Exploring the data set with PCA

Principal Components Analysis (PCA) is an excellent method to visualize similarities and differences between the samples in a data set. PCA can be

invoked through a workflow, by selecting () from the main command bar, or by selecting *Scatter Plot* from the *View* section of the main toolbar. We will use a workflow.

- Select Gene Expression from the Workflows drop-down menu
- Select PCA Scatter Plot from the QA/QC section of the Gene Expression workflow

The PCA scatter plot will open as a new tab (Figure 1).



Figure 5. Viewing the PCA scatter plot. Each point is a sample. Samples are colored by treatment.

In this PCA scatter plot, each point represents a sample in the spreadsheet. Points that are close together in the plot are more similar, while points that are far apart in the plot are more dissimilar.

To better view the data, we can rotate the plot.

- Select () to activate Rotate Mode
- Click and drag to rotate the plot

Rotating the plot allows us to look for outliers in the data on each of the three principal components (PC1-3). The percentage of the total variation explained by each PC is listed by its axis label. The chart label shows the sum percentage of the total variation explained by the displayed PCs.

We can change the plot properties to better visualize the effects of different variables.



- Set Shape to 4. Batch
- Set Size to 3. Time
- Set Connect to 5. Treatment Combination
- Select **OK** (Figure 2)

Plot Rendering Properties	×
Style Ellipsoids Labels Box&Whiskers Titles Color O Fixed	Axes Color Legend Text
2. Treatment Manual	3. Time ~
4. Batch ~ Manual	5. Treatment Combination
Outline Thin ~	Style direct ~ Line Width 1 pixels
Lighting Effects	Drawing Mode
✓ Fogging Transparency 0.00	Normal O Mixed
Save Load	OK Cancel

Figure 6. Configuring plot properties to color by treatment, shape by batch, size by time, and connect by treatment combination

The PCA scatter plot now shows information about treament, batch, and time for each sample (Figure 3).



Figure 7. PCA scatter plot showing treatment, batch, and time information for each sample. A batch effect is clearly visible.

PCA is particularly useful for identifying outliers and batch effects in data sets. We can see a batch effect in this dataset as samples separate by batch. To make this more clear, we can add an ellipses by *Batch*.

- Select () to open the *ConfigurePlot Properties* dialog
- Select Ellipsoids from the tab
- Select Ellipsoids from the tab
 Select Add Ellipse/Ellipsoid
- Select Add Ellipse
- Select Batch from the Categorical Vairable(s) panel and move it to the Group Variable(s) panel
- Select OK
- Select OK to close the dialog

The ellipses help illustrate that the data is spearated by batches (Figure 4).



Figure 8. Ellipses around batch groups show that samples separate by batch

Ways to address the batch effect in the data set will be detailed later in this tutorial.

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Additional Assistance

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

