Tools for high throughput SNP chip data

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Introduction

SNPchip defines classes and methods useful for organizing high throughput genomic data. The classes defined here extend the eSet class in Biobase, utilizing the existing Bioconductor infrastructure for genomic data. This provides a foundation upon which statistical and visualization tools can be further developed. See [2] for additional details.

1 Simple Usage

```r
> library(SNPchip)
> data(sample.snpset)
> sample.snpset

oligoSnpSet (storageMode: lockedEnvironment)
assayData: 5859 features, 5 samples
  element names: calls, callsConfidence, cnConfidence, copyNumber
experimentData: use 'experimentData(object)'
Annotation: pd.mapping50k.xba240
phenoData
An object of class "AnnotatedDataFrame"
sampleNames: NA17101_X_hAF_A1_4000091.CEL, NA17102_X_hAF_A2_4000091.CEL, ..., NA17105_X_hAF_A5_4000091.CEL (5 total)
  varLabels and varMetadata description: none
featureData
An object of class "AnnotatedDataFrame"
  rowNames: SNP_A-1507972, SNP_A-1641761, ..., SNP_A-1759046 (5859 total)
  varLabels and varMetadata description:
    dbsnp_rs_id: dbsnp_rs_id
    chromosome: chrom
    enzyme: enzyme
    (8 total)
Annotation [1] "pd.mapping50k.xba240"
```

For Affymetrix platforms, access to SNP-level annotation such as chromosome and physical position is through RSQLite. See the oligo vignette for additional information regarding available SNP-level Annotation. Accessing RSQLite databases each time chromosome and physical position are needed (such as for making scatterplots of physical position versus copy number) may increase the time of computation, particularly for small objects such as the `sample.snpset`. Therefore, one may wish to
add this information to the \texttt{featureData} slot. Subsequent calls to chromosome and physical position will look in the \texttt{featureData} before searching in the RSQLite database. Because \texttt{sample.snpset} is small and adding this annotation to the \texttt{featureData} slot will greatly increase the speed for graphing the data (the graphs make repeated use of chromosome and position calls), we add this annotation to the \texttt{featureData} for this vignette.

\begin{verbatim}
> if (require("pd.mapping50k.xba240")) {
+ system.time(tmp <- chromosome(sample.snpset))
+ object.size(sample.snpset)
+ featureData(sample.snpset)$chromosome <- chromosome(sample.snpset)
+ featureData(sample.snpset)$position <- position(sample.snpset)
+ system.time(tmp <- chromosome(sample.snpset))
+ }
\end{verbatim}

Note that if one is using a platform for which there is no \texttt{pd.mapping} package available, one must provide the chromosome (character string) and physical position (integer) in the \texttt{featureData} slot using the column labels “chromosome” and “position”, respectively. Subsetting an object inheriting from the class \texttt{SnpLevelSet} is done in the usual way. For instance, here we select SNPs on the first three chromosomes and then generate an object of class \texttt{ParSnpSet} that contains graphical parameters for visualizing the data for this subset:

\begin{verbatim}
> snpset <- sample.snpset[chromosome(sample.snpset) %in%
+ as.character(1:3), 1:4]
> graph.par <- plotSnp(snpset)
> class(graph.par)
[1] "ParSnpSet"
attr(,"package")
[1] "SNPchip"

> graph.par$use.chromosome.size <- TRUE
> graph.par$pch <- "."
> graph.par$cex <- 3
\end{verbatim}

Plot the first few chromosomes for samples 1-4 (note the print() command is needed for producing the figures in the vignette, but just typing the object name is sufficient for plotting):

\begin{verbatim}
> print(graph.par)
\end{verbatim}
The samples are plotted by row. For each sample, the copy number (vertical axis) is plotted against the physical position of the SNP in the chromosome. Here, the chromosome labels are plotted beneath the cytobands.

2 Examples

2.1 Genome-wide plots for multiple samples

A genome-wide view of copy number and genotype calls versus physical position can be made using plotSnp. Here, we plot chromosomes 1-22 and X of samples 1 - 3 in the object sample.snpset:

```r
> graph.par <- plotSnp(sample.snpset[, 1:3], add.cytoband = FALSE)
> graph.par$one.ylim <- TRUE
> graph.par$mar <- c(0.1, 0.1, 2, 0.1)
> graph.par$oma <- c(3, 4, 2, 1)
> graph.par$cex <- 2
> graph.par$abline <- TRUE
> graph.par$cex.lab <- 0.9
> graph.par$add.cytoband <- FALSE

> print(graph.par)
```
Note that we suppress the cytobands in the above plot (the resolution is too poor at this level) by the argument `add.cytoBand` in the function `getPar`. The default plot layout generally works well, but can be adjusted through additional arguments to `par` and layout.

### 2.2 Subsetting for more focused plots

A more focused view of chromosomes 1, 7, 16, 19, and X of sample 2 could be obtained by

```r
> graph.par <- plotSnps(sample.snpset[chromosome(sample.snpset) %in%
+ c(1, 7, 16, 19, "X"), 2])
> graph.par$cex <- 0.8
> graph.par$mar <- rep(0.5, 4)
> graph.par$pch <- c(20, 21, 20)
> graph.par$bty <- "o"
> graph.par$cex.axis <- 1.2
> graph.par$cex.lab <- 1.5
> graph.par$xaxs <- "r"
> graph.par$abline <- TRUE
> graph.par$abline.col <- "black"
> print(graph.par)
```

```r
> print(graph.par)
```
A plot of just the p-arm in sample 2 of chromosome 1.

```r
> data(chromosomeAnnotation)
> parm <- chromosomeAnnotation["1", "centromereStart"]
> snpset <- sample.snpset[chromosome(sample.snpset) == "1" & position(sample.snpset) < parm, 2]
> graph.par <- plotSnp(snpset)
> graph.par$use.chromosome.size <- FALSE
> graph.par$pch <- 21
> graph.par$cex <- 1
> graph.par$ylim <- c(0.4, 9)
> graph.par$cytoband.ycoords <- c(0.5, 0.6)
> print(graph.par)
```

Note that the cytoband is automatically subsetted appropriately. Had we instead specified `use.chromosome.size=TRUE`, the x-axis limits would include the entire chromosome (and cytoband) though only the SNPs on the p-arm would be plotted.

Adding a legend for the genotypes

```r
> data(sample.snpset)
> x <- sample.snpset[chromosome(sample.snpset) == "1", 1]
> gp <- plotSnp(x)
> gp$legend <- c("AA", "AB", "BB")
> gp$legend.col <- unique(gp$col)
```
2.3 Plotting cytoband

To plot the cytoband of chromosome 1,

```r
> plotCytoband("1")
```

NULL

2.4 Smoothing example

Hidden Markov models for objects of class SnpCallSet, SnpCopyNumberSet, and oligoSnpSet are available in the package VanillaICE available at Bioconductor. A more detailed description of the hidden Markov models fit in the VanillaICE package are discussed elsewhere (1). Here we provide an example of a lowess smoother for copy number estimates. The following code chunk first assigns
heterozygous calls to the integer 1 and homozygous calls to the integer zero. It follows that regions of deletions will have homozygous calls of zero. We simulated a deletion of 50 consecutive SNPs and then converted the \texttt{sample.snpset} to a list where each element in the list is an \texttt{oligoSnpSet} object for one chromosome.

\begin{verbatim}
> sim1 <- sample.snpset[chromosome(sample.snpset) %in% 1:5, 1]
> sim1 <- sim1[chromosome(sim1) == "1", ]
> sim1 <- sim1[order(position(sim1)), ]
> copyNumber(sim1)[101:150, 1] <- copyNumber(sim1)[101:150, + 1] - 1
> calls(sim1)[101:150, 1] <- 1
> smoothSet <- smoothSnp(sim1, 1:5, 1:3, span = 1/10)
> highlight <- calls(smoothSet)[, 1] <= 0.1 & copyNumber(smoothSet)[, + 1] <= 1.5

A plot of the smoothed calls versus copynumber can be used to visualize the deletion:

\begin{verbatim}
> op <- par(las = 1, mfrow = c(1, 1), mar = c(5, 4, 0.5, 0.5), oma = rep(0, 4))
> plot(calls(smoothSet)[, 1], copyNumber(smoothSet)[, 1], ylim = range(copyNumber(smoothSet)), pch = ".", + cex = 3, xlab = "% heterozygous calls", ylab = "smooth copy number", + xaxt = "n", xlim = c(-0.05, 30/70 + 0.2))
> axis(1, at = pretty(calls(smoothSet)), labels = pretty(calls(smoothSet)))
> points(calls(smoothSet)[highlight, 1], copyNumber(smoothSet)[highlight, + 1], pch = 20, col = "royalblue", bg = "white")
> par(op)
\end{verbatim}
\end{verbatim}

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{plot.png}
\caption{Plot of smoothed calls versus copynumber.
\label{fig:plot}}
\end{figure}
\end{center}

> graph.par$cex <- 1
> graph.par$use.chromosome.size <- TRUE
> graph.par$main <- "Chromosome 1: Example title"
> graph.par$label.chromosome <- FALSE
> print(graph.par)
> print(graph.par)
2.5 Descriptive and statistical summaries

Descriptive statistics for copy number and genotype calls are provided with the `summary` method. For each chromosome in the `oligoSnpSet`, `summary` calculates the average and standard deviation of the copy number estimates, as well as the % homozygous and heterozygous calls. In addition, `summary` calculates the average copy number, standard deviation, % homozygous and heterozygous across all autosomes in the `oligoSnpSet`. The dimensions of the four matrices are S x C + 1, where S is the number of samples and C is the number of chromosomes in the `oligoSnpSet`.

```r
> x <- summary(sample.snpset, digits = 1)
> str(x)
List of 5
$ avg.CN : num [1:23, 1:5] 2.2 2.2 2.2 2.1 2.1 2.1 2.2 2.1 2.2 2.2 ... 
  ..- attr(*, "dimnames")=List of 2 
  .. ..$ : chr [1:23] "1" "10" "11" "12" ... 
  .. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17103_X_hAF_A3_4000091.CEL" ... 
$ sd.CN : num [1:23, 1:5] 1 0.9 1.1 0.8 0.9 0.8 0.8 0.9 0.8 0.9 ... 
  ..- attr(*, "dimnames")=List of 2 
  .. ..$ : chr [1:23] "1" "10" "11" "12" ... 
  .. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17103_X_hAF_A3_4000091.CEL" ... 
$ prop.Hom : num [1:23, 1:5] 0.7 0.7 0.7 0.6 0.7 0.7 0.7 0.7 0.7 0.7 ... 
  ..- attr(*, "dimnames")=List of 2 
  .. ..$ : chr [1:23] "1" "10" "11" "12" ... 
  .. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17103_X_hAF_A3_4000091.CEL" ... 
$ prop.Het : num [1:23, 1:5] 0.3 0.3 0.3 0.4 0.3 0.3 0.3 0.3 0.3 0.3 ... 
  ..- attr(*, "dimnames")=List of 2 
  .. ..$ : chr [1:23] "1" "10" "11" "12" ... 
  .. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17103_X_hAF_A3_4000091.CEL" ... 
$ prop.NoCall: num [1:23, 1:5] 0 0 0 0 0 0 0 0 0 0 ... 
  ..- attr(*, "dimnames")=List of 2
```

Boxplot by chromosome:
> op <- par(mfrow = c(1, 1), mar = c(4, 4, 3, 1),
+ las = 1)
> boxplot(split(copyNumber(sample.snpset[, 1]),
+ chromosome(sample.snpset)), ylab = "copy number",
+ main = sampleNames(sample.snpset)[1], log = "y")
> par(op)

3 Annotation

3.1 Chromosome-level
The chromosome-level annotation used in the plotting methods can be accessed by `data()` calls:

> data(chromosomeAnnotation)
> chromosomeAnnotation[1:5, ]

<table>
<thead>
<tr>
<th>chromosomeStart</th>
<th>centromereEnd</th>
<th>chromosomeSize</th>
</tr>
</thead>
<tbody>
<tr>
<td>121147476</td>
<td>123387476</td>
<td>245522847</td>
</tr>
<tr>
<td>91748045</td>
<td>94748045</td>
<td>243018229</td>
</tr>
<tr>
<td>90587544</td>
<td>93487544</td>
<td>199505740</td>
</tr>
<tr>
<td>49501045</td>
<td>52501045</td>
<td>191411218</td>
</tr>
<tr>
<td>46441398</td>
<td>49441398</td>
<td>180857866</td>
</tr>
</tbody>
</table>

> data(cytoband)
> cytoband[1:5, ]

<table>
<thead>
<tr>
<th>chrom</th>
<th>chromStart</th>
<th>chromEnd</th>
<th>name</th>
<th>gieStain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>2300000</td>
<td>p36.33</td>
<td>gneg</td>
</tr>
<tr>
<td>2</td>
<td>2300000</td>
<td>5300000</td>
<td>p36.32</td>
<td>gpos25</td>
</tr>
<tr>
<td>3</td>
<td>5300000</td>
<td>7100000</td>
<td>p36.31</td>
<td>gneg</td>
</tr>
<tr>
<td>4</td>
<td>7100000</td>
<td>9000000</td>
<td>p36.23</td>
<td>gpos25</td>
</tr>
<tr>
<td>5</td>
<td>9000000</td>
<td>12300000</td>
<td>p36.22</td>
<td>gneg</td>
</tr>
</tbody>
</table>
This is a static table and will be deprecated once chromosome-level annotation is provided by Bioconductor. If chromosome-level annotation is available in Bioconductor, please send me a note.

3.2 Feature-level

Feature-level annotation for Affymetrix platforms is available in the pd.mapping packages. See the oligo vignette for additional information about available feature-level annotation.

4 Integration with other Bioconductor packages

4.1 oligo

For generating SnpCallSets from .CEL files, see the R package oligo. In particular, the function crlmm in oligo creates an instance of the class SnpCallSetPlus. See the oligoHowTo vignette for details on coercing an object of class SnpCallSetPlus to oligoSnpSet.

4.2 RSNPper

To retrieve additional annotation on the known SNP’s in the region of this simulated deletion, we could use the RSNPper.

```R
> library(RSNPper)
> (dbId <- dbSnpId(annSnpset)[snps[2] == featureNames(annSnpset)])
> dbId <- strsplit(dbId, "rs")[[1]][2]
> print(SNPinfo(dbId))
```

5 Session Information

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

- R version 2.7.0 (2008-04-22), x86_64-unknown-linux-gnu
- Locale: LC_CTYPE=en_US;LC_NUMERIC=C;LC_TIME=en_US;LC_COLLATE=en_US;LC_MONETARY=C;LC_MESSAGES=en_US;LC_PAPER=en_US;LC_NAME=C;LC_ADDRESS=C;LC_TELEPHONE=C;LC_MEASUREMENT=en_US;LC_IDENTIFICATION=C
- Base packages: base, datasets, graphics, grDevices, methods, splines, stats, tools, utils
- Other packages: Biobase 2.0.0, genefilter 1.20.0, oligoClasses 1.2.0, SNPchip 1.4.0, survival 2.34-1
- Loaded via a namespace (and not attached): annotate 1.18.0, AnnotationDbi 1.2.0, DBI 0.2-4, RSQLite 0.6-8

References
