Assign order

Assign feature order using a saved feature list. The features will be filtered to those in the list and will be ordered as they are listed.

Default order

Sample order

☒ Cluster

Cluster by distance metrics to sort based on similarity. Requires at least 3 samples.

☐ Assign order

Order samples by an attribute. Categorical attributes with fewer than 50 categories can be manually ordered by drag and drop. Numeric attributes can be sorted.

Default order



Filtering

☐ Filter samples

Specify the logical operations to filter by. Use AND for inclusion if all conditions pass. Use OR for inclusion if any conditions pass.

include Sample name in SRR592578 OR

AND

Advanced options

Option set

-- Default --

[Configure](#)

Back Finish

Figure 8. Configuring Hierarchical clustering

- Click **Finish** to run with default settings

A *Hierarchical clustering* task node will be added to the pipeline (Figure 3).

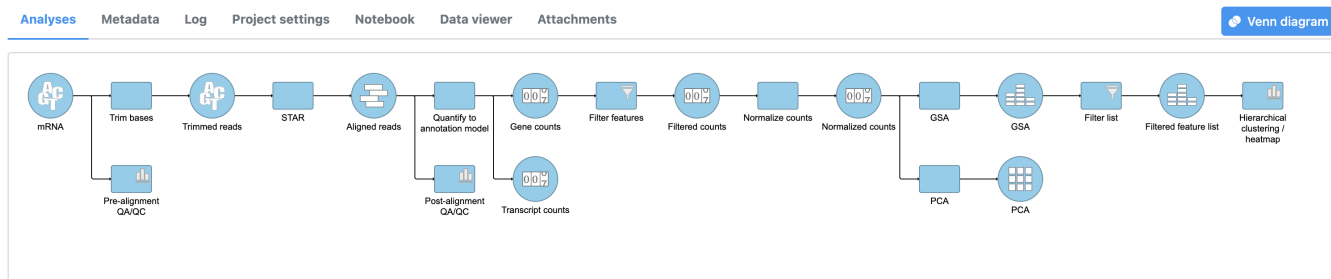


Figure 9. Hierarchical clustering task node

- Double-click the **Hierarchical clustering / heatmap** task node to launch the heatmap

The *Dendrogram view* will open showing a heatmap with the hierarchical clustering results (Figure 4).

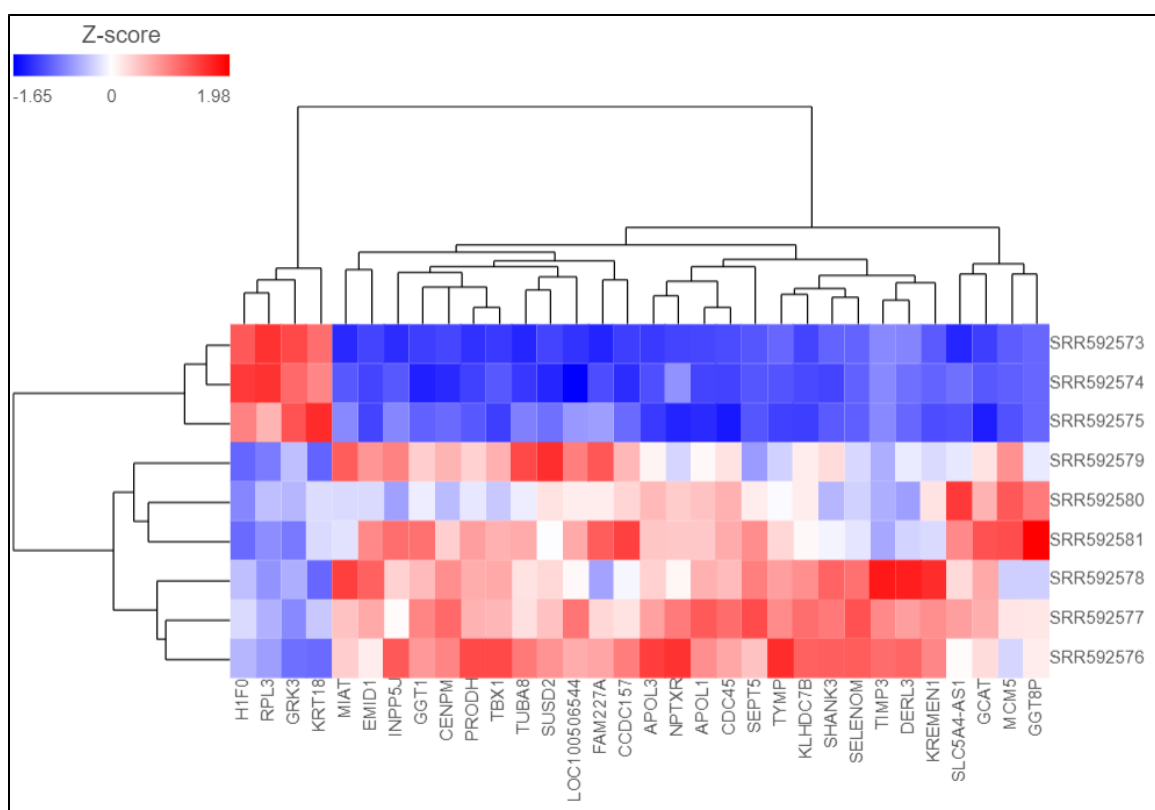


Figure 10. Viewing the hierarchical clustering heat map

Samples are shown on rows and genes on columns. Clustering for samples and genes is shown through the dendrogram trees. More similar samples /genes are separated by fewer branch points of the dendrogram tree.

The heatmap displays standardized expression values with a mean of zero and standard deviation of one.

The heatmap can be customized to improve data visualization using the menu on the *Configuration* panel on the left.

- Expand the *Annotations > Data* card.
- Click on the **gray button** on the right hand side of *Row annot None available*
- In the dialog, click on the **Gene counts** node
- Now set the *Row annot* to **5-AZA Dose**

Samples are now labeled with their *5-AZA Dose* group (Figure 5).

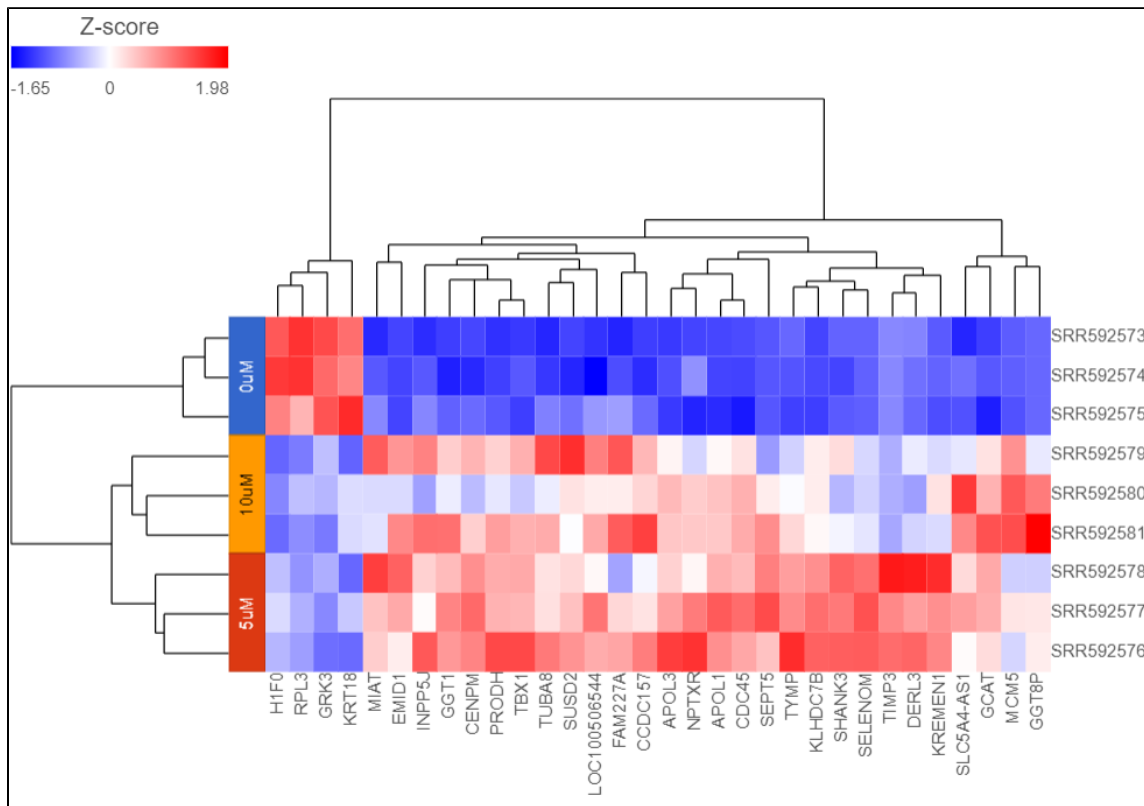
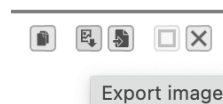


Figure 11. Samples labeled with their 5-AZA Dose group

Samples cluster based on treatment group and the 5M and 10M groups are more similar to each other than to the 0M group.

We can save the heatmap as a publication-quality image.



- Click the **Export image** icon in the top right corner of the plot
- Choose size and resolution using the *Save as SVG* dialog (Figure 6)

Export image



Format SVG image ▼

Size 11.03 x 5.88 cm ▼

Resolution 300 DPI

Save

Cancel

Figure 12. Choosing size and resolution for SVG file

- Select **Save**

The heatmap will be saved as a .svg file and downloaded in your web browser.

For more information about hierarchical clustering and the *Dendrogram view*, please see the [Hierarchical Clustering](#) user guide.

[« Visualizing gene expression in Chromosome view](#) [Performing biological interpretation »](#)

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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