

Application of VAM to Seurat pbmc_small scRNA-seq data using Seurat SCTransform normalization.

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1 Load the VAM package

```
> library(VAM)
```

2 Summary statistics for the pbmc_small scRNA-seq data

This example uses the pbmc_small data set included in the SeuratObject package and a single contrived gene set. Please see the other vignettes for more realistic examples using larger scRNA-seq data sets and gene set collections based on MSigDB.

```
> if (requireNamespace("Seurat", quietly=TRUE)) {  
+   SeuratObject::pbmc_small  
+   gene.names = rownames(SeuratObject::pbmc_small)  
+   gene.names[1:5]  
+ } else {  
+   message("Seurat package not available! Not executing associated vignette content.")  
+ }
```

```
[1] "MS4A1" "CD79B" "CD79A" "HLA-DRA" "TCL1A"
```

3 Apply SCTransform normalization to the data

```
> if (requireNamespace("Seurat", quietly=TRUE)) {  
+   pbmc_sctransform = Seurat::SCTransform(SeuratObject::pbmc_small, verbose=F)  
+   # Compute PCA and UMAP on the normalized values  
+   pbmc_sctransform = Seurat::RunPCA(pbmc_sctransform, npcs=10)  
+   pbmc_sctransform = Seurat::RunUMAP(pbmc_sctransform, dims = 1:10)  
+   Seurat::VariableFeatures(pbmc_sctransform)[1:5]  
+ } else {  
+   message("Seurat package not available! Not executing associated vignette content.")  
+ }
```

```
[1] "PPBP" "NKG7" "GNLY" "PF4" "LYZ"
```

4 Define gene set collection

A gene set collection containing just a single contrived set (containing the top 5 variable genes) will be used for this example.

```

> if (requireNamespace("Seurat", quietly=TRUE)) {
+   gene.set.name = "Test"
+   gene.ids = c("NKG7", "PPBP", "GNLY", "PF4", "GNG11")
+   # Create a collection list for this gene set
+   gene.set.id.list = list()
+   gene.set.id.list[[1]] = gene.ids
+   names(gene.set.id.list)[1] = gene.set.name
+   gene.set.id.list
+   # Create the list of gene indices required by vamForSeurat()
+   (gene.set.collection = createGeneSetCollection(gene.ids=gene.names,
+   gene.set.collection=gene.set.id.list))
+   gene.indices = gene.set.collection[[1]]
+   (gene.names = gene.names[gene.indices])
+ } else {
+   message("Seurat package not available! Not executing associated vignette content.")
+ }

[1] "NKG7" "PPBP" "GNLY" "PF4" "GNG11"

```

5 Execute VAM method

Since the scRNA-seq data has been processed using Seurat, we execute VAM using the `vamForSeurat()` function. We have set `return.dist=T` so that the squared adjusted Mahalanobis distances will be returned in a "VAMdist" Assay.

```

> if (requireNamespace("Seurat", quietly=TRUE)) {
+   pbmc.vam = vamForSeurat(seurat.data=pbmc_sctransform,
+   gene.set.collection=gene.set.collection,
+   center=F, gamma=T, sample.cov=F, return.dist=T)
+ } else {
+   message("Seurat package not available! Not executing associated vignette content.")
+ }

```

Look at the first few entries in the "VAMdist" and "VAMcdf" Assays.

```

> if (requireNamespace("Seurat", quietly=TRUE)) {
+   pbmc.vam@assays$VAMdist@data[1,1:10]
+   pbmc.vam@assays$VAMcdf@data[1,1:10]
+ } else {
+   message("Seurat package not available! Not executing associated vignette content.")
+ }

```

```

ATGCCAGAACGACT CATGGCCTGTGCAT GAACCTGATGAACC TGACTGGATTCTCA AGTCAGACTGCACA
  0.0000000    0.8264096    0.3941335    0.4525661    0.1149294
TCTGATACACGTGT TGGTATCTAAACAG GCAGCTCTGTTTCT GATATAACACGCAT AATGTTGACAGTCA
  0.2308289    0.0000000    0.0000000    0.0000000    0.6322672

```

6 Visualize VAM scores

Visualize VAM scores using `Seurat FeaturePlot()`. The default Assay must first be changed to "VAMcdf".

```

> if (requireNamespace("Seurat", quietly=TRUE)) {
+   Seurat::DefaultAssay(object = pbmc.vam) = "VAMcdf"
+   Seurat::FeaturePlot(pbmc.vam, reduction="tsne", features=gene.set.name)
+ } else {
+   message("Seurat package not available! Not executing associated vignette content.")
+   par(mar = c(0,0,0,0))
+   plot(c(0, 1), c(0, 1), ann = F, bty = 'n', type = 'n', xaxt = 'n', yaxt = 'n')
+   text(x = 0.5, y = 0.5, paste("Seurat package not available!\n",
+                                 "FeaturePlot not generated."),
+        cex = 1.6, col = "black")
+ }

```

