

## 1. Set up the Seurat Object

The Read10X function reads in the output of the cellranger pipeline from 10X, returning a unique molecular identified count matrix. Next use the count matrix to create a Seurat object.

## 2. Pre-processing

- **QC and selecting cells for further analysis:** PercentageFeatureSet(), removing unwanted cells from the dataset.
- **Normalizing the data** NormalizeData(), a global-scaling normalization method.
- Identification of highly variable features (feature selection): FindVariableFeatures(), will be used in downstream analysis.
- **Scaling the data:** linear transformation: ScaleData(), a standard pre-processing step prior to dimensional reduction techniques like PCA. Shifts the expression of each gene, so the mean expression across cells is 0 and the variance across cells is 1. Gives equal weight in downstream analysis.
- **Perform linear dimensional reduction:** perform PCA on the scaled data: RunPCA(). Only the previously determined variable features are used as input.
- **Determine the dimensionality of the dataset:** JackStraw().

## 3. Cluster the cells

FindNeighbors()---construct KNN graph and refine the edge weights between any 2 cells.  
FindClusters()---apply modularity optimization techniques

## 4. Run non-linear dimensional reduction (UMAP/tSNE)

RunUMAP(), goal of these algorithms is to learn the underlying manifold of the data in order to place similar cells together in low-dimensional space.

## 5. Finding differentially expressed features (cluster biomarkers).

FindMarkers(), find markers that define clusters via differential expression.

## 6. Assigning cell type identity to clusters