Primer: Preparing NChannelSet objects with differential expression scores

February 8, 2013

1 Differential expression analysis

The gCMAP package offers a the generate_gCMAP_NChannelSet function to process multiple instances differential expression experiments with two classes (e.g. cases vs controls). For microarray data, the limma package is used to calculate a moderated t-statistic (default). Optionally, a standard t-test can be computed instead. For RNAseq data, the DESeq package is used instead.

Data preprocessing differs considerably between different technologies and array platforms and needs to be performed beforehand. Normalized microarray data and accompanying annotation is passed to generate_gCMAP_NChannelSet as a list of ExpressionSet objects, RNAseq data can be passed as a list of CountDataSet objects instead.

To generate a set of 3 example CountDataSets , we use the makeExampleCountDataSet function from the DESeq package.

```
> library(gCMAP)
> set.seed( 123 )
> cds.list <- lapply( 1:3, function(n) {
+    cds <- makeExampleCountDataSet()
+    featureNames(cds) <- paste("gene",1:10000, sep="_")
+    cds
+ })
> names(cds.list) <- paste("Instance", 1:3, sep="")
> sapply(cds.list, dim)

         Instance1 Instance2 Instance3
Features     10000     10000     10000
Samples          5         5         5

> sapply(cds.list, function(n) pData(n)$condition )

     Instance1 Instance2 Instance3
[1,] "A"       "A"       "A"
[2,] "A"       "A"       "A"
[3,] "B"       "B"       "B"
[4,] "B"       "B"       "B"
[5,] "B"       "B"       "B"
```

By default, each CountDataset object contains counts for 10000 genes from five samples. Each sample is assigned to one of two conditions, A or B, in the phenoData slot of the CountDataSet . The pData column containing group membership information (e.g. "condition") is provided as the control_perturb_col

parameter. The levels associated with control and treatment groups are specified as "control" and "perturb" character strings.

Each of the three `CountDataSet` instances is analyzed individually by `generate_gCMAP_NChannelSet` . To assemble the results into a single NChannelSet, the input `ExpressionSet` or `CountDataSet` objects must contain measurements for the same features (e.g. the vectors returned by "featureNames" must be identical across all instances).

To include information about the instances in the NChannelSet, a 'sample.annotation' data.frame can be provided, containing exactly one row for each element of the input list of `ExpressionSet / CountDataSet` objects.

```
> ## this step takes a little time
> cde <- generate_gCMAP_NChannelSet(cds.list,
+                             uids=1:3,
+                             sample.annotation=NULL,
+                             platform.annotation="Entrez",
+                             control_perturb_col="condition",
+                             control="A",
+                             perturb="B")
> channelNames(cde)

[1] "exprs"  "log_fc" "mod_fc" "p"      "z"
```

For array data, a `NChannelSet` with slots "exprs","z", "p", and "log_fc" is returned, containing the average intenstity across all samples within the instance, z-scores, (raw) p-values and log2 fold changes, respectively. If count data is processed, an additional "mod_fc" channel is returned, providing the moderated fold change, calculated after performing variance-stabilising transformation across all input instances. (Please consult the `DESeq` vignette for details.)

1.1 Storing assayData as BigMatrix objects on disk

When large numbers of instances are processed, the resulting `NChannelSet` objects can require large amounts of memory. If a file name is provided via the "big.matrix" parameter, `generate_gCMAP_NChannelSet` uses the `BigMatrix` package to store data from each channel on disk. In the future, individual channels and / or subsets of the datasets can then be loaded without requiring the full object to be read into memory again.

To highlight this functionality, we derive three (arbitrary) instances from the sample.ExpressionSet object available from tbe `Biobase` package, process them and store the results in a temporary directory.

```
> ## list of ExpressionSets
> data("sample.ExpressionSet") ## from Biobase
> es.list <- list( sample.ExpressionSet[,1:4],
+                 sample.ExpressionSet[,5:8],
+                 sample.ExpressionSet[,9:12])
> ## three instances
> names(es.list) <- paste( "Instance", 1:3, sep=".")
> storage.file <- tempfile()
> storage.file ## filename prefix for BigMatrices

[1] "/tmp/RtmpvLZWUO/file149544ff9e76b"

> de <- generate_gCMAP_NChannelSet(
+     es.list,
+     1:3,
+     platform.annotation = annotation(es.list[[1]]),
```

```
+     control_perturb_col="type",
+     control="Control",
+     perturb="Case",
+     big.matrix=storage.file)
> channelNames(de)

[1] "exprs"  "log_fc" "p"      "z"

> head( assayDataElement(de, "z") )

                          1          2          3
AFFX-MurIL2_at  -1.36808562  0.04333555 -0.7255849
AFFX-MurIL10_at  1.56254427 -0.69203457  0.1589525
AFFX-MurIL4_at  -0.65915229 -0.85080055  0.1804448
AFFX-MurFAS_at  -0.31745996  0.43936805  0.2813885
AFFX-BioB-5_at  -0.08767134  0.15619365 -0.2836740
AFFX-BioB-M_at  -0.32253278  0.82819990 -0.5521458

> dir(dirname( storage.file ))

 [1] "file149544ff9e76b.rdata"           "file149544ff9e76b_exprs"
 [3] "file149544ff9e76b_exprs.desc"      "file149544ff9e76b_exprs.desc.rds"
 [5] "file149544ff9e76b_log_fc"          "file149544ff9e76b_log_fc.desc"
 [7] "file149544ff9e76b_log_fc.desc.rds" "file149544ff9e76b_p"
 [9] "file149544ff9e76b_p.desc"          "file149544ff9e76b_p.desc.rds"
[11] "file149544ff9e76b_z"               "file149544ff9e76b_z.desc"
[13] "file149544ff9e76b_z.desc.rds"
```

For each channel, three files were generated in the temporary directory, identified by their suffices. To demonstrate the use of these disk-based `NChannelSet` objects, we will first delete the object from the current R session and reload it from disk.

Accessing the complete matrix in the assayData slots, e.g. for the "z" channel, returns a BigMatrix object - a pointer to the associated file on disk. Upon subsetting, only the requested part of the dataset is loaded into memory.

```
> ## remove de object from R session and reload
> rm( de )
> de <-get( load( paste( storage.file, "rdata", sep=".")) )
> class( assayDataElement(de, "z") ) ## Bigmatrix

[1] "BigMatrix"
attr(,"package")
[1] "bigmemoryExtras"

> assayDataElement(de, "z")[1:10,] ## load subset

Attaching to on-disk data: /private/tmp/RtmpvLZWUO/file149544ff9e76b_z.desc.rds ...
                          1          2          3
AFFX-MurIL2_at  -1.36808562  0.04333555 -0.7255849
AFFX-MurIL10_at  1.56254427 -0.69203457  0.1589525
AFFX-MurIL4_at  -0.65915229 -0.85080055  0.1804448
AFFX-MurFAS_at  -0.31745996  0.43936805  0.2813885
AFFX-BioB-5_at  -0.08767134  0.15619365 -0.2836740
```

```
AFFX-BioB-M_at  -0.32253278  0.82819990 -0.5521458
AFFX-BioB-3_at  -0.30488232  1.79473755  0.4374636
AFFX-BioC-5_at  -0.29368831  0.34488031  0.0982909
AFFX-BioC-3_at   0.05507180 -1.89130218  0.2943413
AFFX-BioDn-5_at  0.78669240  0.74946863  1.0688364
```

The `memorize` function reads the complete `NChannelSet` into memory. Alternatively, one or more selected channels can be specified with the 'name' parameter.

```
> ## read z-score channel into memory
> dem <- memorize( de, name="z" )
> channelNames(dem)

[1] "z"

> class( assayDataElement(dem, "z") ) ## matrix

[1] "matrix"

>   sessionInfo()

R version 2.15.2 (2012-10-26)
Platform: i386-apple-darwin9.8.0/i386 (32-bit)

locale:
[1] C/en_US.US-ASCII/en_US.US-ASCII/C/en_US.US-ASCII/en_US.US-ASCII

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
 [1] gCMAP_1.1.7          DESeq_1.10.1         lattice_0.20-13
 [4] locfit_1.5-8         GSEABase_1.20.2      graph_1.36.2
 [7] annotate_1.36.0      AnnotationDbi_1.20.3 Biobase_2.18.0
[10] BiocGenerics_0.4.0

loaded via a namespace (and not attached):
 [1] DBI_0.2-5              GSEAlm_1.18.0          IRanges_1.16.4
 [4] Matrix_1.0-10          RColorBrewer_1.0-5     RSQLite_0.11.2
 [7] XML_3.95-0.1           bigmemory_4.3.0        bigmemory.sri_0.1.2
[10] bigmemoryExtras_1.0.0 genefilter_1.40.0      geneplotter_1.36.0
[13] grid_2.15.2           latticeExtra_0.6-24    limma_3.14.4
[16] parallel_2.15.2       splines_2.15.2         stats4_2.15.2
[19] survival_2.37-2       tools_2.15.2           xtable_1.7-0
```