Performing differential expression analysis with DESeq2

After normalizing the data, we can perform differential analysis to identify genes that are differentially expressed based on treatment.

- Click the **Normalized counts** node
- Click **Statistics** in the task menu
- Click **Differential analysis** in the task menu (Figure 1)

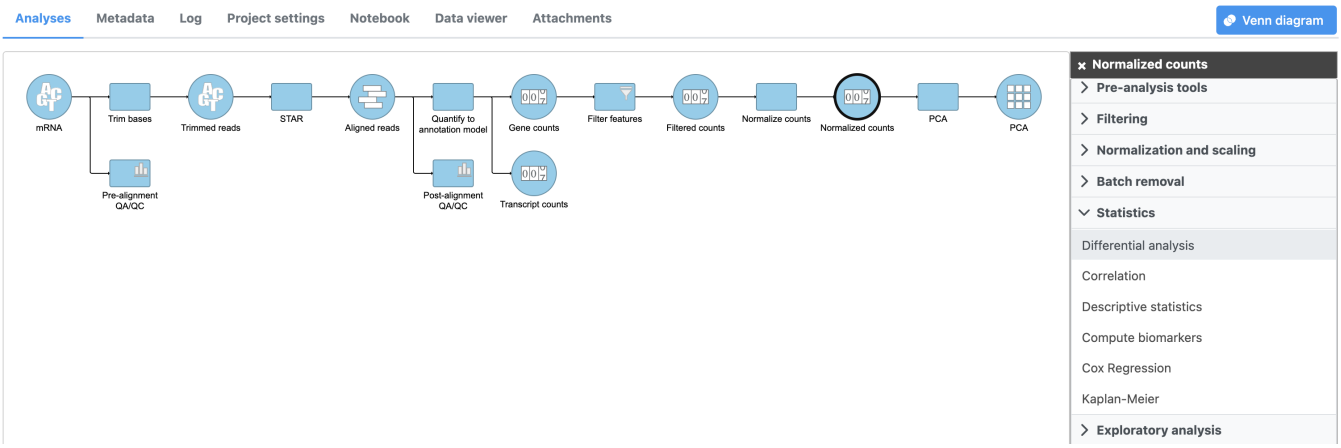

Figure 8. Navigating to the differential analysis options

Select the appropriate differential analysis method (Figure 2). In this tutorial we are going to use DESeq2, but Partek® Flow® offers a number of alternatives. Hover the mouse over the *i* symbol for more information on each differential analysis method, or see our Differential Analysis user guide for a more in-depth look.

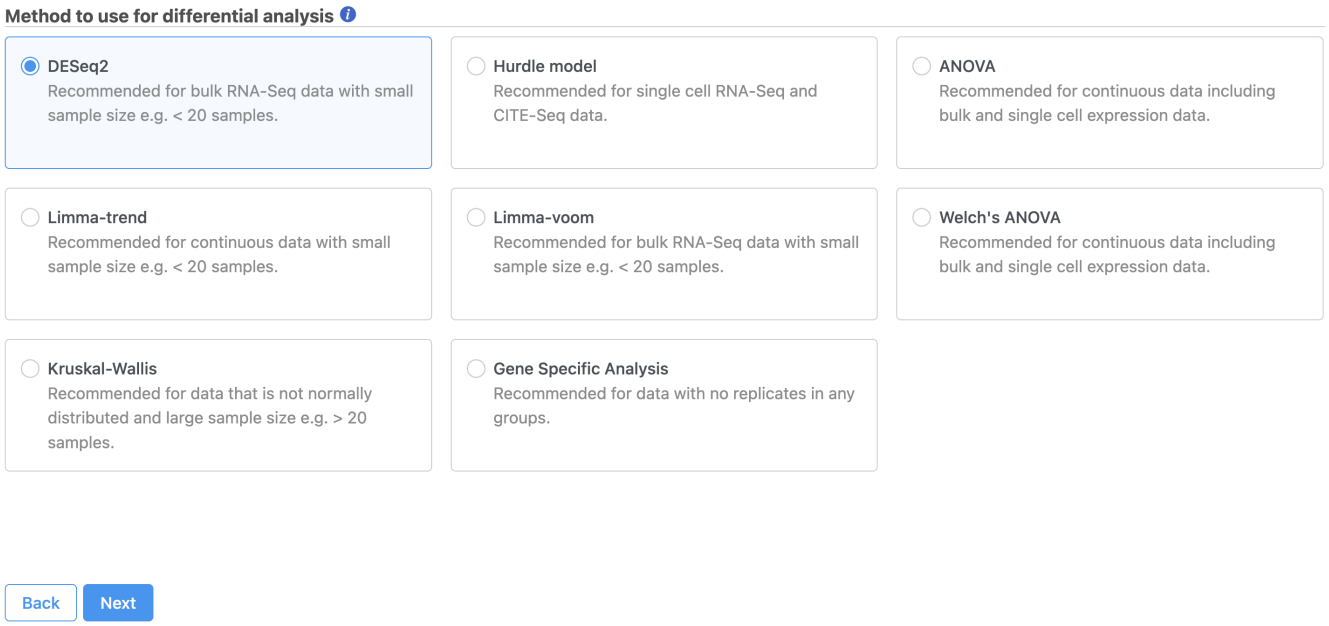

Figure 9. Select the method for differential analysis from the options provided.

- Check *5-AZA Dose* and click **Add factors** to add the attribute to the statistical model.

Select factor(s) for analysis

Categorical factors

☐ 5-AZA Dose

Add factors | Add interaction

Selected factor(s)

Factor	Delete
5-AZA Dose	—

Back | Next

Figure 10. Selecting attributes to include

- Select **Next** to continue with *5-AZA Dose* as the selected attribute

The *Comparisons* page will open (Figure 4).

Define comparisons

Factor 5-AZA Dose

Numerator

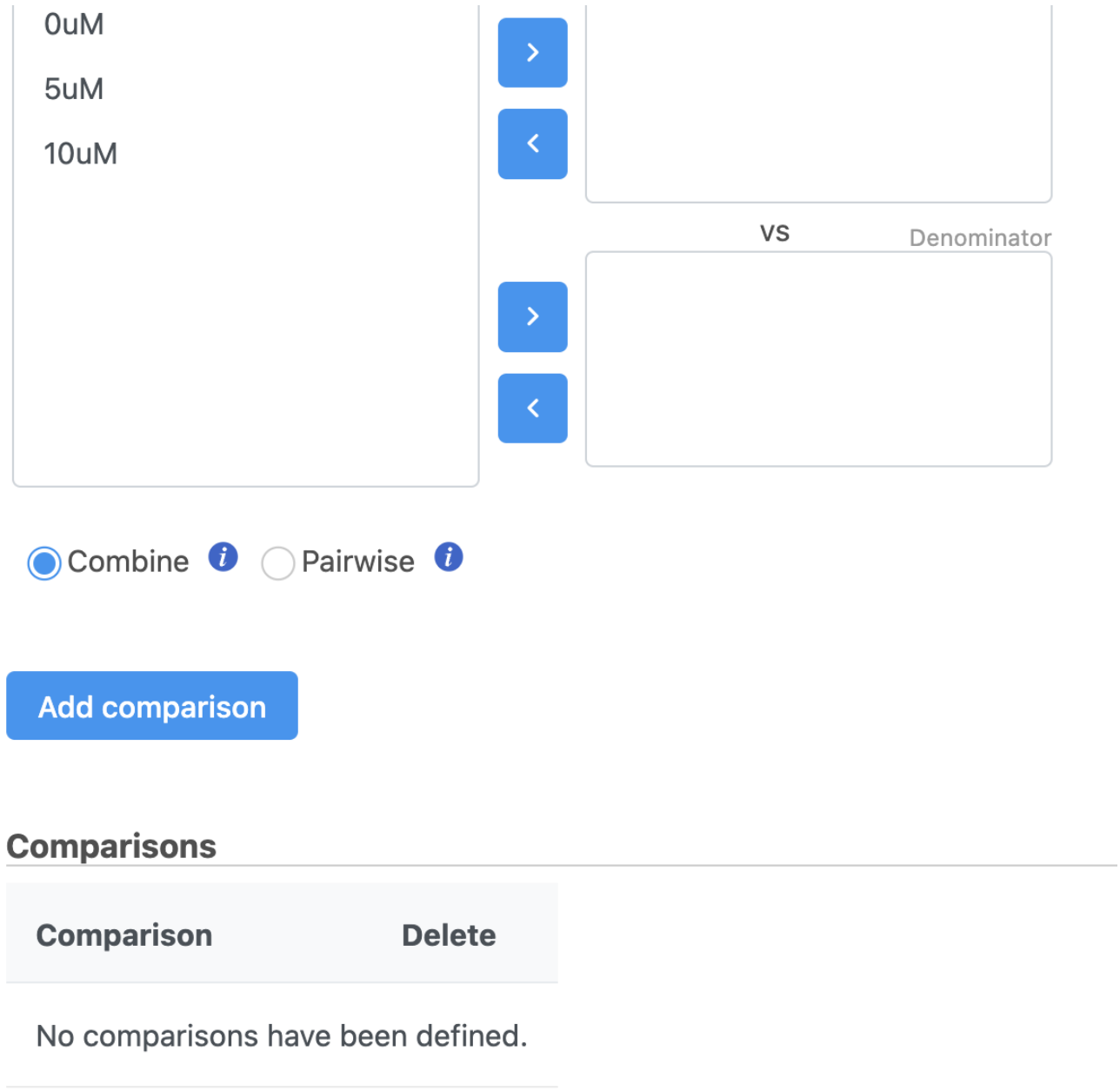

Figure 11. The Comparison selector allows multiple comparisons to be designed and added

It is easiest to think about comparisons as the questions we are asking. In this case, we want to know what are the differentially expressed genes between untreated and treated cells. We can ask this for each dose individually and for both collectively.

The upper box will be the numerator and the lower box will be the denominator in the comparison calculation so we will select the 0M control in the lower box.

- Drag 5M to the upper box
- Drag 0M to the lower box
- Click **Add comparison** to add **5M vs. 0M** to the comparison table (Figure 5)

Define comparisons

Factor 5-AZA Dose

0uM
5uM
10uM

Numerator

5uM

vs

Denominator

0uM

Combine Pairwise

Add comparison

Comparisons

Comparison	Delete
5uM vs. 0uM	—

Figure 12. Designing a comparison to add

- Repeat to create comparisons for **10M vs. 0M** and **10M,5M vs. 0M** (Figure 6)

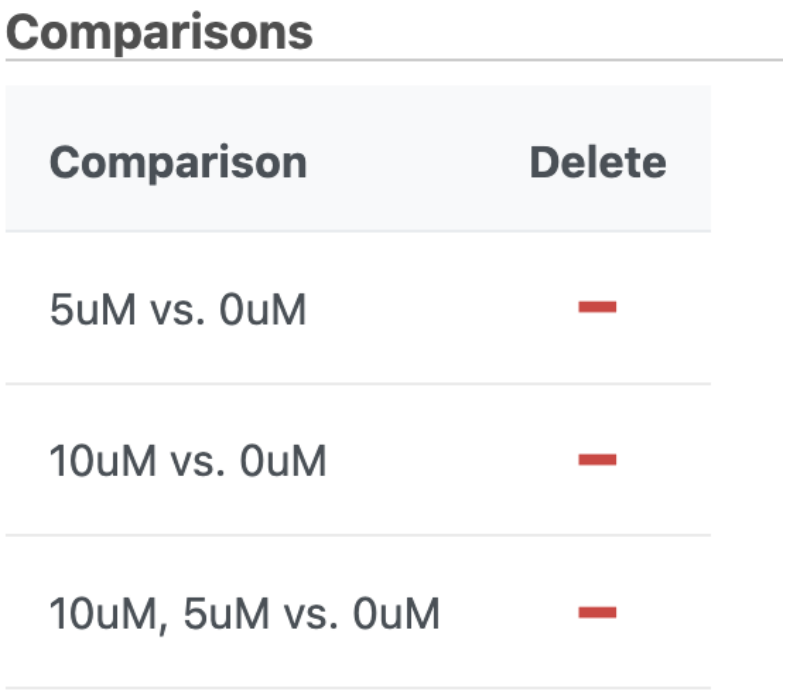

Figure 13. Comparisons for 5uM vs. 0uM, 10uM vs. 0uM, and 5uM:10uM vs. 0uM have been added

- Click **Finish** to perform DESeq2 as configured

A *DESeq2* task node and a *DESeq2* data node will be added to the pipeline (Figure 7).

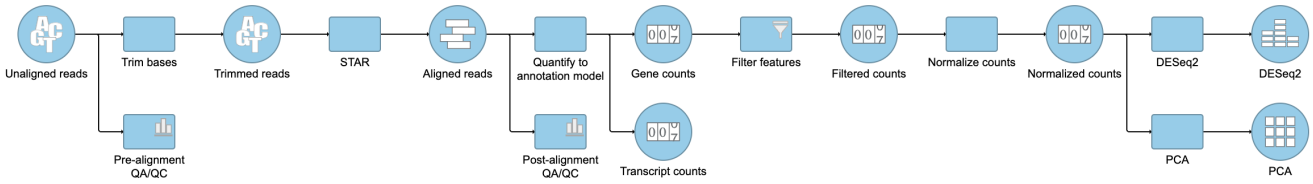

Figure 14. Gene analysis task node and Feature list data nodes

« Exploring the data set with PCA Viewing DESeq2 results and creating a gene list »

Additional Assistance

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