

# Package ‘aCGH’

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**Title** Classes and functions for Array Comparative Genomic  
Hybridization data

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**Description** Functions for reading aCGH data from image analysis output  
files and clone information files, creation of aCGH S3 objects  
for storing these data. Basic methods for accessing/replacing,  
subsetting, printing and plotting aCGH objects.

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**License** GPL-2

**Collate** aCGH.R aCGH.plotting.R aCGH.read.R aCGH.test.R clusterGenome.R  
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human.chrom.info.May04.R mergeLevels.R

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|      |                   |
|------|-------------------|
| aCGH | <i>Class aCGH</i> |
|------|-------------------|

---

## Description

Objects of this class represent batch of arrays of Comparative Genomic Hybridization data. In addition to that, there are slots for representing phenotype and various genomic events associated with aCGH experiments, such as transitions, amplifications, aberrations, and whole chromosomal gains and losses. Currently objects of class aCGH are represented as S3 classes which are named list of lists with functions for accessing elements of that list. In the future, it's anticipated that aCGH objects will be implemented using S4 classes and methods.

## Details

One way of creating objects of class aCGH is to provide the two mandatory arguments to create .aCGH function: `log2.ratios` and `clones.info`. Alternatively aCGH object can be created using [aCGH.read.Sprocs](#) that reads Sproc data files and creates object of type aCGH.

## Value

|                          |  |
|--------------------------|--|
| <code>log2.ratios</code> | Data frame containing the log2 ratios of copy number changes; rows correspond to the clones and the columns to the samples (Mandatory).  |
| <code>clones.info</code> | Data frame containing information about the clones used for comparative genomic hybridization. The number of rows of <code>clones.info</code> has to match the number of rows in <code>log2.ratios</code> (Mandatory). |

|   |  |
|---|--|
| phenotype   | Data frame containing phenotypic information about samples used in the experiment generating the data. The number of rows of phenotype has to match the number of columns in log2.ratios (Optional).   |
| log2.ratios.imputed   | Data frame containing the imputed log2 ratios. Calculate this using <code>impute.lowess</code> function; look at the examples below (Optional).  |
| hmm   | The structure of the hmm element is described in <a href="#">hmm</a> . Calculate this using <a href="#">find.hmm.states</a> function; look at the examples below (Optional).   |
| hmm   | Similar to the structure of the hmm element. Calculate this using <a href="#">mergeHmmStates</a> function; look at the examples below (Optional).  |
| sd.samples  | The structure of the sd.samples element is described in <a href="#">computeSD.Samples</a> . Calculate this using <a href="#">computeSD.Samples</a> function; look at the examples below (Optional). It is prerequisite that the hmm states are estimated first.  |
| genomic.events  | The structure of the genomic.events element is described in <a href="#">find.genomic.events</a> . Calculate this using <a href="#">find.genomic.events</a> function; look also at the examples below. It is prerequisite that the hmm states and sd.samples are computed first. The genomic.events is used widely in variety of plotting functions such as <a href="#">plotHmmStates</a> , <a href="#">plotFreqStat</a> , and <a href="#">plotSummaryProfile</a> . |
| dim.aCGH  | returns the dimensions of the aCGH object: number of clones by number of samples.  |
| num.clones  | number of clones/number of rows of the log2.ratios data.frame.   |
| nrow.aCGH   | same as num.clones.  |
| is.aCGH   | tests if its argument is an object of class aCGH.  |
| num.samples   | number of samples/number of columns of the log2.ratios data.frame.   |
| nrow.aCGH   | same as num.samples.   |
| num.chromosomes   | number of chromosomes processed and stored in the aCGH object.   |
| clone.names   | returns the names of the clones stored in the clones.info slot of the aCGH object.   |
| row.names.aCGH  | same as clone.names.   |
| sample.names  | returns the names of the samples used to create the aCGH object. If the object is created using <a href="#">aCGH.read.Sprocs</a> , these are the file names of the individual arrays.  |
| col.names.aCGH  | same as sample.names.  |
| [.aCGH  | subsetting function. Works the same way as <a href="#">[.data.frame</a> .  |
| Most of the functions/slots listed above have assignment operators ' <code>&lt;-</code> ' associated with them. |  |

### Note

clones.info slot has to contain a list with at least 4 columns: Clone (clone name), Target (unique ID, e.g. Well ID), Chrom (chromosome number, X chromosome = 23 in human and 20 in mouse, Y chromosome = 24 in human and 21 in mouse) and kb (kb position on the chromosome).

### Author(s)

Peter Dimitrov

**See Also**

[aCGH.read.Sprocs](#), [find.hmm.states](#), [computeSD.Samples](#), [find.genomic.events](#), [plotGenome](#), [plotHmmStates](#), [plotFreqStat](#), [plotSummaryProfile](#)

**Examples**

```
## Creating aCGH object from log2.ratios and clone info files
## For alternative way look at aCGH.read.Sprocs help

datadir <- system.file(package = "aCGH")
datadir <- paste(datadir, "/examples", sep="")

clones.info <-
  read.table(file = file.path(datadir, "clones.info.ex.txt"),
             header = TRUE, sep = "\t", quote="", comment.char="")
log2.ratios <-
  read.table(file = file.path(datadir, "log2.ratios.ex.txt"),
             header = TRUE, sep = "\t", quote="", comment.char="")
pheno.type <-
  read.table(file = file.path(datadir, "pheno.type.ex.txt"),
             header = TRUE, sep = "\t", quote="", comment.char="")
ex.acgh <- create.aCGH(log2.ratios, clones.info, pheno.type)

## Printing, summary and basic plotting for objects of class aCGH

data(colorectal)
colorectal
summary(colorectal)
sample.names(colorectal)
phenotype(colorectal)
plot(colorectal)

## Subsetting aCGH object

colorectal[1:1000, 1:30]

## Imputing the log2 ratios

log2.ratios.imputed(ex.acgh) <- impute.lowess(ex.acgh)

## Determining hmm states of the clones
## WARNING: Calculating the states takes some time

##in the interests of time, hmm-finding function is commented out
##instead the states previously save are assigned
##hmm(ex.acgh) <- find.hmm.states(ex.acgh)

hmm(ex.acgh) <- ex.acgh.hmm
hmm.merged(ex.acgh) <-
  mergeHmmStates(ex.acgh, model.use = 1, minDiff = .25)

## Calculating the standard deviations for each array

sd.samples(ex.acgh) <- computeSD.Samples(ex.acgh)

## Finding the genomic events associated with each sample
```

```

genomic.events(ex.acgh) <- find.genomic.events(ex.acgh)

## Plotting and printing the hmm states

plotHmmStates(ex.acgh, 1)
pdf("hmm.states.temp.pdf")
plotHmmStates(ex.acgh, 1)
dev.off()

## Plotting summary of the sample profiles

plotSummaryProfile(colorectal)

```

---

|              |                                    |
|--------------|------------------------------------|
| aCGH.process | <i>Process data in aCGH object</i> |
|--------------|------------------------------------|

---

## Description

This function takes object of class aCGH, and filters clones based on their mapping information and proportion missing. It also average duplicated clones and reports quality statistic.

## Usage

```

aCGH.process(aCGH.obj, chrom.remove.threshold = 24,
             prop.missing = 0.25, sample.quality.threshold = 0.4,
             unmapScreen=TRUE, dupRemove = TRUE)

```

## Arguments

|                          |   |
|--------------------------|---|
| aCGH.obj                 | Object of class aCGH  |
| chrom.remove.threshold   | Chromosomes are ordered and numbered as usual, except for X and Y chromosome, which in for Homo sapiens genome have numbers 23 and 24 respectively, in for Mus musculus 20 and 21, etc. |
| prop.missing             | Clones are screened out and if the proportion missing in the samples is prop.missing they are removed.  |
| sample.quality.threshold | Mark those samples that have their proportion of missing values sample.quality.threshold.   |
| unmapScreen              | Indicator for whether clones with incomplete mapping information should be removed from the dataset. Note that leaving them in may cause plotting routines fail. Defaults to TRUE       |
| dupRemove                | Indicator for whether clones with duplicate names should be averaged and removed from the dataset leaving only one occurrence of each duplicated set. Defaults to TRUE                  |

## Value

Object of class aCGH.

**Author(s)**

Jane Fridlyand, Peter Dimitrov

**See Also**

aCGH

**Examples**

```
datadir <- system.file(package = "aCGH")
datadir <- paste(datadir, "/examples", sep="")

clones.info <-
  read.table(file = file.path(datadir, "clones.info.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
log2.ratios <-
  read.table(file = file.path(datadir, "log2.ratios.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
pheno.type <- read.table(file = file.path(datadir, "pheno.type.ex.txt"), header = TRUE, sep = "\t", quote="", comment.char="")
ex.acgh <- create.aCGH(log2.ratios, clones.info, pheno.type)
ex.acgh <-
  aCGH.process(ex.acgh, chrom.remove.threshold = 23, prop.missing = .25,
sample.quality.threshold = .4, unmapScreen=TRUE, dupRemove = FALSE)
ex.acgh
```

---

aCGH.read.Sprocs

---

*Create object of class "aCGH" from Sproc files*


---

**Description**

This function reads in two-channel Array Comparative Genomic Hybridization Sproc files, flags them for bad quality and missing data, and creates object of class aCGH.

**Usage**

```
aCGH.read.Sprocs(fnames, latest.mapping.file = NULL, maxsd = 0.2,
  minreplic = 2, chrom.remove.threshold = 24,
  prop.missing = 0.25, sample.names = fnames,
  sample.quality.threshold = 0.4,
  cols = c("Log2Rat", "Log2StdDev", "NReplic", "Bad.P"),
  unmapScreen=TRUE, dupRemove = TRUE)
```

**Arguments**

|                     |  |
|---------------------|--|
| fnames              | a vector of character strings containing the file names of each Sproc data file.   |
| latest.mapping.file | The name of an optional file that contains newer clone mapping different from the clone mapping used at the time when the arrays were created. |
| maxsd               | maximum of standard deviation of log2 ratios used in pre-filtering.  |

|                          |   |
|--------------------------|---|
| minreplic                | minimum number of replicates per clone for a single chip used to calculate the log2 ratios.   |
| chrom.remove.threshold   | Chromosomes are ordered and numbered as usual, except for X and Y chromosome, which in for Homo sapiens genome have numbers 23 and 24 respectively, in for Mus musculus 20 and 21, etc.   |
| prop.missing             | Clones are screened out and if the proportion missing in the samples is prop.missing they are removed.  |
| sample.names             | Sample names. If they are missing, the file names are used after stripping the characters after the last dot in the filename if one exists; for example 'myfile.txt' becomes myfile.  |
| sample.quality.threshold | Mark those samples that have their proportion of missing values sample.quality.threshold.   |
| cols                     | character vector of length 4 containing the following Sproc file column names: log2 ratios, std. deviations of the log2 ratios, number of replicates for each clone and flags for bad clones. Defaults to c("Log2Rat", "Log2StdDev", "NReplic", "Bad.P"). Note that all the whitespace characters in the column names will be replaced with dots. |
| unmapScreen              | Indicator for whether clones with incomplete mapping information should be removed from the dataset. Note that leaving them in may cause plotting routines fail. Defaults to TRUE   |
| dupRemove                | Indicator for whether clones with duplicate names should be averaged and removed from the dataset leaving only one occurrence of each duplicated set. Defaults to TRUE  |

**Value**

Object of class aCGH.

**Author(s)**

Jane Fridlyand, Peter Dimitrov

**See Also**

aCGH

**Examples**

```
datadir <- system.file("examples", package = "aCGH")
latest.mapping.file <-
  file.path(datadir, "human.clones.info.Jul03.txt")
ex.acgh <-
  aCGH.read.Sprocs(dir(path = datadir, pattern = "sproc",
    full.names = TRUE), latest.mapping.file,
    chrom.remove.threshold = 23)
ex.acgh

## Testing if creating the object went right. Should all be true.

all(log2.ratios(ex.acgh)[ 1, ] == c(-0.077698 , 0.007389))
clone.name <- "HumArray2H10_T30"
all(log2.ratios(ex.acgh)[ clone.name, ] == c(0.025567 , -0.036908))
```

---

|           |  |
|-----------|--|
| aCGH.test | <i>Testing association of aCGH clones with censored or continuous outcomes</i> |
|-----------|--|

---

## Description

aCGH.test function tests for association of each clone in an univariate manner with censored or continuous outcome by fitting Cox proportional hazards model or linear regression model. There is also an alternative to Cox prop. hazards - testing for differences in survival curves defined by the groups in the outcome variable using the  $G^p$  family of tests.

## Usage

```
aCGH.test(aCGH.obj, rsp, test = c("survdiff", "coxph",
  "linear.regression"), p.adjust.method = "fdr", imputed=TRUE,
  subset = NULL, strt = NULL, ...)
```

## Arguments

|                 |   |
|-----------------|---|
| aCGH.obj        | aCGH object containing clones' log2 ratios.   |
| rsp             | Response variable which is either <a href="#">Surv</a> object from survival package or continuous outcome.  |
| test            | Currently only three values are allowed - "coxph", "survdiff", and "linear.regression", which test for association using Cox proportional hazards model, $G^p$ family of tests ( <a href="#">survdiff</a> ) or linear model.                        |
| p.adjust.method | This is a parameter controlling how the p-values from the univariate tests are going to be adjusted for multiple testing. Default value is Benjamini & Hochberg (1995) FDR method. Please refer to <a href="#">p.adjust</a> function for more help. |
| imputed         | Whether imputed or original log2ratios should be used. Default is TRUE (imputed).   |
| subset          | Specifies subset index of clones to be tested.  |
| strt            | Optional strata variable for splitting the data in different strata.  |
| ...             | Optional parameters passed further along to each of the univariate testing functions.   |

## Value

A data frame similar to the result returned from [mt.maxT](#) function from multtest package with components:

|          |  |
|----------|--|
| index    | Vector of row indices, between 1 and nrow(X), where rows are sorted first according to their adjusted $p$ -values, next their unadjusted $p$ -values, and finally their test statistics. |
| teststat | Vector of test statistics, ordered according to index. To get the test statistics in the original data order, use teststat[order(index)].  |
| rawp     | Vector of raw (unadjusted) $p$ -values, ordered according to index.  |
| adjp     | Vector of adjusted $p$ -values, ordered according to index.  |



**Author(s)**

Peter Dimitrov

**See Also**[aCGH](#), [Surv](#), [mt.maxT](#), [coxph](#), [survdiff](#), [p.adjust](#)

clusterGenome

*clustering and heatmap***Description**

This function clusters samples and shows their heatmap

**Usage**

```
clusterGenome(aCGH.obj,
              response = as.factor(rep("All", ncol(aCGH.obj))),
              chrominfo = human.chrom.info.Jul03, cutoff=1,
              lowCol = "red", highCol = "green", midCol = "black",
              ncolors = 50, byclass = FALSE, showaber = FALSE,
              amplif = 1, homdel = -0.75,
              samplenames = sample.names(aCGH.obj),
              vecchrom = 1:23, titles = "Image Plot",
              methodS = "ward.D", dendPlot = TRUE, imp = TRUE,
              categoricalPheno = TRUE)
```

**Arguments**

|             |   |
|-------------|---|
| aCGH.obj    | object of class aCGH here   |
| response    | phenotype of interest. defaults to the same phenotype assigned to all samples   |
| chrominfo   | a chromosomal information associated with the mapping of the data   |
| cutoff      | maximum absolute value. all the values are floored to +/-cutoff depending on whether they are positive or negative. defaults to 1       |
| ncolors     | number of colors in the grid. input to <a href="#">maPalette</a> . defaults to 50   |
| lowCol      | color for the low (negative) values. input to <a href="#">maPalette</a> . defaults to "red"   |
| highCol     | color for the high (positive) values. input to <a href="#">maPalette</a> . defaults to "green"  |
| midCol      | color for the values close to 0. input to <a href="#">maPalette</a> . defaults to "black"   |
| byclass     | logical indicating whether samples should be clustered within each level of the phenotype or overall. defaults to F                     |
| showaber    | logical indicating whether high level amplifications and homozygous deletions should be indicated on the plot. defaults to F            |
| amplif      | positive value that all observations equal or exceeding it are marked by yellow dots indicating high-level changes. defaults to 1       |
| homdel      | negative value that all observations equal or below it are marked by light blue dots indicating homozygous deletions. defaults to -0.75 |
| samplenames | sample names  |

|                  |   |
|------------------|---|
| vecchrom         | vector of chromosomal indices to use for clustering and to display. defaults to 1:23  |
| titles           | plot title. defaults to "Image Plots"   |
| methodS          | clustering method to cluster samples. defaults to "ward.D"  |
| dendPlot         | logical indicating whether dendrogram needs to be drawn. defaults to T.   |
| imp              | logical indicating whether imputed or original values should be used. defaults to T, i.e. imputed.  |
| categoricalPheno | logical indicating whether phenotype is categorical. Continuous phenotypes are treated as "no groups" except that their values are displayed. defaults to TRUE. |

## Details

This functions is a more flexible version of the [heatmap](#). It can cluster within levels of categorical phenotype as well as all of the samples while displaying phenotype levels in different colors. It also uses any combination of chromosomes that is requested and clusters samples based on these chromosomes only. It draws the chromosomal boundaries and displays high level changes and homozygous deletions. If phenotype is not categorical, its values may still be displayed but groups are not formed and byclass = F. Image plot has the samples reordered according to clustering order.

## See Also

[aCGH heatmap](#)

## Examples

```
data(colorectal)

#cluster all samples using imputed data on all chromosomes (autosomes and X):

clusterGenome(colorectal)

#cluster samples within sex groups based on 3 chromosomes individually.
#use non-imputed data and do not show dendrogram. Indicate amplifications and
#homozygous deletions.

clusterGenome(colorectal, response = phenotype(colorectal)$sex,
               byclass = TRUE, showaber = TRUE, vecchrom = c(4,8,9),
               dendPlot = FALSE, imp = FALSE)

#cluster samples based on each chromosome individually and display age. Show
#gains in red and losses in green. Show aberrations and use values < -1
#to identify homozygous deletions. Do not show dendrogram.

pdf("plotimages.pdf", width = 11, height = 8.5)
for (i in 1:23)
  clusterGenome(colorectal,
                response = phenotype(colorectal)$age,
                chrominfo = human.chrom.info.Jul03,
                cutoff = 1, ncolors = 50, lowCol="green",
                highCol="red", midCol="black", byclass = FALSE,
                showaber = TRUE, homdel = -1, vecchrom = i,
                titles = "Image Plot", methodS = "ward.D",
                dendPlot = FALSE, categoricalPheno = FALSE)
```

```
dev.off()
```

---

colorectal

*Colorectal array CGH dataset*

---

## Description

The colorectal dataset is an object of class [aCGH](#). It represents a collection of 124 array CGH profiles of primary colorectal tumors and their derived attributes. Each sample was measured on the BAC clone DNA microarray with approximate resolution of 1.4 Mb per clone. There were approximately 2400 clones spotted on the array and each clone was printed in triplicates located immediately next to each other. Each array consisted of the 16 (4 by 4) subarrays. The clones were mapped on the July 03 UCSC freeze. There were a number of the discrete and continuous phenotypes associated with the samples such as age, mutation status for various markers, stage, location and so on. All images were quantified and normalized by Dr. Taku Tokuyasu using custom image software SPOT and postprocessing custom software SPROC.

## Usage

```
data(colorectal)
```

## Source

These data were generated at Dr. Fred Waldman's lab at UCSF Cancer Center by K. Nakao and K. Mehta. The manuscript describing the data and the analysis are described in High-resolution analysis of DNA copy number alterations in colorectal cancer by array-based comparative genomic hybridization, *Carcinogenesis*, 2004, Nakao et. al.

## References

Nakao et. al., High-resolution analysis of DNA copy number alterations in colorectal cancer by array-based comparative genomic hybridization, *Carcinogenesis*, 2004 Jain et. al, Fully automatic quantification of microarray image data, *Genome Research*, 2003

## See Also

[aCGH plotGenome](#)

## Examples

```
data(colorectal)
## WARNING: plotting the heatmap takes some time
plot(colorectal)
plotGenome(colorectal[,1:2])
```

computeSD.func

*Function to estimate experimental variability of a sample***Description**

This functions estimate experimental variability of a given sample. This value can be used to rank samples in terms of the quality as well as to derive thresholds for declaring gained and lost clones.

**Usage**

```
computeSD.Samples(aCGH.obj, maxChrom = 22, maxmadUse = .3,
                  maxmedUse = .5, maxState = 3, maxStateChange = 100,
                  minClone = 20)
computeSD.func(statesres, maxmadUse = 0.2, maxmedUse = 0.2,
               maxState = 3, maxStateChange = 100, minClone = 20,
               maxChrom = 22)
```

**Arguments**

|                |  |
|----------------|--|
| aCGH.obj       | Object of class <a href="#">aCGH</a> .   |
| statesres      | The states.hmm object, generally is the output of <a href="#">mergeFunc</a> .  |
| maxmadUse      | Maximum median absolute deviation allowed to controbute to the overall variability calculation.  |
| maxmedUse      | Maximum median value for a state allowed to contribute to the calculation.   |
| maxState       | Maximum number of the states on a given chromosome for the states from that chromosome to be allowed to enter noise variability calculation. |
| maxStateChange | Maximum number of changes from state to state on a given chromosome for that chromosome to enter noise variability calculation.              |
| minClone       | Minimum number of clones in a state for clones in that sate to enter variability calculation.  |
| maxChrom       | Maxiumum chromosomal index (generally only autosomes are used for this calculation).   |

**Details**

Median absolute deviation is estimated in all the states passing the criteria defined by the parameters of the function. Then median of all MADs on individual chromosomes as well as across all chromosomes is taken to estimate chromosomal experimental variability and sample experimental variability.

**Value**

|           |  |
|-----------|--|
| madChrom  | Returns a matrix containing estimated variability for each chromosome for each sample. |
| madGenome | Returns a vector with estimate of experimental varibility for each sample.             |

**Author(s)**

Jane Fridlyand

## References

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

## See Also

[aCGH](#)

---

|          |   |
|----------|---|
| fga.func | <i>Function to compute fraction of genome altered for each sample</i> |
|----------|---|

---

## Description

This function outputs lists containing proportions of the genome that are gained and lost for each sample.

## Usage

```
fga.func(aCGH.obj, thres = 0.25, factor = 2.5,
        samplenames = sample.names(aCGH.obj),
        chrominfo = human.chrom.info.Jul03)
```

## Arguments

|             |   |
|-------------|---|
| aCGH.obj    | An object of aCGH class   |
| thres       | either a vector providing unique threshold for each sample or a vector of the same length as number of samples providing sample-specific threshold. If 'aCGH.obj' has non-null 'sd.samples', then threshold is automatically replaced by tumor-specific sd multiplied by 'factor'. Clone is considered to be gained if it is above the threshold and lost if it is below negative threshold. Defaults to 0.25 |
| factor      | specifies the number by which experimental variability should be multiples. Used only when tumor specific variability in 'aCGH.obj' is not NULL or when factor is greater than 0. Defaults to 2.5.  |
| samplenames | Sample names. Default is sample.names(aCGH.obj)   |
| chrominfo   | A chromosomal information associated with the mapping of the data. Default is human.chrom.info.Jul03 data frame   |

## Value

|       |   |
|-------|---|
| gainP | Vector of proportion of genome gained for each sample |
| lossP | Vector of proportion of genome lost for each sample   |

## Author(s)

Jane Fridlyand, Ritu Roydasgupta

## Examples

```
data(colorectal)

col.fga <- fga.func(colorectal, factor=3,chrominfo=human.chrom.info.Jul03)
cbind(gainP=col.fga$gainP,lossP=col.fga$lossP)[1:5,]
```

---

|                     |   |
|---------------------|---|
| find.genomic.events | <i>Finds the genomic events associated with each of the array CGH samples</i> |
|---------------------|---|

---

## Description

Finds the genomic events associated with each of the array CGH samples. Events include whole chromosomal gains and losses, aberrations, transitions, amplifications and their respective counts and sizes. The hmm states has to be computed before using this function.

## Usage

```
find.genomic.events(aCGH.obj, maxChrom = 23, factor = 5, maxClones = 1,
                    maxlen = 1000, absValSingle = 1, absValRegion = 1,
                    diffVal1 = 1, diffVal2 = .5, maxSize = 10000,
                    pChrom.min = .9, medChrom.min = .1)
```

## Arguments

|              |  |
|--------------|--|
| aCGH.obj     | Object of class <a href="#">aCGH</a> .   |
| maxChrom     | Highest chromosomal number to find events.   |
| factor       | Determines outliers. See <a href="#">findOutliers.func</a> .   |
| maxClones    | Determines aberrations. See <a href="#">findAber.func</a> .  |
| maxLen       | Determines aberrations. See <a href="#">findAber.func</a> .  |
| absValSingle | Determines amplifications. See <a href="#">findAmplif.func</a> .   |
| absValRegion | Determines amplifications. See <a href="#">findAmplif.func</a> .   |
| diffVal1     | Determines amplifications. See <a href="#">findAmplif.func</a> .   |
| diffVal2     | Determines amplifications. See <a href="#">findAmplif.func</a> .   |
| maxSize      | Determines amplifications. See <a href="#">findAmplif.func</a> .   |
| pChrom.min   | Determines whole chromosomal gains and losses. Chromosome should contain no transitions, have its absolute median equal or greater than medChrom.min and at least medChrom.min has to be greater or less than 0. |
| medChrom.min | Determines whole chromosomal gains and losses. Chromosome should contain no transitions, have its absolute median equal or greater than medChrom.min and at least medChrom.min has to be greater or less than 0. |

## Details

The default parameters generally work. Threshold for merging may be changed depending on the expected normal cell contamination and/or expected magnitude of the changes. AIC model generally works, however, may need to be readjusted depending on how liberal or conservative one wants to be in finding genomic events. We recommend BIC criterion with delta = 1 for noisier data.

**Value**

|                           |  |
|---------------------------|--|
| num.transitions           | matrix of dimensions maxChrom by number of samples. It contains number of transitions that were recorded on a given chromosome for a given sample.   |
| num.amplifications        | matrix of dimensions maxChrom by number of samples. It contains number of amplifications that were recorded on a given chromosome for a given sample.  |
| num.aberrations           | matrix of dimensions maxChrom by number of samples. It contains number of focal aberrations that were recorded on a given chromosome for a given sample.   |
| num.outliers              | matrix of dimensions maxChrom by number of samples. It contains number of outliers that were recorded on a given chromosome for a given sample.  |
| num.transitions.binary    | binary matrix of dimensions maxChrom by number of samples. Non-zero entry indicates whether 1 or more transitions were recorded on a given chromosome for a given sample.  |
| num.amplifications.binary | binary matrix of dimensions maxChrom by number of samples. Non-zero entry indicates whether 1 or more amplifications were recorded on a given chromosome for a given sample.   |
| num.aberrations.binary    | binary matrix of dimensions maxChrom by number of samples. Non-zero entry indicates whether 1 or more focal aberrations were recorded on a given chromosome for a given sample.  |
| num.outliers.binary       | binary matrix of dimensions maxChrom by number of samples. Non-zero entry indicates whether 1 or more outliers were recorded on a given chromosome for a given sample.   |
| whole.chrom.gain.loss     | matrix of dimensions maxChrom by number of samples. Positive entry indicates that a given chromosome was gained in a given sample, negative entry indicates that a given chromosome was lost in a given sample, 0 entry is normal chromosome and NA marks chromosomes with one or more transition. |
| size.amplicons            | matrix of dimensions maxChrom by number of samples. Reports size of a given chromosome that is amplified (kb units) in a given sample.   |
| num.amplicons             | matrix of dimensions maxChrom by number of samples. Reports number of disjoint amplicons on a given chromosome for a given sample.   |
| outliers                  | list containing 3 matrices of dimensions number of clones by number of samples. See <a href="#">findOutliers.func</a> .  |
| aberrations               | list containing a matrix of dimensions number of clones by number of samples. See <a href="#">findAber.func</a> .  |
| transitions               | list containing 2 matrices of dimensions number of clones by number of samples. See <a href="#">findTrans.func</a> .   |
| amplifications            | list containing a matrix of dimensions number of clones by number of samples. See <a href="#">findAmplif.func</a> .  |

**See Also**

[aCGH.find.hmm.states.mergeFunc](#) [findAber.func](#) [findTrans.func](#) [findAmplif.func](#) [findOutliers.func](#)

---

|                 |  |
|-----------------|--|
| find.hmm.states | <i>Determines states of the clones</i> |
|-----------------|--|

---

## Description

This function runs unsupervised HMM algorithm and produces the essential state information which is used for the subsequent structure determination.

## Usage

```
hmm.run.func(dat, datainfo = clones.info, vr = 0.01, maxiter = 100,
             aic = TRUE, bic = TRUE, delta = NA, eps = 0.01)
find.hmm.states(aCGH.obj, ...)
```

## Arguments

|          |  |
|----------|--|
| aCGH.obj | object of class <a href="#">aCGH</a> .   |
| dat      | dataframe with clones in the rows and samples in the columns   |
| datainfo | dataframe containing the clones information that is used to map each clone of the array to a position on the genome. Has to contain columns with names Clone/Chrom/kb containing clone names, chromosomal assignment and kb positions respectively |
| vr       | Initial experimental variance  |
| maxiter  | Maximum number of iterations   |
| aic      | TRUE or FALSE variable indicating whether or nor AIC criterion should be used for model selection (see DETAILS)  |
| bic      | TRUE or FALSE variable indicating whether or nor BIC criterion should be used for model selection (see DETAILS)  |
| delta    | numeric vector of penalty factors to use with BIC criterion. If BIC is true, delta=1 is always calculated (see DETAILS)  |
| eps      | parameter controlling the convergence of the EM algorithm.   |
| ...      | All the parameters that can be passed to find.hmm.states except dat and datainfo.  |

## Details

One or more model selection criterion is used to determine number of states on each chromosomes. If several are specified, then a separate matrix is produced for each criterion used. Delta is a fudge factor in BIC criterion:  $\delta BIC(\gamma) = \log RSS(\gamma) + q_\gamma \delta \log n/n$ . Note that delta = NA leads to conventional BIC. (Broman KW, Speed TP (2002) A model selection approach for the identification of quantitative trait loci in experimental crosses (with discussion). J Roy Stat Soc B 64:641-656, 731-775 )

find.hmm.states(aCGH.obj, ...) uses aCGH object instead of log2 ratios matrix dat. Equivalent representation (assuming normally distributed residuals) is to write  $-\loglik(\gamma) = n/2 * \log(RSS)(\gamma)$  and then  $bic = -\loglik + \log(n) * k * \delta / 2$  and  $aic = -\loglik + 2 * k / 2$



**Value**

Two lists of lists are returned. Each list contains information on the states with each of the specified model selection criteria. E.g., if AIC = T, BIC = T and delta = c(1.5), then each list will contain three lists corresponding to AIC, BIC(1) and BIC(1.5) as the 1st, 2nd and 3rd lists respectively. If AIC is used, it always comes first followed by BIC and then deltaBIC in the order of delta vector.

|             |   |
|-------------|---|
| states.hmm  | Each of the sublists contains 2+ 6*n columns where the first two columns contain chromosome and kb positions for each clone in the dataset supplied followed up by 6 columns for each sample where n = number of samples.<br>column 1 = state<br>column 2 = smoothed value for a clone<br>column 3 = probability of being in a state<br>column 4 = predicted value of a state<br>column 5 = dispersion<br>column 6 = observed value |
| nstates.hmm | Each of the sublists contains a matrix with each row corresponding to a chromosome and each column to a sample. The entries indicate how many different states were identified for a given sample on a given chromosome   |

**WARNING**

When algorithm fails to fit an HMM for a given number of states on a chromosome, it prints a warning.

**Author(s)**

Jane Fridlyand

**References**

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

**See Also**

[aCGH](#)

**Examples**

```
datadir <- system.file("examples", package = "aCGH")
latest.mapping.file <-
  file.path(datadir, "human.clones.info.Jul03.txt")
ex.acgh <-
  aCGH.read.Sprocs(dir(path = datadir, pattern = "sproc",
    full.names = TRUE), latest.mapping.file,
    chrom.remove.threshold = 23)
ex.acgh

data(colorectal)
#in the interests of time, we comment the actual hmm-finding function out.
#hmm(ex.acgh) <- find.hmm.states(ex.acgh, aic = TRUE, delta = 1.5)
summary(ex.acgh)
```

---

|               |   |
|---------------|---|
| findAber.func | <i>Function to determines focal aberrations</i> |
|---------------|---|

---

### Description

The function identifies clones that are focal aberrations.

### Usage

```
findAber.func(maxClones = 1, maxLen = 1000, statesres)
```

### Arguments

|           |   |
|-----------|---|
| maxClones | Maximum number of clones assigned to the same state which can be considered to be focal aberrations                                 |
| maxLen    | Maximum length of the region containing clones assigned to the state so that those clones can be considered to be focal aberrations |
| statesres | The states output of the <a href="#">hmm.run.func</a>   |

### Details

The focal aberrations are the one or more clones assigned to the state different from the states of the surrounding clones. They may indicate copy number polymorphisms or interesting high or low focal changes.

### Value

|      |  |
|------|--|
| aber | Binary matrix with a row for each clone and column for each sample. 1 indicates presence of a focal aberrations, 0 lack of such. |
|------|--|

### Author(s)

Jane Fridlyand

### References

"Application of Hidden Markov Models to the analysis of the array CGH data", Fridlyand et.al., JMVA, 2004

---

|                 |  |
|-----------------|--|
| findAmplif.func | <i>Function to determine high level amplifications</i> |
|-----------------|--|

---

### Description

This function identifies high level amplifications by considering the height, the width of an amplicon relative to the surrounding clones. Only narrow peaks much higher than its neighbors are considered as high level amplifications.

**Usage**

```
findAmplif.func(absValSingle = 1, absValRegion = 1.5, diffVal1 = 1,
diffVal2 = 0.5, maxSize = 10000, translen.matr, trans.matr, aber,
outliers, pred, pred.obs, statesres)
```

**Arguments**

|               |  |
|---------------|--|
| absValSingle  | A clone is declared to be an amplification if it is a focal aberration or an outlier and its value exceeds absValSingle                                |
| absValRegion  | A clone is an amplification if if a clone belong to a region with width less than maxSize and observed value for a clones is greater than absValRegion |
| diffVal1      | Clone is an amplification if it is an aberration and greater by diffVal1 than max of the two surrounding stretches                                     |
| diffVal2      | Clone is an amplification if it is an outlier, its observed values is greater by diffVal2 than max of the two surrounding stretches                    |
| maxSize       | The clones may not be declared as amplifications if they belong to the states with spanning more than maxSize  |
| translen.matr | State length matrix. The output of the <a href="#">findTrans.func</a>  |
| trans.matr    | Transition matrix. The output of the <a href="#">findTrans.func</a>  |
| aber          | Aberration matrix. The output of the <a href="#">findAber.func</a>   |
| outliers      | Outliers matrix. The output of the <a href="#">findOutliers.func</a>   |
| pred          | Predicted values matrix. The output of the <a href="#">findOutliers.func</a>   |
| pred.obs      | Predicted values matrix with observed values assigned to the outliers. The output of the <a href="#">findOutliers.func</a>                             |
| statesres     | The states output of the <a href="#">hmm.run.func</a>  |

**Details**

Note that all the distances are in Megabases and all the heights are on log2ratio scale.

**Value**

|               |   |
|---------------|---|
| amplif.matrix | Binary matrix with a row for each clone and column for each sample. "1" indicates amplification |
| ...           |   |

**Author(s)**

Jane Fridlyand

**References**

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

**See Also**

[aCGH](#)

---

|                   |  |
|-------------------|--|
| findOutliers.func | <i>Function to identify outlier clones</i> |
|-------------------|--|

---

## Description

The function identified the clones that are outliers.

## Usage

```
findOutliers.func(thres, factor = 4, statesres)
```

## Arguments

|           |  |
|-----------|--|
| thres     | Estimate of experimental variability, generally, madGenome |
| factor    | Factor indicating how many standard                        |
| statesres | The states output of the <a href="#">hmm.run.func</a>      |

## Details

The outliers are the clones that are dissimilar enough from the clones assigned to the same state. Magnitude of the factor determines how many MADs away from a median a value needs to be to be declared an outlier. Outliers consistent over many samples may indicate technological artifact with that clone or possibly copy number polymorphism.

## Value

|              |   |
|--------------|---|
| outlier      | Binary matrix with a row for each clone and column for each sample. "1" indicates outlier, 0 otherwise.   |
| pred.obs.out | Matrix with a row for each clone and column for each sample. The entries are the median value for the state with outliers excluded for all clones but outliers. The value if the observed value for the outliers. |
| pred.out     | Matrix with a row for each clone and column for each sample. The entries are the median value for the state   |

## Author(s)

Jane Fridlyand

## References

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

## See Also

[aCGH](#)

---

|                |  |
|----------------|--|
| findTrans.func | <i>Funtion identifying the transitions</i> |
|----------------|--|

---

## Description

This function identifies the start and end of the states (regions with the constant estimated copy number).

## Usage

```
findTrans.func(outliers, aber, statesres)
```

## Arguments

|           |   |
|-----------|---|
| outliers  | Binary matrix of the outliers (generally output of the <a href="#">findOutliers.func</a> )      |
| aber      | Binary matrix of the focal aberrations (generally output of the <a href="#">findAber.func</a> ) |
| statesres | The states output of the <a href="#">hmm.run.func</a>   |

## Details

The transitions end is placed at the last non-focal aberration clone of the contiguous region containing clones belonging to the same state and transitions start is placed at the first non-focal aberration clone of the contiguous region containing clones belonging to the same state.

## Value

|                 |   |
|-----------------|---|
| trans.matrix    | Matrix with a row for each clone and column for each sample. The starts of the states are indicated by "1", the end are by "2" and the focal aberrations are coded as "3"   |
| translen.matrix | Matrix with a row for each clone and column for each sample. The entries are the length of the region to which a clone belongs. Zero length is assigned to the focal aberrations. This output may be buggy at the moment. |

## Author(s)

Jane Fridlyand

## References

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004.

## See Also

[aCGH](#)

gainLoss

*Function to compute proportion of gains and losses for each clones***Description**

This function outputs lists containing proportion of gains and losses for each clone.

**Usage**

```
gainLoss(dat, cols, thres=0.25)
```

**Arguments**

|       |  |
|-------|--|
| dat   | log2ratios of the relevant array CGH object          |
| cols  | indeces of the samples to use                        |
| thres | global or tumor-specific threshold. defaults to 0.25 |

**Value**

|       |   |
|-------|---|
| gainP | Vector of proportion gained for each clones |
| lossP | Vector of proportion lost for each clones   |

**Author(s)**

Jane Fridlyand

**See Also**

[plotFreqStat](#)

**Examples**

```
data(colorectal)

## Use mt.maxT function from multtest package to test
## differences in group means for each clone grouped by sex
##use only clones with show gain or loss in at least 10% of the samples
colnames(phenotype(colorectal))
sex <- phenotype(colorectal)$sex
sex.na <- !is.na(sex)
colorectal.na <- colorectal[,sex.na, keep = TRUE ]
factor <- 2.5
minChanged <- 0.1
gainloss <- gainLoss(log2.ratios(colorectal.na), cols=1:ncol(colorectal.na), thres=factor*sd.samples(colorectal.na))
ind.clones.use <- which(gainloss$gainP >= minChanged | gainloss$lossP>= minChanged)
#create filtered dataset
colorectal.na <- colorectal.na[ind.clones.use,keep=TRUE]
dat <- log2.ratios.imputed(colorectal.na)
resT.sex <- mt.maxT(dat, sex[sex.na],test = "t.equalvar", B = 1000)

## Plot the result along the genome
plotFreqStat(colorectal.na, resT.sex, sex[sex.na],factor=factor,titles = c("Male", "Female"))
```

---

|         |  |
|---------|--|
| heatmap | <i>Creates heatmap array CGH objects</i> |
|---------|--|

---

## Description

Clusters samples and produces heatmapp of the observed log2ratios.

## Usage

```
heatmap(x, imp = TRUE, Rowv = NA, Colv = NULL, distfun = dist,
        hclustfun = hclust, add.expr, symm = FALSE,
        revC = identical(Colv, "Rowv"), scale = "none",
        na.rm = TRUE, margins = c(5, 5), ColSideColors,
        RowSideColors, cexRow = 0.2 + 1 / log10(nr),
        cexCol = 0.2 + 1 / log10(nc), labRow = NULL,
        labCol = NULL, main = NULL, xlab = NULL, ylab = NULL,
        verbose = getOption("verbose"), methodR = "ward.D",
        methodC = "ward.D", zlm = c(-0.5, 0.5), ...)
```

## Arguments

|           |  |
|-----------|--|
| x         | object of the <a href="#">aCGH</a> object  |
| imp       | logical variable indicating whether log2.ratios.imputed or log2.ratios slot of <a href="#">aCGH</a> should be used. Defaults to imputed value (TRUE).  |
| Rowv      | determines if and how the row dendrogram should be computed and reordered. Either a 'dendrogram' or a vector of values used to reorder the row dendrogram or 'NA' to suppress any row dendrogram (and reordering) or by default, 'NULL'  |
| Colv      | determines if and how the column dendrogram should be reordered. Has the same options as the Rowv argument above and additionally when x is a square matrix, Colv = "Rowv" means that columns should be treated identically to the rows. |
| distfun   | function used to compute the distance (dissimilarity) between both rows and columns. Defaults to 'dist'.   |
| hclustfun | function used to compute the hierarchical clustering when 'Rowv' or 'Colv' are not dendrograms. Defaults to 'hclust'   |
| add.expr  | expression that will be evaluated after the call to 'image'. Can be used to add components to the plot.  |
| symm      | logical indicating if 'x' should be treated *symm*etrically; can only be true when 'x' is a square matrix.   |
| revC      | logical indicating if the column order should be 'rev'ersed for plotting, such that e.g., for the symmetric case, the symmetry axis is as usual.   |
| scale     | character indicating if the values should be centered and scaled in either the row direction or the column direction, or none. The default is "row" if symm false, and "none" otherwise.   |
| na.rm     | logical indicating whether 'NA's should be removed.  |

|                               |  |
|-------------------------------|--|
| <code>margins</code>          | numeric vector of length 2 containing the margins (see <code>'par(mar= *)'</code> ) for column and row names, respectively.  |
| <code>ColSideColors</code>    | (optional) character vector of length <code>'ncol(x)'</code> containing the color names for a horizontal side bar that may be used to annotate the columns of <code>'x'</code> . |
| <code>RowSideColors</code>    | (optional) character vector of length <code>'nrow(x)'</code> containing the color names for a vertical side bar that may be used to annotate the rows of <code>'x'</code> .      |
| <code>cexRow, cexCol</code>   | positive numbers, used as <code>'cex.axis'</code> in for the row or column axis labeling. The defaults currently only use number of rows or columns, respectively.               |
| <code>labRow, labCol</code>   | character vectors with row and column labels to use; these default to <code>'rownames(x)'</code> or <code>'colnames(x)'</code> , respectively.                                   |
| <code>main, xlab, ylab</code> | main, x- and y-axis titles;  |
| <code>verbose</code>          | logical indicating if information should be printed.   |
| <code>methodR</code>          | method to use for clustering rows. defaults to "ward.D"  |
| <code>methodC</code>          | method to use for clustering columns. defaults to "ward.D"   |
| <code>zlm</code>              | all the values greater or equal than <code>zlm</code> are set to <code>zlm - 0.01</code> . a;; value less or equal to <code>-zlm</code> are set to <code>-zlm + 0.01</code>      |
| <code>...</code>              | additional arguments passed on to <code>'image'</code> , e.g., <code>'col'</code> specifying the colors.   |

### Details

This function is almost identical to the [heatmap](#) in base R. The slight modifications are that (1) a user can specify clustering method for rows and columns; (2) all the values outside specified limits are floored to be 0.01 less than a limit; (3) default values are different. Note that using default option of `imp` (TRUE) produces nicer looking plots as all missing values are removed.

### Value

Invisibly, a list with components

|                      |  |
|----------------------|--|
| <code>crowInd</code> | row index permutation vector as returned by <a href="#">order.dendrogram</a> |
| <code>colInd</code>  | row index permutation vector as returned by <a href="#">order.dendrogram</a> |

### References

heatmap function in base R

### See Also

[aCGH clusterGenome](#)

### Examples

```
#default plotting method for the aCGH object
data(colorectal)
plot(colorectal)

#to produce smoother looking heatmap, use imp = T: this will use imputed
#slot of aCGH object

plot(colorectal, imp = TRUE)
```



---

`human.chrom.info.Jul03`*Basic Chromosomal Information for UCSC Human Genome Assembly  
July 2003 freeze*

---

### Description

This dataset contains basic chromosomal information for UCSC Human Genome Assembly July 2003 freeze. `human.chrom.info.Jul03` is loaded automatically with the aCGH package.

### Usage

`human.chrom.info.Jul03`

### Format

A data frame with 24 observations on the following 3 variables.

**chrom** Chromosomal index, X is coded as 23 and Y as 24.

**length** Length of each chromosome in kilobases.

**centromere** Location of the centromere on the chromosome (kb).

### Details

This file is used for many plotting functions and needs to correspond to [clones.info](#) mapping file. The centromeric location is approximately estimated by taking mid-point between the last fish-mapped clone on the p-arm and the first fish-mapped clone on the q-arm using relevant UCSC freeze. For an alternative freeze, one needs to manually create a 3-column file of the format described above.

### Source

<http://genome.ucsc.edu/cgi-bin/hgText>

---

`human.chrom.info.May04`*Basic Chromosomal Information for UCSC Human Genome Assembly  
May 2004 freeze*

---

### Description

This dataset contains basic chromosomal information for UCSC Human Genome Assembly May 2004 freeze. `human.chrom.info.May04` is loaded automatically with the aCGH package.

### Usage

`human.chrom.info.May04`

**Format**

A data frame with 24 observations on the following 3 variables.

**chrom** Chromosomal index, X is coded as 23 and Y as 24.

**length** Length of each chromosome in kilobases.

**centromere** Location of the centromere on the chromosome (kb).

**Details**

This file is used for many plotting functions and needs to correspond to [clones.info](#) mapping file. The centromeric location is approximately estimated by taking mid-point between the last fish-mapped clone on the p-arm and the first fish-mapped clone on the q-arm using relevant UCSC freeze. For an alternative freeze, one needs to manually create a 3-column file of the format described above.

**Source**

<http://genome.ucsc.edu/cgi-bin/hgText>

---

impute.HMM

---

*Imputing log2 ratios using HMM*


---

**Description**

Imputing log2 ratios using the output of the HMM segmentation

**Usage**

```
impute.HMM(aCGH.obj, chrominfo = human.chrom.info.Jul03, maxChrom =
23, use.BIC = TRUE)
```

**Arguments**

|           |   |
|-----------|---|
| aCGH.obj  | Object of class aCGH.   |
| chrominfo | a chromosomal information associated with the mapping of the data   |
| maxChrom  | Highest chromosome to impute.   |
| use.BIC   | logical parameter; if true impute missing values based on the Hidden Markov Model selected using Bayesian Information Criterion impute missing data, otherwise use AIC. |

**Details**

See details in [aCGH](#) discussion.

**Value**

Computes and returns the imputed log2 ratio matrix of the aCGH object using the output of the Hidden Markov Model segmentation done by invoking [find.hmm.states](#) function.

**See Also**

[aCGH](#), [find.hmm.states](#), [impute.lowess](#).

**Examples**

```
datadir <- system.file(package = "aCGH")
datadir <- paste(datadir, "/examples", sep="")

clones.info <-
  read.table(file = file.path(datadir, "clones.info.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
log2.ratios <-
  read.table(file = file.path(datadir, "log2.ratios.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
ex.acgh <- create.aCGH(log2.ratios, clones.info)

## Imputing the log2 ratios

hmm(ex.acgh) <- find.hmm.states(ex.acgh, aic = TRUE, delta = 1.5)
log2.ratios.imputed(ex.acgh) <- impute.HMM(ex.acgh)
```

---

|               |                             |
|---------------|-----------------------------|
| impute.lowess | <i>Imputing log2 ratios</i> |
|---------------|-----------------------------|

---

**Description**

Imputing log2 ratios

**Usage**

```
impute.lowess(aCGH.obj, chrominfo = human.chrom.info.Jul03, maxChrom =
23, smooth = 0.1)
```

**Arguments**

|           |   |
|-----------|---|
| aCGH.obj  | Object of class aCGH.   |
| chrominfo | a chromosomal information associated with the mapping of the data |
| maxChrom  | Highest chromosome to impute.                                     |
| smooth    | smoothing parameter for the lowess procedure                      |

**Details**

There are two main reasons to impute data. One is that given that imputation is reasonable, one can increase the analytical power and improve results. Another, more practical, is that at the moment many widely used functions in R do not support missing values. While procedures such as kNN imputations is widely used for gene expression data, it is more powerful to take advantage of the genomic structure of the array CGH data and use a smoother. Note that we perform only one pass of smoothing. If there still remain missing values, they are imputed by the median on the chromosome or chromosomal arm where applicable,

**Value**

Computes and returns the imputed log2 ratio matrix of the aCGH object.

**See Also**

[aCGH](#), [impute.HMM](#).

**Examples**

```
datadir <- system.file(package = "aCGH")
datadir <- paste(datadir, "/examples", sep="")

clones.info <-
  read.table(file = file.path(datadir, "clones.info.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
log2.ratios <-
  read.table(file = file.path(datadir, "log2.ratios.ex.txt"),
            header = TRUE, sep = "\t", quote="", comment.char="")
ex.acgh <- create.aCGH(log2.ratios, clones.info)

## Imputing the log2 ratios

log2.ratios.imputed(ex.acgh) <- impute.lowess(ex.acgh)
```

---

|           |   |
|-----------|---|
| mergeFunc | <i>Funtion to merge states based on their state means</i> |
|-----------|---|

---

**Description**

mergeFunc takes the output of `hmm.run.func` (or `find.hmm.states`) with a particular model selection criterion and iteratively merges the states with means closer than a supplied threshold. `mergeHmmStates` is a frontend for `mergeFunc` using [aCGH](#) object.

**Usage**

```
mergeHmmStates(aCGH.obj, model.use = 1, minDiff = 0.25)
mergeFunc(statesres, minDiff = 0.1)
```

**Arguments**

|                        |   |
|------------------------|---|
| <code>aCGH.obj</code>  | Object of class <a href="#">aCGH</a> .  |
| <code>statesres</code> | the sublist of the <code>states.hmm</code> list output from <a href="#">find.hmm.states</a> for a given model selection criterion                 |
| <code>minDiff</code>   | The states whose predicted values are less than <code>minDiff</code> apart are merged into one state and all the predicted values are recomputed. |
| <code>model.use</code> | Model selection criterion to use, See <a href="#">find.hmm.states</a> .   |

**Details**

This function is intended to reduce effect of the possible small magnitude technological artifacts on the structure determination.

**Value**

List containing states.hmm object is returned.

**Author(s)**

Jane Fridlyand

**References**

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

**See Also**

[aCGH](#), [find.hmm.states](#)

---

|             |                    |
|-------------|--------------------|
| mergeLevels | <i>mergeLevels</i> |
|-------------|--------------------|

---

**Description**

Merging of predicted levels for array CGH data and similar.

**Usage**

```
mergeLevels(vecObs,vecPred,pv.thres=0.0001,ansari.sign=0.05,thresMin=0.05,thresMax=0.5,verbose=1)
```

**Arguments**

|             |   |
|-------------|---|
| vecObs      | Vector of observed values, i.e. observed log2-ratios  |
| vecPred     | Vector of predicted values, i.e. mean or median of levels predicted by segmentation algorithm   |
| pv.thres    | Significance threshold for Wilcoxon test for level merging  |
| ansari.sign | Significance threshold for Ansari-Bradley test  |
| thresMin    | merge if segment medians are closer than thresMin , default is 0.05   |
| thresMax    | don't merge if segment medians are further than thresMax (unless needs to be merged for a different reason: wilcoxon test), default is .5 |
| verbose     | if 1, progress is printed   |
| scale       | whether thresholds are on the log2ratio scale and thus need to be converted to the copy number. default is TRUE                           |

**Details**

mergeLevels takes a vector of observed log2-ratios and predicted log2ratios and merges levels that are not significantly distinct.

**Value**

|           |   |
|-----------|---|
| vecMerged | Vector with merged values. One merged value returned for each predicted/observed value  |
| mnNow     | Merged level medians  |
| sq        | Vector of thresholds, the function has searched through to find optimum. Note, these thresholds are based on copy number transformed values |
| ansari    | The p-values for the ansari-bradley tests for each threshold in sq  |

**Note**

vecObs and vecPred must have same length and observed and predicted value for a given probe should have same position in vecObs and vedPred. The function assumes that log2-ratios are supplied

**Author(s)**

Hanni Willenbrock (<Hanni@cbs.dtu.dk>) and Jane Fridlyand (<jfridlyand@cc.ucsf.edu>)

**References**

Willenbrock H, Fridlyand J. (2005). A comparison study: applying segmentation to array CGH data for downstream analyses. Bioinformatics. 2005 Sep 14; [Epub ahead of print]

**Examples**

```
# Example data of observed and predicted log2-ratios
vecObs <- c(rep(0,40),rep(0.6,15),rep(0,10),rep(-0.4,20),rep(0,15))+rnorm(100,sd=0.2)
vecPred <- c(rep(median(vecObs[1:40]),40),rep(median(vecObs[41:55]),15),
  rep(median(vecObs[56:65]),10),rep(median(vecObs[66:85]),20),rep(median(vecObs[86:100]),15))

# Plot observed values (black) and predicted values (red)
plot(vecObs,pch=20)
points(vecPred,col="red",pch=20)

# Run merge function
merge.obj <- mergeLevels(vecObs,vecPred)

# Add merged values to plot
points(merge.obj$vecMerged,col="blue",pch=20)

# Examine optimum threshold
merge.obj$sq
```

---

plotFreqStat

*frequency plots and significance analysis*


---

**Description**

The main application of this function is to plot the frequency of changes.

**Usage**

```
plotFreqStat(aCGH.obj, resT = NULL, pheno = rep(1, ncol(aCGH.obj)),
  chrominfo = human.chrom.info.Jul03,
  X = TRUE, Y = FALSE,
  rsp.uniq = unique(pheno),
  all = length(rsp.uniq) == 1 && is.null(resT),
  titles = if (all) "All Samples" else rsp.uniq,
  cutplot = 0, thres = .25, factor = 2.5, ylm = c(-1, 1),
  p.thres = c(.01, .05, .1), numaut = 22, onepage = TRUE,
  colored = TRUE)
```

**Arguments**

|           |  |
|-----------|--|
| aCGH.obj  | Object of class aCGH   |
| resT      | Data frame having the same structure as the result of applying <code>mt.maxT</code> or <code>mt.minP</code> functions from Bioconductor's <code>multtest</code> package for multiple testing. The result is a data frame including the following 4 components: 'index', 'teststat', 'rawp' and 'adjp'.   |
| pheno     | phenotype to compare.  |
| chrominfo | Chromosomal information. Defaults to <a href="#">human.chrom.info.Jul03</a>  |
| X         | Include X chromosome? Defaults to yes.   |
| Y         | Include Y chromosome? Defaults to no.  |
| rsp.uniq  | rsp.uniq specified the codes for the groups of interest. Default is the unique levels of the phenotype. Not used when all is T.  |
| all       | all specifies whether samples should be analyzed by subgroups (T) or together (F).   |
| titles    | titles names of the groups to be used. Default is the unique levels of the pheno.  |
| cutplot   | only clones with at least cutplot frequency of gain and loss are plotted.  |
| thres     | thres is either a vector providing unique threshold for each sample or a vector of the same length as number of samples (columns in data) providing sample-specific threshold. If aCGH.obj has non-null sd.samples, then thres is automatically replaced by factor times madGenome of aCGH object. Clone is considered to be gained if it is above the threshold and lost if it below negative threshold. Used for plotting the gain/loss frequency data as well as for clone screening and for significance analysis when threshold is TRUE. Defaults to 0.25 |
| factor    | factor specifies the number by which experimental variability should be multiplied. used only when sd.samples(aCGH.obj) is not NULL or when factor is greater than 0. Defaults to 2.5  |
| ylm       | ylm vertical limits for the plot   |
| p.thres   | p.thres vector of p-value ciut-off to be plotted. computed conservatively as the threshold corresponding to a given adjusted p-value.  |
| numaut    | numaut number of the autosomes   |
| onepage   | onepage whether all plots are to be plotted on one page or different pages. When more than 2 groups are compared, we recommend multiple pages.   |
| colored   | Is plotting in color or not? Default is TRUE.  |

## Examples

```
data(colorectal)

## Use mt.maxT function from multtest package to test
## differences in group means for each clone grouped by sex
colnames(phenotype(colorectal))
sex <- phenotype(colorectal)$sex
sex.na <- !is.na(sex)
colorectal.na <- colorectal[,sex.na, keep = TRUE ]
dat <- log2.ratios.imputed(colorectal.na)
resT.sex <- mt.maxT(dat, sex[sex.na], test = "t", B = 1000)

## Plot the result along the genome
plotFreqStat(colorectal.na, resT.sex, sex[sex.na],
             titles = c("Male", "Female"))

## Adjust the p.values from previous exercise with "fdr"
## method and plot them
resT.sex.fdr <- resT.sex
resT.sex.fdr$adjp <- p.adjust(resT.sex.fdr$rawp, "fdr")
plotFreqStat(colorectal.na, resT.sex.fdr, sex[sex.na],
             titles = c("Male", "Female"))

## Derive statistics and p-values for testing the linear association of
## age with the log2 ratios of each clone along the samples

age <- phenotype(colorectal)$age
age.na <- which(!is.na(age))
age <- age[age.na]
colorectal.na <- colorectal[, age.na]
stat.age <- aCGH.test(colorectal.na, age, test = "linear.regression", p.adjust.method = "fdr")

#separate into two groups: < 70 and > 70 and plot frequencies of gain and loss
#for each clone. Note that statistic plotted corresponds to linear coefficient
#for age variable

plotFreqStat(colorectal.na, stat.age, ifelse(age < 70, 0, 1), titles =
             c("Young", "Old"), X = FALSE, Y = FALSE)
```

---

plotGenome

*Plots the genome*

---

## Description

Basic plot of the log2 ratios for each array ordered along the genome.

## Usage

```
plotGenome(aCGH.obj, samples = 1:num.samples(aCGH.obj), naut = 22,
          Y = TRUE, X = TRUE, data = log2.ratios(aCGH.obj),
          chrominfo = human.chrom.info.Jul03,
          yScale = c(-2, 2), samplenames = sample.names(aCGH.obj),
          ylb = "Log2Ratio")
```



**Arguments**

|             |  |
|-------------|--|
| aCGH.obj    | an object of class aCGH  |
| samples     | vector containing indeces of the samples to be plotted.  |
| naut        | number of autosomes in the organism  |
| Y           | TRUE if chromosome Y is to be plotted, FALSE otherwise   |
| X           | TRUE if chromosome X is to be plotted, FALSE otherwise   |
| data        | a matrix containing values to use for plotting. defaults to the log2.ratios(aCGH.obj).                             |
| chrominfo   | a chromosomal information associated with the mapping of the data.   |
| yScale      | Minimum y-scale to use for plotting. Scale is expanded if any of the values exceed the positive or negative limit. |
| samplenames | sample names.  |
| ylb         | label for the Y-axis.  |

**See Also**[aCGH](#)**Examples**

```
#plot samples in the order of descending quality
data(colorectal)
order.quality <- order(sd.samples(colorectal)$madGenome)
pdf("plotGenome.orderByQuality.pdf")
par(mfrow=c(2,1))
for(i in order.quality)
  plotGenome(colorectal, samples = i, Y = FALSE)
dev.off()
```

plotHmmStates

*Plotting the estimated hmm states and log2 ratios for each sample.***Description**

This function displays the estimated hmm states and log2 ratios for each sample.

**Usage**

```
plotHmmStates(aCGH.obj, sample.ind, chr = 1:num.chromosomes(aCGH.obj),
  statesres = hmm.merged(aCGH.obj), maxChrom = 23,
  chrominfo = human.chrom.info.Jul03, yScale = c(-2, 2),
  samplenames = sample.names(aCGH.obj))
```

**Arguments**

|             |  |
|-------------|--|
| aCGH.obj    | object of class aCGH   |
| sample.ind  | index of the sample to be plotted relative to the data matrix (i.e. column index in the file)                                |
| statesres   | matrix containing states informations. defaults to the states selected using the first model selection criterion of aCGH.obj |
| chr         | vector of chromosomes to be plotted  |
| yScale      | specified scale for Y-axis   |
| maxChrom    | highest chromosome to show   |
| chrominfo   | a chromosomal information associated with the mapping of the data  |
| samplenames | vector of sample names   |

**Details**

Each chromosome is plotted on a separate page and contains two figures. The top figure shows the observed log2ratios and the bottom figure shows predicted values for all clones but outliers which show observed values. The genomic events are indicated on both figures as following. The first clone after transition is indicated with solid blue line and the last clone after transitions is shown with dotted green line. Focal aberrations clones are colored orange, amplifications are colored red and outliers are yellow.

**Author(s)**

Jane Fridlyand

**References**

Application of Hidden Markov Models to the analysis of the array CGH data, Fridlyand et.al., *JMVA*, 2004

**See Also**

[aCGH.find.hmm.states.plotGenome](#)

**Examples**

```
data(colorectal)
plotHmmStates(colorectal, 1)
```

---

plotSummaryProfile      *plotSummaryProfile*

---

**Description**

This function display the genomic events and tests for the differences between groups if they are specified.

**Usage**

```
plotSummaryProfile(aCGH.obj,
                   response = as.factor(rep("All", ncol(aCGH.obj))),
                   titles = unique(response[!is.na(response)]),
                   X = TRUE, Y = FALSE, maxChrom = 23,
                   chrominfo = human.chrom.info.Jul03,
                   num.plots.per.page = length(titles),
                   factor = 2.5, posThres=100, negThres=-0.75)
```

**Arguments**

|                    |   |
|--------------------|---|
| aCGH.obj           | an object of aCGH class.  |
| response           | phenotype to compare. defaults to all the samples being analyzed together.  |
| titles             | titles for the groups, defaults to the name of the response   |
| X                  | logical indicating whether X needs to be shown  |
| Y                  | logical indicating whether Y needs to be shown  |
| maxChrom           | this parameter controls how many chromosomes will be plotted, from 1 to max-Chrom   |
| chrominfo          | a chromosomal information associated with the mapping of the data   |
| num.plots.per.page | number of frequency plots per page. Default is the number of groups   |
| factor             | factor specifies the number by which experimental variability should be multiples. Used only when tumor specific variability in aCGH.obj is not NULL. Defaults to 2.5 |
| posThres           | Threshold for gain. Set very high for homozygous deletion   |
| negThres           | Threshold for homozygous deletion   |

**Details**

This function utilizes output of the [find.genomic.events](#) by plotting it and testing between groups. The test are performed using kruskal-wallis rank test.

**See Also**

[aCGH find.genomic.events](#)

**Examples**

```
data(colorectal)

## Plotting summary of the sample profiles
plotSummaryProfile(colorectal)
```

---

states.hmm.func

*A function to fit unsupervised Hidden Markov model*


---

## Description

This function is a workhorse of [find.hmm.states](#). It operates on the individual chromosomes/samples and is not called directly by users.

## Usage

```
states.hmm.func(sample, chrom, dat, datainfo = clones.info, vr = 0.01,
               maxiter = 100, aic = FALSE, bic = TRUE, delta = 1,
               nlists = 1, eps = .01, print.info = FALSE,
               diag.prob = .99)
```

## Arguments

|            |  |
|------------|--|
| sample     | sample identifier  |
| chrom      | chromosome identifier  |
| dat        | dataframe with clones in the rows and samples in the columns   |
| datainfo   | dataframe containing the clones information that is used to map each clone of the array to a position on the genome. Has to contain columns with names Clone/Chrom/kb containing clone names, chromosomal assignment and kb positions respectively |
| vr         | Initial experimental variance  |
| maxiter    | Maximum number of iterations   |
| aic        | TRUE or FALSE variable indicating whether or nor AIC criterion should be used for model selection (see DETAILS)  |
| bic        | TRUE or FALSE variable indicating whether or nor BIC criterion should be used for model selection (see DETAILS)  |
| delta      | numeric vector of penalty factors to use with BIC criterion. If BIC is true, delta=1 is always calculated (see DETAILS)  |
| nlists     | defaults to 1 when aic=TRUE, otherwise > 1   |
| eps        | parameter controlling the convergence of the EM algorithm.   |
| print.info | print.info = T allows diagnostic information to be printed on the screen.  |
| diag.prob  | parameter controlling the construction of the initial transition probability matrix.   |

## See Also

[aCGH](#)

summarize.clones

*Extracting summary information for all clones***Description**

summarize.clones function is the text equivalent of [plotFreqStat](#) function - it summarizes the frequencies of changes for each clone accross tumors and when available assigns statistics. The resulting table can be easily exported.

**Usage**

```
summarize.clones(aCGH.obj, resT = NULL, pheno = rep(1, ncol(aCGH.obj)), rsp.uniq = unique(pheno), th
```

**Arguments**

|          |   |
|----------|---|
| aCGH.obj | aCGH.obj object here  |
| resT     | Data frame having the same structure as the result of applying <a href="#">mt.maxT</a> or <a href="#">mt.minP</a> functions from Bioconductor's multtest package for multiple testing. The result is a data frame including the following 4 components: 'index', 'teststat', 'rawp' and 'adjp'. Default is the unique levels of the phenotype. Not used when all is TRUE.   |
| pheno    | phenotype to compare  |
| rsp.uniq | rsp.uniq specified the codes for the groups of interest. Default is the unique levels of the phenotype. Not used when all is TRUE.  |
| thres    | thres is either a vector providing unique threshold for each sample or a vector of the same length as number of samples (columns in data) providing sample-specific threshold. If aCGH.obj has non-null sd.samples, then threshold is automatically replaced by tumor-specific sd multiplied by factor. Clone is considered to be gained if it is above the threshold and lost if it below negative threshold. Defaults to 0.25 |
| factor   | factor specifies the number by which experimental variability should be multiples. used only when tumor specific variability in aCGH.obj is not NULL. Defaults to 2.5   |
| all      | all specifies whether samples should be analyzed by subgroups (TRUE) or together (FALSE)  |
| titles   | titles names of the groups to be used. Default is the unique levels of the pheno.   |

**Value**

Returns matrix containg the following information for each clones: annotation (same as in clones.info), number and proportion of samples where clone is present,gained and lost; and the same in each group if more than one group. Additionally, if significance comparison has been done, value of the statistic, unadjusted p-value and adjusted p-values are included for each clone.

**Author(s)**

Jane Fridlyand

**See Also**

[plotFreqStat](#), [aCGH](#)

**Examples**

```
data(colorectal)
summarize.clones(colorectal)
```

---

threshold.func

*Function to indicate gain or loss for each clone for each sample*

---

**Description**

This function outputs a matrix containing gain/loss indicator for each clone and sample.

**Usage**

```
threshold.func(dat, posThres, negThres = NULL)
```

**Arguments**

|          |   |
|----------|---|
| dat      | log2ratios of the relevant array CGH object                         |
| posThres | Global or sample-specific threshold for gain                        |
| negThres | Global or sample-specific threshold for loss. Defaults to -posThres |

**Value**

Returns a matrix with a row for each clone and column for each sample. "1" indicates gain and "-1" indicates loss.

**Author(s)**

Jane Fridlyand, Ritu Roydasgupta

**Examples**

```
data(colorectal)

factor <- 3
tbl <- threshold.func(log2.ratios(colorectal), posThres=factor*(sd.samples(colorectal)$madGenome))
rownames(tbl) <- clone.names(colorectal)
colnames(tbl) <- sample.names(colorectal)
tbl[1:5, 1:5]
```

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