

CompGO vignette

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1 Introduction

This package contains functions to accomplish several tasks relating to Gene Ontology enrichment comparison and visualisation given either .bed files or gene lists. It interfaces with rtracklayer and VariantAnnotation to easily annotate .bed files with genes, and with RDAVIDWebService to generate functional annotation charts based on these gene lists. From here, we provide several methods for comparative visualisation, including viewing the GO term hierarchy in two samples using a directed acyclic graph (DAG), performing z-score standardisation (Equation 1) of GO terms returned from DAVID and comparing enrichment via pairwise scatterplots with linear fit, differential z-score (Equation 2) and Jaccard metrics (Equation 3). We also provide functions to enable large-scale clustering, KEGG pathway enrichment comparison and data dimension reduction via PCA. In this vignette, we will comprehensively demonstrate use of this package on the supplied data.

$$z_i = \frac{\log(OR)}{SE(OR)} \quad (1)$$

$$zDiff(x, y) = \frac{\log(OR_x) - \log(OR_y)}{\sqrt{SE(x)^2 + SE(y)^2}} \quad (2)$$

$$J_c = \frac{A \cap B}{A \cup B} \quad (3)$$

2 Annotating .bed files

The first step in this analysis pipeline is to generate individual Gene Ontology data for each dataset. We provide an interface to generate a list of genes from input .bed coordinates

with the function `anotateBedFromDb`, which can take either a system path or a `GRanges` object as input. Here we use the example files provided with the package.

```
> library(CompGO)
> library(TxDb.Mmusculus.UCSC.mm9.knownGene)
> # load all data packages into R:
> data(gata4, mef2a, nkx25, p300, srf, tbx5)
> # Create the GRanges objects:
> gata4.range = GRanges(seqnames=gata4$chromosome,
+   IRanges(start = gata4$start, end = gata4$end))
> mef2a.range = GRanges(seqnames=mef2a$chromosome,
+   IRanges(start = mef2a$start, end = mef2a$end))
> nkx25.range = GRanges(seqnames=nkx25$chromosome,
+   IRanges(start = nkx25$start, end = nkx25$end))
> p300.range = GRanges(seqnames=p300$chromosome,
+   IRanges(start = p300$start, end = p300$end))
> srf.range = GRanges(seqnames=srf$chromosome,
+   IRanges(start = srf$start, end = srf$end))
> tbx5.range = GRanges(seqnames=tbx5$chromosome,
+   IRanges(start = tbx5$start, end = tbx5$end))
> # Note that the window around the .bed region beyond which
> # genes are cutoff is 5kb, this is modifiable
> gata4.annotated = annotateBedFromDb(gRanges = gata4.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> mef2a.annotated = annotateBedFromDb(gRanges = mef2a.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> nkx25.annotated = annotateBedFromDb(gRanges = nkx25.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> p300.annotated = annotateBedFromDb(gRanges = p300.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> srf.annotated = annotateBedFromDb(gRanges = srf.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> tbx5.annotated = annotateBedFromDb(gRanges = tbx5.range,
+   db = TxDb.Mmusculus.UCSC.mm9.knownGene)
> head(gata4.annotated)
```

`GRanges` with 6 ranges and 2 metadata columns:

	seqnames	ranges	strand	tx_id	gene_id
	<Rle>	<IRanges>	<Rle>	<integer>	<CharacterList>
[1]	chr1	[16678537, 16699686]	+	75	17087
[2]	chr1	[16678537, 16699686]	+	76	17087
[3]	chr1	[34068670, 34365497]	+	130	13518

```

[4]      chr1 [34068670, 34365497]      + |      131      13518
[5]      chr1 [34272114, 34286091]      + |      134      13518
[6]      chr1 [34282354, 34319096]      + |      135      13518
---
seqlengths:
      chr1      chr2      chr3 ... chrY_random chrUn_random
197195432 181748087 159599783 ... 58682461 5900358

```

3 Generation of Gene Ontology data

Once we have these annotated GRanges objects, we can derive lists of genes to query DAVID and retrieve a full functional annotation chart. Alternatively, a list of genes can be directly supplied to DAVID. Note that there are several parameters available for tweaking the results returned from DAVID: users can specify p value and count cutoffs; however, by default, no thresholding is performed. Since DAVID requires registration before the use of their Web Service R package, you should register first then substitute the email here for your own. This step can take some time, since it depends upon the latency of DAVID's server.

```

> # These steps require registration with DAVID. To do so, visit
> # http://david.abcc.ncifcrf.gov/webservice/register.htm then replace
> # "name@yourdomain.com" with your email address below.
> gata4.fnAnot = getFnAnot_genome(gata4.annotated$gene_id,
+   email = "name@yourdomain.com", listName="gata4", getKEGG=TRUE)
> mef2a.fnAnot = getFnAnot_genome(mef2a.annotated$gene_id,
+   email = "name@yourdomain.com", listName="mef2a", getKEGG=TRUE)
> nkx25.fnAnot = getFnAnot_genome(nkx25.annotated$gene_id,
+   email = "name@yourdomain.com", listName="nkx25", getKEGG=TRUE)
> p300.fnAnot = getFnAnot_genome(p300.annotated$gene_id,
+   email = "name@yourdomain.com", listName="p300", getKEGG=TRUE)
> srf.fnAnot = getFnAnot_genome(srf.annotated$gene_id,
+   email = "name@yourdomain.com", listName="srf", getKEGG=TRUE)
> tbx5.fnAnot = getFnAnot_genome(tbx5.annotated$gene_id,
+   email = "name@yourdomain.com", listName="tbx5", getKEGG=TRUE)
>
> # once the charts have been retrieved, they can be written
> # to disk for subsequent batch processing like so:
> # write.table(funChart1, "./path/to/text.txt")
> # however, here we continue with the generated charts in R.

```

4 Clustering and PCA

When faced with a large volume of data, like we are here, it is useful to first get an overall perspective on how similar or different each set is to each other set. We include a function to do interactive PCA and dendrogram-style clustering on large volumes of data, but that doesn't work well in a non-interactive environment - here, we just use the constituent functions `plotPCA` and `plotDendrogram`.

```
> # First, put all the charts from the previous step into a list.  
> chartList = list("gata4" = gata4.fnAnot, "mef2a" = mef2a.fnAnot, "nkx25" = nkx25.fnAnot,  
+   "p300" = p300.fnAnot, "srf" = srf.fnAnot, "tbx5" = tbx5.fnAnot)
```

```
> plotDendrogram(chartList)
```

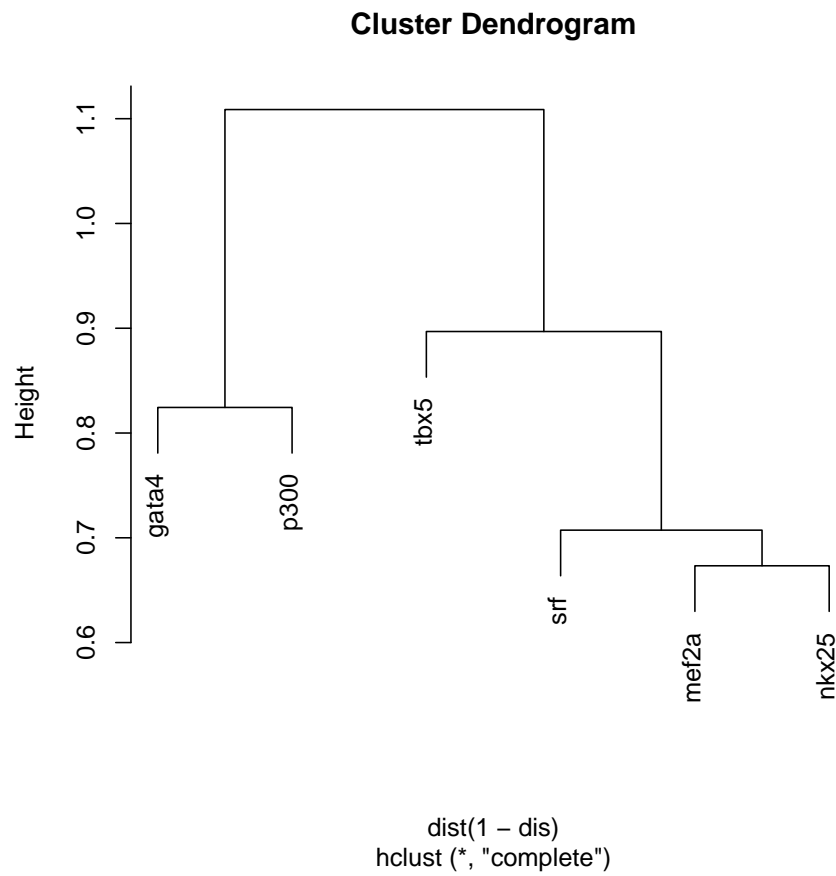


Figure 1: A dendrogram of the sample input data. This gives a good general overview of similarity between individual input sets, later methods show exactly how similar they are (and why).

```
> plotPCA(chartList)
```

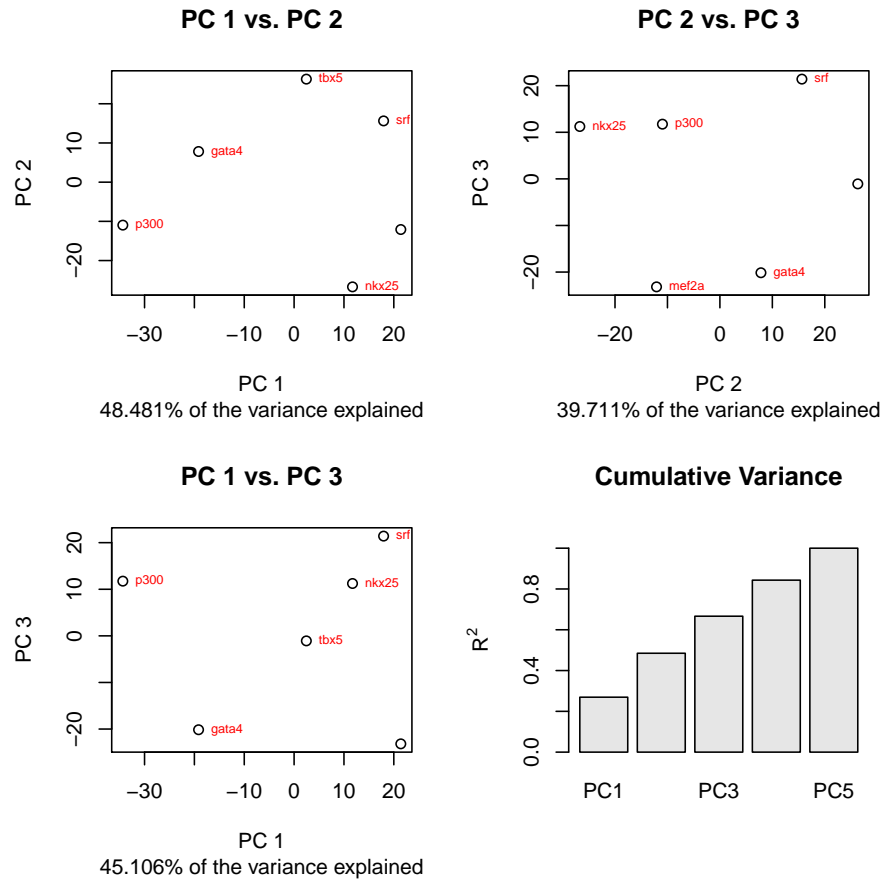


Figure 2: A PCA of the sample input data

5 Result refinement and visualisation

Now that a general overview of similarity has been generated using PCA and clustering, it is helpful to understand the differences between specific transcription factor binding sites. The pairwise plots of z-transformed GO terms is very useful as a metric for overall similarity, while the GO term graph shows the specific differences between two sets.

It is also possible to directly access the comparative Z-score values as a data frame:

```
> compared = compareZscores(p300.fnAnot, mef2a.fnAnot)
> # Accessing a random row of the data.frame
> compared[sample(1:nrow(compared), 1),]
```

	Term	Zscore.A	Zscore.B	ComparedZ
495	GO:0070013~intracellular organelle lumen	0.4296869	3.965095	-1.995372
	Pvalue	PvalueAdj		
495	0.04600231	0.5162481		

Finally, viewKegg uses pathview to compare two lists visually by differentially enriched KEGG pathway elements. As a note, it is recommended that you supply a working directory argument - pathview will download an XML and PNG file there for the pathway, as well as outputting a PNG of the differentially expressed elements themselves.

```
> plotZScores(nkx25.fnAnot, mef2a.fnAnot)
```

```
[1] "0.5785"
```

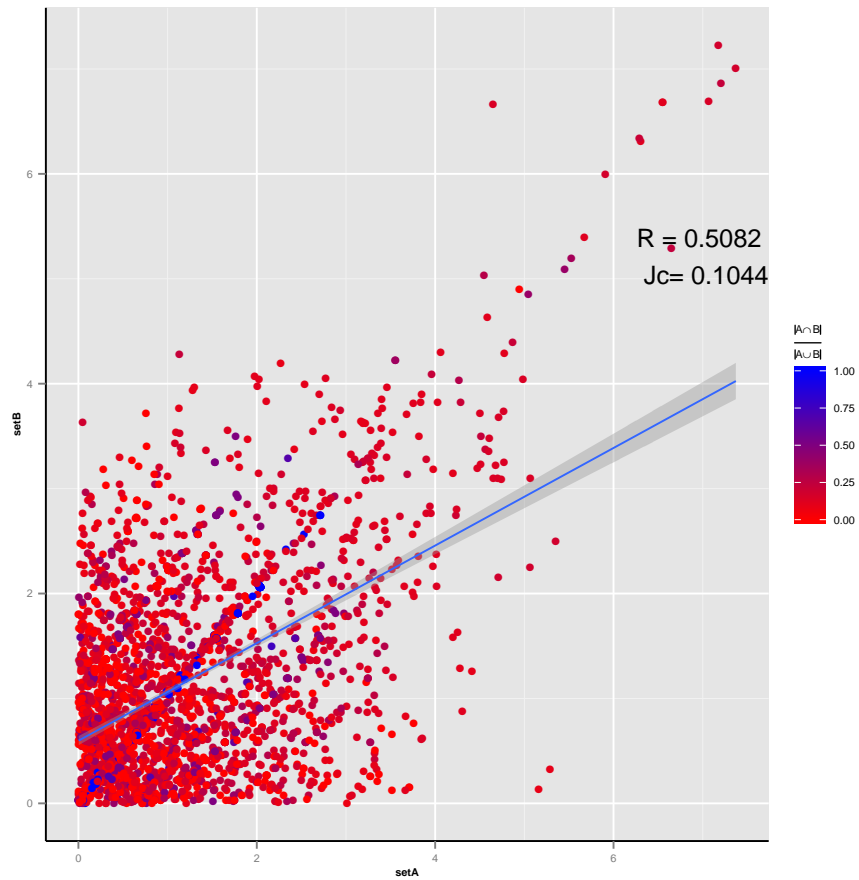


Figure 3: Non-thresholded pairwise plot of Z-scores between Nkx2.5 and Mef2a, two of the more similar sets from the dendrogram. Each point is a GO term, and the colour of each point represents the overlap of genes associated with that term (the Jaccard coefficient). While the Jaccard coefficient remains very low at 0.1, the overall correlation is much higher at 0.508.


```
> plotZScores(p300.fnAnot, mef2a.fnAnot)
```

```
[1] "0.3808"
```

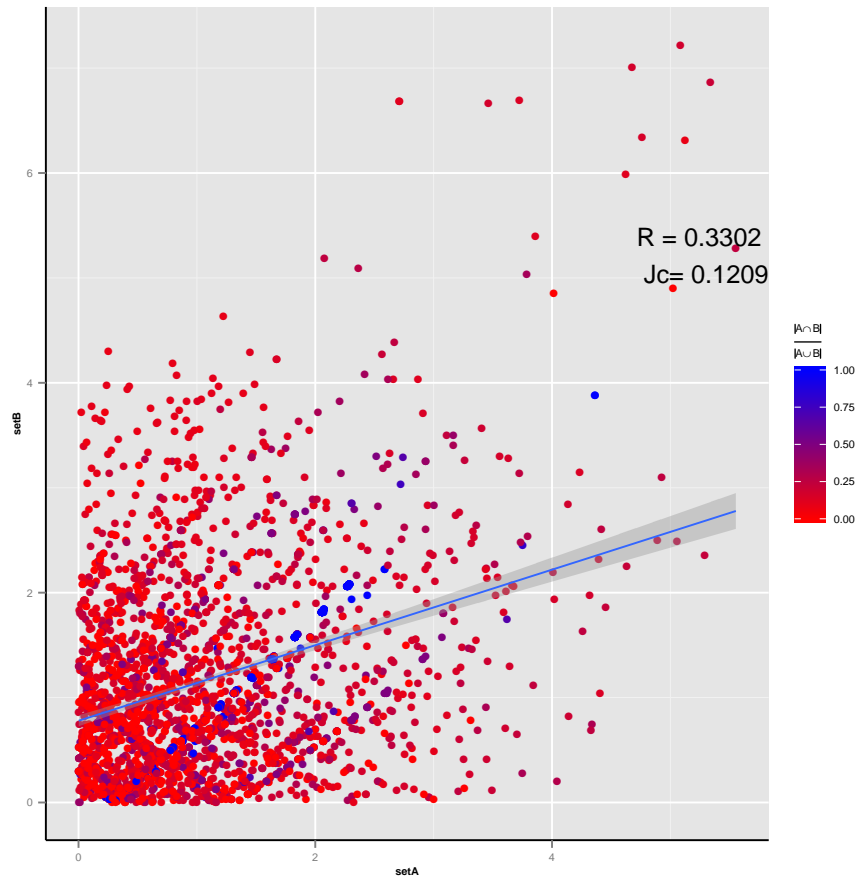


Figure 4: p300 against Mef2a, two of the more different sets. Interestingly, the Jaccard coefficient between these two sets is marginally higher than before, but the overall correlation is reduced to 0.330

```
> plotZRankedDAG(nkx25.fnAnot, mef2a.fnAnot, ont="MF", n=50)
```

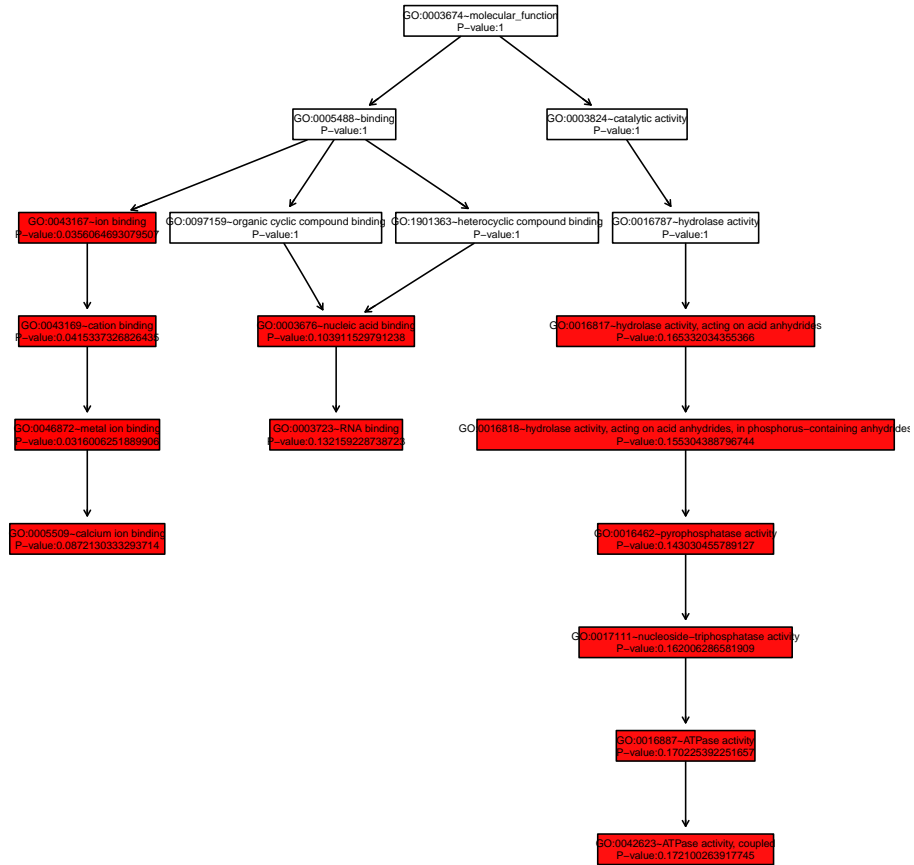


Figure 5: Significant differences between Nkx2.5 and Mef2a in molecular function. Note that in these plots, more saturated node colour represents more significant P-values.

```
> plotZRankedDAG(p300.fnAnot, mef2a.fnAnot, ont="MF", n=50)
```

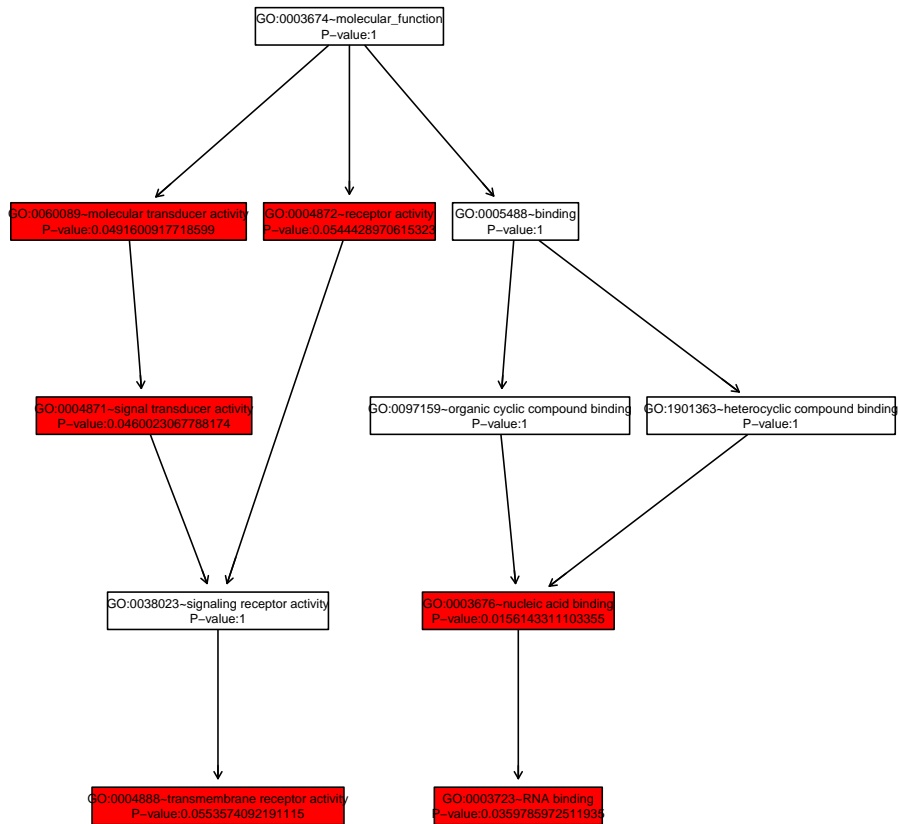


Figure 6: Significant differences between p300 and Mef2a in molecular function.

```
> viewKegg(p300.fnAnot, mef2a.fnAnot, workingDir = "~/Desktop/workingDir/")
```

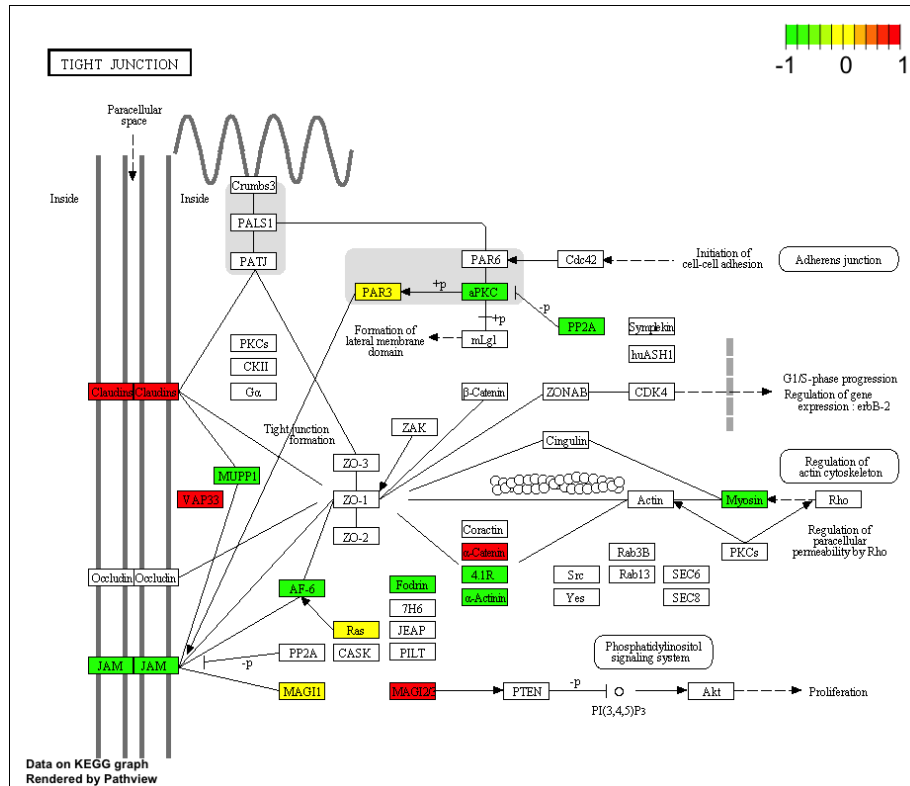


Figure 7: The most differentially enriched KEGG pathway between p300 and mef2a is Tight Junction. Green is enriched in setA, yellow is enriched in both, and red is enriched in setB.