Figure 3A:

1. Launch SCTK version 2.8.1
2. Open Data menu option and go to Import Single Cell Data
3. In the “1. Add sample to import:” tab, select “Upload SingleCellExperiment or Seurat object stored in an RDS file” option.
4. Browse the RDS file “tutorial_pbmc3k_qc.rds” and click “Add to sample list”.
5. In the “2. Create dataset:” tab, click “Import” to import the data.
6. Go to “Normalization & Batch Correction” tab, select “decontXcounts” as input, set scaling factor to 10000, check “Scale data after normalization?”, check “Trim data after scale” with values set to 10 and -10, and run.
7. Open “Feature Selection & Dimensionality Reduction” from the top navigation menu.
8. Select the “Feature Selection” tab. In section “1. Compute variability metric”, select “Seurat - vst” as method and “decontXcounts” as assay. Click “Run” to compute the metrics. In section “2. Select number of variable features”, in “Select variability metric” select “vst - decontXcounts”, set “Select number of highly variable features:” to 2000 and press “Run”.
9. Select the “Dimensionality Reduction” tab. Set “Select Input Matrix:” to “SeuratLogNormalize”, “Select method:” to “Seurat - PCA”, “Select HVG list:” to “HVG_vst2000”, uncheck “Scale” option, set “Number of dimensions:” to 50, uncheck all plot options, set seed value to 42 and Run.
10. Go to “Embedding” tab within “Feature Selection & Dimensionality Reduction”. Set “Select Input Matrix:” to “SeuratLogNormalize_seuratPCA”, “Select method:” to “seuratUMAP”, “reducedDimName” to “seuratUMAP”, “Number of dimensions to use:” to 10, “Set min.dist:” to 0.3, “Set n.neighbors:” to 30, “Set spread:” to 1, “Seed value for reproducibility of result:” to 42.
11. Go to “Clustering” tab in the navigation menu and run with “Select Algorithm” to “louvain”, “Select Input Matrix:” to “seuratUMAP”, “K value” to 8, “Number of components” to 10, “Edge weight type” to “rank”, “Name of Clustering Result:” to “scran_snn_cluster”.

Figure 3B:

1. Launch SCTK version 2.8.1
2. Open Data menu option and go to Import Single Cell Data
3. In the “1. Add sample to import:” tab, select “Upload SingleCellExperiment or Seurat object stored in an RDS file” option.
4. Browse the RDS file “tutorial_pbmc3k_qc.rds” and click “Add to sample list”.
5. In the “2. Create dataset:” tab, click “Import” to import the data.
6. Go to the “Curated Workflows” option in the menu and select “Seurat”.
7. Run the “Normalize Data” tab with “Select assay:” set to “decontXCounts”, “Select normalization method:” to “LogNormalize, “Set scaling factor: ” to 10000.
8. Run the “Highly Variable Genes” tab with “Select HVG method:” to “vst” and “Select number of features to find:” to 2000.

9. Run the “PCA” in the “Dimensionality Reduction” tab with “Select number of components to compute:” to 50 and “Seed value:” to 42. Once computed, set “Select number of components for downstream analysis:” to 10.
10. In the “tSNE/UMAP” tab, select “UMAP” and run with “Select reduction method:” to “pca”, “Set min.dist:” to 0.3, “Set n.neighbors:” to 30, “Set spread:” to 1, “Seed value for reproducibility of result:” to 42.
11. In the “Clustering” tab, run with “Select reduction method:” to “pca”, “Select clustering algorithm:” to “Original Louvain algorithm”, “Set resolution:” to 0.8 and “Group singletons?” to TRUE.
12. In the “Find Markers” tab, set “Select type of markers to identify:” to “markers between all groups”, “Select biological phenotype:” to “Seurat_louvain_Resolution0.8”, “Select test:” to “wilcox” and “Only return positive markers?” to FALSE.
13. Once markers are computed, on the right side “Marker Genes” table, add filters with “extremes greater than 60 and less than 30” and “p_val_adj <= 1e-59”. Select markers “CD3E” and “CD79A”, and open “Feature Plot”.