

PathwaySplice testing for RNA-seq datasets

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1 Introduction

This document gives an introduction to the use of the PathwaySplice R Bioconductor package. This package provides methods for (1) performing pathway analysis that explicitly adjusts for the number of exons or junctions associated with each gene; (2) visualizing selection bias due to different number of exons or junctions for each gene and formally tests for presence of bias using logistic regression; (3) supporting gene sets based on the Gene Ontology terms, as well as more broadly defined gene sets (e.g. MSigDB) or user defined gene sets; (4) identifying the significant genes driving pathway significance and (5) organizing significant pathways with an enrichment map, where pathways with large number of overlapping genes are grouped together in a network graph.

Once installed, the PathwaySplice package can be easily loaded into R using:

```
library(PathwaySplice)
```

2 Use PathwaySplice

PathwaySplice uses the output from DEXSeq or JunctionSeq as input basis. Assume users have gotten the differential exon usage or differential splicing junctions usage results from DEXSeq or JunctionSeq. User needs to convert these results into gene based table, then use this gene based table to perform analysis using the following commands:

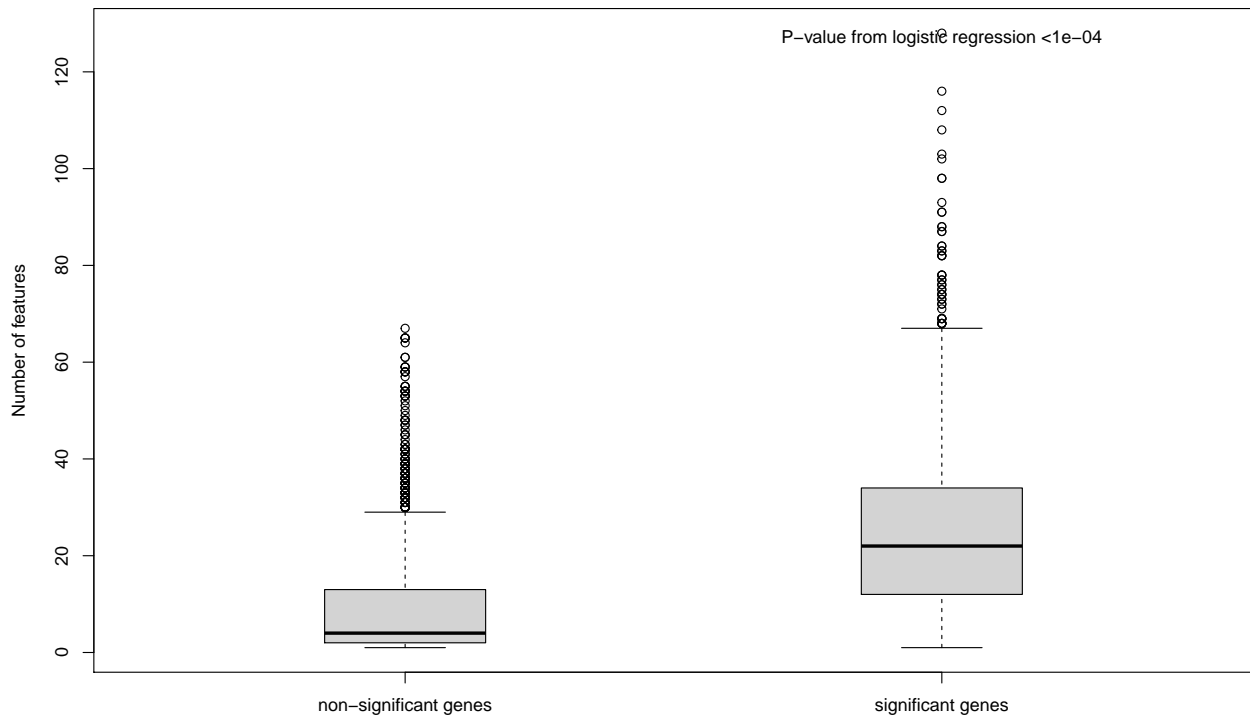
```
featureBasedData <- PathwaySplice::makeFeatureTable(res)
gene.based.table <- makeGeneTable(featureBasedData)
```

For a showcase, PathwaySplice supply a feature based data with this packages, users can load this data using commands:

```
data("featureBasedData")
gene.based.table <- makeGeneTable(featureBasedData)
```

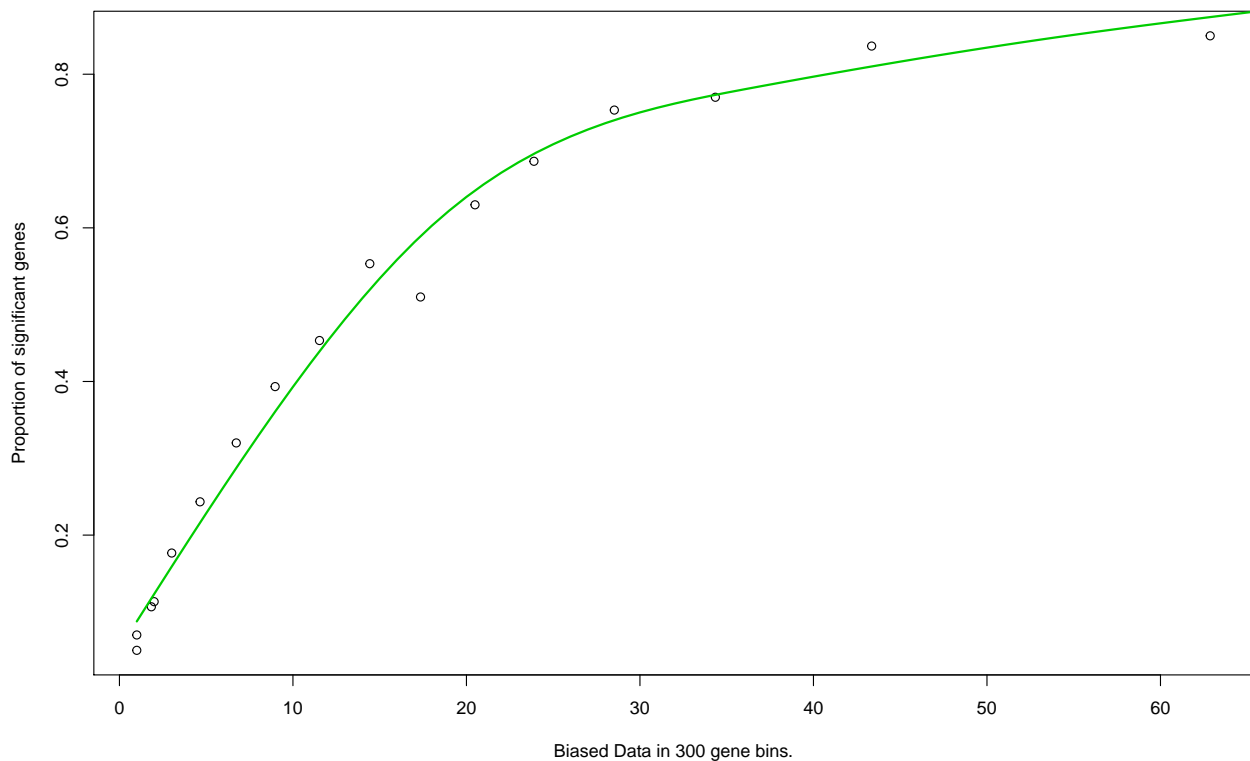
After this step, use can identify bias factor by the function lrTestBias

```
lrTestBias(gene.based.table,boxplot.width=0.3)
```



To perform analysis by adjusting number of feature, users can perform the following step:

```
res <- runPathwaySplice(gene.based.table,genome='hg19',id='ensGene',
  test.cats=c('GO:BP'),
  go.size.limit=c(5,30),method='Wallenius')
```



User can use the results from runPathwaySplice to build up enrichment network using the following commands:

```
output.file.dir <- file.path(tempdir(),"OutputEnmap")
enmap <- enrichmentMap(res,n=3,output.file.dir
                        =output.file.dir,similarity.threshold=0)
```

