sigPathway: Pathway Analysis with Microarray Data

Weil Lai[1], Lu Tian[2], and Peter Park[1,3]

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1. Harvard-Partners Center for Genetics and Genomics, 77 Avenue Louis Pasteur, Boston, MA 02115
2. Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, 680 North Lake Shore Drive, Chicago, IL 60611
3. Children's Hospital Informatics Program, 300 Longwood Avenue, Boston, MA 02115

Contents

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 criteron 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"

>
```

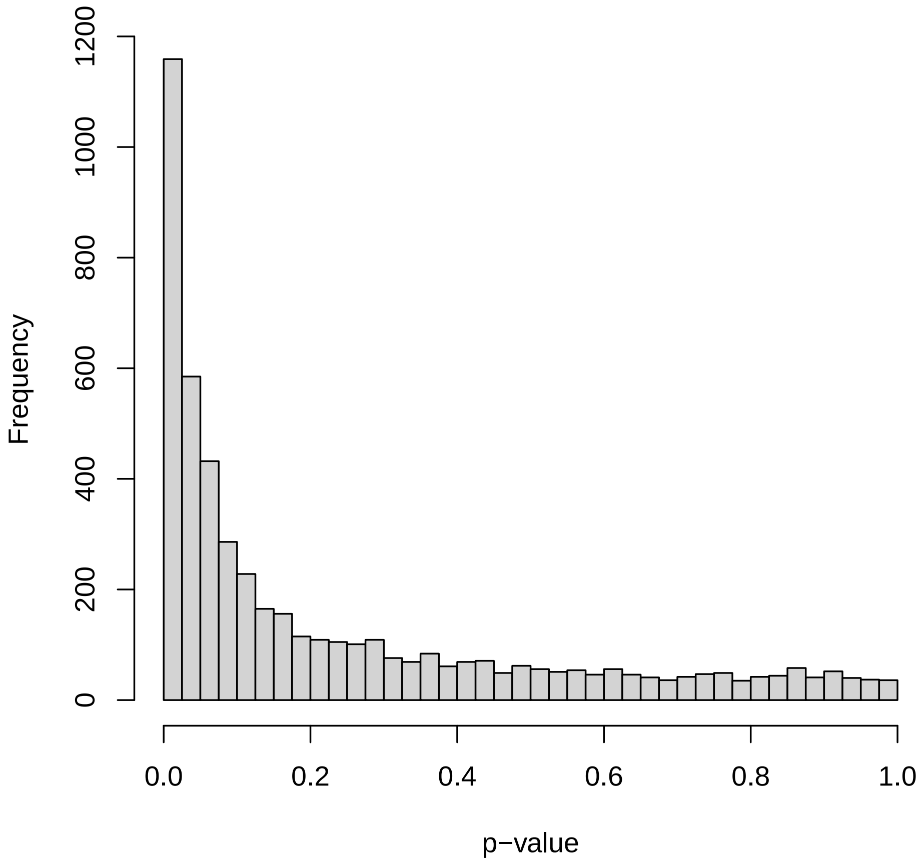
For microarray data, the convention is to use rows and columns to represent probe sets and individual arrays, respectively. To tell the program which column in `tab` belongs to which phenotype, we have created a character vector with `0_NORM` to represent NORM and `1_IBM` to represent IBM. Because `0_NORM` 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 NTk statistics for each selected gene set
Calculating NEk 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, ])
```

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

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

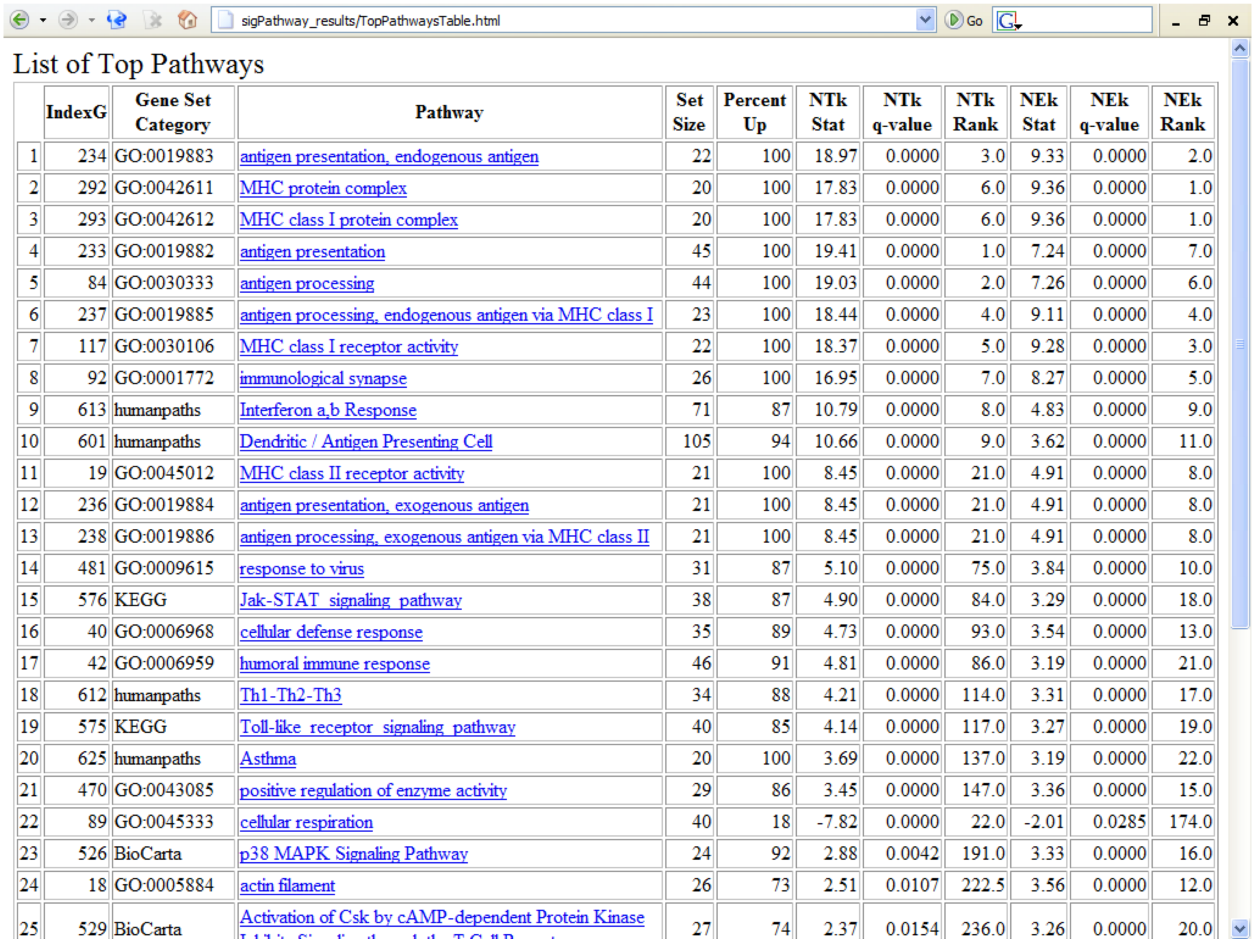
```
208812_x_at major histocompatibility complex, class I, C   13994.429   62945.38
211799_x_at major histocompatibility complex, class I, C    2167.571   23667.38
            StDev_0_NORM StDev_1_IBM T-Statistic      p-value
201891_s_at    5551.0402    7325.835    7.629433 4.155125e-06
216231_s_at    4350.8622    9728.212    9.250160 3.329180e-06
218831_s_at     351.2762    2263.444    3.515833 8.973576e-03
213932_x_at    6463.6639    8931.066   10.485603 1.363431e-07
215313_x_at    5568.5959    9316.500   12.197747 5.615578e-08
208729_x_at     609.5618   11082.266   10.188448 1.804436e-05
209140_x_at    1433.5514    5988.982   24.441414 9.843999e-09
211911_x_at    2499.9600   14031.821    8.832473 3.162204e-05
208812_x_at    1825.2766    8910.416   15.178761 5.386934e-07
211799_x_at     451.3210    8454.758    7.180791 1.749504e-04
```

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:

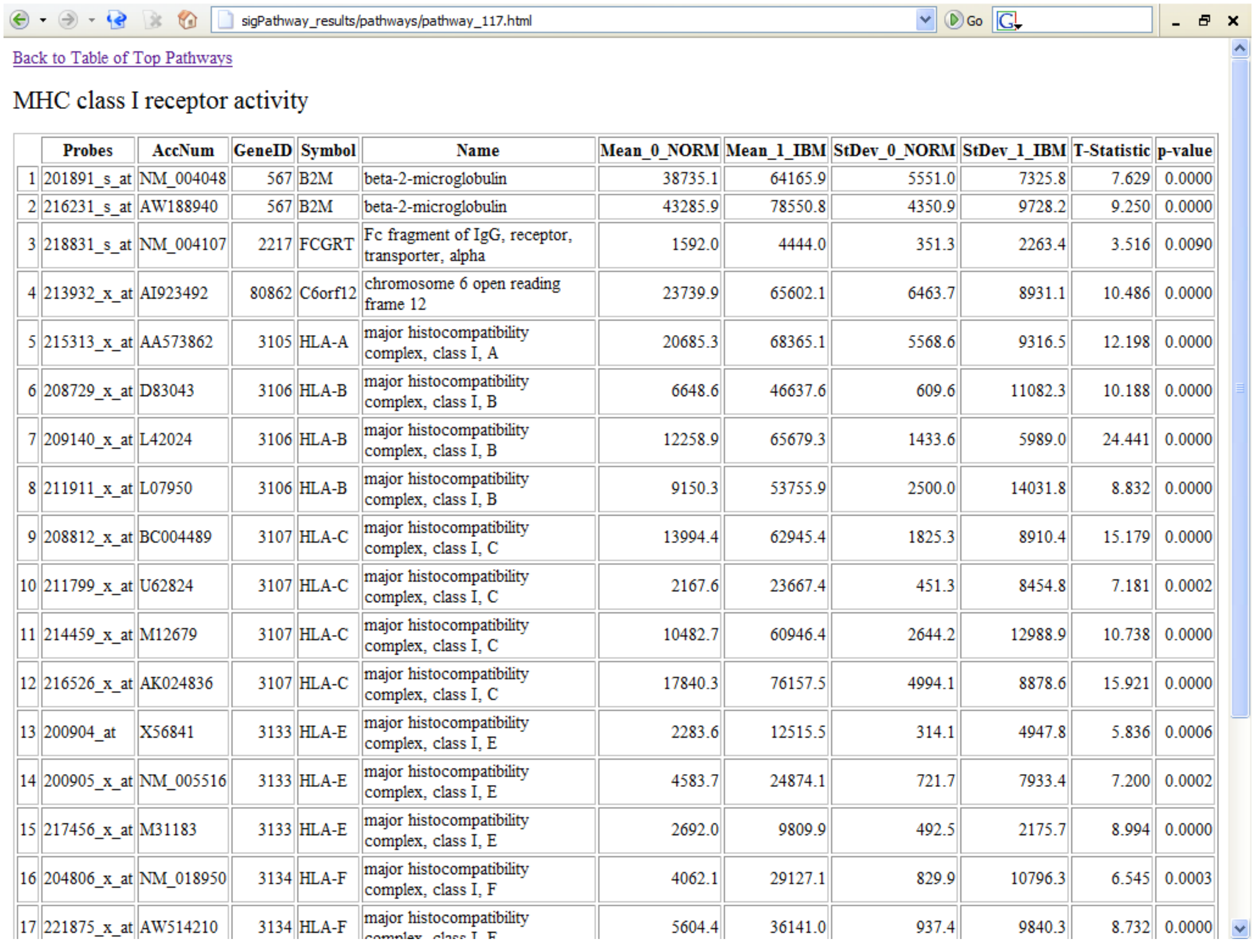
```
> print(sessionInfo())

R version 4.1.1 (2021-08-10)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 20.04.3 LTS

Matrix products: default
BLAS:   /home/biocbuild/bbs-3.14-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.14-bioc/R/lib/libRlapack.so

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_GB              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] stats4    stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
```



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



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_3.13.0    org.Hs.eg.db_3.14.0  AnnotationDbi_1.56.0
[4] IRanges_2.28.0       S4Vectors_0.32.0     Biobase_2.54.0
[7] BiocGenerics_0.40.0  sigPathway_1.62.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.7             rstudioapi_0.13        XVector_0.34.0
 [4] zlibbioc_1.40.0        bit_4.0.4              R6_2.5.1
 [7] rlang_0.4.12           fastmap_1.1.0          blob_1.2.2
[10] httr_1.4.2             GenomeInfoDb_1.30.0    tools_4.1.1
[13] png_0.1-7              DBI_1.1.1              bit64_4.0.5
[16] crayon_1.4.1           GenomeInfoDbData_1.2.7 bitops_1.0-7
[19] vctrs_0.3.8            KEGGREST_1.34.0        RCurl_1.98-1.5
[22] memoise_2.0.0          cachem_1.0.6           RSQLite_2.2.8
[25] compiler_4.1.1         Biostrings_2.62.0      pkgconfig_2.0.3
```

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.