

Dimensionality Reduction and Clustering

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PCA

Next, we will perform some exploratory analysis on the merged mRNA and protein expression data and visualize the data in preparation to identify cell populations. Because the merged count matrix has thousands of features, it is a good idea to reduce the dimensionality of the data for more efficient downstream processing.

- Click the **Merged counts** data node
- Click **Exploratory analysis** in the toolbox
- Click **PCA**
- Click **Finish** to run the PCA with default settings (Figure 1)

PCA

Number of principal components

The number of principal components to calculate.

☐ All

☒ 100

Features contribute

Feature expression can be standardized prior to PCA so that the contribution of each feature does not depend on its variance. Choose "by variance" if you prefer to take variance into account and focus on most variable features.

☐ equally ☒ by variance

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Finish

Figure 15. Run PCA with default settings

388pxA PCA task node will be added to the pipeline under the *Analyses* tab and a circular PCA output data node will be produced (Figure 2).

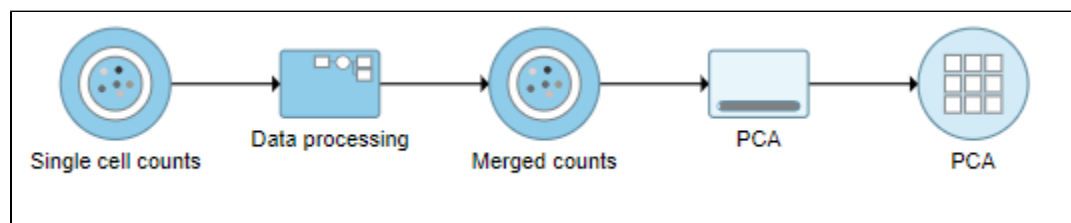


Figure 16. PCA task run on the merged counts data node

Once the task completes, we will inspect the results to decide the optimal number of principal components (PCs) to use in downstream analyses. To do this, we will use a Scree plot.

- Double click the **PCA** data node to open the task report

The PCA plot will open in a new data viewer session. A 3D scatterplot will be displayed on the canvas (Figure 3).

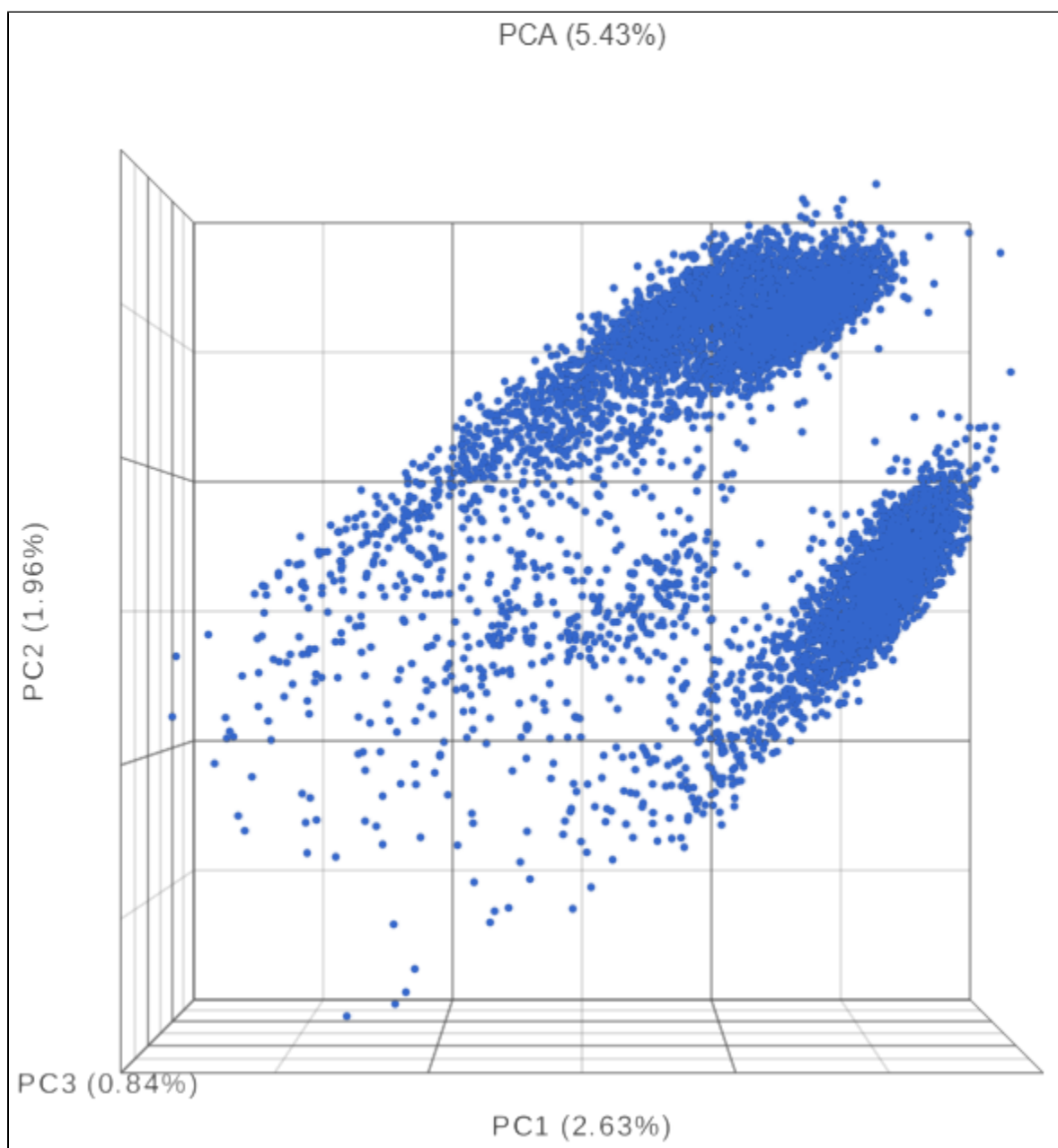


Figure 17. Each dot is a different cell. Cells are clustered based on how similar their expression profile is across the combined mRNA and protein data

- Click and drag the **Scree plot** from **New plot** under *Setup* on the left onto the canvas
- Drop it over the **Replace** option (Figure 4)

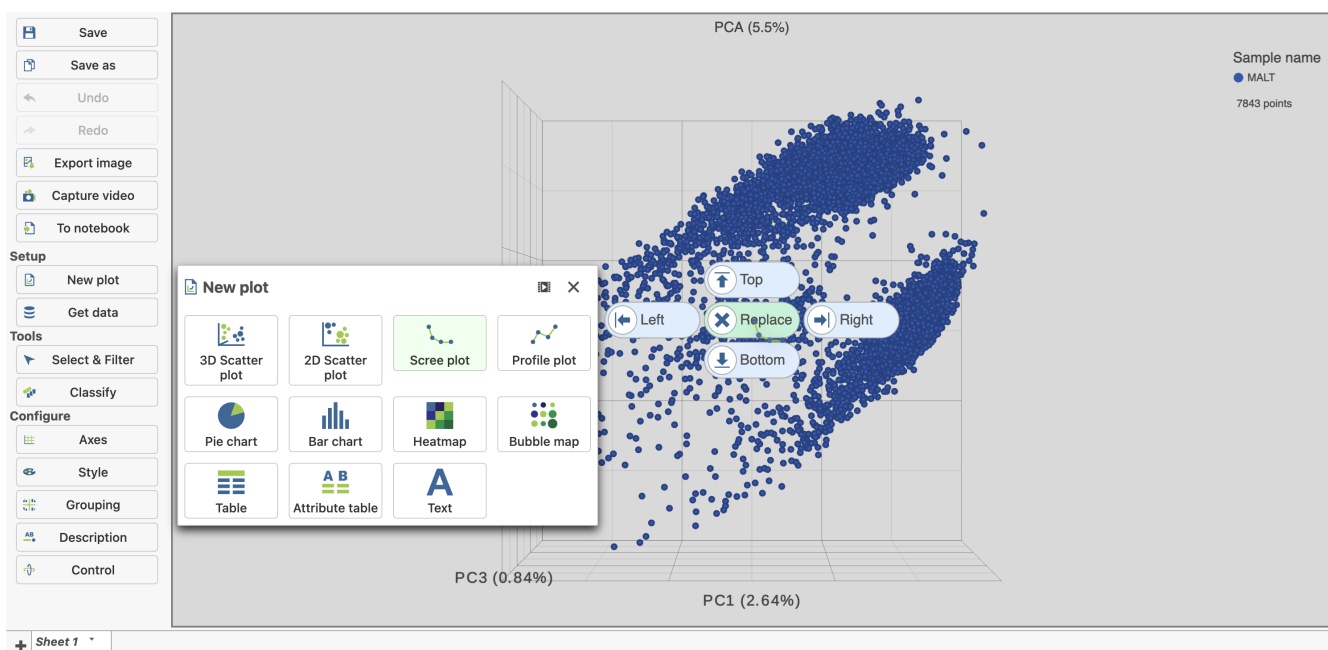


Figure 18. Click and drag the Scree plot to replace the PCA plot on the canvas

- Select **PCA** as data for the new Scree plot (Figure 5)

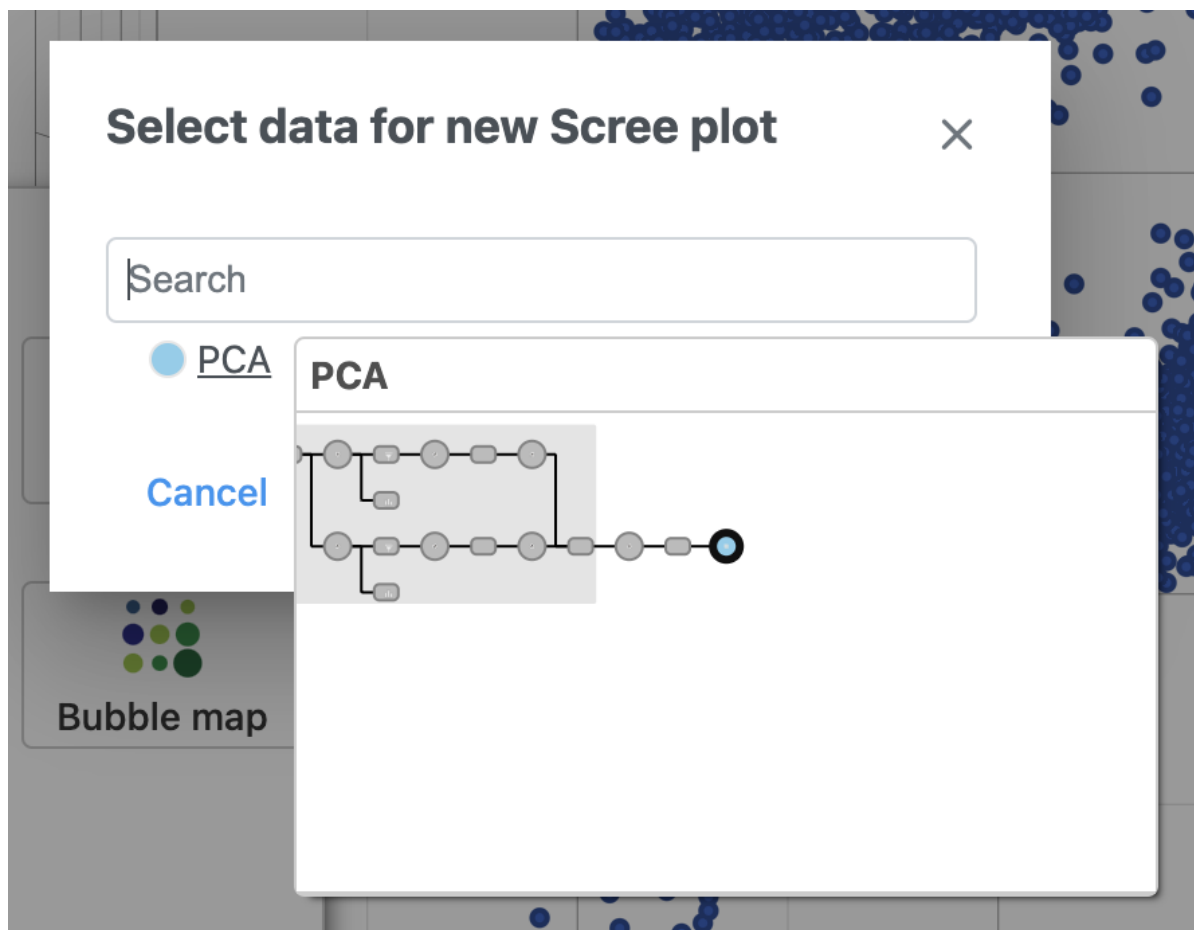


Figure 19. The PCA data node contains the data to draw the Scree plot

The Scree plot (Figure 6) shows the eigenvalues on the y-axis for each of the 100 PCs on the x-axis. The higher the eigenvalue, the more variance explained by each PC. Typically, after an initial set of highly informative PCs, the amount of variance explained by analyzing additional components is minimal. By identifying the point where the Scree plot levels off, you can choose an optimal number of PCs to use in downstream analysis steps like graph-based clustering and UMAP.

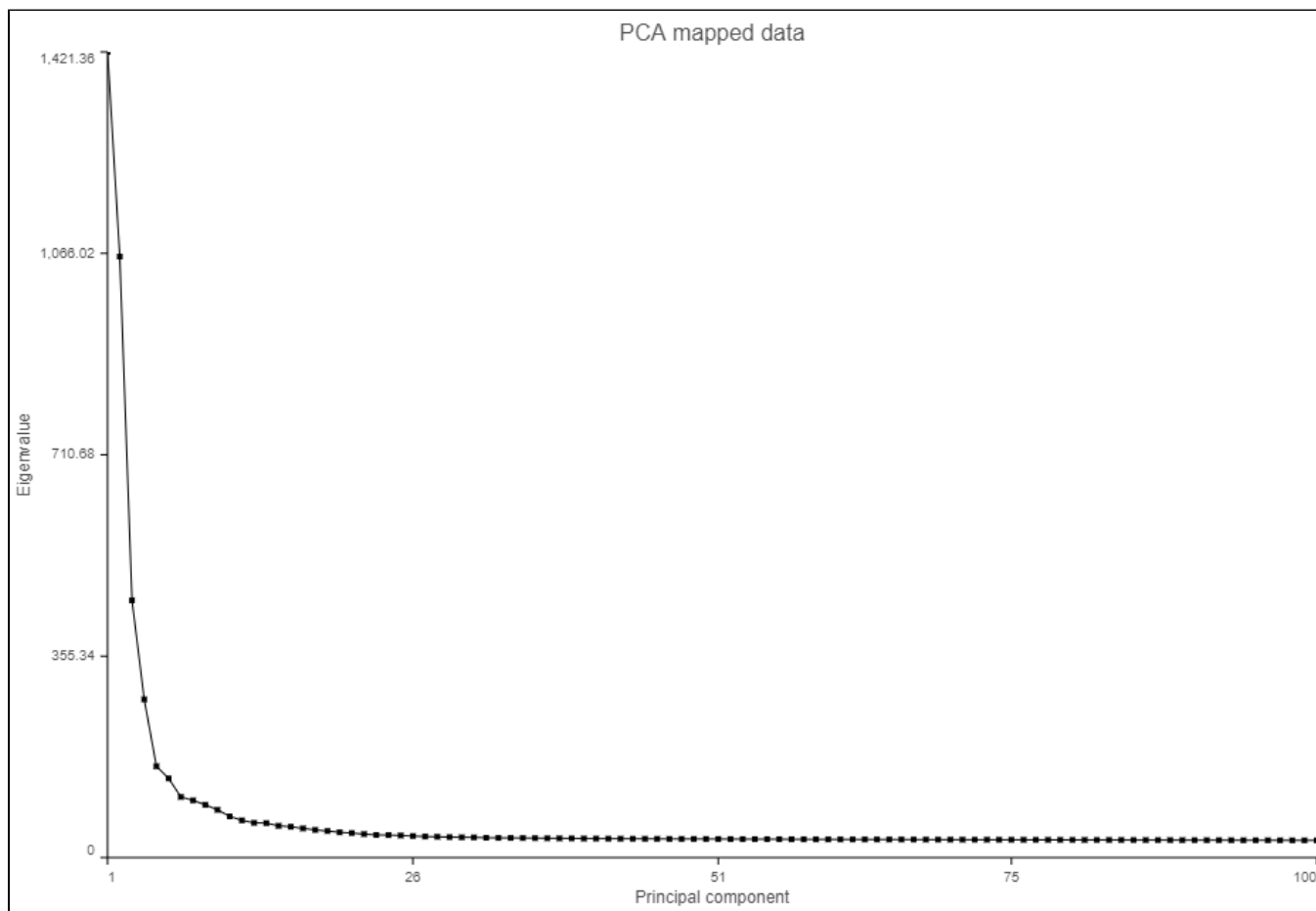


Figure 20. Scree plot shows the amount of variation explained by each principal component

- Click and drag over the first set of PCs to zoom in (Figure 7)

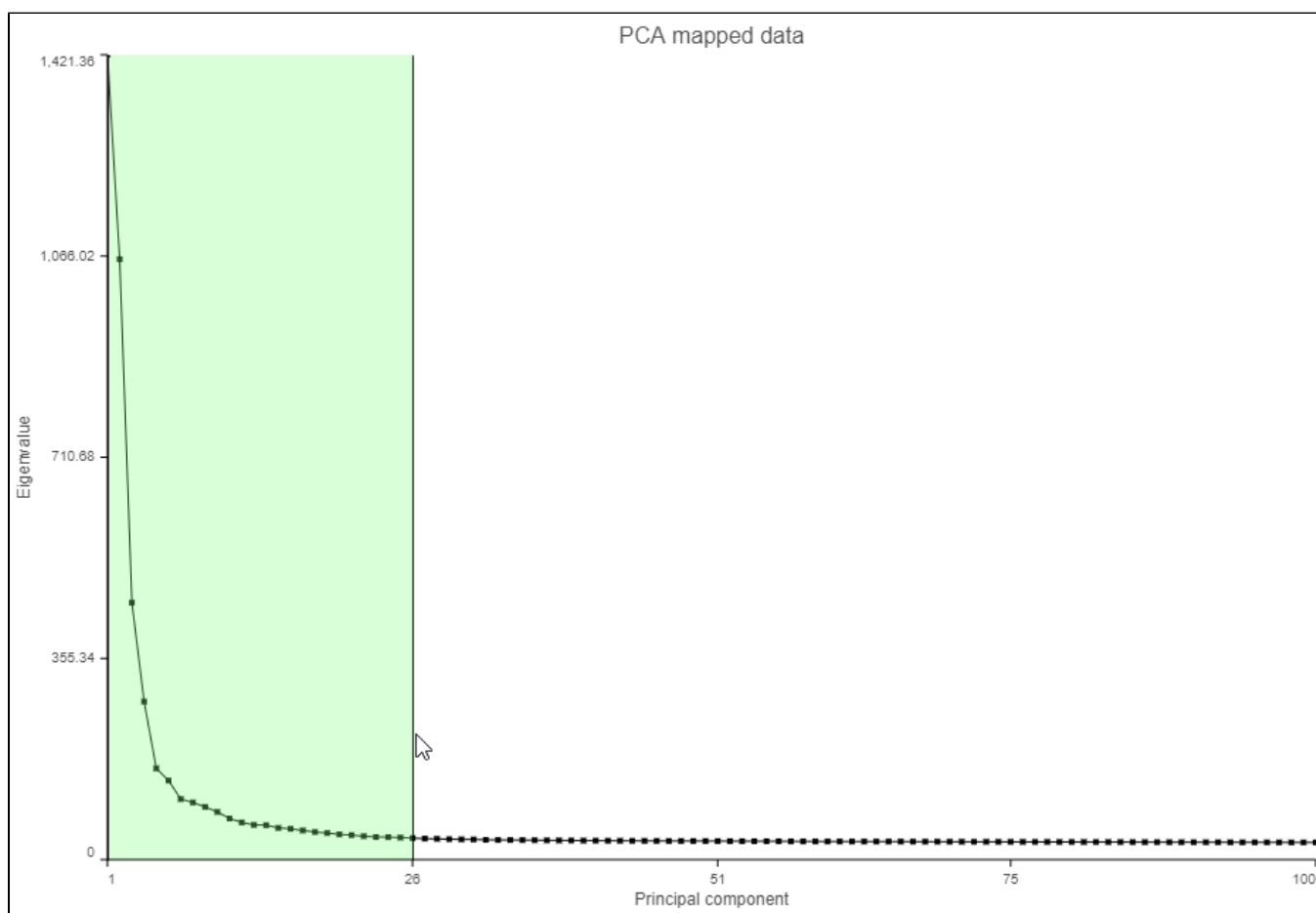


Figure 21. Click and drag on the Scree plot to zoom in and see the first set of principal components

- Mouse over the Scree plot to identify the point where additional PCs offer little additional information (Figure 8)

In this data set, a reasonable cut-off could be set anywhere between around 10 and 30 PCs. We will use 15 in downstream steps.

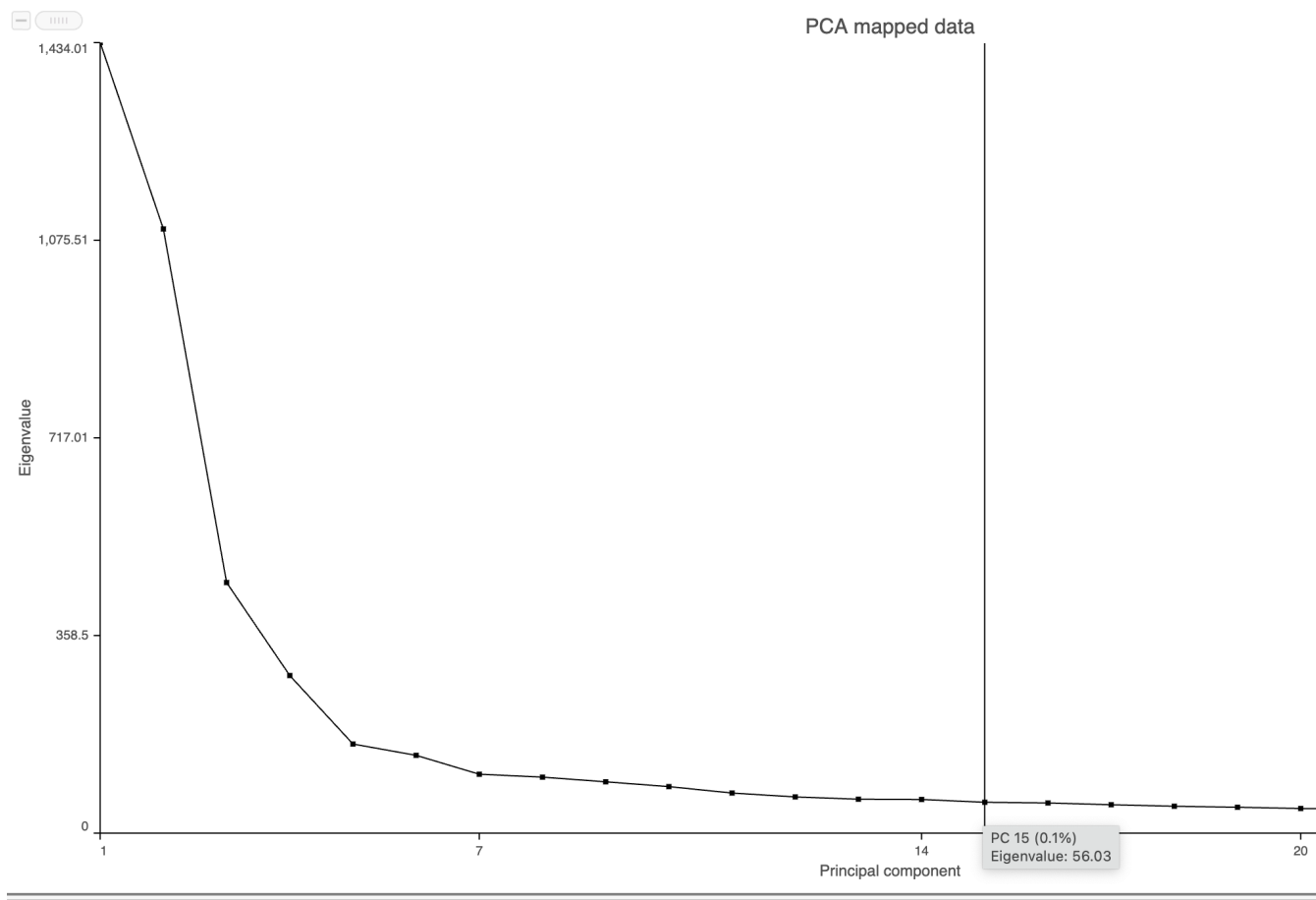


Figure 22. Identifying the optimal number of PCs

Graph-based clustering

We can use Graph-based clustering to group similar cells together in an unsupervised manner.

- Click the **project name** near the top to go back to the *Analyses* tab
- Click the circular **PCA** data node
- Click **Exploratory analysis** in the toolbox
- Click **Graph-based clustering**
- Click to **Compute biomarkers**
- Set the number of principal components to **15** (Figure 9)
- Click **Configure** under *Advanced options* and change the *Resolution* to **1.0**
- Click **Finish** to run the task

Clustering

Clustering algorithm

Three modifications of Louvain clustering algorithm are available (Waltman & Van Eck, 2013). The most recent version is Smart Local Moving approach (SLM).

☒ Louvain ☐ Louvain with refinement ☐ SLM

☒ Compute biomarkers

Queue a "Compute biomarkers" task for the resulting attribute, which will compute the features that are expressed highly when comparing each cluster.

PCA

Number of principal components

The number of principal components to calculate.

☐ All

☒ 15

Advanced options

Option set

-- Default --



[Configure](#)

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Figure 23. Graph-based clustering task set up. Reduce the number of PCs to 15

A *Graph-based clustering* task node will be added to the pipeline under the *Analyses* tab and a circular *Graph-based clusters* output data node will be produced (Figure 10)

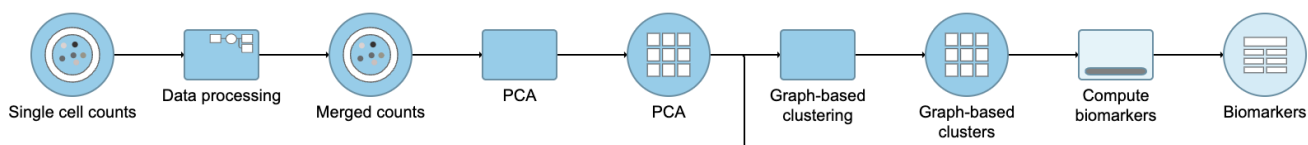


Figure 24. Graph-based clustering task and output data nodes

UMAP

Once the graph-based clustering task has completed, we can visualize the results with a UMAP plot. You could use the same steps here to generate a t-SNE plot. For this tutorial, we will use UMAP, as it is faster on several thousand cells.

- Click the circular **PCA** data node
- Click **Exploratory analysis** in the toolbox
- Click **UMAP**
- Set the number of principal components to **15** (Figure 11)
- Click **Finish** to run the task

Initialize output values

Initialize the low dimensional embedding either at random or using a spectral embedding of the fuzzy 1-skeleton.

Random ▼

PCA

Number of principal components

The number of principal components to calculate.

☐ All

☒ 15 ▲▼

Advanced options

Option set

-- Default -- ▼

[Configure](#)

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[Finish](#)

Figure 25. UMAP task set up. Reduce the number of PCs to 15.

A UMAP task node will be added to the pipeline under the *Analyses* tab and a circular UMAP output data node will be produced (Figure 12)

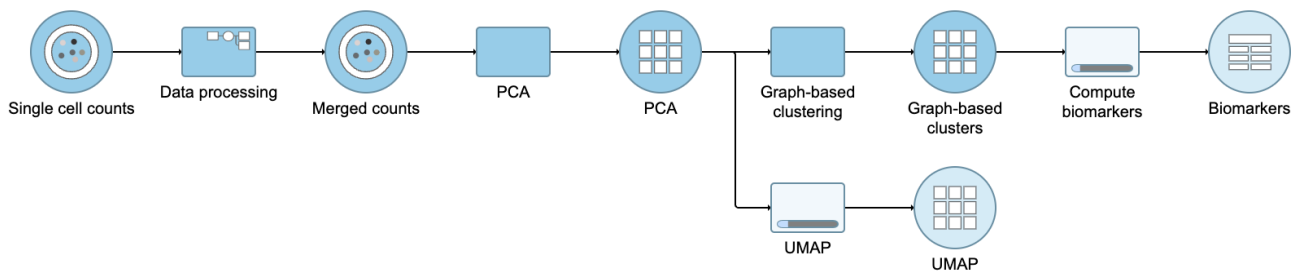


Figure 26. UMAP task and output data node

Notes on Performing Exploratory Analysis with Protein or Gene Expression Data Only

In this tutorial, we have performed exploratory analysis on merged protein and gene expression data, and we will perform classification on the merged data in the next step.

It can be interesting to perform exploratory analysis on the two feature types separately. For example, you might be interested to see how the clustering of the same cells differs between protein expression profiles vs. gene expression profiles.

To perform exploratory analysis on the two feature types separately, select the *Merged counts* data node, click *Pre-analysis tools*, followed by *Split by feature type* from the toolbox. A new task, *Split by feature type*, will be added to the pipeline resulting in two output data nodes: *Antibody capture* (protein data) and *Gene expression* (mRNA data). Both contain the same high-quality cells.

Performing exploratory analysis with gene expression data is the same as for the merged counts. Because there are a large number of genes, you will need to reduce the dimensionality with PCA, choose an optimal number of PCs and perform downstream clustering and visualization (e.g. graph-based clustering and UMAP/t-SNE). Performing exploratory analysis with protein data is different. There is no need to reduce the dimensionality as there are only a handful of features (17 proteins in this case), so you can proceed straight to downstream clustering and visualization. Figure 13 shows an example of how the pipeline might look if the data is split and analyzed separately.

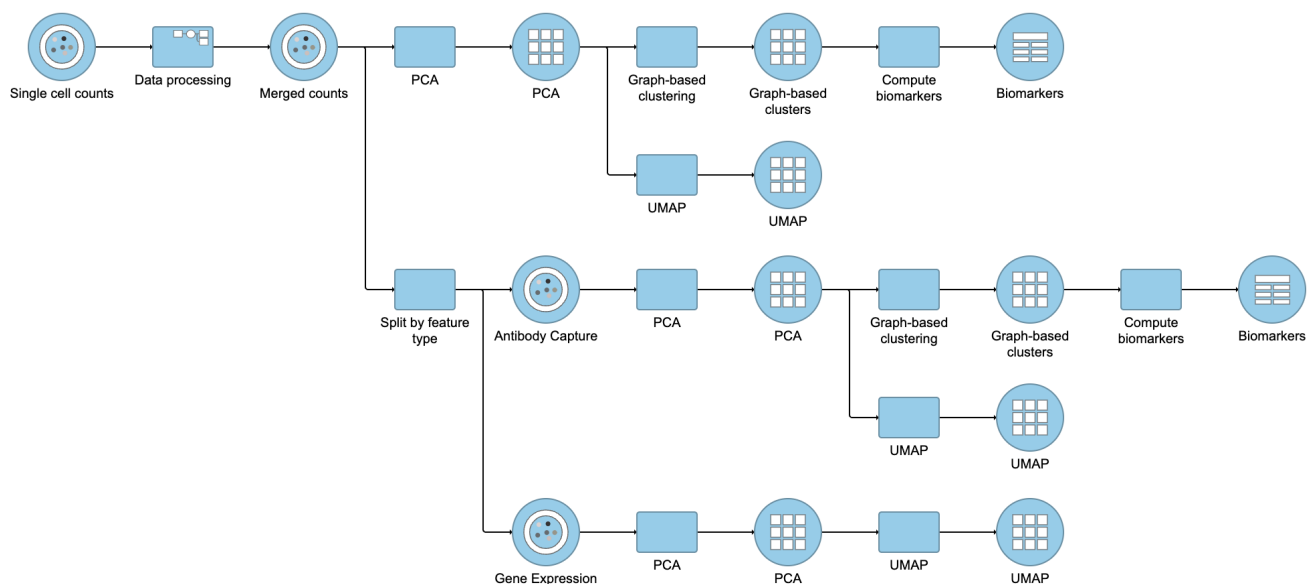


Figure 27. Example of how the pipeline might look if you split the merged counts and perform exploratory analysis for protein and gene expression data separately

You can then use the *Data viewer* to bring together multiple plots for comparison (Figure 14).

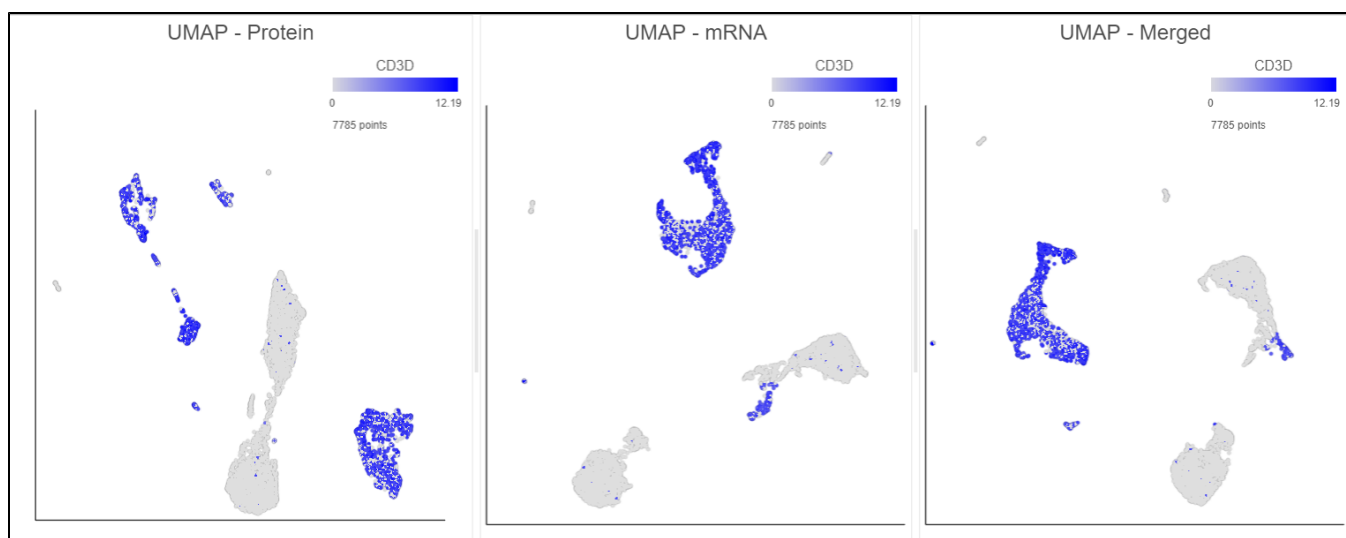


Figure 28. Comparison of 2D UMAP plots for the same cells clustered on protein, mRNA and merged data. All cells are coloured based on their expression of the CD3D gene (in blue). Note, the plots in this figure may differ from the default UMAP plots because these are 2D plots. Default UMAP plots are in 3D.

« Data Processing Classifying Cells »

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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