An Introduction to Bioconductor’s ExpressionSet Class

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1 Introduction

Biobase is part of the Bioconductor project, and is used by many other packages. Biobase contains standardized data structures to represent genomic data. The ExpressionSet class is designed to combine several different sources of information into a single convenient structure. An ExpressionSet can be manipulated (e.g., subsetted, copied) conveniently, and is the input or output from many Bioconductor functions.

The data in an ExpressionSet is complicated, consisting of expression data from microarray experiments (assayData; assayData is used to hint at the methods used to access different data components, as we will see below), ‘meta-data’ describing samples in the experiment (phenoData), annotations and meta-data about the features on the chip or technology used for the experiment (featureData, annotation), information related to the protocol used for processing each sample (and usually extracted from manufacturer files, protocolData), and a flexible structure to describe the experiment (experimentData). The ExpressionSet class coordinates all of this data, so that you do not usually have to worry about the details. However, an ExpressionSet needs to be created in the first place, and creation can be complicated.

In this introduction we learn how to create and manipulate ExpressionSet objects, and practice some basic R skills.

2 Preliminaries

2.1 Installing Packages

If you are reading this document and have not yet installed any software on your computer, visit http://bioconductor.org and follow the instructions for installing R and Bioconductor. Once you have installed R and Bioconductor, you are ready to go with this document. In the future, you might find that you need to install one or more additional packages. The best way to do this is to start an R session and evaluate commands like
> if (!require("BiocManager"))
+    install.packages("BiocManager")
> BiocManager::install("Biobase")

### 2.2 Loading Packages

The definition of the *ExpressionSet* class along with many methods for manipulating *ExpressionSet* objects are defined in the *Biobase* package. In general, you need to load class and method definitions before you use them. When using Bioconductor, this means loading R packages using `library` or `require`.

> library("Biobase")

**Exercise 1**

*What happens when you try to load a package that is not installed?*

When using `library`, you get an error message. With `require`, the return value is `FALSE` and a warning is printed.

### 3 Building an ExpressionSet From .CEL and other files

Many users have access to .CEL or other files produced by microarray chip manufacturer hardware. Usually the strategy is to use a Bioconductor package such as `affyPLM`, `affy`, `oligo`, or `limma`, to read these files. These Bioconductor packages have functions (e.g., `ReadAffy`, `expresso`, or `justRMA` in `affy`) to read CEL files and perform preliminary preprocessing, and to represent the resulting data as an *ExpressionSet* or other type of object. Suppose the result from reading and preprocessing CEL or other files is named `object`, and `object` is different from *ExpressionSet*; a good bet is to try, e.g.,

> library(convert)
> as(object, "ExpressionSet")

It might be the case that no converter is available. The path then is to extract relevant data from `object` and use this to create an *ExpressionSet* using the instructions below.

### 4 Building an ExpressionSet From Scratch

As mentioned in the introduction, the data from many high-throughput genomic experiments, such as microarray experiments, usually consist of several conceptually distinct parts: assay data, phenotypic meta-data, feature annotations and meta-data, and a description of the experiment. We’ll construct each of these components, and then assemble them into an *ExpressionSet*. 
4.1 Assay data

One important part of the experiment is a matrix of ‘expression’ values. The values are usually derived from microarrays of one sort or another, perhaps after initial processing by manufacturer software or Bioconductor packages. The matrix has $F$ rows and $S$ columns, where $F$ is the number of features on the chip and $S$ is the number of samples.

A likely scenario is that your assay data is in a 'tab-delimited' text file (as exported from a spreadsheet, for instance) with rows corresponding to features and columns to samples. The strategy is to read this file into R using the read.table command, converting the result to a matrix. A typical command to read a tab-delimited file that includes column ‘headers’ is

```r
> dataDirectory <- system.file("extdata", package="Biobase")
> exprsFile <- file.path(dataDirectory, "exprsData.txt")
> exprs <- as.matrix(read.table(exprsFile, header=TRUE, sep="\t",
+         row.names=1,
+         as.is=TRUE))
```

The first two lines create a file path pointing to where the assay data is stored; replace these with a character string pointing to your own file, e.g,

```r
> exprsFile <- "c:/path/to/exprsData.txt"
```

(Windows users: note the use of / rather than \; this is because R treats the \ character as an ‘escape’ sequence to change the meaning of the subsequent character). See the help pages for read.table for more detail. A common variant is that the character separating columns is a comma (“comma-separated values”, or “csv” files), in which case the sep argument might be sep="",".

It is always important to verify that the data you have read matches your expectations. At a minimum, check the class and dimensions of geneData and take a peak at the first several rows

```r
> class(exprs)
[1] "matrix" "array"
> dim(exprs)
[1] 500 26
> colnames(exprs)
[1] "A" "B" "C" "D" "E" "F" "G" "H" "I" "J" "K" "L" "M" "N" "O"
[16] "P" "Q" "R" "S" "T" "U" "V" "W" "X" "Y" "Z"
> head(exprs[,1:5])
```
At this point, we can create a minimal `ExpressionSet` object using the `ExpressionSet` constructor:

```r
> minimalSet <- ExpressionSet(assayData=exprs)
```

We'll get more benefit from expression sets by creating a richer object that coordinates phenotypic and other data with our expression data, as outlined in the following sections.

### 4.2 Phenotypic data

Phenotypic data summarizes information about the samples (e.g., sex, age, and treatment status; referred to as ‘covariates’). The information describing the samples can be represented as a table with $S$ rows and $V$ columns, where $V$ is the number of covariates. An example of phenotypic data can be input with

```r
> pDataFile <- file.path(dataDirectory, "pData.txt")
> pData <- read.table(pDataFile,
+   row.names=1, header=TRUE, sep="\t")
> dim(pData)
[1] 26 3
> rownames(pData)
[1] "A" "B" "C" "D" "E" "F" "G" "H" "I" "J" "K" "L" "M" "N" "O"
[16] "P" "Q" "R" "S" "T" "U" "V" "W" "X" "Y" "Z"
> summary(pData)

gender            type        score
 Length:26   Length:26   Min. :0.1000
Class :character Class :character 1st Qu.:0.3275
Mode :character  Mode :character Median :0.4150
                              Mean :0.5369
                              3rd Qu.:0.7650
                              Max. :0.9800
There are three columns of data, and 26 rows. Note that the number of rows of phenotypic data match the number of columns of expression data, and indeed that the row and column names are identically ordered:

```r
> all(rownames(pData)==colnames(exprs))
[1] TRUE
```

This is an essential feature of the relationship between the assay and phenotype data; ExpressionSet will complain if these names do not match.

Phenotypic data can take on a number of different forms. For instance, some covariates might reasonably be represented as numeric values. Other covariates (e.g., gender, tissue type, or cancer status) might better be represented as factor objects (see the help page for factor for more information). It is especially important that the phenotypic data are encoded correctly; the colClasses argument to read.table can be helpful in correctly inputing (and ignoring, if desired) columns from the file.

**Exercise 2**
What class does read.table return?

**Exercise 3**
Determine the column names of pData. Hint: apropos("name").

```r
> names(pData)
[1] "gender" "type" "score"
```

**Exercise 4**
Use sapply to determine the classes of each column of pData. Hint: read the help page for sapply.

```r
> sapply(pData, class)

    gender    type    score
"character" "character" "numeric"
```

**Exercise 5**
What is the sex and Case/Control status of the 15th and 20th samples? And for the sample(s) with score greater than 0.8.
InVESTigators often find that the meaning of simple column names does not provide enough information about the covariate – What is the cryptic name supposed to represent? What units are the covariates measured in? We can create a data frame containing such meta-data (or read the information from a file using read.table) with

```r
> metadata <- data.frame(labelDescription =
+                                      c("Patient gender",
+                                        "Case/control status",
+                                        "Tumor progress on XYZ scale"),
+                                      row.names = c("gender", "type", "score"))
```

This creates a data.frame object with a single column called labelDescription, and with row names identical to the column names of the data.frame containing the phenotypic data. The column labelDescription must be present; other columns are optional.

Bioconductor’s Biobase package provides a class called AnnotatedDataFrame that conveniently stores and manipulates the phenotypic data and its metadata in a coordinated fashion. Create and view an AnnotatedDataFrame instance with:

```r
> phenoData <- new("AnnotatedDataFrame",
+                   data=pData, varMetadata=metadata)
> phenoData
```

An object of class 'AnnotatedDataFrame'

rowNames: A B ... Z (26 total)
varLabels: gender type score
varMetadata: labelDescription

Some useful operations on an AnnotatedDataFrame include sampleNames, pData (to extract the original pData data.frame), and varMetadata. In addition, AnnotatedDataFrame objects can be subset much like a data.frame:
> head(pData(phenoData))

    gender type score
A Female Control 0.75
B Male Case 0.40
C Male Control 0.73
D Male Case 0.42
E Female Case 0.93
F Male Control 0.22

> phenoData[c("A","Z"),"gender"]

An object of class 'AnnotatedDataFrame'
  rowNames: A Z
  varLabels: gender
  varMetadata: labelDescription

> pData(phenoData[phenoData$score>0.8,])

    gender type score
E Female Case 0.93
G Male Case 0.96
X Male Control 0.98
Y Female Case 0.94

4.3 Annotations and feature data

Meta-data on features is as important as meta-data on samples, and can be very large and diverse. A single chip design (i.e., collection of features) is likely to be used in many different experiments, and it would be inefficient to repeatedly collect and coordinate the same meta-data for each ExpressionSet instance. Instead, the idea is to construct specialized meta-data packages for each type of chip or instrument. Many of these packages are available from the Bioconductor web site. These packages contain information such as the gene name, symbol and chromosomal location. There are other meta-data packages that contain the information that is provided by other initiatives such as GO and KEGG. The annotate and AnnotationDbi packages provide basic data manipulation tools for the meta-data packages.

The appropriate way to create annotation data for features is very straightforward: we provide a character string identifying the type of chip used in the experiment. For instance, the data we are using is from the Affymetrix hgu95av2 chip:

> annotation <- "hgu95av2"

It is also possible to record information about features that are unique to the experiment (e.g., flagging particularly relevant features). This is done by creating or modifying an AnnotatedDataFrame like that for phenoData but with row names of the AnnotatedDataFrame matching rows of the assay data.
4.4 Experiment description

Basic description about the experiment (e.g., the investigator or lab where the experiment was done, an overall title, and other notes) can be recorded by creating a MIAME object. One way to create a MIAME object is to use the new function:

```r
> experimentData <- new("MIAME",
+   name="Pierre Fermat",
+   lab="Francis Galton Lab",
+   contact="pfermat@lab.not.exist",
+   title="Smoking-Cancer Experiment",
+   abstract="An example ExpressionSet",
+   url="www.lab.not.exist",
+   other=list(
+     notes="Created from text files"
+   )
+ )
```

Usually, new takes as arguments the class name and pairs of names and values corresponding to different slots in the class; consult the help page for MIAME for details of available slots.

4.5 Assembling an ExpressionSet

An ExpressionSet object is created by assembling its component parts and calling the ExpressionSet constructor:

```r
> exampleSet <- ExpressionSet(assayData=exprs,
+    phenoData=phenoData,
+    experimentData=experimentData,
+    annotation="hgu95av2")
```

Note that the names on the right of each equal sign can refer to any object of appropriate class for the argument. See the help page for ExpressionSet for more information.

We created a rich data object to coordinate diverse sources of information. Less rich objects can be created by providing less information. As mentioned earlier, a minimal expression set can be created with

```r
> minimalSet <- ExpressionSet(assayData=exprs)
```

Of course this object has no information about phenotypic or feature data, or about the chip used for the assay.

5 ExpressionSet Basics

Now that you have an ExpressionSet instance, let’s explore some of the basic operations. You can get an overview of the structure and available methods for ExpressionSet objects by reading the help page:
When you print an ExpressionSet object, a brief summary of the contents of the object is displayed (displaying the entire object would fill your screen with numbers):

> exampleSet

ExpressionSet (storageMode: lockedEnvironment)
assayData: 500 features, 26 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: A B ... Z (26 total)
  varLabels: gender type score
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: hgu95av2

5.1 Accessing Data Elements

A number of accessor functions are available to extract data from an ExpressionSet instance. You can access the columns of the phenotype data (an AnnotatedDataFrame instance) using $:

> exampleSet$gender[1:5]

[1] "Female" "Male" "Male" "Male" "Female"

> exampleSet$gender[1:5] == "Female"

[1] TRUE FALSE FALSE FALSE TRUE

You can retrieve the names of the features using featureNames. For many microarray datasets, the feature names are the probe set identifiers.

> featureNames(exampleSet)[1:5]

[1] "AFFX-MurIL2_at" "AFFX-MurIL10_at" "AFFX-MurIL4_at"
[4] "AFFX-MurFAS_at" "AFFX-BioB-5_at"

The unique identifiers of the samples in the data set are available via the sampleNames method. The varLabels method lists the column names of the phenotype data:

> sampleNames(exampleSet)[1:5]
Extract the expression matrix of sample information using `exprs`:

```r
> mat <- exprs(exampleSet)
> dim(mat)
[1] 500 26
```

### 5.1.1 Subsetting

Probably the most useful operation to perform on `ExpressionSet` objects is subsetting. Subsetting an `ExpressionSet` is very similar to subsetting the expression matrix that is contained within the `ExpressionSet`, the first argument subsets the features and the second argument subsets the samples. Here are some examples: Create a new `ExpressionSet` consisting of the 5 features and the first 3 samples:

```r
> vv <- exampleSet[1:5, 1:3]
> dim(vv)
Features Samples
  5   3
> featureNames(vv)
[1] "AFFX-MurIL2_at" "AFFX-MurIL10_at" "AFFX-MurIL4_at"
[4] "AFFX-MurFAS_at" "AFFX-BioB-5_at"
> sampleNames(vv)
[1] "A" "B" "C"
```

Create a subset consisting of only the male samples:

```r
> males <- exampleSet[, exampleSet$gender == "Male"]
> males
ExpressionSet (storageMode: lockedEnvironment)
assayData: 500 features, 15 samples
element names: exprs
protocolData: none
phenoData
  sampleNames: B C ... X (15 total)
  varLabels: gender type score
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: hgu95av2

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6 What was used to create this document

The version number of R and the packages and their versions that were used to generate this document are listed below.

- R version 4.2.0 RC (2022-04-19 r82224), x86_64-pc-linux-gnu
- Running under: Ubuntu 20.04.4 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.15-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.15-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: Biobase 2.56.0, BiocGenerics 0.42.0
- Loaded via a namespace (and not attached): compiler 4.2.0, tools 4.2.0