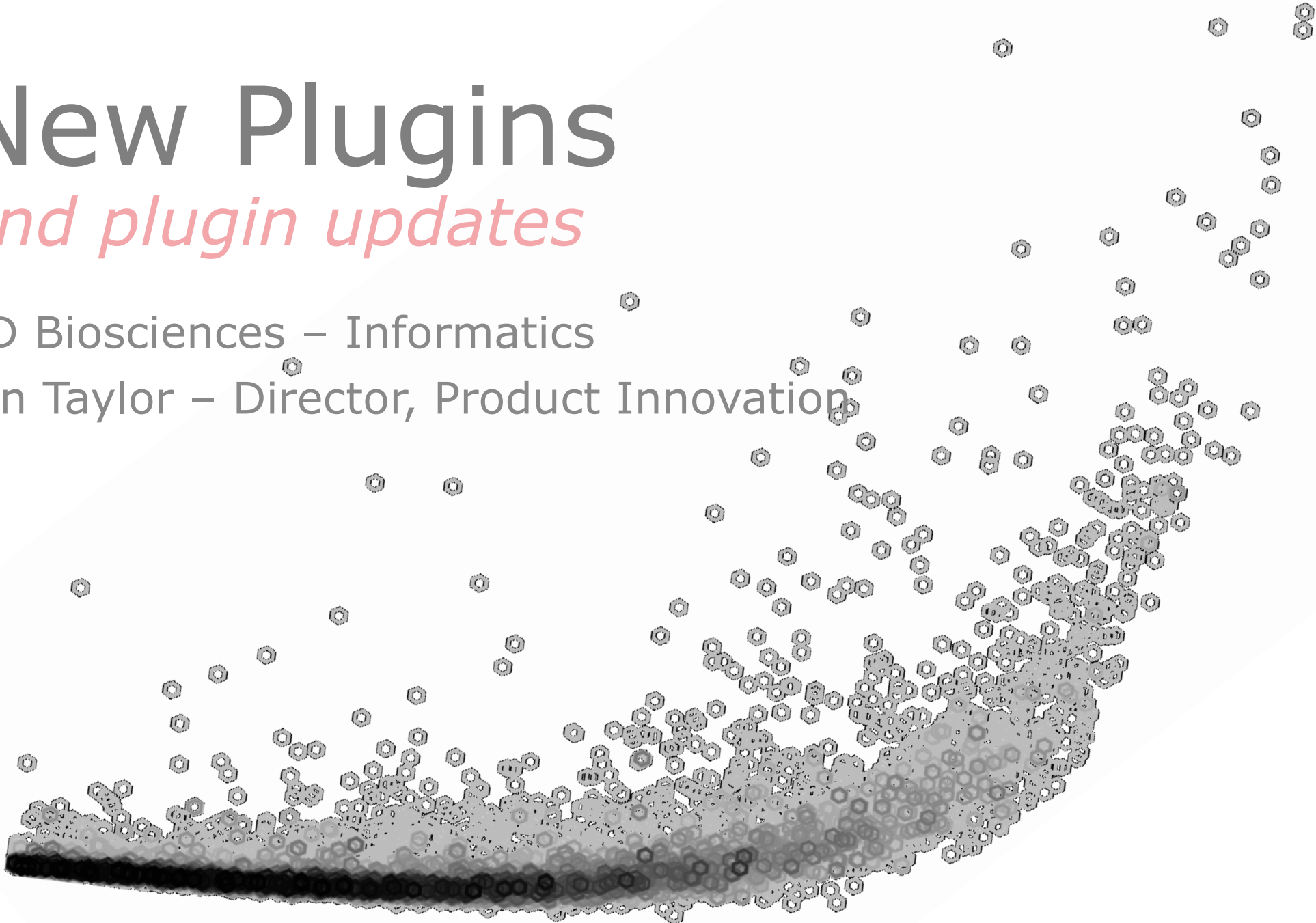


# New Plugins *and plugin updates*

BD Biosciences – Informatics

Ian Taylor – Director, Product Innovation



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# Agenda

## What's New?:

- TriMap
- ViolinBox Updates
- Seurat Updates
- iCellR
- CytoNorm
- BatchLR
- ClusterExplorer Updates
- CBA

Dimensionality Reduction

Visualizations

scRNA Pipeline

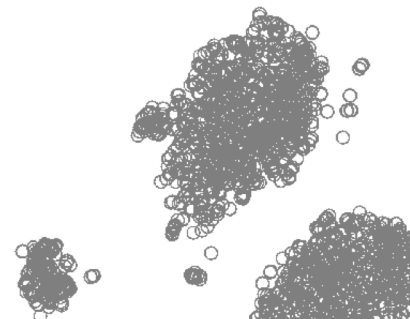
Differential Expression in SeqGeq

Batch Effect Normalization for FlowJo

Batch Effect Correction for SeqGeq

Visualize Clusters in tSNE

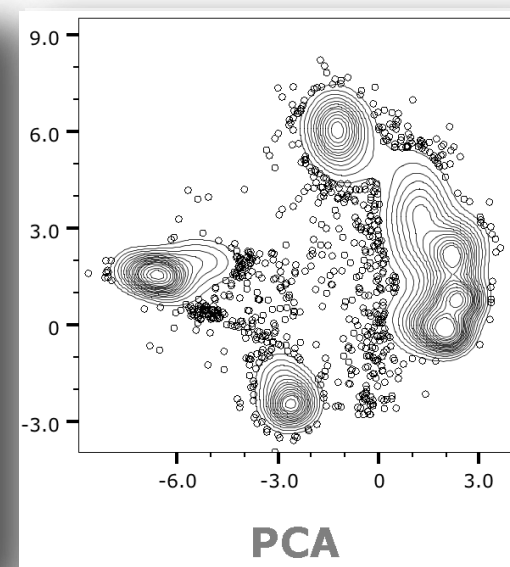
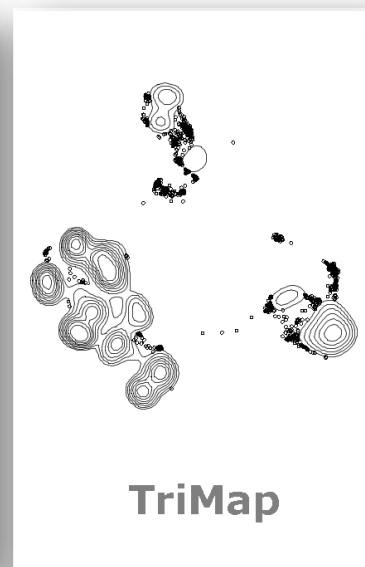
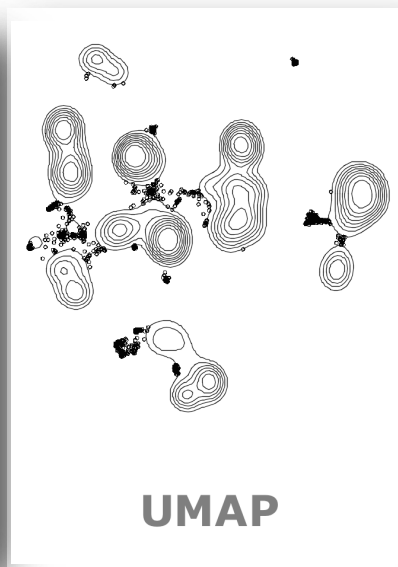
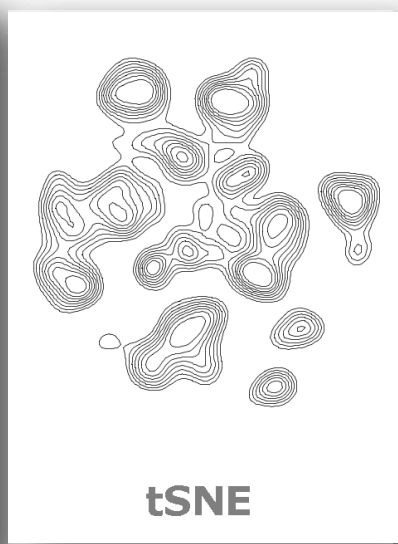
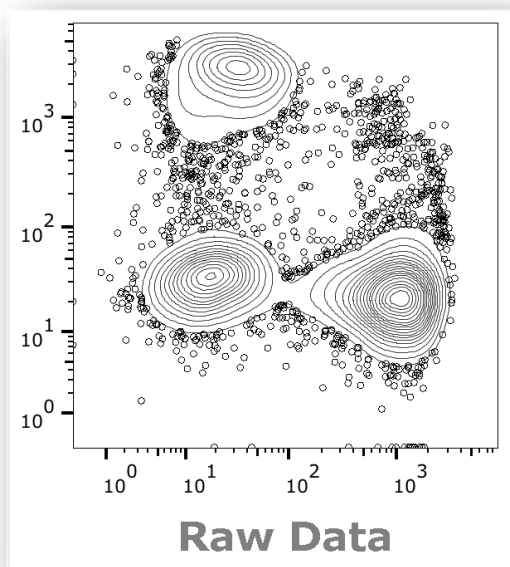
Analyze CBA Kits in FlowJo



# Dimensionality Reduction

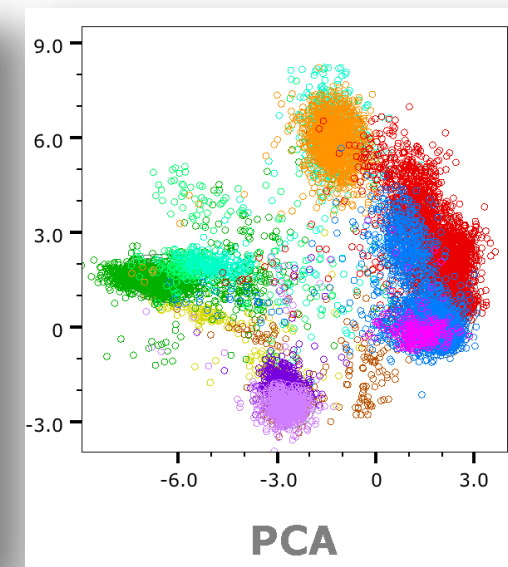
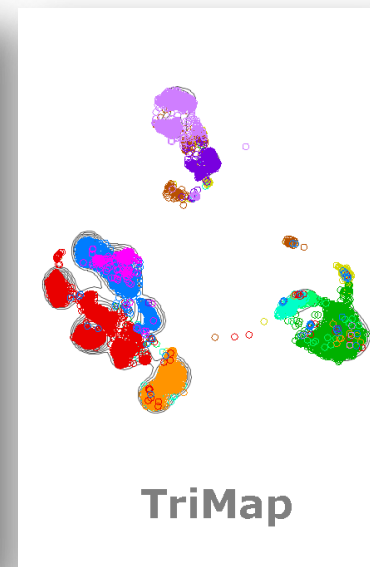
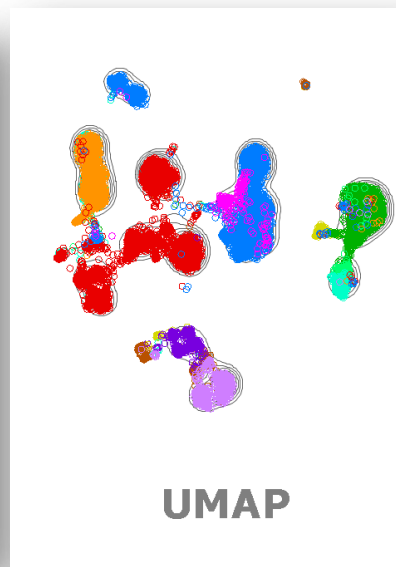
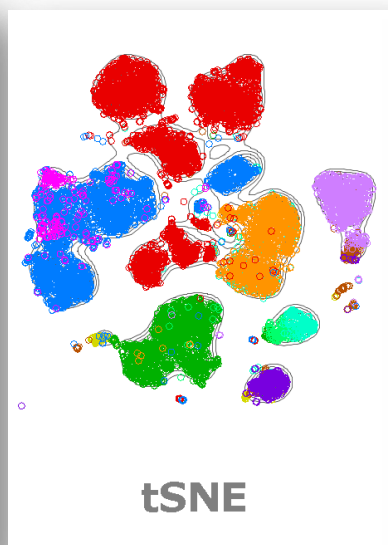
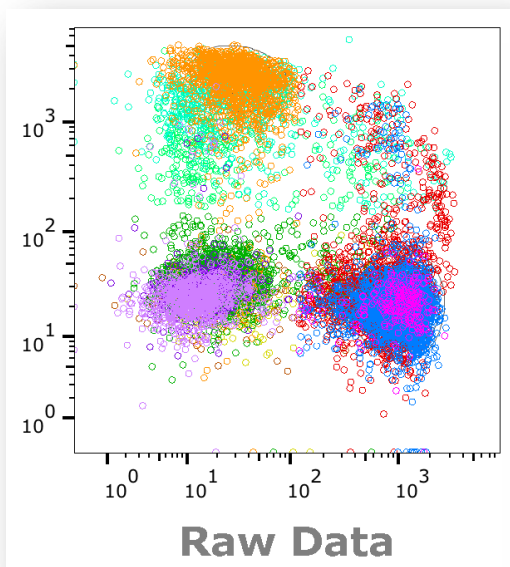


# Dimensionality Reduction

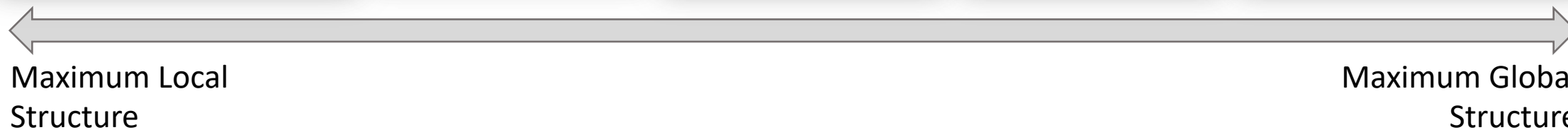




# Dimensionality Reduction



- Bn
- Bm
- Treg
- T4
- T8
- NK
- pMono
- iMono
- cMono
- pDC
- mDC
- Quality Cells



# ViolinBox v2.7

Rain-Cloud /  
Plot Type

Figure  
Orientation

Additional  
Outputs

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ViolinPlots 2.7

Select parameters to be plotted

Collections: All

Parameter Sets

All (428)

Parameters

(Ab)

CD8 (Ab)  
CD56 (Ab)  
CD272 (Ab)  
Tim3 (Ab)  
CD19 (Ab)  
CD185 (Ab)  
CD183 (Ab)  
CD14 (Ab)  
CD33 (Ab)

Select a Catagorical Parameter for Cluster Identification (Optional)

Parameters

Sa  
TPSAB1  
SampleID

☒ Violin ☒ Box-n-Whisker ☒ Jitter

☐ Flip ☐ Overlay ☒ Dark Theme ☒ PDF

☒ Stats Table ☐ Heatmap ☒ Scaled Heatmap

To learn more about RainCloud plots or to site the creator, check out this link:  
[github.com/RainCloudPlots/RainCloudPlots](https://github.com/RainCloudPlots/RainCloudPlots)

For any questions or help using this plugin, reach out:  
[seqgeq@bd.com](mailto:seqgeq@bd.com)

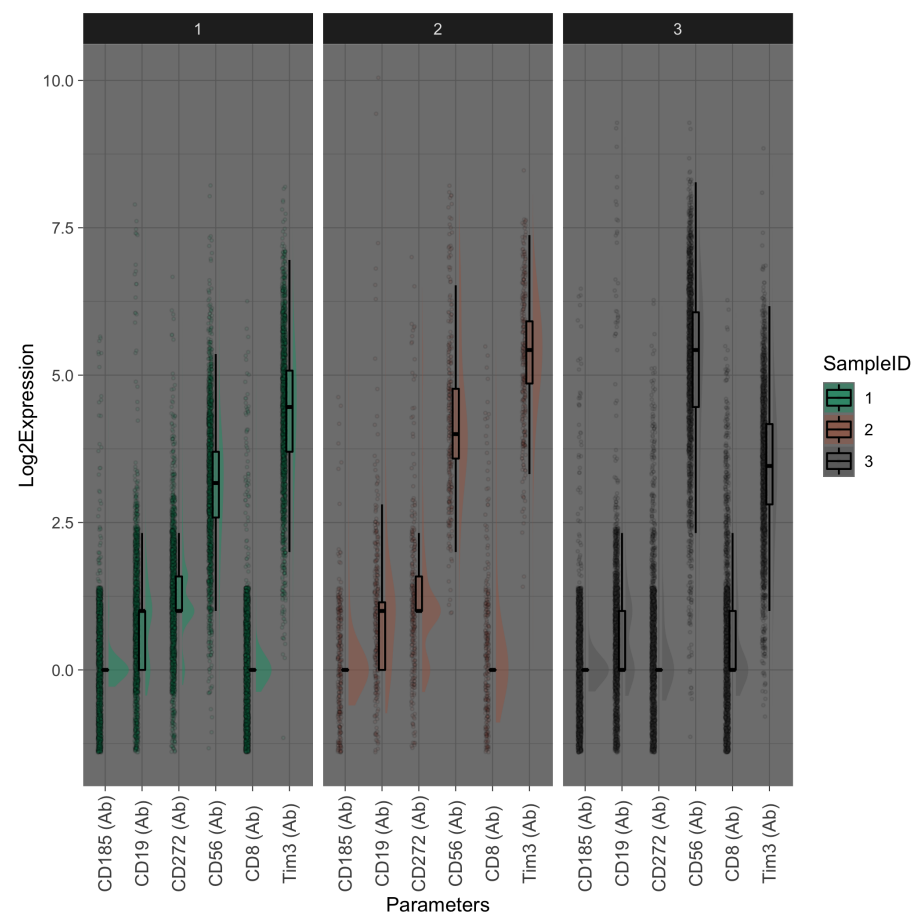
Cancel OK

Choose  
Parameters

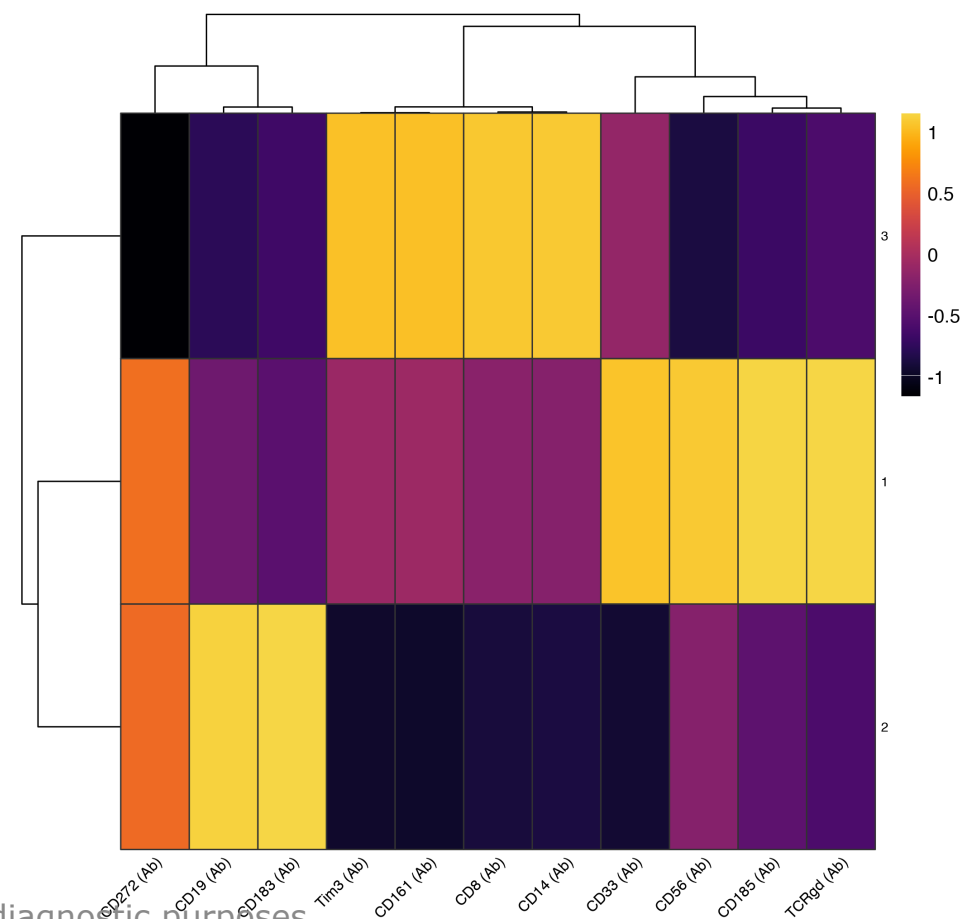
Choose  
"Factor"

Choose  
Output(s)

# Figure Outputs



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# Seurat v3.1

Choose  
Parameters

The screenshot shows the Seurat v2.7 GUI with the following settings:

- Collections:** All
- Parameter Sets:** All (427)
- Parameters:** CPA3, IL2, NCAM1, ADGRG3, DEFA3, CD8 (Ab), IGHG3\_secreted, IL12RB1
- Seurat Plugin:**
  - Basic Seurat Clustering Pipeline.
  - QC Cells based on simple thresholds.
  - Dimensionality Reduction.
  - Graph Based KNN Clustering.
  - Differential Expression analysis.
- QC:** ☐ QC
- % Genes:** 5
- % Cells:** 10
- Library Filter:** ☐ Library Filter
- scTransform data:** ☒ scTransform data
- Log normalize data:** ☐ Log normalize data
- Run tSNE:** ☐ Run tSNE
- Perplexity:** 10
- Run UMAP:** ☒ Run UMAP
- DE Stat Model:** Negative Binomial
- Model:** huPBMC (selected from a dropdown menu showing None, huPBMC, mmLiver, mmLN\_Spleen)

**Citation**

Seurat is developed and maintained by the Satija lab and is released under the GNU Public License (GPL 3.0) [www.cell.com/cell/fulltext/S0092-8674\(19\)30559-8](http://www.cell.com/cell/fulltext/S0092-8674(19)30559-8)

Buttons: Cancel, OK

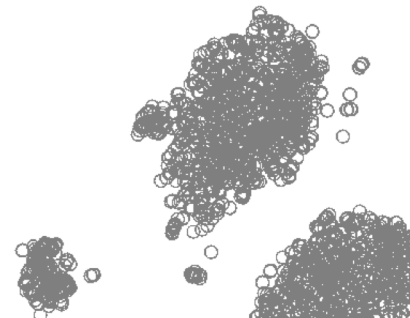
Normalization

DimRedux

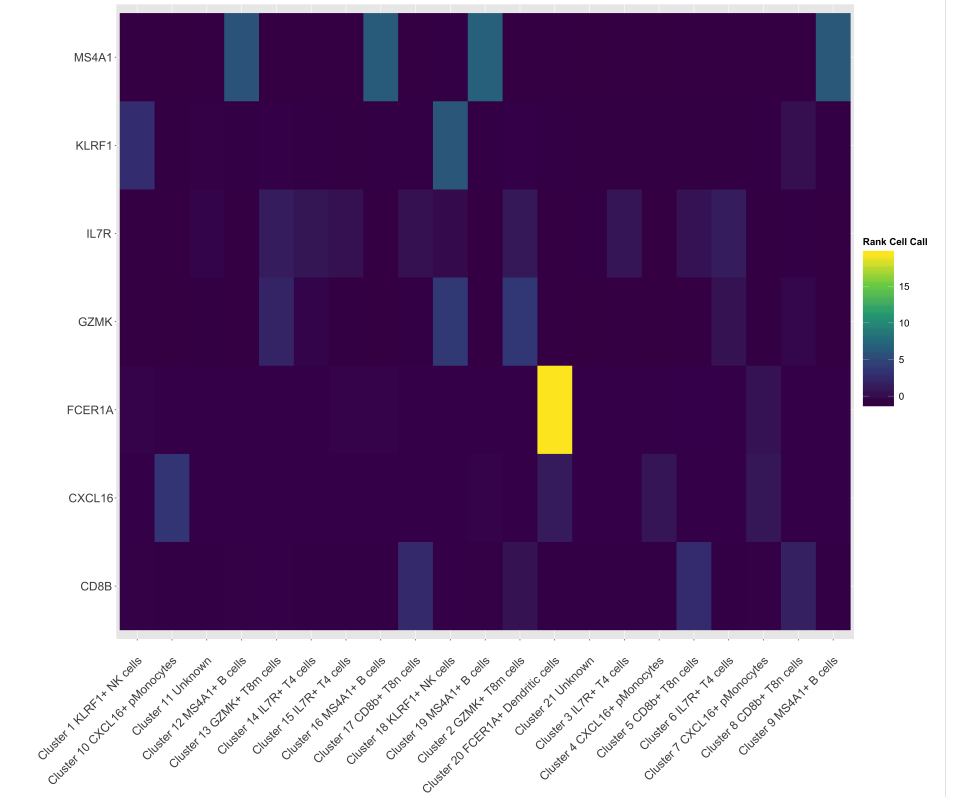
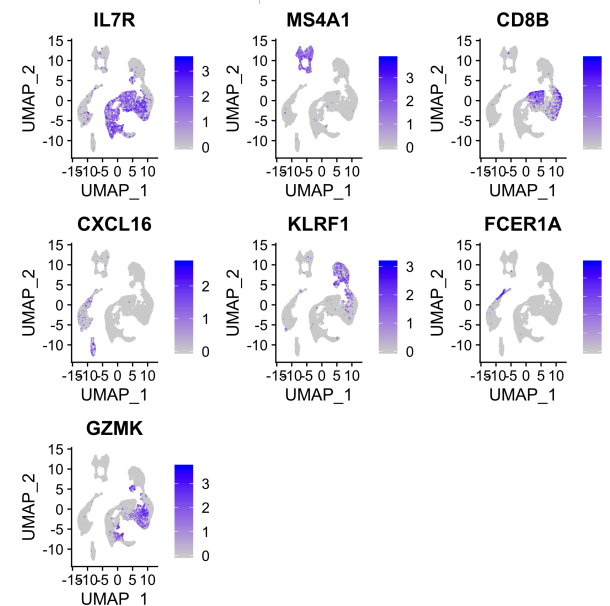
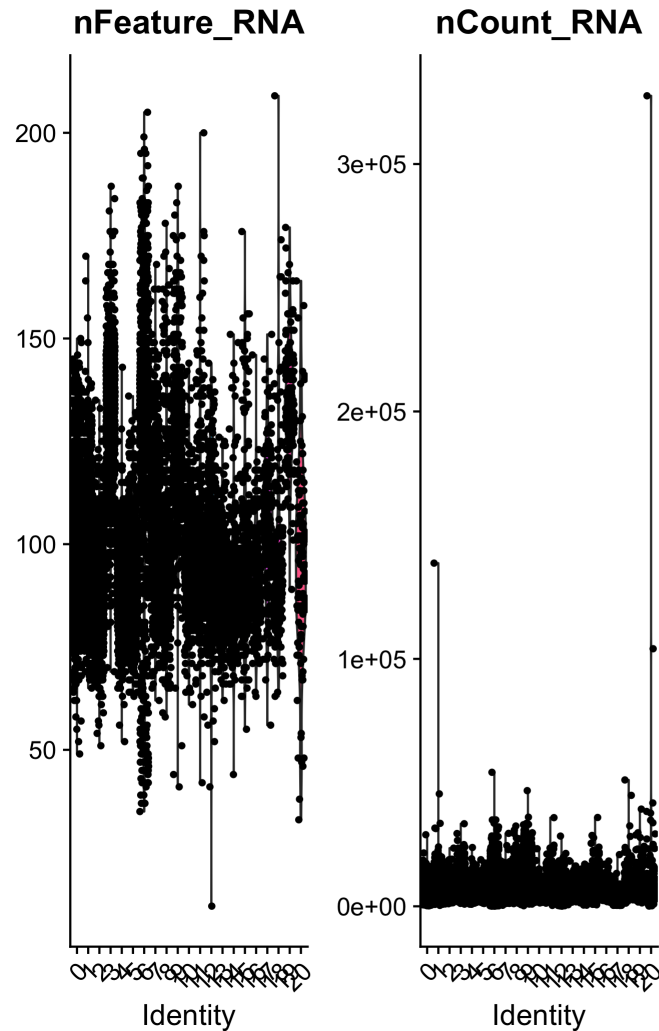
Cell  
Classification

Note: Now utilizing the latest  
Seurat R package (v3.1)

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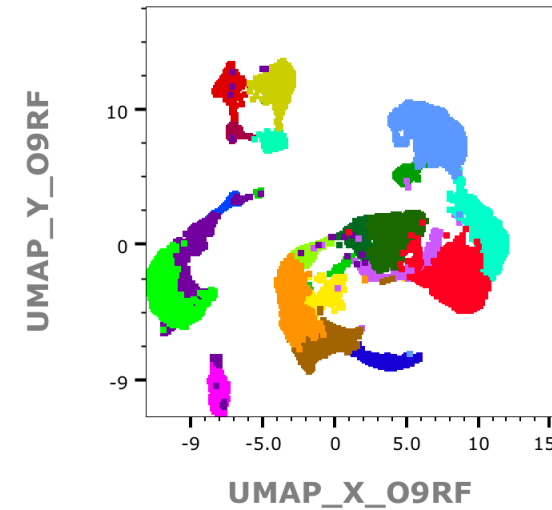
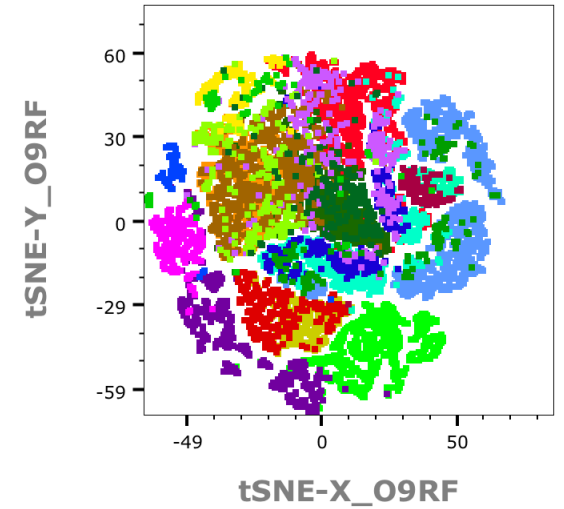
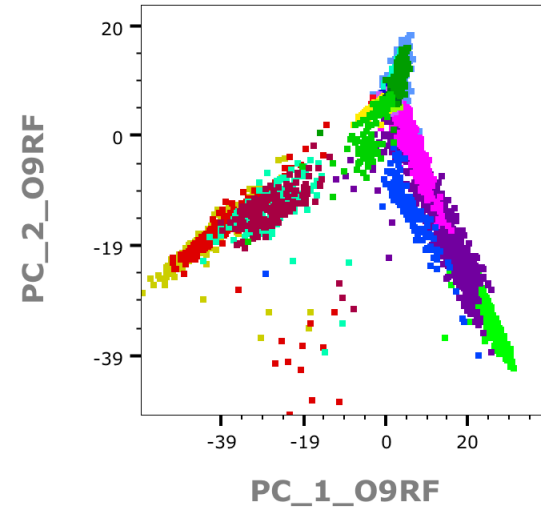
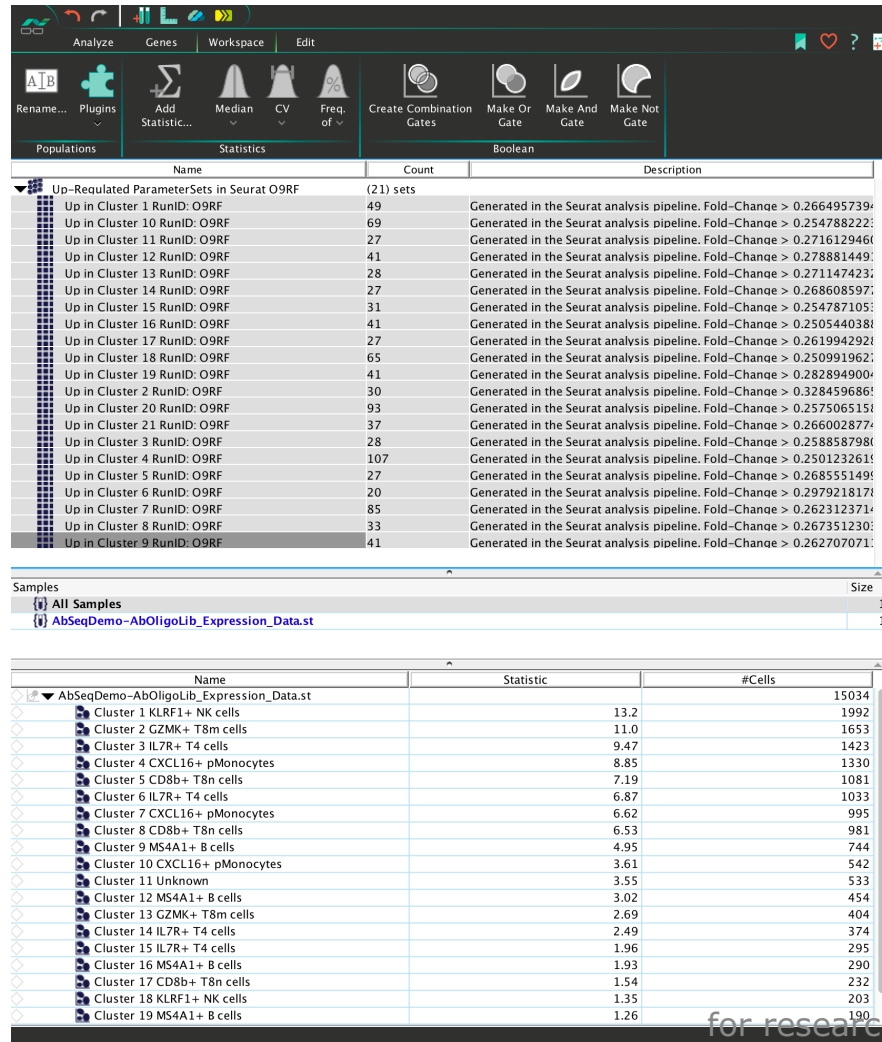


# Summary Graphics



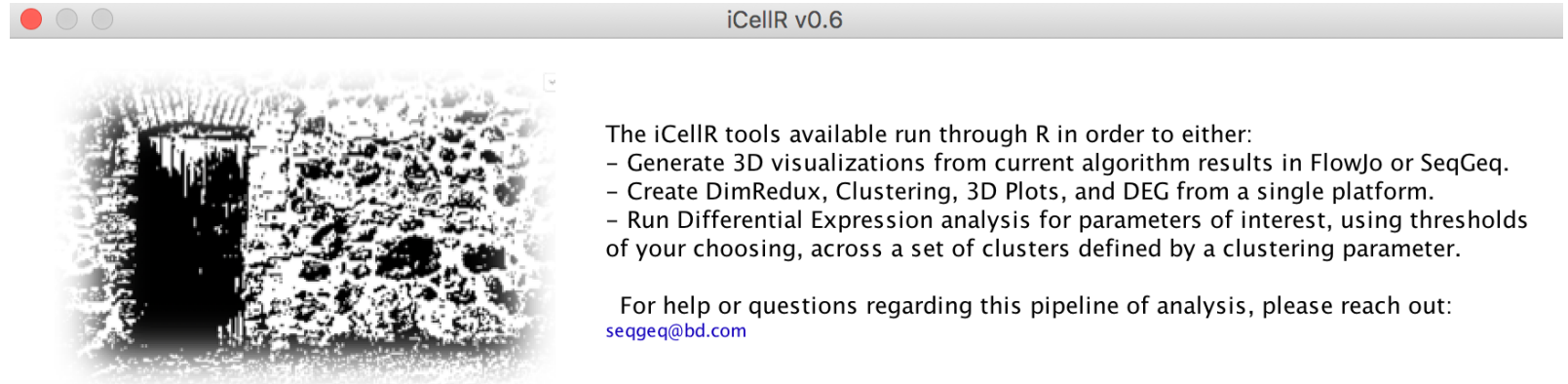
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# Other Outputs



- Cluster 1 KLRF1+ NK cells
- Cluster 2 GZMK+ T8m cells
- Cluster 3 IL7R+ T4 cells
- Cluster 4 CXCL16+ pMonocytes
- Cluster 5 CD8b+ T8n cells
- Cluster 6 IL7R+ T4 cells
- Cluster 7 CXCL16+ pMonocytes
- Cluster 8 CD8b+ T8n cells
- Cluster 9 MS4A1+ B cells
- Cluster 10 CXCL16+ pMonocytes
- Cluster 11 Unknown
- Cluster 12 MS4A1+ B cells
- Cluster 13 GZMK+ T8m cells
- Cluster 14 IL7R+ T4 cells
- Cluster 15 IL7R+ T4 cells
- Cluster 16 MS4A1+ B cells
- Cluster 17 CD8b+ T8n cells
- Cluster 18 KLRF1+ NK cells
- Cluster 19 MS4A1+ B cells
- Cluster 20 FCER1A+ Dendritic cells
- Cluster 21 Unknown

# iCellR v0.6



The **Differential Expression** section of your iCellR will calculate parameters differentially expressed according to a Kruskal-Wallis (KW) h-Test, within a selected categorical parameter corresponding to the clusters of interest (Phenograph, SampleID, etc).

**Select Parameters**  
Choose the set of parameters to consider with regard to differential expression in a set of clusters selected:

Collections:

Parameter Sets

All (482)  
Up in Cluster 1 RunID: O9RF (47 of 49 Valid)  
Up in Cluster 10 RunID: O9RF (69 of 69 Valid)  
Up in Cluster 11 RunID: O9RF (25 of 27 Valid)  
Up in Cluster 12 RunID: O9RF (38 of 41 Valid)  
Up in Cluster 13 RunID: O9RF (27 of 28 Valid)

Parameters

CPA3  
IL2  
NCAM1  
ADCRG3  
DEFA3  
CD8 (Ab)

**Select a Clustering Category**  
Choose a parameter identifying clusters to use for differential expression analysis:

Parameters

seur  
Seurat\_Clusters\_O9RF

**Enter Thresholds for Differential Expression**

Fold-Change:

q-Value:

☐ Save the R script and output messages

To start learning more about the Kruskal-Wallis H Test used to calculate differential expression check out the Laerd Statistical manual page, here:  
[statistics.laerd.com/spss-tutorials/kruskal-wallis-h-test-using-spss-statistics.php](https://statistics.laerd.com/spss-tutorials/kruskal-wallis-h-test-using-spss-statistics.php)

**Differential Expression** **iCellR Pipeline** **3D Plot Only**

This section of the iCellR will allow you to:

- Create 3D plots defined within one of FlowJo's bioinformatics platforms.
- Color dots in the 3D visualization via a quaternary parameter.

**X-Axis**  
Parameters  
umap  
UMAP\_X\_O9RF  
UMAP\_Y\_O9RF

**Y-Axis**  
Parameters  
umap  
UMAP\_X\_O9RF  
UMAP\_Y\_O9RF

**Z-Axis**  
Parameters  
cd19  
CD19 (Ab)  
CD196 (Ab)  
CD197 (Ab)  
CD194 (Ab)

**Color Parameter:** Primary Categorical used for coloring 3D plot.  
Choose one parameter: (Optional)  
Parameters  
seur  
Seurat\_Clusters\_O9RF

**Cell Size Parameter:** Parameter Used to influence the size of cells in 3D plots: (Optional)  
Parameters  
ms  
TYMS  
MS4A1

**Cell Naming Parameter:** Secondary Categorical Used as cell annotation during 3D mouse-over: (Optional)  
Parameters  
CPA3  
IL2  
NCAM1  
ADCRG3

☐ Dark Theme

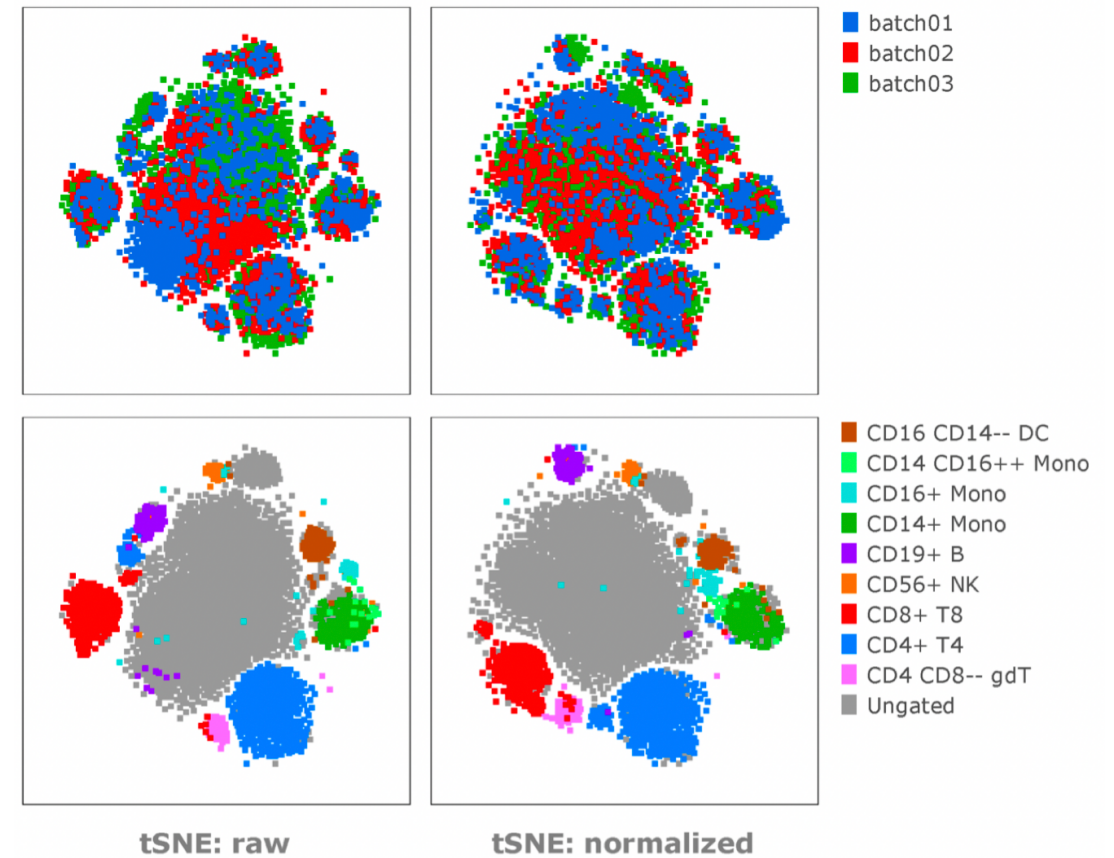
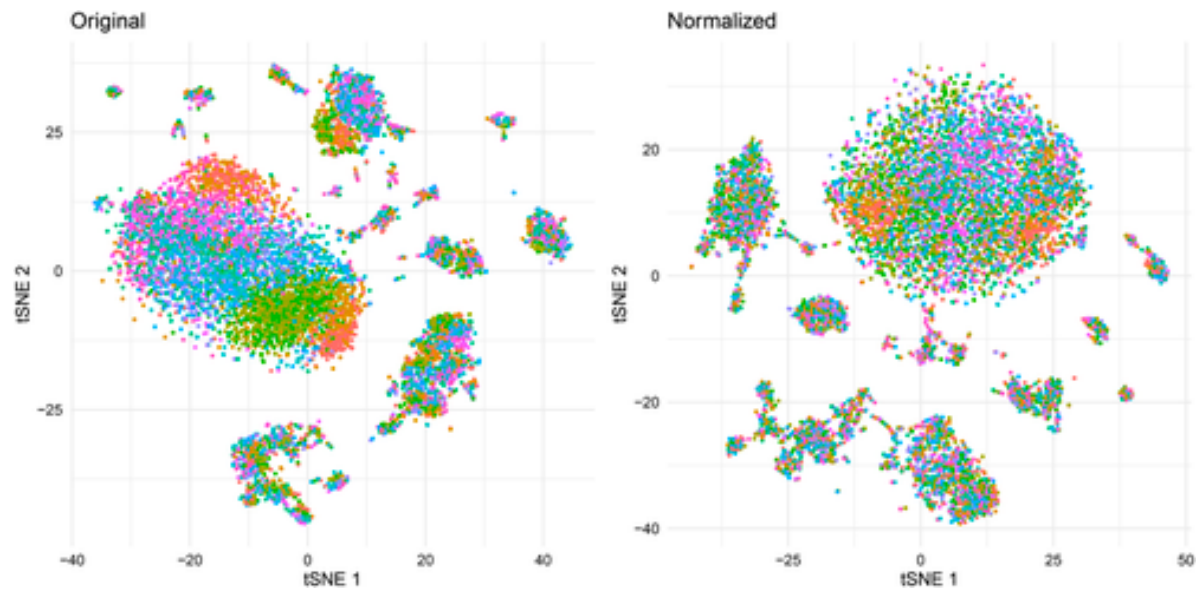
For more information about the tools coming out of BD Life Sciences, explore the Informatics home page, here:  
[docs.flowjo.com/seqgeq](https://docs.flowjo.com/seqgeq)



4

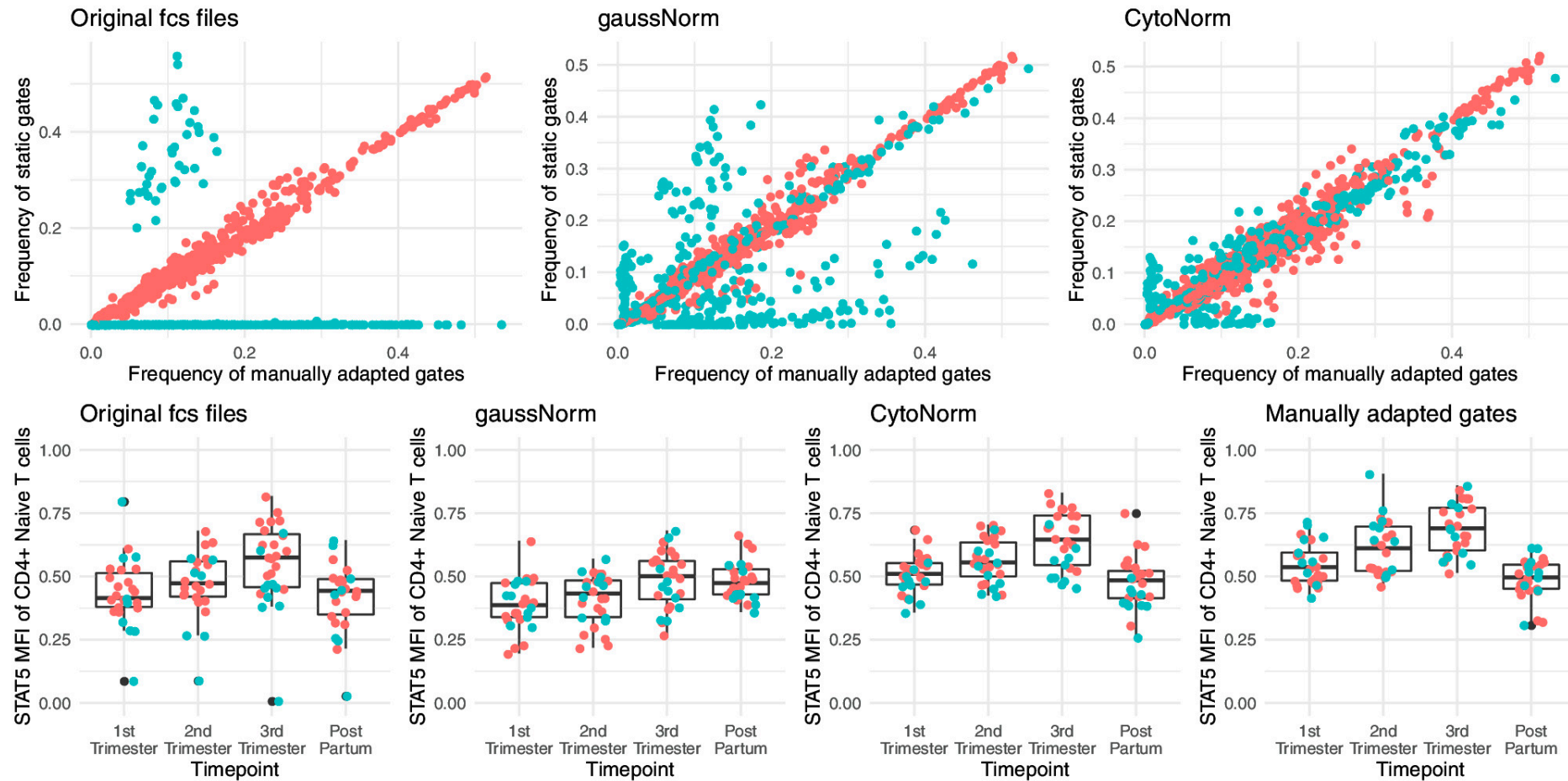


# CytoNorm



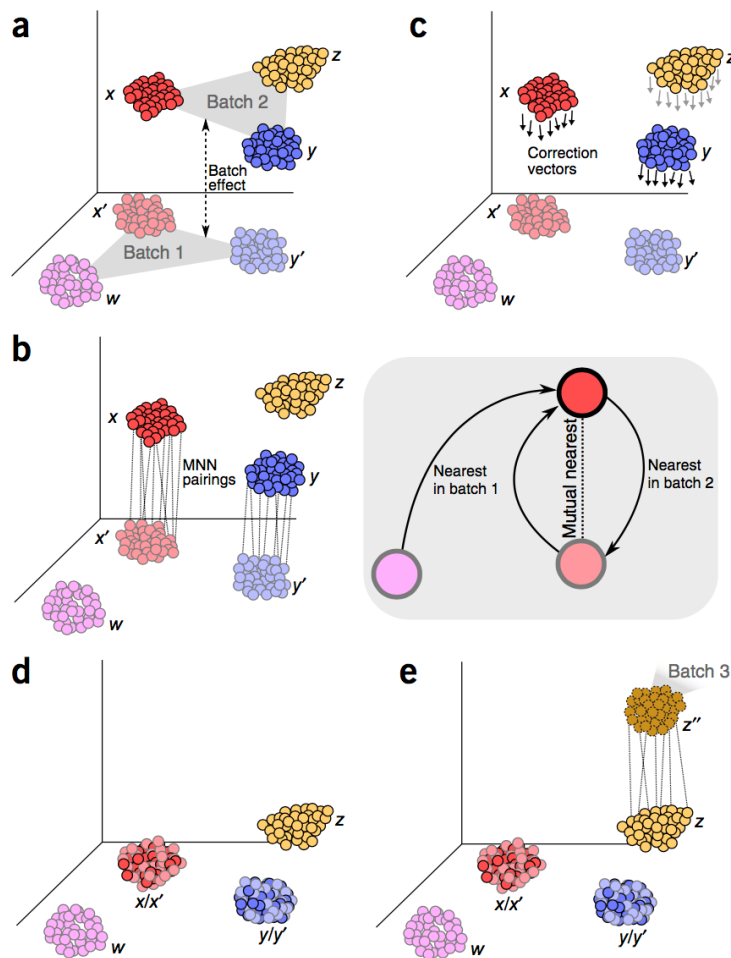
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# Compare



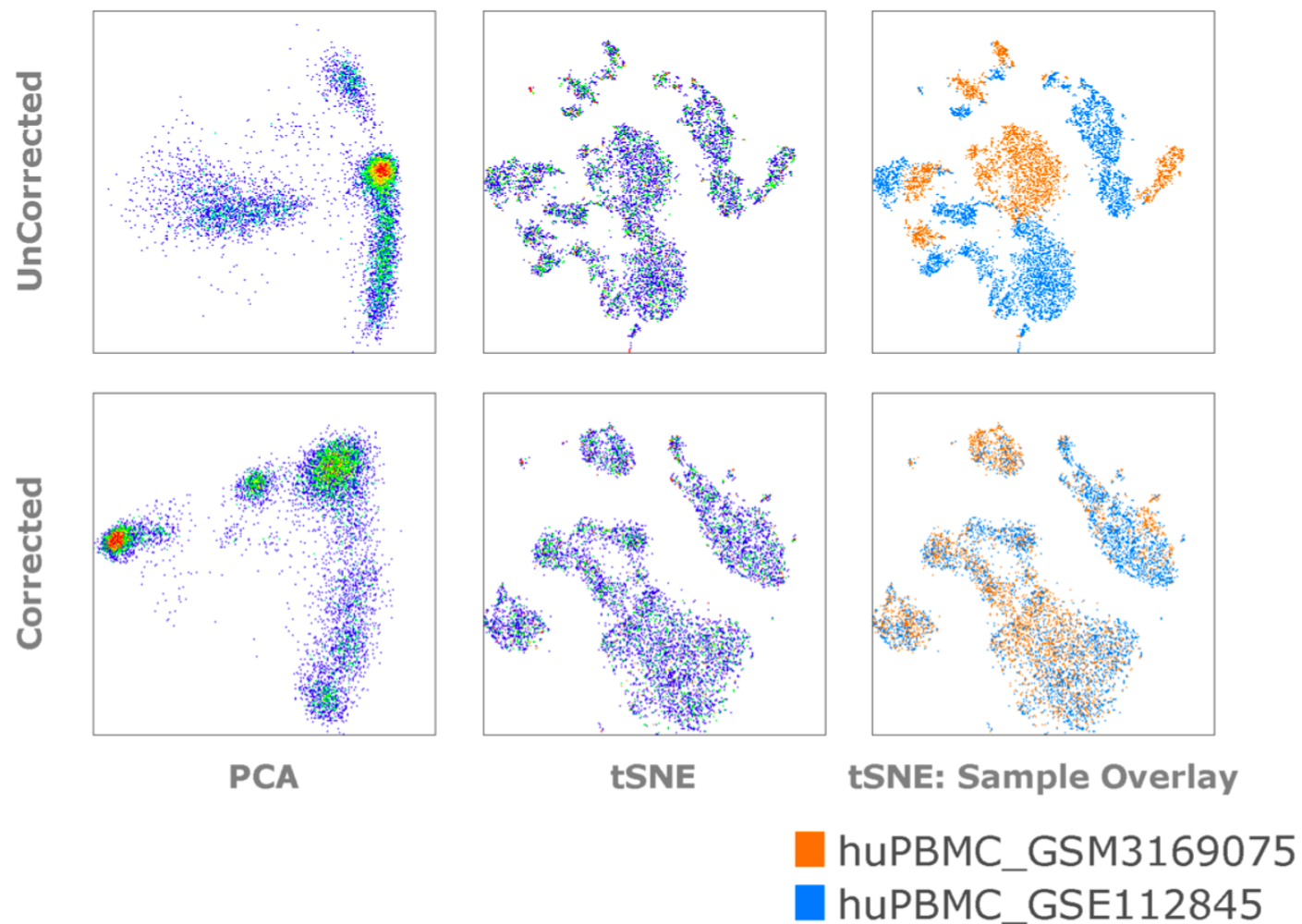
for research use only - not for diagnostic purposes

# BatchLR

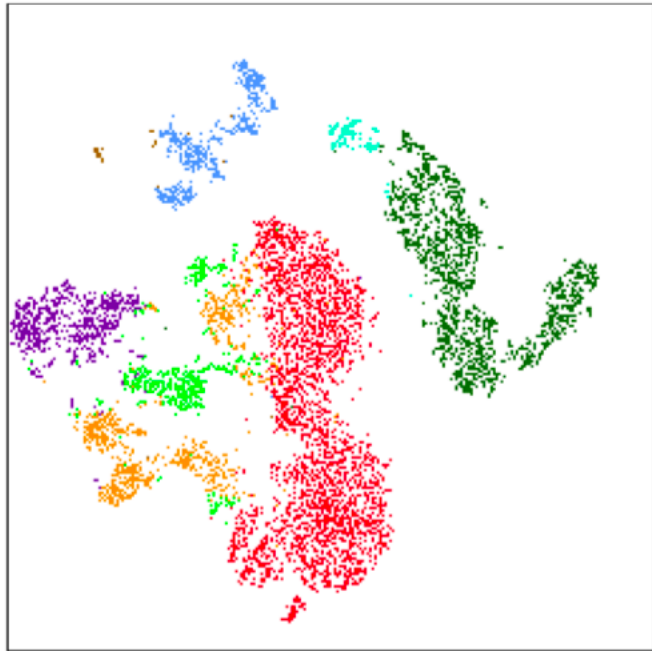


**Figure 1** Schematics of batch-effect correction by MNN. **(a)** Batch 1 and batch 2 in high dimensions with an almost orthogonal batch effect difference between them. **(b)** The algorithm identifies matching cell types by finding MNN pairs of cells (gray box). **(c)** Batch-correction vectors are calculated between the MNN pairs. **(d)** Batch 1 is regarded as the reference, and batch 2 is integrated into it by subtraction of correction vectors. **(e)** The integrated data are considered the reference, and the procedure is repeated for integration of any new batch.

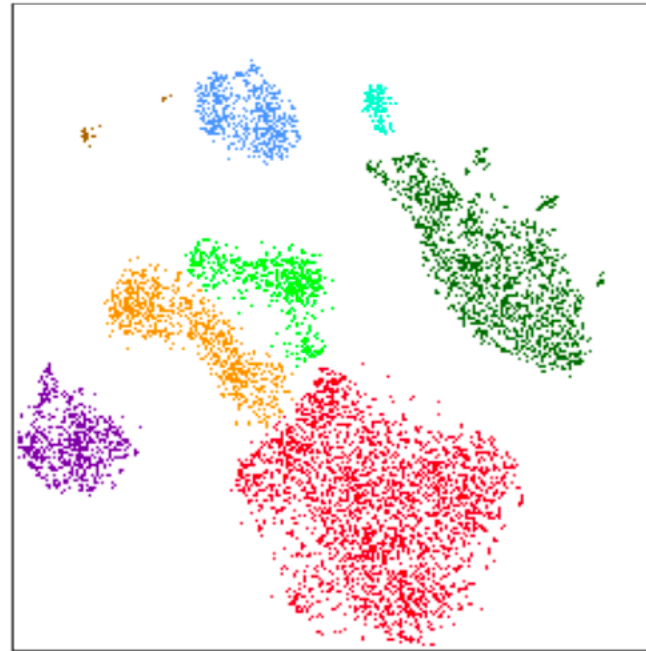
# Comparing PBMCs



# Comparing PBMCs



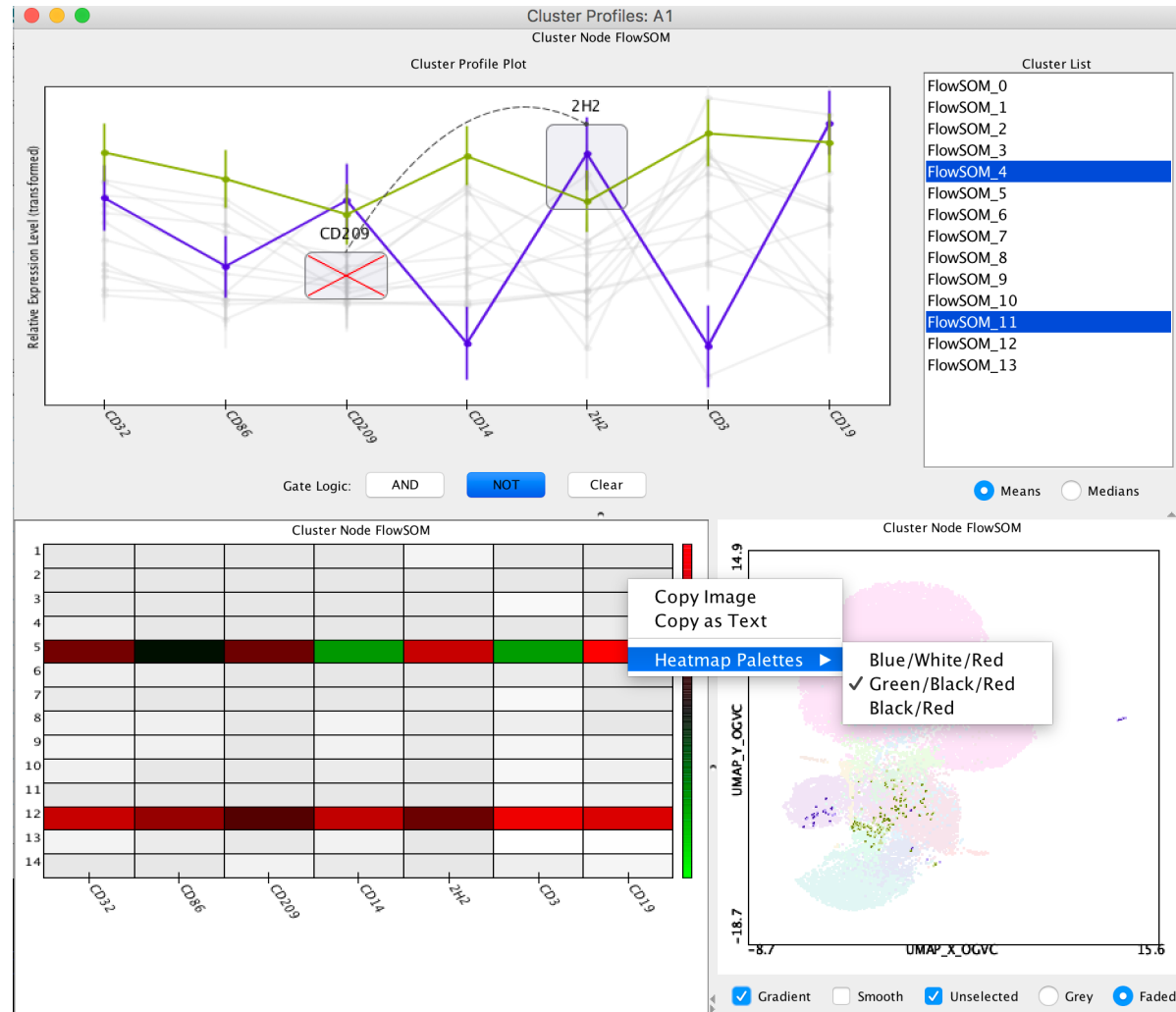
**Raw tSNE**



**MNN tSNE**

- NonClassical MO
- NK Cells
- DCs
- Classical MO
- CD8 Tmem
- CD8 T
- CD4 T Cells
- B Cells

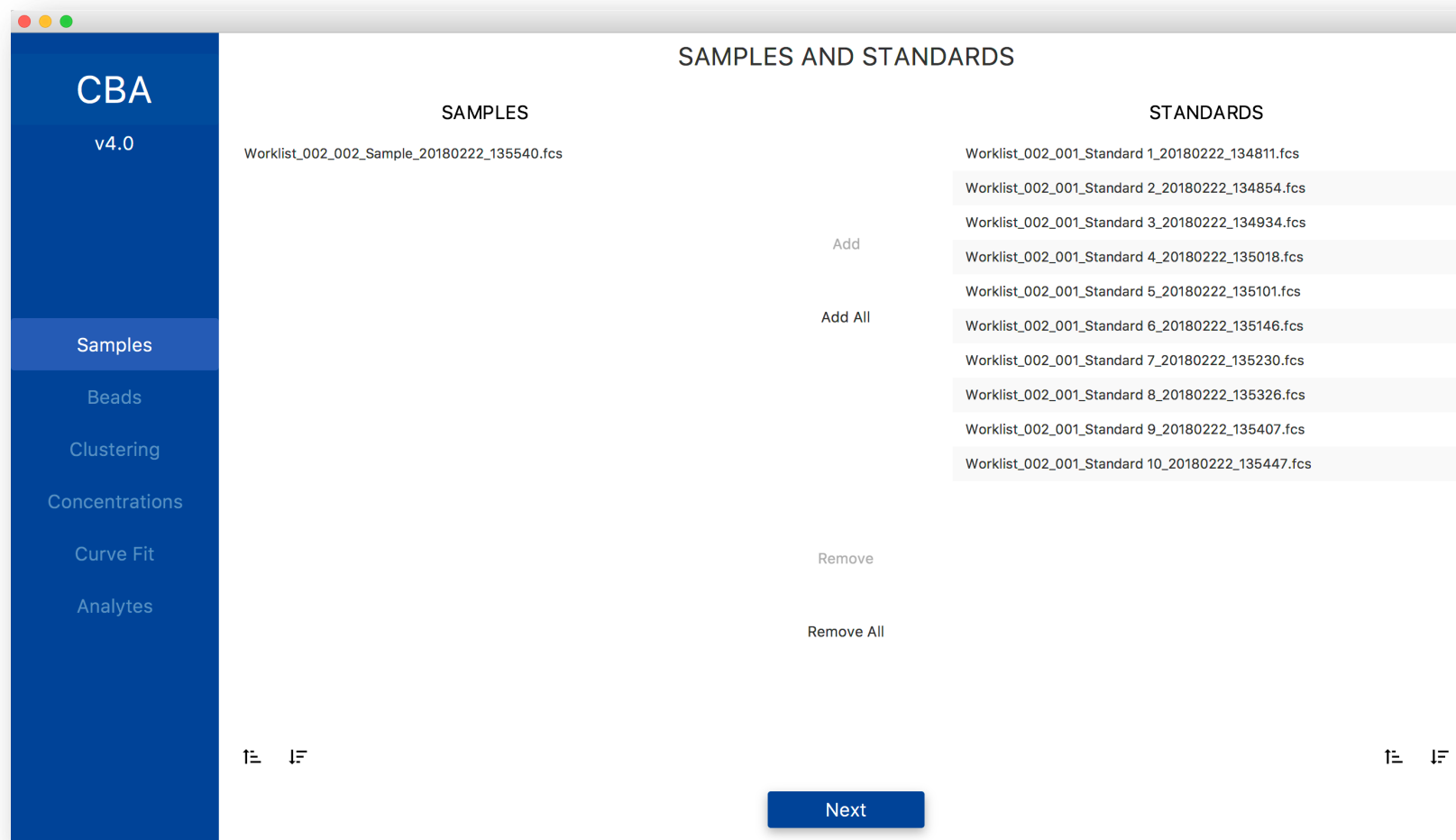
# ClusterExplorer



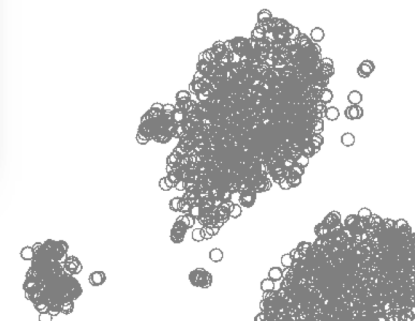
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# CBA



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# Thank You!



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