

Biobase development and the new **eSet**

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Revised 4 September, 2006 – **featureData** slot. Revised 20 April 2007 – minor wording changes; **verbose** and other arguments passed through **updateObject** example; introduce a second variant of **initialize** illustrating its use as a copy constructor.

1 Introduction

These notes help *developers* who are interested in using and extending the **eSet** class hierarchy, and using features in *Biobase*. The information here is not useful to regular users of *Biobase*.

This document illustrates the *Biobase* structures and approaches that make it it easy for developers to creatively use and extend the **eSet** class hierarchy.

The document starts with a brief description of the motivation for change, and a comparison of the old (before August, 2006) and new **eSets** and related functionality (e.g., the *Versioned* class and **updateObject** methods). We then illustrate how **eSet** can be extended to handle additional types of data, and how new methods can exploit the **eSet** class hierarchy. We conclude with a brief summary of lessons learned, useful developer-related side-effects of efforts to revise **eSet**, and possible directions for future development.

2 Comparing old and new

What is an **eSet**?

- Coordinate high through-put (e.g., gene expression) and phenotype data.
- Provide common data container for diverse Bioconductor packages.

Motivation for change (August, 2006).

- What was broken? Complex data structure. Inconsistent object validity. No straight-forward way to extend **eSet** to new data types.
- What forward-looking design goals did we have? Flexible storage model. Class hierarchy to promote code reuse and facilitate extension to new data objects. Methods for updating serialized instances.

Key features in the redesign.

- Simplified data content.
- Structured class hierarchy .
- Alternative storage modes.

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- More validity checking.
- Conversion of example data in *Biobase*, and many other data sets elsewhere in Bioconductor, to *ExpressionSet*.
- *Versioned* class information (in the development branch).
- `updateObject` methods (in the development branch).

3 A quick tour

3.1 The eSet object: high-throughput experiments

Purpose.

- Coordinate and contain high-throughput genomic data.

Structure: virtual base class.

```
> getClass("eSet")
```

```
Virtual Class "eSet" [package "Biobase"]
```

Slots:

```
Name:      assayData      phenoData      featureData
Class:      AssayData AnnotatedDataFrame AnnotatedDataFrame
```

```
Name:      experimentData      annotation      protocolData
Class:      MIAME              character AnnotatedDataFrame
```

```
Name:      __classVersion__
Class:      Versions
```

Extends:

```
Class "VersionedBiobase", directly
```

```
Class "Versioned", by class "VersionedBiobase", distance 2
```

```
Known Subclasses: "ExpressionSet", "NChannelSet", "MultiSet", "SnpSet"
```

- `assayData`: high-throughput data.
- `phenoData`: sample covariates.
- `featureData`: feature covariates.
- `experimentData`: experimental description.
- `annotation`: assay description.
- See below, and `?eSet-class`

3.1.1 assayData: high-throughput data

Purpose.

- Efficiently and flexibly contain high-volume data.

Structure: *list*, *environment*, or *lockEnvironment* class union.

- Each element of *list* / *environment* / *lockEnvironment* is a matrix
- Rows: *features*, e.g., gene names.
- Columns: *samples* represented on each chip.
- All matrices must have the same dimensions, row names, and column names.
- Subclasses determine which matrices *must* be present.
- See `?"AssayData-class"`

3.1.2 phenoData: sample covariates

Purpose.

- Contain and document sample covariates.

Structure: *AnnotatedDataFrame*.

- **data**: *data.frame*.
 - Rows: sample identifiers.
 - Columns: measured covariates.
- **varMetadata**: *data.frame*.
 - Rows: measured covariate labels.
 - Columns: covariate descriptors.
- See `?"AnnotatedDataFrame-class"`

3.1.3 featureData: feature covariates

Purpose.

- Contain and document feature covariates specific to the experiment; use the **annotation** slot for chip-level descriptions.

Structure: *AnnotatedDataFrame*.

- **data**: *data.frame*.
 - Rows: feature identifiers. These match row names of **assayData**.
 - Columns: measured covariates.
- **varMetadata**: *data.frame*.
 - Rows: measured covariate labels.
 - Columns: covariate descriptors.
- See `?"AnnotatedDataFrame-class"`

3.1.4 experimentData: experiment description

Purpose.

- Summarize where and how the experiment was performed.

Structure: *MIAME*

- **title**: experiment title.
- **name**: experimenter name(s).
- **preprocessing**: list of pre-processing steps.
- Additional slots.
- See ?"MIAME-class".

3.1.5 annotation: assay description

Purpose.

- Link experiment to annotation package.

Structure: *character*

- Label identifying annotation package.

3.2 Important eSet methods

Initialization.

- **eSet** is VIRTUAL, initialize via subclass `callNextMethod`

Accessors (get, set).

- `assayData(obj)`; `assayData(obj) <- value`: access or assign `assayData`
- `phenoData(obj)`; `phenoData(obj) <- value`: access or assign `phenoData`
- `experimentData(obj)`; `experimentData(obj) <- value`: access or assign `experimentData`
- `annotation(obj)`; `annotation(obj) <- value`: access or assign `annotation`

Subsetting.

- `obj[i, j]`: select genes *i* and samples *j*.
- `obj$name`; `obj$name <- value`: retrieve or assign covariate name in `phenoData`

3.2.1 Additional eSet methods

- `show`.
- `storageMode`: influence how `assayData` is stored.
- `updateObject`: update `eSet` objects to their current version.
- `validObject`: ensure that `eSet` is valid.

The `validObject` method is particularly important to `eSet`, ensuring that `eSet` contains consistent structure to data.

```
> getValidity(getClass("eSet"))

function (object)
{
  msg <- validMsg(NULL, isValidVersion(object, "eSet"))
  dims <- dims(object)
  if (ncol(dims) > 0) {
    if (any(dims[1, ] != dims[1, 1]))
      msg <- validMsg(msg, "row numbers differ for assayData members")
    if (any(dims[2, ] != dims[2, 1]))
      msg <- validMsg(msg, "sample numbers differ for assayData members")
    msg <- validMsg(msg, assayDataValidMembers(assayData(object)))
    if (dims[1, 1] != dim(featureData(object))[[1]])
      msg <- validMsg(msg, "feature numbers differ between assayData and featureData")
    if (!identical(featureNames(assayData(object)), featureNames(featureData(object))))
      msg <- validMsg(msg, "featureNames differ between assayData and featureData")
    if (dims[2, 1] != dim(phenoData(object))[[1]])
      msg <- validMsg(msg, "sample numbers differ between assayData and phenoData")
    if (!identical(sampleNames(assayData(object)), sampleNames(phenoData(object))))
      msg <- validMsg(msg, "sampleNames differ between assayData and phenoData")
    if (dim(phenoData(object))[[1]] != dim(protocolData(object))[[1]])
      msg <- validMsg(msg, "sample numbers differ between phenoData and protocolData")
    if (!identical(sampleNames(phenoData(object)), sampleNames(protocolData(object))))
      msg <- validMsg(msg, "sampleNames differ between phenoData and protocolData")
  }
  if (is.null(msg))
    TRUE
  else msg
}
<environment: namespace:Biobase>
```

The validity methods for `eSet` reflect our design goals. All `assayData` members must have identical row and column dimensions and `featureNames`. The names and numbers of samples must be the same in `assayData` and `phenoData` slots. Validity methods are defined for the classes underlying each slot as well. For instance, the validity methods for `AnnotatedDataFrame` check that variables used in `pData` are at least minimally described in `varMetadata`.

3.3 Subclasses of `eSet`

Biobase defines three classes that extend `eSet`. `ExpressionSet` (discussed further below) is meant to contain microarray gene expression data. *SnpSet* is a preliminary class to contain SNP data; other classes in development (e.g., in *oligo*) may provide alternative implementations for SNP data. *MultiSet* is an `ExpressionSet`-like class, but without restriction on the names (though not structure) of elements in the `assayData` slot.

3.3.1 `ExpressionSet`

Purpose:

- Contain gene expression data.

Required `assayData` members.

- `exprs`, a matrix of expression values.

Important methods.

- Initialization (additional details below):

```
> obj <- new("eSet", phenoData = new("AnnotatedDataFrame"),  
+   experimentData = new("MIAME"), annotation = character(),  
+   exprs = new("matrix"))
```

- `exprs(obj)`, `exprs(obj) <- value`: get or set `exprs`; methods defined for *ExpressionSet*, *AssayData*.

3.3.2 *MultiSet* and *SnpSet*

MultiSet.

- Purpose: flexibly contain a collection of expression data matrices.
- Required `assayData` members: none.

SnpSet.

- Purpose: contain genomic SNP calls.
- Required `assayData` members: `call`, `callProbability`.

4 Comments on `assayData`: high-throughput data storage

The `assayData` slot is meant to store high-throughput data. The idea is that the slot contains identically sized matrices containing expression or other data. All matrices in the slot must have the same dimension, and are structured so that rows represent ‘features’ and columns represent ‘samples’. Validity methods enforce that row and column names of slot elements are identical.

For technical reasons, creating instance of *AssayData* is slightly different from the way this is usually done in R. Normally, one creates an instance of a class with an expression like `new("ExpressionSet", ...)`, with the `...` representing additional arguments. *AssayData* objects are created with

```
> assayDataNew("environment", elt)
```

where `elt` might be a matrix of expression values. For the curious, the reason for this setup stems from our desire to have a class that **is** a list or environment, rather than a class that has a slot that contains a list or environment. The **is** relationship is desirable to avoid unnecessary function calls to access slots, and requires that a class **contain** the base type (e.g., *environment*. Until recently an R object could not **contain** an *environment*.

The `assayData` slot of *ExpressionSet* objects must contain a matrix named `exprs`. Nonetheless, the *ExpressionSet* validity method tries to be liberal – it guarantees that the object has an `exprs` element, but allows for other elements too. The prudent developer wanting consistent additional data elements should derive a class from *ExpressionSet* that enforces the presence of their desired elements.

The *AssayData* class allows for data elements to be stored in three different ways (see `?storageMode` and `?storageMode<-"` for details): as a `list`, `environment`, or `lockedEnvironment`. Developers are probably familiar with `list` objects; a drawback is that `exprs` elements may be large, and some operations on lists in R may trigger creation of many copies of the `exprs` element. This can be expensive in both space and time. Environments are nearly unique in R, in that they are passed by

reference rather than value. This eliminates some copying, but has the unfortunately consequence that side-effects occur – modifications to an environment inside a function influence the value of elements outside the function. For these reasons, environments can be useful as ‘read only’ arguments to functions, but can have unexpected consequences when functions modify their arguments. Locked environments implemented in *Biobase* try to strike a happy medium, allowing pass by reference for most operations but triggering (whole-environment) copying when elements in the environment are modified. The locking mechanism is enforced by only allowing known ‘safe’ operations to occur, usually by channeling user actions through the accessor methods:

```
> data(sample.ExpressionSet)
> storageMode(sample.ExpressionSet)

[1] "lockedEnvironment"

> tryCatch(assayData(sample.ExpressionSet)$exprs <- log(exprs(sample.ExpressionSet)),
+   error = function(err) cat(conditionMessage(err)))

cannot change value of locked binding for 'exprs'

> exprs(sample.ExpressionSet) <- log(exprs(sample.ExpressionSet))
```

The `setReplaceMethod` for `exprs` (and `assayData`) succeeds by performing a deep copy of the entire environment. Because this is very inefficient, the recommended paradigm to update an element in a `lockedEnvironment` is to extract it, make many changes, and then reassign it. Developers can study `assayData` methods to learn more about how to lock and unlock environment bindings. *Biobase* allows the experienced user to employ (and run the risks of) environments, but the expectation is that most user objects are constructed with the default `lockedEnvironment` or `list`.

A longer term consideration in designing *AssayData* was to allow more flexible methods of data storage, e.g., through database-hosted arrays. This is facilitated by using generic functions such as `exprs()` for data access, so that classes derived from *AssayData* can provide implementations appropriate for their underlying storage mode.

5 Extending eSet

A designer wanting to implement `eSet` for a particular type of data creates a class that ‘contains’ `eSet`. The steps for doing this are described below. One example of such a class is `ExpressionSet`, designed to hold a matrix of gene expression values in the `assayData` slot.

```
> getClass("ExpressionSet")

Class "ExpressionSet" [package "Biobase"]

Slots:

Name:      assayData      phenoData      featureData
Class:     AssayData AnnotatedDataFrame AnnotatedDataFrame

Name:      experimentData      annotation      protocolData
Class:     MIAME               character AnnotatedDataFrame

Name:      __classVersion__
Class:     Versions
```

```

Extends:
Class "eSet", directly
Class "VersionedBiobase", by class "eSet", distance 2
Class "Versioned", by class "eSet", distance 3

> getValidity(getClass("ExpressionSet"))

function (object)
{
  msg <- validMsg(NULL, isValidVersion(object, "ExpressionSet"))
  msg <- validMsg(msg, assayDataValidMembers(assayData(object),
    c("exprs")))
  if (is.null(msg))
    TRUE
  else msg
}
<environment: namespace:Biobase>

```

The data structure of an `ExpressionSet` is identical to that of `eSet`, and in fact is inherited (without additional slot creation) from `eSet`. The main difference is that the validity methods of `eSet` are augmented by a method to check that the `assayData` slot contains an entity named `"exprs"`. A valid `ExpressionSet` object must also satisfy all the validity requirements of `eSet`, but the developer does not explicitly invoke validity checking of the parts of the data structure inherited from `eSet`.

5.1 Implementing a new class: a *SwirlSet* example

We want the *Swirl* data set (see the *SW* two color data set that motivates this class) to contain four elements in the `assayData` slot: `R`, `G`, `Rb`, `Gb`. To derive a class from `eSet` for this data, we create a class, and provide initialization and validation methods.

We create a class as follows:

```
> setClass("SwirlSet", contains = "eSet")
```

```
[1] "SwirlSet"
```

Notice that there are no new data elements in *SwirlSet* compared with `eSet`. The `initialize` method is written as

```
> setMethod("initialize", "SwirlSet", function(.Object,
+   R = new("matrix"), G = new("matrix"), Rb = new("matrix"),
+   Gb = new("matrix"), ...) {
+   callNextMethod(.Object, R = R, G = G, Rb = Rb, Gb = Gb,
+   ...)
+ })
```

```
[1] "initialize"
```

A slightly different `initialize` method allows the user to specify either the `assayData` or the `assayData` content. In advanced use, this has the advantage that `initialize` can be used as a ‘copy constructor’ to update several slots simultaneously.

```
> setMethod("initialize", "SwirlSet", function(.Object,
+   assayData = assayDataNew(R = R, G = G, Rb = Rb,
+   Gb = Gb), R = new("matrix"), G = new("matrix"),
```



```
+   Rb = new("matrix"), Gb = new("matrix"), ...) {
+   if (!missing(assayData) && any(!missing(R), !missing(G),
+     !missing(Rb), !missing(Gb))) {
+     warning("using 'assayData'; ignoring 'R', 'G', 'Rb', 'Gb'")
+   }
+   callNextMethod(.Object, assayData = assayData, ...)
+ })
```

```
[1] "initialize"
```

The structure of the `initialize` method is a bit different from those often seen in R. Often, `initialize` has only `.Object` as a named argument, or, if there are other named arguments, they correspond to slot names. Here our `initialize` method accepts four arguments, named after the `assayData` elements. Inside the `initialize` method, the named arguments are passed to the next initialization method in the hierarchy (i.e., `initialize` defined for `eSet`). The `eSet initialize` method then uses these arguments to populate the data slots in `.Object`. In particular, `eSet` places all arguments other than `phenoData`, `experimentData`, and `annotation` into the `assayData` slot. The `eSet initialize` method then returns the result to the `initialize` method of `SwirlSet`, which returns a `SwirlSet` object to the user:

```
> new("SwirlSet")

SwirlSet (storageMode: lockedEnvironment)
assayData: 0 features, 0 samples
  element names: G, Gb, R, Rb
phenoData
  sampleNames:
  varLabels and varMetadata description: none
featureData
  featureNames:
  fvarLabels and fvarMetadata description: none
experimentData: use 'experimentData(object)'
Annotation:
```

General programming guidelines emerge from experience with the `initialize` method of `eSet` and derived classes. First, an appropriate strategy is to name only those data elements that will be manipulated directly by the `initialize` method. For instance, the definition above did *not* name `phenoData` and other `eSet` slots by name. To do so is not incorrect, but would require that they be explicitly named (e.g., `phenoData=phenoData`) in the `callNextMethod` code. Second, the arguments `R`, `G`, `Rb`, `Rg` are present in the `initialize` method to provide defaults consistent with object construction; the ‘full’ form of `callNextMethod`, replicating the named arguments, is required in the version of R in which this class was developed. Third, named arguments can be manipulated before `callNextMethod` is invoked. Fourth, the return value of `callNextMethod` can be captured...

```
> setMethod("initialize", "MySet", function(.Object, ...) {
+   .Object <- callNextMethod(.Object, ...)
+ })
> .
```

and manipulated before being returned to the user. Finally, it is the responsibility of the developer to ensure that a valid object is created; `callNextMethod` is a useful way to exploit correctly designed `initialize` methods for classes that the object extends, but the developer is free to use other techniques to create valid versions of their class.

A validity method might complete our new class. A validity method is essential to ensure that the unique features of *SwirlSet* – our reason for designing the new class – are indeed present. We define our validity method to ensure that the `assayData` slot contains our four types of expression elements:

```
> setValidity("SwirlSet", function(object) {
+   assayDataValidMembers(assayData(object), c("R",
+       "G", "Rb", "Gb"))
+ })

Class "SwirlSet" [in ".GlobalEnv"]

Slots:

Name:      assayData      phenoData      featureData
Class:     AssayData AnnotatedDataFrame AnnotatedDataFrame

Name:      experimentData      annotation      protocolData
Class:     MIAME               character AnnotatedDataFrame

Name:      __classVersion__
Class:     Versions

Extends:
Class "eSet", directly
Class "VersionedBiobase", by class "eSet", distance 2
Class "Versioned", by class "eSet", distance 3
```

Notice that we do not have to explicitly request that the validity of other parts of the *SwirlSet* object are valid; this is done for us automatically. Objects are checked for validity when they are created, but not when modified. This is partly for efficiency reasons, and partly because object updates might transiently make them invalid. So a good programming practice is to ensure validity after modification, e.g.,

```
> myFancyFunction <- function(obj) {
+   assayData(obj) <- fancyAssaydData
+   phenoData(obj) <- justAsFancyPhenoData
+   validObject(obj)
+   obj
+ }
```

Assigning `fancyAssaydData` might invalidate the object, but `justAsFancyPhenoData` restores validity.

6 Versioned

One problem encountered in the Bioconductor project is that data objects stored to disk become invalid as the underlying class definition changes. For instance, earlier releases of *Biobase* contain a sample *eSet* object. But under the changes discussed here, *eSet* is virtual and the stored object is no longer valid. The challenge is to easily identify invalid objects, and to provide a mechanism for updating old objects to their new representation.

Biobase introduces the *Versioned* and *VersionedBiobase* classes to facilitate this. These classes are incorporated into key *Biobase* class definitions. *Biobase* also defines the `updateObject` generic and methods for conveniently updating old objects to their new representation.

```
> data(sample.ExpressionSet)
> classVersion(sample.ExpressionSet)
```

R	Biobase	eSet	ExpressionSet
"2.10.0"	"2.5.5"	"1.3.0"	"1.0.0"

```
> obj <- updateObject(sample.ExpressionSet)
```

The version information for this object is a named list. The first two elements indicate the version of R and Biobase used to create the object. The latter two elements are contained in the class prototype, and the class prototype is consulted to see if the instance of an object is 'current'. These lists can be subsetted in the usual way, e.g.,

```
> isCurrent(sample.ExpressionSet)[c("eSet", "ExpressionSet")]
```

eSet	ExpressionSet
TRUE	TRUE

Versioned classes, `updateObject` and related methods simplify the long-term maintenance of data objects. Take the fictitious *MySet* as an example.

```
> setClass("MySet", contains = "eSet", prototype = prototype(new("VersionedBiobase",
+   versions = c(classVersion("eSet"), MySet = "1.0.0"))))
```

```
[1] "MySet"
```

```
> obj <- new("MySet")
> classVersion(obj)
```

R	Biobase	eSet	MySet
"2.10.0"	"2.6.1"	"1.3.0"	"1.0.0"

This is a new class, and might undergo changes in its structure at some point in the future. When these changes are introduced, the developer will change the version number of the class in its prototype (the last line, below):

```
> setClass("MySet", contains = "eSet", prototype = prototype(new("VersionedBiobase",
+   versions = c(classVersion("eSet"), MySet = "1.0.1"))))
```

```
[1] "MySet"
```

```
> isCurrent(obj)
```

S4	R	Biobase	eSet	MySet
TRUE	TRUE	TRUE	TRUE	FALSE

and add code to update to the new version

```
> setMethod("updateObject", signature(object = "MySet"),
+   function(object, ..., verbose = FALSE) {
+     if (verbose)
+       message("updateObject(object = 'MySet')")
+     object <- callNextMethod()
+     if (isCurrent(object)["MySet"])
+       return(object)
+     if (!isVersioned(object))
```

```

+         new("MySet", assayData = updateObject(assayData(object),
+         ..., verbose = verbose), phenoData = updateObject(phenoData(object),
+         ..., verbose = verbose), experimentData = updateObject(experimentData(object),
+         ..., verbose = verbose), annotation = updateObject(annotation(object),
+         ..., verbose = verbose))
+     else {
+         classVersion(object)["MySet"] <- classVersion("MySet")["MySet"]
+         object
+     }
+ })

```

```
[1] "updateObject"
```

The code after `if(!isVersioned)` illustrates one way of performing ‘radical surgery, creating a new up-to-date instance by updating all slots. The `else` clause represents more modest changes, using methods to update stale information. `updateObject` then returns a new, enhanced object:

```

> classVersion(updateObject(obj))

      R   Biobase      eSet      MySet
"2.10.0" "2.6.1" "1.3.0" "1.0.1"

```

As in the example, versioning helps in choosing which modifications to perform – minor changes for a slightly out-of-date object, radical surgery for something more ancient. Version information might also be used in methods, where changing class representation might facilitate more efficient routines.

6.1 *Versioned* versus *VersionedBiobase*

The information on R and *Biobase* versions is present in `eSet` derived classes because `eSet` contains *VersionedBiobase*. On the other hand, *AnnotatedDataFrame* contains *Versioned*, and has only information about its own class version.

```

> classVersion(new("AnnotatedDataFrame"))

AnnotatedDataFrame
      "1.1.0"

```

The rationale for this is that *AnnotatedDataFrame* is and will likely remain relatively simple, and details about R and *Biobase* are probably irrelevant to its use. On the other hand, some aspects of `eSet` and the algorithms that operate on them are more cutting edge and subject to changes in R or *Biobase*. Knowing the version of R and *Biobase* used to create an instance might provide valuable debugging information.

6.2 Adding *Versioned* information to your own classes

The key to versioning your own classes is to define your class to **contain** *Versioned* or *VersionedBiobase*, and to add the version information in the prototype. For instance, to add a class-specific version stamp to *SwirlSet* we would modify the class definition to

```

> setClass("SwirlSet", contains = "eSet", prototype = prototype(new("VersionedBiobase",
+     versions = c(classVersion("eSet"), SwirlSet = "1.0.0"))))

[1] "SwirlSet"

```

```
> classVersion(new("SwirlSet"))
```

```
      R   Biobase      eSet SwirlSet  
"2.10.0" "2.6.1" "1.3.0" "1.0.0"
```

See additional examples in the *Versioned* help page.

It is also possible to add arbitrary information to particular instances.

```
> obj <- new("SwirlSet")  
> classVersion(obj)["MyID"] <- "0.0.1"  
> classVersion(obj)
```

```
      R   Biobase      eSet SwirlSet      MyID  
"2.10.0" "2.6.1" "1.3.0" "1.0.0" "0.0.1"
```

```
> classVersion(updateObject(obj))
```

```
      R   Biobase      eSet SwirlSet      MyID  
"2.10.0" "2.6.1" "1.3.0" "1.0.0" "0.0.1"
```

There is additional documentation about these classes and methods in *Biobase*.

7 Summary

This document summarizes *Biobase*, outlining strategies that developers using *Biobase* may find useful. The main points are to introduce the **eSet** class hierarchy, to illustrate how developers can effectively extend this class, and to introduce class versions as a way of tracking and easily updating objects. It is anticipated that **eSet**-derived classes will play an increasingly important role in *Biobase* development.

8 Session Information

The version number of R and packages loaded for generating the vignette were:

- R version 2.10.0 Patched (2009-10-27 r50222), i386-apple-darwin9.8.0
- Locale: C/en_US.UTF-8/C/C/C/C
- Base packages: base, datasets, grDevices, graphics, methods, stats, tools, utils
- Other packages: Biobase 2.6.1