

Bioconductor's aCGH package

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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)
```

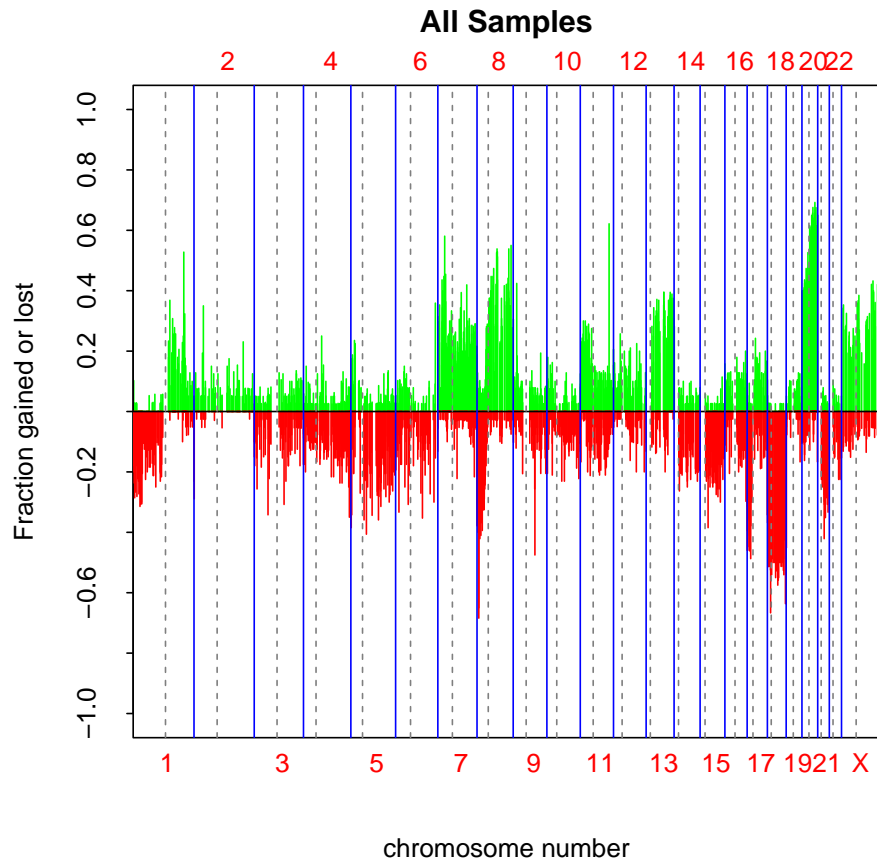


Figure 1: Basic Frequency Plot

```

> 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/RtmpTgwcQE/Rinst37f1fcb3594/aCGH/examples/sprocCR40.txt
Trying to read /tmp/RtmpTgwcQE/Rinst37f1fcb3594/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)
```

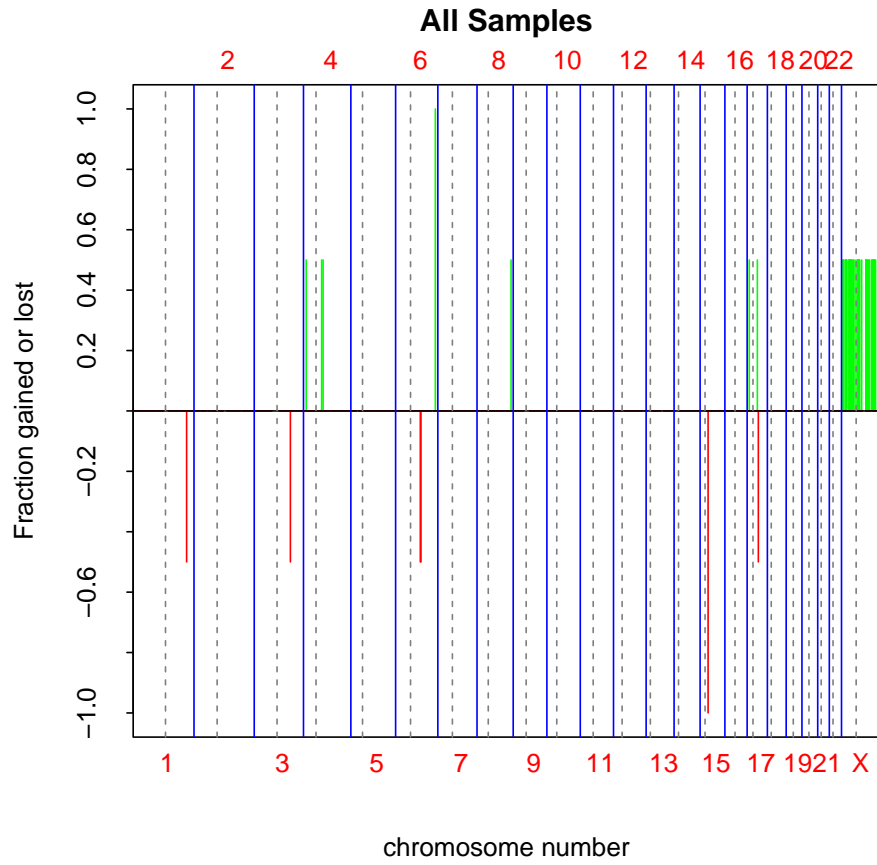


Figure 2: Basic plot for batch of aCGH Sproc files

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)
```

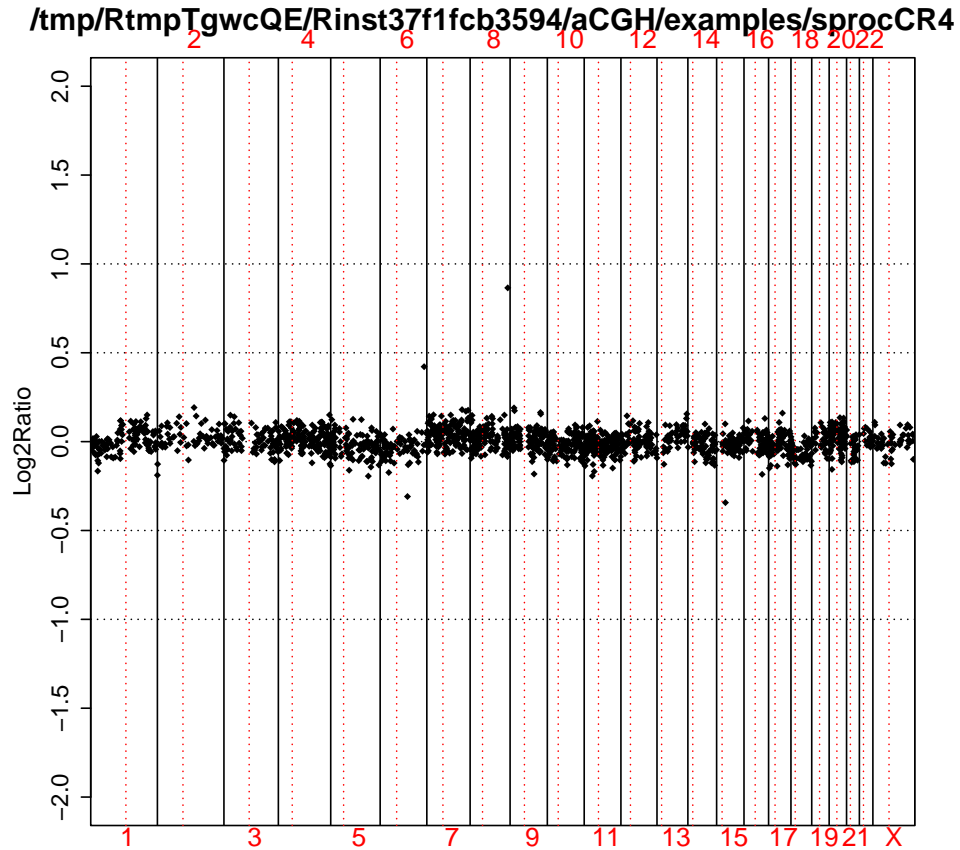


Figure 3: Basic plot for the ordered log2 ratios along the genome

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)
```

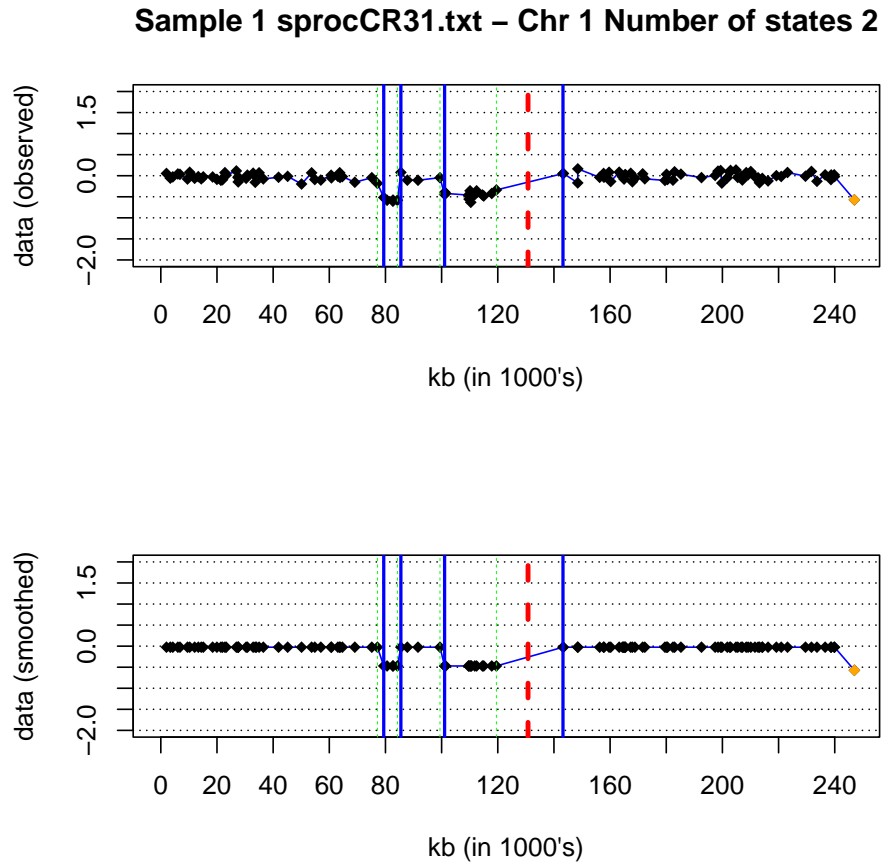


Figure 4: Plotting the hmm states found for colorectal data set.

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)
```

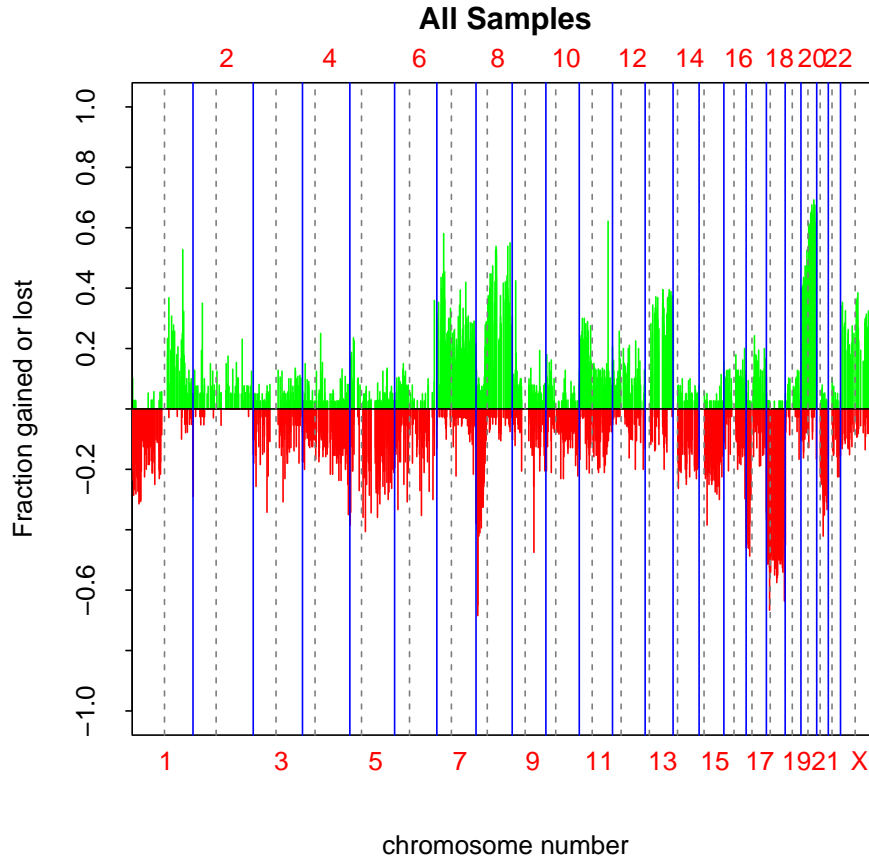


Figure 5: Overall frequency plot of the tumor profiles

`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)

```

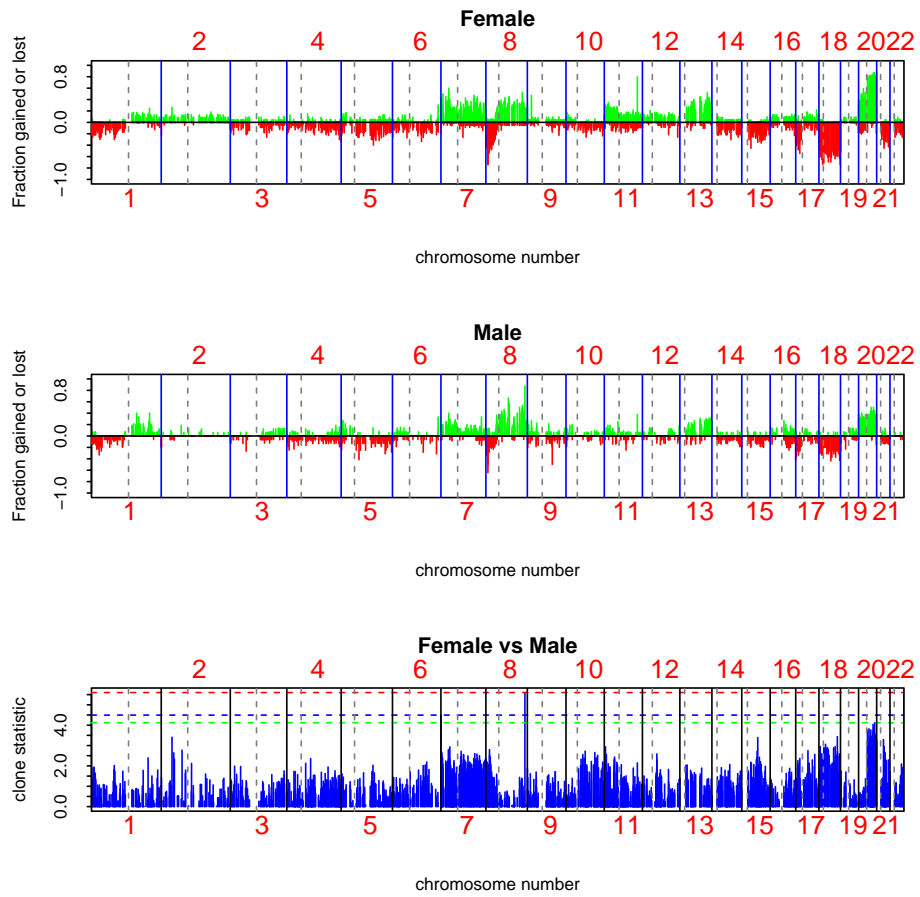


Figure 6: Frequency plots of the samples with respect to the sex groups

```

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

```

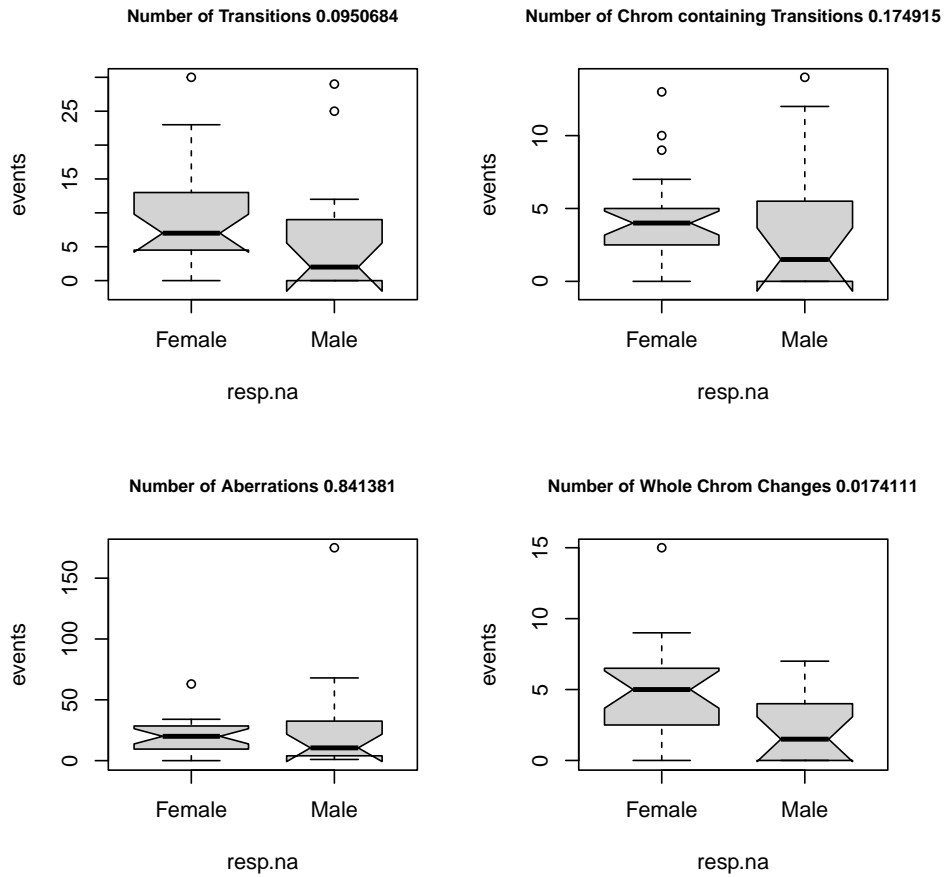


Figure 7: Plotting summary of the tumor profiles

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=190
b=210	b=220	b=230	b=240	b=250	b=260	b=270	b=280	b=290
b=310	b=320	b=330	b=340	b=350	b=360	b=370	b=380	b=390
b=410	b=420	b=430	b=440	b=450	b=460	b=470	b=480	b=490
b=510	b=520	b=530	b=540	b=550	b=560	b=570	b=580	b=590
b=610	b=620	b=630	b=640	b=650	b=660	b=670	b=680	b=690
b=710	b=720	b=730	b=740	b=750	b=760	b=770	b=780	b=790
b=810	b=820	b=830	b=840	b=850	b=860	b=870	b=880	b=890
b=910	b=920	b=930	b=940	b=950	b=960	b=970	b=980	b=990

```
>
```

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

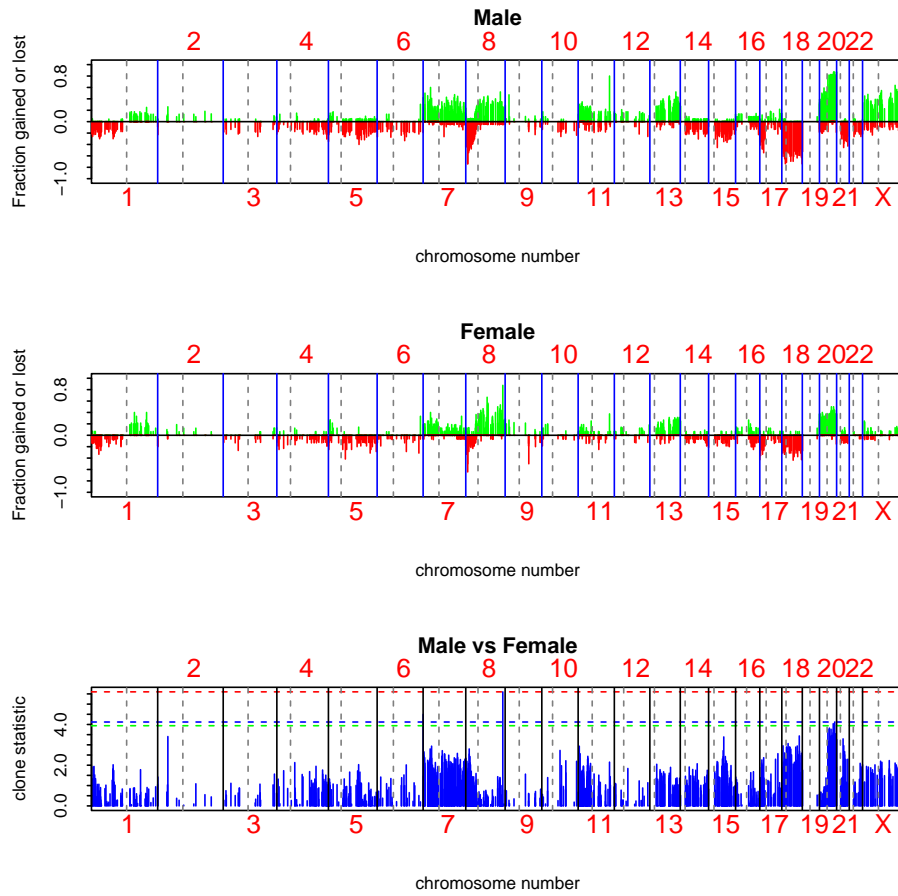


Figure 8: Frequency plots of the samples with respect to the sex groups for clones gained or lost in at least 10% of the samples

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] 2.9775748 3.0424816+ 1.2160169+ 2.7641612+ 1.0069022+ 3.7145128
 [7] 2.7499731 4.7898765 7.6406136+ 6.6980257 8.1810839 7.5974047+
[13] 22.9596330+ 19.4638316 20.5909864+ 1.4243680+ 27.8433741+ 0.6336224
[19] 17.2540390 4.3883096+ 10.6576363 6.4003901 17.1260591+ 3.1394250
[25] 2.0583710 0.2235671+ 30.4518002 12.7138287 15.9088183 2.6636138+
[31] 9.8758910+ 6.3032023 3.9747153 21.8658826+ 9.6692580 7.7447935
[37] 0.5417930 4.5946822+ 2.9634995+ 2.5609706+

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

      index teststat      rawp      adjp
1972 1972 -3.979983 6.892023e-05 0.08737502
1491 1491 -3.926918 8.604138e-05 0.08737502
1490 1490 -3.697062 2.181090e-04 0.10521176
69    69 -3.682757 2.307247e-04 0.10521176
1967 1967 -3.653178 2.590147e-04 0.10521176
1158 1158 -3.479270 5.027813e-04 0.17019146
1973 1973 -3.433760 5.952702e-04 0.17271340
206   206 3.355900 7.910722e-04 0.19119316
208   208 3.286445 1.014605e-03 0.19119316
1729 1729 -3.277590 1.046975e-03 0.19119316
```

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

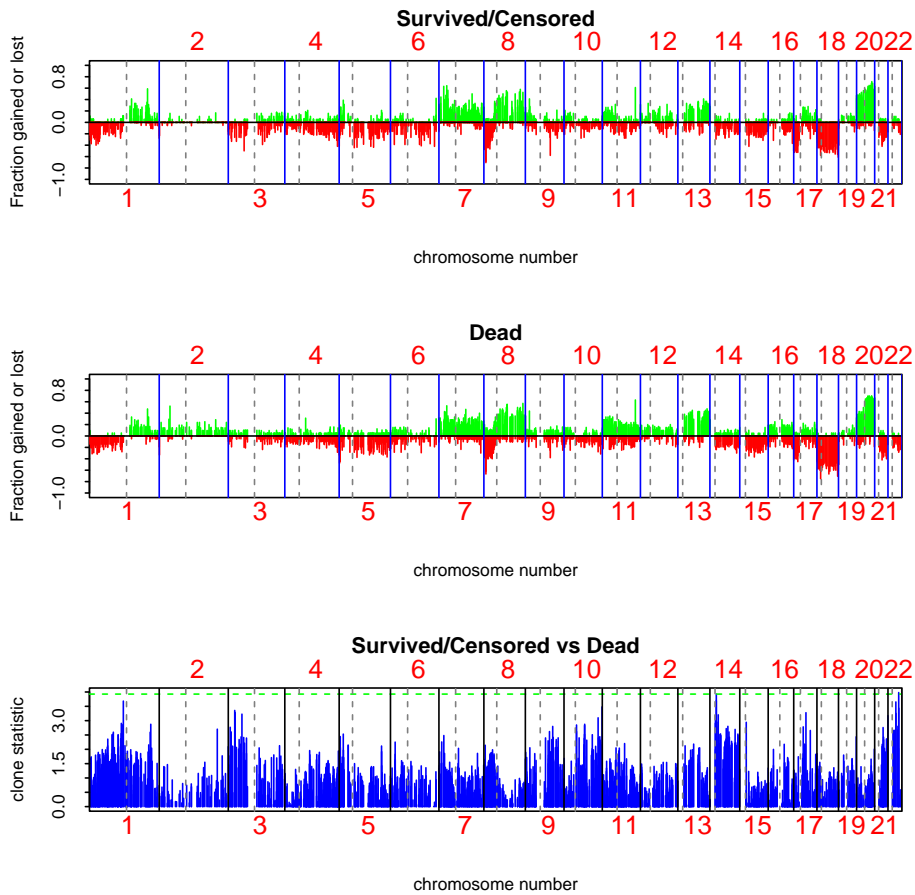


Figure 9: Frequency plots of the samples with respect to survival.

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)

```

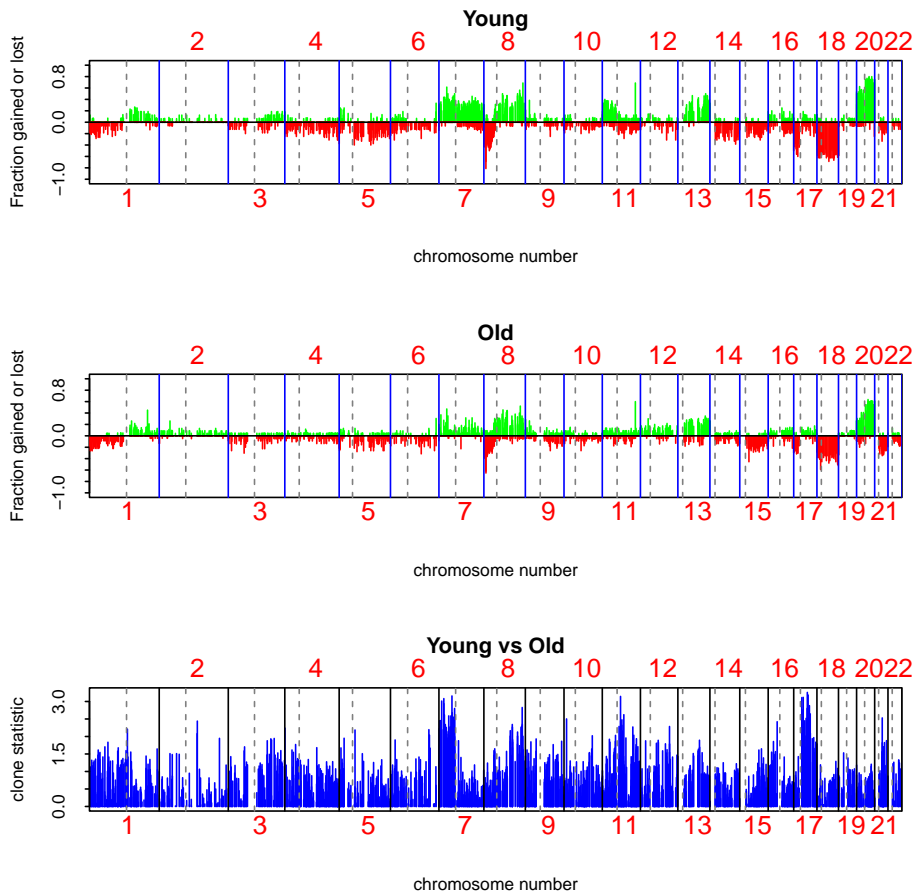


Figure 10: Frequency plots of the samples with respect to age.

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).

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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)
>

```

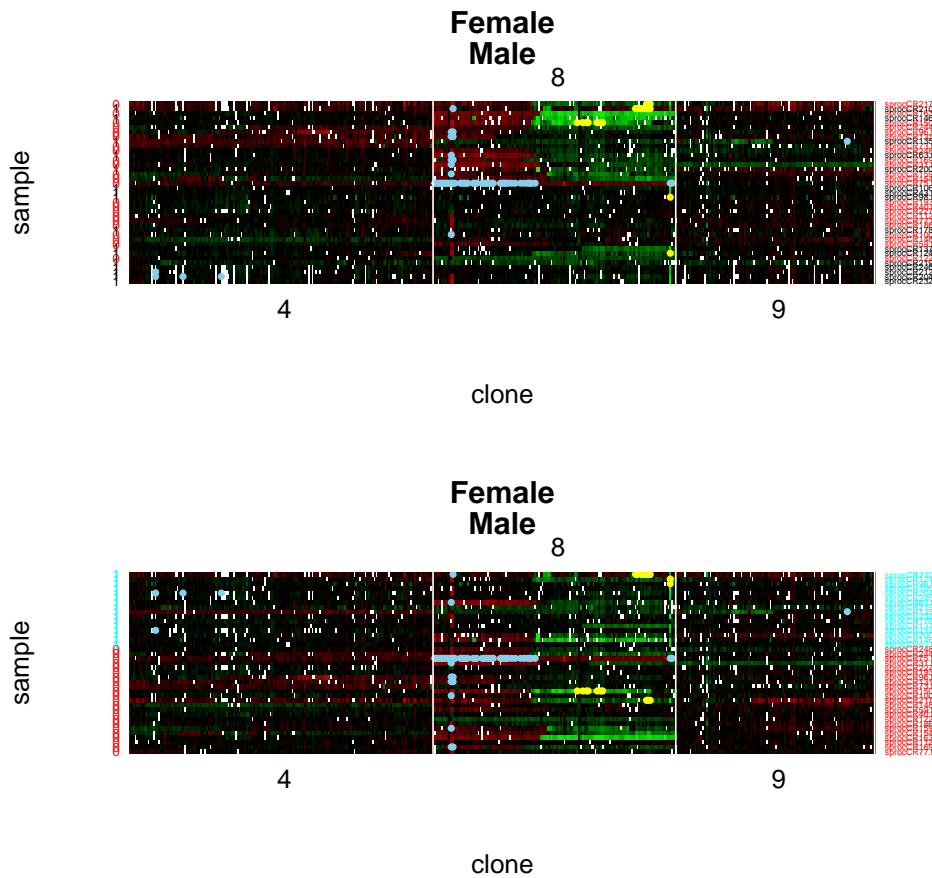


Figure 11: Clustering of the samples by sex