

PatientGeneSets package

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April 14, 2011

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1 Overview

Patient-oriented methods are a novel approach to gene-set analysis which calculate gene-set scores for each sample, then combine them across samples. By comparison, gene-oriented methods calculate a score for each gene across all samples, then combine them into gene-set scores. We find that for cancer mutation data, patient-oriented methods often perform better on both real and simulated data.

The `PatientGeneSets` package has functions which perform gene-set analyses for cancer mutation data. It can be used to compute p-values and q-values for 4 patient-oriented methods described in [2], as well as the gene-oriented method described in [10] (see also [8]), which is in the `limma` package and performs a Wilcoxon test. It can also be used to do the simulations described in [2]. The user has a choice between calculating q-values based on the FDR-control method in [1] or [11]. This package is closely related to the `CancerMutationAnalysis` package, available at <http://bcb.dfci.harvard.edu/~gp/software/CancerMutationAnalysis/cma.htm> ([6]), which provides methods for gene-level analysis of cancer mutation data. In particular, the `cma.scores` function and the data format are nearly identical, but the `PatientGeneSets` package does not consider the two-phase design when performing gene-set analysis.

This vignette represents an introduction on the `PatientGeneSets` package. The primary function `do.gene.set.analysis`, which implements the gene-set analysis methods. The function

`sim.data.p.values` performs simulations using either the permutation null or the passenger null (see [2]). The function `cma.data` calculates scores for each gene across all samples, as in [9] and [12]. The functions `extract.sims.method` and `combine.sims` are used to manipulate the objects resulting from `sim.data.p.values`.

2 Glioblastoma dataset

We use the glioblastoma dataset from [7]. When typing `data(Parsons)`, 4 objects are loaded: `CoverageBrain`, `EventsBySampleBrain`, `GeneSizes08` and `MutationsBrain`. For this example, we use KEGG annotations ([3], [5], [4]). from the Bioconductor package `KEGG.db` by using the `KEGGPATHID2EXTID` object.

```
> library(PatientGeneSets)
> data(Parsons)
> ls()

[1] "CoverageBrain"      "EventsBySampleBrain" "GeneSizes08"
[4] "MutationsBrain"

> library(KEGG.db)
> KEGGPATHID2EXTID
```

PATHID2EXTID map for KEGG (object of class "AnnDbBimap")

Since the genes in the glioblastoma dataset are annotated by gene-names, while `KEGGPATHID2EXTID` uses EntrezGene identifiers, we also provide a vector mapping the identifiers to the names, which we obtained by using the `biomaRt` package.

```
> data(ID2name)
> head(ID2name)

      10      100      1000      10000      10007      1001
"NAT2"  "ADA"  "CDH2"  "AKT3" "GNPDA1" "CDH3"
```

3 Implementing the gene-set analysis methods

The function `do.gene.set.analysis` returns a data-frame with p-values and q-values for all the methods selected. By default, all the methods are implemented; however this takes several minutes. Setting the `gene.method` parameter to `TRUE` implements the gene-oriented method. The other method parameters refer to the patient-oriented methods: `perm.null.method` refers to the permutation null without heterogeneity, `perm.null.het.method` to the permutation null with heterogeneity, `pass.null.method` to the passenger null without heterogeneity, and `pass.null.het.method` to the passenger null with heterogeneity. See [2] for more details on all these methods.

The gene-sets we use correspond to the KEGG annotations for endometrial cancer, non-small cell lung cancer, and alanine, aspartate and glutamate metabolism i.e. `hsa05213`, `hsa05223`, and `hsa00250`:

```
> as.character(KEGGPATHNAME2ID[c("Endometrial cancer", "Non-small cell lung cancer",
+ "Alanine, aspartate and glutamate metabolism")])
```

```

Alanine, aspartate and glutamate metabolism
      "00250"
      Endometrial cancer
      "05213"
Non-small cell lung cancer
      "05223"

```

```

> resultsBrain <- do.gene.set.analysis(EventsBySample = EventsBySampleBrain,
+   GeneSizes = GeneSizes08, GeneSets = KEGGPATHID2EXTID[c("hsa05213",
+     "hsa05223", "hsa00250")], Coverage = CoverageBrain, ID2name = ID2name,
+   gene.method = FALSE, perm.null.method = TRUE, perm.null.het.method = FALSE,
+   pass.null.method = TRUE, pass.null.het.method = FALSE)

```

```

[1] "Permutation null w/o heterogeneity"
[1] "Thu Apr 14 00:47:22 2011"
[1] "Passenger null w/o heterogeneity"
[1] "Thu Apr 14 00:47:22 2011"
[1] "Thu Apr 14 00:47:24 2011"

```

The resulting object is a data-frame, with NAs for the methods which were not implemented:

```

> resultsBrain

      p.values.gene q.values.gene p.values.perm.null q.values.perm.null
hsa05213          NA             1          9.019202e-16          2.705761e-15
hsa05223          NA             1          1.644843e-15          2.467264e-15
hsa00250          NA             1          3.105934e-01          3.105934e-01
      p.values.perm.null.het q.values.perm.null.het p.values.pass.null
hsa05213                   NA                       1          2.108377e-10
hsa05223                   NA                       1          2.735935e-11
hsa00250                   NA                       1          6.340137e-01
      q.values.pass.null p.values.pass.null.het q.values.pass.null.het
hsa05213          3.162565e-10                NA                       1
hsa05223          8.207804e-11                NA                       1
hsa00250          6.340137e-01                NA                       1

```

3.1 Calculating gene scores

In order to implement the gene-oriented method, we need to have gene-level scores. We can obtain a variety of scores using the `cma.scores` function. The functions in this package are designed to use the `logLRT` scores.

```

> GeneScores <- cma.scores(cma.data = MutationsBrain, number.genes = nrow(GeneSizes08))
> head(GeneScores)

```

```

      CaMP neglogPg  logLRT logitBinomialPosteriorDriver
A2M@@408          0.7113996 2.279349 1.845074                NA
A4GALT@@21470     1.2286366 3.232244 2.798146                NA
ABCA10@@388       0.9279687 2.597908 2.163654                NA
ABCA4@@140        0.5650629 2.097952 1.663669                NA

```

ABCA7@@194	0.9745022	2.673914	2.239663	NA
ABCB6@@2071	1.2044322	3.146882	2.712739	NA
	PoissonlogBF	PoissonPosterior	Poissonlmlik0	Poissonlmlik1
A2M@@408	NA	NA	NA	NA
A4GALT@@21470	NA	NA	NA	NA
ABCA10@@388	NA	NA	NA	NA
ABCA4@@140	NA	NA	NA	NA
ABCA7@@194	NA	NA	NA	NA
ABCB6@@2071	NA	NA	NA	NA

4 Simulating data

We now demonstrate the use of the `sim.data.p.values` function, which simulates datasets under either the permutation or passenger null (see [2]), depending on whether `pass.null` is set to `TRUE` or `FALSE`, and calculates the p-values and q-values for those datasets for the selected methods. The simulations may also include spiked-in gene-sets, by using the `perc.samples` and `spiked.set.sizes` options. For example, if one desires to have two spiked-in gene-sets, both having 50 genes, but one having the probability of being altered in any given sample of 0.75 and the other of 0.95, then these parameters should be set to `perc.samples = c(75, 90)` and `spiked.set.sizes = 50`. The spiked-in gene-sets are artificially created with hypothetical genes (for more details on how they are generated, see [2]). To simulate the data without spiked-in sets, under the permutation or passenger null hypotheses, the parameters should be set as following: `perc.samples = NULL`, `spiked.set.sizes = NULL`. The object outputted by `sim.data.p.values` is of the class `SetMethodsSims`. Note that this code takes several minutes to run:

```
> set.seed(831984)
> resultsSim <- sim.data.p.values(EventsBySample = EventsBySampleBrain,
+   Mutations = MutationsBrain, GeneSizes = GeneSizes08, Coverage = CoverageBrain,
+   GeneSets = KEGGPATHID2EXTID[c("hsa05213", "hsa05223", "hsa00250")],
+   ID2name = ID2name, nr.iter = 2, pass.null = TRUE, perc.samples = c(75,
+     95), spiked.set.sizes = c(50), show.iter = TRUE, gene.method = FALSE,
+   perm.null.method = TRUE, perm.null.het.method = FALSE, pass.null.method = TRUE,
+   pass.null.het.method = FALSE)

[1] "Currently simulating data: Iteration #1"
[1] "Thu Apr 14 00:47:26 2011"
[1] "Currently simulating data: Iteration #2"
[1] "Thu Apr 14 00:48:05 2011"
[1] ""
[1] "Implement methods: Iteration #1"
[1] "Permutation null w/o heterogeneity"
[1] "Thu Apr 14 00:48:44 2011"
[1] "Passenger null w/o heterogeneity"
[1] "Thu Apr 14 00:48:44 2011"
[1] "Thu Apr 14 00:48:46 2011"
[1] ""
[1] "Implement methods: Iteration #2"
[1] "Permutation null w/o heterogeneity"
```

```
[1] "Thu Apr 14 00:48:46 2011"
[1] "Passenger null w/o heterogeneity"
[1] "Thu Apr 14 00:48:46 2011"
[1] "Thu Apr 14 00:48:48 2011"
```

```
> resultsSim
```

```
Simulation results for gene-set analysis of mutations
```

```
Data-generating mechanism : Passenger null
Number of simulations      : 2
Number of gene-sets       : 5
  Original      : 3
  Spiked-in    : 2
Spiked-in sets           :
  Probability of being mutated in a set : 0.75 0.95
  Length (as number of genes)          : 50
```

```
> slotNames(resultsSim)
```

```
[1] "null.dist"      "perc.samples"   "spiked.set.sizes" "GeneSizes"
[5] "GeneSets"      "Coverage"       "EventsBySample"  "Mutations"
[9] "Scores"        "results"
```

```
> resultsSim@null.dist
```

```
[1] "Passenger null"
```

4.1 Manipulating the SetMethodsSims objects

We provide 2 functions to help manipulate the `SetMethodsSims` objects which result from the `sim.data.p.values` function: `extract.sims.method` and `combine.sims`. The `extract.sims.method` function is used to obtain a single data frame with the p-values or q-values from one of the specific methods. For instance, the command to get the p-values for the permutation null with no heterogeneity method is:

```
> extract.sims.method(resultsSim, "p.values.perm.null")
```

```
          [,1]      [,2]
hsa05213 1.628379e-02 3.150681e-01
hsa05223 1.951091e-02 5.847419e-01
hsa00250 5.545376e-01 2.693547e-01
gene.set.50.75 6.645712e-10 2.550107e-14
gene.set.50.95 7.502657e-18 4.783445e-16
```

The function `combine.sims` may be used to combine 2 simulations:

```
> combine.sims(resultsSim, resultsSim)
```

Simulation results for gene-set analysis of mutations

Data-generating mechanism : Passenger null
Number of simulations : 4
Number of gene-sets : 5
 Original : 3
 Spiked-in : 2
Spiked-in sets :
 Probability of being mutated in a set : 0.75 0.95
 Length (as number of genes) : 50

References

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