

sigPathway: Pathway Analysis with Microarray Data

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1 Introduction

sigPathway is an R package that performs pathway (gene set) analysis on microarray data. It calculates two gene set statistics, the NT_k (Q1) and NE_k (Q2), by permutation, ranks the pathways based on the magnitudes of the two statistical tests, and estimates q-values for each pathway (Tian et al., 2005). The program permutes the rows and columns of the expression matrix for NT_k and NE_k , respectively. In this vignette, we demonstrate how the user can use this package to identify statistically significant pathways in their data and export the results to HTML for browsing.

2 Data

In Tian et al. (2005), microarray data from patients with diabetes, inflammatory myopathies, and Alzheimers' data sets were analyzed. To save disk space, a small portion of the inflammatory myopathies data set has been included with *sigPathway* as an example data set. Expression values and annotations for this data set are stored in the `MuscleExample` workspace. This workspace contains the following R objects:

tab a filtered numeric matrix containing expression values from 7/13 normal (NORM) and 8/23 inclusion body myositis (IBM) samples. The row and column names of the matrix correspond to Affymetrix probe set IDs and sample IDs, respectively. The 5000 probe sets in this matrix represent the most variable probe sets (by expression value) in the 15 arrays.

phenotype a character vector with 0_NORM to represent NORM and 1_IBM to represent IBM

G a pathway annotation list containing the pathway's source, title, and associated probe set IDs

To load this data set, type 'data(MuscleExample)' after loading the *sigPathway* package.

The pathways annotated in **G** were curated from Gene Ontology, KEGG, BioCarta, BioCyc, and SuperArray. Each element *within* **G** is a list describing a pathway with the following sub-elements:

src a character vector containing either the pathway ID (for Gene Ontology) or the name of the pathway database

title a character vector containing the pathway name

probes a character vector containing probe set IDs that are associated with the pathway (by mapping them to Entrez Gene IDs)

The full inflammatory myopathway data set and pathway annotations for other, selected Affymetrix microarray platforms are available at <http://www.chip.org/~ppark/Supplements/PNAS05.html>. For example, the more comprehensive pathway annotation list for the Affymetrix HG-U133A platform is called *GenesetsU133a*. For arrays not listed on the website (or for scenarios such as linkage analysis), the user can make his/her own pathway annotations and use them in *sigPathway* as long as the pathway annotations are arranged in the above format.

3 Example

In this section, we show the R code necessary to conduct pathway analysis with *sigPathway* on an example data set.

First, we load *sigPathway* and the example data set into memory. If we are dealing with the full data set, we could remove probe sets that have expression values less than the trimmed mean in all of the arrays. We assume that the probe sets with lower expression values across all arrays are not of interest. The trimmed mean was used as the filtering criterion in Tian et al. (2005). The probe sets in the example data set were selected for their variance across 15 arrays (not shown).

```
> library(sigPathway)
> data(MuscleExample)
> ls()

[1] "G"          "phenotype" "tab"

>
```

For microarray data, the convention is to use rows and columns to represent probe sets and individual arrays, respectively. To tell the program which column in **tab** belongs to which phenotype, we have created a character vector with 0_NORM to represent NORM and 1_IBM to represent IBM. Because 0_NORM comes before 1_IBM in alphanumeric order, the program internally treats NORM as 0 and IBM as 1. Alternatively, we could have simply used the numerals 0 and 1 to represent NORM and IBM. Note that the row names for **tab** are probe set IDs.

```
> dim(tab)
```

```
[1] 5000 15
```

```
> print(tab[501:504, 1:3])
```

	GEIM1.IBM.S	GEIM7.IBM.S	GEIM20.IBM.S
217466_x_at	3203	4085	23736
211939_x_at	28250	32293	36890
203932_at	6452	3596	13392
200715_x_at	20792	12647	18865

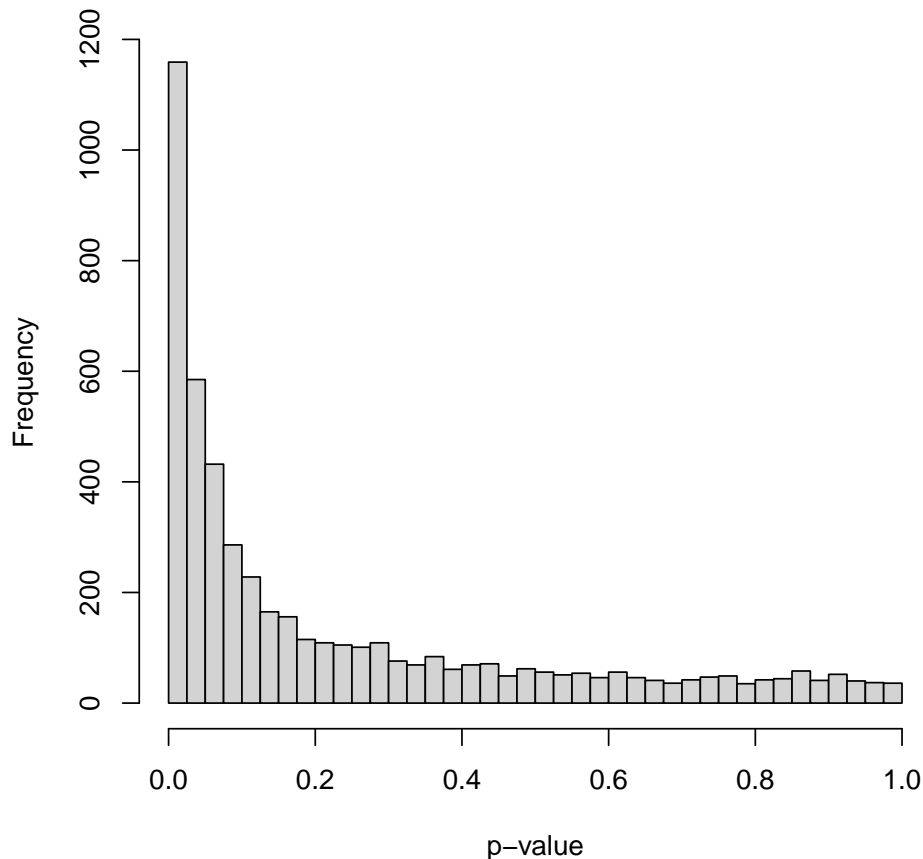
```
> table(phenotype)
```

```
phenotype
0_NORM  1_IBM
      7      8
```

```
>
```

How much do IBM and NORM samples differ? Let us plot the unadjusted p-values for each probe set from the 2 group (sample) t-test, assuming unequal variances and using the Welch approximation to estimate the appropriate degrees of freedom.

```
> statList <- calcTStatFast(tab, phenotype, ngroups = 2)
> hist(statList$pval, breaks = seq(0,1,0.025), xlab = "p-value",
+       ylab = "Frequency", main = "")
```



The two different types of samples are certainly very different by the probe set level, but what pathways are driving the differences? With our pathway annotations, we calculate the NT_k and NE_k statistics for each gene set, and rank the top pathways based on the magnitude of the two statistics. The result is stored in a list (`res.muscle`), of which we will later use to write results to HTML.

```
> set.seed(1234)
> res.muscle <-
+   runSigPathway(G, 20, 500, tab, phenotype, nsim = 1000,
+                 weightType = "constant", ngroups = 2, npath = 25,
+                 verbose = FALSE, allpathways = FALSE, annotpkg = "hgu133a.db",
+                 alwaysUseRandomPerm = FALSE)
```

Selecting the gene sets

Calculating NT_k statistics for each selected gene set

Calculating NE_k statistics for each selected gene set

Summarizing the top 25 pathways from each statistic

Done! Use the `writeSigPathway()` function to write results to HTML

The `set.seed` function is used here only for the purpose of getting the exact results when regenerating this vignette from its source files.

Because there can be many thousands of pathways represented in the pathway annotations, we have chosen to analyze pathways that contain at least 20 probe sets as represented in `tab`. We also exclude pathways represented by more than 500 probe sets because larger pathways tend to be non-specific. These two values were the ones used in Tian et al. (2005). To save space, our pathway annotation list has already been filtered with the above criteria. So, all of the 626 pathways in `G` will be considered in the calculations.

The run time of the NT_k and NE_k is approximately linearly proportional to `nsim`, or the maximum number of permutations. When `alwaysUseRandomPerm` is set to `FALSE` (the default value), the program will use a smaller `nsim` for the NE_k calculations and switch to using complete permutation if the total number of unique permutations for the phenotype is less than `nsim`.

We are setting `weightType` to 'constant' because of the additional time required to calculate variable weights for NE_k . If the histogram of unadjusted p-values (of the probe sets) is nearly horizontal, and we later observe high q-values (i.e., approaching 1) for the top ranked pathways, then setting `weightType` to 'variable' would help lower some of the NE_k q-values.

To rank the pathways, the program adds up the ranks corresponding to the magnitudes of NT_k and NE_k . When `npath` is set to 25 and `allpathways` to `FALSE`, the program considers the top 25 pathways for each gene set statistic before summing the individual ranks. If `allpathways` is set to `TRUE`, then all pathways are ranked for each gene set statistic before summing the individual ranks. Here, `allpathways` is set to `FALSE` because we are interested in observing pathways that are consistently highly ranked for each gene set statistic.

Also, please note that out of the numerous input parameters to `runSigPathway`, `annotpkg` is optional because it refers to a Bioconductor metadata package that may not already be present on your installation of R. In our example, 'hgu133a.db' refers to the BioConductor metadata package of the Affymetrix HG-U133A platform. By specifying 'hgu133a.db' for `annotpkg`, `runSigPathway` will include the accession number, Entrez Gene ID, gene symbol, and gene name of probe sets associated with each pathway in the list of top pathways.

Printed below is a table of the top 10 pathways, the set size, the NT_k and NE_k statistics, and the statistics' ranks and q-values. This table is accessible through the following command:

```
> print(res.muscle$df.pathways[1:10, ])
```

Index	Gene Set	Category	Pathway	Set Size	Percent Up
1	234	G0:0019883	antigen presentation, endogenous antigen	22	0.00
2	292	G0:0042611	MHC protein complex	20	0.00
3	293	G0:0042612	MHC class I protein complex	20	0.00
4	233	G0:0019882	antigen presentation	45	0.00
5	84	G0:0030333	antigen processing	44	0.00
6	237	G0:0019885			
7	117	G0:0030106			
8	92	G0:0001772			
9	613	humanpaths			
10	601	humanpaths			

6	antigen processing, endogenous antigen via MHC class I					23	0.00
7	MHC class I receptor activity					22	0.00
8	immunological synapse					26	0.00
9	Interferon a,b Response					71	12.68
10	Dendritic / Antigen Presenting Cell					105	5.71
	Ntk Stat	Ntk q-value	Ntk Rank	Nek Stat	Nek q-value	Nek Rank	
1	18.97	0	3	9.33	0	2	
2	17.83	0	6	9.36	0	1	
3	17.83	0	6	9.36	0	1	
4	19.41	0	1	7.24	0	7	
5	19.03	0	2	7.26	0	6	
6	18.44	0	4	9.11	0	4	
7	18.37	0	5	9.28	0	3	
8	16.95	0	7	8.27	0	5	
9	10.79	0	8	4.83	0	9	
10	10.66	0	9	3.62	0	11	

The positive signs on the gene set statistics indicate that the corresponding pathways are more highly expressed in IBM compared to NORM. Had we defined 1 for NORM and 0 for IBM, the interpretation would remain the same, but we would expect the signs for the gene set statistics to be flipped.

Detailed information about each probe set in each pathway on the list of top pathways are stored in the `list.gPS`, an element within `res.muscle`. `list.gPS` is a list containing data frames describing the probe sets for each top pathway. For example, let us view the annotations and test statistics for 10 probe sets in the *MHC class I receptor activity* pathway.

```
> print(res.muscle$list.gPS[[7]][1:10, ])
```

Probes	AccNum	GeneID	Symbol	Name	Mean_0_NORM	Mean_1_IBM
201891_s_at	201891_s_at	NM_004048	567 B2M	beta-2-microglobulin	38735.143	64165.88
216231_s_at	216231_s_at	AW188940	567 B2M	beta-2-microglobulin	43285.857	78550.75
218831_s_at	218831_s_at	NM_004107	2217 FCGRT	Fc fragment of IgG receptor and transporter	1592.000	4444.00
213932_x_at	213932_x_at	AI923492	3105 HLA-A	major histocompatibility complex, class I, A	23739.857	65602.12
215313_x_at	215313_x_at	AA573862	3105 HLA-A	major histocompatibility complex, class I, A	20685.286	68365.12
208729_x_at	208729_x_at	D83043	3106 HLA-B	major histocompatibility complex, class I, B	6648.571	46637.62
209140_x_at	209140_x_at	L42024	3106 HLA-B	major histocompatibility complex, class I, B	12258.857	65679.25
211911_x_at	211911_x_at	L07950	3106 HLA-B	major histocompatibility complex, class I, B	9150.286	53755.88
208812_x_at	208812_x_at	BC004489	3107 HLA-C			
211799_x_at	211799_x_at	U62824	3107 HLA-C			

208812_x_at	major histocompatibility complex, class I, C	13994.429	62945.38
211799_x_at	major histocompatibility complex, class I, C	2167.571	23667.38
	StDev_0_NORM	StDev_1_IBM	T-Statistic
	p-value		
201891_s_at	5551.0402	7325.835	7.629433
216231_s_at	4350.8622	9728.212	9.250160
218831_s_at	351.2762	2263.444	3.515833
213932_x_at	6463.6639	8931.066	10.485603
215313_x_at	5568.5959	9316.500	12.197747
208729_x_at	609.5618	11082.266	10.188448
209140_x_at	1433.5514	5988.982	24.441414
211911_x_at	2499.9600	14031.821	8.832473
208812_x_at	1825.2766	8910.416	15.178761
211799_x_at	451.3210	8454.758	7.180791

A much more intuitive method to browse through the results is to write the results to HTML, which can then be read by an Internet browser program (e.g., Mozilla Firefox, Microsoft Internet Explorer). Writing the results can be achieved with the `writeSigPathway` function. Please refer to the help file of `writeSigPathway` for more details on how to save to results to a specific directory. Figures 1 and 2 show examples of the HTML output after running `writeSigPathway` and opening the corresponding HTML file in an Internet browser.

4 Notes

This vignette was compiled with the following settings:

```
> print(sessionInfo())
```

```
R version 4.0.3 (2020-10-10)
```

```
Platform: x86_64-pc-linux-gnu (64-bit)
```

```
Running under: Ubuntu 18.04.5 LTS
```

```
Matrix products: default
```

```
BLAS: /home/biocbuild/bbs-3.12-bioc/R/lib/libRblas.so
```

```
LAPACK: /home/biocbuild/bbs-3.12-bioc/R/lib/libRlapack.so
```

```
locale:
```

```
[1] LC_CTYPE=en_US.UTF-8
```

```
LC_NUMERIC=C
```

```
[3] LC_TIME=en_US.UTF-8
```

```
LC_COLLATE=C
```

```
[5] LC_MONETARY=en_US.UTF-8
```

```
LC_MESSAGES=en_US.UTF-8
```

```
[7] LC_PAPER=en_US.UTF-8
```

```
LC_NAME=C
```

```
[9] LC_ADDRESS=C
```

```
LC_TELEPHONE=C
```

```
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
```

```
attached base packages:
```

```
[1] parallel stats4 stats graphics grDevices utils datasets
```

```
[8] methods base
```

```
other attached packages:
```

sigPathway_results/TopPathwaysTable.html

List of Top Pathways

IndexG	Gene Set Category	Pathway	Set Size	Percent Up	NTk Stat	NTk q-value	NTk Rank	NEk Stat	NEk q-value	NEk Rank
1	234 GO:0019883	antigen presentation, endogenous antigen	22	100	18.97	0.0000	3.0	9.33	0.0000	2.0
2	292 GO:0042611	MHC protein complex	20	100	17.83	0.0000	6.0	9.36	0.0000	1.0
3	293 GO:0042612	MHC class I protein complex	20	100	17.83	0.0000	6.0	9.36	0.0000	1.0
4	233 GO:0019882	antigen presentation	45	100	19.41	0.0000	1.0	7.24	0.0000	7.0
5	84 GO:0030333	antigen processing	44	100	19.03	0.0000	2.0	7.26	0.0000	6.0
6	237 GO:0019885	antigen processing, endogenous antigen via MHC class I	23	100	18.44	0.0000	4.0	9.11	0.0000	4.0
7	117 GO:0030106	MHC class I receptor activity	22	100	18.37	0.0000	5.0	9.28	0.0000	3.0
8	92 GO:0001772	immunological synapse	26	100	16.95	0.0000	7.0	8.27	0.0000	5.0
9	613 humanpaths	Interferon a,b Response	71	87	10.79	0.0000	8.0	4.83	0.0000	9.0
10	601 humanpaths	Dendritic / Antigen Presenting Cell	105	94	10.66	0.0000	9.0	3.62	0.0000	11.0
11	19 GO:0045012	MHC class II receptor activity	21	100	8.45	0.0000	21.0	4.91	0.0000	8.0
12	236 GO:0019884	antigen presentation, exogenous antigen	21	100	8.45	0.0000	21.0	4.91	0.0000	8.0
13	238 GO:0019886	antigen processing, exogenous antigen via MHC class II	21	100	8.45	0.0000	21.0	4.91	0.0000	8.0
14	481 GO:0009615	response to virus	31	87	5.10	0.0000	75.0	3.84	0.0000	10.0
15	576 KEGG	Jak-STAT signaling pathway	38	87	4.90	0.0000	84.0	3.29	0.0000	18.0
16	40 GO:0006968	cellular defense response	35	89	4.73	0.0000	93.0	3.54	0.0000	13.0
17	42 GO:0006959	humoral immune response	46	91	4.81	0.0000	86.0	3.19	0.0000	21.0
18	612 humanpaths	Th1-Th2-Th3	34	88	4.21	0.0000	114.0	3.31	0.0000	17.0
19	575 KEGG	Toll-like receptor signaling pathway	40	85	4.14	0.0000	117.0	3.27	0.0000	19.0
20	625 humanpaths	Asthma	20	100	3.69	0.0000	137.0	3.19	0.0000	22.0
21	470 GO:0043085	positive regulation of enzyme activity	29	86	3.45	0.0000	147.0	3.36	0.0000	15.0
22	89 GO:0045333	cellular respiration	40	18	-7.82	0.0000	22.0	-2.01	0.0285	174.0
23	526 BioCarta	p38 MAPK Signaling Pathway	24	92	2.88	0.0042	191.0	3.33	0.0000	16.0
24	18 GO:0005884	actin filament	26	73	2.51	0.0107	222.5	3.56	0.0000	12.0
25	529 BioCarta	Activation of Csk by cAMP-dependent Protein Kinase	27	74	2.37	0.0154	236.0	3.26	0.0000	20.0

Figure 1: List of Top Pathways in Inclusion Body Myositis versus Normal

sigPathway_results/pathways/pathway_117.html

[Back to Table of Top Pathways](#)

MHC class I receptor activity

	Probes	AccNum	GeneID	Symbol	Name	Mean_0_NORM	Mean_1_IBM	StDev_0_NORM	StDev_1_IBM	T-Statistic	p-value
1	201891_s_at	NM_004048	567	B2M	beta-2-microglobulin	38735.1	64165.9	5551.0	7325.8	7.629	0.0000
2	216231_s_at	AW188940	567	B2M	beta-2-microglobulin	43285.9	78550.8	4350.9	9728.2	9.250	0.0000
3	218831_s_at	NM_004107	2217	FCGRT	Fc fragment of IgG, receptor, transporter, alpha	1592.0	4444.0	351.3	2263.4	3.516	0.0090
4	213932_x_at	AI923492	80862	C6orf12	chromosome 6 open reading frame 12	23739.9	65602.1	6463.7	8931.1	10.486	0.0000
5	215313_x_at	AA573862	3105	HLA-A	major histocompatibility complex, class I, A	20685.3	68365.1	5568.6	9316.5	12.198	0.0000
6	208729_x_at	D83043	3106	HLA-B	major histocompatibility complex, class I, B	6648.6	46637.6	609.6	11082.3	10.188	0.0000
7	209140_x_at	L42024	3106	HLA-B	major histocompatibility complex, class I, B	12258.9	65679.3	1433.6	5989.0	24.441	0.0000
8	211911_x_at	L07950	3106	HLA-B	major histocompatibility complex, class I, B	9150.3	53755.9	2500.0	14031.8	8.832	0.0000
9	208812_x_at	BC004489	3107	HLA-C	major histocompatibility complex, class I, C	13994.4	62945.4	1825.3	8910.4	15.179	0.0000
10	211799_x_at	U62824	3107	HLA-C	major histocompatibility complex, class I, C	2167.6	23667.4	451.3	8454.8	7.181	0.0002
11	214459_x_at	M12679	3107	HLA-C	major histocompatibility complex, class I, C	10482.7	60946.4	2644.2	12988.9	10.738	0.0000
12	216526_x_at	AK024836	3107	HLA-C	major histocompatibility complex, class I, C	17840.3	76157.5	4994.1	8878.6	15.921	0.0000
13	200904_at	X56841	3133	HLA-E	major histocompatibility complex, class I, E	2283.6	12515.5	314.1	4947.8	5.836	0.0006
14	200905_x_at	NM_005516	3133	HLA-E	major histocompatibility complex, class I, E	4583.7	24874.1	721.7	7933.4	7.200	0.0002
15	217456_x_at	M31183	3133	HLA-E	major histocompatibility complex, class I, E	2692.0	9809.9	492.5	2175.7	8.994	0.0000
16	204806_x_at	NM_018950	3134	HLA-F	major histocompatibility complex, class I, F	4062.1	29127.1	829.9	10796.3	6.545	0.0003
17	221875_x_at	AW514210	3134	HLA-F	major histocompatibility complex, class I, F	5604.4	36141.0	937.4	9840.3	8.732	0.0000

Figure 2: MHC class I receptor activity

```
[1] hgu133a.db_3.2.3      org.Hs.eg.db_3.12.0  AnnotationDbi_1.52.0
[4] IRanges_2.24.0       S4Vectors_0.28.0    Biobase_2.50.0
[7] BiocGenerics_0.36.0  sigPathway_1.58.0
```

loaded via a namespace (and not attached):

```
[1] Rcpp_1.0.5      digest_0.6.27  DBI_1.1.0      RSQLite_2.2.1
[5] rlang_0.4.8     blob_1.2.1     vctrs_0.3.4    tools_4.0.3
[9] bit64_4.0.5     bit_4.0.4      compiler_4.0.3 pkgconfig_2.0.3
[13] memoise_1.1.0
```

References

Lu Tian, Steven A Greenberg, Sek Won Kong, Josiah Altschuler, Isaac S Kohane, and Peter J Park. Discovering statistically significant pathways in expression profiling studies. *Proc Natl Acad Sci U S A*, 102(38):13544–13549, Sep 2005.