Data Processing

Split matrix

The *Single cell counts* data node contains two different types of data, mRNA expression and protein expression. So that we can process these two different types of data separately, we will split the data by data type.

- Click the **Single cell counts** data node
- Click **Pre-analysis tools** in the toolbox
- Click **Split by feature type**

A rectangular task node will be created along with two circular data nodes, one for each data type (Figure 1). The labels for these data types are determined by features.csv file used when processing the data with Cell Ranger. Here, our data is labeled *Gene Expression*, for the mRNA data, and *Antibody Capture*, for the protein data.

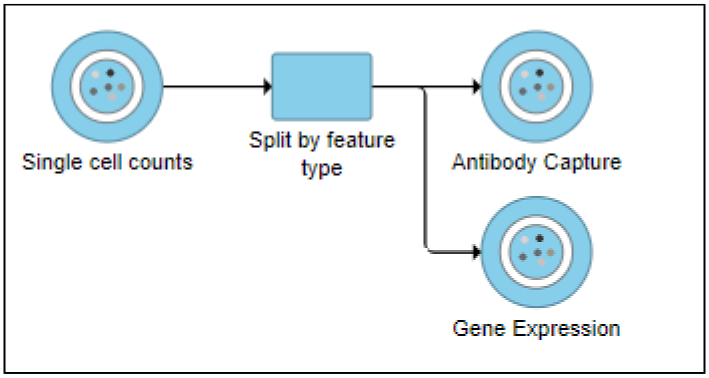


Figure 18. Split by feature type produces two data nodes, one for each data type

Filter low-quality cells

An important step in analyzing single cell RNA-Seq data is to filter out low-quality cells. A few examples of low-quality cells are doublets, cells damaged during cell isolation, or cells with too few counts to be analyzed. In a CITE-Seq experiment, protein aggregation in the antibody staining reagents can cause a cell to have a very high number of counts. These are low-quality cells that can be excluded. Additionally, if all cells in a data set are expected to show a baseline level of expression for one of the antibodies used, it may be appropriate to filter out cells with very low counts or a low number of detected features. You can do this in Partek Flow using the *Single cell QA/QC* task.

We will start with the protein data.

- Click the **Antibody Capture** data node
- Click **QA/QC** in the toolbox
- Click **Single Cell QA/QC**

This produces a *Single-cell QA/QC* task node (Figure 2).

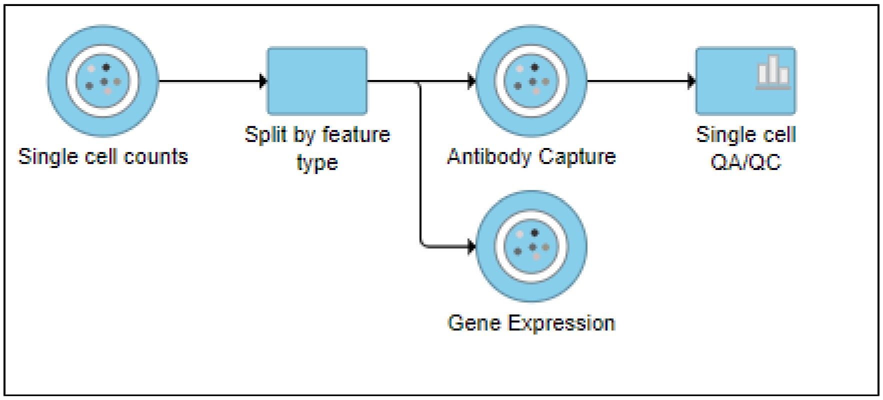


Figure 19. Single cell QA/QC produces a task node

- Double-click the **Single cell QA/QC** task node to open the task report

The *Single cell QA/QC* report opens in a new data viewer session. There are interactive violin plots showing the most commonly used quality metrics for each cell: the total count per cell and the number of detected features per cell (Figure 3). Each point on the plots is a cell and the violins illustrate the distribution of values for the y-axis metric.

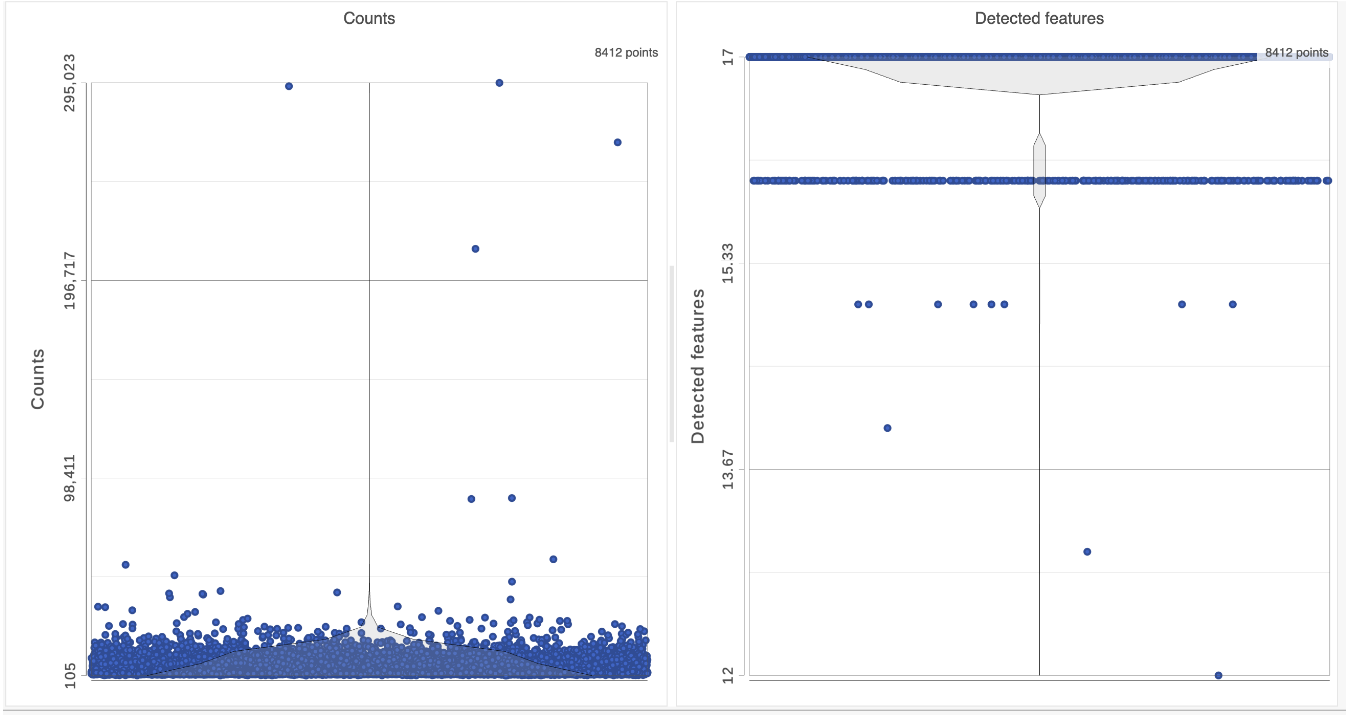
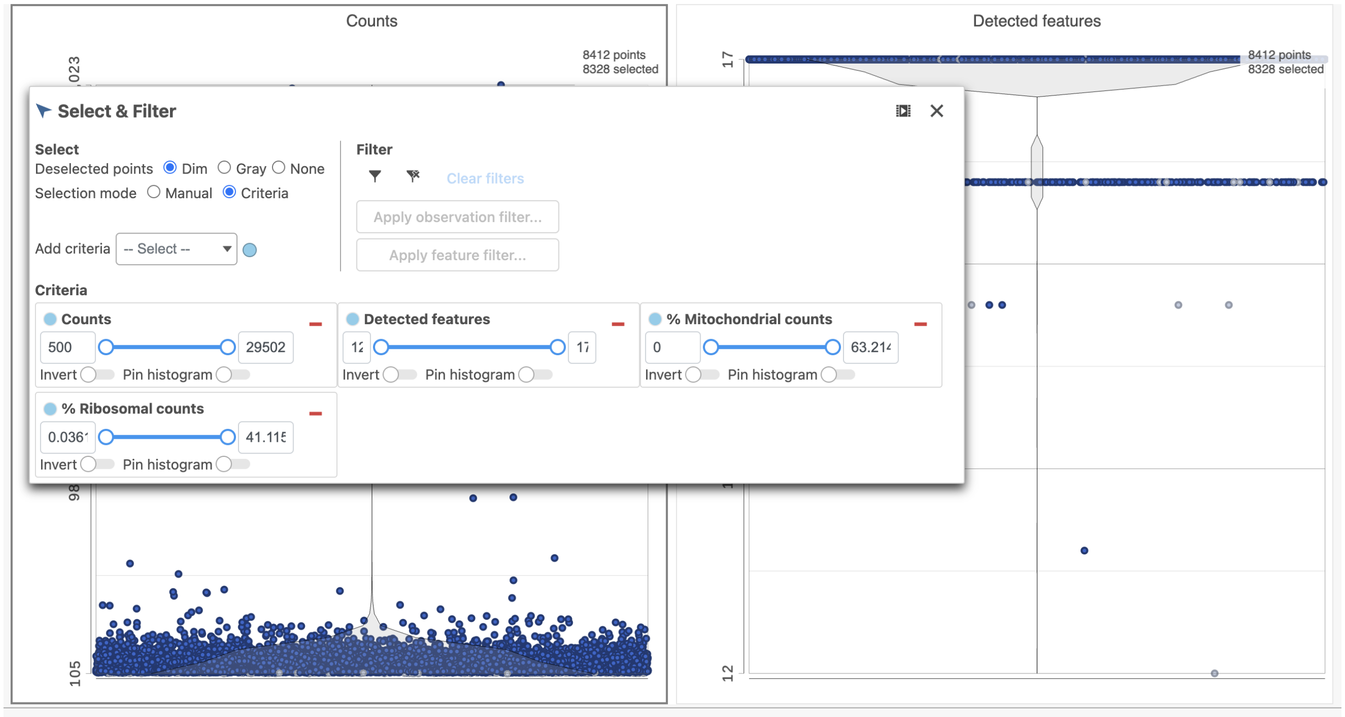


Figure 20. Each cell is shown as a point on the plot.

For this analysis, we will set a maximum counts threshold to exclude potential protein aggregates and, because we expect every cell to be bound by several antibodies, we will also set a minimum counts threshold.

- Select one of the plots on the canvas
- In the **Select & Filter** icon on the left under *Tools*, set the *Counts* threshold to keep cells between **500** and **20000** (Figure 4)



Select & Filter

Select

Deselected points ● Dim ○ Gray ○ None

Selection mode ○ Manual ● Criteria

Add criteria -- Select --

Filter

Clear filters

Apply observation filter...

Apply feature filter...

Criteria

Counts	500	29502
Detected features	12	17
% Mitochondrial counts	0	63.214
% Ribosomal counts	0.0361	41.115

Invert ○ Pin histogram ○

Figure 21. Filtering low quality cells based on protein expression data

- Click ▼ under *Filter* on the right
- Click **Apply observation filter...**
- Select the **Antibody Capture** data node as input in the pipeline preview (Figure 5)
- Click **Select**

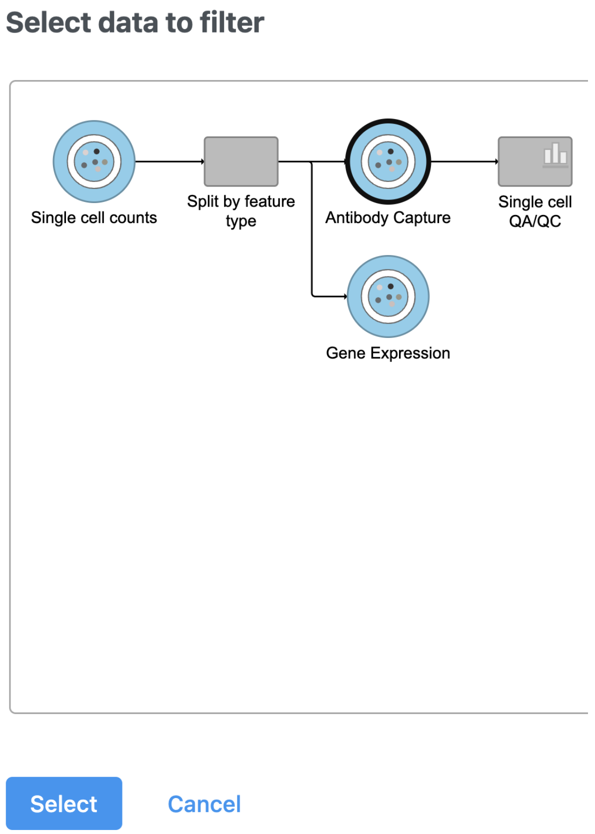

Select data to filter

Figure 22. After the Apply filter button is selected, you will be presented with a preview of your pipeline. You need to select the appropriate data node to apply the filtering to. In this case, the Antibody capture node

You will see a message telling you a new task has been enqueued.

- Click **OK** to dismiss the message
- Click the **project name** at the top to go back to the *Analyses* tab
- Your browser may warn you that any unsaved changes to the data viewer session will be lost. Ignore this message and proceed to the *Analyses* ta

A new task, *Filter counts*, is added to the *Analyses* tab. This task produces a new *Filter counts* data node.

Next, we can repeat this process for the *Gene Expression* data node.

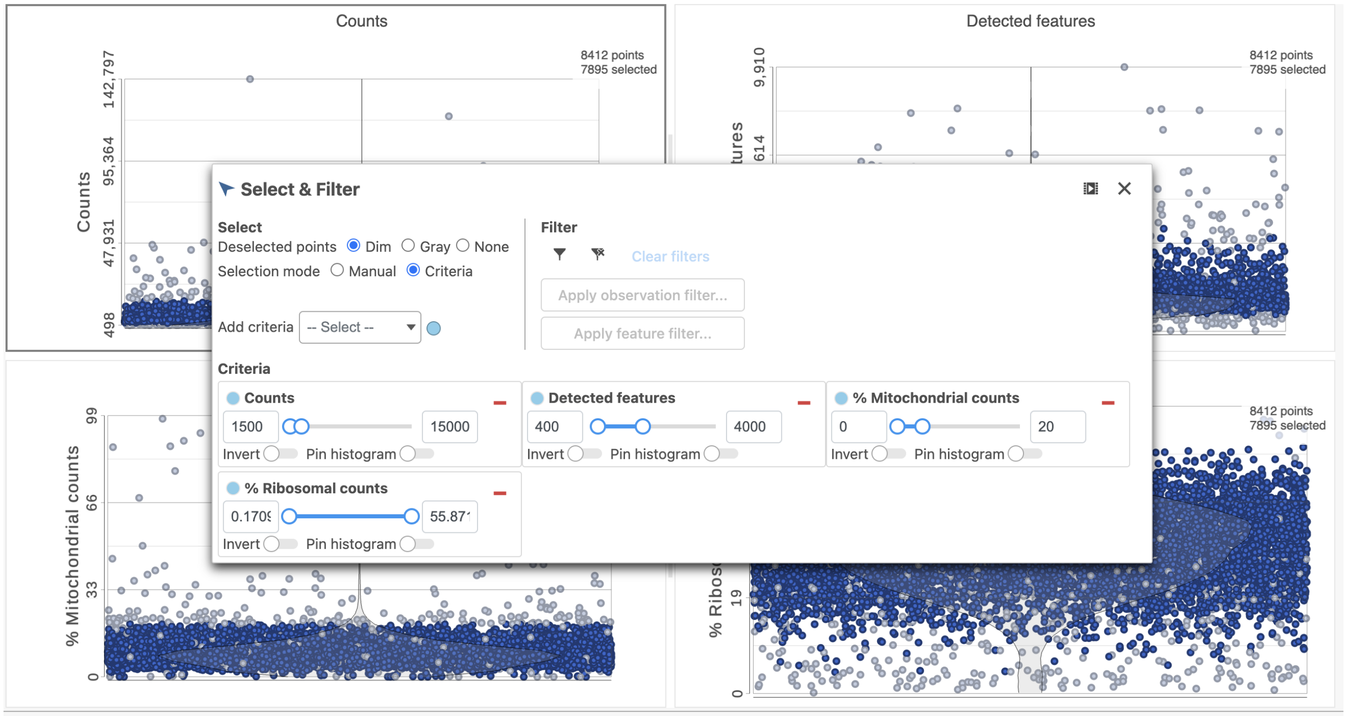
- Click the **Gene Expression** data node
- Click the **QA/QC** section in the toolbox
- Click **Single Cell QA/QC**

This produces a *Single-cell QA/QC* task node

- Double-click the **Single cell QA/QC** task node to open the task report

The task report lists the number of counts per cell, the number of detected features per cell, the percentage of mitochondrial reads per cell, and the percentage of ribosomal counts per cell in four violin plots (Figure 6). For this analysis, we will set maximum and minimum thresholds for total counts and detected genes to exclude potential doublets and a maximum mitochondrial reads percentage filter to exclude potential dead or dying cells. There is no need to apply a filter based on the percentage of ribosomal counts in this tutorial.

- In the *Selection* card on the right, set the *Counts* threshold to keep cells between **1500** and **15000**
- Set the *Detected features* to keep cells between **400** and **4000**
- Set the % *Mitochondrial counts* to keep cells between **0%** and **20%** (Figure 6)



Select & Filter

Select

Deselected points ● Dim ○ Gray ○ None

Selection mode ○ Manual ● Criteria

Add criteria -- Select --

Filter

Clear filters

Apply observation filter...

Apply feature filter...

Criteria

Criterion	Min	Max
Counts	1500	15000
Detected features	400	4000
% Mitochondrial counts	0	20
% Ribosomal counts	0.170ξ	55.87'

Invert ○ Pin histogram ○

Figure 23. Filtering low quality cells based on gene expression data

- Click under *Filter* on the right
- Click **Apply observations filter**
- Select the **Gene Expression** data node as input in the pipeline preview
- Click **Select**
- Click **OK** to dismiss the message about the task being enqueued
- Click the **project name** at the top to go back to the *Analyses* tab
- Your browser may warn you that any unsaved changes to the data viewer session will be lost. Ignore this message and proceed to the *Analyses* ta

A new task, *Filter counts*, is added to the *Analyses* tab. This task produces a new *Filter counts* data node (Figure 7)

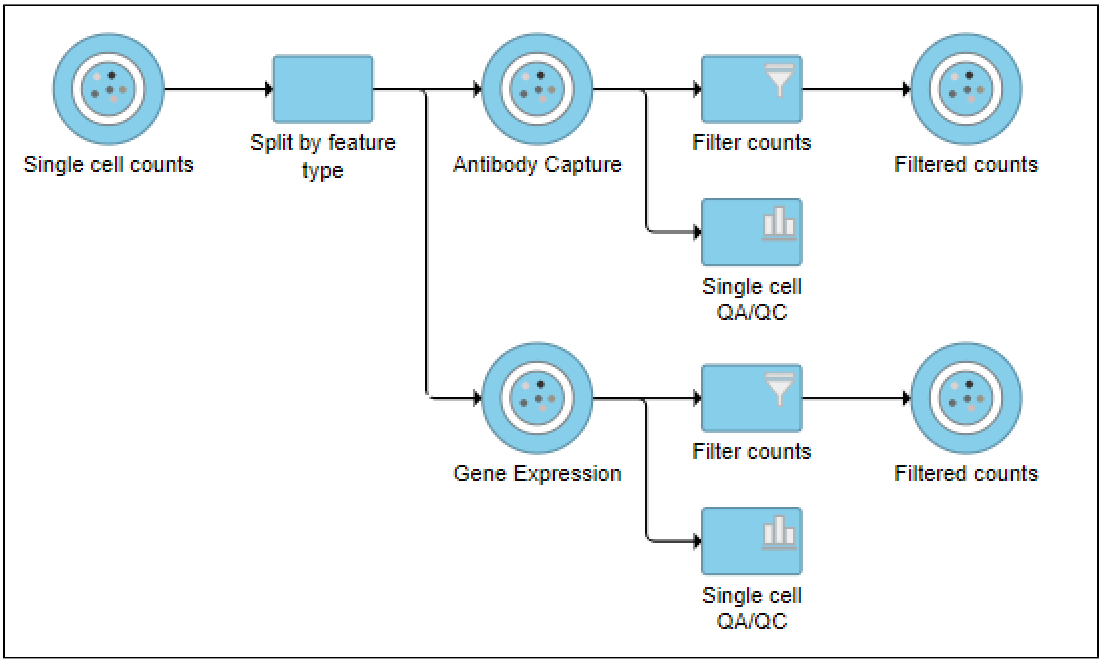


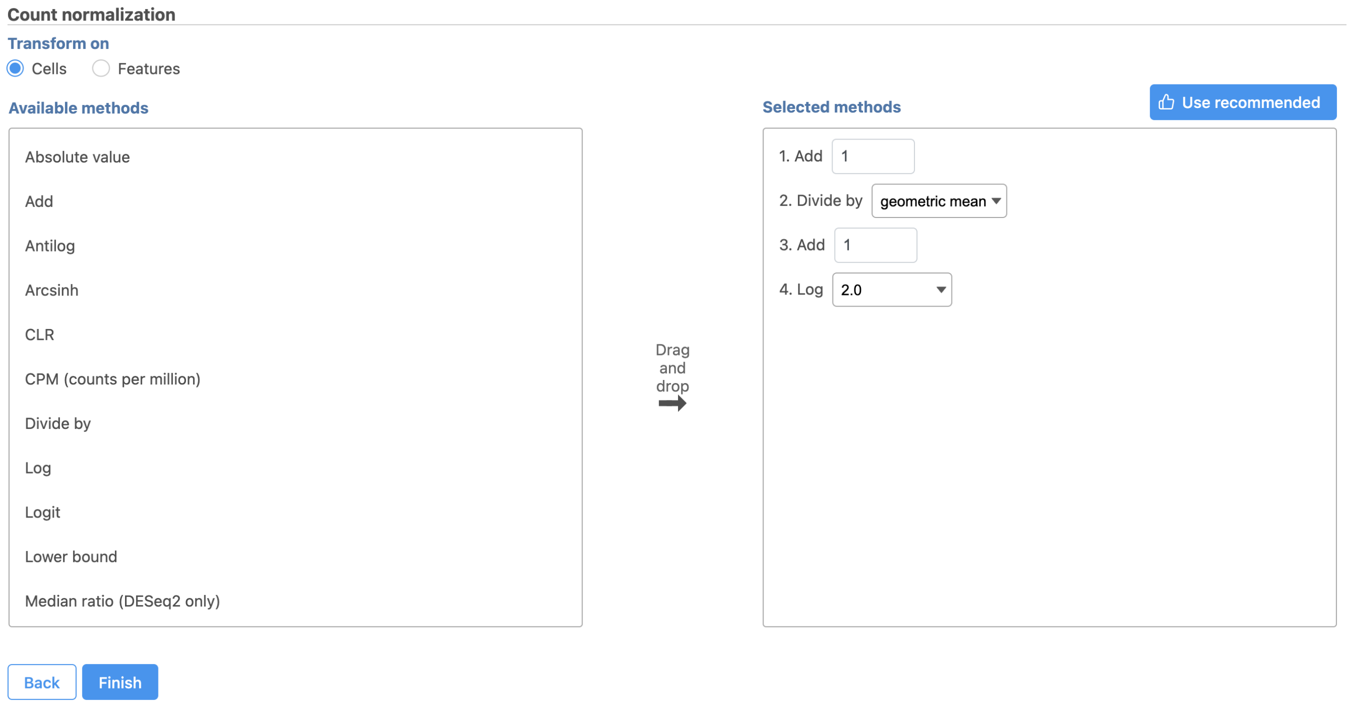
Figure 24. Antibody Capture and Gene Expression data have been filtered to remove low quality cells

Normalization

After excluding low-quality cells, we can normalize the data.

We will start with the protein data.

- Click the **Filtered counts** data node produced by filtering the *Antibody Capture* data node
- Click **Normalization and scaling** in the toolbox
- Click **Normalization**
- Click the green Use recommended button
- Click **Finish** to run (Figure 8)



Count normalization

Transform on: ● Cells ○ Features

Selected methods:
1. Add 1
2. Divide by geometric mean
3. Add 1
4. Log 2.0

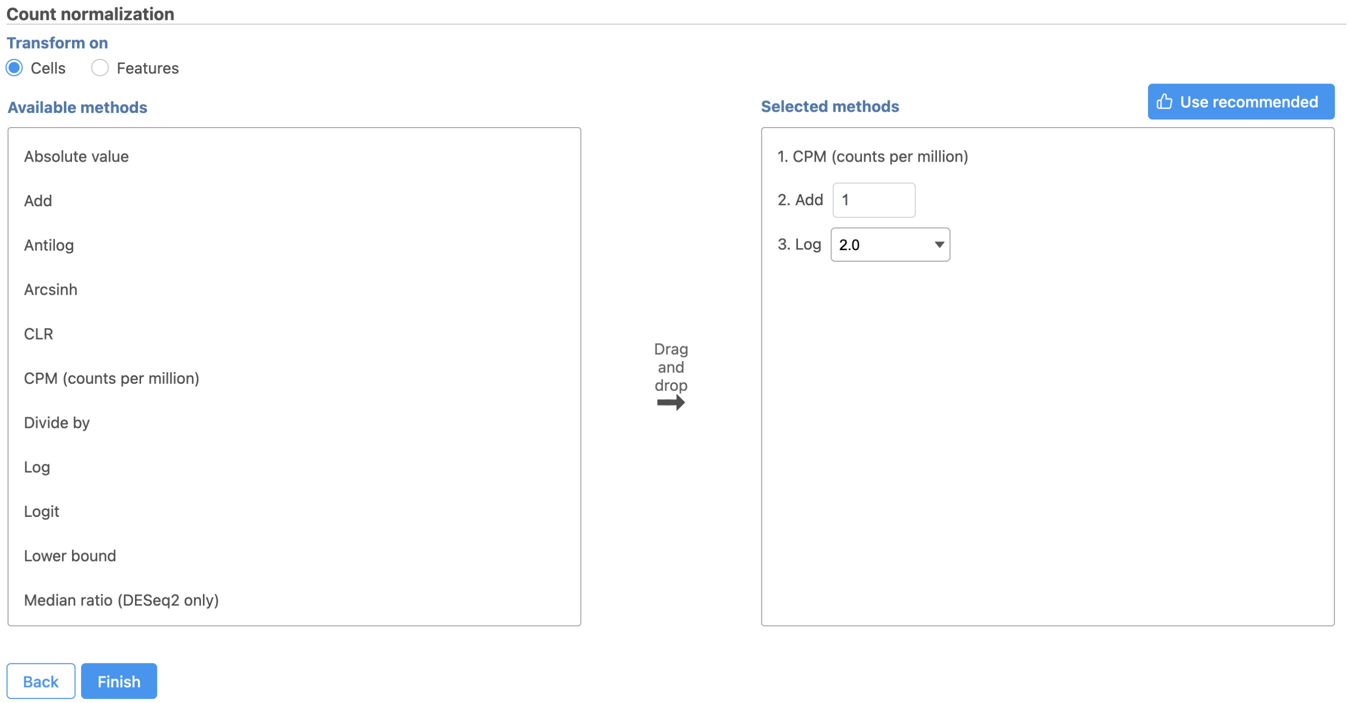
Figure 25. Recommended normalization for protein count data

The recommended normalization for protein data includes the following steps: Add 1, Divide by Geometric mean, Add 1, Log base 2. This is a variant of Centered log-ratio (CLR), which was used to normalize antibody capture protein counts data in the paper that introduced CITE-Seq [1] and in subsequent publications on similar assays [2. 3]. CLR normalization includes the following steps: Add 1, Divide by Geometric mean, Add 1, log base e. Normalizing the protein data to base 2 instead of e allows for better integration with gene expression data further downstream. If you would prefer to use CLR, click and drag CLR from the panel on the left to the right. If you do choose to use CLR, we recommend making sure the gene expression data is normalized to the base e, to allow for smoother integration further downstream.

Normalization produces a *Normalized counts* data node on the *Antibody Capture* branch of the pipeline.

Next, we can normalize the mRNA data. We will use the recommended normalization method in Partek Flow, which accounts for differences in library size, or the total number of UMI counts, per cell, adds 1 and log2 transforms the data.

- Click the **Filtered counts** data node produced by filtering the *Gene Expression* data node
- Click the **Normalization and scaling** section in the toolbox
- Click **Normalization**
- Click the Use recommended button
- Click **Finish** to run (Figure 9)



Count normalization

Transform on

● Cells ○ Features

Available methods

Absolute value
Add
Antilog
Arcsinh
CLR
CPM (counts per million)
Divide by
Log
Logit
Lower bound
Median ratio (DESeq2 only)

Drag and drop ➡

Selected methods

Use recommended

1. CPM (counts per million)
2. Add: 1
3. Log: 2.0

Back Finish

Figure 26. Recommended normalization for single cell gene expression data

Normalization produces a *Normalized counts* data node on the *Gene Expression* branch of the pipeline (Figure 10).

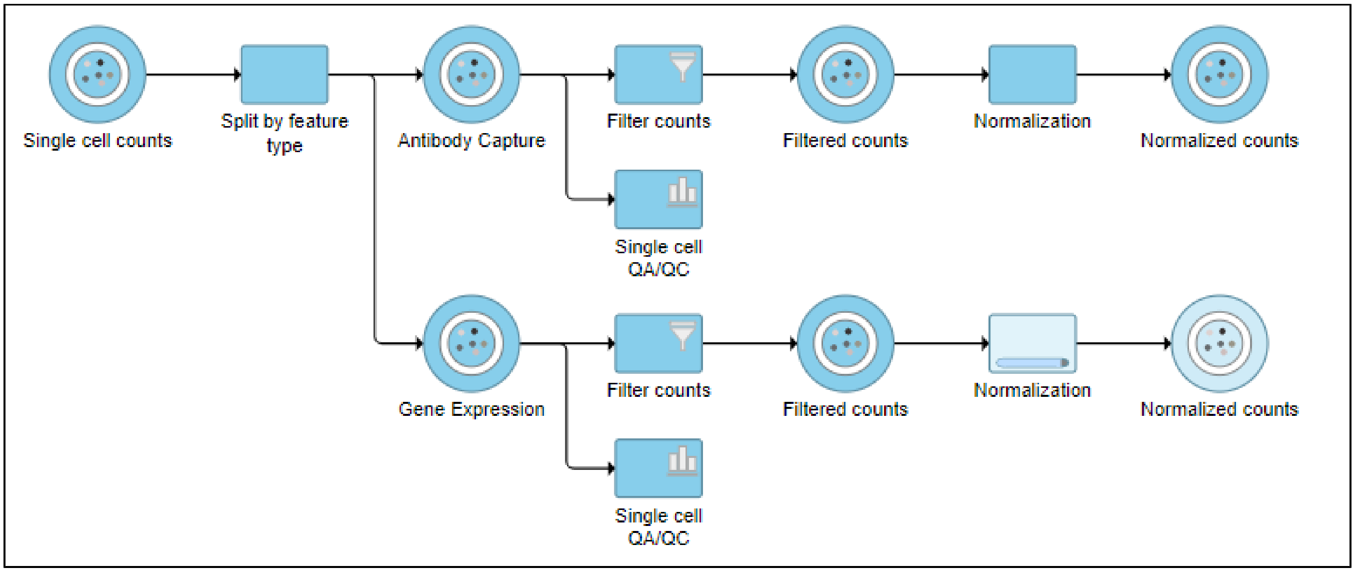


Figure 27. The two normalization tasks produce Normalized counts data nodes

Merge Protein and mRNA data

For quality filtering and normalization, we needed to have the two data types separate as the processing steps were distinct. For downstream analysis, we want to be able to analyze protein and mRNA data together. To bring the two data types back together, we will merge the two normalized counts data nodes.

- Click the **Normalized counts** data node on the *Antibody Capture* branch of the pipeline
- Click **Pre-analysis tools** in the toolbox
- Click **Merge matrices**
- Click **Select data node** to launch the data node selector

Data nodes that can be merged with the *Antibody Capture* branch *Normalized counts* data node are shown in color (Figure 11).

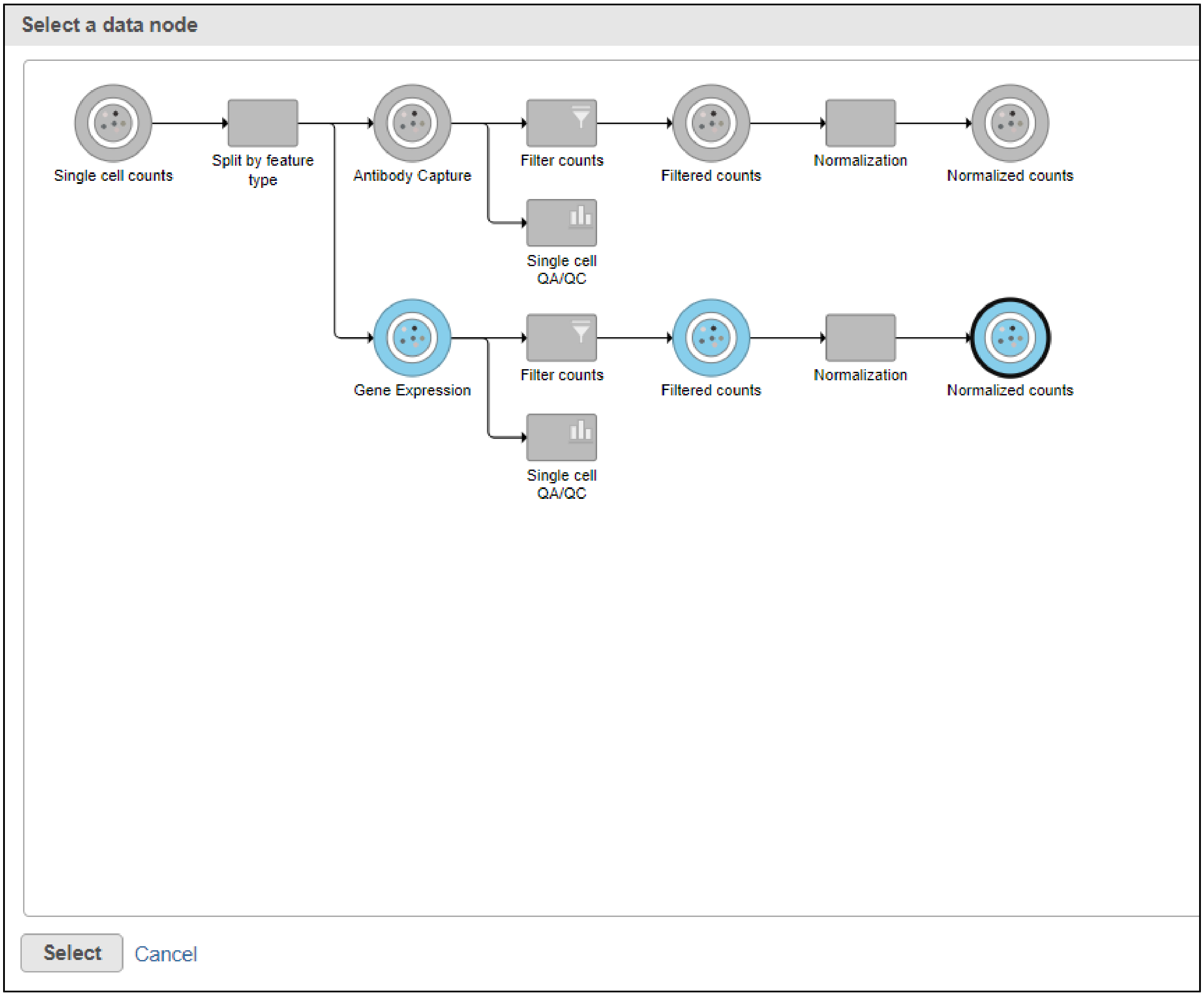


Figure 28. Select the normalizated gene expression counts to merge the protein counts with

- Click the **Normalized counts** data node on the *Gene Expression* branch of the pipeline (Figure 11)
- Click **Select**
- Click **Finish** to run the task

The output is a *Merged counts* data node (Figure 12). This data node will include the normalized counts of our protein and mRNA data. The intersection of cells from the two input data nodes is retained so only cells that passed the quality filter for both protein and mRNA data will be included in the *Merged counts* data node.

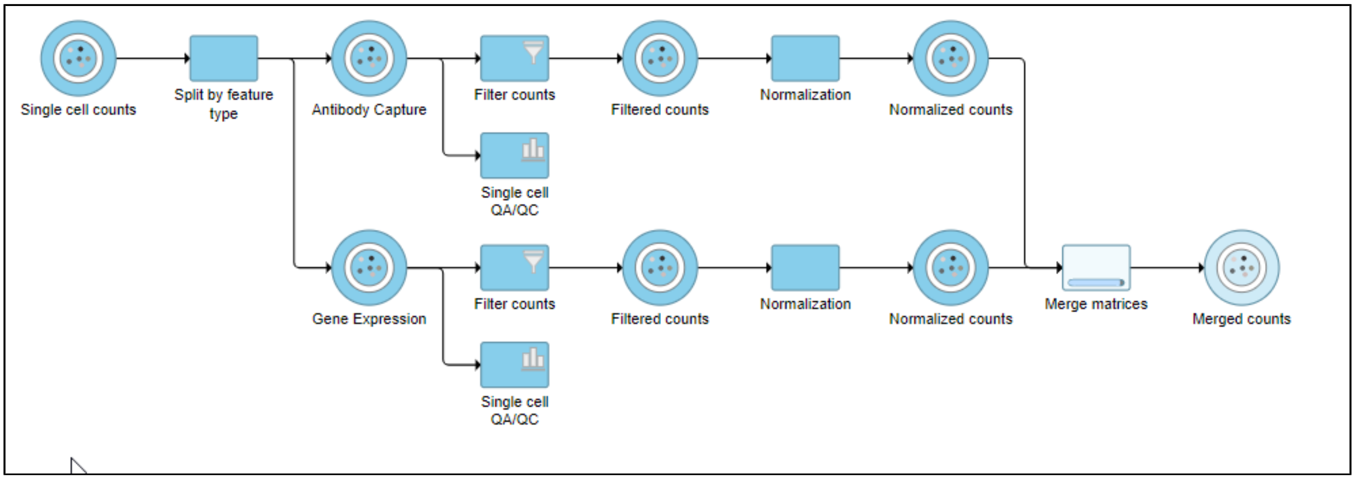


Figure 29. Merged counts output

Collapsing tasks to simplify the pipeline

To simplify the appearance of the pipeline, we can group task nodes into a single collapsed task. Here, we will collapse the filtering and normalization steps.

- Right-click the **Split by feature type** task node
- Choose **Collapse tasks** from the pop-up dialog (Figure 13)

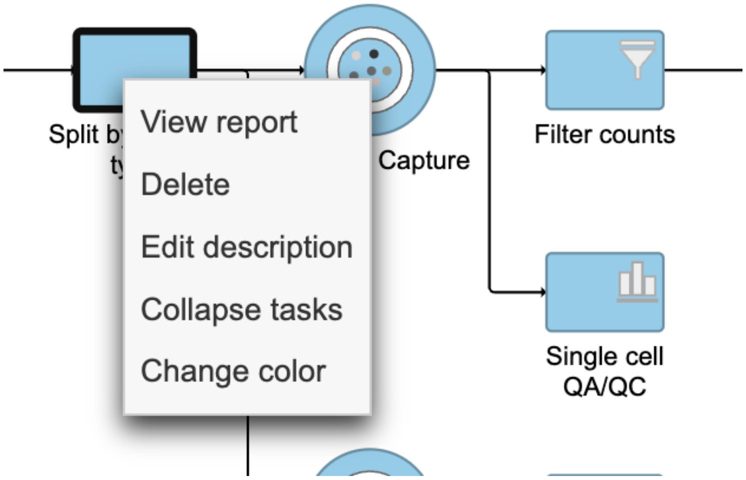


Figure 30. Choosing the first task node to generate a collapsed task

Tasks that can be selected for the beginning and end of the collapsed section of the pipeline are highlighted in purple (Figure 14). We have chosen the *Split matrix* task as the start and we can choose *Merge matrices* as the end of the collapsed section.

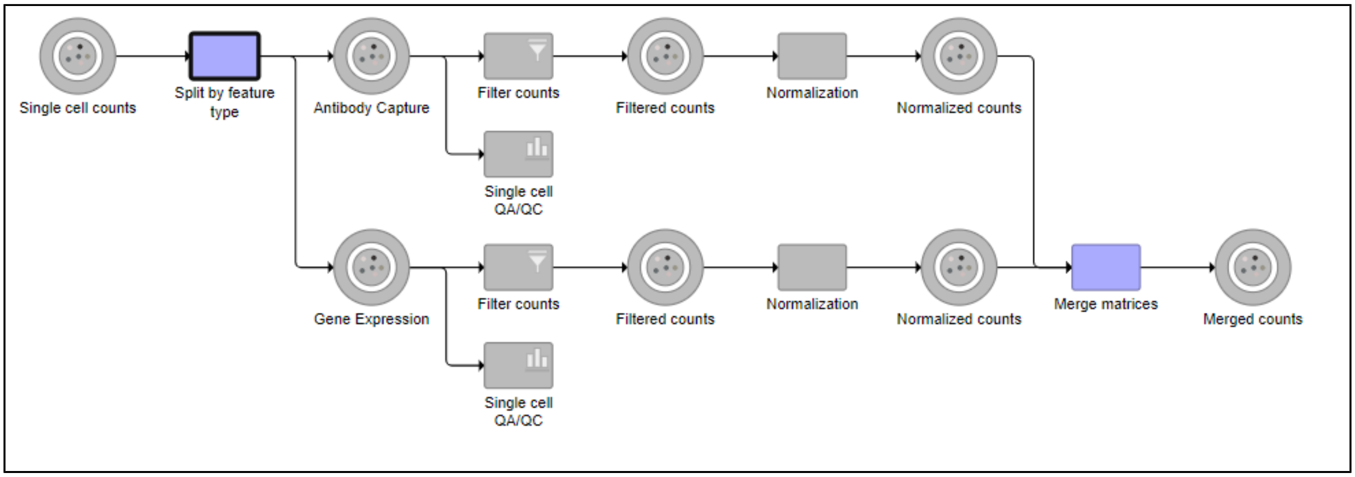
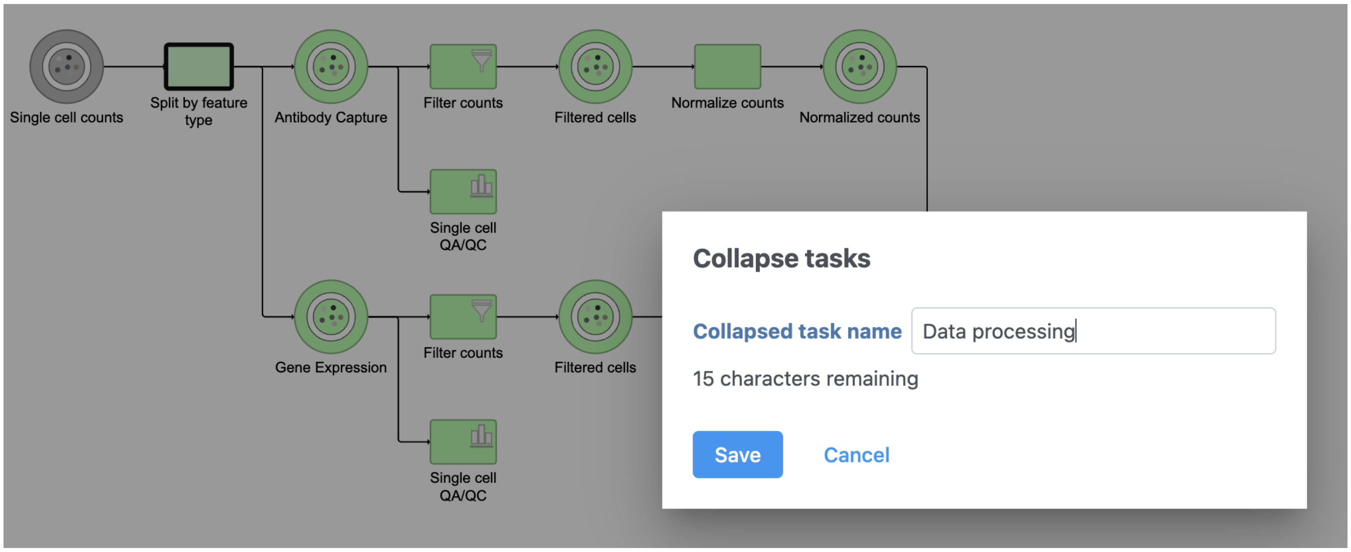


Figure 31. Tasks that can be the start or end of a collapsed task are shown in purple

- Click the **Merge matrices task** to choose it as the end of the collapsed section
- Name the Collapsed task **Data processing**
- Click **Save** (Figure 15)



Collapse tasks

Collapsed task name: Data processing

15 characters remaining

Figure 32. Naming the collapsed task

The new collapsed task, *Data processing*, appears as a single rectangular task node (Figure 16).

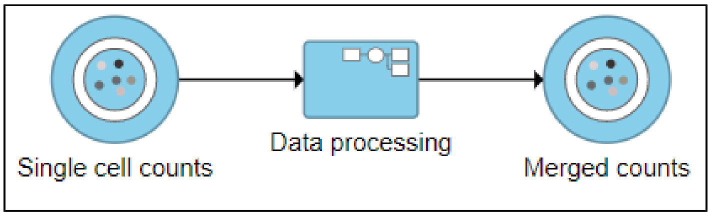


Figure 33. Collapsed tasks are represented by a single task node

To view the tasks in *Data processing,* we can expand the collapsed task.

- Double-click **Data processing** to expand it or right-click and choose **Expand collapsed task**

When expanded, the collapsed task is shown as a shaded section of the pipeline with a title bar (Figure 17).

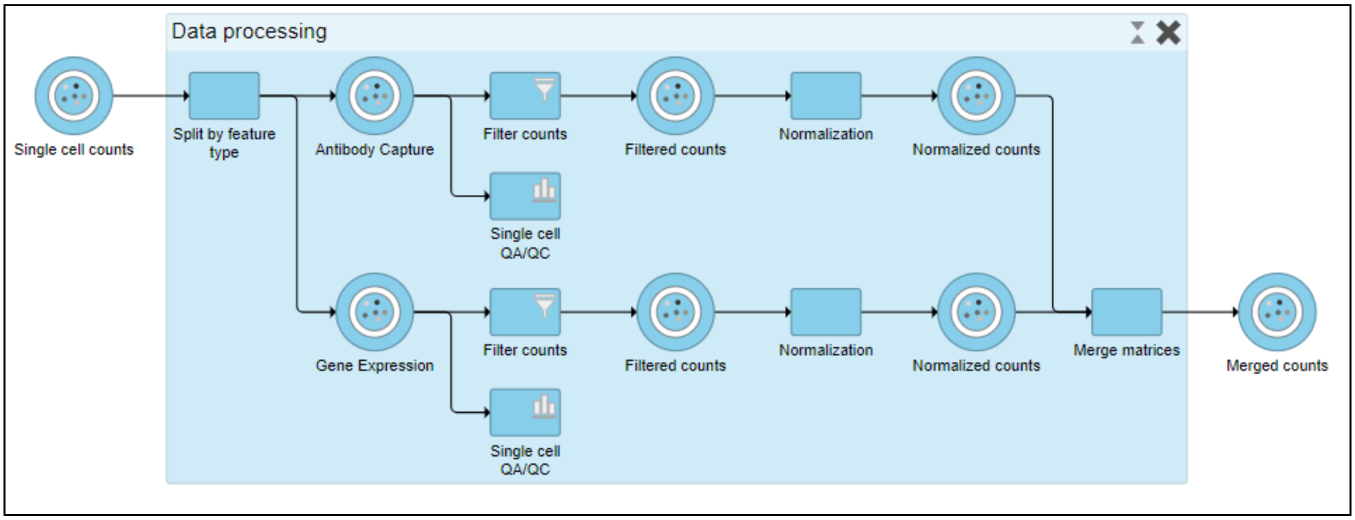


Figure 34. Expanding a collapsed task to show its components

To re-collapse the task, you can double click the title bar or click the ⧗ icon in the title bar. To remove the collapsed task, you can click the ✖. Please note that this will not remove tasks, just the grouping.

- Double-click the *Data processing* title bar to re-collapse

References

[1] Stoeckius, M., Hafemeister, C., Stephenson, W., Houck-Loomis, B., Chattopadhyay, P. K., Swerdlow, H., ... & Smibert, P. (2017). Simultaneous epitope and transcriptome measurement in single cells. Nature methods, 14(9), 865.

[2] Stoeckius, M., Zheng, S., Houck-Loomis, B., Hao, S., Yeung, B. Z., Mauck, W. M., ... & Satija, R. (2018). Cell hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. Genome biology, 19(1), 224.

[3] Mimitou, E., Cheng, A., Montalbano, A., Hao, S., Stoeckius, M., Legut, M., ... & Satija, R. (2018). Expanding the CITE-seq tool-kit: Detection of proteins, transcriptomes, clonotypes and CRISPR perturbations with multiplexing, in a single assay. bioRxiv, 466466.

« Importing Feature Barcoding Data   Dimensionality Reduction and Clustering »

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Your Rating: ☆☆☆☆☆ Results: ★★★★⯪ 10 rates