

# R Notebook

Code ▼

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downloading and loading packages:

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```
install.packages('patchwork')
```

```
Error in install.packages : Updating loaded packages
```

Step 1: loading the TSV file

	cell_091.133 <int>	cell_177.113 <int>	cell_289.088 <int>	cell_205.268 <int>	cell_162.063 <int>	cell_183.039 <int>
A1BG	0	0	0	0	0	0
A1CF	0	0	0	1	0	0
A2M	0	0	0	0	0	0
A2ML1	0	0	0	0	0	0
A3GALT2	0	0	0	0	0	0
A4GALT	0	0	0	0	0	0

6 rows | 1-7 of 2000 columns

Step 2: creating Seurat object

project is titled 'pdac1'. the Seurat object created here retains genes which are expressed in at least 3 cells, and it retains cells that express at least 200 features (genes)

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```
pdac1 <- CreateSeuratObject(counts = data, project = "pdac1", min.cells = 3, min.features = 200)
pdac1
```

```
An object of class Seurat
13248 features across 633 samples within 1 assay
Active assay: RNA (13248 features, 0 variable features)
```

step 3: label mitochondrial genes

This code creates a column named "percent.mt" and isolates all the mitochondrial expression

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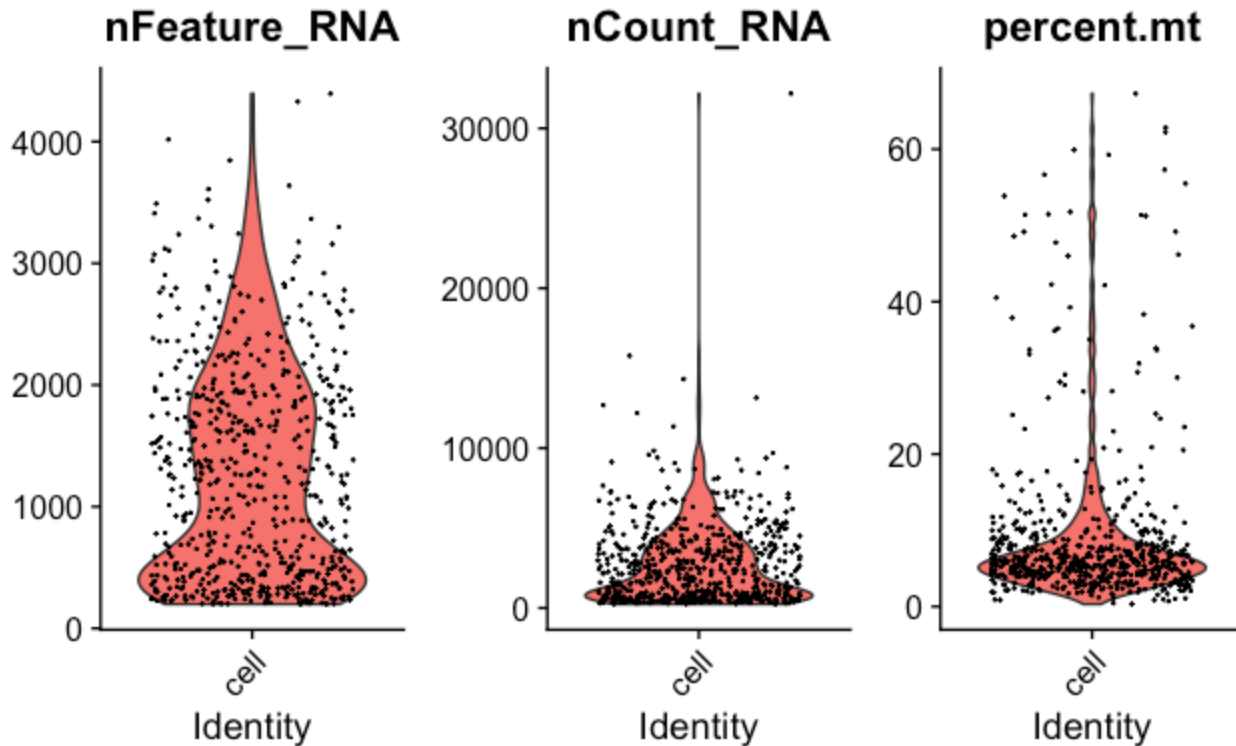
```
pdac1[["percent.mt"]] <- PercentageFeatureSet(object = pdac1, pattern = "^MT-")
```

step 4: visualize the distribution:

the code below creates a violin plot, which visualizes the feature data, count data, and percent of mitochondrial genes

[Hide](#)

```
VlnPlot(object = pdac1, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3)
```



step 5: filter data

the code below uses the 'subset' function to select data which has features between 200 and 2500, while the percent mitochondrial expression is less than 5.

[Hide](#)

```
pdac1 <- subset(x = pdac1, subset = nFeature_RNA > 200 & nFeature_RNA < 2500 & percent.mt < 5)
```

step 6: normalize data

normalizing the data using Log method, and scaling the data by a vector of 10000

[Hide](#)

```
pdac1 <- NormalizeData(object = pdac1, normalization.method = "LogNormalize", scale.factor = 10000)
```

Performing log-normalization

```
0%   10   20   30   40   50   60   70   80   90  100%
[----|----|----|----|----|----|----|----|----|----|
*****|
```

step 7: calculate gene variation

the “FindVariableFeatures” function allows R to look through the seurat object and use the vst method to filter out the top 2000 features/gene which have the most amount of variation across all the cells in the data

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```
pdac1 <- FindVariableFeatures(object = pdac1, selection.method = "vst", nfeatures = 2000)
```

Calculating gene variances

```
0%   10   20   30   40   50   60   70   80   90  100%
[----|----|----|----|----|----|----|----|----|----|
*****|
```

Calculating feature variances of standardized and clipped values

```
0%   10   20   30   40   50   60   70   80   90  100%
[----|----|----|----|----|----|----|----|----|----|
*****|
```

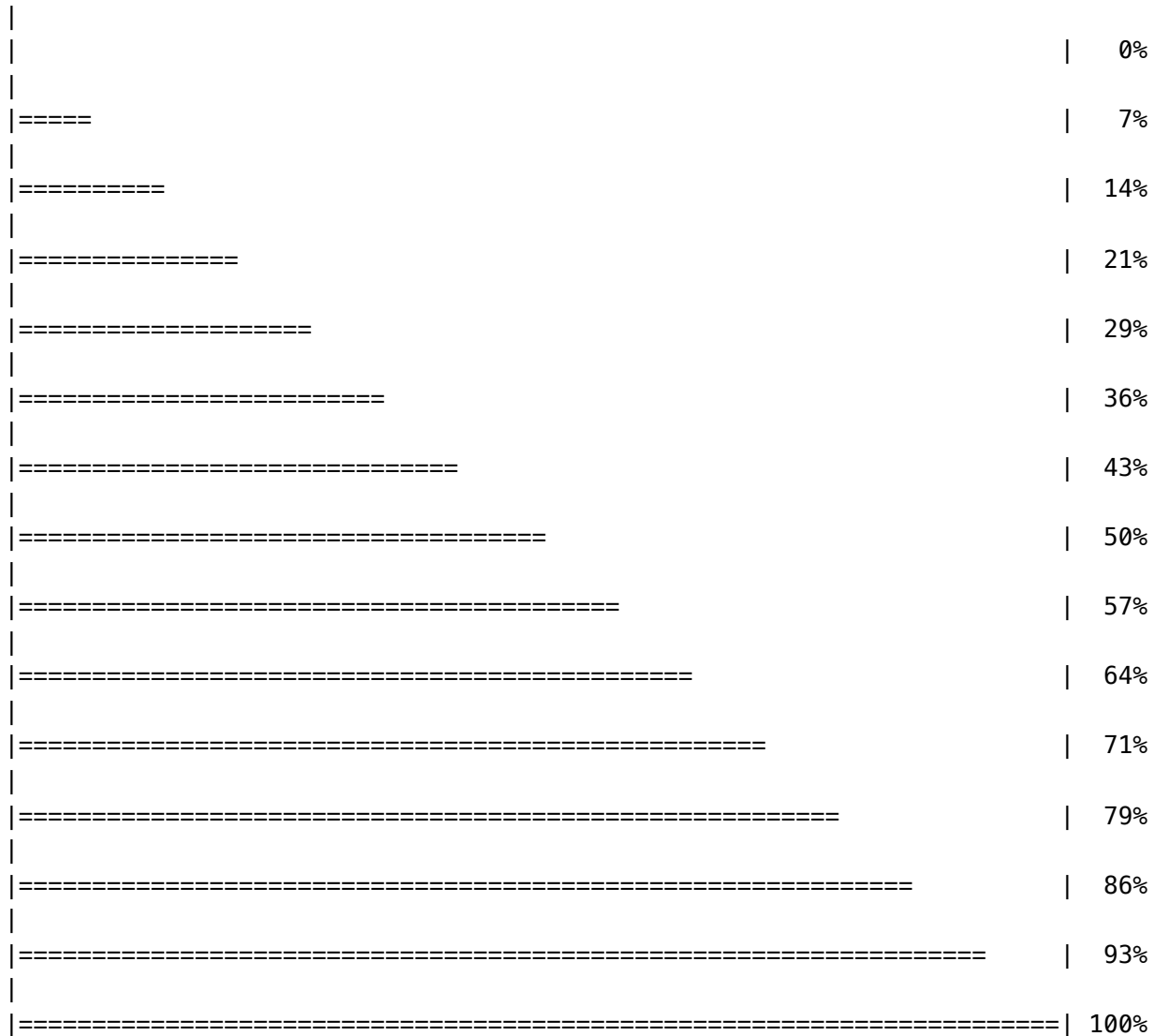
step 8: scale data

scaling all the count data stored in the seurat object for all the genes (rows)

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```
all.genes <- rownames(x = pdac1)
pdac1 <- ScaleData(object = pdac1, features = all.genes)
```

## Centering and scaling data matrix



## step 9: PCA

performing principal component analysis on the `seurat` object, specifically using the features that show high variability

[Hide](#)

```
pdac1 <- RunPCA(object = pdac1, features = VariableFeatures(object = pdac1))
```

## PC\_ 1

Positive: SPINK1, TM4SF1, DU0XA2, AQP3, APOL1, SOX4, GC, TSPAN8, SLPI, MMP7  
 DMBT1, DSTN, AGR2, SLC2A1, GABRP, ITGA2, EFNA1, AKR1B10, CYB5A, F3  
 CES1, TFF3, AKR1C3, ANKRD36C, MPZL2, FXD3, FHL2, BAIAP2L1, EIF4A2, LIPH

Negative: LAPTM5, TYROBP, ALOX5AP, FPR1, S100A9, BASP1, RGS2, S100A8, SLC2A3, LCP1  
 HCLS1, BCL2A1, FCGR3A, CSF3R, G0S2, FCGR3B, ARRB2, ST20, CD53, C5AR1  
 ARHGDIB, FCGR2A, ITGB2, OSM, FCER1G, SAMSN1, TNFRSF1B, AIF1, SLA, CD37

## PC\_ 2

Positive: G0S2, CXCL8, CXCR2, S100A8, ST20, BCL2A1, S100A9, HCAR3, FFAR2, IL1R2  
 HCAR2, CMTM2, CSF3R, PROK2, FPR2, MMP9, SOD2, RIPOR2, FPR1, TLR4  
 CXCR1, MME, PLEK, ALPL, FAM177B, RHOB, MGAM, MMP25, AQP9, ALOX5AP

Negative: MS4A7, SLC02B1, HLA-DQB1, HLA-DPA1, TGFBI, HLA-DQA1, HLA-DRA, CD163, HLA-DQA  
 2, HLA-DRB1

HLA-DPB1, HLA-DRB5, LGALS1, C1QC, VIM, HLA-DQB2, NPC2, FCGR1A, C1QB, SDS  
 APOE, GPR183, CTSB, C1QA, CSF1R, CD74, MS4A4A, CPVL, CD4, GPX1

## PC\_ 3

Positive: AQP3, DU0XA2, GC, TFF3, CES1, DMBT1, HLA-DRB1, AKR1B10, HLA-DRB5, CRP  
 HK2, CRISP3, NR4A1, SPINK1, CD74, CPM, ANKRD36, CTSS, FCGBP, REG1A  
 ANKRD36C, SGK1, F5, CYB5A, TSPAN8, AKR1C3, ANKRD36B, AGR2, CA12, ALDOB

Negative: COL6A1, SERPINB5, COL6A2, COL17A1, MIA, MGP, CRABP2, SNCG, WNT11, PLAUI  
 TIMP3, IGFBP2, S100P, DCBLD2, PCDH7, HMGA2, PODXL, LY6D, FSTL1, HIST1H2BD  
 VCAM1, TOMM70, TRIM29, RNASE1, DMKN, LYPD3, KRT16, PRODH, ZSCAN9, MUC16

## PC\_ 4

Positive: CD2, SEPT6, CCL5, SPOCK2, IL2RB, CD3D, CD8A, CD7, CD8B, KLRC1  
 GZMB, RGS1, KLRC2, CD247, NPIPA7, IRF4, NPIPA3, NPIPA8, NPIPA2, SLAMF7  
 PRF1, FYN, CXCR3, RPL3, ZNF683, SIT1, TRAF3, LCK, CTLA4, NPIPA1

Negative: S100A8, S100A9, PLAUR, SOD2, BCL2A1, FPR1, BASP1, ST20, IL1RN, FCN1  
 SLC11A1, FCGR3B, AQP9, S100P, C5AR1, FCGR3A, SLC2A3, G0S2, S100A12, CSF3R  
 PTGS2, MMP9, CXCL8, FAM177B, EMP1, RAB31, FFAR2, FCGR2A, SMAP2, GCA

## PC\_ 5

Positive: ATP8A1, PALM, SLC02A1, FGF2, REEP4, COL4A2, RBM43, RAD51D, ACE, HEG1  
 RASIP1, CYP1B1, SPARC, PCDH17, IGF2, PLEKHG1, SLC4A7, HTRA1, TCF4, AQP1  
 PTPRG, ZNF419, EFEMP1, MKL2, SLFN11, PODXL, ADGRL1, TTC27, ERMARD, FN1

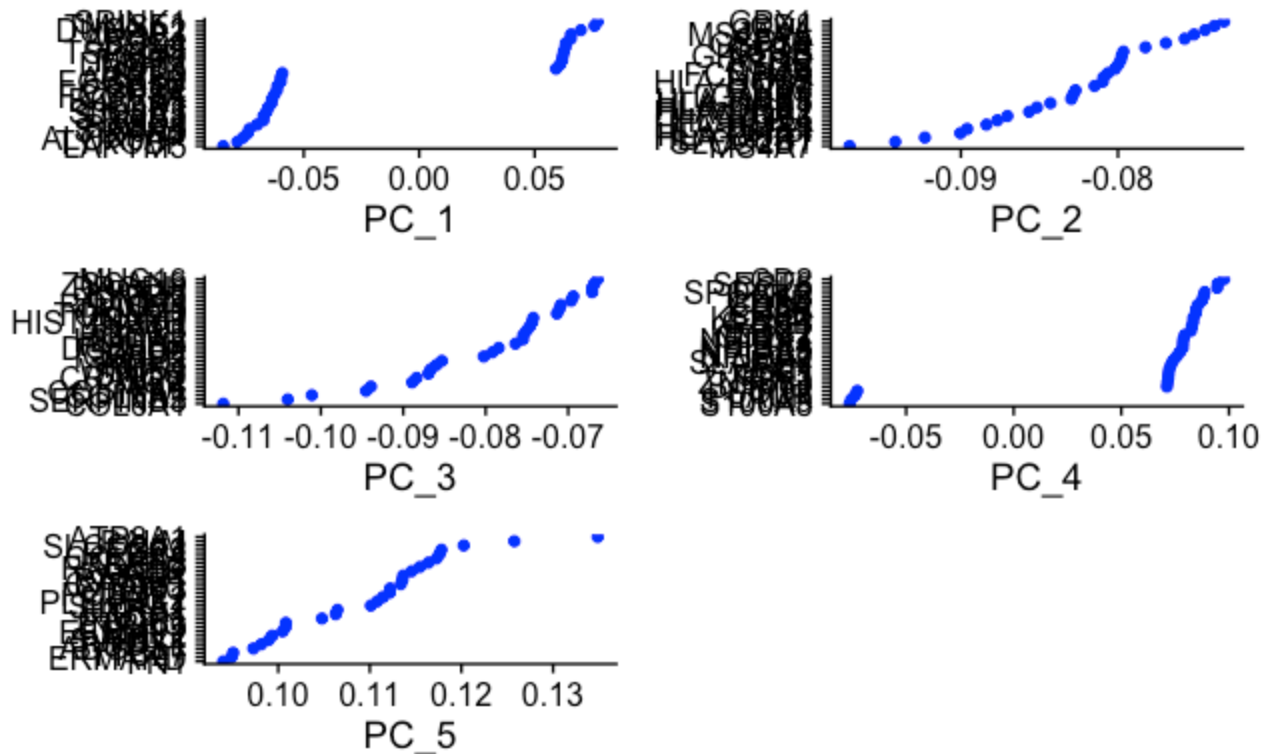
Negative: MSX2, MKI67, LPXN, ADCY7, TOP2A, RNF214, NUSAP1, SNAI2, UBE2C, CCNA2  
 CGRRF1, KIF14, CXCL14, MIS18BP1, CEP55, NCAPD2, NEURL1B, RACGAP1, CAD, RFWD3  
 HMMR, PRODH, UBE2T, UBE2T, UBE2T, NPIPA1, DIAPH3, NGEF, LGALS1, NPIPA3, MCM7

step 10: visualize PCA data

visualizing the 5 PC generated above by using 'dims 1:5'

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```
VizDimLoadings(object = pdac1, dims = 1:5, reduction = "pca")
```



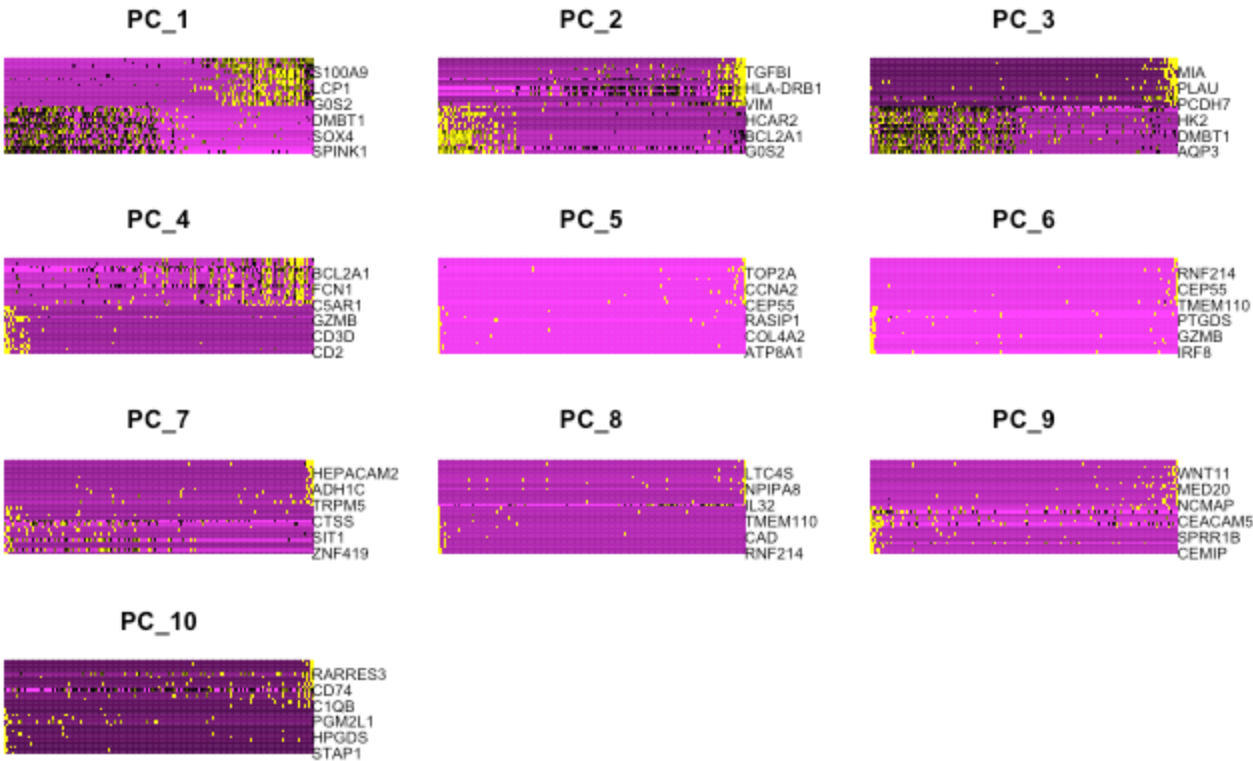
**the results of visualizing the variation from PC1 indicate that there could potentially be at least 2 different types of cells that are present as the data clusters in 2 groups**

step 11: PCA heatmaps

dims = 1:10 specifies that I want 10 PCs and cells = 200 is for 200 cells

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```
DimHeatmap(object = pdac1, dims = 1:10, cells = 200, balanced = TRUE)
```



step 12: dimensionality

num.replicate = 100 specifies that I want 100 replicates, and dims = 1:20 computes p-values for first 20 PCs

	orig.ident	nCount_R...	nFeature_RNA	percent.mt	RNA_snn_res.0.5	seurat_clus
	<fctr>	<dbl>	<int>	<dbl>	<fctr>	<fctr>
cell_091.133	cell	32176	1993	0.3170065	1	1
cell_143.259	cell	6459	2233	4.2885896	1	1
cell_057.153	cell	5026	2247	3.0839634	0	0
cell_169.242	cell	5416	2398	4.8929099	0	0
cell_058.010	cell	6806	2301	2.7622686	3	3
cell_189.209	cell	4386	2119	2.8727770	0	0
cell_023.156	cell	5060	2273	4.8616601	0	0
cell_018.217	cell	5391	2212	2.5041736	0	0
cell_312.307	cell	5615	2432	4.3811220	0	0
cell_318.085	cell	8606	2477	4.5317221	3	3

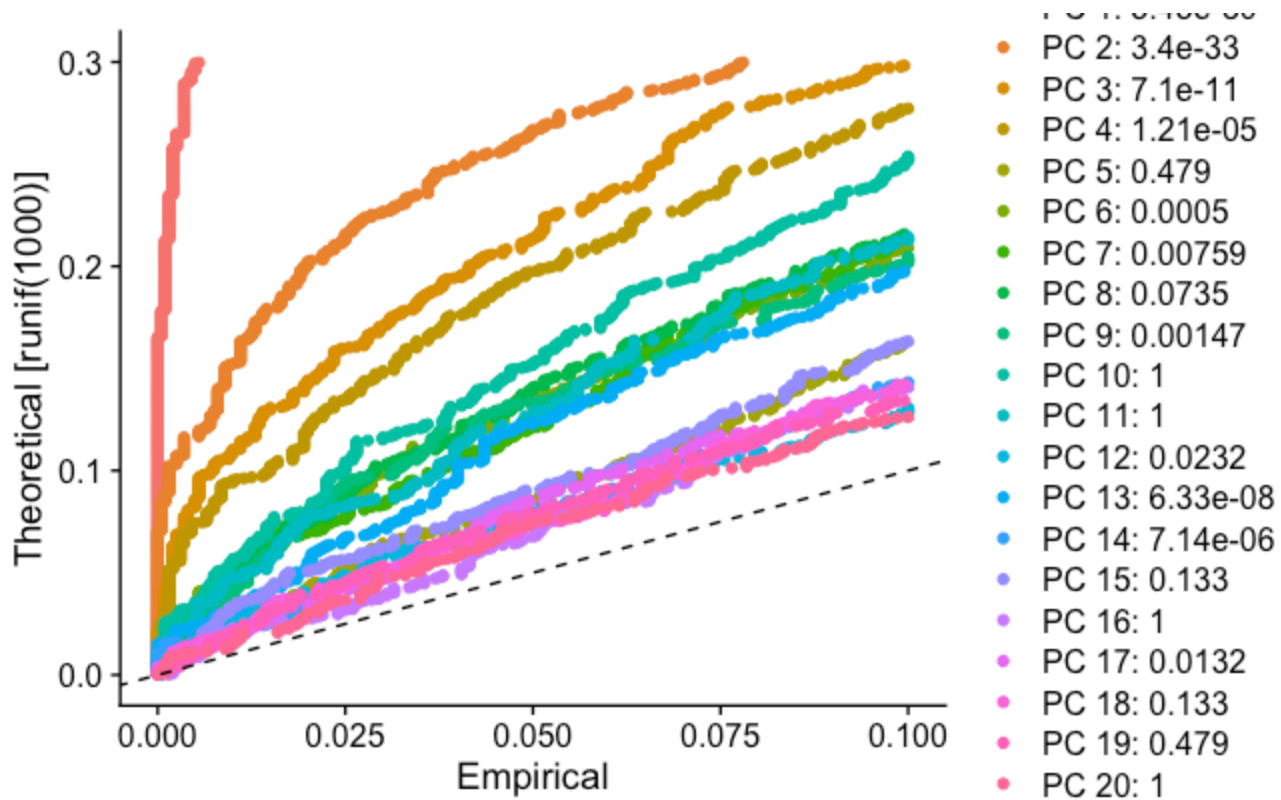
1-10 of 10 rows

plotting the results from JackStraw

This code below plots the results from JackStraw and gives the resulting p-value, which can tell us which PCs are showing significant variation in our data

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```
JackStrawPlot(object = pdac1, dims = 1:20)
```



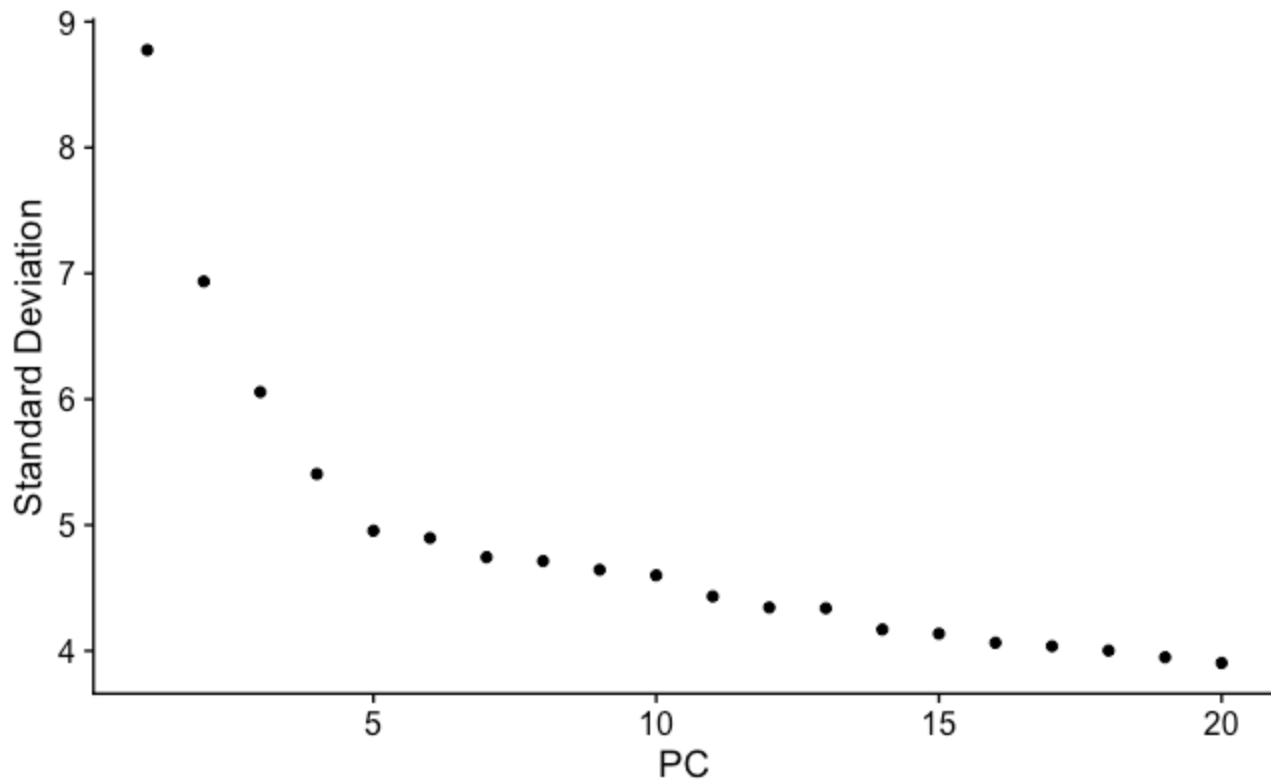
graphing the results with Elbow plot:

Allows us to look at the amount of SD explained by each PC

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```
ElbowPlot(object = pdac1)
```





step 13: clustering

first 9 PCs capture most of the variation, so we can use that by specifying 1:9. The resolution = 0.5 can be increased if we want more clusters (or vice versa)

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```
pdac1 <- FindNeighbors(object = pdac1, dims = 1:9)
```

Computing nearest neighbor graph  
Computing SNN

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```
pdac1 <- FindClusters(object = pdac1, resolution = 0.5)
```

Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 212

Number of edges: 5475

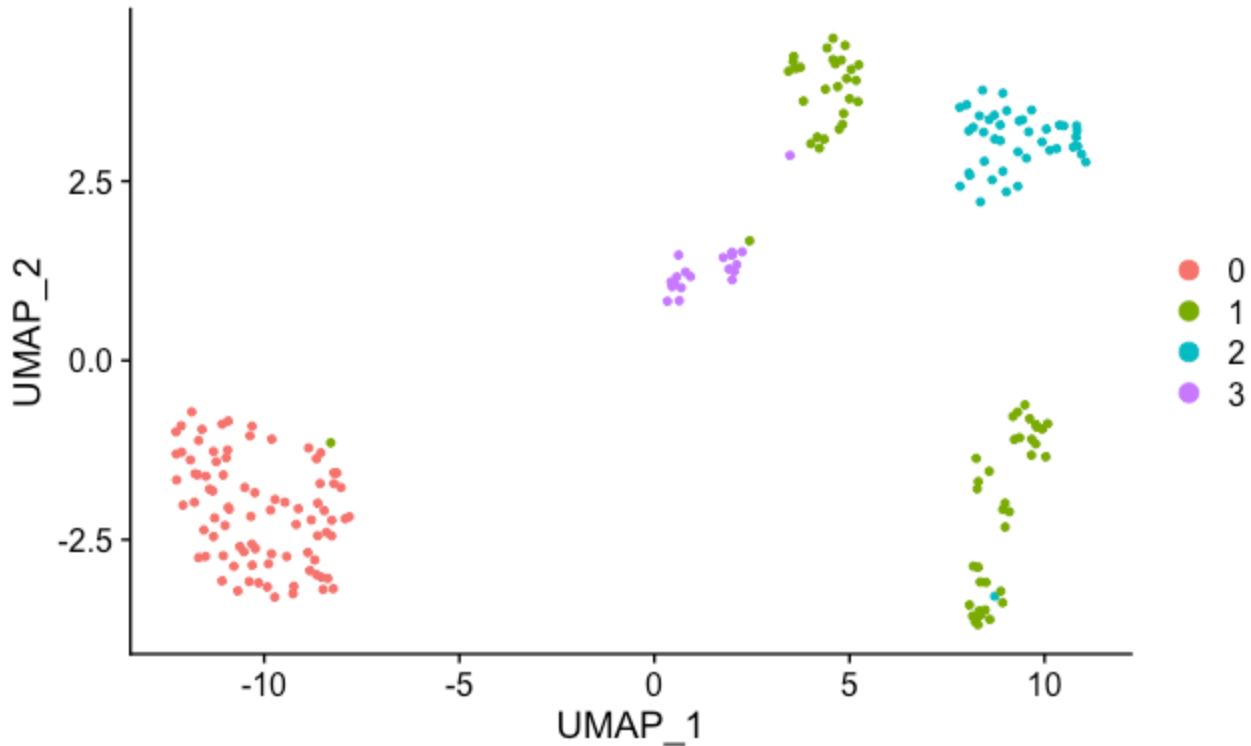
Running Louvain algorithm...

```
0% 10 20 30 40 50 60 70 80 90 100%
[----|----|----|----|----|----|----|----|----|----|
*****|
```

```
Maximum modularity in 10 random starts: 0.7875
Number of communities: 4
Elapsed time: 0 seconds
```

step 14: UMAP on first 9 dimensions

installing UMAP; running first 9 dimensions; plotting the first 9 dimensions



**4 distinct clusters are visible in the UMAP (visibly there are 5 but two of them are grouped in 1)**

step 15: identify markers

the minimum number of cells that express the gene in at least 1 cluster is set at 25% (as per the Seurat markdown - I wasn't sure if this is something that needs to be altered). The log fold change threshold for the gene to be considered is also set at 0.25. In `slice_max`, I put `n=1`, specifying that I want 1 marker per cluster, which will then be used for plotting.

Hide

```
pdac1.markers <- FindAllMarkers(object = pdac1, only.pos = TRUE, min.pct = 0.25, logfc.threshold = 0.25)
```

Calculating cluster 0

	0 % ~calculating
+	1 % ~04s
++	2 % ~03s
++	3 % ~03s
+++	4 % ~03s
+++	5 % ~03s
++++	6 % ~03s
++++	7 % ~03s
+++++	8 % ~03s
+++++	9 % ~03s
++++++	10% ~03s
++++++	11% ~03s
+++++++	12% ~03s
+++++++	14% ~03s
++++++++	15% ~03s
++++++++	16% ~03s
++++++++	17% ~03s
++++++++	18% ~03s
++++++++	19% ~03s
++++++++	20% ~03s
++++++++	21% ~03s
++++++++	22% ~02s
++++++++	23% ~02s
++++++++	24% ~02s
++++++++	25% ~02s
++++++++	26% ~02s
++++++++	27% ~02s
++++++++	28% ~02s
++++++++	29% ~02s
++++++++	30% ~02s
++++++++	31% ~02s
++++++++	32% ~02s
++++++++	33% ~02s
++++++++	34% ~02s
++++++++	35% ~02s
++++++++	36% ~02s
++++++++	38% ~02s
++++++++	39% ~02s
++++++++	40% ~02s
++++++++	41% ~02s
++++++++	42% ~02s
++++++++	43% ~02s
++++++++	44% ~02s
++++++++	45% ~02s
++++++++	46% ~02s
++++++++	47% ~02s
++++++++	48% ~02s
++++++++	49% ~02s
++++++++	50% ~02s
++++++++	51% ~01s
++++++++	52% ~01s

```
| ++++++ | 53% ~01s
| ++++++ | 54% ~01s
| ++++++ | 55% ~01s
| ++++++ | 56% ~01s
| ++++++ | 57% ~01s
| ++++++ | 58% ~01s
| ++++++ | 59% ~01s
| ++++++ | 60% ~01s
| ++++++ | 61% ~01s
| ++++++ | 62% ~01s
| ++++++ | 64% ~01s
| ++++++ | 65% ~01s
| ++++++ | 66% ~01s
| ++++++ | 67% ~01s
| ++++++ | 68% ~01s
| ++++++ | 69% ~01s
| ++++++ | 70% ~01s
| ++++++ | 71% ~01s
| ++++++ | 72% ~01s
| ++++++ | 73% ~01s
| ++++++ | 74% ~01s
| ++++++ | 75% ~01s
| ++++++ | 76% ~01s
| ++++++ | 77% ~01s
| ++++++ | 78% ~01s
| ++++++ | 79% ~01s
| ++++++ | 80% ~01s
| ++++++ | 81% ~01s
| ++++++ | 82% ~01s
| ++++++ | 83% ~01s
| ++++++ | 84% ~00s
| ++++++ | 85% ~00s
| ++++++ | 86% ~00s
| ++++++ | 88% ~00s
| ++++++ | 89% ~00s
| ++++++ | 90% ~00s
| ++++++ | 91% ~00s
| ++++++ | 92% ~00s
| ++++++ | 93% ~00s
| ++++++ | 94% ~00s
| ++++++ | 95% ~00s
| ++++++ | 96% ~00s
| ++++++ | 97% ~00s
| ++++++ | 98% ~00s
| ++++++ | 99% ~00s
| ++++++ | 100% elapsed=03s
```

Calculating cluster 1

	0 % ~calculating
+	1 % ~02s
++	2 % ~01s
++	3 % ~01s
+++	5 % ~01s
+++	6 % ~01s
++++	7 % ~01s
++++	8 % ~01s
+++++	9 % ~01s
++++++	10% ~01s
++++++	11% ~01s
+++++++	12% ~01s
+++++++	14% ~01s
++++++++	15% ~01s
++++++++	16% ~01s
++++++++	17% ~01s
++++++++	18% ~01s
++++++++	19% ~01s
++++++++	20% ~01s
++++++++	22% ~01s
++++++++	23% ~01s
++++++++	24% ~01s
++++++++	25% ~01s
++++++++	26% ~01s
++++++++	27% ~01s
++++++++	28% ~01s
++++++++	30% ~01s
++++++++	31% ~01s
++++++++	32% ~01s
++++++++	33% ~01s
++++++++	34% ~01s
++++++++	35% ~01s
++++++++	36% ~01s
++++++++	38% ~01s
++++++++	39% ~01s
++++++++	40% ~01s
++++++++	41% ~01s
++++++++	42% ~00s
++++++++	43% ~00s
++++++++	44% ~00s
++++++++	45% ~00s
++++++++	47% ~00s
++++++++	48% ~00s
++++++++	49% ~00s
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++++++++	51% ~00s
++++++++	52% ~00s
++++++++	53% ~00s
++++++++	55% ~00s
++++++++	56% ~00s
++++++++	57% ~00s

```
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|+++++++| 59% ~00s
|+++++++| 60% ~00s
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|+++++++| 68% ~00s
|+++++++| 69% ~00s
|+++++++| 70% ~00s
|+++++++| 72% ~00s
|+++++++| 73% ~00s
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|+++++++| 77% ~00s
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|+++++++| 89% ~00s
|+++++++| 90% ~00s
|+++++++| 91% ~00s
|+++++++| 92% ~00s
|+++++++| 93% ~00s
|+++++++| 94% ~00s
|+++++++| 95% ~00s
|+++++++| 97% ~00s
|+++++++| 98% ~00s
|+++++++| 99% ~00s
|+++++++| 100% elapsed=01s
```

Calculating cluster 2

	0 % ~calculating
+	1 % ~01s
++	3 % ~00s
++	4 % ~00s
+++	5 % ~00s
++++	6 % ~00s
++++	8 % ~01s
+++++	9 % ~01s
++++++	10% ~01s
++++++	12% ~01s
++++++	13% ~01s
++++++	14% ~01s
++++++	15% ~01s
++++++	17% ~01s
++++++	18% ~01s
++++++	19% ~01s
++++++	21% ~01s
++++++	22% ~01s
++++++	23% ~01s
++++++	24% ~01s
++++++	26% ~01s
++++++	27% ~01s
++++++	28% ~01s
++++++	29% ~00s
++++++	31% ~00s
++++++	32% ~00s
++++++	33% ~00s
++++++	35% ~00s
++++++	36% ~00s
++++++	37% ~00s
++++++	38% ~00s
++++++	40% ~00s
++++++	41% ~00s
++++++	42% ~00s
++++++	44% ~00s
++++++	45% ~00s
++++++	46% ~00s
++++++	47% ~00s
++++++	49% ~00s
++++++	50% ~00s
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++++++	54% ~00s
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++++++	56% ~00s
++++++	58% ~00s
++++++	59% ~00s
++++++	60% ~00s
++++++	62% ~00s
++++++	63% ~00s
++++++	64% ~00s

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|+++++++| 71% ~00s
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|+++++++| 81% ~00s
|+++++++| 82% ~00s
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|+++++++| 85% ~00s
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|+++++++| 87% ~00s
|+++++++| 88% ~00s
|+++++++| 90% ~00s
|+++++++| 91% ~00s
|+++++++| 92% ~00s
|+++++++| 94% ~00s
|+++++++| 95% ~00s
|+++++++| 96% ~00s
|+++++++| 97% ~00s
|+++++++| 99% ~00s
|+++++++| 100% elapsed=01s
```

Calculating cluster 3



	0 % ~calculating
+	1 % ~02s
++	2 % ~02s
++	3 % ~02s
+++	4 % ~02s
+++	5 % ~02s
++++	6 % ~02s
++++	7 % ~02s
+++++	8 % ~02s
+++++	9 % ~02s
++++++	10% ~02s
++++++	11% ~02s
+++++++	12% ~02s
+++++++	13% ~02s
++++++++	14% ~02s
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++++++++	51% ~01s

```
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| ++++++ | 68% ~01s
| ++++++ | 69% ~01s
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| ++++++ | 71% ~01s
| ++++++ | 72% ~01s
| ++++++ | 73% ~01s
| ++++++ | 74% ~01s
| ++++++ | 75% ~01s
| ++++++ | 76% ~01s
| ++++++ | 77% ~01s
| ++++++ | 78% ~01s
| ++++++ | 79% ~01s
| ++++++ | 80% ~01s
| ++++++ | 81% ~00s
| ++++++ | 82% ~00s
| ++++++ | 83% ~00s
| ++++++ | 84% ~00s
| ++++++ | 85% ~00s
| ++++++ | 86% ~00s
| ++++++ | 87% ~00s
| ++++++ | 88% ~00s
| ++++++ | 89% ~00s
| ++++++ | 90% ~00s
| ++++++ | 91% ~00s
| ++++++ | 92% ~00s
| ++++++ | 93% ~00s
| ++++++ | 94% ~00s
| ++++++ | 95% ~00s
| ++++++ | 96% ~00s
| ++++++ | 97% ~00s
| ++++++ | 98% ~00s
| ++++++ | 99% ~00s
| ++++++ | 100% elapsed=02s
```

[Hide](#)

```
pdac1.markers %>%
  group_by(cluster) %>%
  slice_max(n = 1, order_by = avg_log2FC)
```

p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fctr>	gene <chr>
1.260561e-30	3.830777	0.882	0.094	1.669991e-26	0	SPP1
1.053404e-11	2.997340	0.369	0.027	1.395550e-07	1	RGS1
1.967397e-29	5.863167	0.744	0.030	2.606407e-25	2	G0S2
2.631740e-15	4.856750	0.368	0.005	3.486529e-11	3	MGP

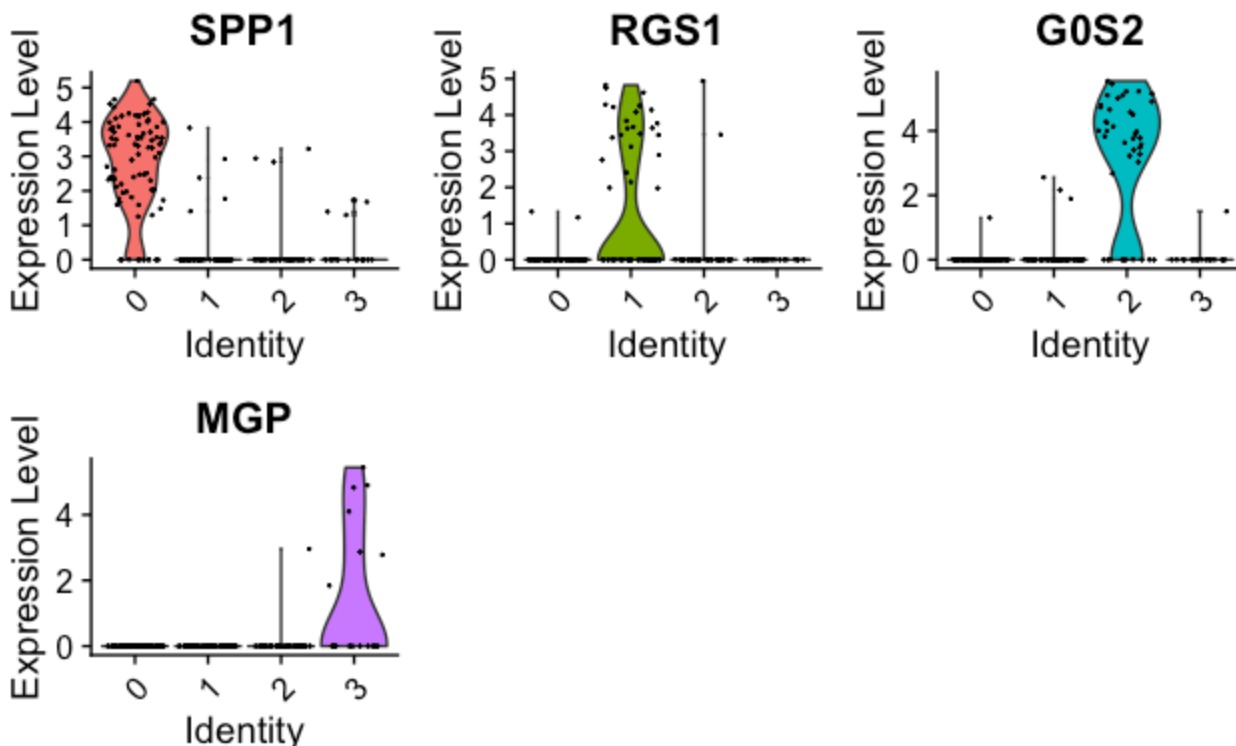
4 rows

step 16: violin plot from 1 feature from each cluster

I used the features obtained above to create this violon plot to see the distribution

Hide

```
VlnPlot(object = pdac1, features = c("SPP1", "RGS1", "G0S2", "MGP"))
```



step 17: feature plot with the same features as step 16

Feature plot has been constructed with the same features as used in the creation of the violon plot above.

Hide

```
FeaturePlot(object = pdac1, features = c("SPP1", "RGS1", "G0S2", "MGP"))
```

