EMBO03 Lab 2: Introduction to Bioconductor Annotation Packages

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One of the largest challenges in analyzing genomic data is associating the experimental data with the available biological *metadata*, e.g., sequence, gene annotation, chromosomal maps, literature. Bioconductor provides two main packages for this purpose: `annotate` (end-user) and `AnnBuilder` (developer). These packages can be used for querying databases such as GenBank, GO, LocusLink, and PubMed, from R, and for processing the query results in R.

In this lab, we focus on annotation resources for Affymetrix chips. Since Affymetrix chips have a standard layout, Bioconductor can provide annotation data packages for the main types of Affymetrix chips, e.g., hu6800, hgu33, hgu95, mgu74, and rgu34 series. These data packages are built using the `AnnBuilder` package. By contrast, there is no standard array design for two-color spotted microarray experiments; each lab tends to have its own custom design. In this case, specific annotation data packages will have to be created for each facility using `AnnBuilder`. Once annotation data packages are constructed to provide mappings between different sets of gene identifiers, the tools in `annotate` can be used in a similar manner for both platforms.

1 Mappings between different gene identifiers

A main task in annotation is to associate manufacturer (e.g., for Affymetrix chips) or in-house (e.g., for custom cDNA spotted arrays) probe identifiers to other available identifiers (e.g., PMID, GenBank accession number).

In this lab, we will use the Bioconductor annotation data packages to map between Affymetrix identifiers and identifiers for biological metadata available on the WWW. Because these data sources are very large, are constantly evolving, and are similar across species, we have adopted the strategy of distributing data in regularly updated R packages. Each mapping is contained in an *environment*. For our purposes, an environment is simply a *hash table*. It provides a very fast way of looking up *values* for specific text *keys*. This implementation is not the best and we hope to develop real hash tables for R but that will take some time.

We will again use the Golub et al. (1999) dataset as a case study and will explore annotation resources for the Affymetrix HU6800 chip used in this study. The Bioconductor annotation data package for the HU6800 chip is `hu6800` and can be downloaded from the "Data packages" section of the Bioconductor website. To load the packages needed for this lab

```
> library(Biobase)
> library(annotate)
> library(tkWidgets)
> library(geneplotter)
> library(golubEsets)
> library(hu6800)
> library(GO)
```

Alternately, since all these packages are required by the `EMBO03` course package, simply use

```
> library(EMBO03)

Loading required package: annotate
Loading required package: geneplotter
Loading required package: affy

Attaching package 'affy':


        The following object(s) are masked from package:base :

         barplot boxplot colnames hist history image ncol nrow

Loading required package: marrayTools
Loading required package: marrayClasses
Creating a new generic function for "print" in package
marrayClasses
Creating a new generic function for "rbind" in package
marrayClasses
Creating a new generic function for "cbind" in package
marrayClasses

Attaching package 'marrayClasses':
        The following object(s) are masked from package:base :

         cbind print rbind

Loading required package: stepfun
Loading required package: marrayInput
Loading required package: marrayPlots
Creating a new generic function for "plot" in package
.GlobalEnv
Loading required package: GO
Loading required package: hu6800
```

After loading the `hu6800` package, we have access to a number of different datasets (environments). These provide the many different mappings from Affymetrix identifiers to other probe identifiers, such as chromosomal location (in the `hu6800CHR` environment) and PMID (in the `hu6800PMID` environment). Quality control data, counts, etc., for the mappings are available by calling the function `hu6800()` and by examining the help page, `?hu6800`.

```
> hu6800()

Quality control information for hu6800:

Date built:  Thu Feb 13 11:00:27 2003
Number of probes: 7129
Probe number missmatch: None
Probe missmatch: None
Mappings found for probe based rda files:
         hu6800ACCNUM found 7092 of 7129
         hu6800CHRLOC found 6467 of 7129
         hu6800CHRORI found 6467 of 7129
         hu6800CHR found 6882 of 7129
         hu6800ENZYME found 1067 of 7129
         hu6800GENENAME found 6913 of 7129
         hu6800GO found 5731 of 7129
         hu6800GRIF found 3533 of 7129
         hu6800LOCUSID found 6918 of 7129
         hu6800MAP found 6873 of 7129
         hu6800PATH found 1448 of 7129
         hu6800PMID found 6837 of 7129
         hu6800SUMFUNC found 6087 of 7129
         hu6800SYMBOL found 6913 of 7129
         hu6800UNIGENE found 6834 of 7129
Mappings found for non-probe based rda files:
          hu6800AFFYCOUNTS found 2149
         hu6800ENZYME2AFFY found 469
         hu6800GO2AFFY found 2149
         hu6800GO2ALLAFFY found 2149
         hu6800PATH2AFFY found 120
         hu6800PMID2AFFY found 30866
```

The main R functions we will use for dealing with environments are `ls` (to list the names of objects in a specified environment) and `get` (to search for an R object with a given name and return its value, if found, in the specified environment). We obtain the Affymetrix identifiers using `ls` and note that there are 7,129 identifiers for the HU6800 chip (this matches the Golub `exprSets`).

```
> affyID <- ls(env = hu6800CHR)
> length(affyID)

[1] 7129
```

We now arbitrarily select the probe set with Affymetrix identifier `"U18237_at"` and obtain a number of other IDs corresponding to this probe set. Note that there may be several PMIDs, i.e., PubMed abstracts, associated with a given gene.

```
> mygene <- affyID[4001]
> mygene

[1] "U18237_at"

> get(mygene, env = hu6800ACCNUM)

[1] "U18237"

> get(mygene, env = hu6800LOCUSID)

[1] 23456

> get(mygene, env = hu6800SYMBOL)

[1] "ABCB10"

> get(mygene, env = hu6800GENENAME)

[1] "ATP-binding cassette, sub-family B (MDR/TAP), member 10"
```

```
> get(mygene, env = hu6800SUMFUNC)

[1] NA

> get(mygene, env = hu6800UNIGENE)

[1] "Hs.1710"

> get(mygene, env = hu6800CHR)

[1] "1"

> get(mygene, env = hu6800CHRLOC)

        1
225346509

> get(mygene, env = hu6800CHRORI)

  1
"-"

> get(mygene, env = hu6800MAP)

[1] "1q42"

> get(mygene, env = hu6800PMID)

[1] 10922475 10748049  7766993

> get(mygene, env = hu6800GO)

[1] NA
```

In some cases, we need to obtain data on several genes at once. We wrote a special function for this purpose: `multiget`.

```
> fivegenes <- affyID[6:10]
> fivegenes

[1] "AB000409_at"   "AB000410_s_at" "AB000449_at"   "AB000450_at"
[5] "AB000460_at"

> multiget(fivegenes, env = hu6800PMID)
```

```
$"AB000409_at"
[1] 10859165  9155018

$"AB000410_s_at"
 [1] 12244119 12189194 12164330 12119232 12117782 12034821 11992556 11927502
 [9] 11902834 11837743 10449904 10233168  9681819  9348312  9321410  9223306
[17]  9223305  9207108  9197244  9190902  9187114

$"AB000449_at"
[1] 9344656

$"AB000450_at"
[1] 9344656

$"AB000460_at"
[1] 9734812
```

Instead of relying on the general R functions for environments (e.g., `get`), the development version of the `annotate` package also provides new and more user-friendly functions for accessing specific identifiers.

```
> getSYMBOL(fivegenes, data = "hu6800")

  AB000409_at AB000410_s_at   AB000449_at   AB000450_at   AB000460_at
      "MKNK1"        "OGG1"        "VRK1"        "VRK2"      "C4orf8"

> getLL(fivegenes, data = "hu6800")

  AB000409_at AB000410_s_at   AB000449_at   AB000450_at   AB000460_at
         8569          4968          7443          7444          8603

> getPMID(fivegenes, data = "hu6800")

$"AB000409_at"
[1] 10859165  9155018

$"AB000410_s_at"
 [1] 12244119 12189194 12164330 12119232 12117782 12034821 11992556 11927502
 [9] 11902834 11837743 10449904 10233168  9681819  9348312  9321410  9223306
[17]  9223305  9207108  9197244  9190902  9187114

$"AB000449_at"
[1] 9344656
```

```
$"AB000450_at"
[1] 9344656

$"AB000460_at"
[1] 9734812

> gg <- getGO(fivegenes, data = "hu6800")
> getGOdesc(gg[[2]], "MF")

$"GO:0008534"
[1] "purine-specific oxidized base lesion DNA N-glycosylase"

$"GO:0003684"
[1] "damaged DNA binding"

$"GO:0004519"
[1] "endonuclease"
```

A variety of information about the probes can now easily be obtained by, for example, querying PubMed, GenBank, and LocusLink, as described below. In addition, one can also use R to compute on these data and perform a number of quality control and exploratory analyses.

```
> whChrom <- multiget(affyID, env = hu6800CHR)
> table(unlist(whChrom))

  1  10  11  12  13  14  15  16  17  18  19   2  20  21  22   3   4   5   6   7 
673 250 414 419 107 235 181 269 440  98 422 443 150 100 171 365 275 294 419 326 
  8   9   X   Y 
236 254 325  26 

> vv <- sapply(whChrom, length)
> table(vv)

vv
   1    2 
7119   10 

> whChrom[vv == 2]

$"D49410_at"
[1] "X" "Y"

$"HG2868-HT3012_s_at"
```

```
[1] "X" "Y"

$"HG3936-HT4206_at"
[1] "X" "Y"

$"J03592_at"
[1] "X" "Y"

$"L39064_rna1_at"
[1] "X" "Y"

$"M16279_at"
[1] "X" "Y"

$"U11090_at"
[1] "X" "Y"

$"U13706_at"
[1] "X" "Y"

$"U82668_rna1_at"
[1] "X" "Y"

$"X17648_at"
[1] "X" "Y"
```

This yields the distribution of probe sets by chromosome. Note that there are 10 genes that have been assigned two chromosome locations. We might want to explore these further by examining resources at the NCBI.

2 Querying PubMed

The National Library of Medicine (NLM) provides a great deal of information on biological data. We have only just started to explore these resources. We have already developed tools for interacting with PubMed, but need to develop further functionality on MeSH and other available resources. A mapping has been done between LocusLink and PubMed. This associates specific articles with specific genes. The articles can be queried interactively using tools available in R and other systems. Full text abstracts are readily available; in some cases full text articles are also available (we don't have the tools to make use of the later). We next demonstrate how to interact with PubMed using functions in `annotate`.

Bioconductor functions for querying PubMed and other WWW databases from R rely on the `browseURL` function and the R `XML` package to parse query results. For more details, please consult articles in R News (Gentleman & Gentry (2002), *R News* 2(2); Temple Lang (2001), *R News* 1(1)).

```
> browseURL("www.r-project.org")
```

A `pubMedAbst` class structure was defined for handling PubMed abstracts in R. The slots are

```
> slotNames("pubMedAbst")

[1] "authors"      "abstText"     "articleTitle" "journal"      "pubDate"
[6] "abstUrl"
```

The basic engine for talking to PubMed is the function `pubmed`. Given a vector of PMIDs, the function either has a browser display a URL showing the results of the PubMed query for those identifiers (`disp="browser"`) or creates an `XMLdoc` object with the same data (`disp="data"`).

```
> pmids <- getPMID(mygene, data = "hu6800")
> pubmed(pmids, disp = "browser")
> absts1 <- pubmed(pmids, disp = "data")
```

The function `pm.getabst` provides a simpler way to download the specified PubMed abstracts (stored in XML) and create a list of `pubMedAbst` objects. The following commands can be used to store the PubMed abstracts for 5 genes in a list of objects of class `pubMedAbst`, to compute the number of abstracts retrieved for each gene, and to print the abstract(s) for the first gene. We can see, for example, that one of the genes has 21 abstracts associated with it.

```
> absts2 <- pm.getabst(fivegenes, "hu6800")

Loading required package: XML

> lapply(absts2, length)

$"AB000409_at"
[1] 2

$"AB000410_s_at"
[1] 21

$"AB000449_at"
[1] 1
```

```
$"AB000450_at"
[1] 1

$"AB000460_at"
[1] 1

> absts2[[1]]

[[1]]
list()
attr(,"authors")
[1] "R Cuesta"     "G Laroia"     "RJ Schneider"
attr(,"abstText")
[1] "Inhibition of protein synthesis during heat shock limits accumulation of unfolded
attr(,"articleTitle")
[1] "Chaperone hsp27 inhibits translation during heat shock by binding eIF4G and facili
attr(,"journal")
[1] "Genes Dev"
attr(,"pubDate")
[1] "Jun 2000"
attr(,"abstUrl")
[1] "No URL Provided"
attr(,"class")
[1] "pubMedAbst"

[[2]]
list()
attr(,"authors")
[1] "R Fukunaga" "T Hunter"
attr(,"abstText")
[1] "We have developed a novel expression screening method for identifying protein kina
attr(,"articleTitle")
[1] "MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression sc
attr(,"journal")
[1] "EMBO J"
attr(,"pubDate")
[1] "Apr 1997"
attr(,"abstUrl")
[1] "No URL Provided"
attr(,"class")
[1] "pubMedAbst"
```

The functions `pm.titles` and `pm.abstGrep` can then be used to extract the titles from a set of PubMed abstracts and for regular expression matching on these abstracts, respectively. For the genes with Affy IDs `fivegenes`, the following commands extract the abstract titles and search the abstracts for the word "protein", with lower case "p" or upper case "P".

```
> pm.titles(absts2)

[[1]]
[1] "Chaperone hsp27 inhibits translation during heat shock by binding eIF4G and facili
[2] "MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression sc

[[2]]
 [1] "Conditional targeting of the DNA repair enzyme hOGG1 into mitochondria."
 [2] "Inter-individual variation, seasonal variation and close correlation of OGG1 and 
 [3] "A limited association of OGG1 Ser326Cys polymorphism for adenocarcinoma of the lu
 [4] "Protection of human lung cells against hyperoxia using the DNA base excision repa
 [5] "The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer ris
 [6] "Human OGG1 undergoes serine phosphorylation and associates with the nuclear matri
 [7] "hOGG1 Ser(326)Cys polymorphism and modification by environmental factors of stoma
 [8] "Association of the hOGG1 Ser326Cys polymorphism with lung cancer risk."
 [9] "Reciprocal \"flipping\" underlies substrate recognition and catalytic activation 
[10] "Expression of 8-oxoguanine DNA glycosylase is reduced and associated with neurofi
[11] "Structure and chromosome location of human OGG1."
[12] "Expression and differential intracellular localization of two major forms of huma
[13] "Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involve
[14] "Augmented expression of a human gene for 8-oxoguanine DNA glycosylase (MutM) in B
[15] "Opposite base-dependent reactions of a human base excision repair enzyme on DNA c
[16] "Molecular cloning and functional expression of a human cDNA encoding the antimuta
[17] "Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of Saccha
[18] "Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase."
[19] "A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a
[20] "Cloning of a human homolog of the yeast OGG1 gene that is involved in the repair 
[21] "Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosyla

[[3]]
[1] "Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2

[[4]]
[1] "Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2

[[5]]
[1] "The primary structure and genomic organization of five novel transcripts located c
```

```
> sapply(absts2, function(x) pm.abstGrep("[Pp]rotein", x))

$"AB000409_at"
[1] TRUE TRUE

$"AB000410_s_at"
 [1]  TRUE FALSE FALSE  TRUE FALSE  TRUE FALSE FALSE FALSE FALSE FALSE FALSE
[13]  TRUE FALSE  TRUE  TRUE  TRUE FALSE  TRUE  TRUE  TRUE

$"AB000449_at"
[1] TRUE

$"AB000450_at"
[1] TRUE

$"AB000460_at"
[1] TRUE
```

The new function `pmAbst2html` from the development version of `annotate` takes a list of `pubMedAbst` objects and generates an HTML report with the titles of the abstracts and links to their full page on PubMed. The report will be stored in the file `pm.html` in the working directory.

```
> pmAbst2html(absts2[[2]],filename="pm.html")
```

3 Querying GenBank

Another source of data is GenBank. We can interact with GenBank in much the same way as with PubMed; the main function for this purpose is `genbank`.

```
> gbacc <- multiget(fivegenes, hu6800ACCNUM)
> genbank(gbacc, disp = "browser")
> gb <- genbank(gbacc[1], disp = "data")
```

4 Querying LocusLink

Finally, we consider interactions with LocusLink to obtain sequence and other biological information on probe sets of interest.

```
> llid <- getLL(fivegenes, data = "hu6800")
> locuslinkByID(llid)
```

5 HTML reports

The function `ll.htlmpage` generates HTML reports, with one row per gene and a clickable entry which opens the LocusLink webpage for that gene. Additional information can be displayed using the `othernames` argument as shown below. Such HTML reports provide an easy way to communicate results with other researchers working on the same analysis.

```
> tengenes <- affyID[1:10]
> llid <- getLL(tengenes, data = "hu6800")
> symb <- getSYMBOL(tengenes, data = "hu6800")
> map <- multiget(tengenes, hu6800MAP)
> res <- data.frame(tengenes, cbind(unlist(symb), unlist(map)))
> names(res) <- c("Affy ID", "Gene symbol", "Chromosomal location")
> ll.htmlpage(llid, filename = "ll.html", title = "HTML report for 10 genes",
+     othernames = res, table.head = c("LocusID", names(res)),
+     table.center = TRUE)
```

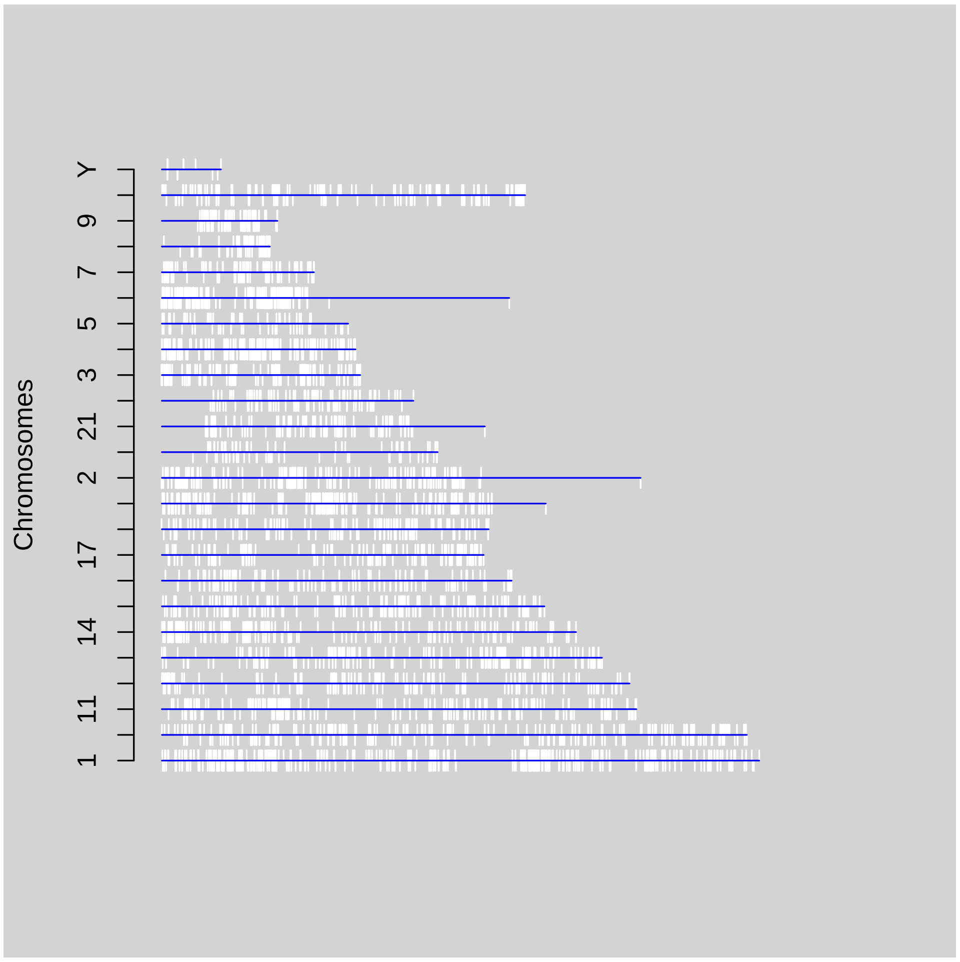
6 Plotting genomic data

The classes `chromLoc` and `chromLocation` are used to keep track of location information for a single gene and a set of genes (entire genome), respectively. The object `hu6800ChrClass` from the `EMBO03` course package is an instance of the class `chromLocation` for the Affymetrix HU6800 chip. It was constructed using the ”byChroms” vignette in the `geneplotter` package.

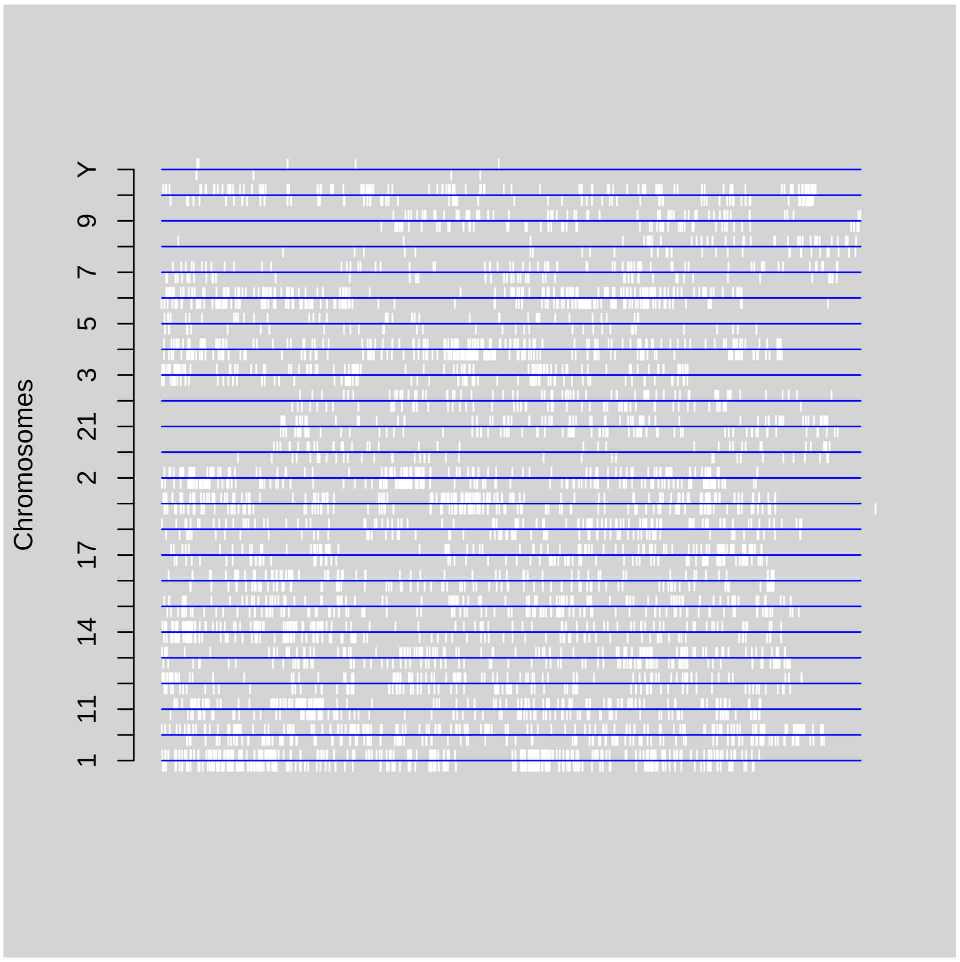
```
> slotNames("chromLoc")

[1] "chrom"    "position" "strand"

> data(hu6800ChrClass)
> species(hu6800ChrClass)

[1] "Human"

> chromNames(hu6800ChrClass)

 [1] "1"  "10" "11" "12" "13" "14" "15" "16" "17" "18" "19" "2"  "20" "21" "22"
[16] "3"  "4"  "5"  "6"  "7"  "8"  "9"  "X"  "Y"
```

The `geneplotter` package provides two main functions for plotting genomic data: `cPlot` and `alongChrom`. These functions operate on instances of the class `chromLocation`. With `cPlot`, we can render all chromosomes the same length or we can scale them by their relative lengths. Note that the `mva` package in the next release of R (1.7.0) will have new functions for heat maps and dendrograms.

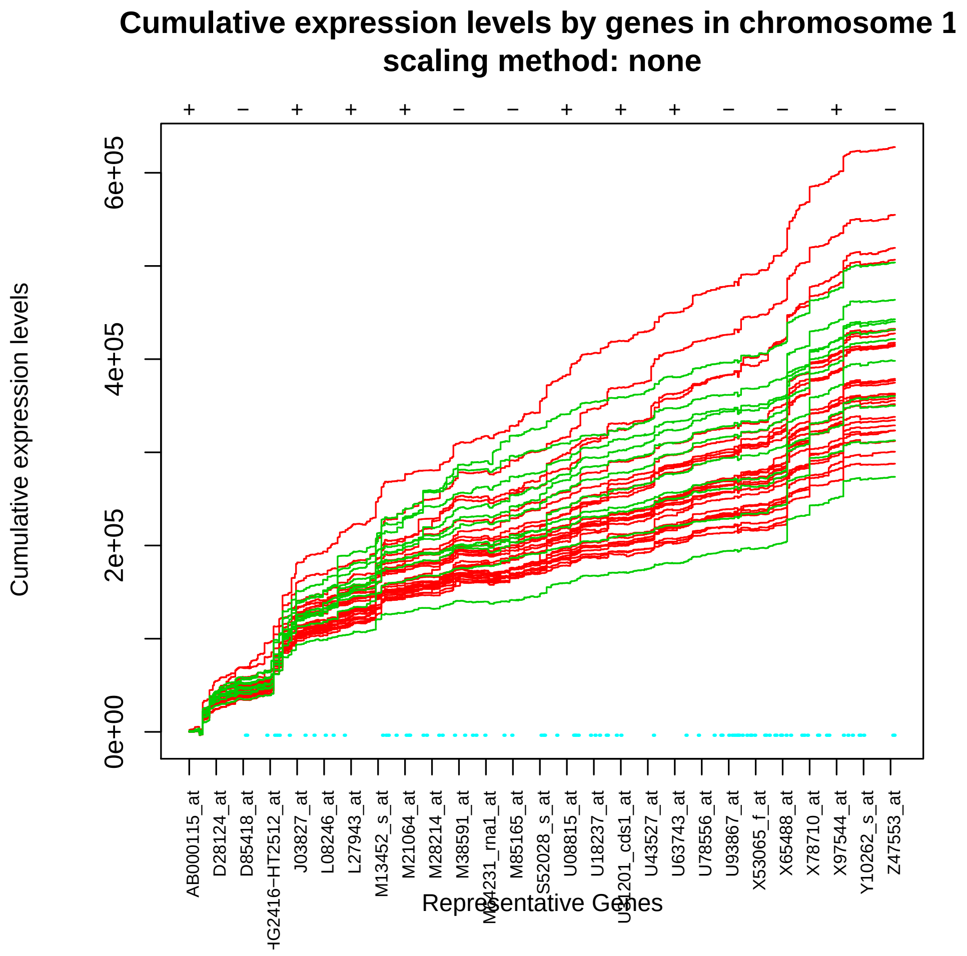
```
> cPlot(hu6800ChrClass, scale = "relative")
```



```
> cPlot(hu6800ChrClass, scale = "max")
```

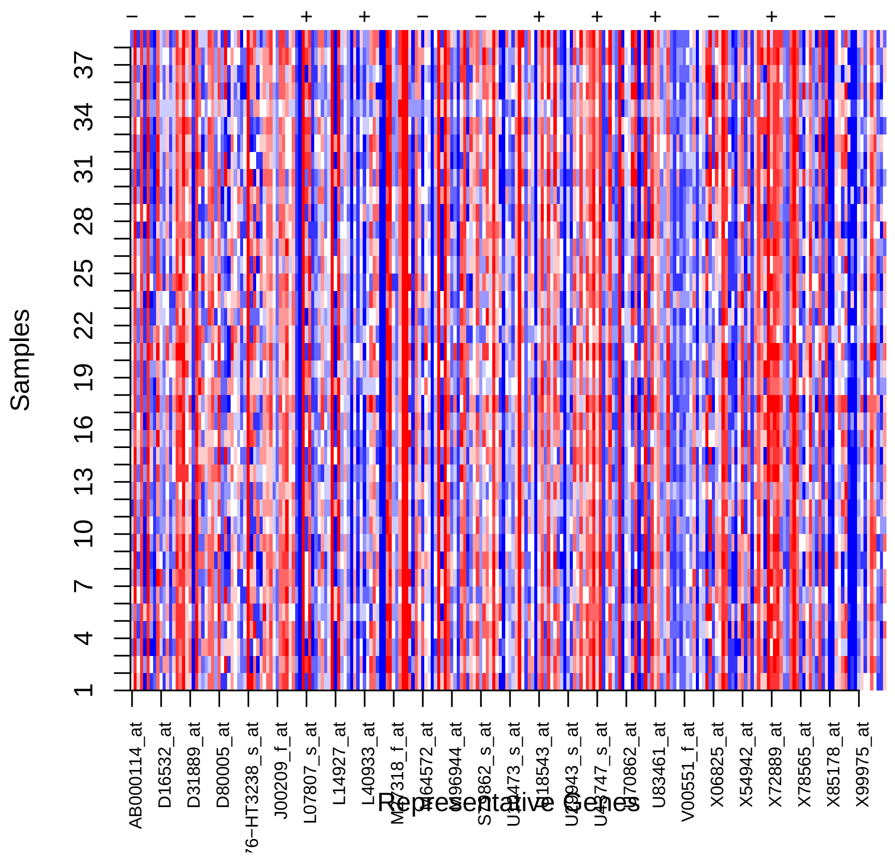


```
> data(golubTrain)
> cols <- as.numeric(pData(golubTrain)$ALL.AML) + 1
> alongChrom(golubTrain, chrom = 1, specChrom = hu6800ChrClass,
+     colors = cols)

<environment: 0xb829220>
```



```
> alongChrom(golubTrain, chrom = 22, specChrom = hu6800ChrClass,
+     plotFormat = "image")
```

Expression levels by genes in chromosome 22
scaling method: none


Samples
AB000114_at
D16532_at
D31889_at
D80005_at
HT3238_s_at
J00209_f_at
L07807_s_at
L14927_at
L40933_at
Representative Genes
U83461_at
V00551_f_at
X06825_at
X54942_at
X72889_at
X78565_at
X85178_at
X99975_at