Bioconductor's PADOG package

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1 Overview

This package implements the ***P**athway **A**nalysis with **D**own-weighting of **O**verlapping **G**enes* (**PADOG**) algorithm described in Tarca et al. (2012). The method can be applied to analyze any type of gene sets yet in here it is illustarted using KEGG pathways. The method computes a gene set score as the mean of absolute values of weighted moderated gene t-scores. The gene weights are chosen to favor genes appearing in few pathways versus genes that appear in many pathways. The significance of pathway scores is evaluated using sample/array labels permutation that preserve the gene-gene correlation structure. The package also contains a benchmark for gene set analysis in general and allows a new gene set analysis method to be benchmarked against PADOG or other exsisting methods (e.g. GSA). The benchmark uses 24 different data sets, each involving a disease (e.g. Colorectal Cancer) for which there is a KEGG pathway with the same name. The only assumption we make (proven to hold in Tarca et al. (2012)) is that the KEGG's pathway with the same name as the disease under the study should be found significant and/or ranked near the top by gene set analysis methods when analyzing a dataset that compares normal with diseased samples.

2 Pathway / gene set analysis with PADOG package

This document provides basic introduction on how to use the `PADOG` package. For extended description of the methods used by this package please consult Tarca et al. (2012) and Tarca et al. (2013).

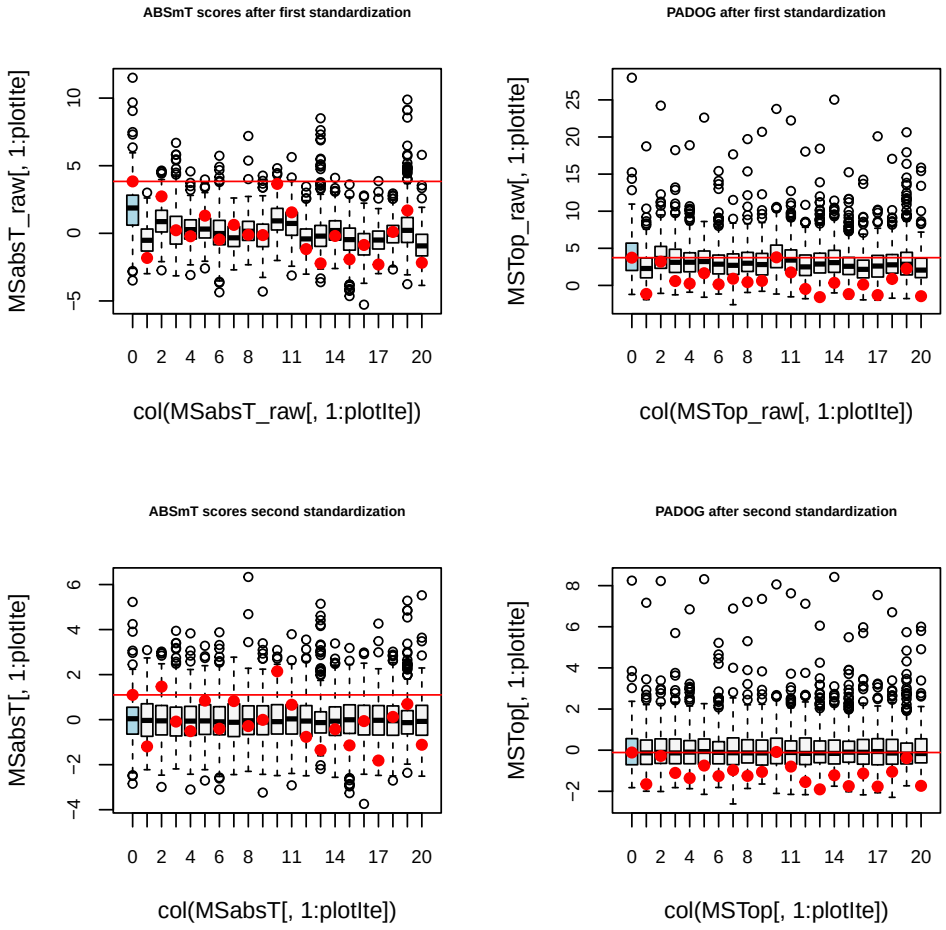
We demonstrate the functionality of this package using a colorectal cancer dataset obtained using Affymetrix GeneChip technology and available through GEO (GSE9348) and incorporated in the `KEGGdzPathwaysGEO` package. This experiment contains 12 normal samples and 70 colorectal cancer samples and is described in Hong et al. (2010). The RMA preprocessed data using the `affy` package is the entry point for the `padog` function:

```
> library(PADOG)
> set = "GSE9348"
> data(list = set, package = "KEGGdzPathwaysGEO")
```

```
> #write a function to extract required info into a list
> getdataaslist = function(x) {
+     x = get(x)
+     exp = experimentData(x)
+     dataset = exp@name
+     disease = notes(exp)$disease
+     dat.m = exprs(x)
+     ano = pData(x)
+     design = notes(exp)$design
+     annotation = paste(x@annotation, ".db", sep = "")
+     targetGeneSets = notes(exp)$targetGeneSets
+     list = list(dataset, disease, dat.m, ano, design, annotation, targetGeneSets)
+     names(list) = c("dataset", "disease", "dat.m", "ano", "design", "annotation",
+         "targetGeneSets")
+     return(list)
+ }
> dlist = getdataaslist(set)
> #run padog function on KEGG pathways
> #use NI=1000 for accurate results and run in parallel to speed up (see below)
> myr = padog(
+     esetm = dlist$dat.m,
+     group = dlist$ano$Group,
+     paired = dlist$design == "Paired",
+     block = dlist$ano$Block,
+     targetgs = dlist$targetGeneSets,
+     annotation = dlist$annotation,
+     gslist = "KEGGRESTpathway",
+     organism = "hsa",
+     verbose = FALSE,
+     Nmin = 3,
+     NI = 50,
+     plots = TRUE,
+     dseed = 1
+ )
> myr[1:15,-c(4,5)]
```

	Name	ID	Size	PmeanAbsT	Ppadog
03008	<NA>	03008	73	0.0200	2e-04
04976	<NA>	04976	77	0.0002	2e-04
00670	<NA>	00670	34	0.0400	2e-04
00601	<NA>	00601	28	0.0400	2e-04
01523	<NA>	01523	30	0.1000	2e-04
04981	<NA>	04981	28	0.1000	2e-04
04923	<NA>	04923	57	0.0400	2e-04
00450	<NA>	00450	17	0.1200	2e-04
01521	<NA>	01521	78	0.1000	2e-04

```
03013 <NA> 03013  101    0.0400  2e-02
05206 <NA> 05206  159    0.0200  2e-02
00920 <NA> 00920   10    0.0600  2e-02
00910 <NA> 00910   17    0.1000  2e-02
00500 <NA> 00500   31    0.1800  2e-02
05222 <NA> 05222   92    0.1200  2e-02
```



Note that for this colorectal cancer dataset it is reasonbale to expect that the KEGG's Colorectal cancer pathway will be found significant and/or ranked close to the top. PmeanAbsT corresponds to the p-value obtained without using gene weights and hence the result is worse (higher p-value) compared to Ppadog obtained by using the gene weights that are inversly related to how often the genes apear accross all gene sets to be analyzed. The plot created when `plots=TRUE` in the call to `padog` shows how gene weighting improves the gene set analysis for the traget pathway set via the `targetgs` argument. Figure above shows the distribution of pathway/gene set scores (y axis) for PADOG and ABSmT (which is PADOG without weights) after the first standardization (row randomization) and after second standardization (between gene sets standardization). The x axis represents the number of iterations. Iteration 0 uses true class labels, all others used randomly permuted labels. The target pathway (set via the `targetgs` argument) in this dataset is the *Colorectal*

Cancer pathway (KEGG ID 05210). Its score is shown with a red bullet throughout all 4 panels, and a red horizontal line marks its level when obtained with the true class labels ($ite = 0$, x-axis). The box plots of scores obtained with the true class labels are also highlighted in blue. With PADOG, after the second standardization, the target pathway scores obtained from permutations are less frequently above the red line (0/20) (more extreme) than for ABSmT (5/20). Over 1,000 iterations, p_{PADOG} was estimated to be 0.018 while p_{ABSmT} worse, i.e. 0.138.

To run PADOG in parallel, you need to have package `doParallel` installed, and set `parallel = TRUE` in the call to `padog`:

```
> #you can control the number of cores to use via argument 'ncr'
> myr2 = padog(
+     esetm = dlist$dat.m,
+     group = dlist$ano$Group,
+     paired = dlist$design == "Paired",
+     block = dlist$ano$Block,
+     targetgs = dlist$targetGeneSets,
+     annotation = dlist$annotation,
+     gslist = "KEGGRESTpathway",
+     organism = "hsa",
+     verbose = FALSE,
+     Nmin = 3,
+     NI = 50,
+     plots = TRUE,
+     dseed = 1,
+     parallel = TRUE
+ )
> # verify that the result is the same which is a built-in feature
> all.equal(myr, myr2)

[1] TRUE
```

3 Benchmark of gene set analysis methods

The entire collection of 24 datasets available in `KEGGdzPathwaysGEO` package that can be used to benchmark PADOG against existing approaches is given in Table 1:
To illustrate how to compare PADOG against a user defined gene set analysis method we create a function called `randomF` that assignes random uniform P-values to gene sets. The user defined function has to take in 3 arguments:

1. `set`: the name of a dataset available in from the KEGGdzPathwaysGEO package;
2. `mygslist`: a list with elements being vectors of gene ids for a given geneset
3. `minsize`: minimum number of genes in a geneset to be considered for analysis

The output should be a dataframe with columns: ID, P, Rank, Dataset, Method for the geneset(s) considered to be relevant in that dataset (targetGeneSets).

Table 1: **The 24 datasets used in the benchmark of pathway analysis methods**

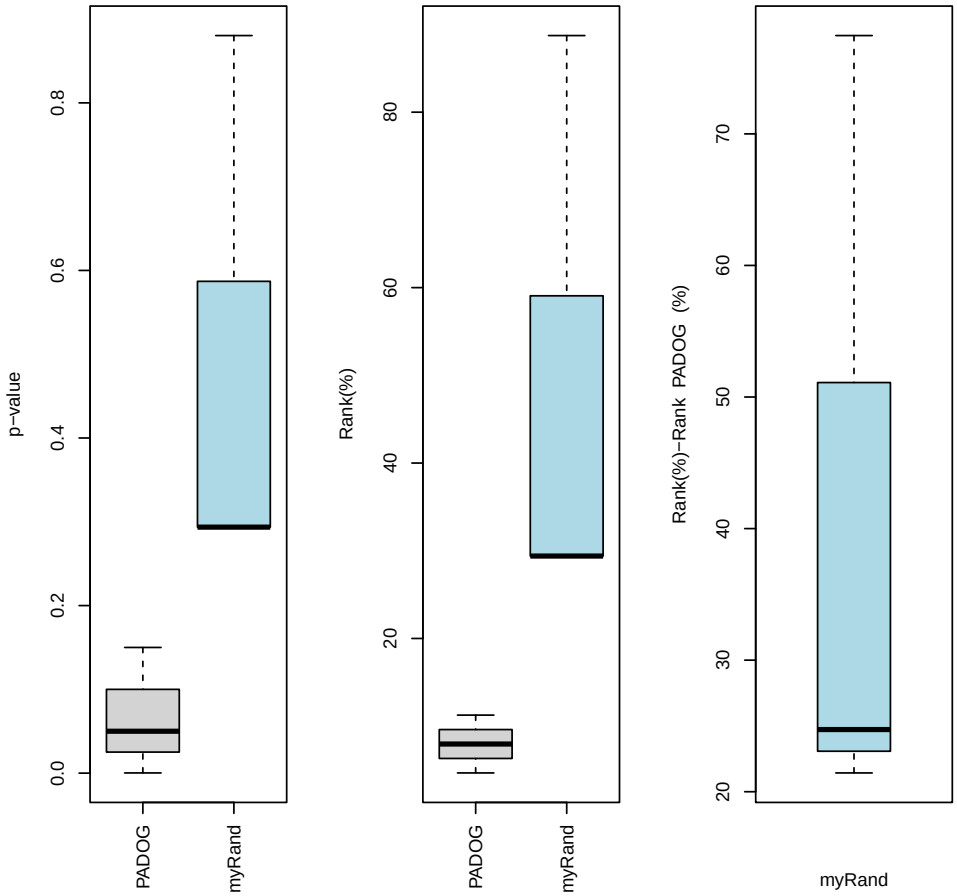
GEOID	Pubmed	Ref.	Disease/Target pathway	KEGGID	Tissue
GSE1297	14769913	Blalock et al. (2004)	Alzheimer's Disease	hsa05010	Hippocampal CA1
GSE5281	17077275	Liang et al. (2007)	Alzheimer's Disease	hsa05010	Brain, Entorhinal Cortex
GSE5281	17077275	Liang et al. (2007)	Alzheimer's Disease	hsa05010	Brain, hippocampus
GSE5281	17077275	Liang et al. (2007)	Alzheimer's Disease	hsa05010	Brain, Primary visual cortex
GSE20153	20926834	Zheng et al. (2010)	Parkinson's disease	hsa05012	Lymphoblasts
GSE20291	15965975	Zhang et al. (2005)	Parkinson's disease	hsa05012	Postmortem brain putamen
GSE8762	17724341	Runne et al. (2007)	Huntington's disease	hsa05016	Lymphocytes (blood)
GSE4107	17317818	Hong et al. (2007)	Colorectal Cancer	hsa05210	Mucosa
GSE8671	18171984	Sabates-Bellver et al. (2007)	Colorectal Cancer	hsa05210	Colon
GSE9348	20143136	Hong et al. (2010)	Colorectal Cancer	hsa05210	Colon
GSE14762	19252501	Wang et al. (2009)	Renal Cancer	hsa05211	Kidney
GSE781	14641932	Lenburg et al. (2003)	Renal Cancer	hsa05211	Kidney
GSE15471	19260470	Badea et al. (2008)	Pancreatic Cancer	hsa05212	Pancreas
GSE16515	19732725	Pei et al. (2009)	Pancreatic Cancer	hsa05212	Pancreas
GSE19728		-	Glioma	hsa05214	Brain
GSE21354		-	Glioma	hsa05214	Brain, Spine
GSE6956	18245496	Wallace et al. (2008)	Prostate Cancer	hsa05215	Prostate
GSE6956	18245496	Wallace et al. (2008)	Prostate Cancer	hsa05215	Prostate
GSE3467	16365291	He et al. (2005)	Thyroid Cancer	hsa05216	Thyroid
GSE3678		-	Thyroid Cancer	hsa05216	Thyroid
GSE9476	17910043	Stirewalt et al. (2008)	Acute myeloid leukemia	hsa05221	Blood, Bone marrow
GSE18842	20878980	Sanchez-Palencia et al. (2010)	Non-Small Cell Lung Cancer	hsa05223	Lung
GSE19188	20421987	Hou et al. (2010)	Non-Small Cell Lung Cancer	hsa05223	Lung
GSE3585	17045896	Barth et al. (2006)	Dilated cardiomyopathy	hsa05414	Heart

The 24 datasets used to compare the pathway analysis methods were obtained from GEO.

```
> randFun = function(dseed, mname = "myRand") {
+     #a helper function to pass additional variables to your method
+     getdataaslist = getdataaslist
+     return(function(set, mygslist, minsize) {#your method function
+         set.seed(dseed)
+         #this loads the dataset
+         data(list = set, package = "KEGGdzPathwaysGEO")
+         #extract the required info using the function defined earlier
+         dlist = getdataaslist(set)
+         #get rid of duplicates probesets per ENTREZ ID by keeping the probeset
+         #with smallest p-value (computed using limma)
+         aT1 = filteranot(esetm = dlist$dat.m, group = dlist$ano$Group,
+             paired = dlist$design == "Paired", block = dlist$ano$Block,
+             annotation = dlist$annotation)
+         #create an output dataframe for this toy method with random gene set p-values
+         mygslistSize = unlist(lapply(mygslist, function(x) {
+             length(intersect(aT1$ENTREZID, x))
+         }))
```

```
+         res = data.frame(ID = names(mygslist), P = runif(length(mygslist)),
+             Size = mygslistSize, stringsAsFactors = FALSE)
+         res$FDR = p.adjust(res$P,"fdr")
+         #drop genesets with less than minsize genes in the current dataset
+         res = res[res$Size >= minsize,]
+         #compute ranks
+         res$Rank = rank(res$P) / dim(res)[1]*100
+         #needed to compare ranks between methods; must be the same as given
+         #in mymethods argument "list(myRand="
+         res$Method = mname
+         #needed because comparisons of ranks between methods is paired at dataset level
+         res$Dataset = dlist$dataset
+         #output only result for the targetGeneSets
+         #which are gene sets expected to be relevant in this dataset
+         return(res[res$ID %in% dlist$targetGeneSets,])
+         }
+     )
+ }
> randomF = randFun(1)
> #run the analysis on all 24 datasets and compare the new method "myRand" with
> #PADOG and GSA (if installed) (chosen as reference since is listed first in the
> #existingMethods)
> #if the package doParallel is installed datasets are analyzed in parallel.
> #out = compPADOG(datasets = NULL, existingMethods = c("GSA","PADOG"),
> #    mymethods = list(myRand = randomF), gslist = "KEGGRESTpathway",
> #    Nmin = 3, NI = 1000, plots = TRUE, verbose=FALSE,
> #    parallel = TRUE, dseed = 1, pkgs = NULL)
>
> #compare myRand against PADOG on 3 datasets only
> #mysets = data(package = "KEGGdzPathwaysGEO")$results[,"Item"]
> mysets = c("GSE9348","GSE8671","GSE1297")
> out = compPADOG(datasets = mysets, existingMethods = c("PADOG"),
+     mymethods = list(myRand = randomF),
+     gslist = "KEGGRESTpathway", Nmin = 3, NI = 40, plots = TRUE,
+     verbose=FALSE, parallel = TRUE, dseed = 1, pkgs = NULL)
> print(out)

$summary
       Method    p geomean        p med % p.value<0.05 % q.value<0.05
PADOG   PADOG 0.012331....         0.05          33.33          33.33
myRand myRand 0.423372.... 0.293605....             0              0
         rank mean     rank med p Wilcox.     p LME coef. LME
PADOG  7.967032.... 7.967032....         1 1.0000000   0.00000
myRand 49.17582.... 29.39560....         1 0.9653858  41.20879

$ranks
```

```
$ranks$PADOG
[1] 11.263736  4.670330  7.967033

$ranks$myRand
[1] 88.73626 29.39560 29.39560


$pvalues
$pvalues$PADOG
[1] 0.15000 0.00025 0.05000

$pvalues$myRand
[1] 0.8803191 0.2936055 0.2936055


$qvalues
$qvalues$PADOG
[1] 0.947318612 0.005352941 0.587096774

$qvalues$myRand
[1] 0.9886705 0.9145159 0.9145159

>
```



Details about the meaning of the columns in the out table are given in Tarca et al. (2012). The better the method, the smaller the p-values and ranks for the target pathways, since these are supposted to be significant to their respective datasets.

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