# Single Cell RNA-Seq Analysis in Partek® Flow®

HANDS-ON TRAINING

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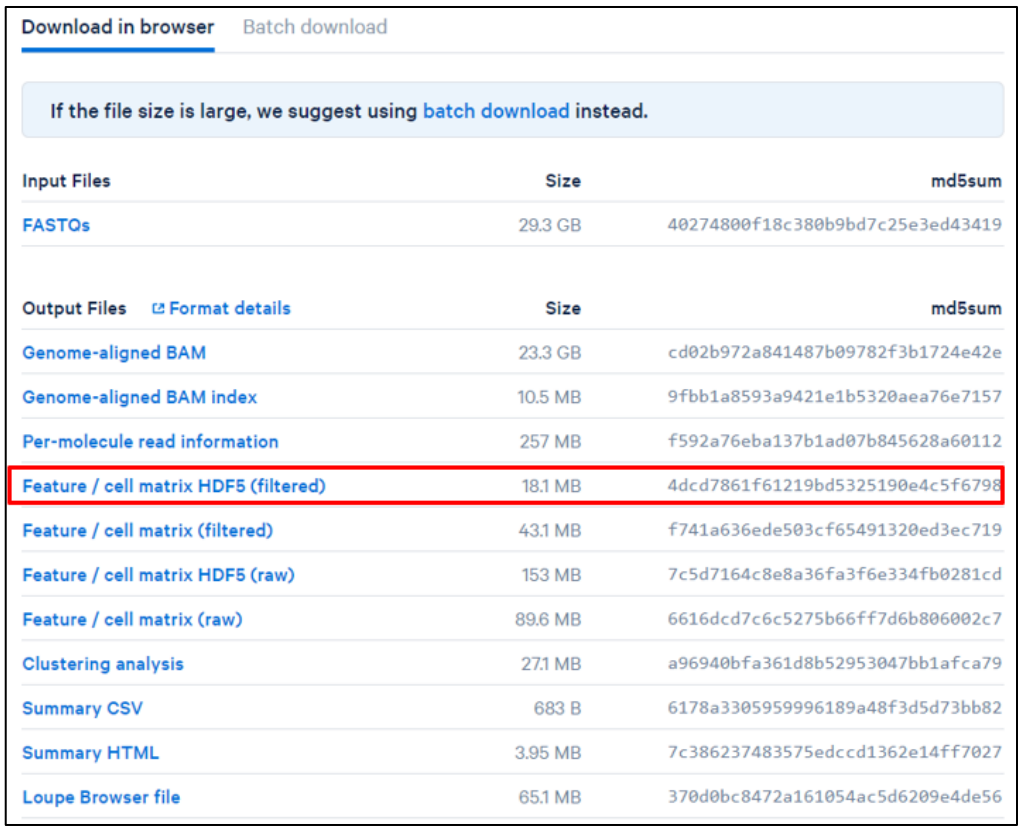
Build 10.0.23.0130

# Experiment Description

- 1k peripheral blood mononuclear cells (PBMCs) from a healthy donor
  - Any peripheral blood cell having a round nucleus
- Partek Flow supports file types: bcl, fastq, bam, h5, txt etc
- Choose **filtered** feature/ cell matrix in .h5 format from CellRanger output
- *Goal for today: Identify different blood cell populations*



Download in browser | Batch download

If the file size is large, we suggest using batch download instead.

| Input Files | Size | md5sum |
|---|---|---|
| FASTQs | 29.3 GB | 40274800f18c380b9bd7c25e3ed43419 |

| Output Files (Format details) | Size | md5sum |
|---|---|---|
| Genome-aligned BAM | 23.3 GB | cd02b972a841487b09782f3b1724e42e |
| Genome-aligned BAM index | 10.5 MB | 9fbb1a8593a9421e1b5320aea76e7157 |
| Per-molecule read information | 257 MB | f592a76eba137b1ad07b845628a60112 |
| Feature / cell matrix HDF5 (filtered) | 18.1 MB | 4dcd7861f61219bd5325190e4c5f6798 |
| Feature / cell matrix (filtered) | 43.1 MB | f741a636ede503cf65491320ed3ec719 |
| Feature / cell matrix HDF5 (raw) | 153 MB | 7c5d7164c8e8a36fa3f6e334fb0281cd |
| Feature / cell matrix (raw) | 89.6 MB | 6616dcd7c6c5275b66ff7d6b806002c7 |
| Clustering analysis | 27.1 MB | a96940bfa361d8b52953047bb1afca79 |
| Summary CSV | 683 B | 6178a3305959996189a48f3d5d73bb82 |
| Summary HTML | 3.95 MB | 7c386237483575edccd1362e14ff7027 |
| Loupe Browser file | 65.1 MB | 370d0bc8472a161054ac5d6209e4de56 |

# Import data

## Login

- Open a web browser (Chrome, Firefox, Safari etc.)
- Go to the server URL
- Log in with your username and password
- This will open to the Partek Flow homepage

## Create project

- Click **New Project** and enter project name
- Click **Add data**
- Select scRNA-seq>10X Genomics Cell Ranger count h5
- Click **Next**
- User file browser to select file
- Select feature annotation file
- Click **Finish**

# Single Cell QA/QC

## Run QA/QC task

- Click the **Single cell counts** data node
- Select **Single Cell QA/QC** from the **QA/QC** section of the task menu

## View report and filter cells

- Double click on **Single cell QA/QC** report to open it
- Click **Select & Filter** in Tools section to specify filter criteria
- Set Detected features **min** to **200**
- Set %Mitochondrial counts **max** to **15**
- Click **Filter include**
- Click **Apply observation filter**
- Choose the **Single cell counts** data node and click **Select**
- Click **Save** in the data viewer to save the filter session

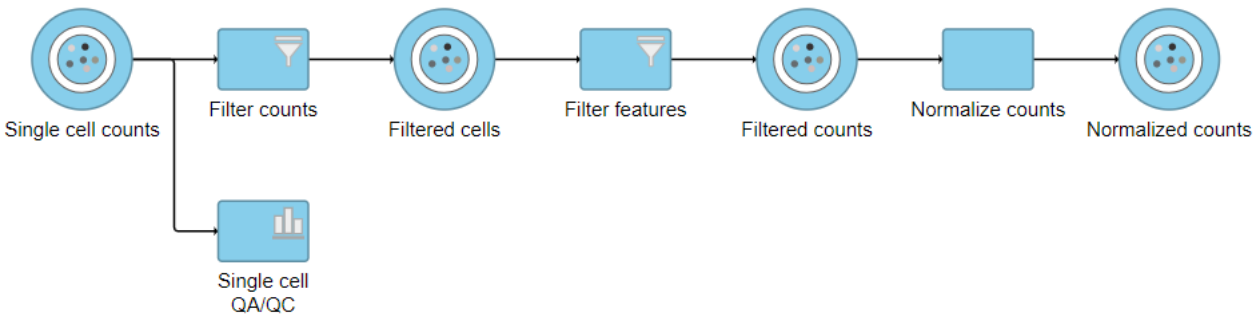
# Filter Features & Normalization

## Filter genes

- Click **Filter features** in Filtering section on the menu
- Choose **Noise reduction**
- Filter criteria: Exclude **value <=0 in at least 99%** of the cells
- Click **Finish**

## Normalization

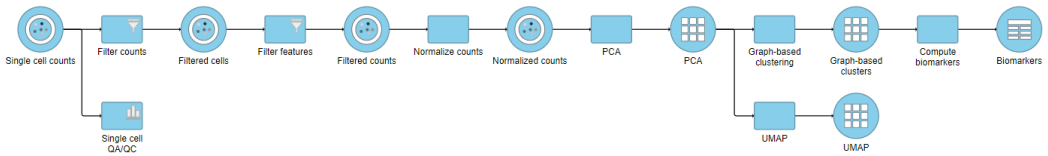
- Click on **Filtered count node** > **Normalization in** Normalization and scaling section on the menu
- Click **Use recommended**
- Click **Finish**



# Exploratory analysis

## Dimension reduction

- Click the **Normalized counts data** node
- Click **PCA** in the **Exploratory analysis** section of the task menu
- Click **Finish** to run with default settings
- Double click on **PCA** node to view report
- Select **PCA** node, choose **UMAP** task on the menu
- Click **Finish** with default settings
- Select **PCA** node, choose **Graph-based clustering** task
- Click **Finish** with default settings
- Double click on **Graph-based clusters** node to view the report
- Double click on **Biomarkers** node to view the report



## View Gene Expression in UMAP

- Double click **UMAP** to open the report
- Click **Get data,** search for **Graph-based clusters** node, click on the node, choose **Graph-based** annotation, drag and drop to **Color** UMAP
- From **Get data** dialog, search for **Biomarkers** data node, drag the node and drop to the **Bottom** of **UMAP** plot to add the biomarker table in the viewer
- Click **Duplicate plot** in UMAP plot to make a copy of UMAP
- Drag a gene from the biomarker table to drop on one of the UMAP plot to color the cells
  - Use one gene to color cells
  - Use two or three genes to color cells
  - Use gene list to color cells

| Cell Type | Gene Markers |
|---|---|
| T-cells | CD3D, CD3E |
| Cytotoxic cells | NKG7, GNLY |
| B cells | CD79A, CD79B (list) |
| Monocytes | CD68, CD14 |

## Classify Cell Types

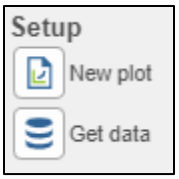
- Click on **Select & Filter** in Tools menu
- Drag and drop legend from plots to add criteria
  - Use graph-based annotation
  - Use gene expression
- Search attributes or gene to add criteria
  - Click on the circle next to the criteria drop-down box to select correct data node
  - No limitation on the number of rules
  - And operation is performed among multiple rules
  - Cells meet the criteria will be selected and highlighted in the plots
- Once cells are selected, click on **Classify** in Tools menu, choose **Classify selection** to give a name to the selected cells
  - Always **Save** the analysis
  - Click on **Style > Color by > New classification** to preview the cell types before apply classification
  - Only click **Apply classifications** when all cell types are identified
- Once classification is applied, it will appear in Meta data tab. This cell attribute is project level, which means all data nodes in the project and see it.

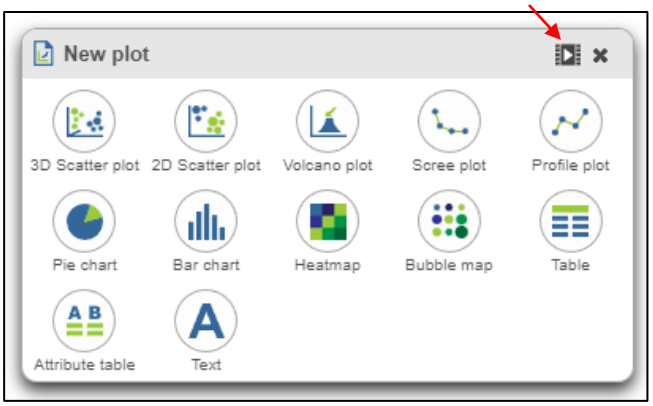
## Identifying Differentially Expressed Genes

- Click the **Normalized counts** node
- Click **Statistics** then the **Differential analysis** section of the task menu
- Choose ***ANOVA*** as the method and click **Next**
- Choose **Cell type** and click **Add factors**
- Click **Next**
- Choose to compare **B cell** vs **T cell**, click **Add comparison**
- Click **Finish**
- Double click the **ANOVA** data node to open the ANOVA report
- Click the  icon next to a gene under **View** to open data viewer
- Using the **Style** configuration option under *Summary*, add violins or box Whiskers on the plot
  - Duplicate the plot and change Y-axis to view multiple genes
  - Drag and drop group name on X-axis to change order
- Click the  icon to invoke volcano plot
  - Use on Style > Label by to display selected gene name
  - Use Select & Filter to search for genes
- Use Gene list filter panel on ANOVA report to generate gene list based on specified criteria

# Plot Types

- Use **New plot** or **Get data** under *Setup* to add content to your *Data Viewer* session
- ***Tip: watch the help videos to navigate each of the panel options***

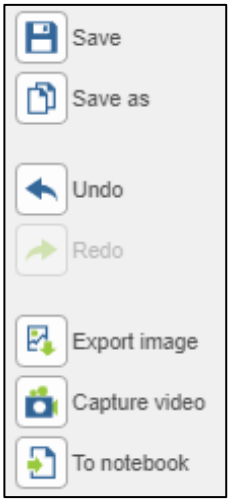



- The plot types:
  - *Text*. Inserts a text box. Useful for captions and notes
  - *Table*. Inserts a table. E.g. table of biomarkers per cluster
  - *Heatmap.* Inserts a heat map, e.g. result of hierarchical clustering
  - *Bubble map.* Uses 3 dimensions to display groups by summary statistics resulting from the hierarchical clustering/ heatmap task
  - *3D Scatter Plot*. Inserts a 3D scatterplot. This is a plot showing three data features at the same time.
  - *2D Scatter Plot*. Inserts a 2D scatter plot. This is a plot showing two data features at the same time.
  - *Volcano plot*. Visualize significance and the magnitude of changes in features within a comparison
  - *Scree plot.* This plot shows information content per principal component.
  - *Attribute summary table*. Insert a table which shows attributes
  - *Histogram.* Histogram of a variable
  - *Profile plot.* Bar or line chart on one or multiple variables
  - *Pie chart.* Inserts a pie chart. Useful for visualization of categorical variables.

# Genaral Data Viewer Controls

- General control buttons are on the left




•*Save*. Saves the session to the *Data Viewer* tab.

•*Save As*... Saves the session under a new name

•*Undo (right-click to see/select multiple steps)*

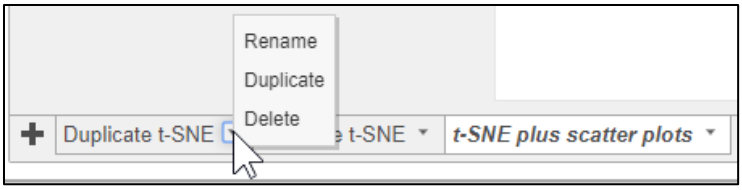
•*Redo (right-click to see/select multiple steps)*

•*Export Image*. Saves the entire canvas as an image to the local computer. Supported formats are .png, .svg and .pdf

•*Capture video*. Record video, like 3D scatterplots, in various formats

•*To Notebook*. Sends the entire canvas to the Notebook.

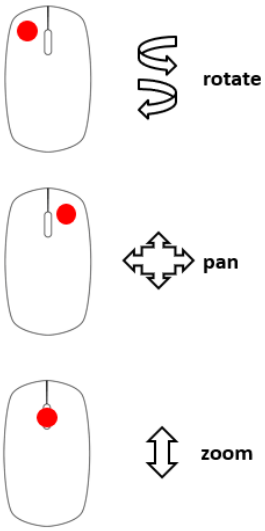
- Sheets are shown in the lower left corner. To add a blank sheet select the plus icon. For sheet options, click on the arrowhead



# Customizing the Data Viewer Appearance

- Click on arrow icon to collapse or expand 
- Plots are separated by grippers. Mouse over a gripper to see options
  - Click and drag: resize the plot
- To reset the view of the plot, use the  control on the right of each plot
- To move a plot to a new position, use the handle at the top

- Mouse operation on a plot:



# In-plot controls

- Plot tools are located in the upper right corner and appear upon a mouseover

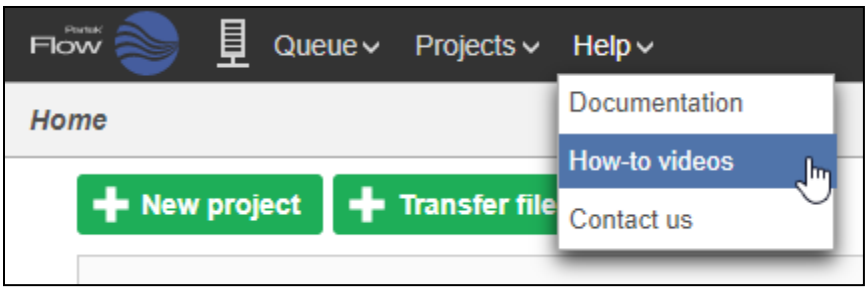
- Horizontal series
  - *Duplicate plot*. Makes an exact replica
  - *Save Image*. Saves the selected plot only as an image to the local computer. Supported formats are .png and .svg
  - *Send to Notebook Page*. Sends the selected plot to the Notebook
  - *Fullscreen plot/Exit Fullscreen mode*. Plot is expanded to fill the canvas
  - *Remove plot*. Removes the selected plot from the canvas. *Data Viewer* session remains open

- Vertical series
  - *Export data*. Download a .txt file with data use to draw a scatter plot (2D & 3D scatter plots only)
  - *Pointer mode*. Click to select data points
  - *Lasso mode.* Draws a lasso to select data points
  - *Invert Selection*. Unselects the currently selected cells, and selects the others
  - *Reset View*. Resets plot rotation and zoom
  - *Toggle Legend*. Turns legend on and off
  - *Toggle Axes Autoscale.* Scales the axes with respect to the visible data points (e.g. after some data points have been filtered out)
  - *Transpose*. Switch the axes

# Getting Help

### Self-learning

- Partek Flow documentation https://documentation.partek.com/display/FLOWDOC/Partek+Flow+Documentation
- Step by step tutorials + practice data sets https://documentation.partek.com/display/FLOWDOC/Tutorials
- Recorded webinars https://www.partek.com/webinars/
- Live training event recordings https://documentation.partek.com/display/FLOWDOC/Live+Training+Event+Recordings
- Partek blog page https://www.partek.com/blog/
- Tips and tricks on Partek Flow are regulary tweeted https://twitter.com/Partek_Inc
- *How-to videos* are accessable from the **Help** menu




### Technical Support

- Open a support ticket at partek.com/support