ChromHeatMap

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1 Introduction

The **ChromHeatMap** package provides functions for visualising expression data in a genomic context, by generating heat map images in which data is plotted along a given chromosome for all the samples in a data matrix.

These functions rely on the existence of a suitable **AnnotationDbi** package which provides chromosome location information for the probe- or gene-level identifiers used in your data set. The data themselves must be in either an ExpressionSet, or a data matrix with row names corresponding to probe or gene identifiers and columns corresponding to samples. While the **ChromHeatMap** package was originally designed for use with microarray data, given an appropriate **AnnotationDbi** package it can also be used to visualise data from next-generation sequencing experiments.

The output heatmap can include sample clustering, and data can either be plotted for each strand separately, or both strands combined onto a single heat map. An idiogram showing the cytogenetic banding pattern of the chromosome will be plotted for supported organisms (at the time of writing: *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*; please contact the maintainer to request additions).

Once a heat map has been plotted, probes or genes of interest can be identified interactively. These identifiers may then be mapped back to gene symbols and other annotation via the **AnnotationDbi** package.

2 Data preparation

Expression data in the form of a data matrix must initially be mapped onto its corresponding chromosome coordinates. This is done using the `makeChrStrandData`:

```
> library('ALL')
> data('ALL')
> selSamples <- ALL$mol.biol %in% c('ALL1/AF4', 'E2A/PBX1')
> ALLs <- ALL[, selSamples]
> library('ChromHeatMap')
> chrdata<-makeChrStrandData(exprs(ALLs), lib='hgu95av2')
```

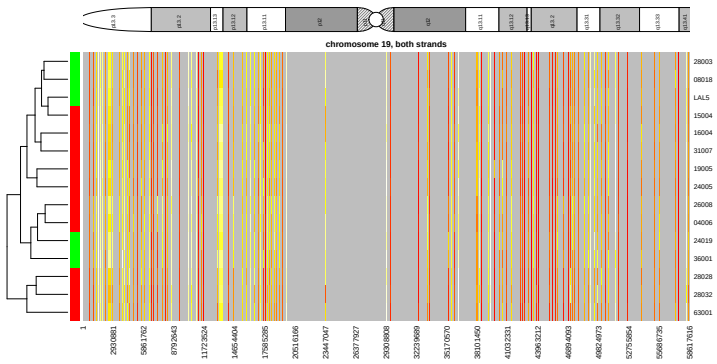
The output *chrdata* object here contains the expression data indexed by coordinate. Note that the `makeChrStrandData` function is based on the `Makesense` function in the **geneplotter** package, removing the internal call to lowess to avoid smoothing the data (which is undesirable in this case). The `makeChrStrandData` function is used specifically because it incorporates information on both the start and end chromosome coordinates for each locus. This allows the `plotChrMap` function to accurately represent target widths on the chromosome plot.

3 Plotting the heat map

Once the data has been prepared, a single call to `plotChrMap` will generate the chromosome heat map. There are many options available for this plot, and only a couple of them are illustrated here. Here we generate a whole-chromosome plot (chromosome 19), with both strands combined into a single heat map:

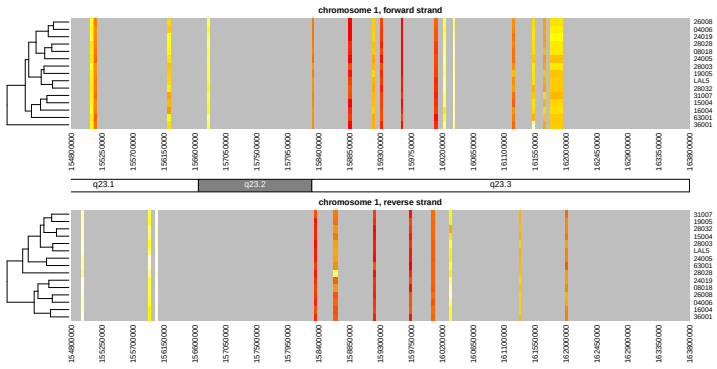
```
> groupcol <- ifelse( ALLs$mol.biol == 'ALL1/AF4', 'red', 'green' )
> plotChrMap(chrdata, 19, strands='both', RowSideColors=groupcol)
```

```
ChrMapPlot
Number of features plotted: 157
```



Chromosomes can be subsetted by cytoband or start/end coordinates along the chromosome. The following illustrates how one might plot the strands separately (this is the default behavior):

```
> plotmap<-plotChrMap(chrdata, 1, cytoband='q23', interval=50000, srtCyto=0, cexCyto=1.2)
```



Other options include subsetting of samples, adding a color key to indicate sample subsets, deactivating the sample-based clustering and so on. See the help pages for `plotChrMap` and `drawMapDendro` for details.

Note that the default colors provided by the `heat.colors` function are not especially attractive or informative; consider using custom-defined colors, for example by using the **RColorBrewer** package.

The output of the `plotChrMap` function can be subsequently used with the `grabChrMapProbes` function which enables the user to identify the probes or genes responsible for heatmap bands of interest.

Note that the `layout` and `par` options for the current graphics device are *not* reset following generation of the image. This is so that the `grabChrMapProbes` function can accurately identify the region of interest when the user interactively clicks on the diagram.

4 Interactive probe/gene identification

Often it will be of interest to determine exactly which probes or genes are shown to be up- or down-regulated by the `plotChrMap` heat map. This can be done using the `grabChrMapProbes` function. This takes the output of the `plotChrMap` function, asks the user to mouse-click the heatmap on either side of the bands of interest and returns a character vector of the locus identifiers in that region. These can then be passed to the **AnnotationDbi** function `mget` to identify which genes are being differentially expressed.

```
> probes <- grabChrMapProbes( plotmap )
> genes <- unlist(mget(probes, envir=hgu95av2SYMBOL, ifnotfound=NA))
```

Note that due to the way the expression values are plotted, genes which lie very close to each other on the chromosome may have been averaged to give a signal that could be usefully plotted at screen resolution. In such cases the locus identifiers will be returned concatenated, separated by semicolons (e.g. "`37687_i_at;37688_f_at;37689_s_at`"). Typically this is easily solved by zooming in on a region of interest, using either the "cytoband" or "start" and "end" options to `plotChrMap`. See also the "interval" option for another approach to this problem.

5 Session information

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

```
R version 4.2.0 RC (2022-04-19 r82224)
Platform: x86_64-pc-linux-gnu (64-bit)
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

locale:
```

```
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_GB              LC_COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats4    stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] hgu95av2.db_3.13.0   org.Hs.eg.db_3.15.0  ChromHeatMap_1.50.0
 [4] annotate_1.74.0      XML_3.99-0.9         AnnotationDbi_1.58.0
 [7] IRanges_2.30.0       S4Vectors_0.34.0     ALL_1.37.0
[10] Biobase_2.56.0       BiocGenerics_0.42.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.8.3                compiler_4.2.0
 [3] restfulr_0.0.13             GenomeInfoDb_1.32.0
 [5] XVector_0.36.0              MatrixGenerics_1.8.0
 [7] bitops_1.0-7                tools_4.2.0
 [9] zlibbioc_1.42.0             bit_4.0.4
[11] lattice_0.20-45             RSQLite_2.2.12
[13] memoise_2.0.1               pkgconfig_2.0.3
[15] png_0.1-7                   rlang_1.0.2
[17] Matrix_1.4-1                DelayedArray_0.22.0
[19] DBI_1.1.2                   cli_3.3.0
[21] parallel_4.2.0              yaml_2.3.5
[23] fastmap_1.1.0               GenomeInfoDbData_1.2.8
[25] rtracklayer_1.56.0          httr_1.4.2
[27] Biostrings_2.64.0           vctrs_0.4.1
[29] grid_4.2.0                  bit64_4.0.5
[31] R6_2.5.1                    BiocParallel_1.30.0
[33] blob_1.2.3                  matrixStats_0.62.0
[35] GenomicAlignments_1.32.0    Rsamtools_2.12.0
[37] GenomicRanges_1.48.0        SummarizedExperiment_1.26.0
[39] KEGGREST_1.36.0             xtable_1.8-4
[41] RCurl_1.98-1.6              cachem_1.0.6
[43] crayon_1.5.1                rjson_0.2.21
[45] BiocIO_1.6.0
```