

```
install.packages("Seurat")
```

```
library(Seurat)
```

```
library(dplyr)
```

```
SR_hs00.data <- Read10X(data.dir = "data/SR_hs00/")
```

```
SR_hs60.data <- Read10X(data.dir = "data/SR_hs60/")
```

```
SR_hs00.seurat <- CreateSeuratObject(counts = SR_hs00.data, project = "SR_hs")
```

```
SR_hs00.seurat$stim <- "hs00"
```

```
SR_hs60.seurat <- CreateSeuratObject(counts = SR_hs60.data, project = "SR_hs")
```

```
SR_hs60.seurat$stim <- "hs60"
```

```
heat_shock <- merge(SR_hs00.seurat, y = SR_hs60.seurat, add.cell.ids =  
c("hs00", "hs60"), project = "heat_shock")
```

```
heat_shock[["percent.mt"]] <- PercentageFeatureSet(heat_shock, pattern = "^MT-")
```

```
VlnPlot(heat_shock, features = c("nFeature_RNA", "nCount_RNA",  
"percent.mt"), ncol = 3)
```

```
heat_shock <- subset(heat_shock, subset = nFeature_RNA > 0 & nFeature_RNA  
< 12000 & percent.mt < 10)
```

```
heat_shock <- NormalizeData(heat_shock, normalization.method =  
"LogNormalize", scale.factor = 10000)
```

```
heat_shock <- FindVariableFeatures(heat_shock, selection.method = "vst",  
nfeatures = 12000)
```

```
top10 <- head(VariableFeatures(heat_shock), 10)
```

```
plot1 <- VariableFeaturePlot(heat_shock)
```

```
plot2 <- LabelPoints(plot = plot1, points = top10, repel = TRUE)
```

```
plot2
```

```
all.genes <- rownames(heat_shock)
```

```
heat_shock <- ScaleData(heat_shock, features = all.genes)
```

```
heat_shock <- RunPCA(heat_shock, features = VariableFeatures(object =  
heat_shock))
```

```
print(heat_shock[["pca"]], dims = 1:10, nfeatures = 10)
```

```
DimPlot(heat_shock, dims = 1:2, reduction = "pca", group.by = "stim",  
cols = c("blue", "red"))
```

```
ElbowPlot(heat_shock)
```

```
heat_shock <- FindNeighbors(heat_shock, dims = 1:6)
```

```
heat_shock <- FindClusters(heat_shock, resolution = 0.5)
```

```
heat_shock <- RunUMAP(heat_shock, dims = 1:6)
```

```
DimPlot(heat_shock, reduction = "umap")
```

```
DimPlot(heat_shock, reduction = "umap", group.by = "stim", cols =  
c("blue", "red"))
```

```
heat_shock <- JoinLayers(heat_shock)
```

```
cluster1.0.markers <- FindMarkers(heat_shock, ident.1 = 1)  
head(cluster1.0.markers, n = 10)
```

```
cluster3.0.markers <- FindMarkers(heat_shock, ident.1 = 3)  
head(cluster3.0.markers, n = 10)
```

```
FeaturePlot(heat_shock, features = c("ACTB", "GAPDH"))
```

```
FeaturePlot(heat_shock, features = c("NSD3", "MAN1A2", "AKAP9",  
"TNPO3", "RTF1", "PLEKHB2", "SARNP", "TNRC6B", "ELF2", "CTBP2"))
```

```
FeaturePlot(heat_shock, features = c("HSPA1A", "HSPA1B"))
```

```
FeaturePlot(heat_shock, features = c("FOSL1", "ID1"))
```

```
heat_shock.markers <- FindAllMarkers(heat_shock, only.pos = TRUE)
```

```
heat_shock.markers %>%
```

```
  group_by(cluster) %>%
```

```
  dplyr::filter(avg_log2FC > 1)
```

```
heat_shock.markers %>%
```

```
  group_by(cluster) %>%
```

```
  dplyr::filter(avg_log2FC > 1) %>%
```

```
  slice_head(n = 10) %>%
```

```
  ungroup() -> top10
```

```
DoHeatmap(heat_shock, features = top10$gene)
```