

Bioconductor's aCGH package

Jane Fridlyand¹ and Peter Dimitrov²

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1. Department of Epidemiology and Biostatistics, and Comprehensive Cancer Center, University of California, San Francisco, jfridlyand@cc.ucsf.edu
2. Division of Biostatistics, University of California, Berkeley, dimitrov@stat.berkeley.edu

Contents

| | | |
|----------|--|-----------|
| 1 | Overview | 1 |
| 2 | Data | 2 |
| 3 | Examples | 2 |
| 3.1 | Creating aCGH object from log2.ratios and clone info files | 2 |
| 3.2 | Filtering and imputation for objects of class aCGH | 2 |
| 3.3 | Printing, summary and basic plotting (fig. 1) for objects of class aCGH | 3 |
| 3.4 | Reading Sproc files | 5 |
| 3.5 | Basic plot for batch of aCGH Sproc files. (fig. 2) | 6 |
| 3.6 | Subsetting example | 7 |
| 3.7 | Basic plot for the ordered log2 ratios along the genome | 8 |
| 3.8 | Computing and plotting hmm states | 9 |
| 3.9 | Plotting summary of the tumor profiles | 11 |
| 3.10 | Overall frequency plot (fig. 5) | 11 |
| 3.11 | Testing association of clones with categorical, censored or continuous outcomes. | 14 |
| 3.12 | Clustering samples | 24 |
| 4 | Acknowledgements | 25 |

1 Overview

This document presents an overview of the aCGH package, which provides wide basic functions for reading, analyzing and plotting array Comparative Genomic Hybridization data (Snijders et al. (2001)). Specific example for reading data in is using output of the custom freely available programs, SPOT and SPROC (Jain et al. (2002)). These programs provide image quantification and pre-processing. Outputs of all the other image processing software need to be combined into a single file containing observed values for each clone and samples and then read in as a matrix.

2 Data

The data used in the example was generated in in lab of Dr. Fred Waldman at UCSF Comprehensive Cancer Center (Nakao et al. (2004)). Array CGH has been done on 125 colorectal fresh-frozen primary tumors and the associations with various phenotypes were analyzed. To reduce running time, only 40 samples are used in the examples.

3 Examples

3.1 Creating aCGH object from log2.ratios and clone info files

Each array CGH object has to contain the log2ratios representing relative copy number along with the mapping information including but not limited to clone name, chromosome and kb relative to the chromosome. Optionally there may be phenotypes associated with each sample.

```
> library(aCGH)
> datadir <- system.file(package = "aCGH")
> datadir <- paste(datadir, "/examples", sep="")
> clones.info <-
+   read.table(file = file.path(datadir, "clones.info.ex.txt"),
+             header = T, sep = "\t", quote="", comment.char="")
> log2.ratios <-
+   read.table(file = file.path(datadir, "log2.ratios.ex.txt"),
+             header = T, sep = "\t", quote="", comment.char="")
> pheno.type <-
+   read.table(file = file.path(datadir, "pheno.type.ex.txt"),
+             header = T, sep = "\t", quote="", comment.char="")
> ex.acgh <- create.aCGH(log2.ratios, clones.info, pheno.type)
```

Note that when working with your own data, you will need to specify absolute path to those files of the path relative to your working folder. For instance, if you are working in the folder *Project1* your data files are placed in the subfolder *Project1/Data*, then `datadir = "Data"` if you are using relative path.

3.2 Filtering and imputation for objects of class aCGH

Here we remove unmapped clones and clones mapping to Y chromosome, screen out clones missing in more than 25

```
> ex.acgh <-
+   aCGH.process(ex.acgh, chrom.remove.threshold = 23, prop.missing = .25, sample.quality.th
```

Here we impute missing observations using lowess approach. Note that occasionally, majority of the observations on chromosome Y may be missing causing imputing function to fail. Therefore, by default, the largest chromosome to be imputed is indexed as `maxChrom=23` (X). Here we specify imputation for all chromosomes ; however, in this example there are no data on chromosome Y.

```
> log2.ratios.imputed(ex.acgh) <- impute.lowess(ex.acgh, maxChrom=24)
```

```
Processing chromosome 1
Processing chromosome 2
Processing chromosome 3
Processing chromosome 4
Processing chromosome 5
Processing chromosome 6
Processing chromosome 7
Processing chromosome 8
Processing chromosome 9
Processing chromosome 10
Processing chromosome 11
Processing chromosome 12
Processing chromosome 13
Processing chromosome 14
Processing chromosome 15
Processing chromosome 16
Processing chromosome 17
Processing chromosome 18
Processing chromosome 19
Processing chromosome 20
Processing chromosome 21
Processing chromosome 22
Processing chromosome 23
```

3.3 Printing, summary and basic plotting (fig. 1) for objects of class aCGH

```
> data(colorectal)
> colorectal

aCGH object
Call: aCGH.read.Sprocs(sproclist[1:40], "human.clones.info.Jul03.csv",
  chrom.remove.threshold = 23)

Number of Arrays 40
Number of Clones 2031

> summary(colorectal)

aCGH object
Call: aCGH.read.Sprocs(sproclist[1:40], "human.clones.info.Jul03.csv",
  chrom.remove.threshold = 23)

Number of Arrays 40
Number of Clones 2031
Imputed data exist
HMM states assigned
samples standard deviations are computed
```

genomic events are assigned
phenotype exists

```
> plot(colorectal)
```

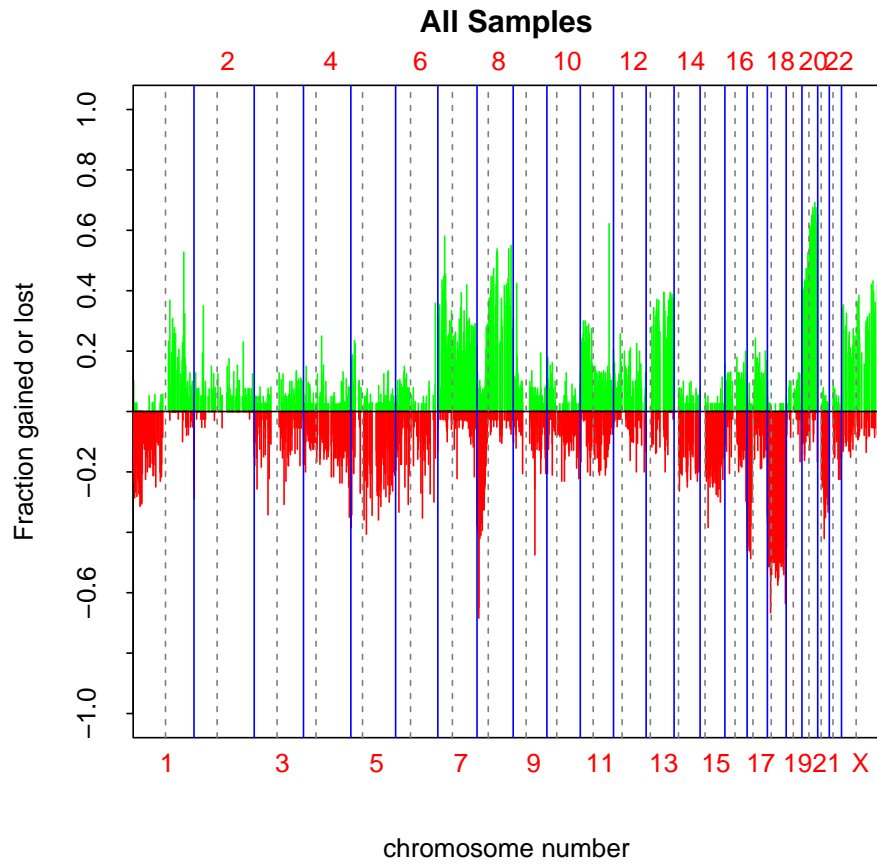


Figure 1: Basic Frequency Plot

```

> sample.names(colorectal)

[1] "sprocCR31.txt" "sprocCR40.txt" "sprocCR43.txt" "sprocCR59.txt"
[5] "sprocCR63.txt" "sprocCR73.txt" "sprocCR75.txt" "sprocCR77.txt"
[9] "sprocCR96.txt" "sprocCR98.txt" "sprocCR100.txt" "sprocCR106.txt"
[13] "sprocCR112.txt" "sprocCR122.txt" "sprocCR124.txt" "sprocCR131.txt"
[17] "sprocCR135.txt" "sprocCR137.txt" "sprocCR146.txt" "sprocCR148.txt"
[21] "sprocCR150.txt" "sprocCR154.txt" "sprocCR159.txt" "sprocCR163.txt"
[25] "sprocCR169.txt" "sprocCR178.txt" "sprocCR180.txt" "sprocCR186.txt"
[29] "sprocCR193.txt" "sprocCR200.txt" "sprocCR204.txt" "sprocCR210.txt"
[33] "sprocCR212.txt" "sprocCR217.txt" "sprocCR219.txt" "sprocCR227.txt"
[37] "sprocCR232.txt" "sprocCR244.txt" "sprocCR246.txt" "sprocCR248.txt"

> phenotype(colorectal)[1:4,]

  id age sex stage loc          hist diff gstm1 gstt1 nqo K12 K13 MTHFR ERCC1
1  31  70   0     1   0 Adenocarcinoma   1     0     1   1   1   2     2     1
2  40  71   0     1   1 Adenocarcinoma   1     1     1   1   2     2     2     2
3  43  59   1     1   0 Adenocarcinoma  NA     1     1   1   2     2     2     1
4  59  72   0     2   1 Adenocarcinoma   1     1     1   1   2     2     1    NA
  bat26 bat25 D5S346 D17S250 D2S123          mi2          LOH k12
1     0     0     0     0     0          0/1 unstable loci negative  1
2     0     0     1     1     1 >2 loci unstable, (NCI def) negative  0
3     0     0     0     0     0          0/1 unstable loci negative  0
4     0     0     0     0     0          0/1 unstable loci negative  0
  K12AA k13 K13AA M677 M1298 p16 p14 mlh1 BAT26 mlh1c          mi misum
1   GTT   0     .   1     0   1   0   1     0     0 0/1 unstable loci  0
2     .   0     .   1     0   0   0   0     0     0 >2 loci unstable  3
3     .   0     .   1     0   2   0   0     0     0 0/1 unstable loci  0
4     .   0     .   0     1   0   1   0     0     0 0/1 unstable loci  0

  CGHSTAT
1 Complete
2 Complete
3 Complete
4 Not Done

```

3.4 Reading Sproc files

Here we demonstrate reading of the sproc files and combining them into one array CGH object. Sproc file format is specific to the custom SPROC processing software at UCSF Cancer Center.

```

> 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)

```

```
Trying to read /tmp/Rtmp2p1eEX/Rinst1e7b3b29e8a2e8/aCGH/examples/sprocCR40.txt
Trying to read /tmp/Rtmp2p1eEX/Rinst1e7b3b29e8a2e8/aCGH/examples/sprocCR43.txt
```

Averaging duplicated clones

| | |
|-----------------|-----------|
| CTB-102E19 | 692 693 |
| CTB-112F7 | 1692 1693 |
| CTB-142024 | 1640 1641 |
| CTB-339E12 | 1633 1634 |
| CTB-36F16 | 1220 1221 |
| DMPC-HFF#1-61H8 | 1662 1663 |
| GS1-20208 | 662 663 |
| RP1-97B16 | 256 257 |
| RP11-119J20 | 409 410 |
| RP11-13C20 | 153 154 |
| RP11-149G12 | 815 816 |
| RP11-172D2 | 825 826 |
| RP11-175H20 | 821 822 |
| RP11-176L22 | 183 184 |
| RP11-188C10 | 817 818 |
| RP11-1L22 | 147 148 |
| RP11-204M16 | 785 786 |
| RP11-238H10 | 850 851 |
| RP11-23G2 | 176 177 |
| RP11-247E23 | 178 179 |
| RP11-268N2 | 813 814 |
| RP11-30M1 | 166 167 |
| RP11-39A8 | 158 159 |
| RP11-47E6 | 170 171 |
| RP11-72C6 | 1006 1007 |
| RP11-83014 | 819 820 |
| RP11-94M13 | 873 874 |

```
> ex.acgh
```

aCGH object

```
Call: aCGH.read.Sprocs(dir(path = datadir, pattern = "sproc", full.names = TRUE),
  latest.mapping.file, chrom.remove.threshold = 23)
```

Number of Arrays 2

Number of Clones 1952

3.5 Basic plot for batch of aCGH Sproc files. (fig. 2)

```
> plot(ex.acgh)
```

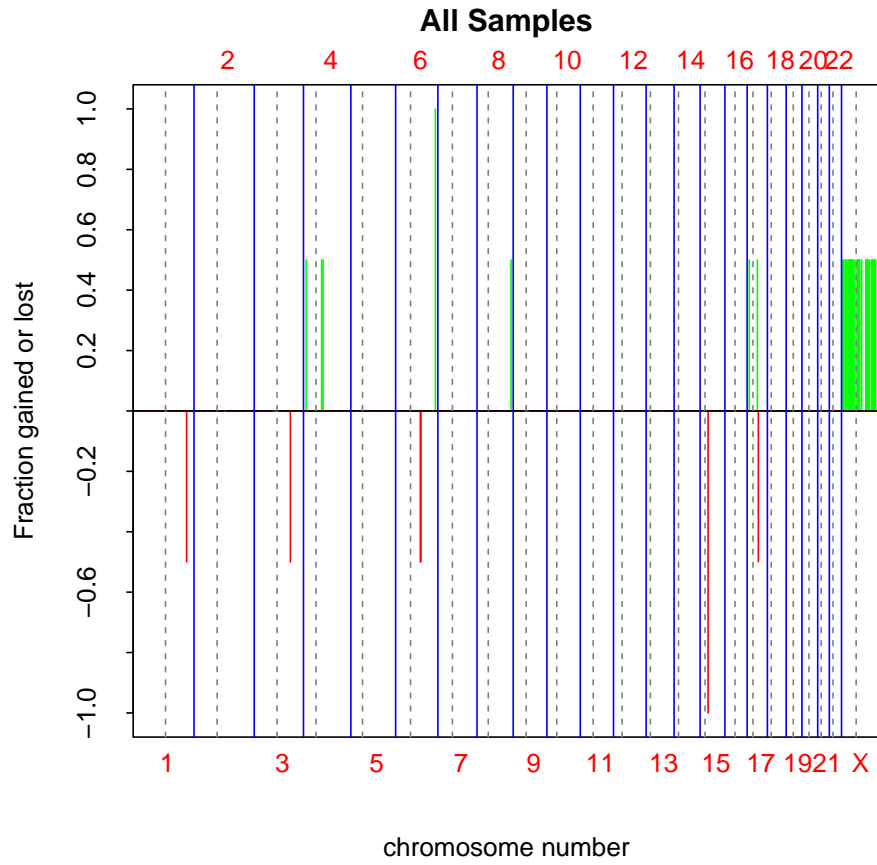


Figure 2: Basic plot for batch of aCGH Sproc files

3.6 Subsetting example

```
> cr <- colorectal[,1:3]
```

3.7 Basic plot for the ordered log2 ratios along the genome

The relative copy number is plotted along the genome with clones placed in the genomic order. We are plotting sample 2 here. (fig. 3). Chromosome Y is explicitly excluded.

```
> plotGenome(ex.acgh, samples=2, Y = FALSE)
```

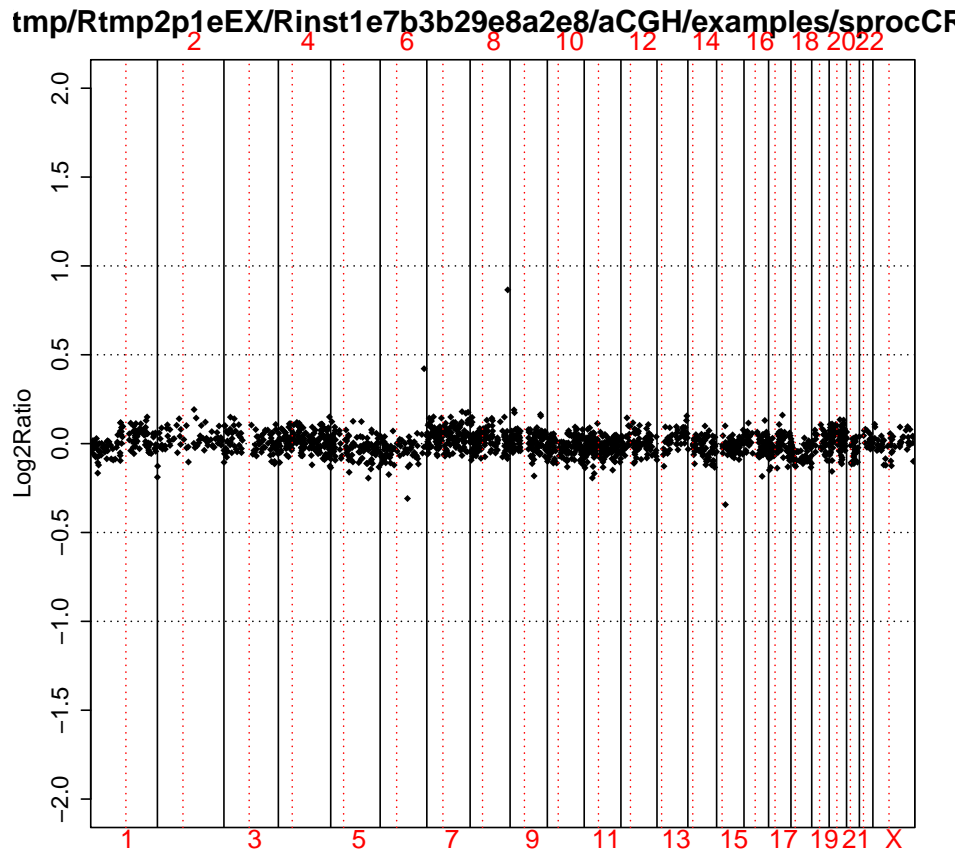


Figure 3: Basic plot for the ordered log2 ratios along the genome

3.8 Computing and plotting hmm states

Unsupervised hidden markov model is repeatedly fitted to each chromosome for varying number of states (2, ..., 5). The number of states is determined after all fits are done using model selection criterion such as AIC, BIC or delta-BIC. The model with minimal penalized negative log-likelihood is chosen for each selection criterion. Note, that some of the model fits are going to fail and are not going to be used in the final selection. Meanwhile, error message warning of the model fit failing will be printed during hmm runs. The user should ignore those particular messages and related warnings.

For a given sample, each chromosome is plotted on a separate page along with its smoothed values (fig. 4). The genomic events such as transitions, focal aberrations and amplifications are indicated. The outliers are also marked.

```
> ## Determining hmm states of the clones. In the interest of time,  
> ##we have commented this step out and used pre-computed results.  
>  
> ##hmm(ex.acgh) <- find.hmm.states(ex.acgh)  
> hmm(ex.acgh) <- ex.acgh.hmm  
> ## Merging hmm states  
>  
> hmm.merged(ex.acgh) <-  
+   mergeHmmStates(ex.acgh, model.use = 1, minDiff = .25)  
> ## Calculating the standard deviations for each array. Standard error is  
> ##calculated for each region and then averaged across regions. The final  
> ##SDs for each samples are contained in sd.samples(exa.acgh)$madGenome.  
>  
> sd.samples(ex.acgh) <- computeSD.Samples(ex.acgh)  
> ## Finding the genomic events associated with each sample using  
> ##results of the partitioning into the states.  
>  
> genomic.events(ex.acgh) <- find.genomic.events(ex.acgh)
```

Finding outliers

Finding focal low level aberrations

Finding transitions

Finding focal amplifications

Processing chromosome 1

Processing chromosome 2

Processing chromosome 3

Processing chromosome 4

Processing chromosome 5

Processing chromosome 6

Processing chromosome 7

Processing chromosome 8

Processing chromosome 9

Processing chromosome 10

Processing chromosome 11

Processing chromosome 12

```
Processing chromosome 13
Processing chromosome 14
Processing chromosome 15
Processing chromosome 16
Processing chromosome 17
Processing chromosome 18
Processing chromosome 19
Processing chromosome 20
Processing chromosome 21
Processing chromosome 22
Processing chromosome 23
```

```
>
> ## Plotting and printing the hmm states either to the screen or into the
> ##postscript file. Each chromosome for each sample is plotted on a separate
> ##page
>
> ##postscript("hmm.states.temp.ps");plotHmStates(ex.acgh, sample.ind=1);dev.off()
```

```
> plotHmmStates(colorectal, sample.ind = 1, chr = 1)
```

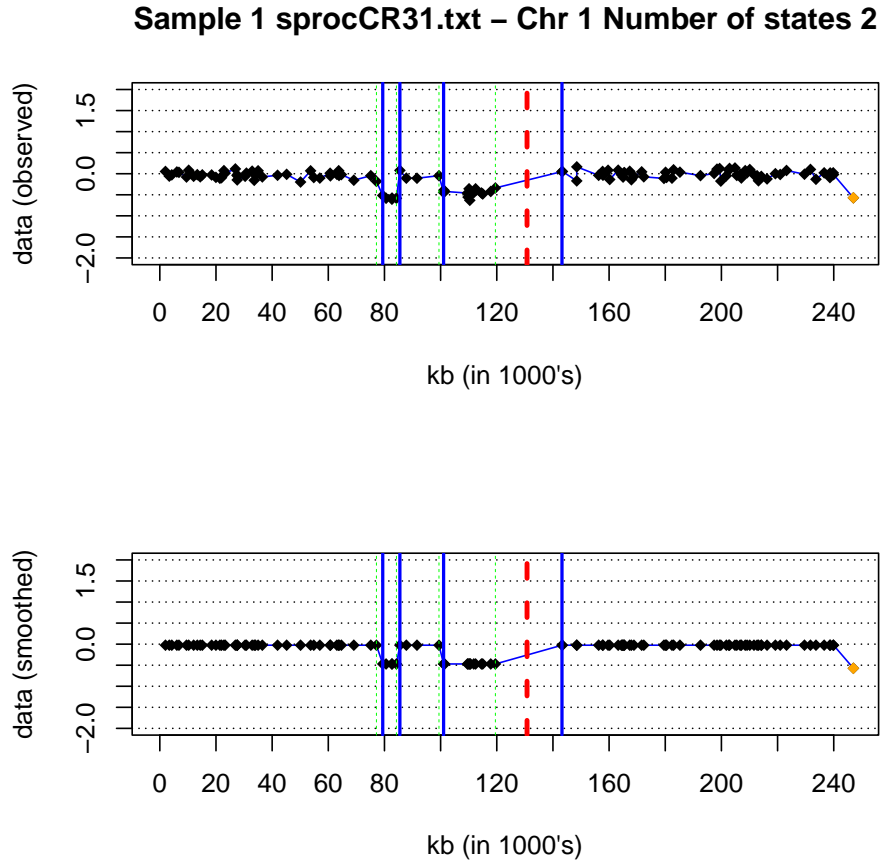


Figure 4: Plotting the hmm states found for colorectal data set.

3.9 Plotting summary of the tumor profiles

Here the distribution of various genomic events as well as their frequency by location is displayed. Run the function `plotSummaryProfile(colorectal)` which produces multi-page figure. Necessary to write out as ps or pdf files.

3.10 Overall frequency plot (fig. 5)

```
> plotFreqStat(colorectal, all = T)
```

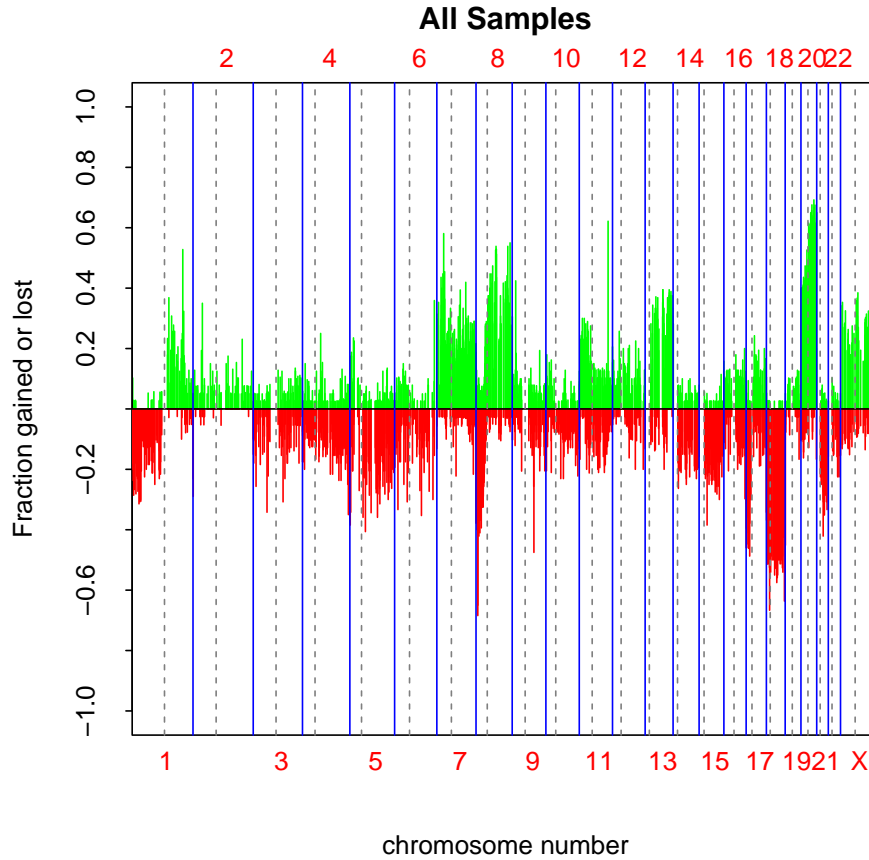


Figure 5: Overall frequency plot of the tumor profiles

summarize.clones() function is the text equivalent of plotFreqStat() - it summarizes the frequencies of changes for each clone across tumors and includes results of statistical comparisons for each clone when available.

```
> summarize.clones(colorectal)[1:10 ,]
```

| | Clone | Target | Chrom | kb | NumPresent.All | NumGain.All |
|---|------------|------------------|-------|-------|----------------|-------------|
| 2 | RP11-82D16 | HumArray2H11_C9 | 1 | 2009 | 39 | 4 |
| 3 | RP11-62M23 | HumArray2H10_N30 | 1 | 3368 | 35 | 1 |
| 4 | RP11-11105 | HumArray2H10_B18 | 1 | 4262 | 38 | 1 |
| 5 | RP11-51B4 | HumArray2H10_Q30 | 1 | 6069 | 35 | 0 |
| 6 | RP11-60J11 | HumArray2H10_T30 | 1 | 6817 | 36 | 1 |
| 7 | RP11-813J5 | HumArray2H10_B19 | 1 | 9498 | 30 | 0 |
| 8 | RP11-19901 | HumArray2H10_W30 | 1 | 10284 | 39 | 1 |
| 9 | RP11-188F7 | HumArray2H9_C14 | 1 | 12042 | 36 | 1 |

| | | | | | | |
|----|-------------|-----------------|-----------------|--------------|--------------|---|
| 10 | RP11-178M15 | HumArray2H9_F14 | 1 | 13349 | 35 | 1 |
| 11 | RP11-219F4 | HumArray2H9_I14 | 1 | 14391 | 39 | 1 |
| | | NumLost.All | PropPresent.All | PropGain.All | PropLost.All | |
| 2 | | 7 | 0.98 | 0.10 | 0.18 | |
| 3 | | 7 | 0.88 | 0.03 | 0.20 | |
| 4 | | 9 | 0.95 | 0.03 | 0.24 | |
| 5 | | 10 | 0.88 | 0.00 | 0.29 | |
| 6 | | 7 | 0.90 | 0.03 | 0.19 | |
| 7 | | 8 | 0.75 | 0.00 | 0.27 | |
| 8 | | 5 | 0.98 | 0.03 | 0.13 | |
| 9 | | 4 | 0.90 | 0.03 | 0.11 | |
| 10 | | 4 | 0.88 | 0.03 | 0.11 | |
| 11 | | 7 | 0.98 | 0.03 | 0.18 | |

threshold.func() function gives the clone by sample matrix of gains and losses. "1" indicates gain and "-1" indicates loss.

```
> 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]
```

| | sprocCR31.txt | sprocCR40.txt | sprocCR43.txt | sprocCR59.txt |
|------------|---------------|---------------|---------------|---------------|
| RP11-82D16 | 0 | 0 | 0 | -1 |
| RP11-62M23 | 0 | 0 | 0 | -1 |
| RP11-11105 | 0 | 0 | 0 | -1 |
| RP11-51B4 | 0 | NA | 0 | -1 |
| RP11-60J11 | 0 | 0 | 0 | -1 |
| | sprocCR63.txt | | | |
| RP11-82D16 | 1 | | | |
| RP11-62M23 | 0 | | | |
| RP11-11105 | 1 | | | |
| RP11-51B4 | 0 | | | |
| RP11-60J11 | 0 | | | |

fga.func() function gives the fraction of genome altered for each sample.

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

| | gainP | lossP |
|------|-------------|-------------|
| [1,] | 0.220098155 | 0.184029096 |
| [2,] | 0.025559893 | 0.004990002 |
| [3,] | 0.006184865 | 0.002350805 |
| [4,] | 0.107402285 | 0.148058176 |
| [5,] | 0.143115647 | 0.137430523 |

3.11 Testing association of clones with categorical, censored or continuous outcomes.

Use `mt.maxT` function from `multtest` package to test differences in group means for each clone grouped by sex. Plot the result along the genome displaying the frequencies of gains and losses as well as height of the statistic corresponding to each clone (figs. 6 and 7.). The p-value can be adjusted and the horizontal lines indicate chosen level of significance.

```
> colnames(phenotype(colorectal))
```

```
[1] "id"      "age"      "sex"      "stage"    "loc"      "hist"     "diff"
[8] "gstm1"   "gstt1"    "nqo"      "K12"      "K13"      "MTHFR"    "ERCC1"
[15] "bat26"   "bat25"    "D5S346"   "D17S250" "D2S123"   "mi2"      "LOH"
[22] "k12"     "K12AA"    "k13"      "K13AA"    "M677"     "M1298"    "p16"
[29] "p14"     "mlh1"     "BAT26"    "mlh1c"    "mi"       "misum"    "CGHSTAT"
```

```
> sex <- phenotype(colorectal)$sex
```

```
> sex.na <- !is.na(sex)
```

```
> index.clones.use <- which(clones.info(colorectal)$Chrom < 23)
```

```
> colorectal.na <- colorectal[ index.clones.use, sex.na , keep=TRUE]
```

```
> dat <- log2.ratios.imputed(colorectal.na)
```

```
> resT.sex <- mt.maxT(dat, sex[sex.na], test = "t.equalvar", B = 1000)
```

| | | | | | | | | | |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| b=10 | b=20 | b=30 | b=40 | b=50 | b=60 | b=70 | b=80 | b=90 | b=100 |
| b=110 | b=120 | b=130 | b=140 | b=150 | b=160 | b=170 | b=180 | b=190 | b=200 |
| b=210 | b=220 | b=230 | b=240 | b=250 | b=260 | b=270 | b=280 | b=290 | b=300 |
| b=310 | b=320 | b=330 | b=340 | b=350 | b=360 | b=370 | b=380 | b=390 | b=400 |
| b=410 | b=420 | b=430 | b=440 | b=450 | b=460 | b=470 | b=480 | b=490 | b=500 |
| b=510 | b=520 | b=530 | b=540 | b=550 | b=560 | b=570 | b=580 | b=590 | b=600 |
| b=610 | b=620 | b=630 | b=640 | b=650 | b=660 | b=670 | b=680 | b=690 | b=700 |
| b=710 | b=720 | b=730 | b=740 | b=750 | b=760 | b=770 | b=780 | b=790 | b=800 |
| b=810 | b=820 | b=830 | b=840 | b=850 | b=860 | b=870 | b=880 | b=890 | b=900 |
| b=910 | b=920 | b=930 | b=940 | b=950 | b=960 | b=970 | b=980 | b=990 | b=1000 |

```
> plotFreqStat(colorectal.na, resT.sex, sex[sex.na], factor=3, titles =  
+ c("Female", "Male"), X = FALSE, Y = FALSE)
```

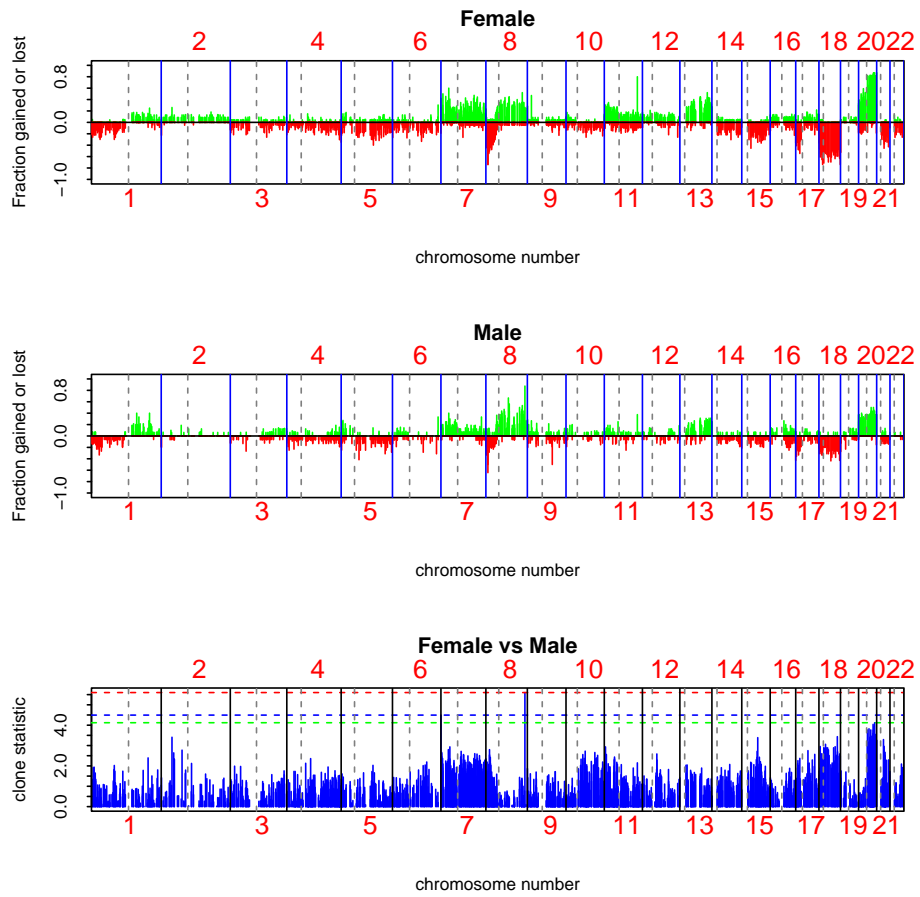


Figure 6: Frequency plots of the samples with respect to the sex groups

```

> plotSummaryProfile(colorectal, response = sex,
+                   titles = c("Female", "Male"),
+                   X = FALSE, Y = FALSE, maxChrom = 22)

```

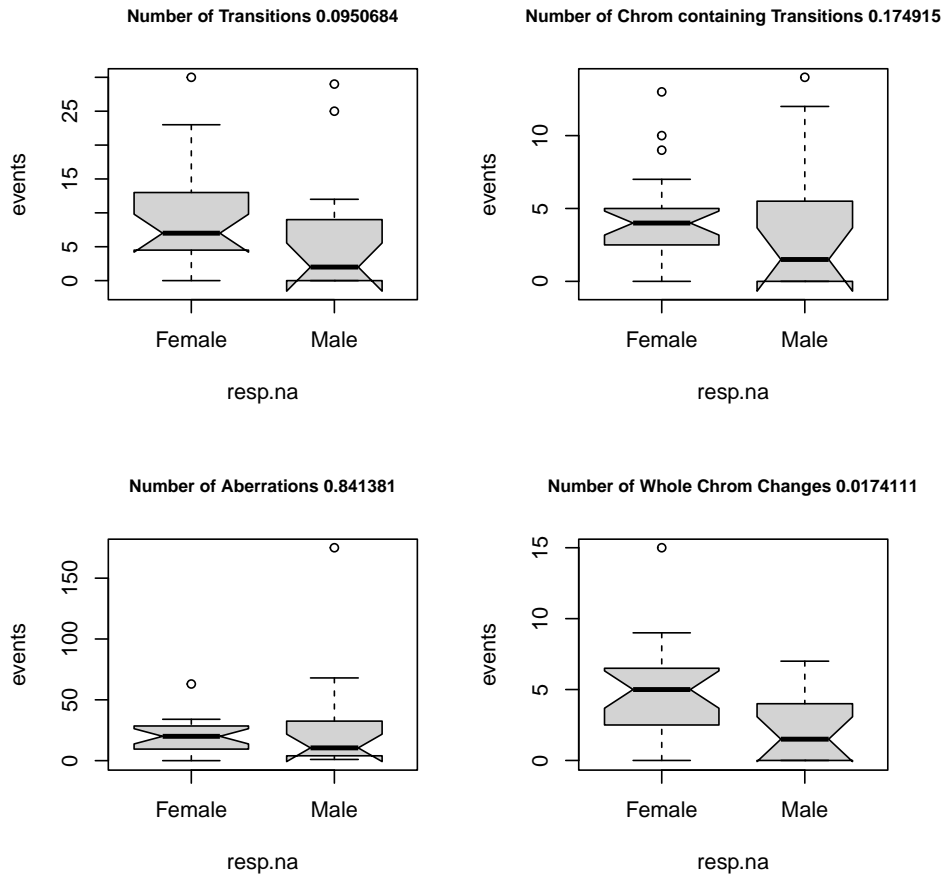


Figure 7: Plotting summary of the tumor profiles

Testing association of clones with categorical outcome for autosomal clones that are gained or lost in at least 10% of the samples. Note that the same dataset should be provided for creating *resT* object and for plotting. Pay attention that HMM-related objects including sample variability do not get subsetted at the moment. Note that currently two-stage subsetting does not work for HMM slots, i.e. two conditions (change and autosomal) need to be done in one iteration.

```
> factor <- 3
> minChanged <- 0.1
> gainloss <- gainLoss(log2.ratios(colorectal)[,sex.na], cols=1:length(which(sex.na)), thres=(
> ind.clones.use <- which(gainloss$gainP >= minChanged | gainloss$lossP >= minChanged & clones.
> colorectal.na <- colorectal[ind.clones.use,sex.na, keep=TRUE]
> dat <- log2.ratios.imputed(colorectal.na)
> resT.sex <- mt.maxT(dat, sex[sex.na], test = "t.equalvar", B = 1000)
```

| b=10 | b=20 | b=30 | b=40 | b=50 | b=60 | b=70 | b=80 |
|-------|-------|-------|-------|-------|-------|-------|-------|
| b=110 | b=120 | b=130 | b=140 | b=150 | b=160 | b=170 | b=180 |
| b=210 | b=220 | b=230 | b=240 | b=250 | b=260 | b=270 | b=280 |
| b=310 | b=320 | b=330 | b=340 | b=350 | b=360 | b=370 | b=380 |
| b=410 | b=420 | b=430 | b=440 | b=450 | b=460 | b=470 | b=480 |
| b=510 | b=520 | b=530 | b=540 | b=550 | b=560 | b=570 | b=580 |
| b=610 | b=620 | b=630 | b=640 | b=650 | b=660 | b=670 | b=680 |
| b=710 | b=720 | b=730 | b=740 | b=750 | b=760 | b=770 | b=780 |
| b=810 | b=820 | b=830 | b=840 | b=850 | b=860 | b=870 | b=880 |
| b=910 | b=920 | b=930 | b=940 | b=950 | b=960 | b=970 | b=980 |

```
>
```

```
> plotFreqStat(colorectal.na, resT.sex, sex[sex.na], factor=factor, titles = c("Male", "Female"))
```

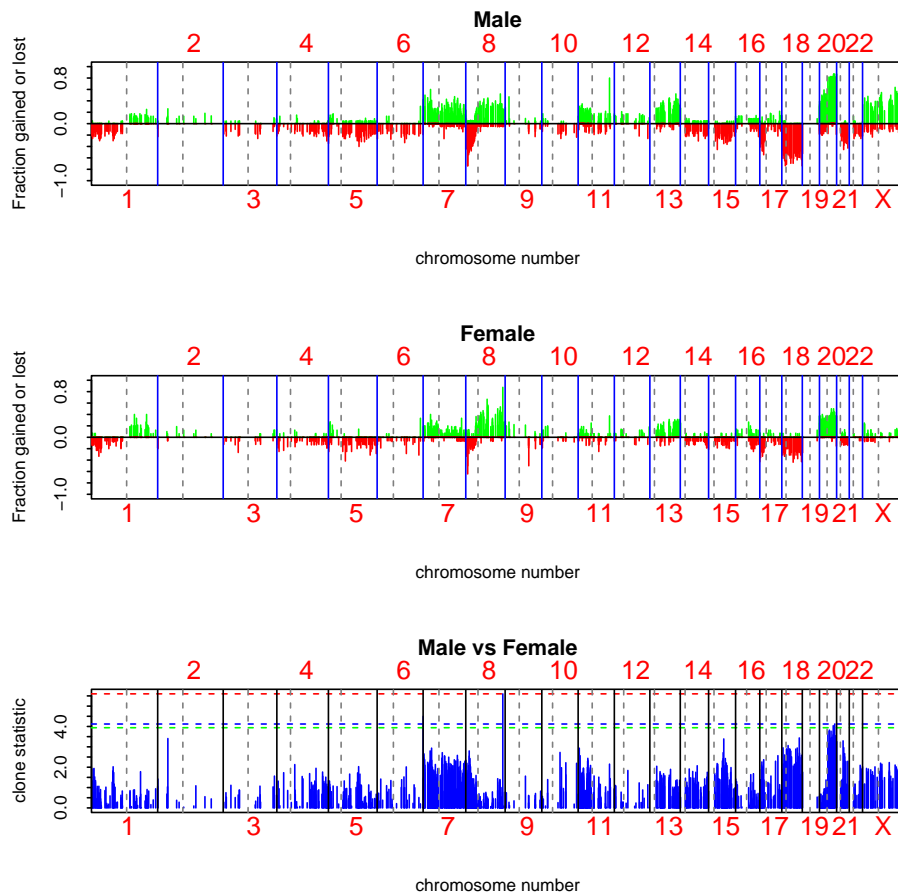


Figure 8: Frequency plots of the samples with respect to the sex groups for clones gained or lost in at least 10% of the samples

Testing association of clones with censored outcomes. Since there was no survival data available, we simulate data for a simple example to demonstrate creation and usage of basic survival object. We create an object equivalent to `resT` object that was created earlier. In the figure the samples are separated into dead and alive/censored groups for ease of visualization. Nevertheless, statistic is computed and assessed for significance using proper survival object.

```
> time <- rexp(ncol(colorectal), rate = 1 / 12)
> events <- rbinom(ncol(colorectal), size = 1, prob = .5)
> surv.obj <- Surv(time, events)
> surv.obj

 [1] 74.02083331+  5.95306477+  8.68961544+ 11.88257949+  1.43506355
 [6]  6.70363768  3.25655403+  0.95942133+  3.31130973+ 10.64956213
[11]  3.46683766  8.20443705+  3.71331033  3.90712540+ 15.95365280+
[16]  2.89520468 16.45853287+  1.64353515+  9.38366490+  7.84946258
[21] 14.38251745+  7.30763362 54.14313457  4.43769598 10.93644873
[26]  0.05562599+ 17.05728350 16.44047923+  8.90169448+  8.76937027+
[31] 27.12010834  9.15936517 12.88647894  5.07142863  2.19405773+
[36]  6.36397484  1.16908230  3.04071650+ 16.70654176  1.84300923

> stat.coxph <-
+ aCGH.test(colorectal, surv.obj, test = "coxph",
+           p.adjust.method = "fdr")
> stat.coxph[1:10 ,]

      index teststat      rawp      adjp
1765 1765 -3.109330 0.001875119 0.8248326
1764 1764 -2.984303 0.002842248 0.8248326
1763 1763 -2.786785 0.005323373 0.8248326
1518 1518 -2.759745 0.005784648 0.8248326
1988 1988  2.635521 0.008400827 0.8248326
137  137 -2.600083 0.009320117 0.8248326
126  126 -2.572051 0.010109794 0.8248326
1366 1366 -2.568643 0.010209752 0.8248326
1550 1550 -2.525102 0.011566471 0.8248326
1766 1766 -2.521789 0.011675975 0.8248326
```

```
> plotFreqStat(colorectal, stat.coxph, events, titles =
+             c("Survived/Censored", "Dead"), X = FALSE, Y = FALSE)
```

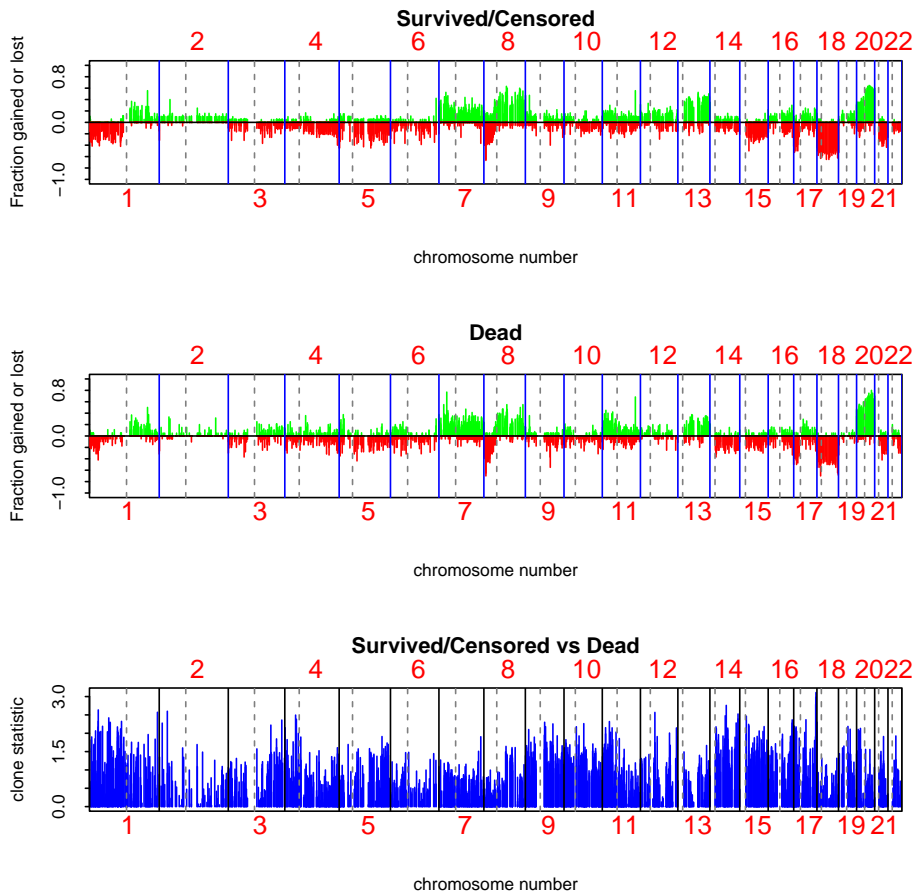


Figure 9: Frequency plots of the samples with respect to survival.

Deriving statistics and p-values for testing the linear association of age with the log2 ratios of each clone along the tumors. Here we repeat above two examples but using significance of linear regression coefficient as a measure of association between genomic variable and continuous outcome.

```
> 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")
> stat.age[1:10 ,]
```

| index | teststat | rawp | adjp |
|-------|----------|------|------|
|-------|----------|------|------|

```

1735 1735 3.259187 0.002399741 0.9952687
1739 1739 3.184326 0.002941084 0.9952687
685 685 -3.158061 0.003157117 0.9952687
1251 1251 3.144471 0.003274723 0.9952687
1718 1718 3.118281 0.003513183 0.9952687
1714 1714 3.112281 0.003570080 0.9952687
642 642 -3.082287 0.003867826 0.9952687
639 639 -3.012157 0.004658116 0.9952687
643 643 -2.937882 0.005659632 0.9952687
1744 1744 2.881404 0.006552898 0.9952687

```

```

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

```

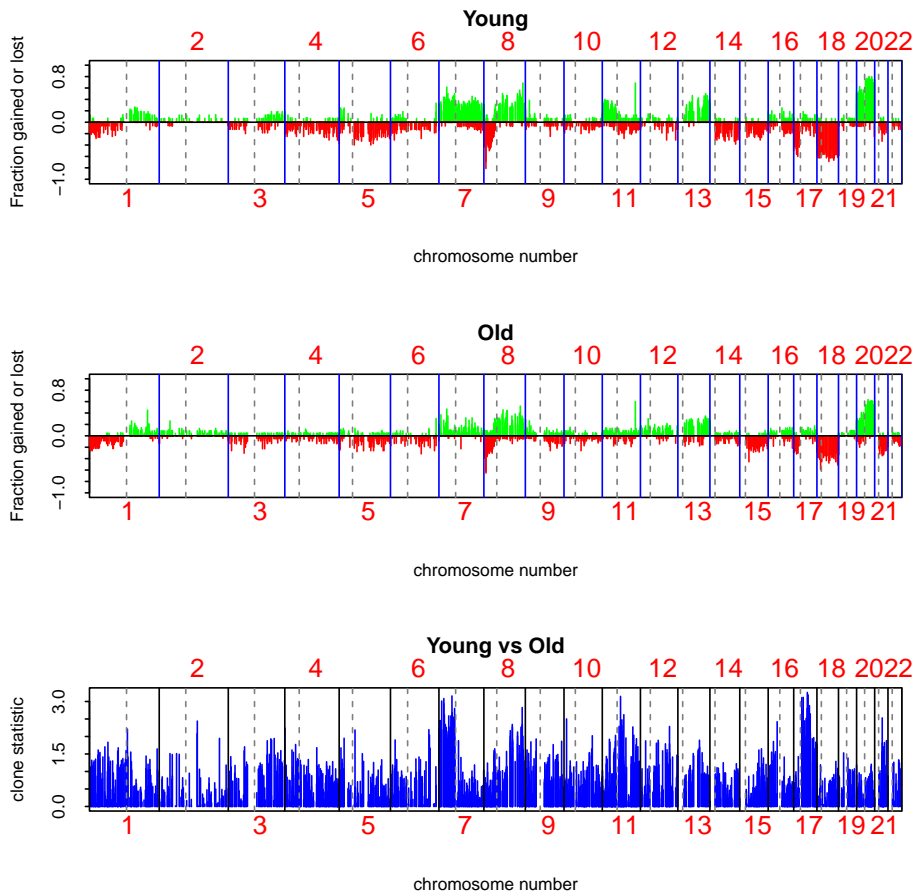


Figure 10: Frequency plots of the samples with respect to age.

Here we show example of how to create a table of results which can be later exported into other programs via *write.table*. First, Males vs Females:

```

> sex <- phenotype(colorectal)$sex

```

```

> sex.na <- !is.na(sex)
> index.clones.use <- which(clones.info(colorectal.na)$Chrom < 23)
> colorectal.na <- colorectal[ index.clones.use,sex.na , keep=TRUE]
> dat <- log2.ratios.imputed(colorectal.na)
> resT.sex <- mt.maxT(dat, sex[sex.na], test = "t.equalvar", B = 1000)

```

```

b=10      b=20      b=30      b=40      b=50      b=60      b=70      b=80
b=110     b=120     b=130     b=140     b=150     b=160     b=170     b=180
b=210     b=220     b=230     b=240     b=250     b=260     b=270     b=280
b=310     b=320     b=330     b=340     b=350     b=360     b=370     b=380
b=410     b=420     b=430     b=440     b=450     b=460     b=470     b=480
b=510     b=520     b=530     b=540     b=550     b=560     b=570     b=580
b=610     b=620     b=630     b=640     b=650     b=660     b=670     b=680
b=710     b=720     b=730     b=740     b=750     b=760     b=770     b=780
b=810     b=820     b=830     b=840     b=850     b=860     b=870     b=880
b=910     b=920     b=930     b=940     b=950     b=960     b=970     b=980

```

```

> sex.tbl <- summarize.clones(colorectal.na, resT.sex, sex[sex.na], titles = c("Male", "Female"))
> sex.tbl[1:5,]

```

| Clone | Target | Chrom | kb | NumPresent.All | NumGain.All | NumLost.All |
|--------------|------------------|-------|------|----------------|-------------|-------------|
| 2 RP11-82D16 | HumArray2H11_C9 | 1 | 2009 | 38 | 4 | 7 |
| 3 RP11-62M23 | HumArray2H10_N30 | 1 | 3368 | 34 | 1 | 7 |
| 4 RP11-11105 | HumArray2H10_B18 | 1 | 4262 | 37 | 1 | 9 |
| 5 RP11-51B4 | HumArray2H10_Q30 | 1 | 6069 | 34 | 0 | 10 |
| 6 RP11-60J11 | HumArray2H10_T30 | 1 | 6817 | 35 | 1 | 7 |

| | PropPresent.All | PropGain.All | PropLost.All | NumPresent.Male | NumGain.Male |
|---|-----------------|--------------|--------------|-----------------|--------------|
| 2 | 0.97 | 0.11 | 0.18 | 23 | 1 |
| 3 | 0.87 | 0.03 | 0.21 | 20 | 1 |
| 4 | 0.95 | 0.03 | 0.24 | 23 | 0 |
| 5 | 0.87 | 0.00 | 0.29 | 19 | 0 |
| 6 | 0.90 | 0.03 | 0.20 | 20 | 0 |

| | NumLost.Male | PropPresent.Male | PropGain.Male | PropLost.Male | NumPresent.Female |
|---|--------------|------------------|---------------|---------------|-------------------|
| 2 | 5 | 1.00 | 0.04 | 0.22 | 15 |
| 3 | 5 | 0.87 | 0.05 | 0.25 | 14 |
| 4 | 7 | 1.00 | 0.00 | 0.30 | 14 |
| 5 | 7 | 0.83 | 0.00 | 0.37 | 15 |
| 6 | 4 | 0.87 | 0.00 | 0.20 | 15 |

| | NumGain.Female | NumLost.Female | PropPresent.Female | PropGain.Female |
|---|----------------|----------------|--------------------|-----------------|
| 2 | 3 | 2 | 0.94 | 0.20 |
| 3 | 0 | 2 | 0.88 | 0.00 |
| 4 | 1 | 2 | 0.88 | 0.07 |
| 5 | 0 | 3 | 0.94 | 0.00 |
| 6 | 1 | 3 | 0.94 | 0.07 |

| | PropLost.Female | stat | rawp | adjp |
|---|-----------------|-----------|-------|------|
| 2 | 0.13 | 1.3456684 | 0.185 | 1 |
| 3 | 0.14 | 1.2966513 | 0.214 | 1 |

| | | | | |
|---|------|-----------|-------|---|
| 4 | 0.14 | 0.7545065 | 0.445 | 1 |
| 5 | 0.20 | 1.9207531 | 0.066 | 1 |
| 6 | 0.20 | 0.5052960 | 0.640 | 1 |

3.12 Clustering samples

Here we cluster samples while displaying phenotypes as well as within phenotypes using chromosomes 4, 8 and 9 and display the phenotype labels, in this case, sex. We also indicate high level amplifications and 2-copy deletions with yellow and blue colors. (fig. 11).

4 Acknowledgements

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```

> par(mfrow=c(2,1))
> clusterGenome(colorectal.na, response = sex[sex.na],
+               titles = c("Female", "Male"),
+               byclass = FALSE, showaber = TRUE, vecchrom = c(4,8,9),
+               dendPlot = FALSE, imp = FALSE)
> clusterGenome(colorectal.na, response = sex[sex.na],
+               titles = c("Female", "Male"),
+               byclass = TRUE, showaber = TRUE, vecchrom = c(4,8,9),
+               dendPlot = FALSE, imp = FALSE)
>

```

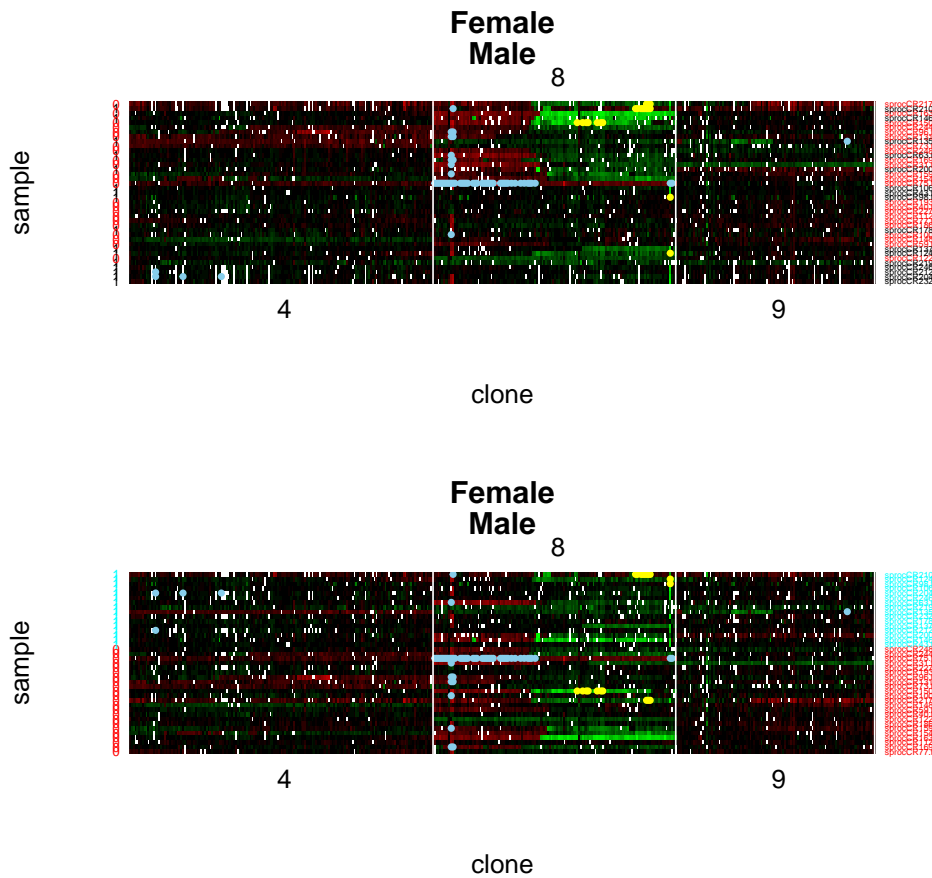


Figure 11: Clustering of the samples by sex