

Seurat v5: Cheat Sheet

Adapted from: https://satijalab.org/seurat/articles/seurat5_essential_commands
https://satijalab.org/seurat/articles/essential_commands.html

Load libraries and set working directory

```
library(Seurat)
setwd("/path to working directory/")
```

Create Seurat object and save

```
data.counts ← Read10X("/path to filtered_feature_bc_matrix/")
data ← CreateSeuratObject(counts = data.counts)
saveRDS(data, "data.rds")
```

Cell, feature, and layer names

# Cell names colnames(data)	# Number of cells and features num_cells ← ncol(data) num_features ← nrow(data)
# Feature names rownames(data)	# List of object layers Layers(data)

Identity class labels

# View current ident Idents(data)	# Set ident Idents(data) ← "ex: seurat_annotations"
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Subset and split Seurat objects

# Subset based on identity class subset(x = data, idents = c("CD8A", "CD3E"))	# Subset based on expression level subset(x = data, subset = CD8A > 2.5)	# Split based on metadata condition sample_list ← SplitObject(data, split.by = "condition")
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Metadata

Retrieve specific values from the metadata

```
data$nCount_RNA
data[[c("percent.mito", "nFeature_RNA")]]
```

Add metadata

```
random_group_labels ← sample(x=c("g1", "g2"), size = ncol(x=data), replace = T)
data$groups ← random_group_labels
```

Visualization

Dimensional reduction plot

```
DimPlot(object = data, reduction = "umap") # can also plot as pca
DimPlot(object = data, reduction = "umap", split.by = "groups") # split by group
```

Dimensional reduction plot with cells colored by quantitative feature

```
FeaturePlot(object = data, features = c("CD8A", "CD3E"))
```

Violin and Ridge plots to look at expression of genes of interest

```
VlnPlot(object = data, features = c("CD8A", "CD3E"))
RidgePlot(object = data, features = c("CD8A", "CD3E"))
```