# GMD Vignette:

Generic histogram construction, generic distance measure, cluster analysis with evaluation and visualization

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**Abstract**

The purpose of this GMD Vignette is to show how to get up and running with the R package GMD. *GMD* denotes **Generalized Minimum Distance between histograms**, which is a true metric that measure the distance between the shapes of any two discrete numerical distributions (*e.g.* histograms).

The vignette includes a brief introduction, an example to illustrate the concepts and the implementation of GMD and case studies that were carried out with applications using classical data sets (*e.g. iris*) and high-throughput sequencing data (*e.g. ChIP-seq*) from biology experiments. The appendix on page 15 contains an overview of package functionality, and examples using primary functions in histogram construction (the `ghist` function), distance measuring (the `gdist` function), cluster analysis with evaluation (the `''elbow''` method in the `css` function) and visualization (the `heatmap.3` function).

**Keywords:** histogram, distance, metric, non-parametric, cluster analysis, hierarchical clustering, sum-of-squares, heatmap.3

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# 1 Introduction

Similarly to the Earth Mover's distance, `Generalized Minimum distance of Distributions (GMD)` (based on MDPA - Minimum Difference of Pair Assignment [3]) is a true metric that measures the similarity of shapes between two histograms $A$ and $B$ by counting the necessary "shifts" of elements between bins that have to be performed to transform distribution $A$ into distribution $B$.

The `GMD` package provides classes and methods for computing `GMD` in R [5]. The algorithm has been implemented in C to interface with R for efficient computation. The package also includes downstream cluster analysis in function `css` (A.4 on page 24) that use a pairwise distance matrix to make partitions given variant criteria, inclusing the "elbow" rule as discussed in [7] or desired number of clusters. In addition, the function `heatmap.3` (A.5 on page 27) integrates the visualization of the hierarchical clustering in dendrogram, the distance measure in heatmap and graphical representations of summary statistics of the resulting clusters or the overall partition. For more flexibility, the function `heatmap.3` also takes plugin functions defined by end-users for variant summary statistics.

The motivation to write this package was born with the project [7] on characterizing Transcription Start Site (TSS) landscapes using high-throughput sequencing data, where a non-parametric distance measure was developped to assess the similarity among distributions of high-throughput sequencing reads from biological experiments.

The package is available on CRAN. The source code is available at http://cran.r-project.org/web/packages/GMD/ under GPL license.

# 2 Minimal Example: "Hello, GMD!"

`hello-GMD.R` (fig. 1) is a minimal example to load and check the sanity of your `GMD` installation. It also includes code for viewing the package information and this "Vignette", checking data sets provided by `GMD`, starting a demo and listing the citation of `GMD`.

hello-GMD.R

```
##' Check GMD's sanity and start up
##' @name hello-GMD

## GMD at CRAN, for source code download and installation
## http://cran.r-project.org/web/packages/GMD/index.html

## load GMD
library(GMD)

## version of GMD and description
packageVersion("GMD")
packageDescription("GMD")

## view GMD vignette
vignette("GMD-vignette",package="GMD")

## list the available data sets in GMD
data(package="GMD")

## list all the objects in the GMD
ls("package:GMD")

## help info on GMD
help(package="GMD")

## run a demo
demo("GMD-demo")

## cite GMD in publications
citation(package="GMD")

```

**Figure 1:** Source code of "hello-GMD.R".

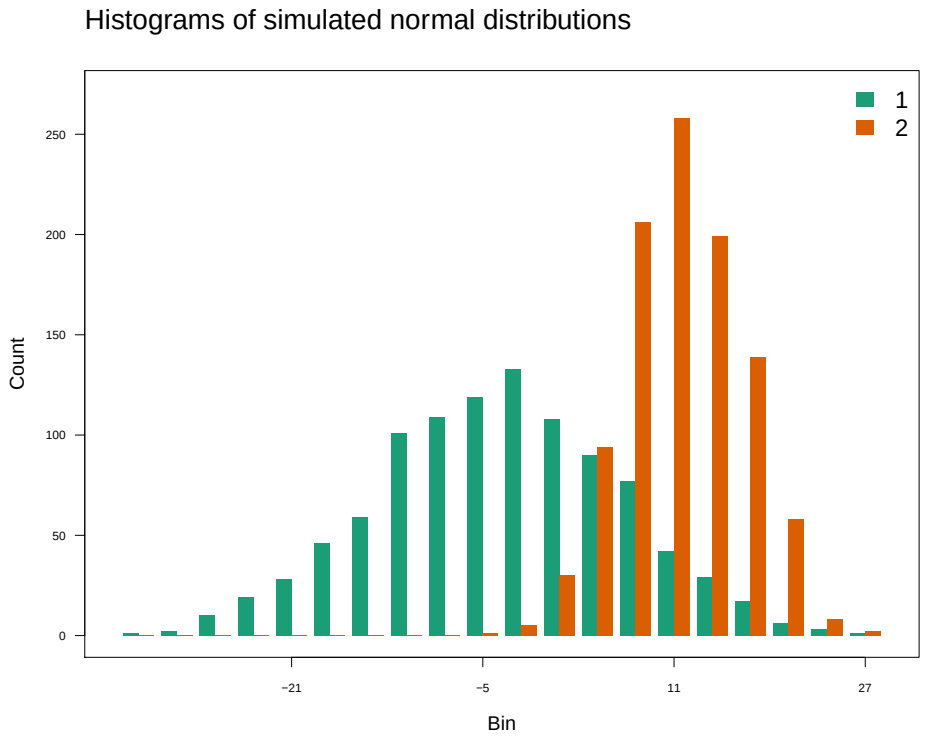
# 3 An example to understand GMD

This example, based on simulated data, is designed to illustrate the concepts and the implementation of `GMD` by stepping through the computations in detail.

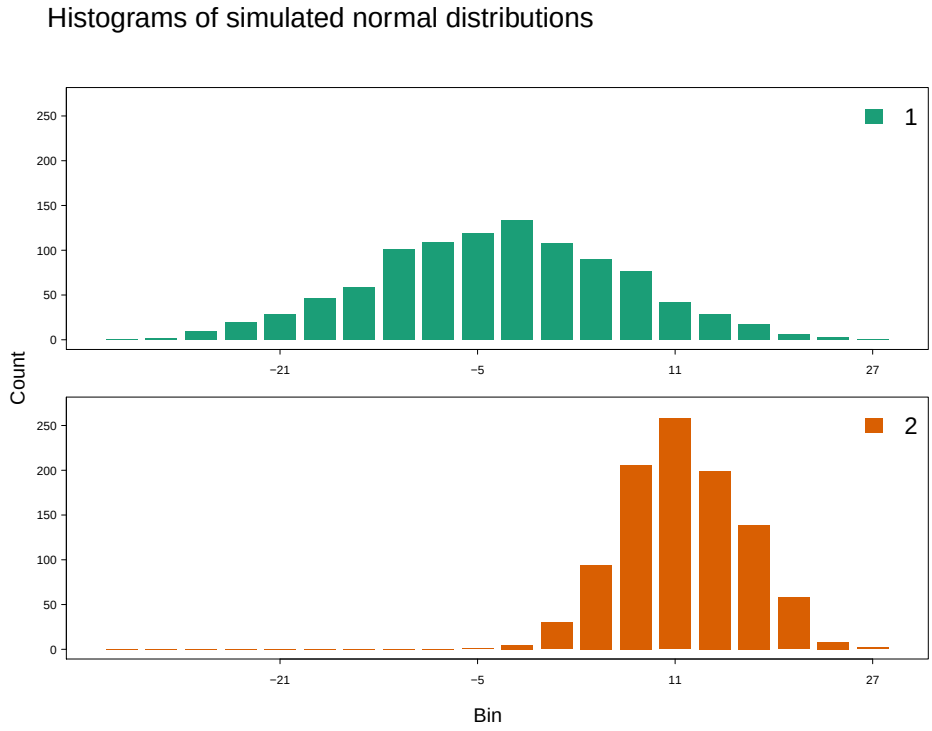
## 3.1 Histogram: construction and visualization

```
> ## ------------------------------------------------------------------------
> ## chunk1: Load library and simulate data
> ## ------------------------------------------------------------------------
> require("GMD") # load library


gdata: read.xls support for 'XLS' (Excel 97-2004) files ENABLED.

gdata: read.xls support for 'XLSX' (Excel 2007+) files ENABLED.


> ## create two normally-distributed samples
> ## with unequal means and unequal variances
> set.seed(2012)
> x1 <- rnorm(1000,mean=-5, sd=10)
> x2 <- rnorm(1000,mean=10, sd=5)

> ## ------------------------------------------------------------------------
> ## chunk2: Construct histograms
> ## ------------------------------------------------------------------------
> ## create common bins
> n <- 20 # desired number of bins
> breaks <- gbreaks(c(x1,x2),n) # bin boundaries
> ## make two histograms
> v1 <- ghist(x1,breaks=breaks,digits=0)
> v2 <- ghist(x2,breaks=breaks,digits=0)

> ## ------------------------------------------------------------------------
> ## chunk3: Save histograms as multiple-histogram (`mhist') object
> ## ------------------------------------------------------------------------
> x <- list(v1,v2)
> mhist.obj <- as.mhist(x)
```

```
> ## ------------------------------------------------------------------------
> ## chunk4: Visualize a `mhist' object
> ## ------------------------------------------------------------------------
> ## plot histograms side-by-side
> plot(mhist.obj,mar=c(1.5,1,1,0),main="Histograms of simulated normal distributions")
```



```
> ## plot histograms as subplots, with corresponding bins aligned
> plot(mhist.obj,beside=FALSE,mar=c(1.5,1,1,0),
+      main="Histograms of simulated normal distributions")
```



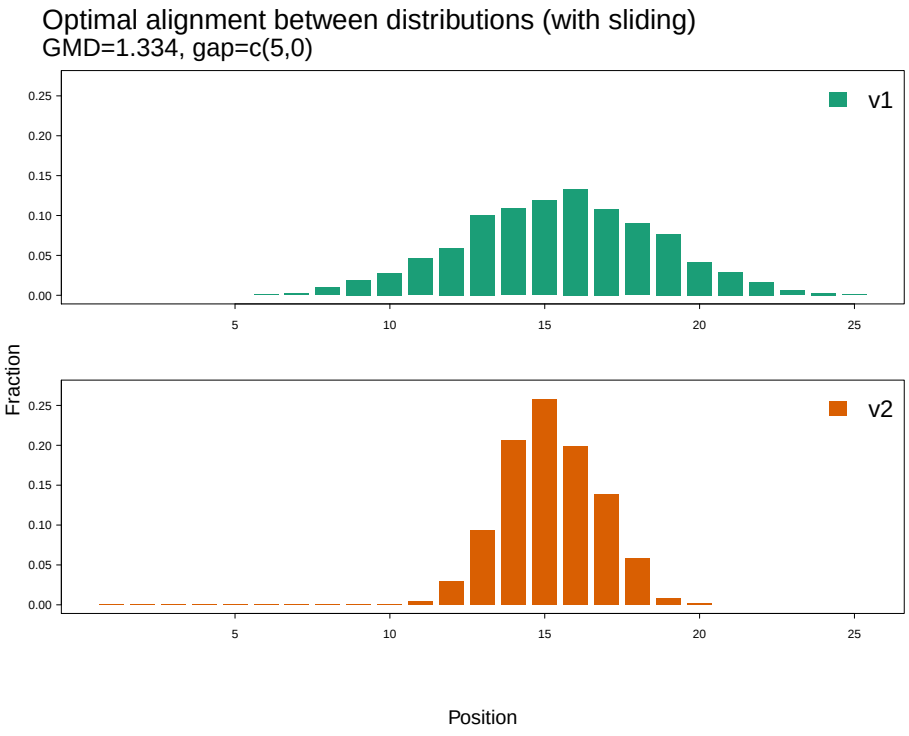

## 3.2 Histogram: distance measure and alignment

Here we measure the *GMD* distance between shapes of two histograms with option *sliding* on.

```
> ## ------------------------------------------------------------------------
> ## chunk5: Measure the pairwise distance betwwen two histograms by GMD
> ## ------------------------------------------------------------------------
> gmdp.obj <- gmdp(v1,v2,sliding=TRUE)
> print(gmdp.obj)                       # print a brief version by default


[1] 1.334


> print(gmdp.obj,print.mode="detailed") # print a detailed version


GM-Distance: 1.334

Sliding: TRUE

Number of hits: 1

Gap:
        v1        v2
Hit1        5        0


Resolution: 1


> print(gmdp.obj,print.mode="full")     # print a full version


Distribution of v1:
1 2 10 19 28 46 59 101 109 119 133 108 90 77 42 29 17 6 3 1
(After normalization)
0.001 0.002 0.01 0.019 0.028 0.046 0.059 0.101 0.109 0.119 0.133 0.108 0.09 0.077 0.042 0.029 0.017 0.006 0.003 0.001

Distribution of v2:
0 0 0 0 0 0 0 0 0 1 5 30 94 206 258 199 139 58 8 2
(After normalization)
0 0 0 0 0 0 0 0 0 0.001 0.005 0.03 0.094 0.206 0.258 0.199 0.139 0.058 0.008 0.002

GM-Distance: 1.334

Sliding: TRUE

Number of hits: 1

Gap:
        v1        v2
Hit1        5        0


Resolution: 1
```

Now, let's have a look at the alignment by `GMD`, with a distance 1.334 and a "shift" of 5 in the $1^{st}$ distribution. It is important to note that the specific features (the values in this case) of the original bins in the histograms are ignored with *sliding* on. To keep original bin-to-bin correspondence, please set *sliding* to `FALSE` (see examples in section 4.2 on page 12).

```
> ## ------------------------------------------------------------------------
> ## chunk6: Show alignment
> ## ------------------------------------------------------------------------
> plot(gmdp.obj,beside=FALSE)
```



# 4 Case study

## 4.1 CAGE: measuring the dissimilarities among TSSDs

Studies have demonstrated that the spatial distributions of read based sequencing reads from different platforms often indicate functional properties and expression profiles (reviewed in [6] and [8]). Analyzing the distributions of DNA reads from the different platforms is therefore often meaningful. To do this systematically, a measure of similarity between distributions is necessary. Such measures should ideally be true metrics, have few parameters as possible, be computationally efficient and also make biological sense to end-users. Case studies were made in section 4.1 and 4.2 to demanstrate the applicaitons of `GMD` using distributions of CAGE and ChIP-seq sequencing reads.

In this section we demonstrate how `GMD` is applied to measure the dissimilarities among TSSDs, histograms of transcription start site (TSS) that are made of CAGE tags, with option *sliding* on. The spatial properties of TSSDs vary widely between promoters and have biological implications in both regulation and function. The raw data were produced by CAGE and downloaded from FANTOM3 ([2]) and CAGE sequence reads were preprocessed as did in [7]. The following codes `case-cage.R` (fig. 2) are sufficient to perform both pairwise GMD calculation by function `gmdp` and to construct a GMD distance matrix by function `gmdm`. A handful of options are available for control and flexibility, particularly, the option `sliding` is enabled by default to allow partial alignment.

case-cage.R

```
require("GMD") # load library
data(cage)     # load data

## measure pairwise distance
x <- gmdp(cage[["Pfkfb3 (T02R00AEC2D8)"]],cage[["Csf1 (T03R0672174D)"]])
print(x)                      # print a brief version by default
print(x, print.mode="full")  # print a full version by default

## show alignment
plot(x,labels=c("Pfkfb3","Csf1"),beside=FALSE)

## show another alignment
plot(gmdp(cage[["Hig1 (T09R0743763C)"]],cage[["Cd72 (T04R028B8BC9)"]]),
     labels=c("Hig1 (T09R0743763C)","Cd72 (T04R028B8BC9)"),
     beside=FALSE)

## construct a distance matrix and visualize it
short.labels <- gsub("(.+) \\(.+","\\1",names(cage)) # get short labels
x <- gmdm(cage[1:6],labels=short.labels[1:6])
plot(x)
```

**Figure 2:** Source code of "case-cage.R".

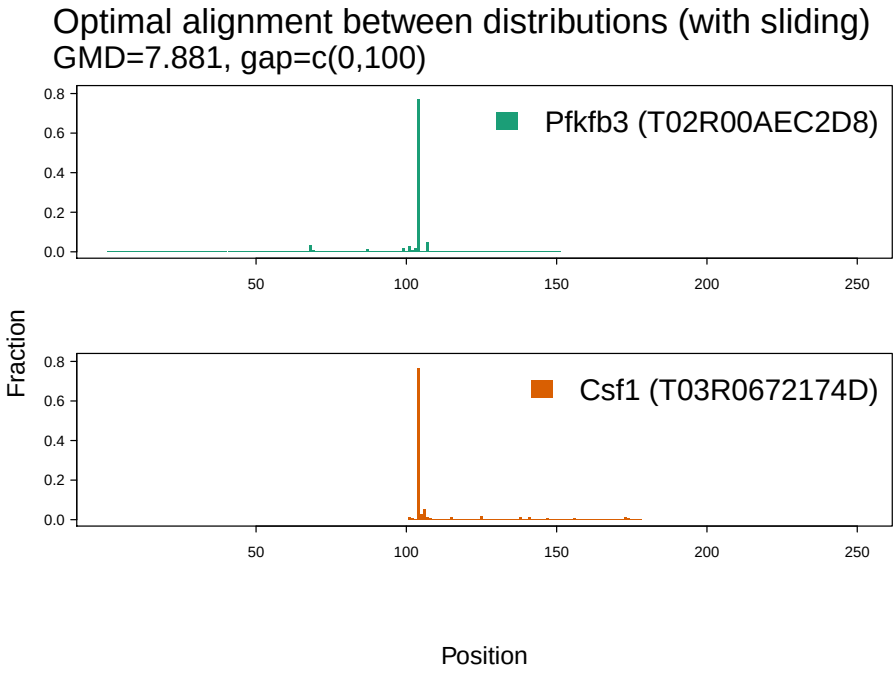



**Figure 3:** Graphical output 1 of source code "case-cage.R".

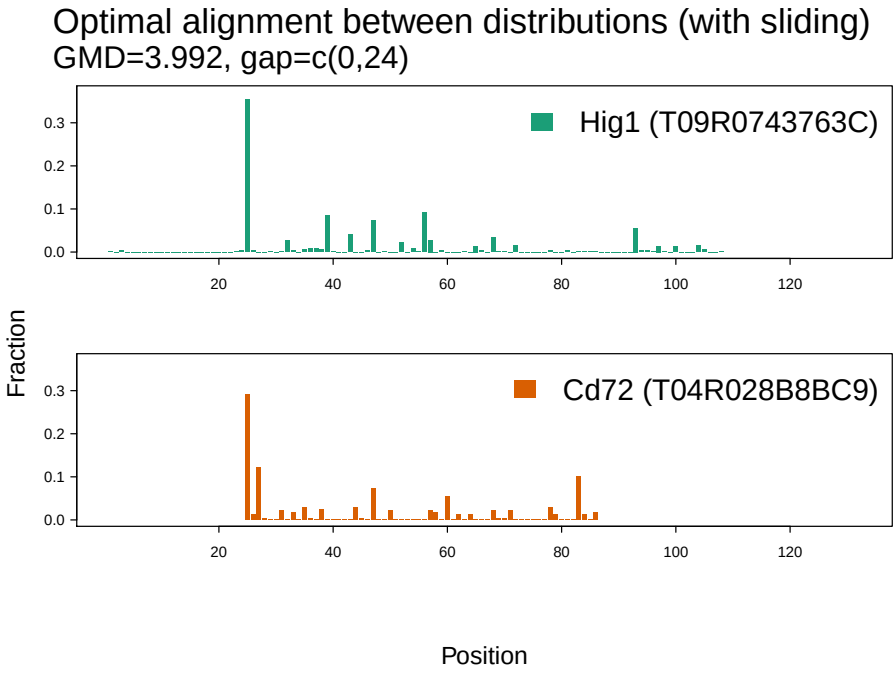



**Figure 4:** Graphical output 2 of source code "case-cage.R".

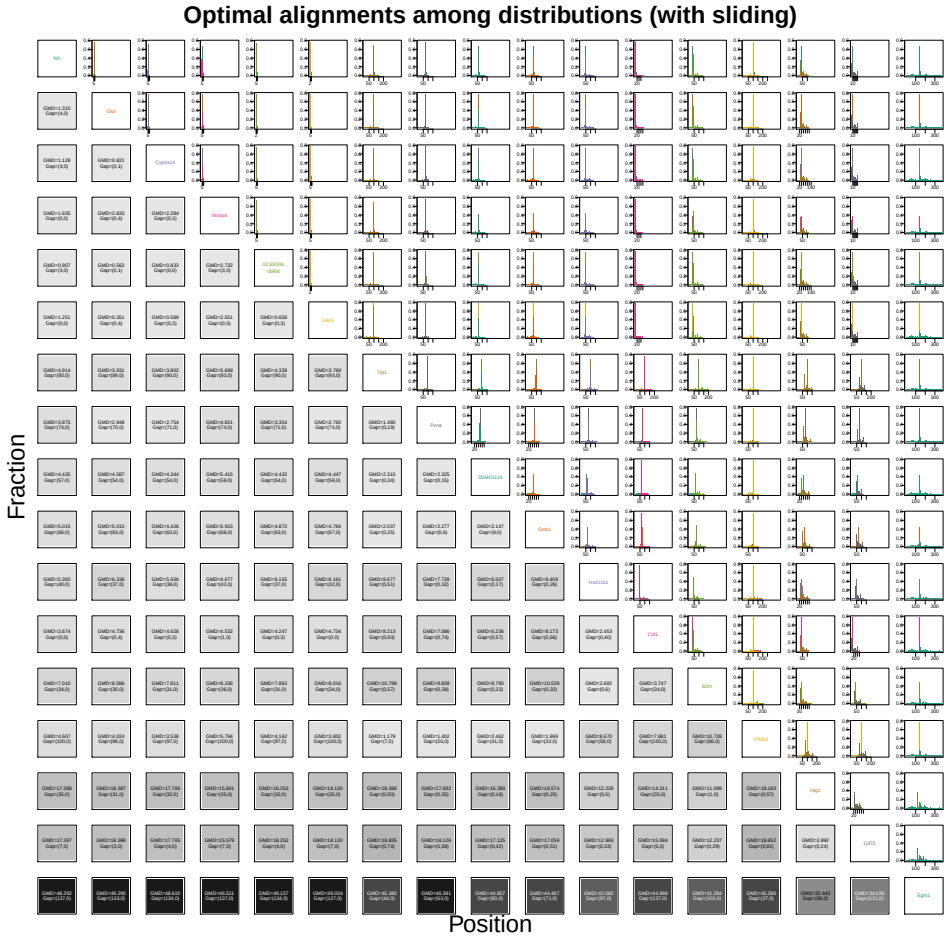



**Figure 5:** Graphical output 1 of source code "case-cage.R".

## 4.2 ChIP-seq: measuring the similarities among histone modification patterns

In this section we demonstrate how `GMD` is applied to measure the dissimilarities among histone modifications represented by ChIP-seq reads. Distinctive patterns of chromatin modifications around the TSS are associated with transcription regulation and expression variation of genes. Comparing the chromatin modification profiles (originally produced by [1] and [4], and preprocessed by [7]), the `sliding` option is disabled for fixed alignments at the TSSs and the flanking regions. The GMD measure indicates how well profiles are co-related to each other. In addition, the downstream cluster analysis is visualized with function `heatmap.3` that use GMD distance matrix to generate clustering dengdrograms and make partitions given variant criteria, including the `''elbow''` rule (discussed in [7]) or desired number of clusters.

case-chipseq.R

```
require("GMD")      # load library
data(chipseq_mES)   # load data
data(chipseq_hCD4T) # load data

## pairwise distance and alignment based on GMD metric
plot(gmdm(chipseq_mES,sliding=FALSE))

## clustering on spatial distributions of histone modifications
x <- gmdm(chipseq_hCD4T,sliding=FALSE,resolution=10)
heatmap.3(x,revC=TRUE)

## Determine the number of clusters by "Elbow" criterion
main <- "Heatmap of ChIP-seq data (human CD4+ T cells)"
dist.obj <- gmdm2dist(x)
css.multi.obj <- css.hclust(dist.obj,hclust(dist.obj))
elbow.obj <- elbow.batch(css.multi.obj,ev.thres=0.90,inc.thres=0.05)
heatmap.3(dist.obj, main=main, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k)

## more strict threshold
elbow.obj <- elbow.batch(css.multi.obj,ev.thres=0.75,inc.thres=0.1)
heatmap.3(dist.obj, main=main, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k)

## side plots
normalizeVector <- function(v){v/sum(v)} # a function to normalize a vector
dev.new(width=12,height=8)

## summary of row clusters
expr1 <- list(quote(op <- par(mar = par("mar")*2)),
              quote(plot(mhist.summary(as.mhist(i.x)),if.plot.new=FALSE)),
              quote(par(op))
              )

## summary of row clustering
expr2 <- list(quote(tmp.clusters <- cutree(hclust(dist.row),k=kr)),
              quote(tmp.css <- css(dist.row,tmp.clusters)),
              quote(print(tmp.css)),
              quote(tmp.wev <- tmp.css$wss/tmp.css$tss),
              quote(names(tmp.wev) <- as.character(unique(tmp.clusters))),
              quote(tmp.wev <- tmp.wev[order(unique(tmp.clusters))]),
              quote(barplot(tmp.wev,main="Cluster Explained Variance", xlab="Cluster",
                            ylab="EV",col="white",border="black",
                            ylim=c(0,max(tmp.wev)*1.1),cex.main=1)))
expr3 <- list(quote(op <- par(mar = par("mar")*2)),
              quote(plot.elbow(css.multi.obj,elbow.obj,if.plot.new=FALSE)),
              quote(par(op))
              )

heatmap.3(dist.obj, main=main, cex.main=1.25, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k,
          keysize=1,mapsize=4.5,row.data=lapply(chipseq_hCD4T,normalizeVector),
          plot.row.clusters=TRUE,plot.row.clusters.list=list(expr1),
          plot.row.clustering=TRUE,plot.row.clustering.list=list(expr2,expr3))

```

**Figure 6:** Source code of "case-chipseq.R".

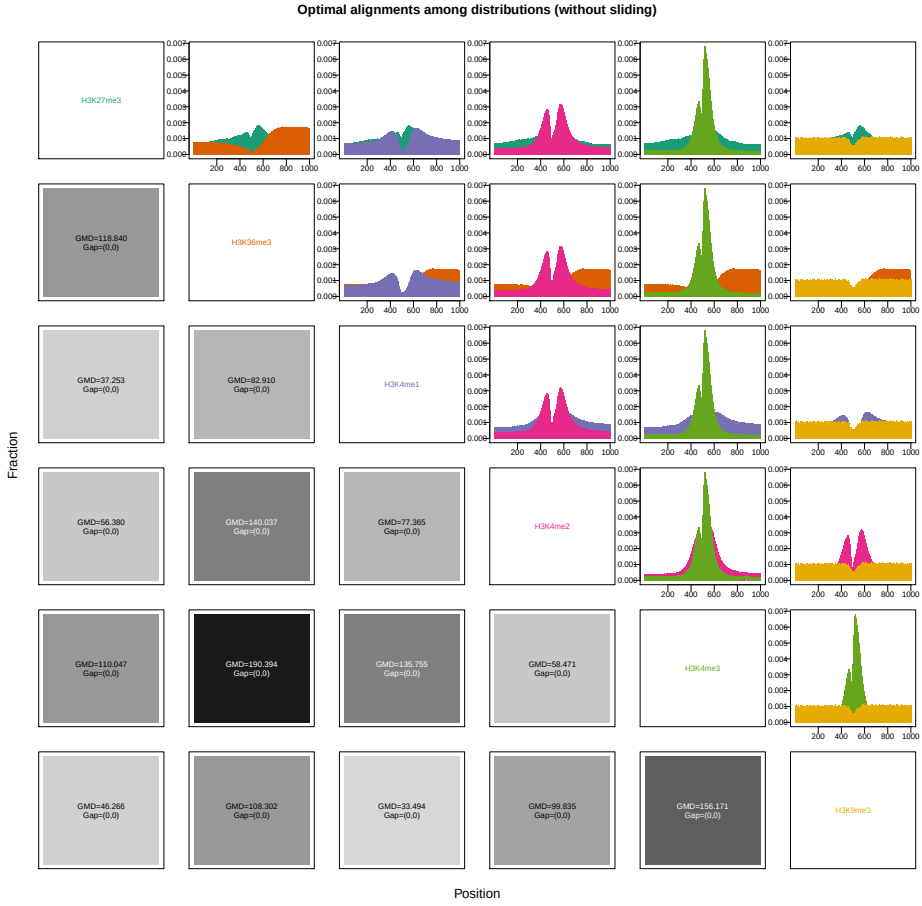



**Figure 7:** Graphical output 1 of source code "case-chipseq.R".

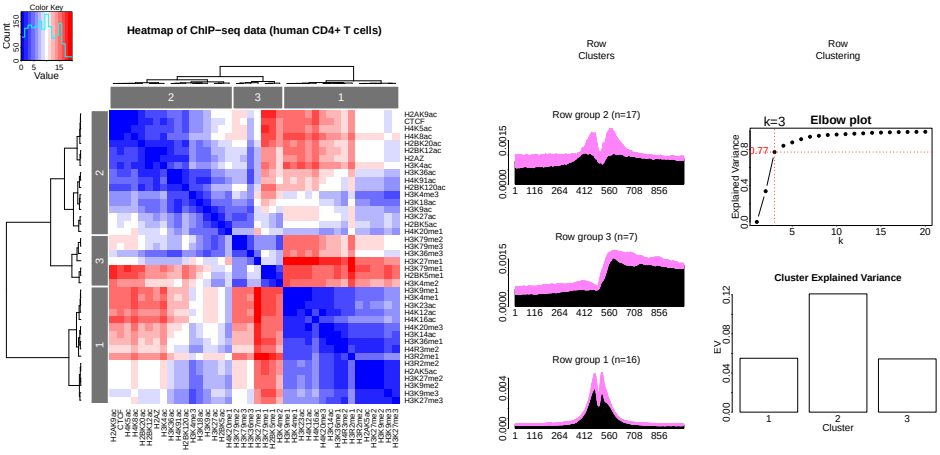



**Figure 8:** Graphical output 1 of source code "case-chipseq.R".

# A Functionality

## A.1 An overview

**Table 1.** Functions of the `GMD` R package

| Function | Description |
|---|---|
| `ghist` | Generalized Histogram Computation and Visualization |
| `gdist` | Generalized Distance Matrix Computation |
| `css` | Computing Clustering Sum-of-Squares and evaluating the clustering by the ‘‘`elbow`’’ method |
| `heatmap.3` | Enhanced Heatmap Representation with Dendrogram and Partition |
| `gmdp` | Computation of GMD on a pair of histograms |
| `gmdm` | Computation of GMD Matrix on a set of histograms |

## A.2 ghist: Generic construction and visualization of histograms

### A.2.1 Examples using simulated data

example-ghist.R (fig. 9) is an example on how to obtain histogram from raw data and make visualization.

example-ghist.R

```
## load library
require("GMD")

## create two normally-distributed samples
## with unequal means and unequal variances
set.seed(2012)
v1 <- rnorm(1000,mean=-5, sd=10)
v2 <- rnorm(1000,mean=10, sd=5)

## create common bins
n <- 20 # desired number of bins
breaks <- gbreaks(c(v1,v2),n) # bin boundaries
x <-
  list(ghist(v1,breaks=breaks,digits=0),
       ghist(v2,breaks=breaks,digits=0))
mhist.obj <- as.mhist(x)

## plot histograms side-by-side
plot(mhist.obj,mar=c(1.5,1,1,0),main="Histograms of simulated normal distributions")

## plot histograms as subplots,
## with corresponding bins aligned
plot(mhist.obj,beside=FALSE,mar=c(1.5,1,1,0),
     main="Histograms of simulated normal distributions")
```

**Figure 9:** Source code of "example-ghist.R".

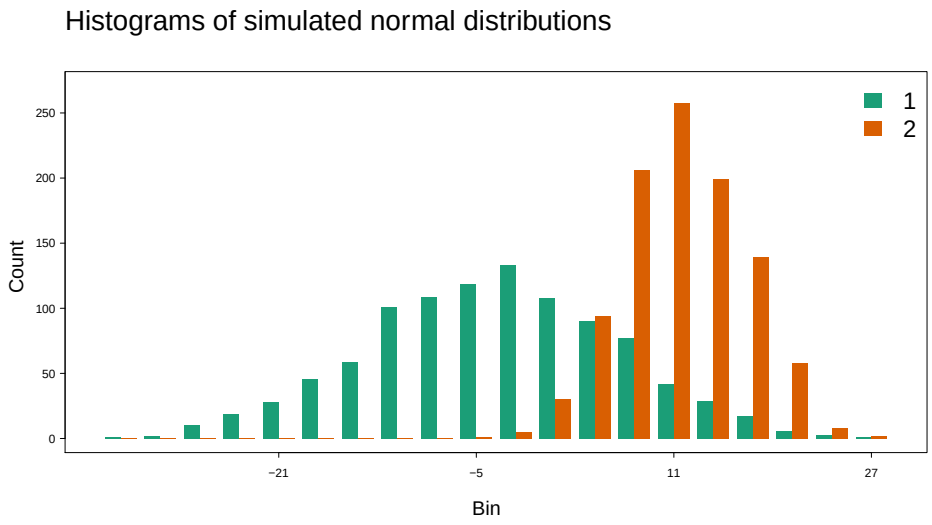



**Figure 10:** Graphical output 1 of source code "example-ghist.R".

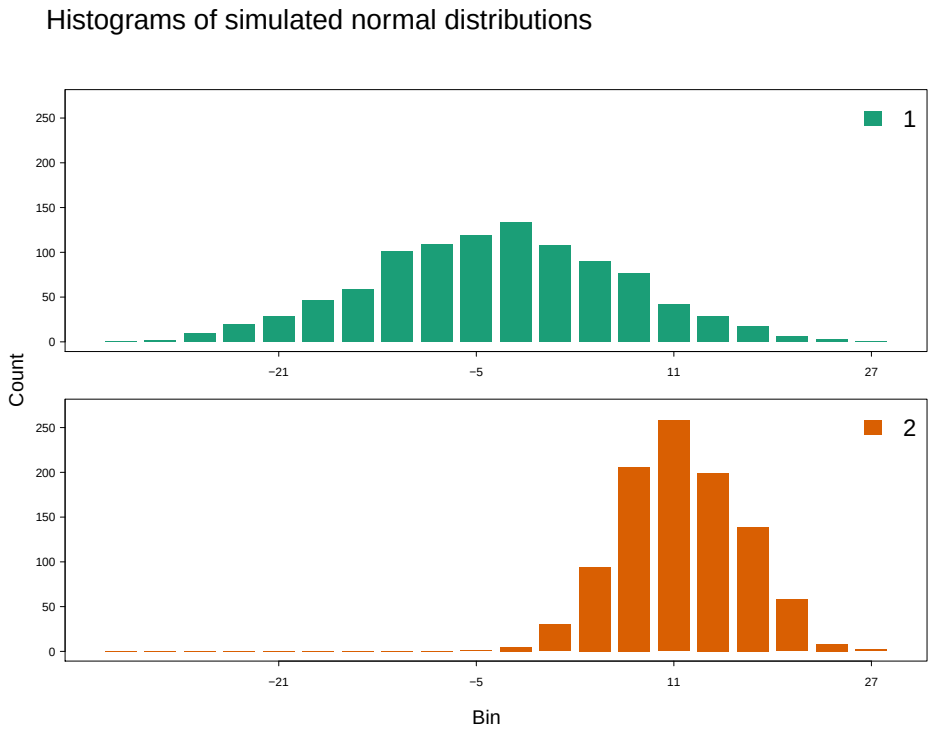



**Figure 11:** Graphical output 1 of source code "example-ghist.R".

### A.2.2 Examples using `iris` data

`case-iris.R` (fig. 12) is a study on how to obtain and visualize histograms, using Fisher's *iris* data set.

case-iris.R

```
## load library
require("GMD")

## load data
data(iris)

## create common bins
n <- 30                                     # the number of bins
breaks <- gbreaks(iris[,"Sepal.Length"],n) # the boundary of bins

## create a list of histograms
Sepal.Length <-
  list(setosa=ghist(iris[iris$Species=="setosa","Sepal.Length"],breaks=breaks),
       versicolor=ghist(iris[iris$Species=="versicolor","Sepal.Length"],breaks=breaks),
       virginica=ghist(iris[iris$Species=="virginica","Sepal.Length"],breaks=breaks)
       )

## convert to a `hist' object
x <- as.mhist(Sepal.Length)

## get bin-wise summary statistics
summary(x)

## visualize the histograms
plot(x,beside=FALSE,
     main="Histogram of Sepal.Length of iris",xlab="Sepal.Length (cm)")
```

**Figure 12:** Source code of "case-iris.R".

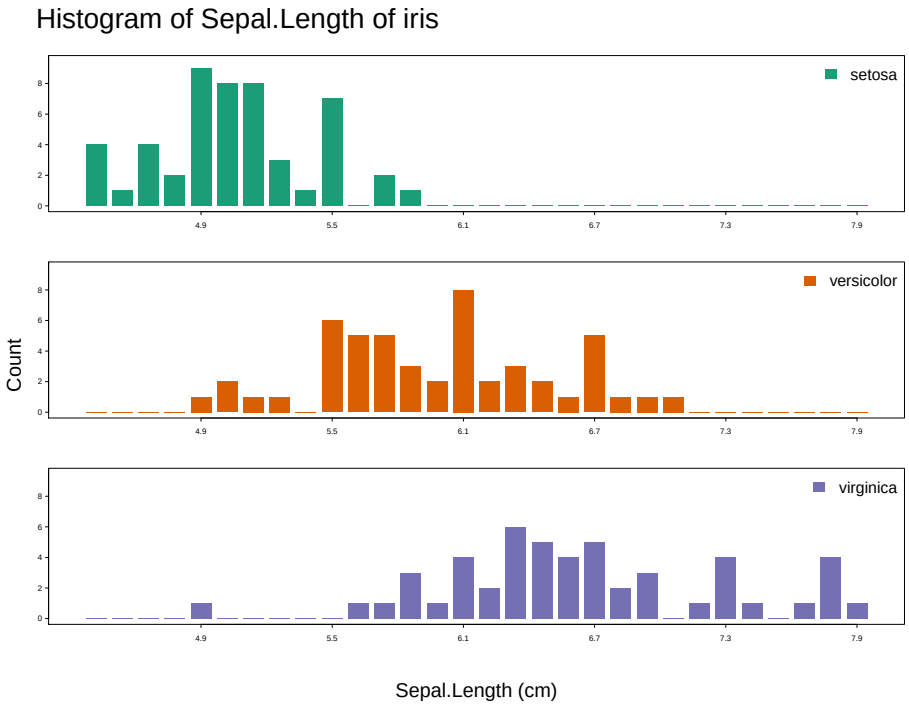



**Figure 13:** Graphical output 1 of source code "case-iris.R".

### A.2.3 Examples using `nottem` data

`case-nottem.R` (fig. 14) is a study on how to draw histograms side-by-side and to compute and visualize a bin-wise summary histogram, using air temperature data at Nottingham Castle.

case-nottem.R

```
## load library
require("GMD")

## load data
data(nottem)

class(nottem)      # a time-series (ts) object
x <- ts2df(nottem) # convert ts to data.frame
mhist1 <- as.mhist(x[1:3,])

## plot multiple discrete distributions side-by-side
plot(mhist1,xlab="Month",ylab="Degree Fahrenheit",
     main="Air temperatures at Nottingham Castle")

## make summary statistics for each bin
mhist2 <- as.mhist(x)
ms <- mhist.summary(mhist2)
print(ms)

## plot bin-wise summary statistics with
## confidence intervals over the bars
plot(ms, main="Mean air temperatures at Nottingham Castle (1920-1939)",
     xlab="Month", ylab="Degree Fahrenheit")
```

**Figure 14:** Source code of "case-nottem.R".

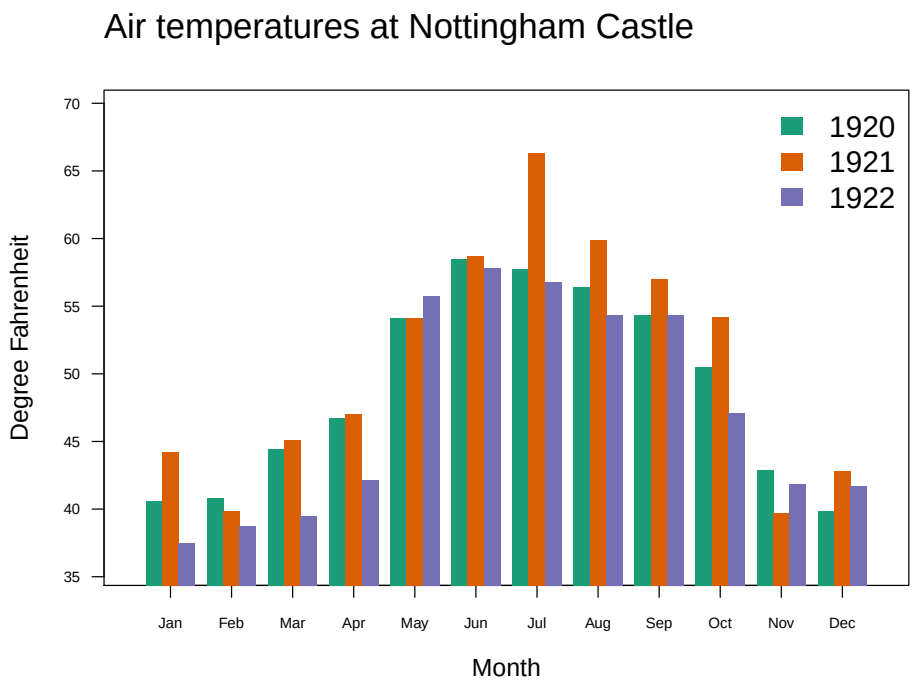



**Figure 15:** Graphical output 1 of source code "case-nottem.R".

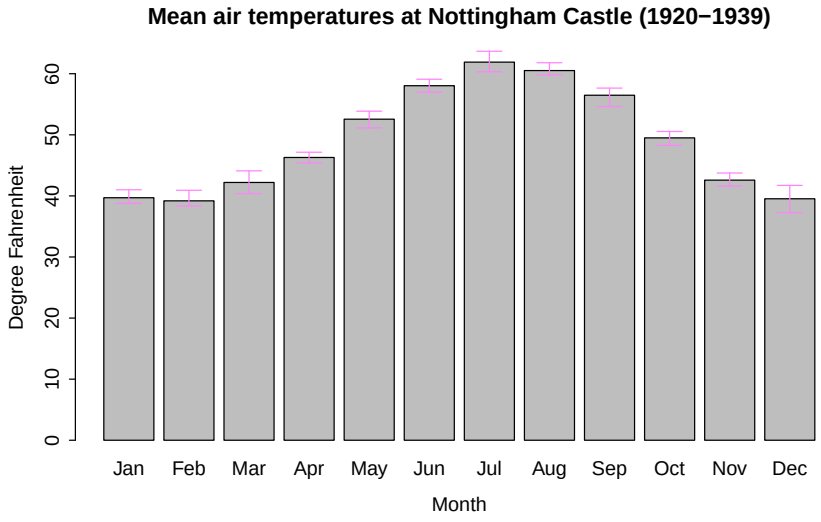



**Figure 16:** Graphical output 2 of source code "case-nottem.R".

## A.3 gdist: Generic construction and visualization of distances

`example-gdist.R` (fig. 17) is an example on how to measure distances using user-defined metric, such as *correlation distance* and *GMD*.

example-gdist.R

```
## load library
require("GMD")
require(cluster)

## compute distance using Euclidean metric (default)
data(ruspini)
x <- gdist(ruspini)

## see a dendrogram result by hierarchical clustering
dev.new(width=12, height=6)
plot(hclust(x),
     main="Cluster Dendrogram of Ruspini data",
     xlab="Observations")

## convert to a distance matrix
m <- as.matrix(x)

## convert from a distance matrix
d <- as.dist(m)
stopifnot(d == x)

## Use correlations between variables "as distance"
data(USJudgeRatings)
dd <- gdist(x=USJudgeRatings,method="correlation.of.variables")
dev.new(width=12, height=6)
plot(hclust(dd),
     main="Cluster Dendrogram of USJudgeRatings data",
     xlab="Variables")
```

**Figure 17:** Source code of "example-gdist.R".

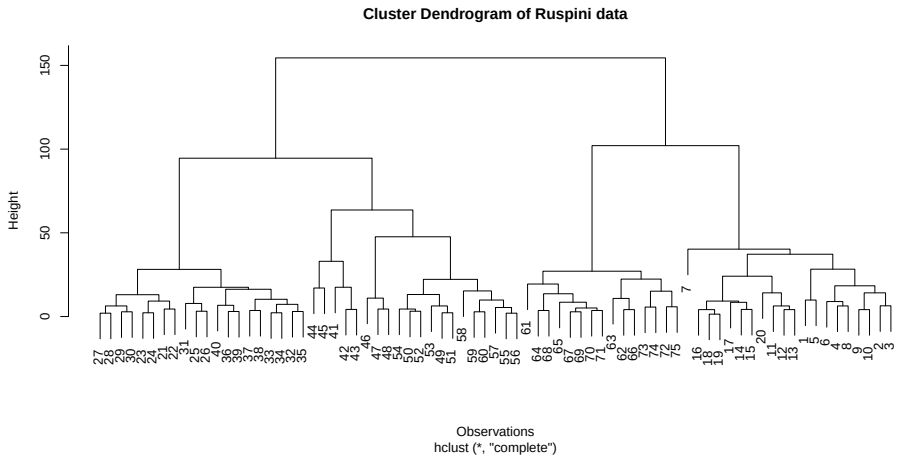



**Figure 18:** Graphical output 1 of source code "example-gdist.R".

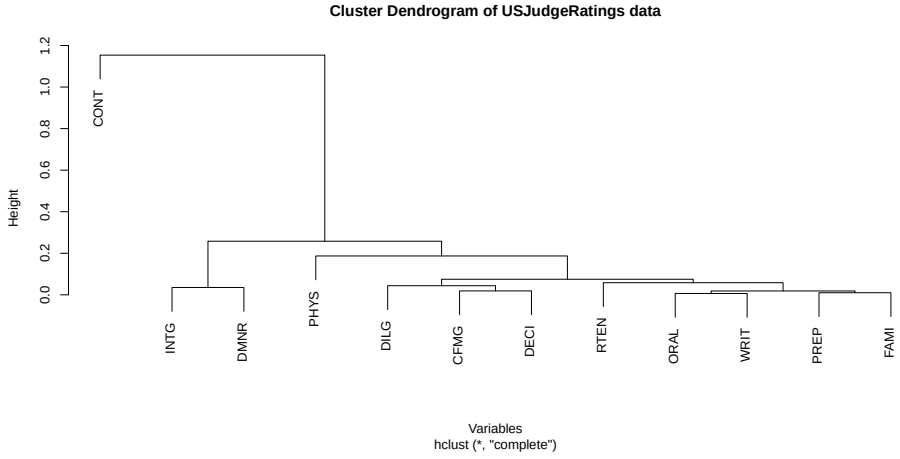



**Figure 19:** Graphical output 2 of source code "example-gdist.R".

## A.4 `css`: Clustering Sum-of-Squares and the ‘‘`elbow`’’ plot: determining the number of clusters in a data set

A good clustering yields clusters where the total within-cluster sum-of-squares (WSSs) is small (i.e. cluster cohesion, measuring how closely related are objects in a cluster) and the total between-cluster sum-of-squares (BSSs) is high (i.e. cluster separation, measuring how distinct or well-separated a cluster is from other clusters). `example-css.R` (fig. 20) is an example on how to make correct choice of $k$ using **“elbow criterion”**. A good $k$ is selected according to the thresholds of explained variances and the changes of the explained variances. The optimal choice of $k$ will strike a balance between maximum compression of the data using a single cluster, and maximum accuracy by assigning each data point to its own cluster. More important, an ideal $k$ should also reflect subject-specific meanings. Here we present a way to measure such performance of a clustering model, using squared Euclidean distances. The evaluation is based on pairwise distance matrix and therefore more generic in a way that doesn't involve means of raw data, which are often not available or hard to obtain.

example-css.R

```
## load library
require("GMD")

## simulate data around 12 points in Euclidean space
pointv <- data.frame(x=c(1,2,2,4,4,5,5,6,7,8,9,9),y=c(1,2,8,2,4,4,5,9,9,8,1,9))
set.seed(2012)
mydata <- c()
for (i in 1:nrow(pointv)){
  mydata <- rbind(mydata,cbind(rnorm(10,pointv[i,1],0.1),rnorm(10,pointv[i,2],0.1)))
}
mydata <- data.frame(mydata); colnames(mydata) <- c("x","y")
plot(mydata,type="p",pch=21, main="Simulated data")

## determine a "good" k using elbow
dist.obj <- dist(mydata[,1:2])
hclust.obj <- hclust(dist.obj)
css.obj <- css.hclust(dist.obj,hclust.obj)
elbow.obj <- elbow.batch(css.obj)
print(elbow.obj)

## make partition given the "good" k
k <- elbow.obj$k; cutree.obj <- cutree(hclust.obj,k=k)
mydata$cluster <- cutree.obj

## draw a elbow plot and label the data
dev.new(width=12, height=6)
par(mfcol=c(1,2),mar=c(4,5,3,3),omi=c(0.75,0,0,0))
plot(mydata$x,mydata$y,pch=as.character(mydata$cluster),col=mydata$cluster,cex=0.75,
     main="Clusters of simulated data")
plot.elbow(css.obj,elbow.obj,if.plot.new=FALSE)

## clustering with more relaxed thresholds (, resulting a smaller "good" k)
elbow.obj2 <- elbow.batch(css.obj,ev.thres=0.90,inc.thres=0.05)
mydata$cluster2 <- cutree(hclust.obj,k=elbow.obj2$k)

dev.new(width=12, height=6)
par(mfcol=c(1,2), mar=c(4,5,3,3),omi=c(0.75,0,0,0))
plot(mydata$x,mydata$y,pch=as.character(mydata$cluster2),col=mydata$cluster2,cex=0.75,
     main="Clusters of simulated data")
plot.elbow(css.obj,elbow.obj2,if.plot.new=FALSE)
```

**Figure 20:** Source code of "example-css.R".

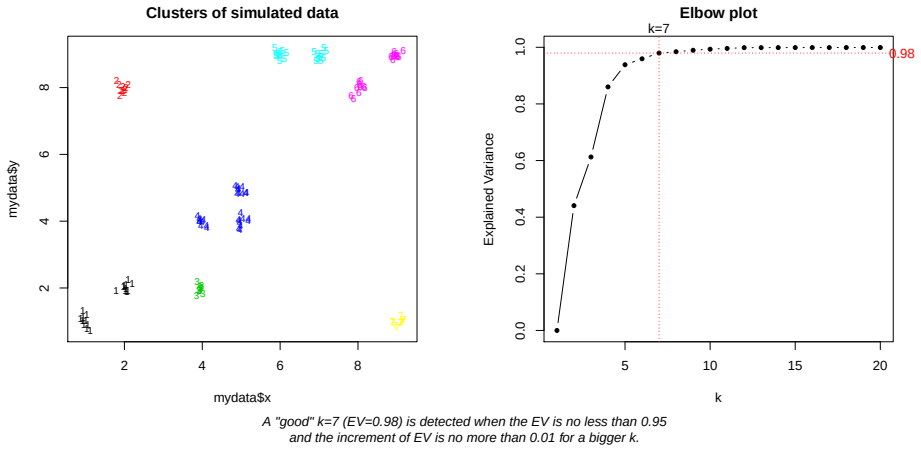



**Figure 21:** Graphical output 1 of source code "example-css.R".

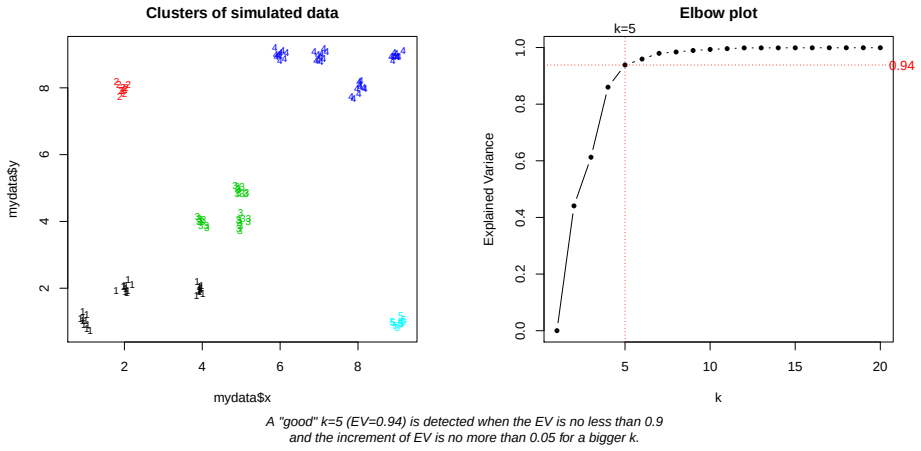



**Figure 22:** Graphical output 2 of source code "example-css.R".

## A.5 `heatmap.3`: Visualization in cluster analysis with evaluation

### A.5.1 Examples using `mtcars` data

`example-heatmap3a.R` (fig. 23) is an example on how to make a heatmap with summary visualization of observations.

example-heatmap3a.R

```
require("GMD")          # load library
data(mtcars)            # load data
x  <- as.matrix(mtcars) # data as a matrix

dev.new(width=10,height=8)
heatmap.3(x)                                # default, with reordering and dendrogram
heatmap.3(x, Rowv=FALSE, Colv=FALSE)        # no reordering and no dendrogram
heatmap.3(x, dendrogram="none")             # reordering without dendrogram
heatmap.3(x, dendrogram="row")              # row dendrogram with row (and col) reordering
heatmap.3(x, dendrogram="row", Colv=FALSE) # row dendrogram with only row reordering
heatmap.3(x, dendrogram="col")              # col dendrogram
heatmap.3(x, dendrogram="col", Rowv=FALSE) # col dendrogram with only col reordering
heatmapOut <-
  heatmap.3(x, scale="column")              # sacled by column
names(heatmapOut)                           # view the list that is returned
heatmap.3(x, scale="column", x.center=0)   # colors centered around 0
heatmap.3(x, scale="column",trace="column")# trun "trace" on

## coloring cars (row observations) by brand
brands <- sapply(rownames(x), function(e) strsplit(e," ")[[1]][1])
names(brands) <- c()
brands.index <- as.numeric(as.factor(brands))
RowIndividualColors <- rainbow(max(brands.index))[brands.index]
heatmap.3(x, scale="column", RowIndividualColors=RowIndividualColors)

## coloring attributes (column features) randomly (just for a test :)
heatmap.3(x, scale="column", ColIndividualColors=rainbow(ncol(x)))

## add a single plot for all row individuals
dev.new(width=12,height=8)
expr1 <- list(quote(plot(row.data[rowInd,"hp"],1:nrow(row.data),xlab="hp",ylab="",yaxt="n',
                         main="Gross horsepower")),
              quote(axis(2,1:nrow(row.data),rownames(row.data)[rowInd],las=2)))
heatmap.3(x, scale="column", plot.row.individuals=TRUE, row.data=x,
          plot.row.individuals.list=list(expr1))

## add a single plot for all col individuals
dev.new(width=12,height=8)
expr2 <- list(quote(plot(colMeans(col.data)[colInd], xlab="", ylab="Mean",xaxt="n",
                         main="Mean features",cex=1,pch=19)),
              quote(axis(1,1:ncol(col.data),colnames(col.data)[colInd],las=2)))
heatmap.3(x, scale="column", plot.col.individuals=TRUE, col.data=x,
          plot.col.individuals.list=list(expr2))

## add another single plot for all col individuals
dev.new(width=12,height=8)
expr3 <- list(quote(op <- par(mar = par("mar")*1.5)),
              quote(mytmp.data <- apply(col.data,2,function(e) e/sum(e))),
              quote(boxplot(mytmp.data[,colInd], xlab="", ylab="Value",
                         main="Boxplot of normalized column features")),
              quote(par(op)))
heatmap.3(x, scale="column", plot.col.individuals=TRUE, col.data=x,
          plot.col.individuals.list=list(expr3))
```



**Figure 23:** Source code of "example-heatmap3a.R".

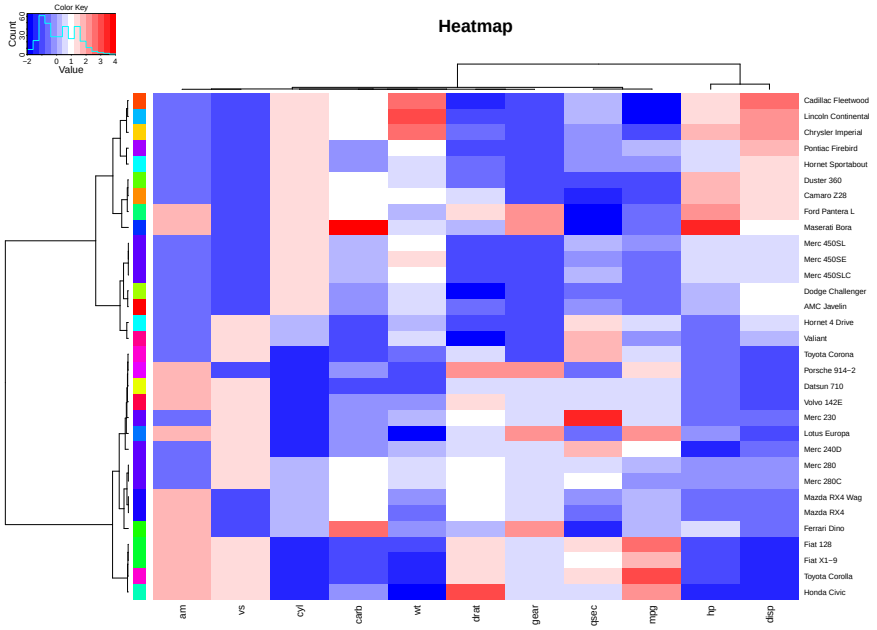



**Figure 24:** Graphical output 1 of source code "example-heatmap3a.R".

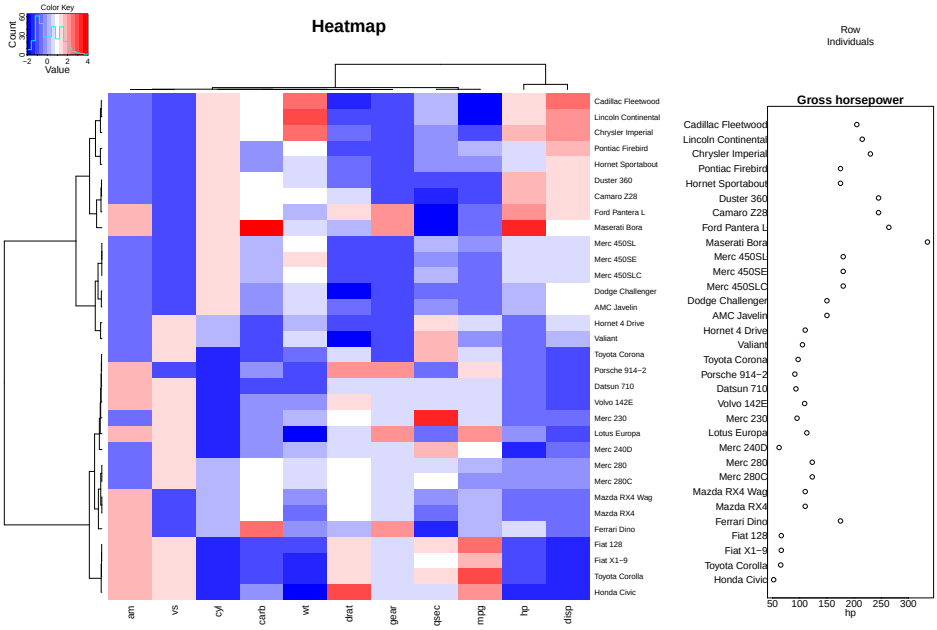



**Figure 25:** Graphical output 2 of source code "example-heatmap3a.R".

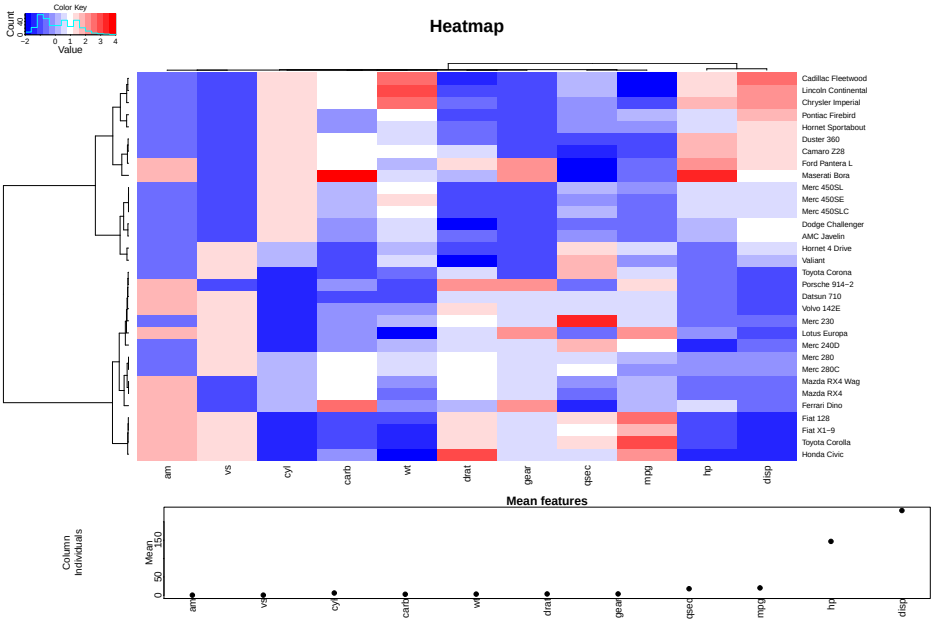



**Figure 26:** Graphical output 3 of source code "example-heatmap3a.R".

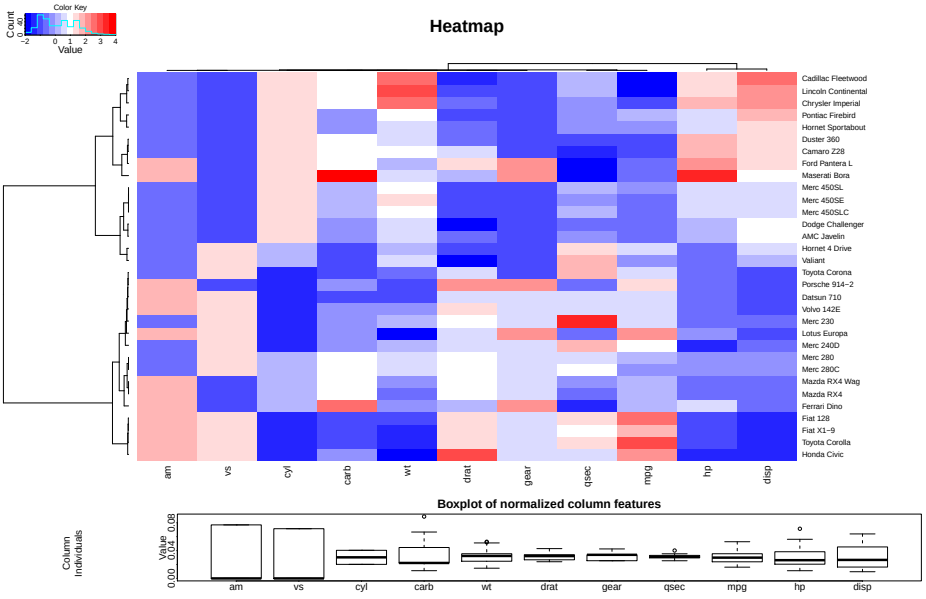



**Figure 27:** Graphical output 4 of source code "example-heatmap3a.R".

### A.5.2 Examples using `ruspini` data

`example-heatmap3b.R` (fig. 28) is an example on how to make a heatmap with summary visualization of clusters.

example-heatmap3b.R

```
## load library
require("GMD")
require(cluster)

## load data
data(ruspini)

## heatmap on a `dist' object
x <- gdist(ruspini)
main <- "Heatmap of Ruspini data"
dev.new(width=10,height=10)
heatmap.3(x, main=main, mapratio=1) # default with a title and a map in square!
heatmap.3(x, main=main, revC=TRUE)  # reverse column for a symmetric look
heatmap.3(x, main=main, kr=2, kc=2) # show partition by predefined number of clusters

## show partition by elbow
css.multi.obj <- css.hclust(x,hclust(x))
elbow.obj <- elbow.batch(css.multi.obj,ev.thres=0.90,inc.thres=0.05)
heatmap.3(x, main=main, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k)
heatmap.3(x, main=main, sub=sub("\n"," ",attr(elbow.obj,"description")), cex.sub=1.25,
          revC=TRUE,kr=elbow.obj$k, kc=elbow.obj$k) # show elbow info as subtitle

## side plot for every row clusters
dev.new(width=10,height=10)
expr1 <- list(quote(plot(do.call(rbind,i.x),xlab="x",ylab="y",
                         xlim=range(ruspini$x),ylim=range(ruspini$y),)))
heatmap.3(x, main=main, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k, trace="none",
          row.data=as.list(data.frame(t(ruspini))),
          plot.row.clusters=TRUE,plot.row.clusters.list=list(expr1))

## side plot for every col clusters
dev.new(width=10,height=10)
expr2 <- list(quote(plot(do.call(rbind,i.x),xlab="x",ylab="y",
                         xlim=range(ruspini$x),ylim=range(ruspini$y),)))
heatmap.3(x, main=main, revC=TRUE, kr=elbow.obj$k, kc=elbow.obj$k, trace="none",
          col.data=as.list(data.frame(t(ruspini))),
          plot.col.clusters=TRUE,plot.col.clusters.list=list(expr2))

```

**Figure 28:** Source code of "example-heatmap3b.R".

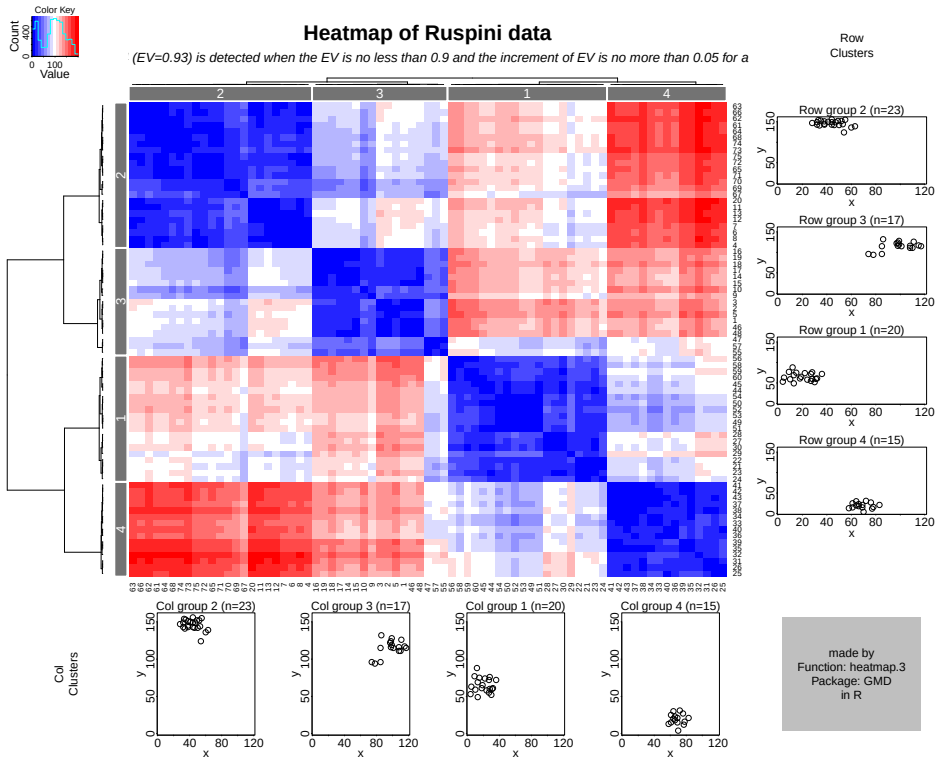



**Figure 29:** Graphical output 1 of source code "example-heatmap3b.R".

# B Data

## B.1 GMD dataset overview

```
> data(package="GMD")


Data sets in package 'GMD':

cage                    CAGE Data
cagel                   CAGE Data
chipseq_hCD4T           ChIP-seq Data
chipseq_mES             ChIP-seq Data
```

## B.2 CAGE data: cage and cagel

```
> help(cage)


> data(cage)
> class(cage)


[1] "list"


> length(cage)


[1] 20


> names(cage)


 [1] "NA (T01F029805F8)"            "Glul (T01F092C2995)"
 [3] "Cyp4a14 (T04R06C91673)"       "Stxbp4 (T11R05607FD4)"
 [5] "D230039L06Rik (T01F0AA465EB)" "Gas5 (T01F09995479)"
 [7] "Rab5c (T11R05FBC6C4)"         "BC003940 (T11R072A6CB0)"
 [9] "Tpt1 (T14F04079189)"          "Pcna (T02R07DE319B)"
[11] "D0H4S114 (T18R020553F0)"      "Gsto1 (T19F02D03566)"
[13] "Hsd11b1 (T01R0B8305BD)"       "Csf1 (T03R0672174D)"
[15] "B2m (T02F0743FF05)"           "Alox5ap (T05F08BCF2C4)"
[17] "Pfkfb3 (T02R00AEC2D8)"        "Hig1 (T09R0743763C)"
[19] "Cd72 (T04R028B8BC9)"          "Egln1 (T08R0769239F)"


> data(cagel)
> names(cagel)


 [1] "NA (T17F05912B83)"            "Tpt1 (T14F04079189)"
 [3] "B2m (T02F0743FF05)"           "Grn (T11F0615F289)"
 [5] "2600001A11Rik (T12R043A2595)" "Rbbp7 (T0XF091A7ACA)"
 [7] "Rpl41 (T10R07AB7138)"         "H2afy (T13R034ACF47)"
 [9] "Dscr1l1 (T17F02802885)"       "Ckap4 (T10R0504CE97)"
[11] "Rab5c (T11R05FBC6C4)"         "Pfkfb3 (T02R00AEC2D8)"
[13] "Csf1 (T03R0672174D)"          "Ctsb (T14F0348EDBA)"
[15] "Crim1 (T17F04928998)"         "3930401E15Rik (T18R02CDD141)"
[17] "Rai17 (T14F014BF473)"         "Hig1 (T09R0743763C)"
[19] "Apbb2 (T05R03E329C8)"         "Ptn (T06R0230806E)"
[21] "Tmeff2 (T01F030EC757)"        "Mrps6 (T16F0583C906)"
[23] "Hsd11b1 (T01R0B8305BD)"       "D0H4S114 (T18R020553F0)"
[25] "4930430J02Rik (T09F036E80C6)" "Phtf2 (T05R0125E896)"
[27] "Trpv2 (T11F03B4EBD8)"         "Slco3a1 (T07R03AC06B9)"
[29] "Scd1 (T19R029B5186)"          "Ctxn (T08R0040864A)"
[31] "5730596K20Rik (T19F006DFC1A)" "Arbp (T05F06BBE13B)"
[33] "Klhl5 (T05F03CCA673)"         "Gsto1 (T19F02D03566)"
[35] "NA (T02R07EF5EDA)"            "Srpk1 (T17R019F4A41)"
[37] "Nudt7 (T08F06C3B651)"         "Tnfrsf10b (T14F03AB1306)"
[39] "Egln1 (T08R0769239F)"         "9630050M13Rik (T02F002EC972)"
[41] "BC003940 (T11R072A6CB0)"      "Ppia (T11F00604AFF)"
[43] "Alox5ap (T05F08BCF2C4)"       "Pcna (T02R07DE319B)"
[45] "Gch1 (T14R02602138)"          "Yap1 (T09R0079F3FF)"
[47] "Vrk1 (T12F06010C9B)"          "Cd72 (T04R028B8BC9)"
[49] "Wdtc1 (T04R07DAFEDC)"         "Centg2 (T01F055392D1)"
```

## B.3 ChIP-seq data: chipseq_mES and chipseq_hCD4T

```
> help(chipseq)
```

```
> data(chipseq_mES)
> class(chipseq_mES)


[1] "list"


> length(chipseq_mES)


[1] 6


> names(chipseq_mES)


[1] "H3K27me3" "H3K36me3" "H3K4me1"  "H3K4me2"  "H3K4me3"  "H3K9me3"


> data(chipseq_hCD4T)
> names(chipseq_hCD4T)


 [1] "CTCF"      "H2AK5ac"   "H2AK9ac"   "H2AZ"      "H2BK120ac" "H2BK12ac"
 [7] "H2BK20ac"  "H2BK5ac"   "H2BK5me1"  "H3K14ac"   "H3K18ac"   "H3K23ac"
[13] "H3K27ac"   "H3K27me1"  "H3K27me2"  "H3K27me3"  "H3K36ac"   "H3K36me1"
[19] "H3K36me3"  "H3K4ac"    "H3K4me1"   "H3K4me2"   "H3K4me3"   "H3K79me1"
[25] "H3K79me2"  "H3K79me3"  "H3K9ac"    "H3K9me1"   "H3K9me2"   "H3K9me3"
[31] "H3R2me1"   "H3R2me2"   "H4K12ac"   "H4K16ac"   "H4K20me1"  "H4K20me3"
[37] "H4K5ac"    "H4K8ac"    "H4K91ac"   "H4R3me2"
```

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