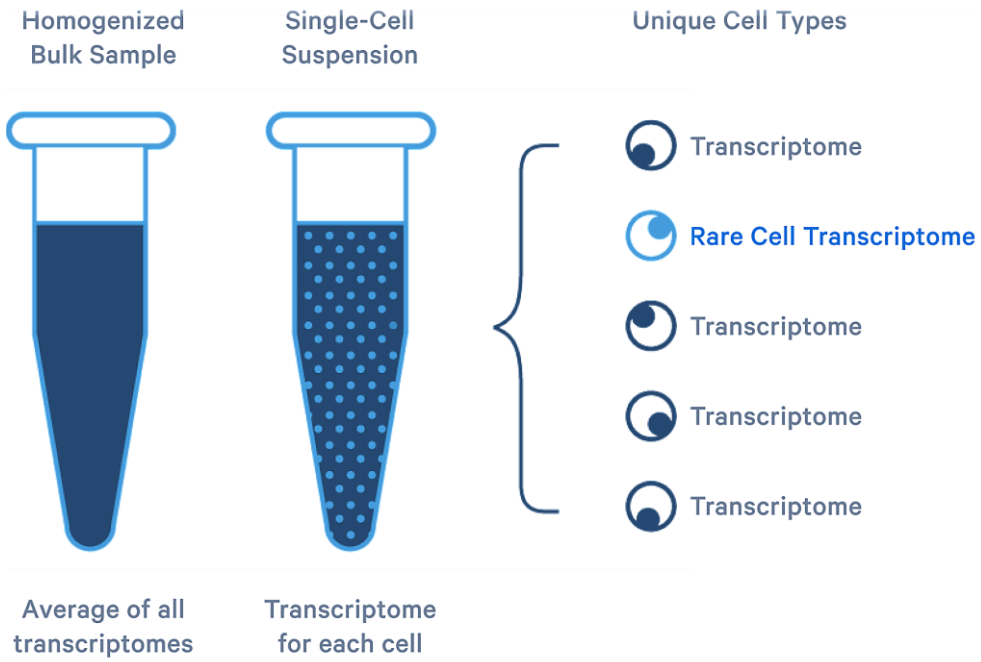
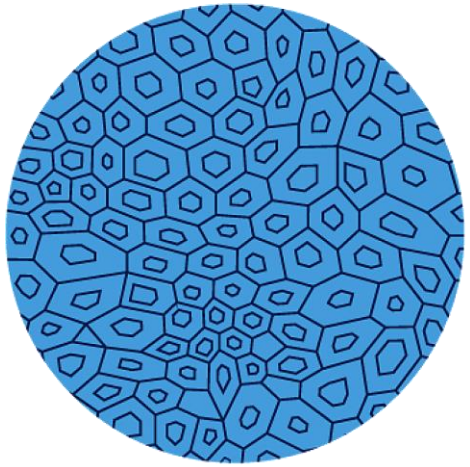
Partek Flow scRNA-Seq Analysis

Tanya

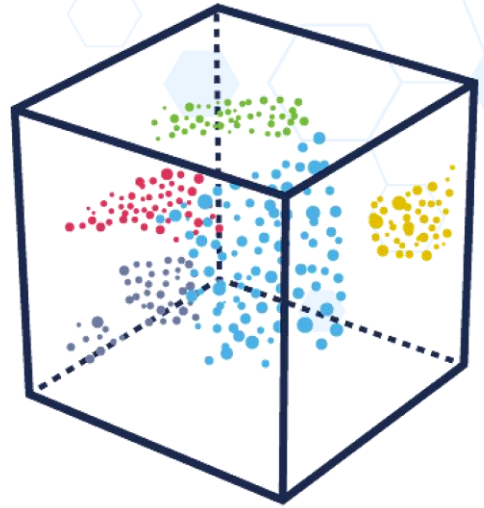
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Introduction of Single-cell Analysis





Tissue Specimen with a spatial relationship between cells.

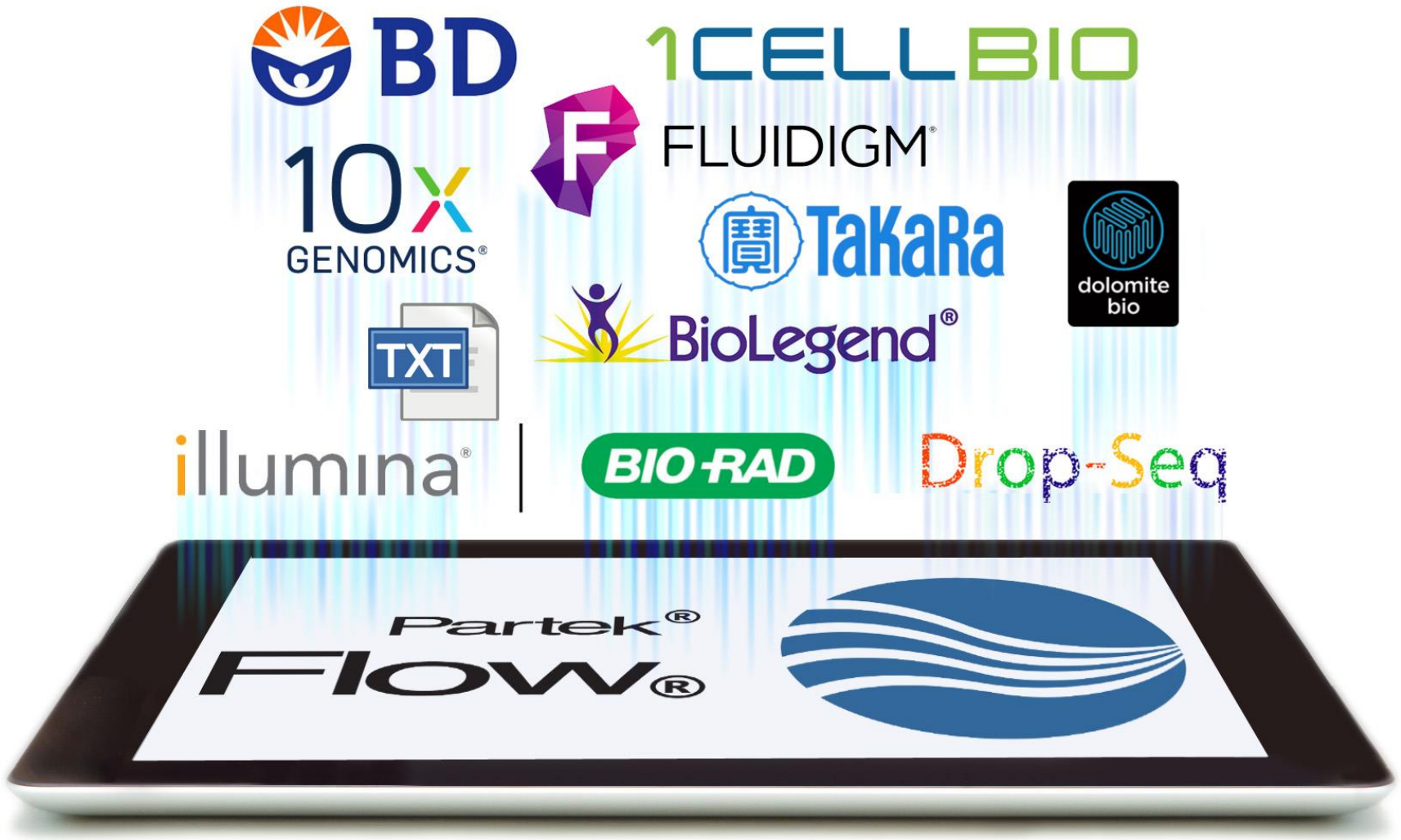


Relationship between cells by similarity of gene expression.

https://www.10xgenomics.com/single-cell-technology

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Supports All Major Single Cell Platforms

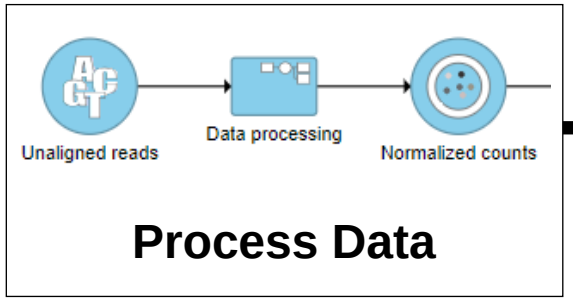


Support for Wide Variety of Single Cell Technologies

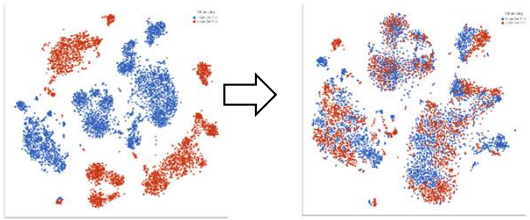
- ✓ Single Cell RNA-Seq
- ✓ Whole Transcriptome Single Cell RNA-Seq
- ✓ Gene & Protein Expression
- ✓ ECCITE-Seq
- ✓ Spatial Transcriptomics
- ✓ Trajectory analysis

Data Processing and Analysis, All in One Place

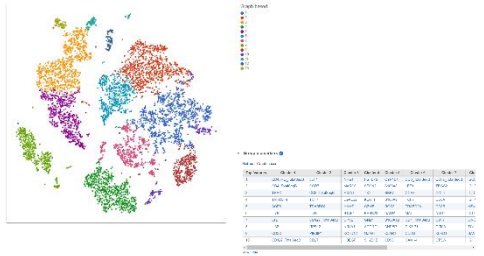
FASTQ, BAM, or Counts



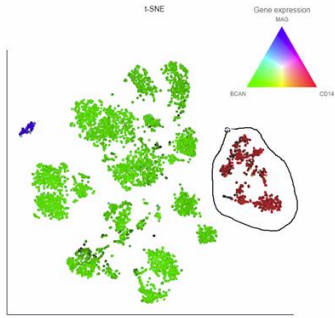
Batch correction



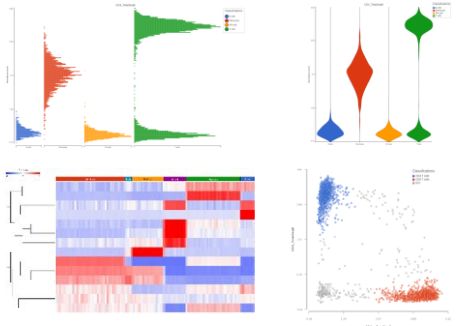
Clustering



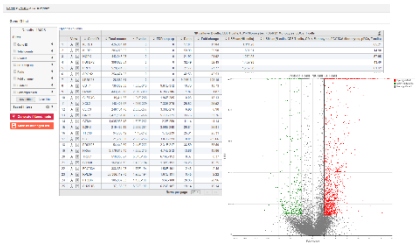
Interactive Visual Analysis



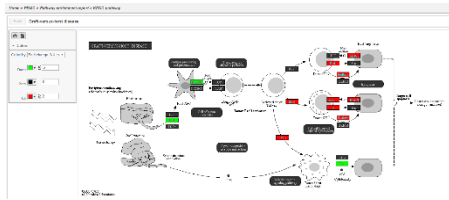
Data Visualization



Differential Analysis



Pathway Analysis



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Demo

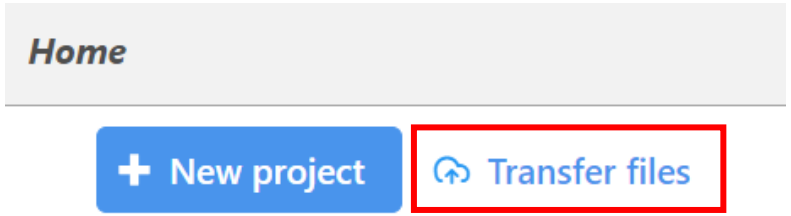
Experiment Description

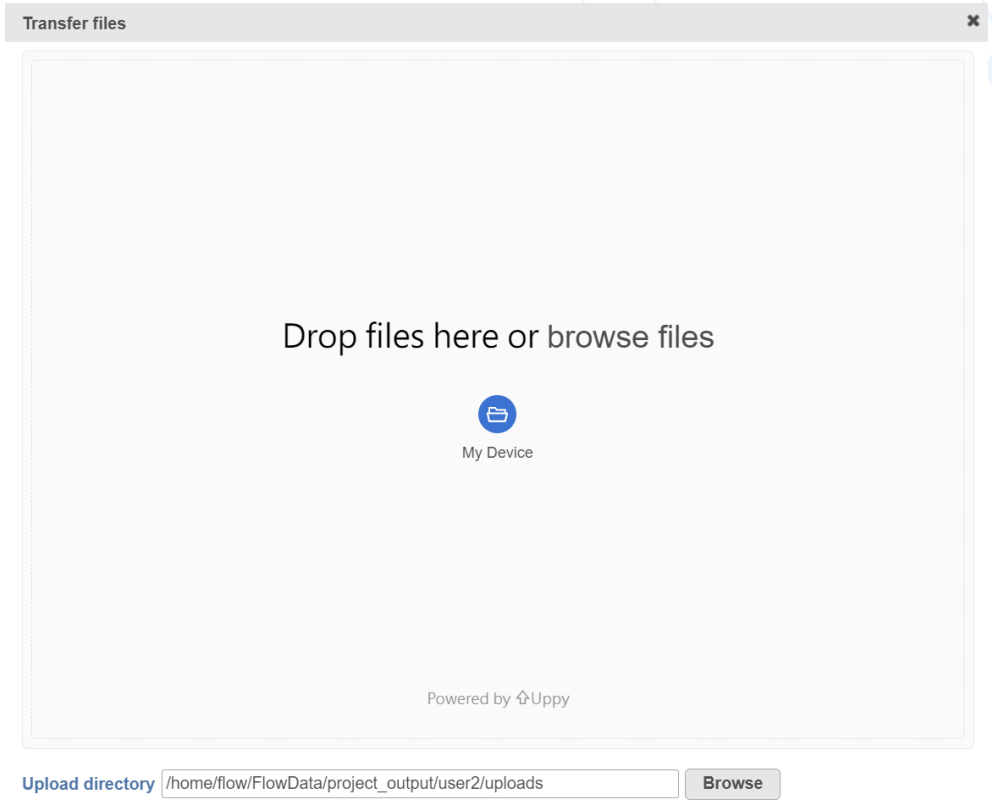
- 5k peripheral blood mononuclear cells (PBMCs) from a healthy donor
  - Any peripheral blood cell having a round nucleus
- Downloaded from 10X Genomics' dataset repository
  - http://cf.10xgenomics.com/samples/cell-exp/3.0.2/5k_pbmc_v3/5k_pbmc_v3_filtered_feature_bc_matrix.h5
- Partek Flow supports file types: bcl, fastq, bam, h5, txt etc.
- Goal: **Identify different blood cell populations**

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Transfer files

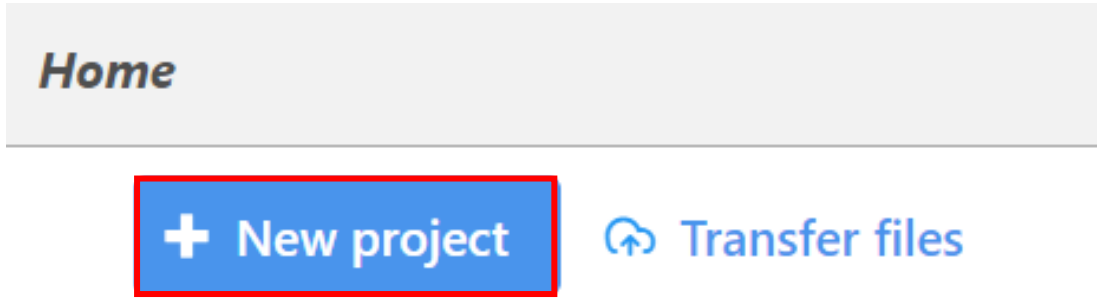
- To move files from your local computer to the Partek server, please **Transfer files** first



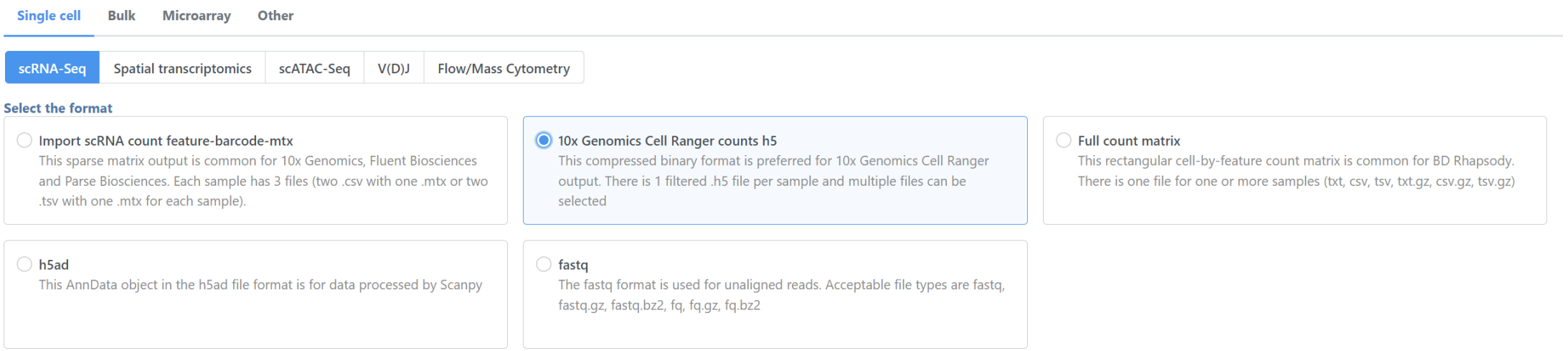


Create a new project

- Click **New project** from home page



Import your own data

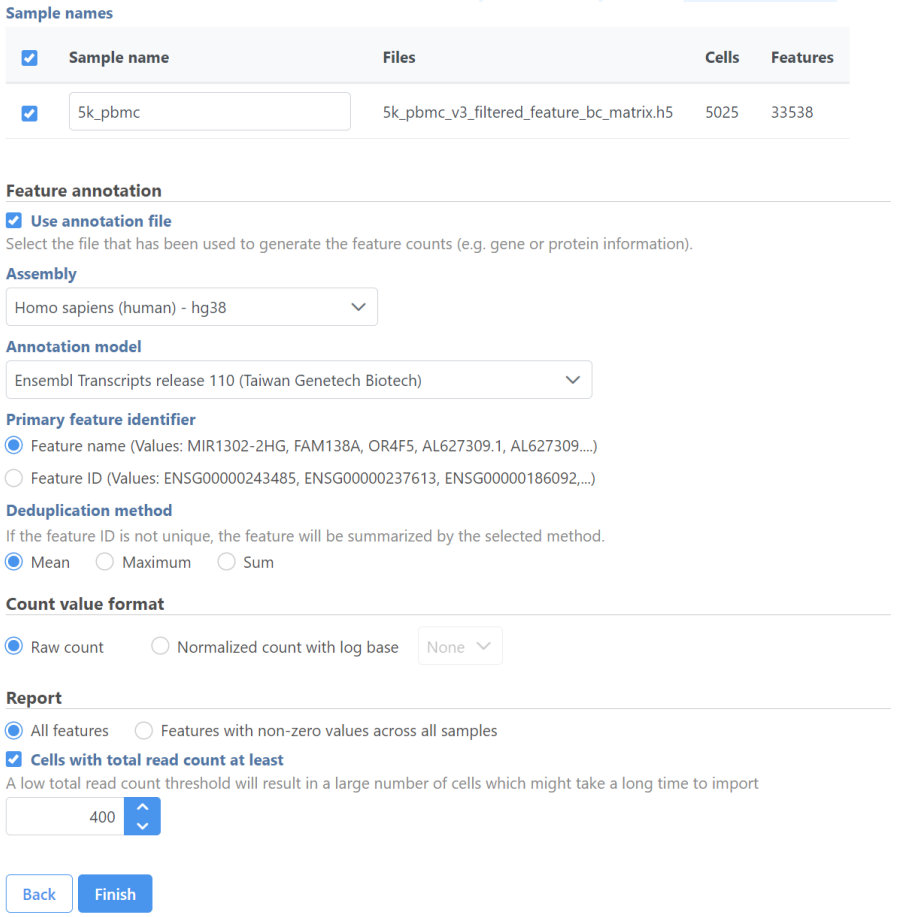


If you want to import your own data

- Select the format
- Select all files and click **Next**

Specify Annotation

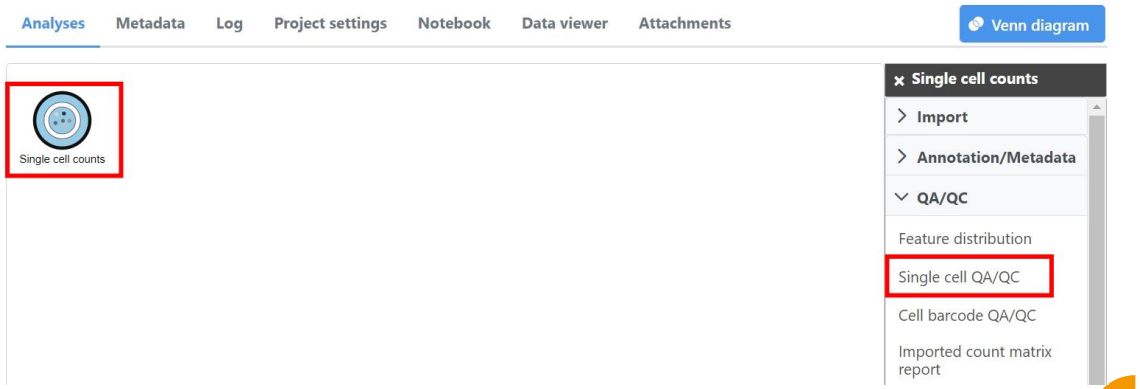
- Set **Sample name** to 5k_pbmc
- Click the **Use annotation file** checkbox and set the annotation
  - Assembly: Homo sapiens (human) - hg38
  - Gene annotation: Ensembl transcripts release 110
- Click **Finish** to import sample



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Single Cell QA/AC

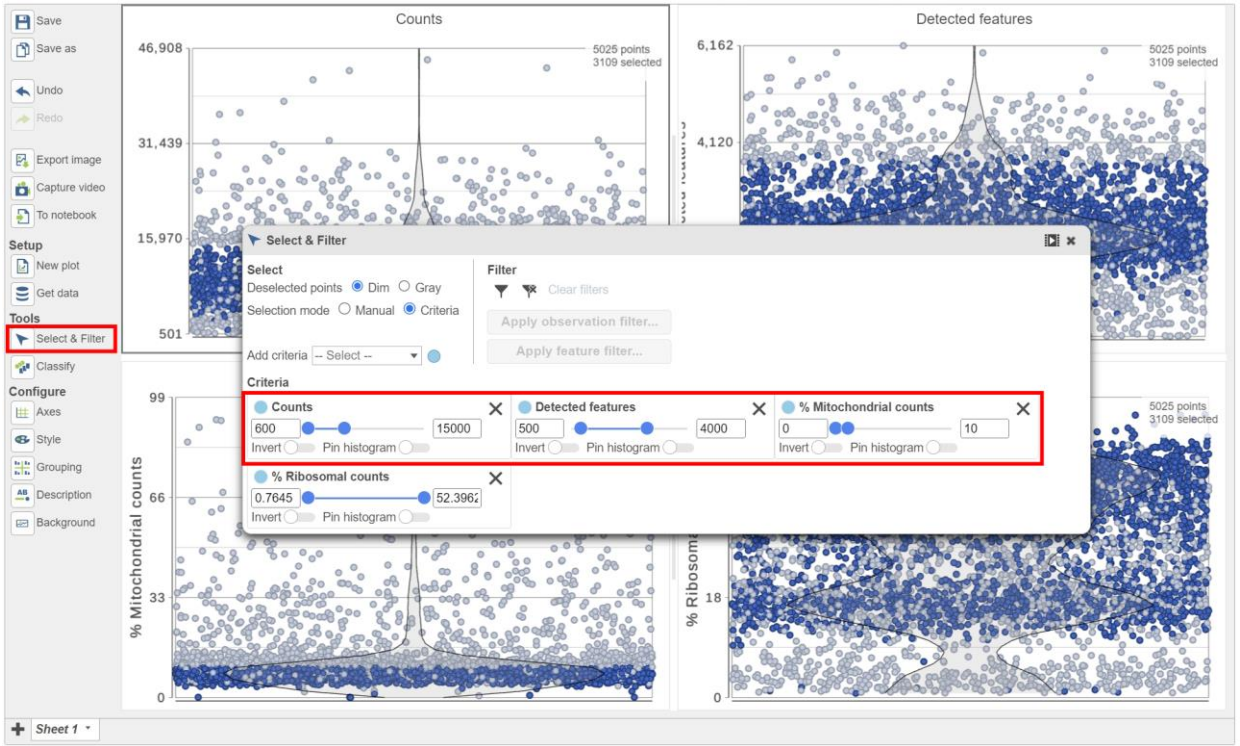
- Go to the **Analyses** tab
- The **Single cell counts** data node appears after the data imported
- Click the data node
- Select **Single Cell QA/QC** from the QA/QC section of the task menu



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Single Cell QA/AC

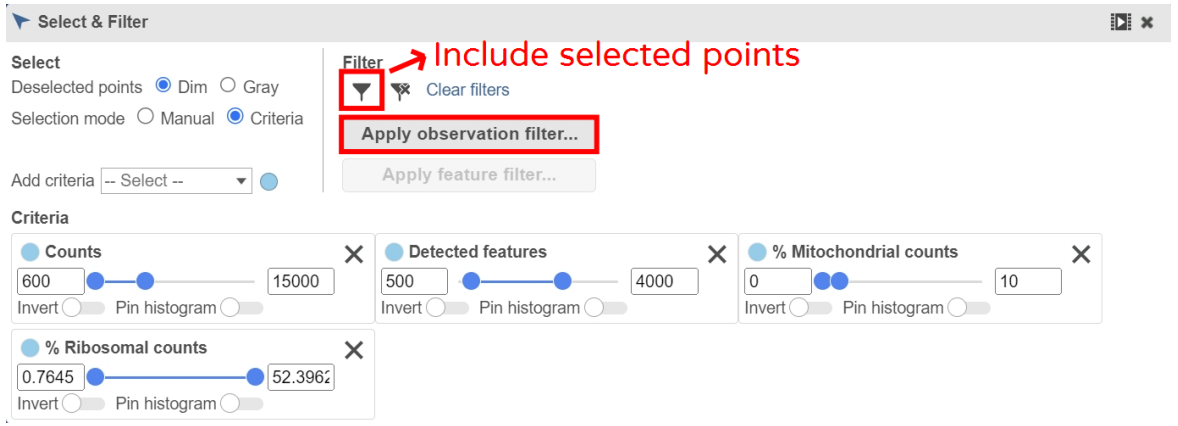
- Double click the **Single Cell QA/QC** task node to open the task report
- Use the **Select & Filter** card to set the Min and Max thresholds:
  - Counts: 600 – 15000
  - Detected features: 500 – 4000
  - Mitochondrial counts 0 – 10



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Single Cell QA/AC

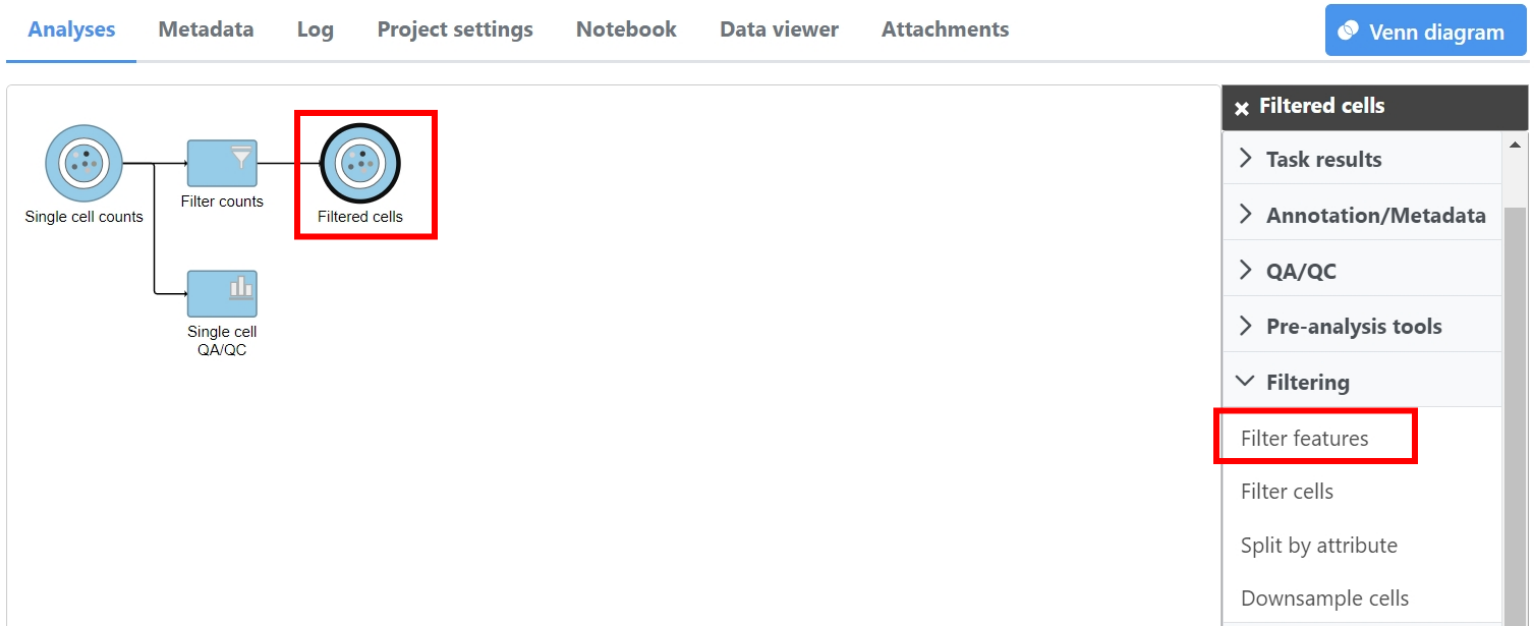
- Select **Include selected points** button
- Select **Apply observation filter…**
- Select the circular **Single cell counts** data node to filter
- Click **OK** on the message in the middle of the screen and click the project name to go back to the Analyses tab
  - This runs the Filter cells task and outputs a new Single cell data node



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Applying a Noise reduction filter

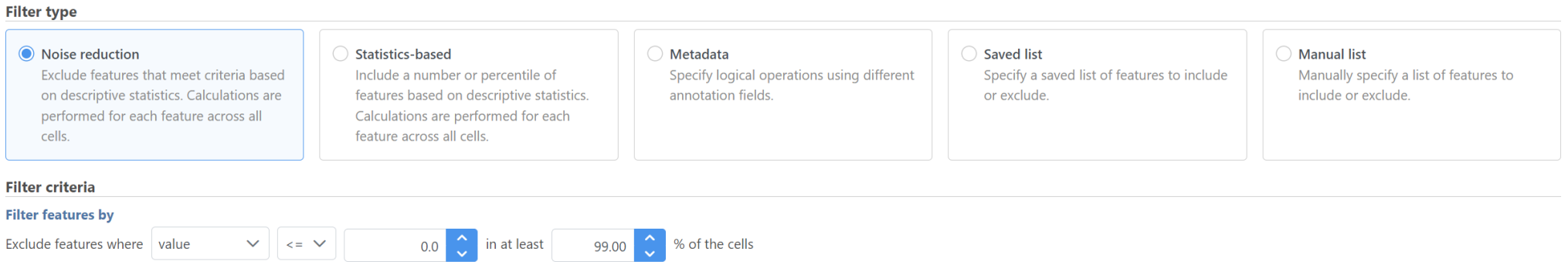
- Click the **Filtered cells** data node
- Click **Filter features** in the **Filtering** section of the task menu



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Applying a Noise reduction filter

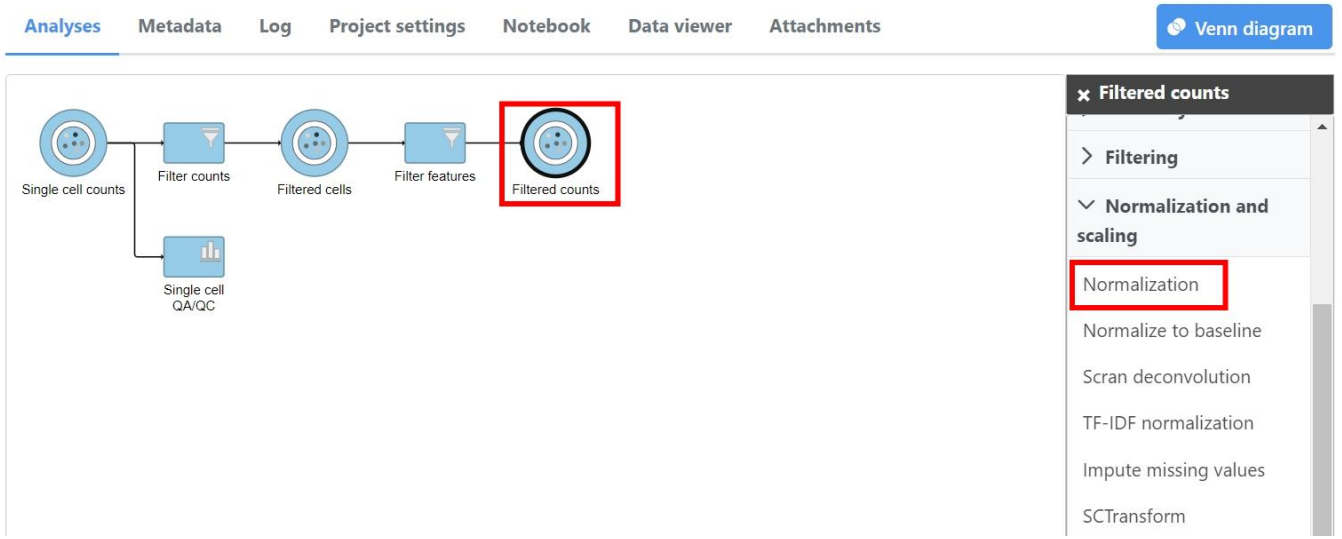
- Click the **Noise reduction filter** checkbox
- Create the following filter using the drop-downs and text boxes
  - Exclude features where value <= 0 in at least 99% of the cells
- Click **Finish** to apply the filer



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Normalizing counts

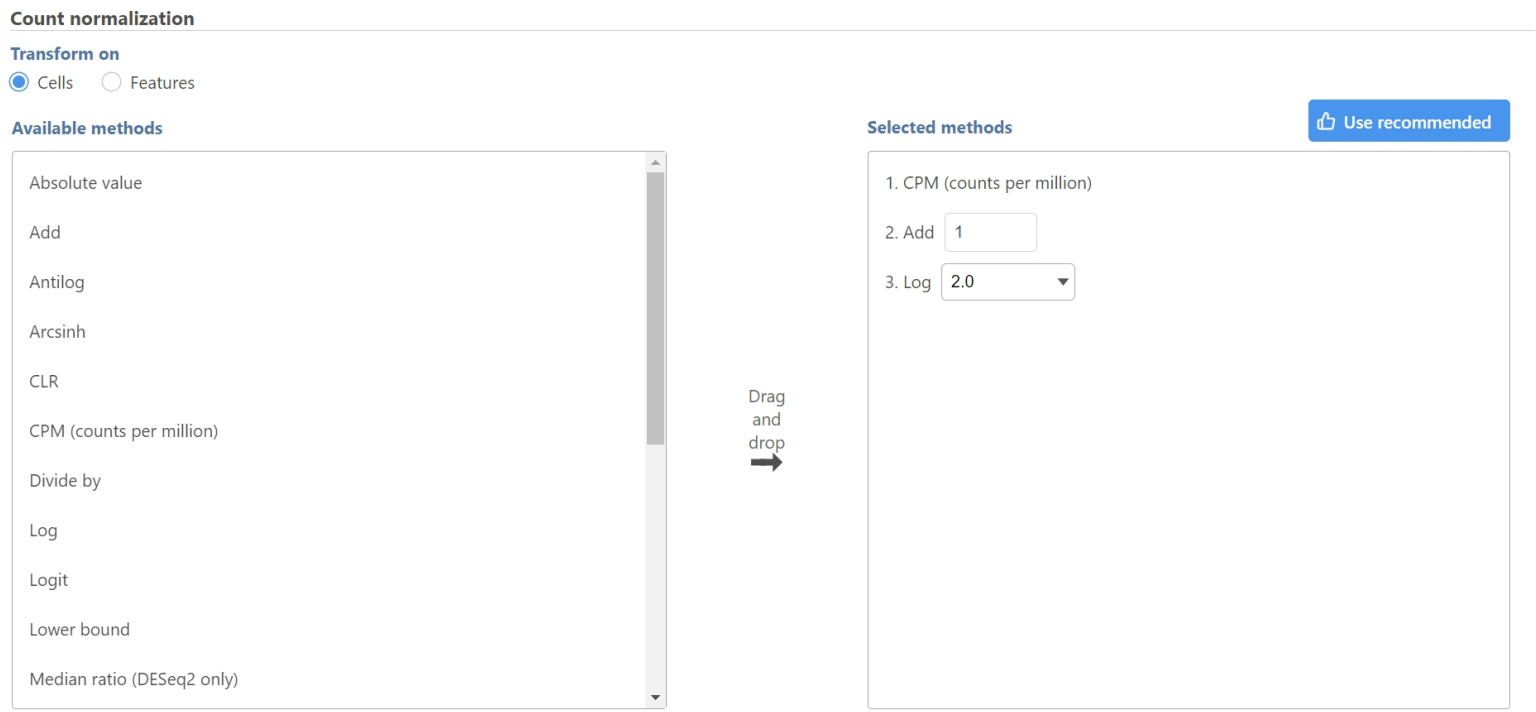
- Click the **Filtered counts** node
- Click **Normalization** in the **Normalization and scaling** section of the task menu



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Normalizing counts

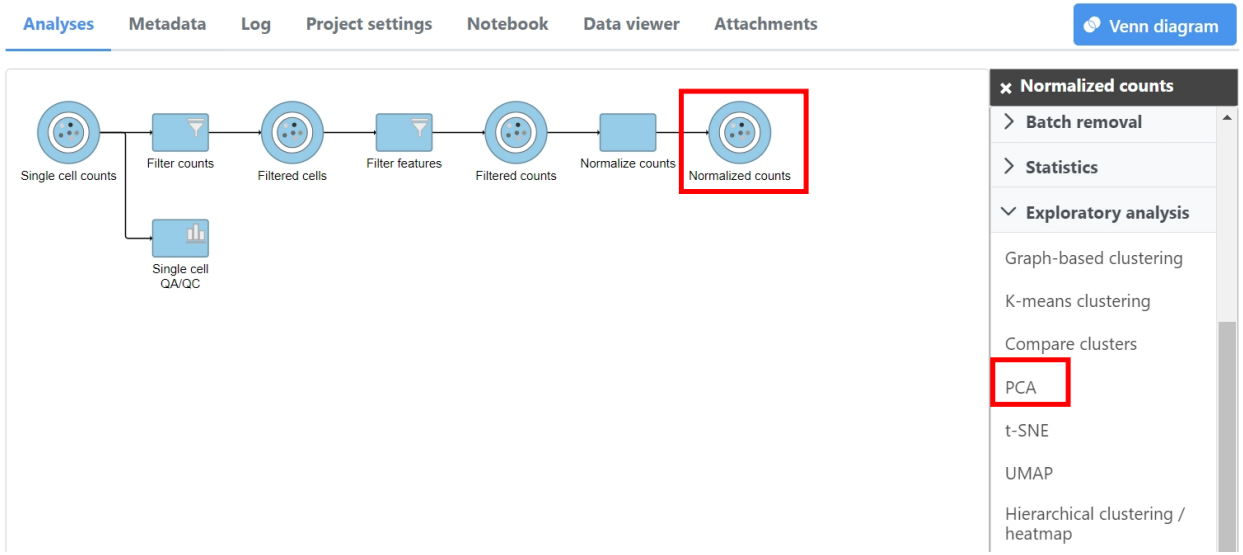
- Click on the **Recommended** button
- Click **Finish** to run

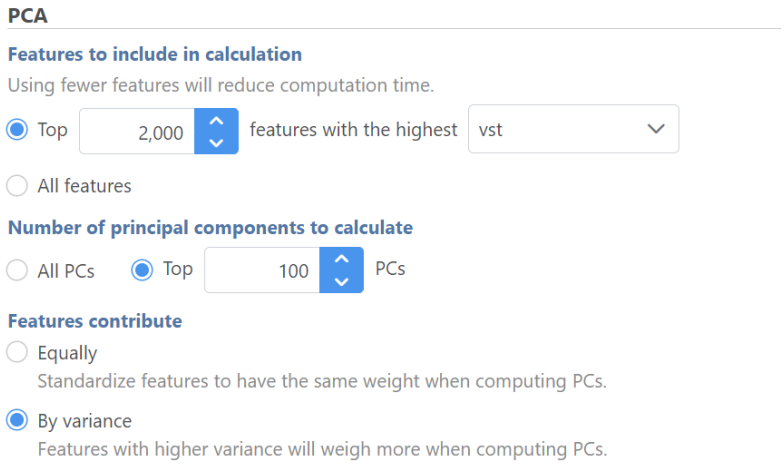


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Performing Principal Components Analysis

- Click the **Normalized counts** data node
- Click **PCA** in the **Exploratory analysis** section
- Click **Finish** to run with default settings

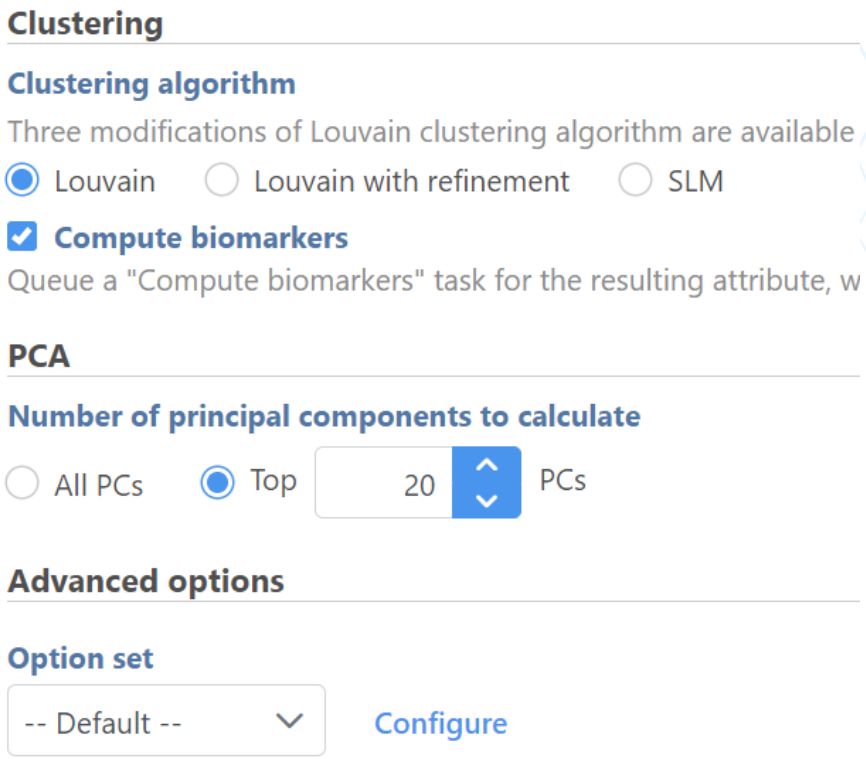




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Performing Graph-based Clustering

- Click the **PCA** data node
- Click **Graph-based clustering** in the **Exploratory analysis** section of the task menu
- Click **Finish** to run with default settings



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Graph-based Clustering Results

- Double-click the **Graph-based clusters** data node to open the Task report
- The *Maximum modularity* is a measure of the quality of the clustering result. Higher modularity (close to 1) indicates a better result
- The *Cluster statistics* shows the number of clusters, cluster size and the percentage of cells in each cluster

Cluster results

Maximum modularity: 0.848268

Cluster statistics

Total number of clusters 5

Cluster	Size	Size %
1	1272	40.91%
2	618	19.88%
3	448	14.41%
4	395	12.71%
5	376	12.09%

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Biomarkers Results

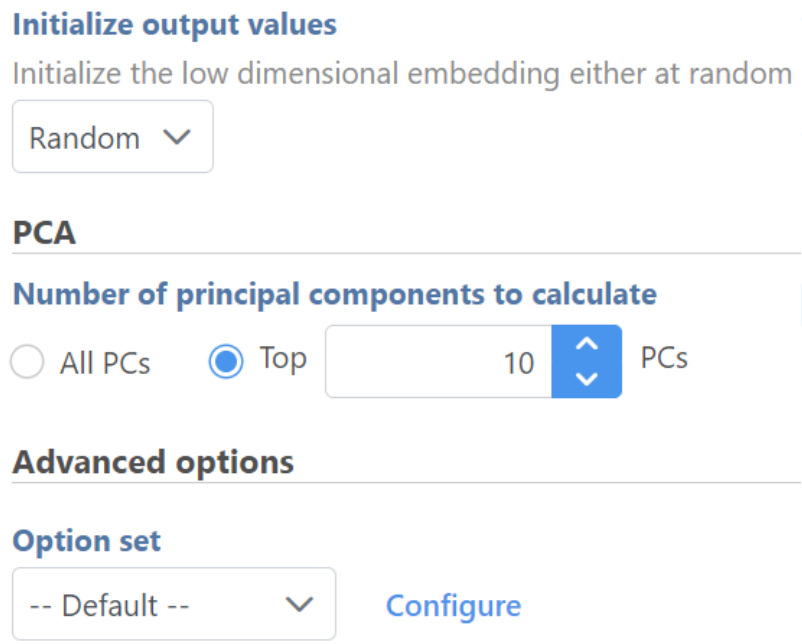
- Double-click the **Biomarkers** data node

Biomarkers for Graph-based

Top features	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
1	TRABD2A	S100A8	TNFRSF4	IGKC	FGFBP2
2	LEF1	S100A9	LMNA	IGHM	GNLY
3	CCR7	S100A12	AQP3	IGHD	GZMH
4	TCF7	LYZ	IL32	TCL1A	NKG7
5	TPT1	FCN1	KLRB1	MS4A1	KLRD1
6	RPL35A	CD14	MAF	CD79A	ADGRG1
7	RPS15A	VCAN	IL7R	VPREB3	KLRF1
8	RPS27A	MNDA	NPDC1	JCHAIN	PRSS23
9	LRRN3	CSTA	SYNE2	SPIB	SPON2
10	CD3E	SERPINA1	NSG1	BANK1	PRF1

Perform UMAP

- Click the **Graph-based clusters** data node
- Click **UMAP** in the **Exploratory analysis** section
- Click **Finish** to run the UMAP task with default settings



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Identifying Cell Types

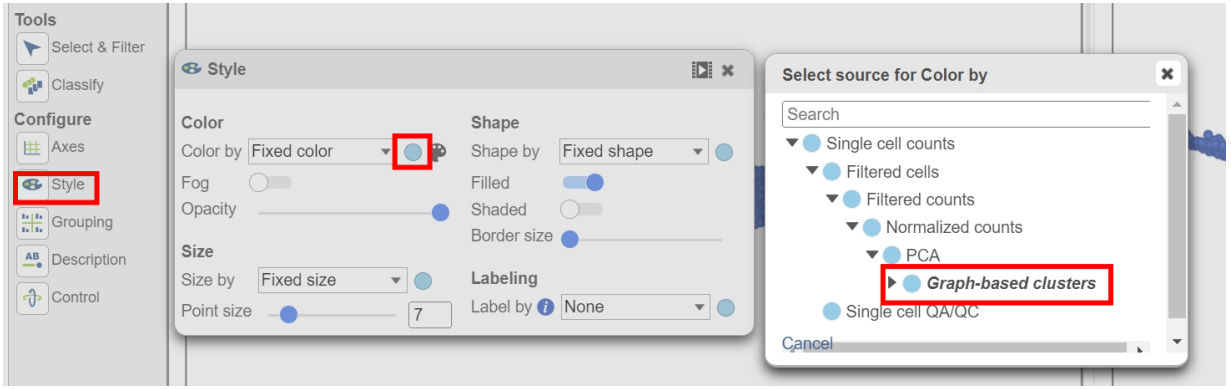
- We'll be using a combination of methods to identify some cell types commonly found in PBMCs. Namely:
  - Unbiased clustering (Graph-based)
  - Visualizing expression using
    - Canonical gene markers
    - Gene lists
  - Lassoing cell populations on the plot

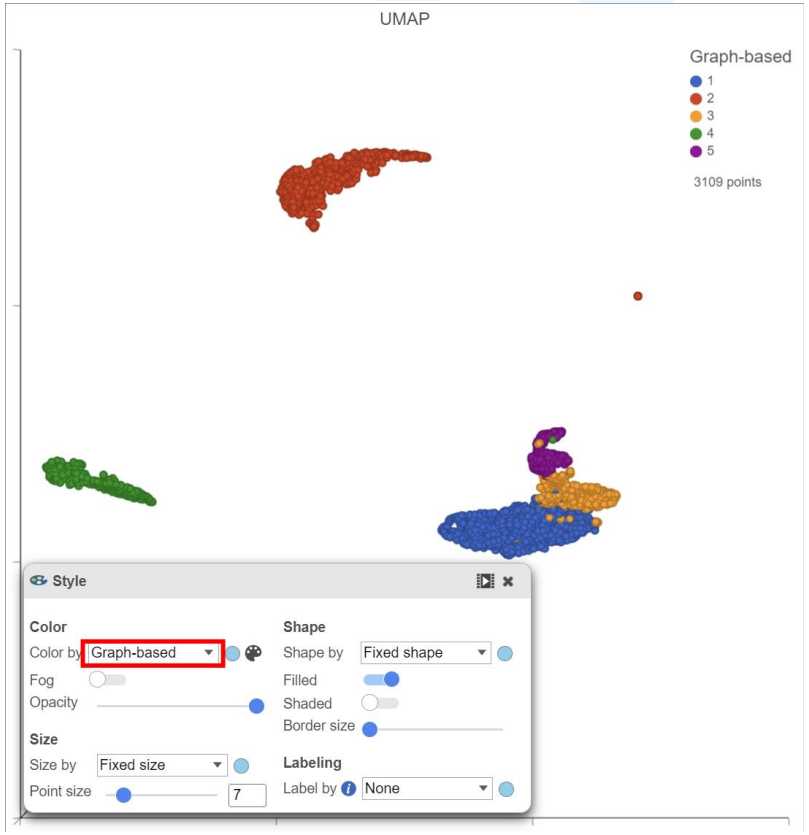
Cell Type	Gene Markers
T-cells	CD3D, CD3E
Cytotoxic cells	NKG7, GNLY
B cells	CD79A, CD79B (list)
Monocytes	CD68, CD14

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Classify T cells

- Duplicate the UMAP plot by clicking 
- Color one of the plots using Graph-based classification
  - Click **Style** and **Select source for Color by** as Graph-based clusters
  - Set **Color by** as **Graph-based**

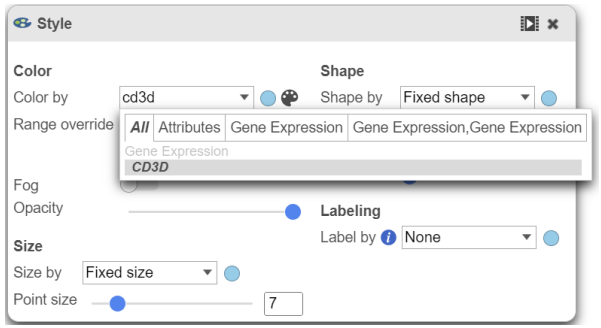


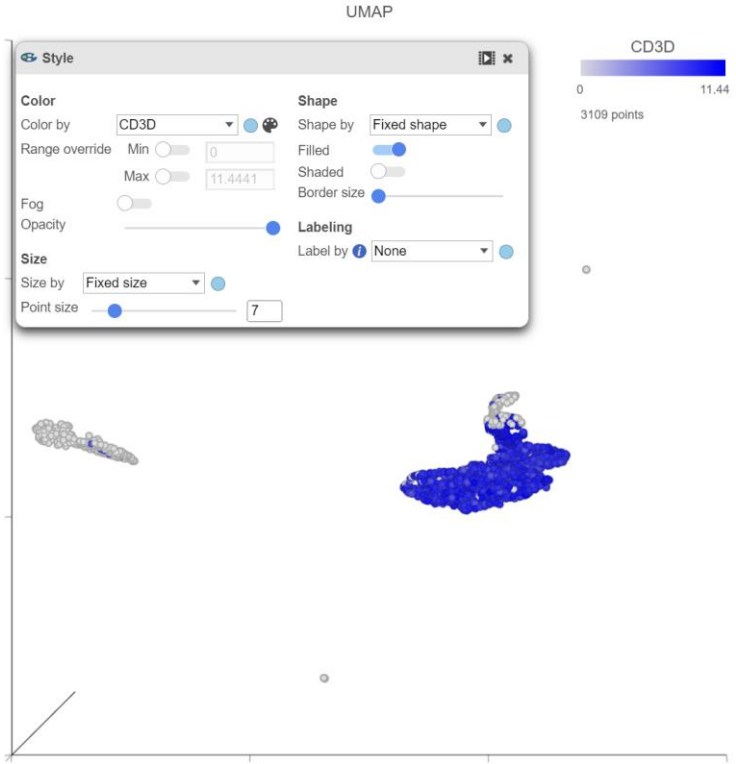


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Classify T cells

- Click on the other UMAP plot
- Color the plot using a gene marker, CD3D
  - Click **Style** and **Select source for Color by** as Normalized counts
  - Enter **CD3D** in the box



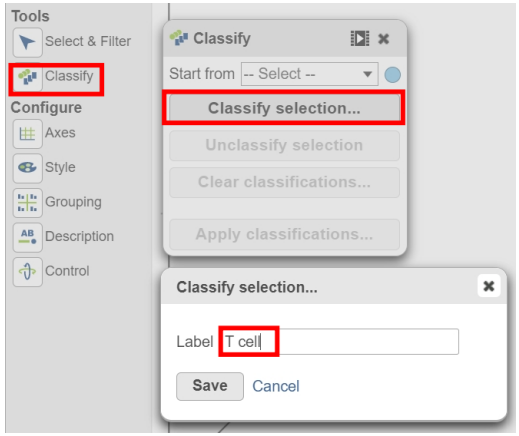


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Classify T cells

- Click **Select & Filter**
- Add criteria as **Graph-based** and choose 1 and 3
- Click **Classify** and **Classify selection…**
- Specify the name of selected cells as **T cell** and click **Save**



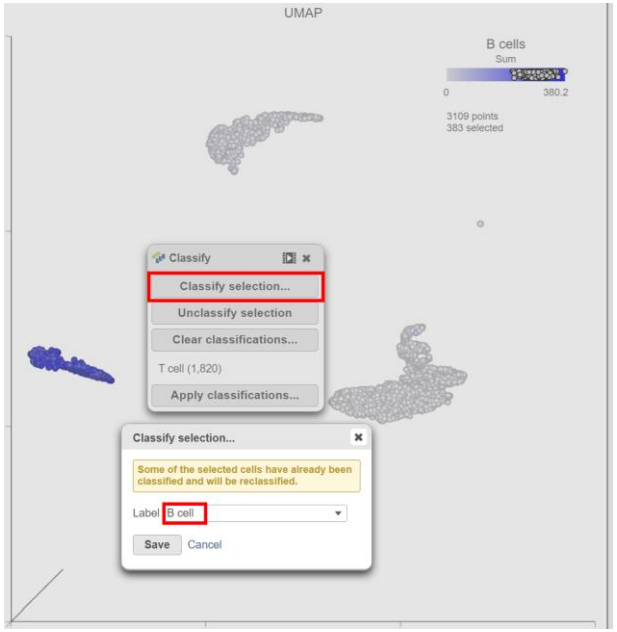


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Classify B cells

- Select the 2nd UMAP plot, choose Color by **Feature list** and select **B cells**
- Use lasso tool to select the cells with high expression
- Click on **Classify selection** to name selected cells as **B cell**

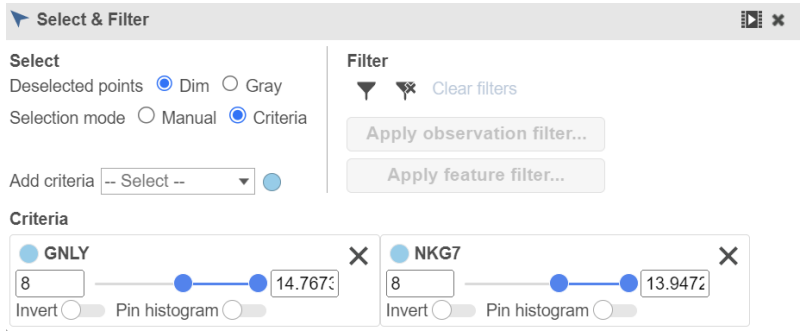


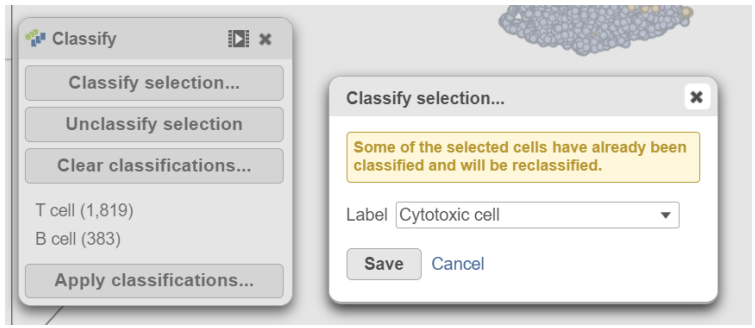


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Classify Cytotoxic cells

- Click **Select & Filter**
- Set **Select source for Color by** as Normalized counts
- Find the NKG7 and specify the min as 8
- Add GNLY and specify the min as 8
- Click **Classify selection** to name it as **Cytotoxic cell**
- Any number of genes can be used to build the rule



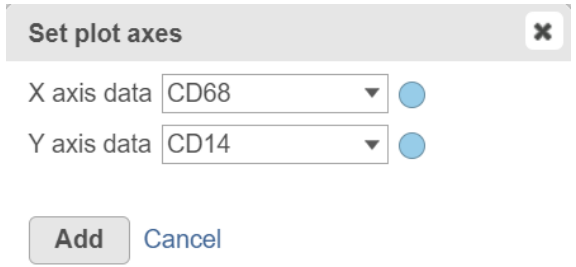


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Classify Monocytes

- Click and drag the **Normalized counts** data node onto the canvas and replace the second UMAP, add a 2D scatter plot
- Set CD68 as X axis, and CD14 as Y axis

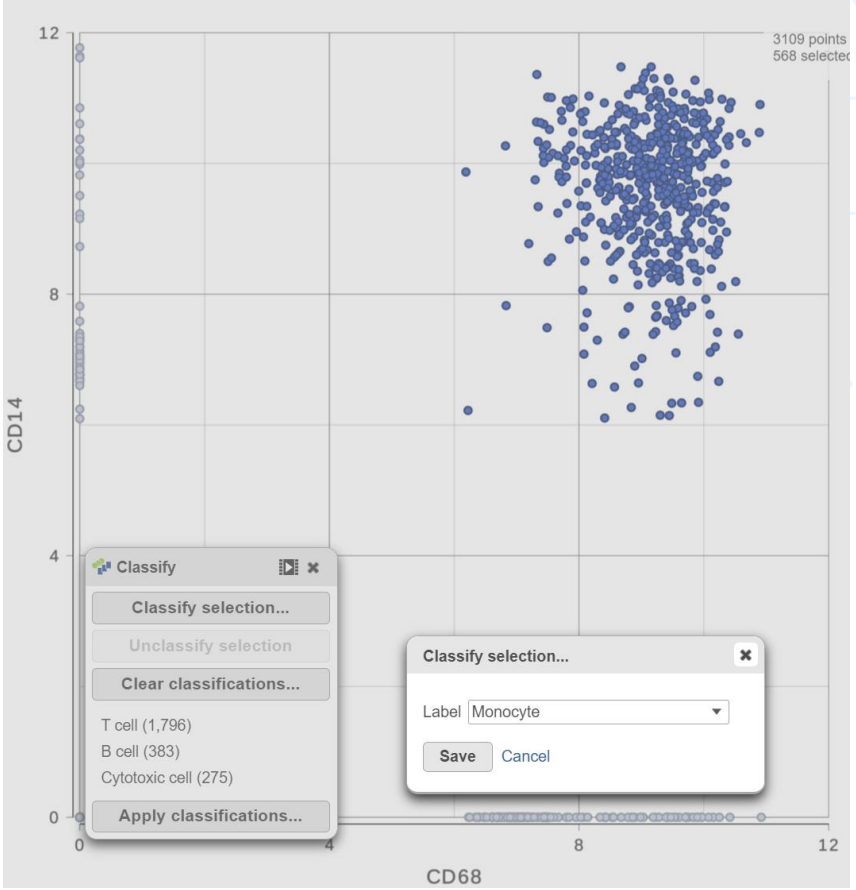




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Classify Monocytes

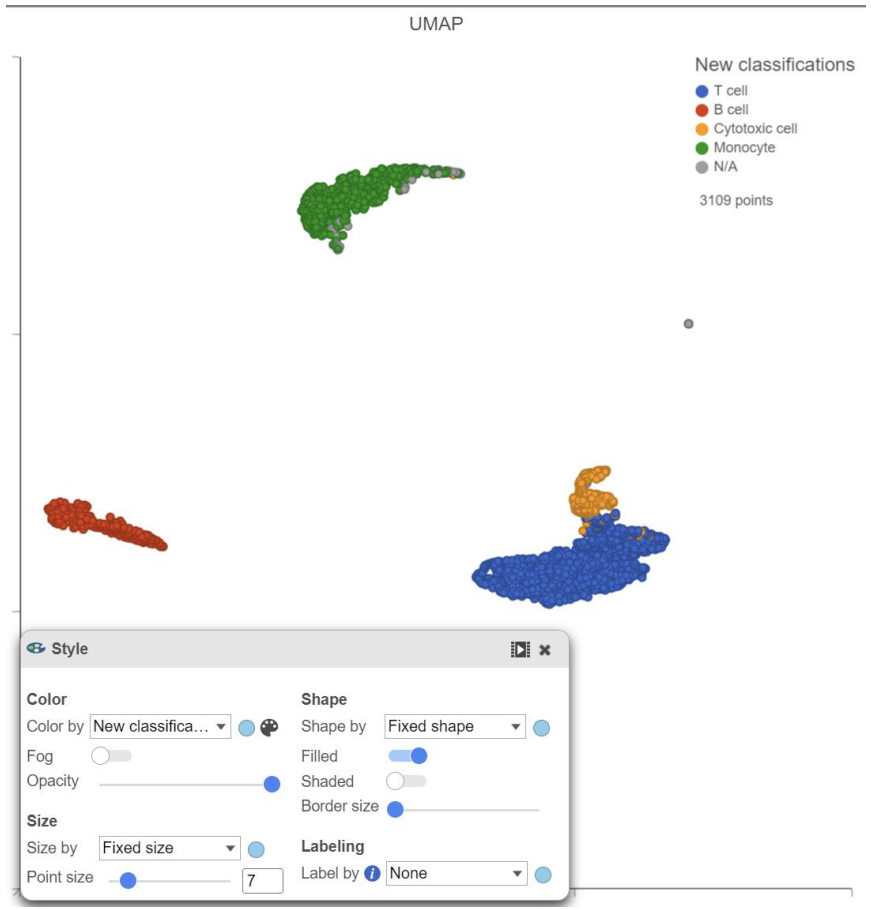
- Use lasso tool to select cells with high expression on both genes (upper-right corner)
- Click **Classify selection**, name it as **Monocyte** and **Save**



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Viewing Classifications

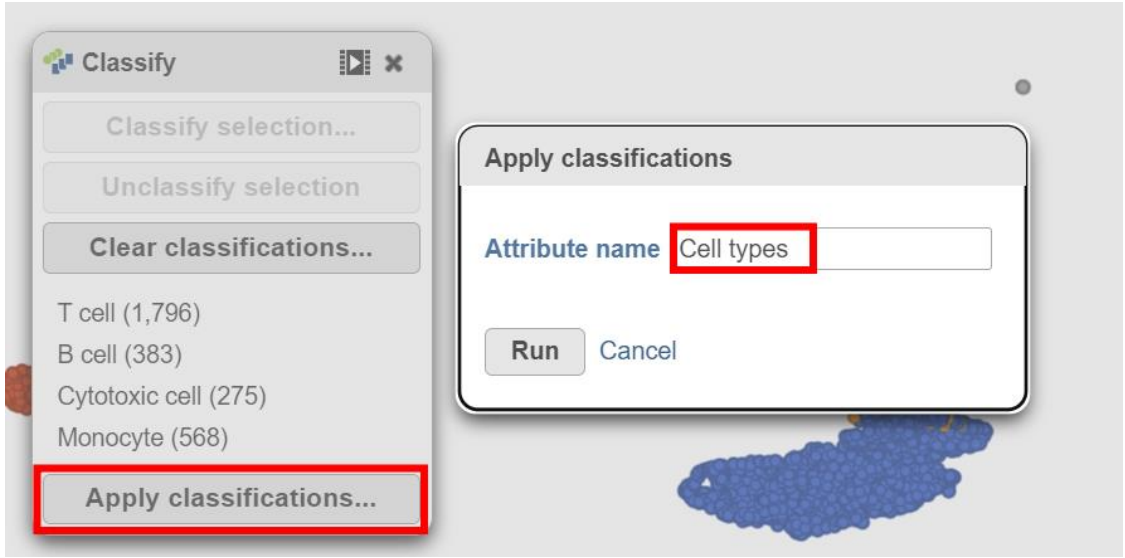
- Click on the UMAP plot, choose Color by **New classifications**



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Viewing Classifications

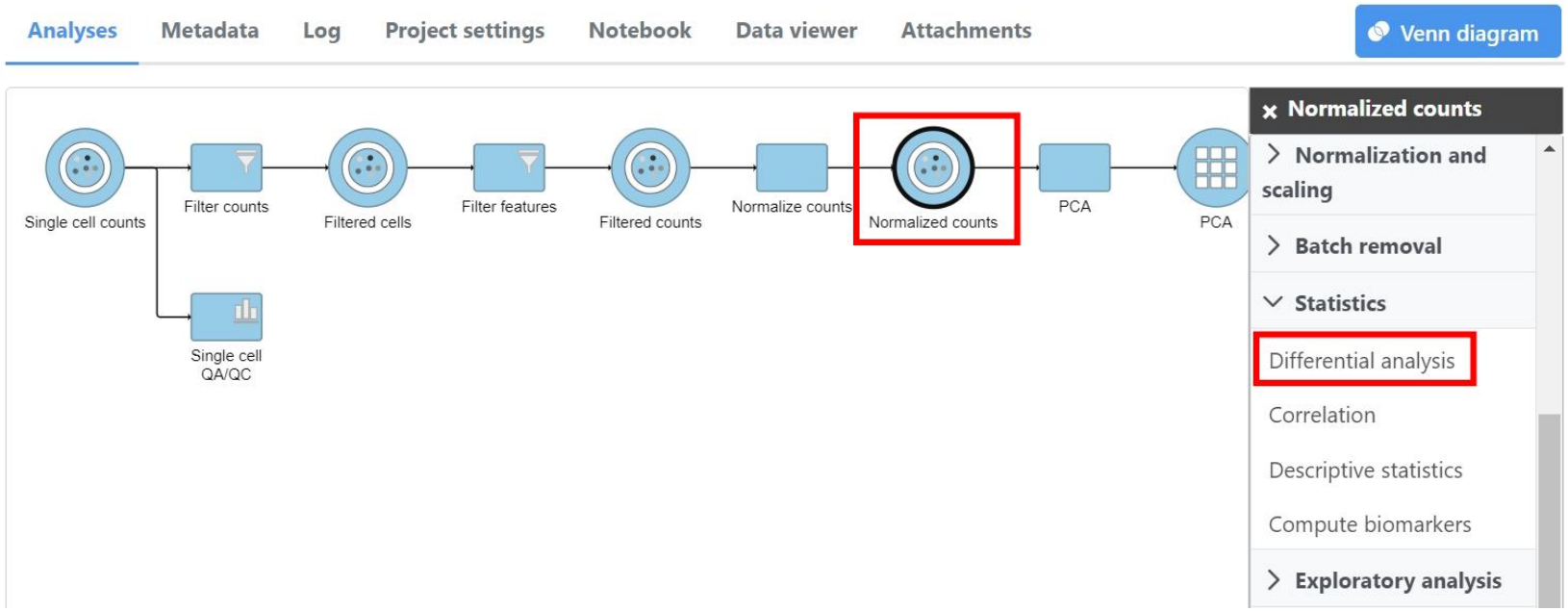
- Click **Apply classification…** button in Classification card to generate a new data node
- Name the new attribute **Cell types**
- Click **Run**



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Identifying Differentially Expressed Genes

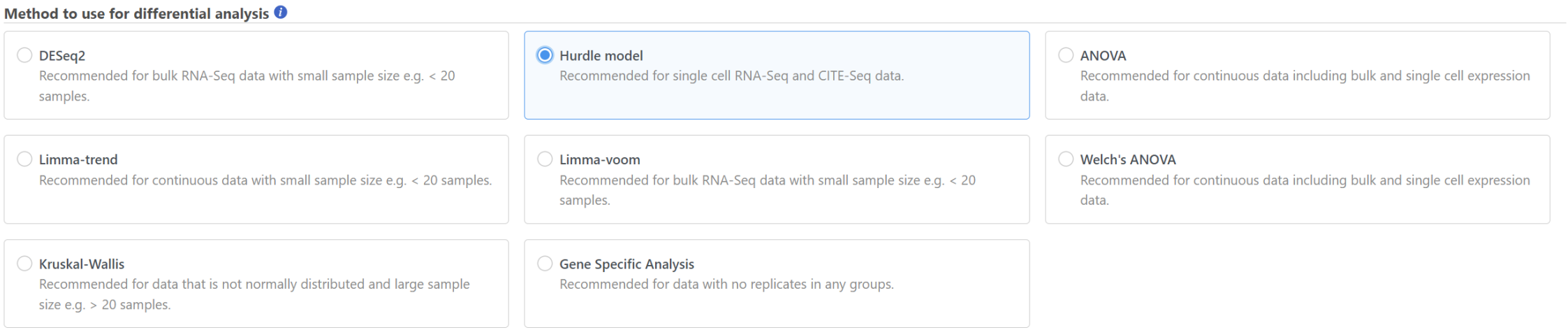
- Click the **Normalized cells** data node
- Click **Differential analysis** in the **Statistics** section of the task menu



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Identifying Differentially Expressed Genes

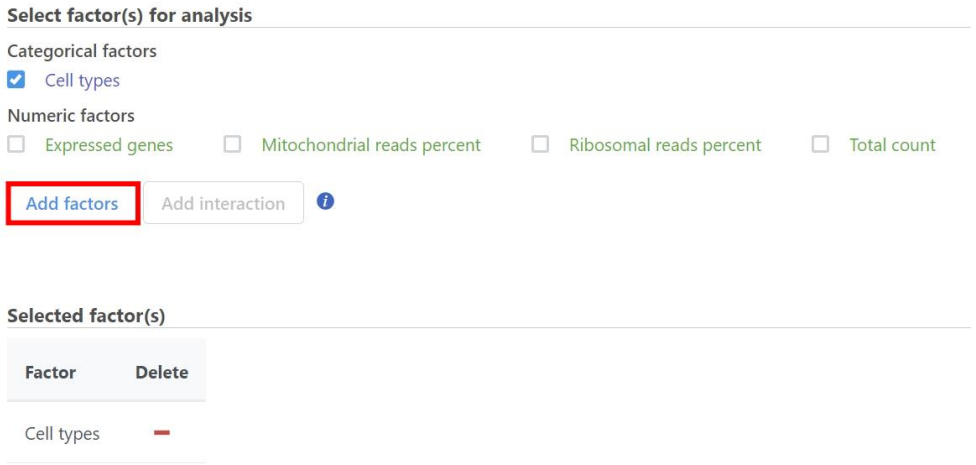
- Choose **Hurdle** and click **Next**

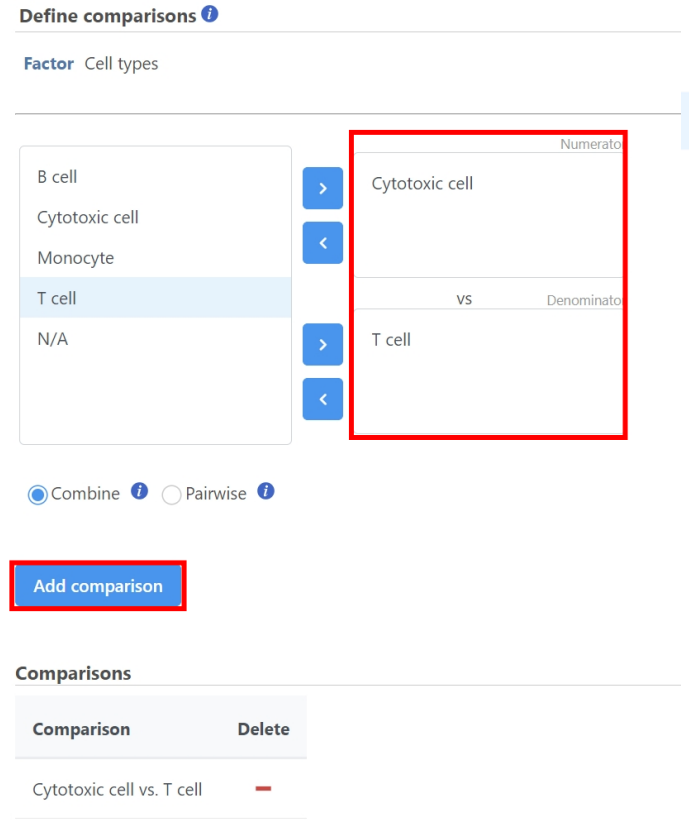


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Identifying Differentially Expressed Genes

- Choose **Cell types** and click **Next**
- Choose to compare Cytotoxic cell vs T cells, click **Add comparison**
- Click **Finish**





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Viewing GSA Results

- Double click the **T cell vs Cytotoxic cell** data node
- Genes are listed starting with the lowest p-value

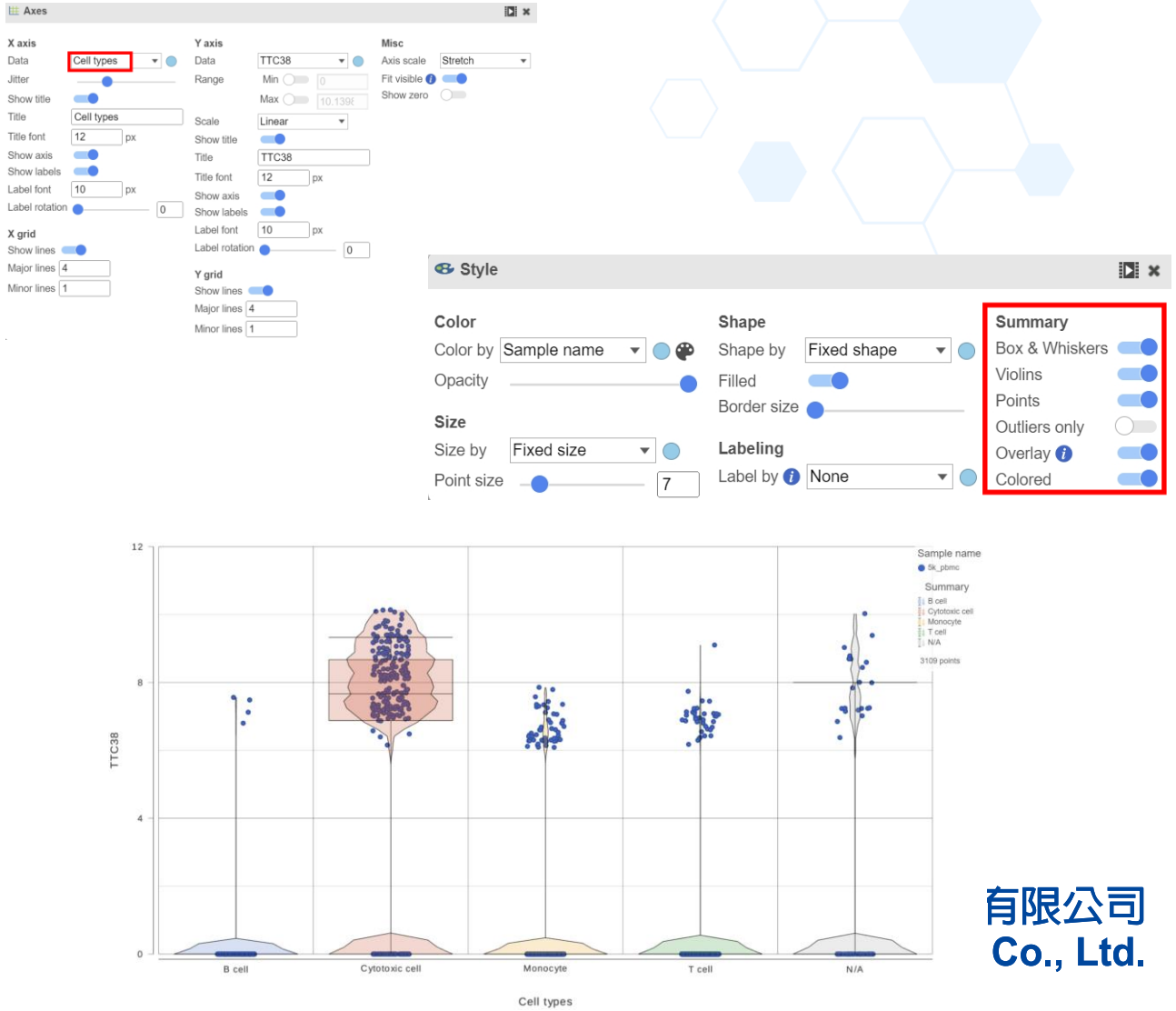
T cell vs Cytotoxic cell

	View	Gene ID	Gene name	P-value	FDR step up	Ratio	Fold change	LSMean(T cell)	LSMean(Cytotoxic cell)	Pct(T cell)	Pct(Cytotoxic cell)
1		PDGFD	PDGFD	0	0	0.38	-2.62	1.02	2.67	3.9E-3	0.20
2		PRELID1	PRELID1	0	0	0.13	-7.69	18.78	144.34	0.57	0.87
3		PREX1	PREX1	0	0	0.20	-4.90	2.23	10.91	0.16	0.45
4		PRF1	PRF1	0	0	1.6E-3	-624.95	1.97	1,232.67	0.13	0.98
5		ARHGEF3	ARHGEF3	0	0	0.26	-3.79	3.18	12.04	0.23	0.48
6		ARHGDIB	ARHGDIB	0	0	0.71	-1.42	548.70	777.43	0.99	0.99
7		ARHGDIA	ARHGDIA	0	0	0.27	-3.68	18.11	66.70	0.56	0.76
8		PRKCA	PRKCA	0	0	6.14	6.14	14.83	2.42	0.53	0.17
9		PRKCB	PRKCB	0	0	0.23	-4.33	9.98	43.17	0.45	0.68
10		PRKCH	PRKCH	0	0	0.26	-3.82	12.67	48.35	0.50	0.71
11		PRDX5	PRDX5	0	0	0.18	-5.56	15.57	86.61	0.54	0.80
12		ERH	ERH	0	0	0.39	-2.55	18.59	47.48	0.58	0.72
13		PRMT2	PRMT2	0	0	0.39	-2.54	37.56	95.47	0.68	0.80
14		ARHGAP18	ARHGAP18	0	0	0.49	-2.05	1.14	2.34	0.03	0.17
15		PRR5	PRR5	0	0	0.09	-10.81	2.59	28.01	0.19	0.62

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Viewing GSA Results

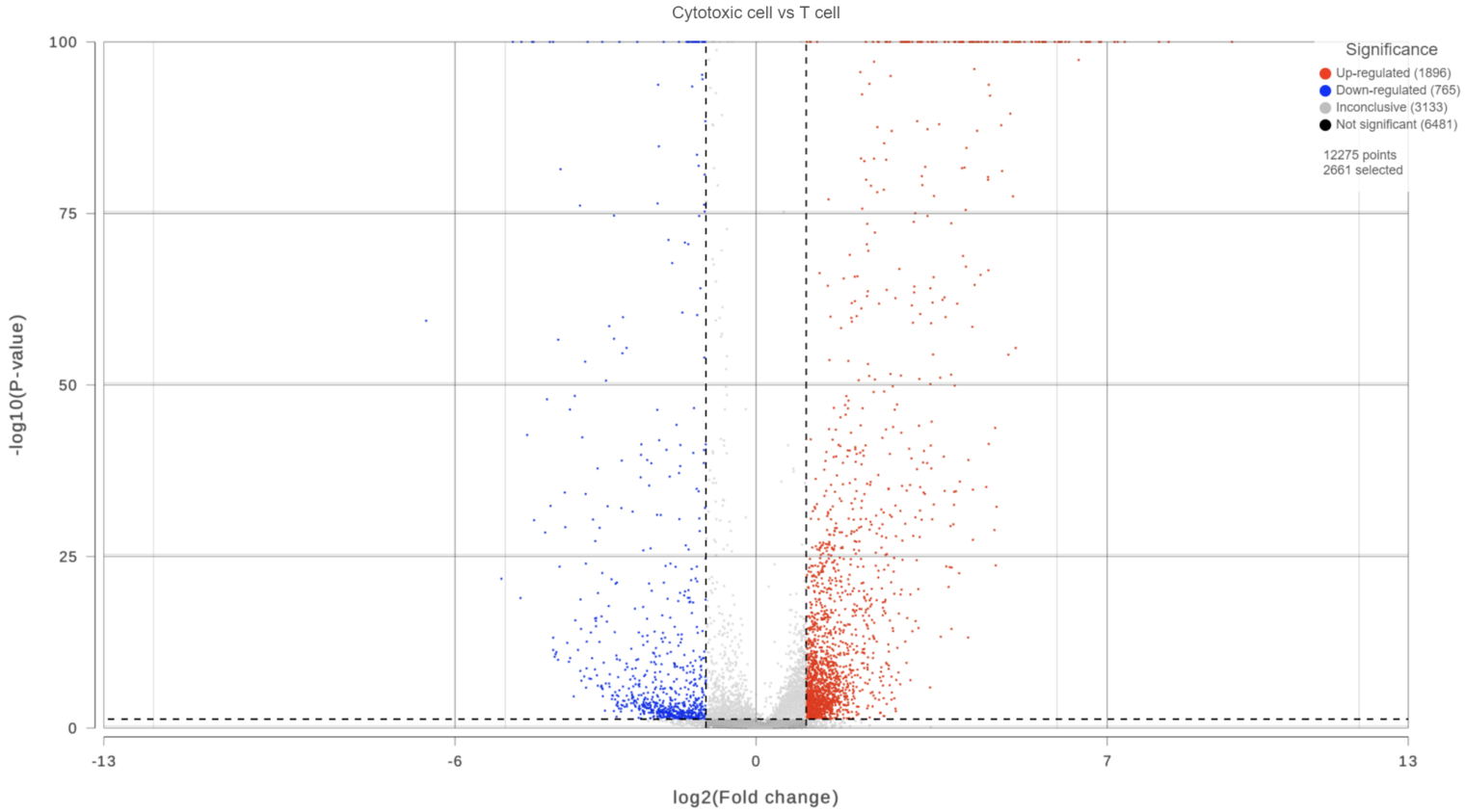
- Click the icon next to a gene under View to open dot plot
- Set **Cell types** as X axis
- The plot can be added violins or box Whiskers in **Summary** session from **Style**



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Co., Ltd.

Viewing GSA Results

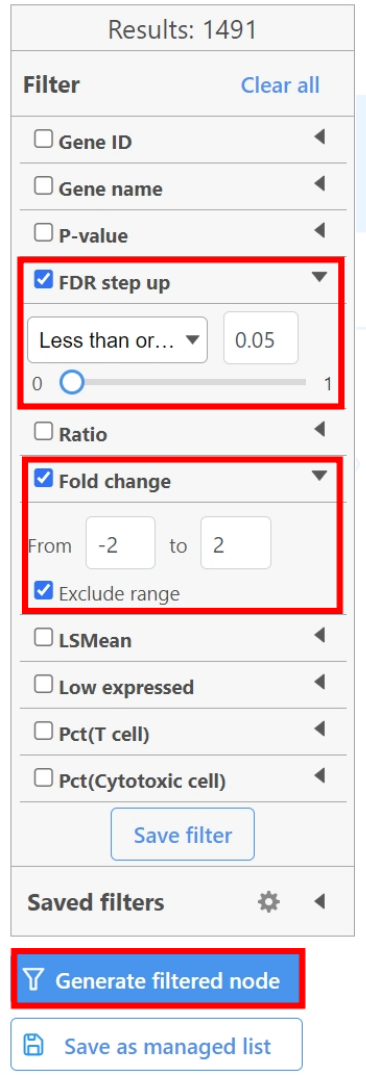
- Click the icon to invoke volcano plot



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etech Biotech Co., Ltd.

Identify Significantly DEG

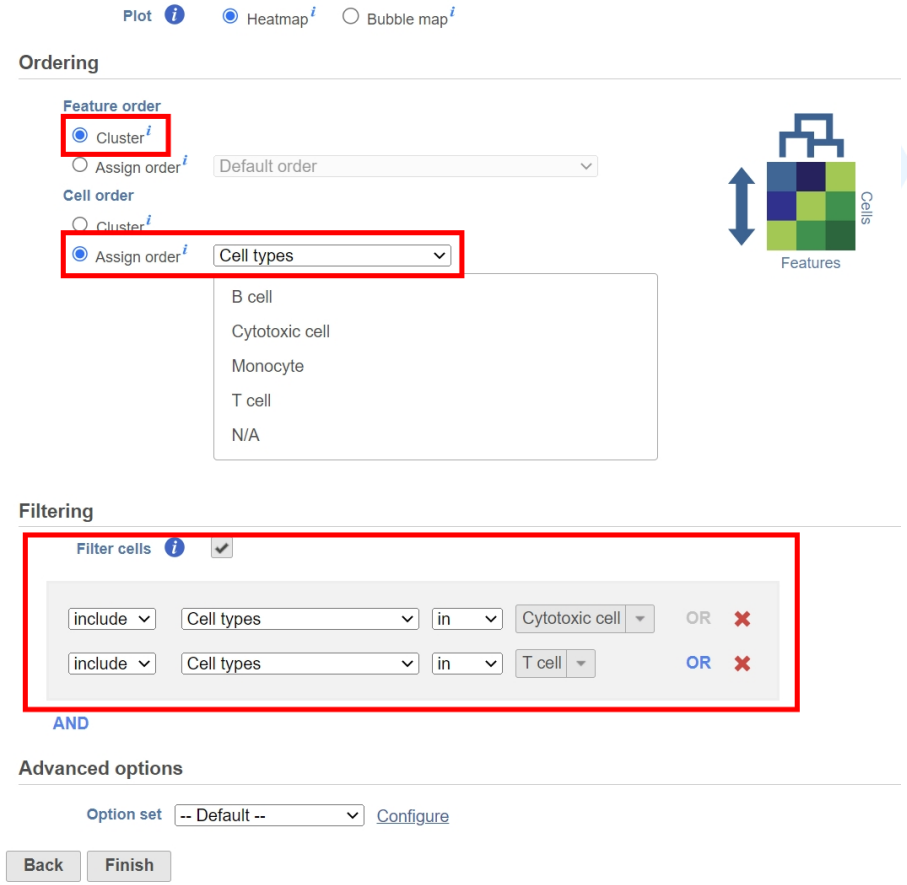
- Use the **Filter** on the left-hand side of the table
  - FDR step up: less than or equal to 0.05
  - Fold change: exclude range -2 to 2
- Click **Generate filtered node** to run the filter task



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Configuring Hierarchical Clustering

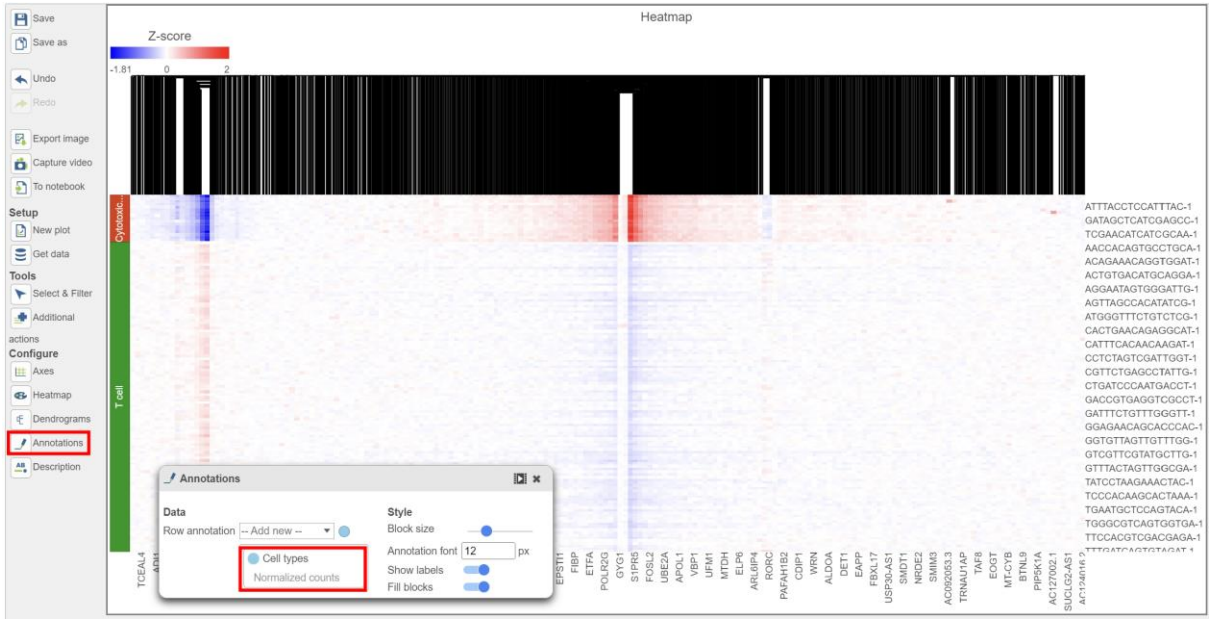
- Click the **Filtered feature list** data node
- Click **Hierarchical clustering / heat map** in the **Exploratory analysis** section of the task menu
- Check Cluster for Feature order
- Check **Filter cells** and set to *Include Cell types in T cells OR Include Cell types in Cytotoxic cells*



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Hierarchical Clustering Results

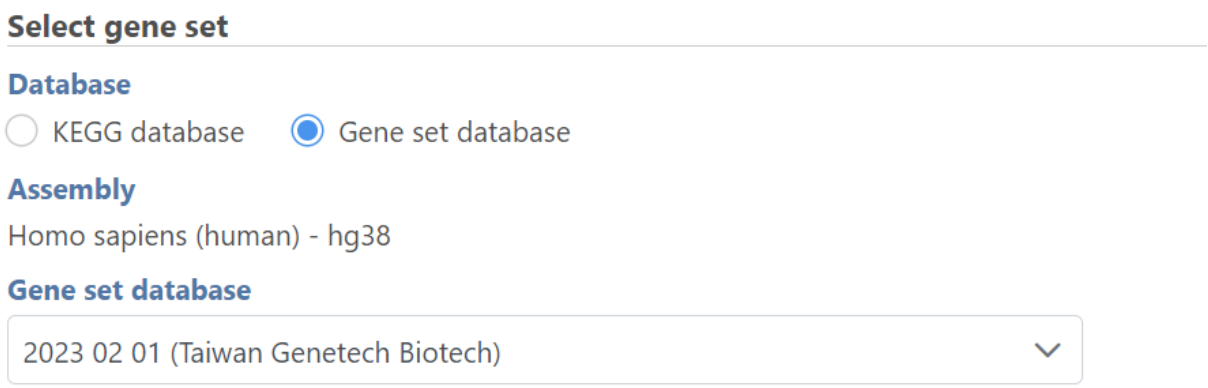
- Double-click on the **Hierarchical clustering / heat map** data node to view the result
- Use **Annotations** to annotate the cell types



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Biological Interpretation

- Click the Filtered feature list data node
- Click **Gene set enrichment** in the **Biological interpretation** section of the task menu
- Select **Gene set database** and choose the database
- Click **Finish**



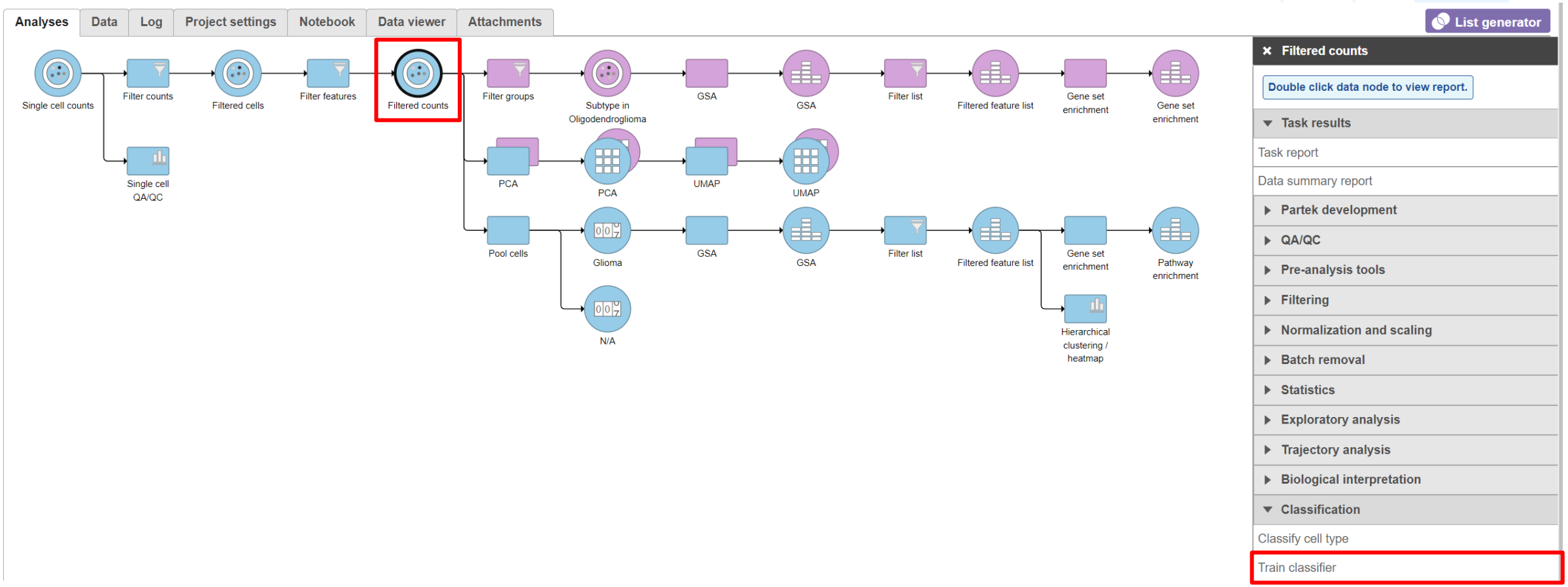
Biological Interpretation

- Double-click on the **Gene set enrichment** data node to view the report

Gene set	Description	Type	Enrichment score	P-value	FDR step up	Rich factor	Genes in set	Genes in list	Genes not in list	Genes in list, not in set	Genes not in list, not in set
GO:0070062	extracellular exosome	cellular component	121.88	1.17E-53	2.26E-49	0.28	1,310	369	941	1,057	8,376
GO:0043230	extracellular organelle	cellular component	119.56	1.19E-52	5.75E-49	0.28	1,321	369	952	1,057	8,365
GO:1903561	extracellular vesicle	cellular component	119.56	1.19E-52	5.75E-49	0.28	1,321	369	952	1,057	8,365
GO:0065010	extracellular membrane-bounded organelle	cellular component	119.56	1.19E-52	5.75E-49	0.28	1,321	369	952	1,057	8,365
GO:0031982	vesicle	cellular component	100.13	3.27E-44	1.26E-40	0.23	2,046	476	1,570	950	7,747
GO:0002376	immune system process	biological process	84.06	3.1E-37	1E-33	0.26	1,199	313	886	1,113	8,431
GO:0002682	regulation of immune system process	biological process	68.71	1.45E-30	4E-27	0.26	1,044	269	775	1,157	8,542
GO:0030055	cell-substrate junction	cellular component	66.64	1.15E-29	2.67E-26	0.38	322	122	200	1,304	9,117
GO:0005925	focal adhesion	cellular component	66.56	1.24E-29	2.67E-26	0.38	318	121	197	1,305	9,120

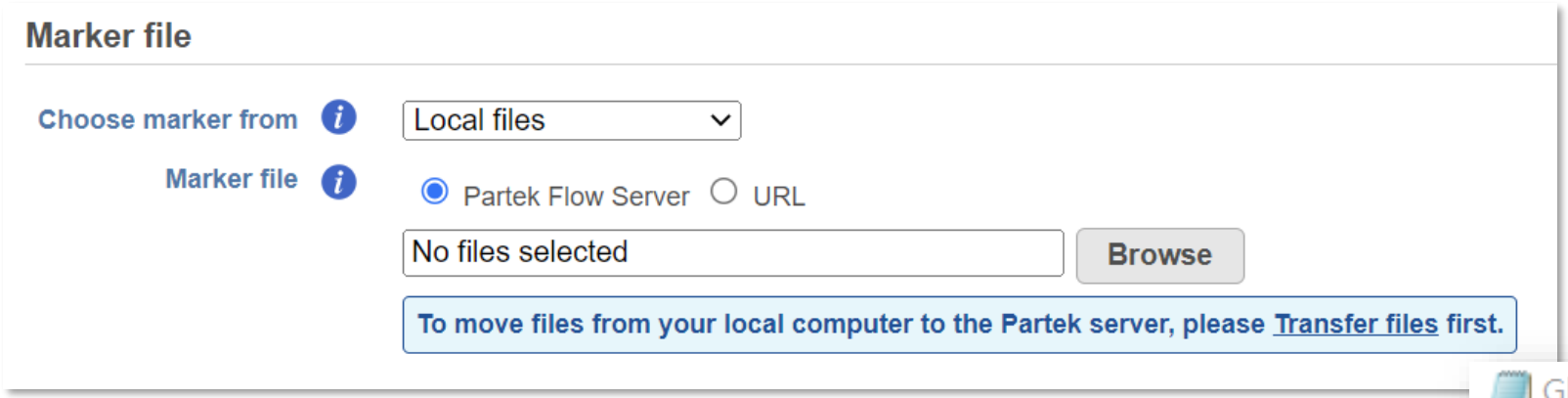
Appendix – Garnett Classifier

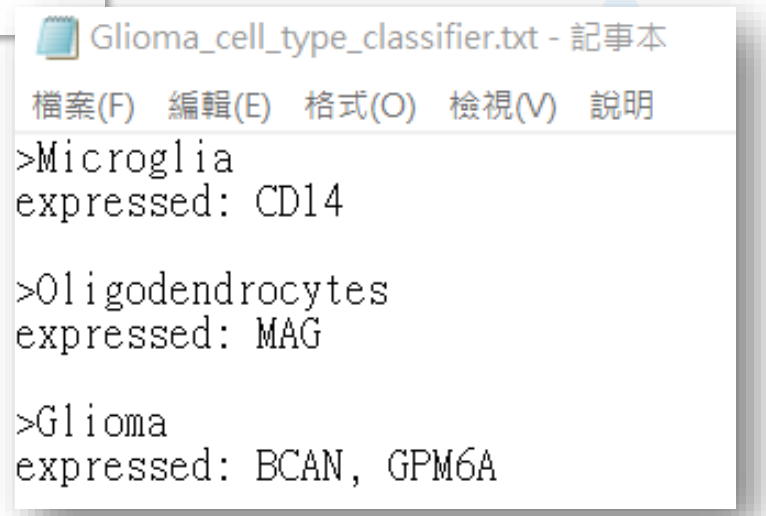
Train Classifier



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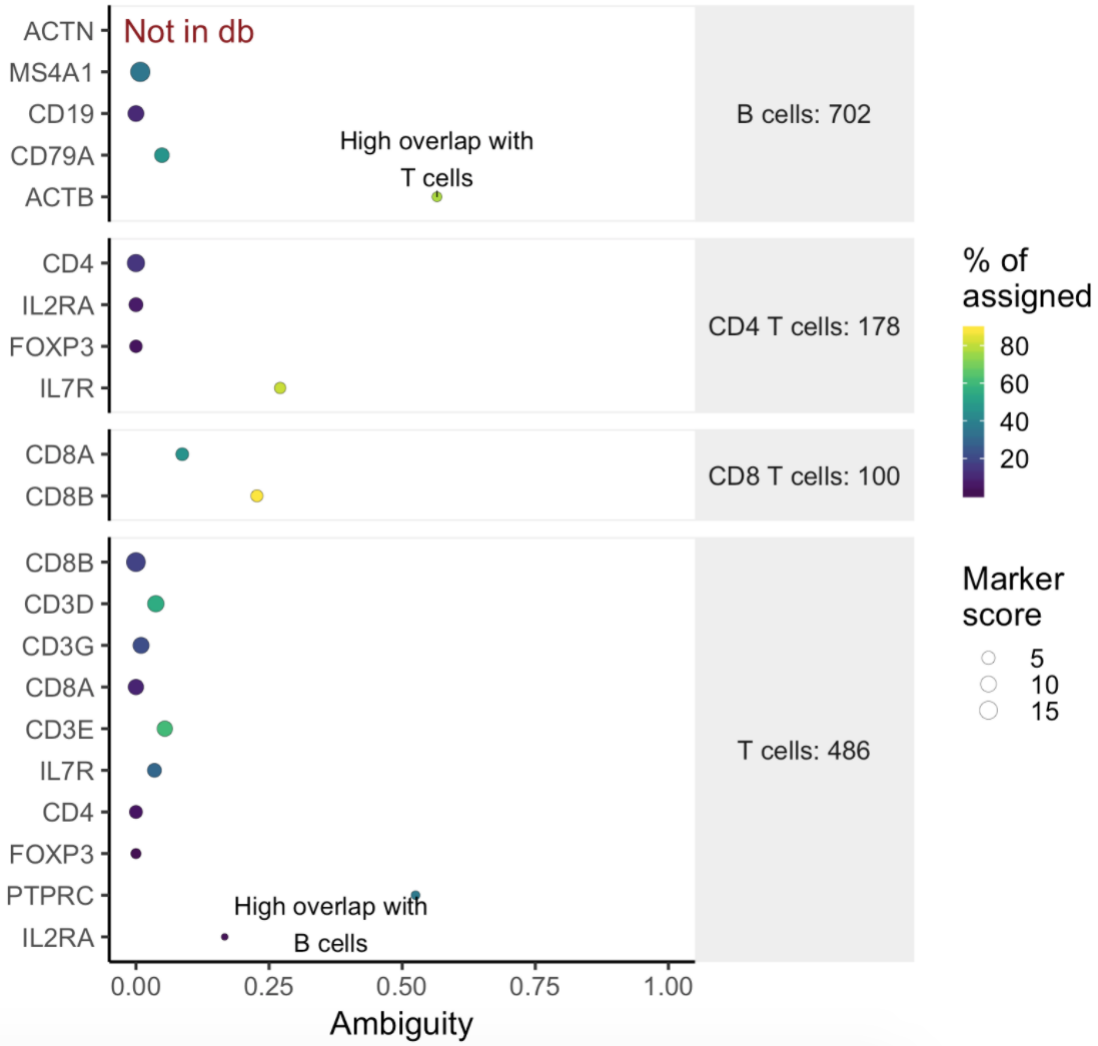
Train Classifier





There has to be a space character after the colon
and that there has to be a space character after the comma.

Train Classifier Results



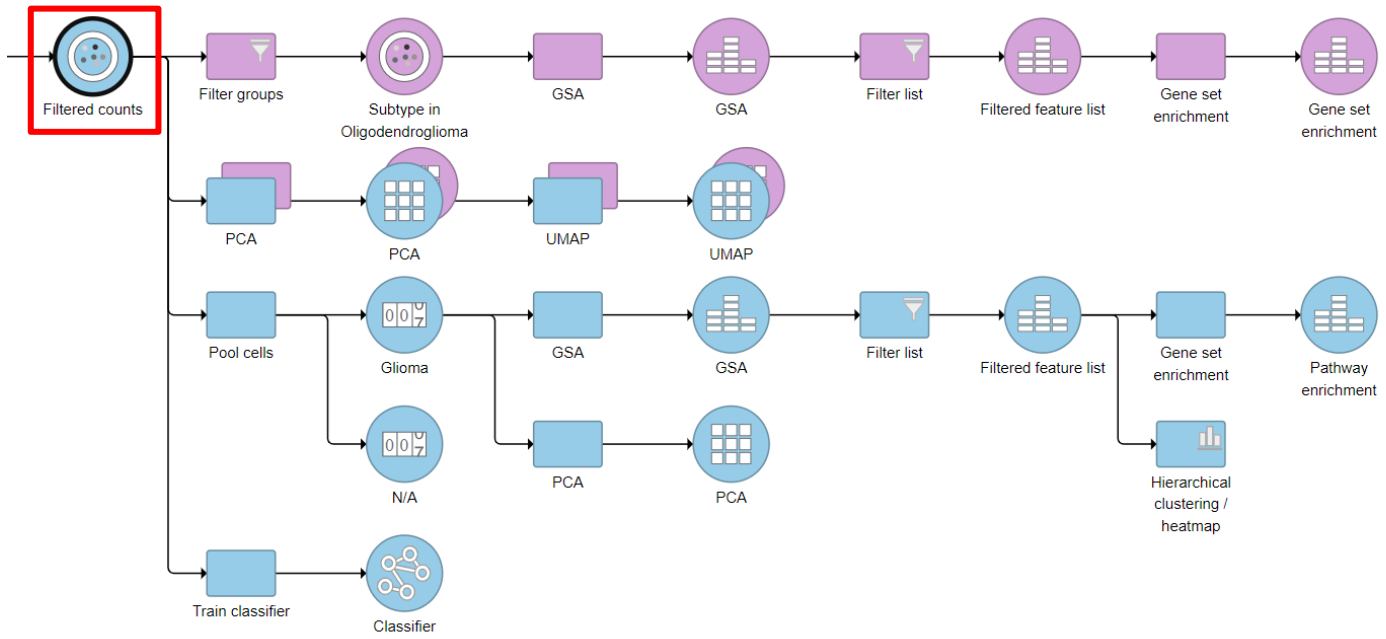
- Double click the **Classifier** data node
- Ambiguity scores are calculated for each of the markers which indicates how many cells receive ambiguous labels when this marker is included

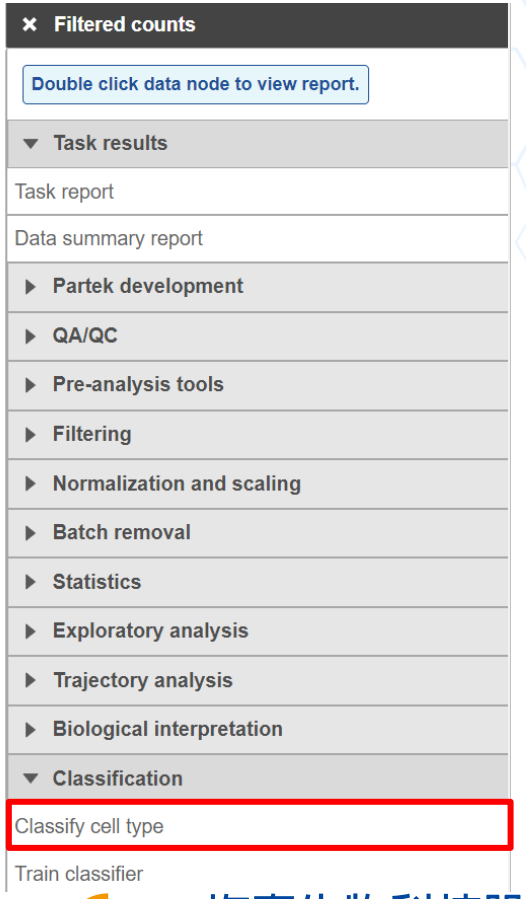
Train Classifier Results

- The classification gene table may give a hint to which genes are chosen as the relevant genes for distinguishing between different cell types

Feature	Glioma	Microglia	Oligodendrocytes	Unknown
(Intercept)	-39.80	9.48	14.21	16.11
BCAN	2.63	-1.00	-0.80	-0.83
GPM6A	2.43	-0.60	-0.96	-0.87
CD14	0.82	1.96	-1.48	-1.30
MAG	0.52	-0.50	2.71	-2.73

Classify Cell Type





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Classify Cell Type – Project classifiers

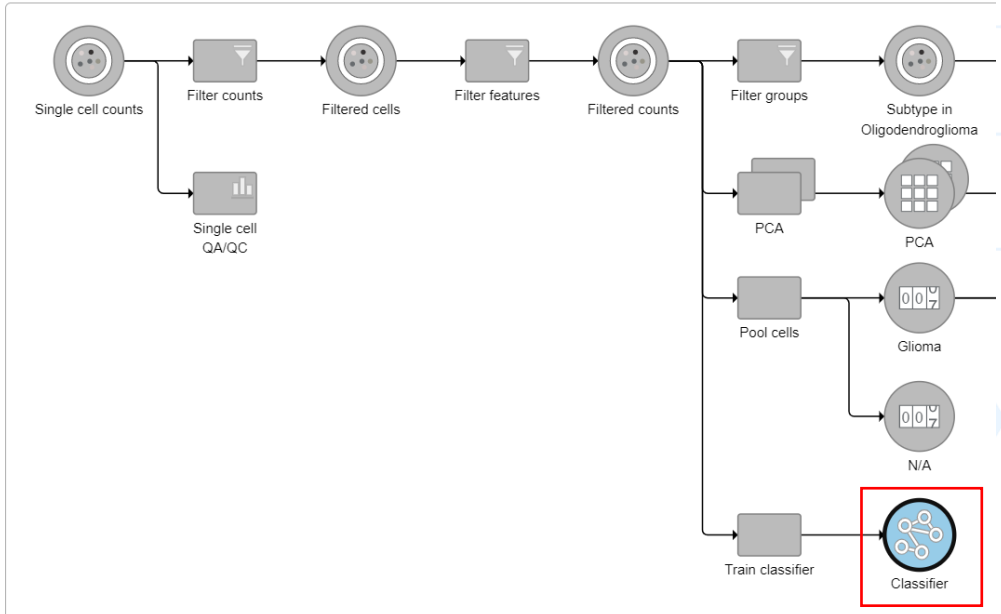
Choose classifier from Project classifiers

Garnett classifier

Select data node Clear selection

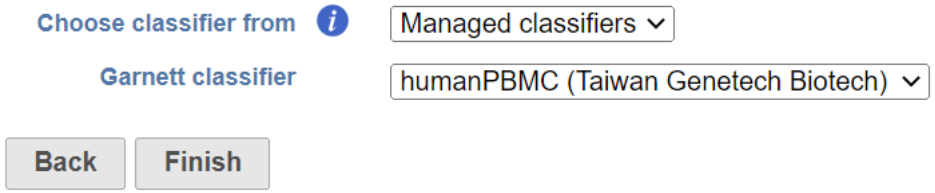
Classifier [Train classifier - 0.2.14]

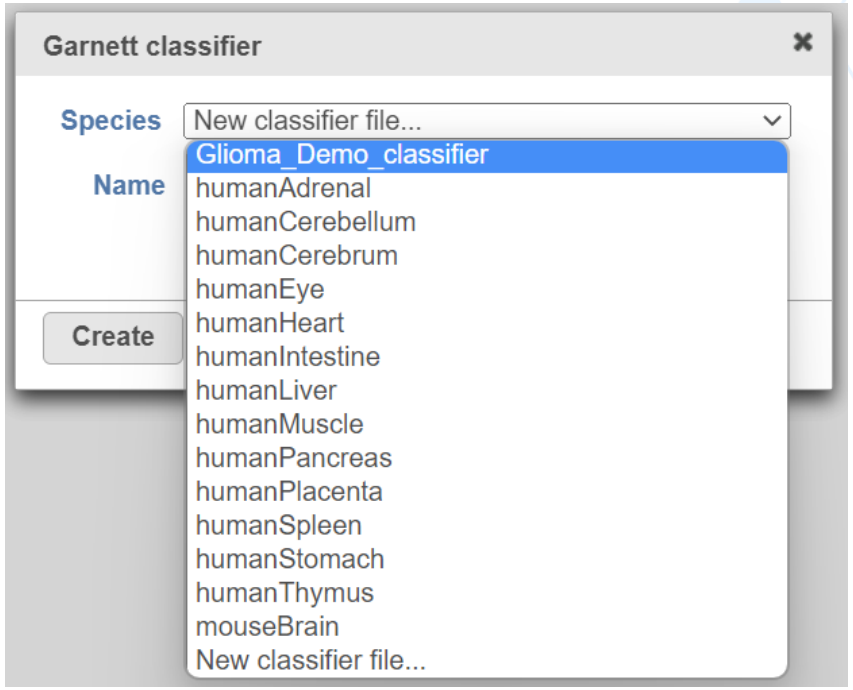
Back Finish



Select Cancel

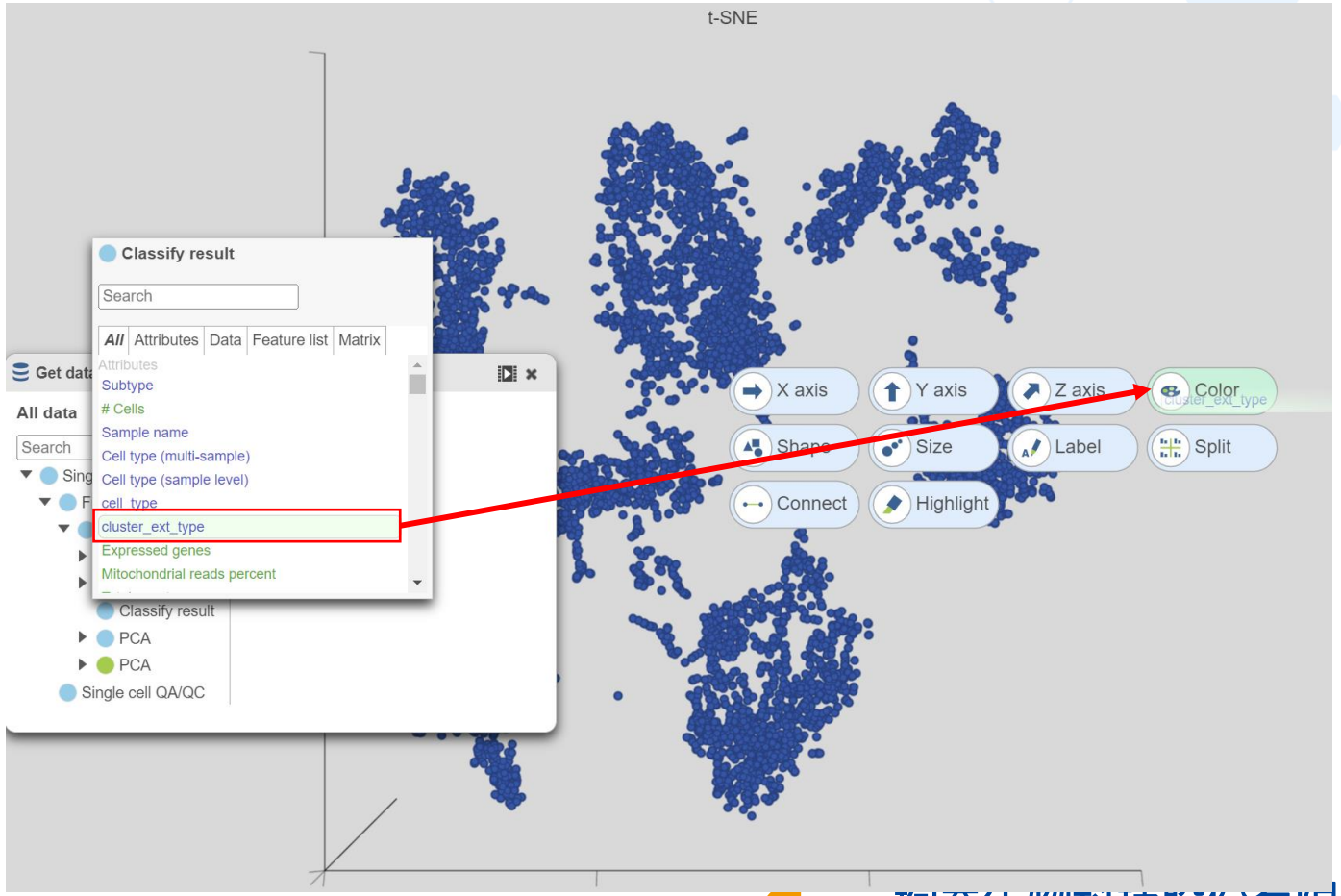
Classify Cell Type – Managed classifiers



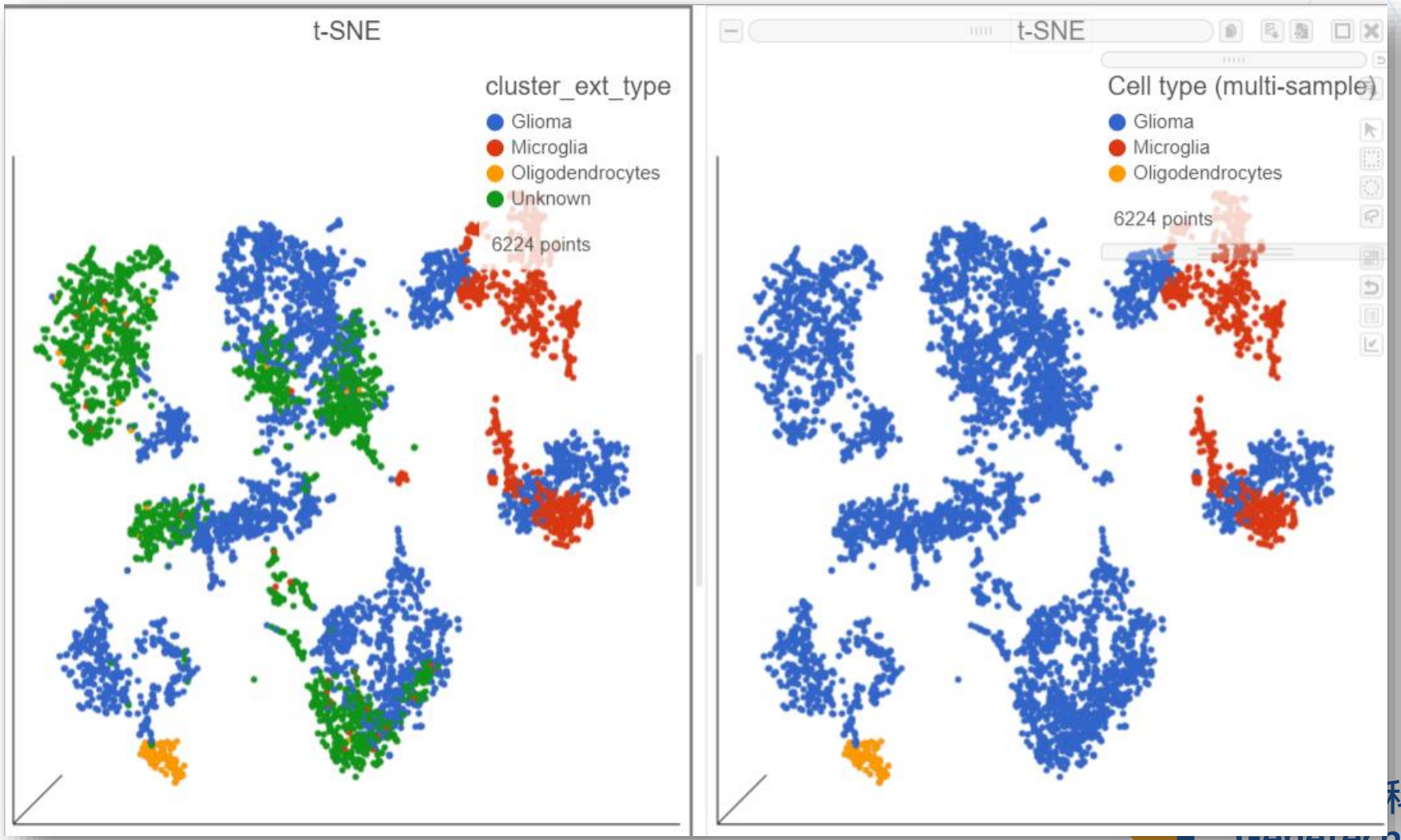


Classification Results

- **“cell_type"** is the cell type assignments directly from Garnett model.
- **"cluster_ext_type"** is the cell type that's determined by expanding cell type assignments to nearby cells using Louvain clustering.



Garnett Classifiers vs. Manual Classification

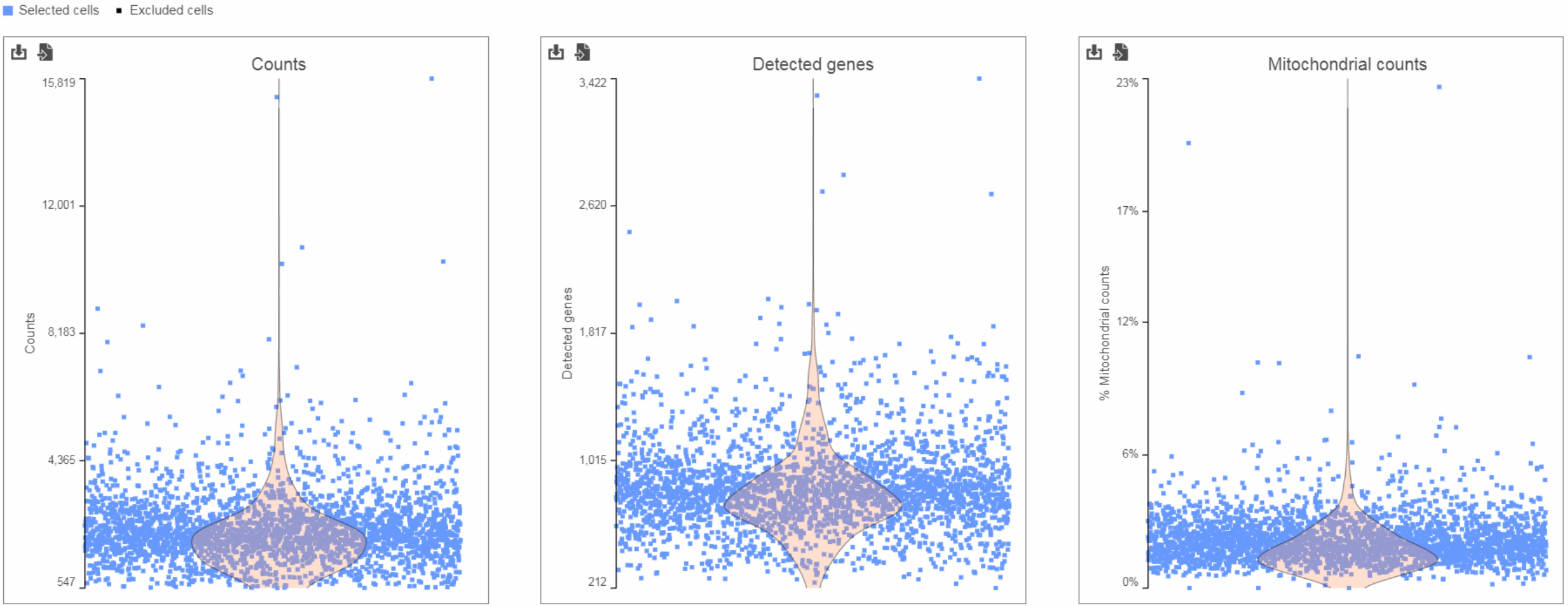


Plot Interpretation

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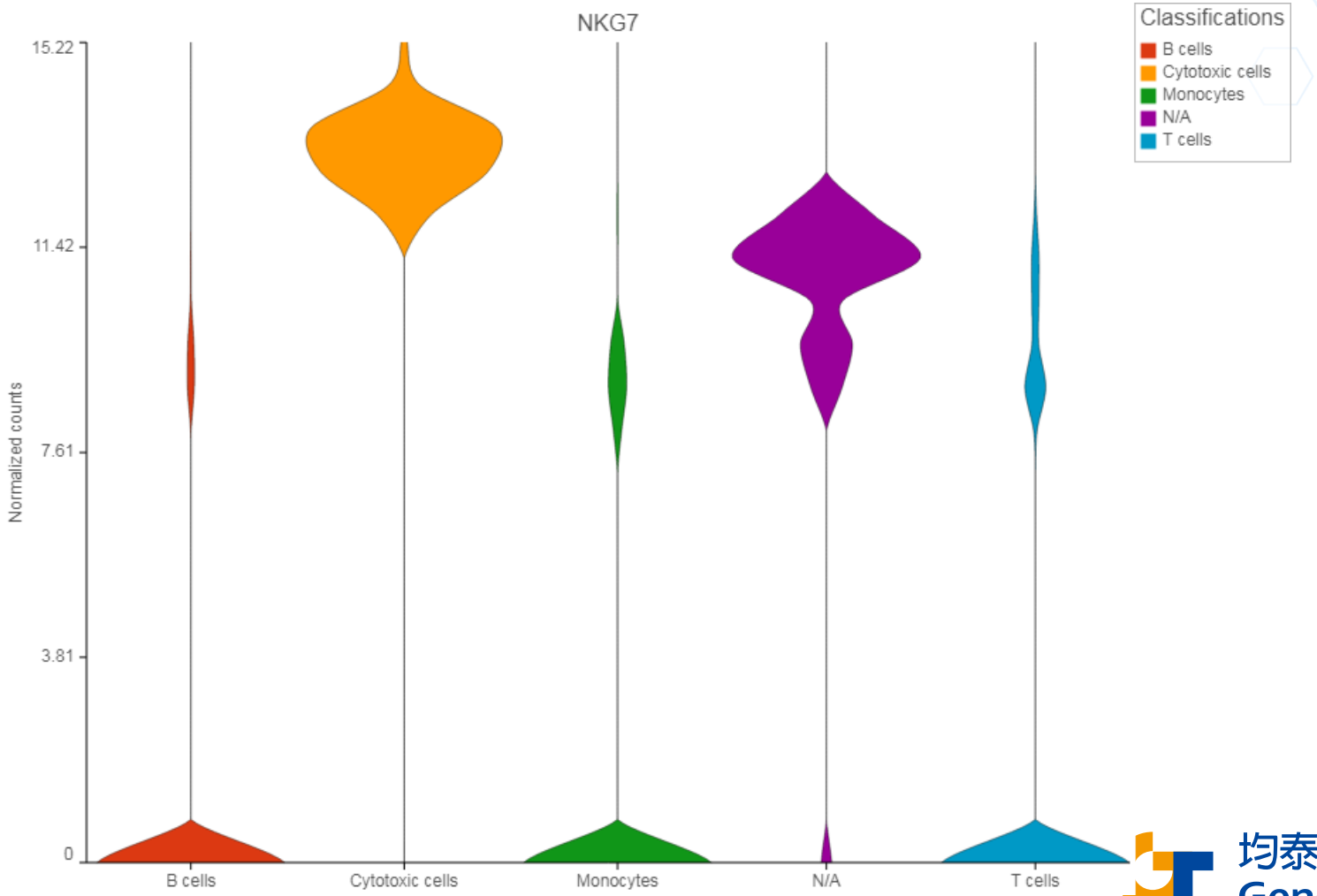
Single cell QA/QC report - Violin Plot

由左至右分別代表細胞中的read數量、基因數量以及Mitochondria gene表達量
X軸沒有意義，目的是為了避免有兩個以上的cells有相同的count重疊看不出來；Y軸代表total count；每個點代表一個細胞
Violin plot 越寬代表密度越大，可以由這張圖明顯看到cell集中於哪個數量區域，並進一步留下較有生物意義的細胞



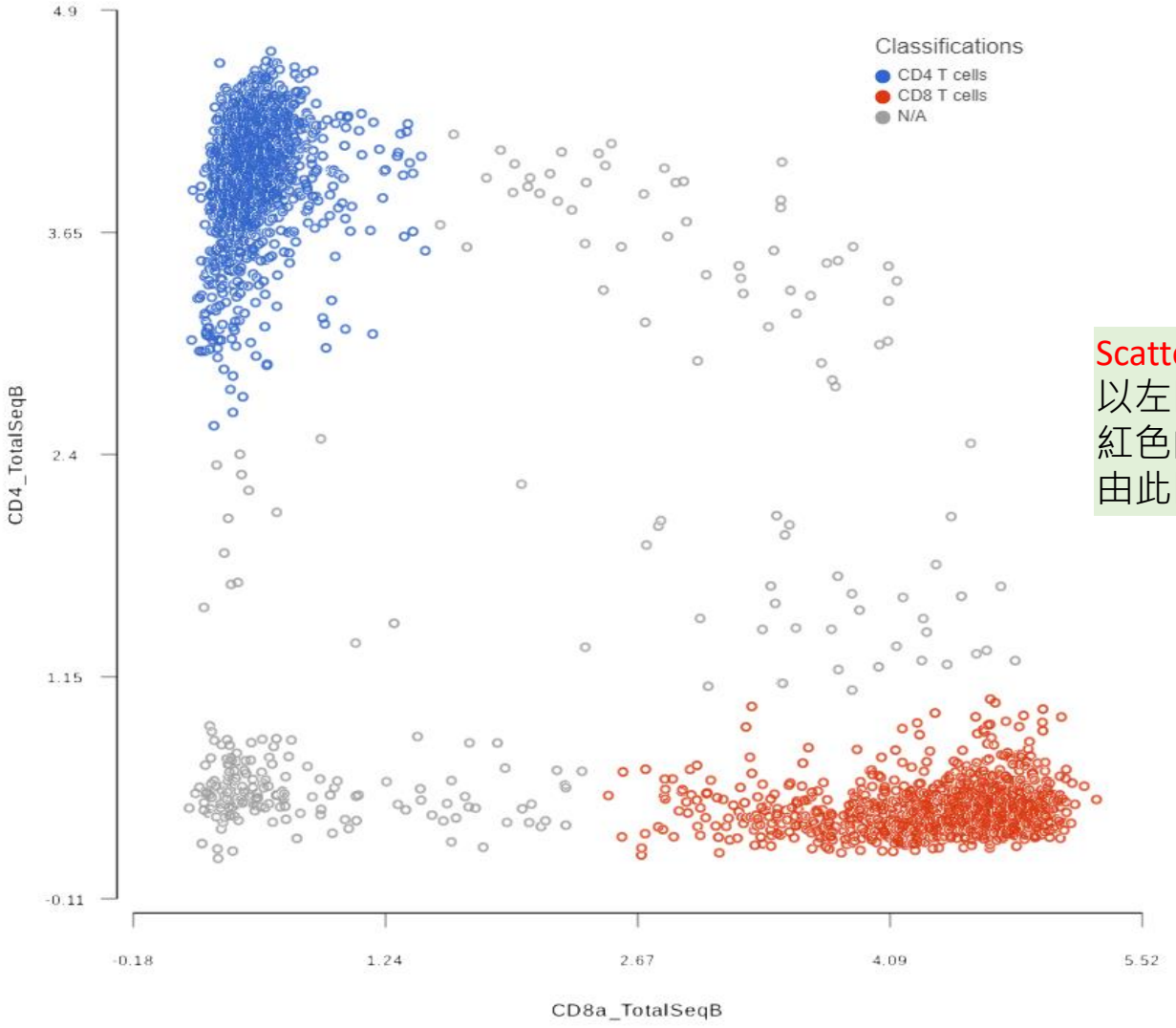
Feature Plot

X軸為不同的細胞類別，Y軸為Normalized後的 Read count數；客戶可自行將細胞分類，
並透過Feature Plot了解特定基因在不同類別中的RNA表現量



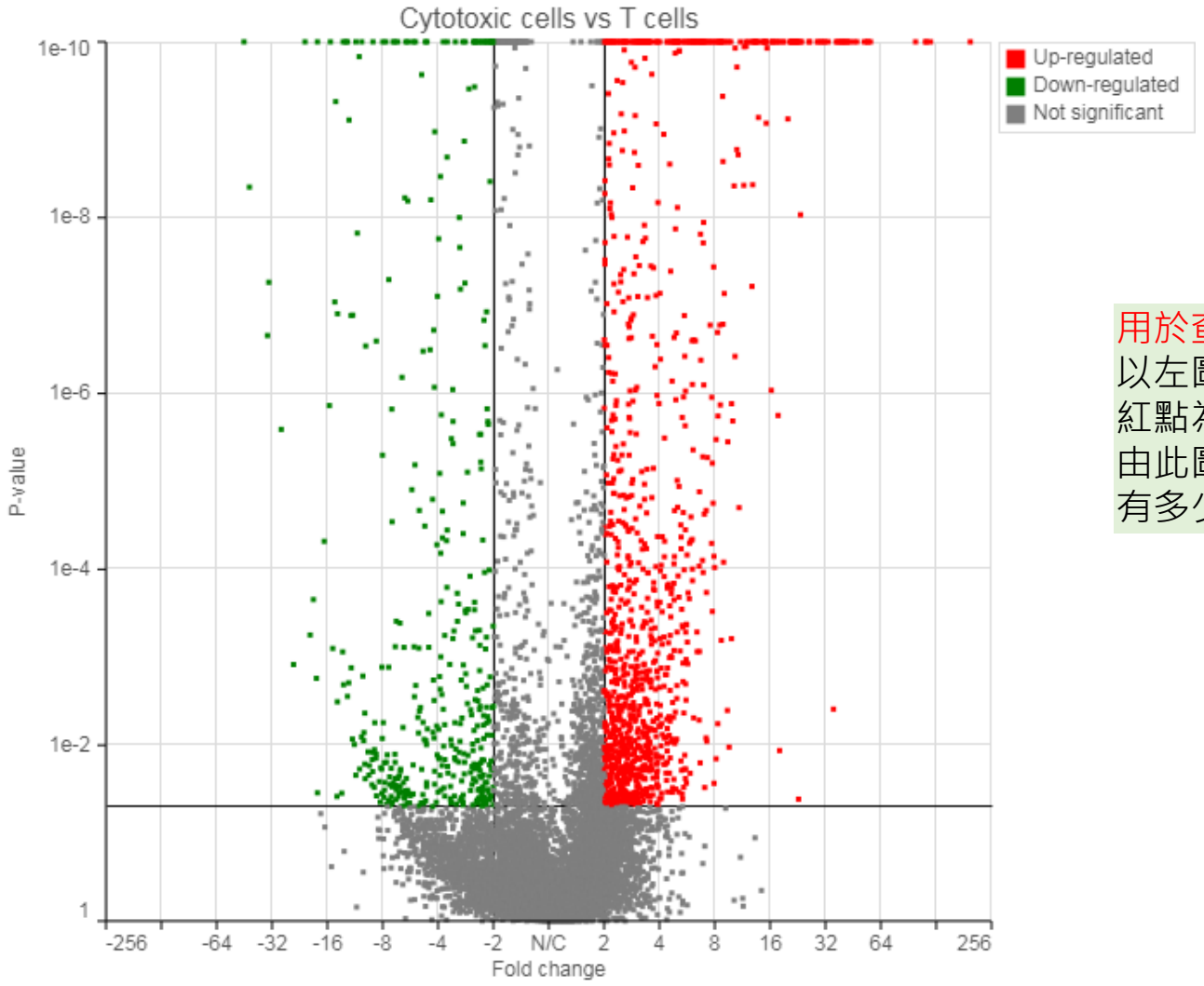
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Scatter Plot



Scatter Plot可以看出不同Biomarker在不同種類的細胞是否具有相關性
以左圖說明， XY軸分別是CD8 及CD4兩種biomarker 表達量，
紅色的CD8 T-cell 群有高表達CD8及低表達CD4的特性，CD4 T-cell 群則反之；
由此圖可知這兩個Biomakers能有效分出藍色及紅色這兩個種類的細胞

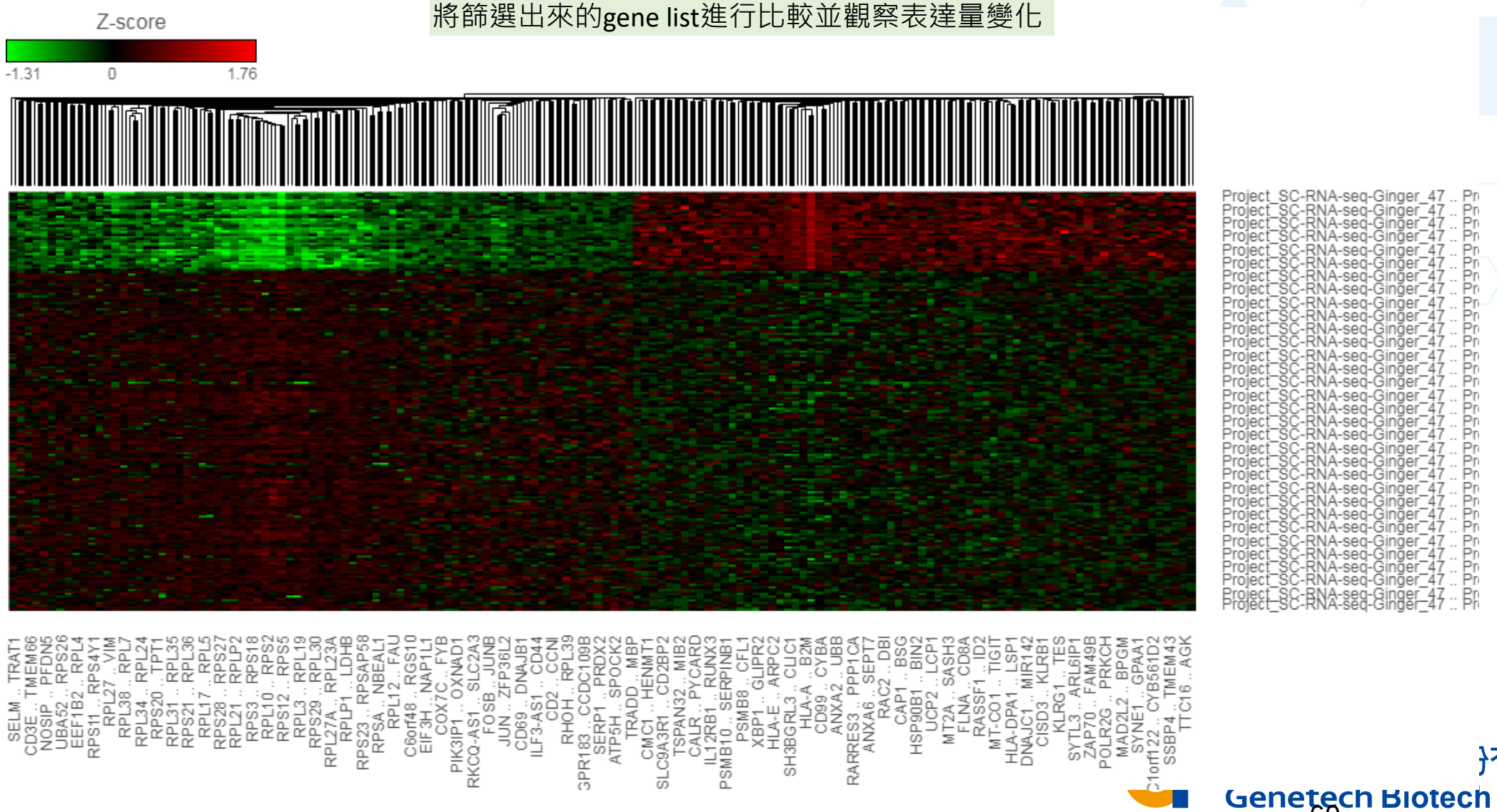
Volcano Plot



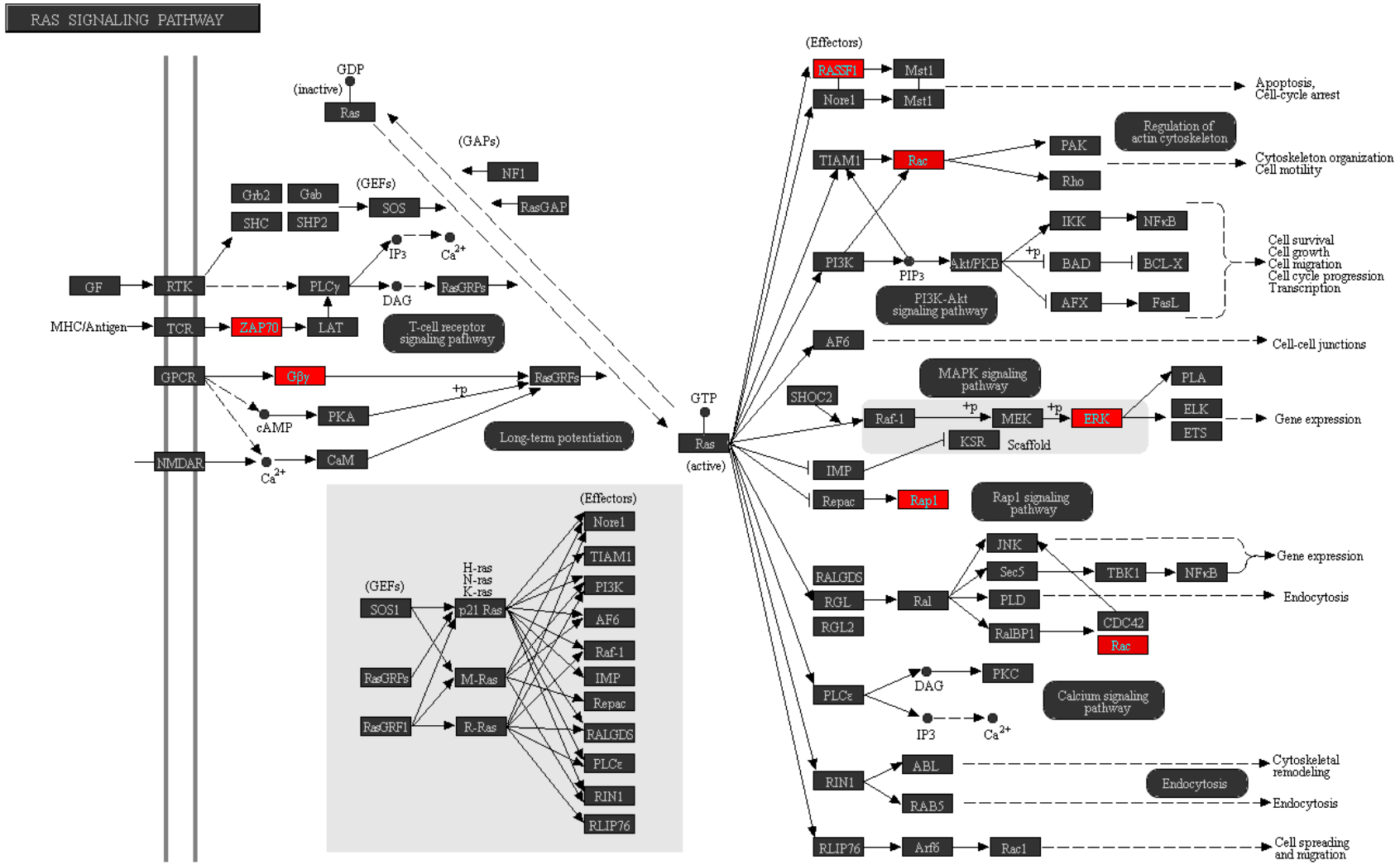
用於查看特定細胞群中高表達基因及低表達基因的數量
以左圖說明，X軸為Fold change，Y軸為P-value；
紅點為Up-regulated gene，綠點為Down-regulated gene
由此圖可看出cytotoxic cells 和 T-cells 這兩個種類的細胞群相比之後，有多少up-regulated, down-regulated 及 un-change 的基因

Heatmap

將篩選出來的gene list進行比較並觀察表達量變化



KEGG Pathway result



挑出感興趣的Gene list，
並分析這些基因是否影響特定pathway

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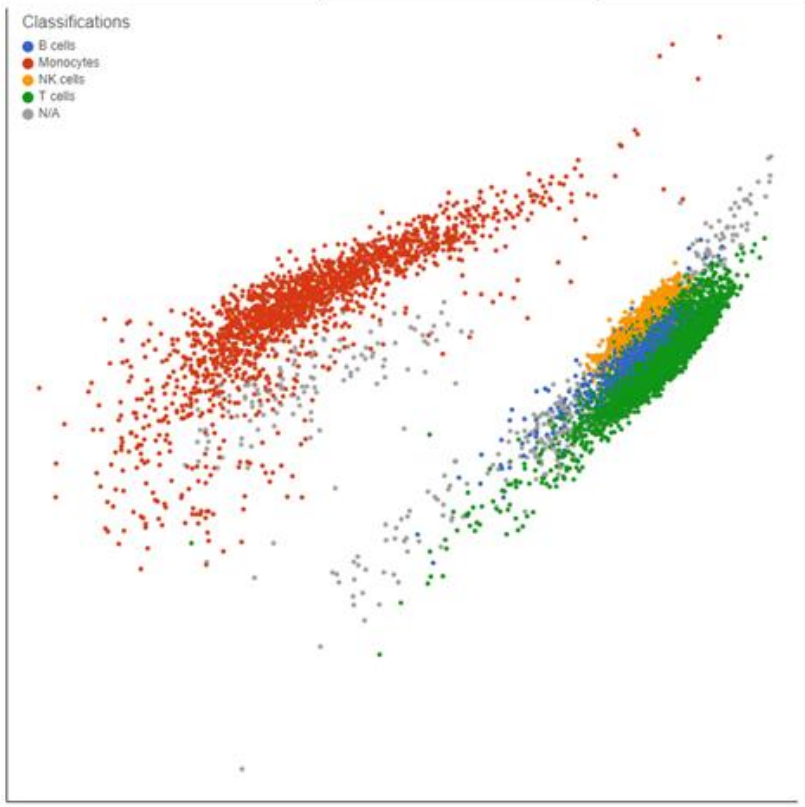
Dimensionality Reduction: PCA, t-SNE, UMAP

細胞分群後的圖表呈現，因每個細胞皆有上千、萬個基因，相等於上千、萬個維度，必須透過降維才能比較各個細胞間不同基因表達量的相關性

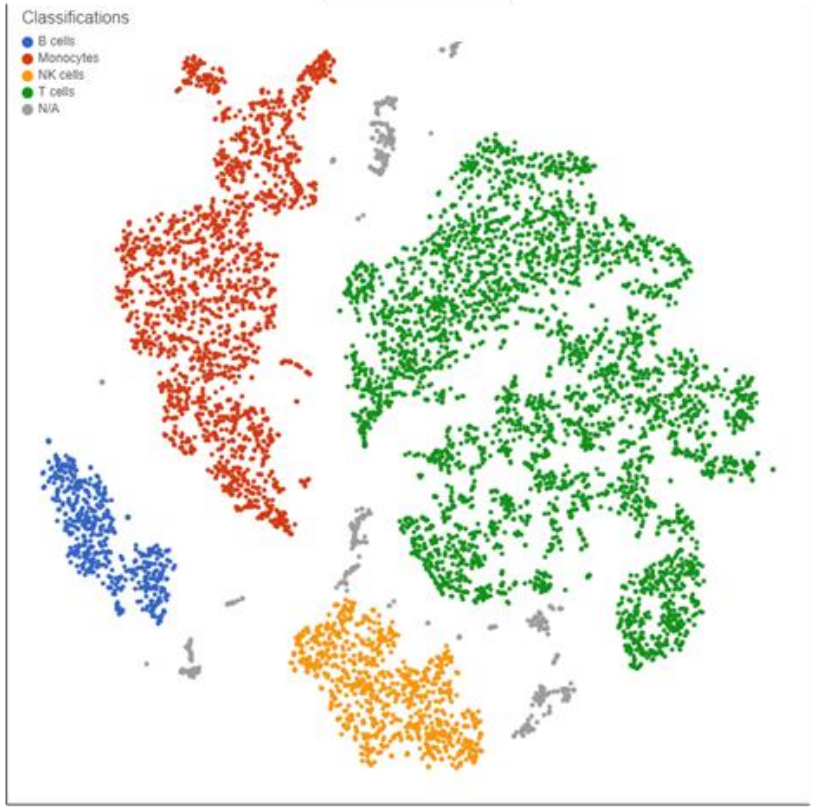
PCA, t-SNE, UMAP分別為三種不同的降維方法，是依照各細胞的基因表達量來分群，同一群的細胞所表達的基因越相似

Partek Flow 提供2D及3D的呈現方式，讓使用者更有效了解樣品中不同細胞的相關性

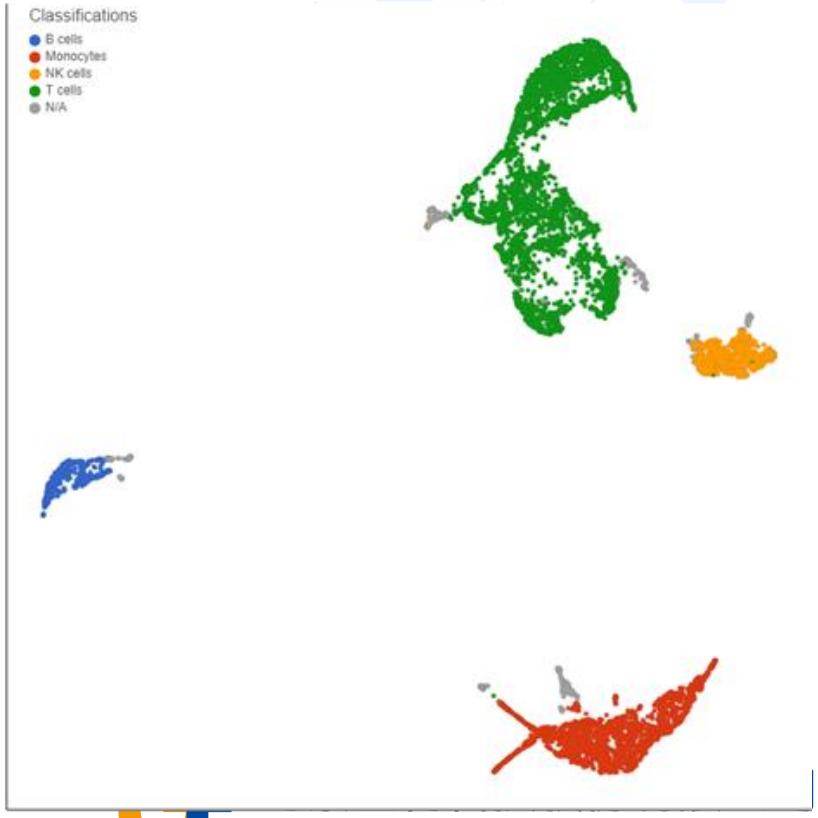
PCA



t-SNE



UMAP

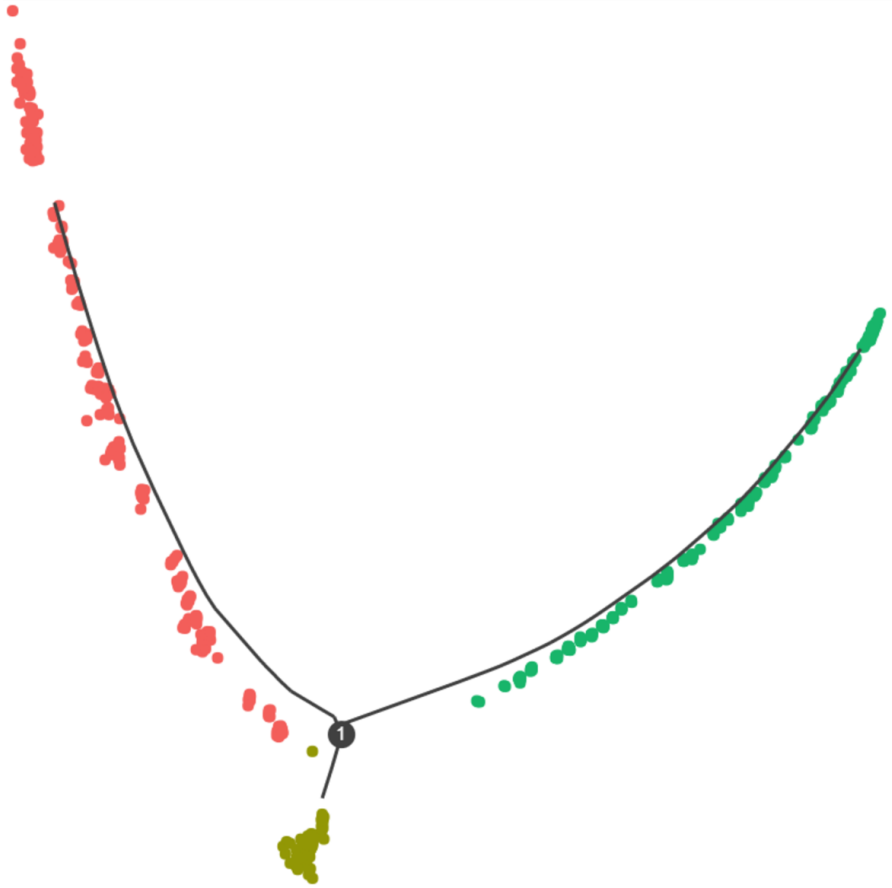


View each in 2D or 3D

Run Trajectory Analysis with Monocle

透過Trajectory分析，將不同的細胞群依照基因的表達量來預測發育細胞的分化軌跡或細胞的演化過程
Identify States:根據表現量的分佈建構出細胞分化過程的樹狀結構
Calculate Pseudotime: 了解每個細胞在該樹狀結構中的位置，可進一步進行差異分析探索細胞分化過程的重要基因，常用於發育相關研究

Identify States



Calculate Pseudotime

