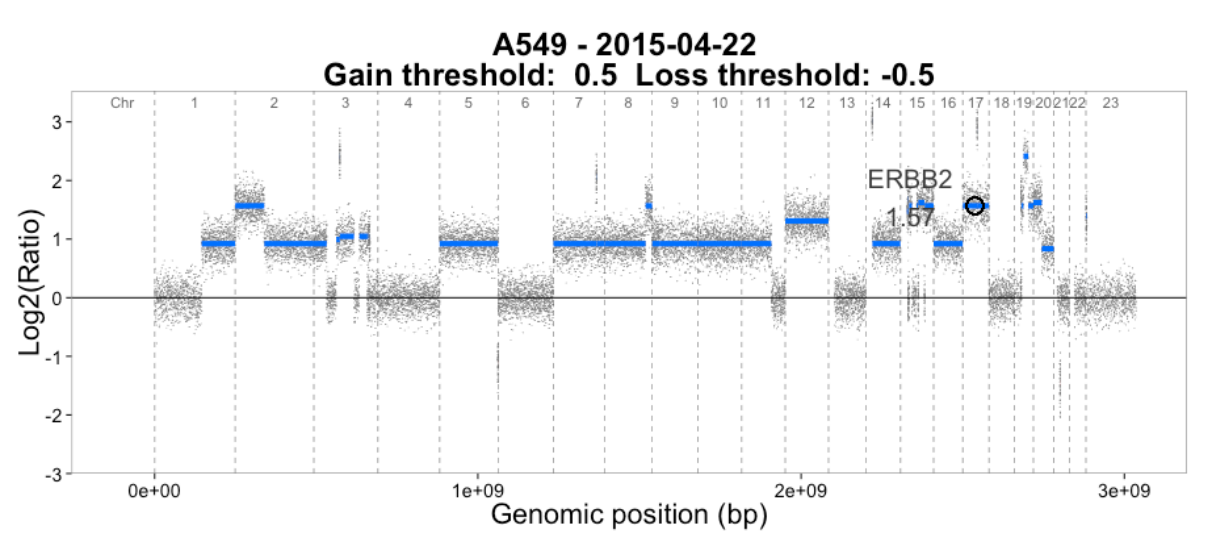
Array-based Genomic Profiles



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In this module

- Cancer & genome, a very brief overview
- Array-based CGH Technologies
- Genomic Profile analysis
- “Do it yourself!”

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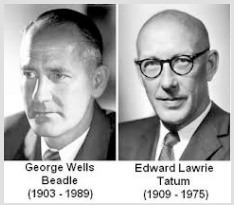
Cancer et génomique : vers une médecine de précision

« […] malignant tumours might be the consequence of a certain abnormal chromosome constitution » Theodor Boveri (1862-1915)

« the evidence shows clearly that the characters of wild animals and plants, as well as those of domesticated races, are inherited both in the wild and in domesticated forms according to the Mendel's Law » Thomas H Morgan (1866-1945)



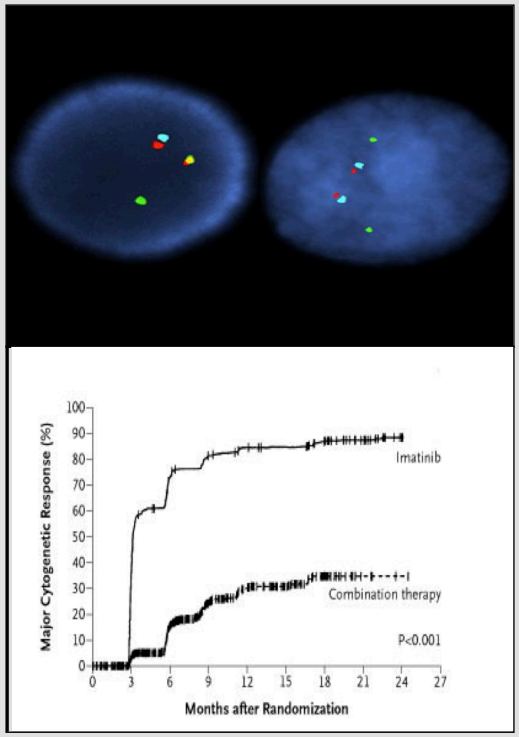
The one gene-one enzyme paradigm.
Genetic Control of Biochemical Reactions in Neurospora. Beadle & Tatum, PNAS 1941

The effect of mutated Hras on cell lines (fibroblast NCI/3T3)
Mechanism of activation of a human oncogene. Tabin C et al. Nature 1982

Early 2000s'
Relations between molecular alterations and treatment efficacy

BCR-Abl and imatinib

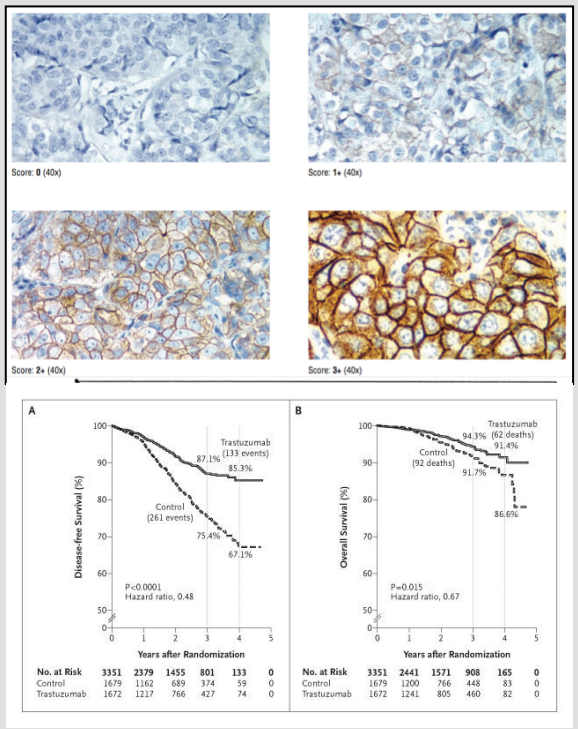
BRC-Abl FISH



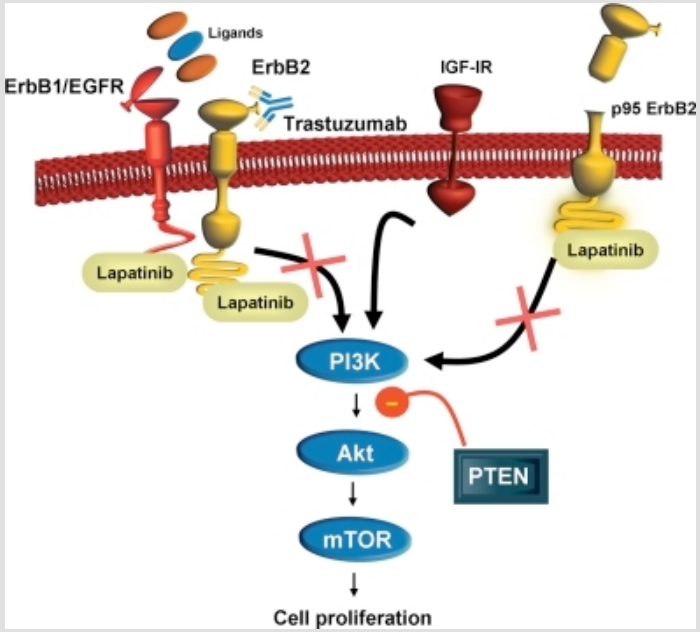
O'Brien et al. N Engl J Med. 2003

ERBB2 and trastuzumab

Her2/neu IHC



Romond et al. N Engl J Med 2005

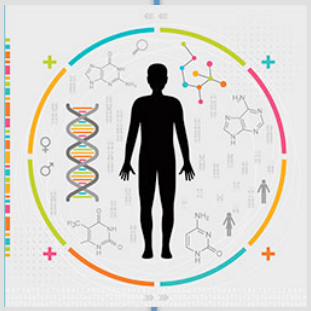


From Vogel et al. Jpn J Clin Oncol. 2010

Matching alterations to targeted therapies

EGFR – ERBB2 – KRAS – BRAF – ALK – MET …

Inhibitors



Precision Medicine

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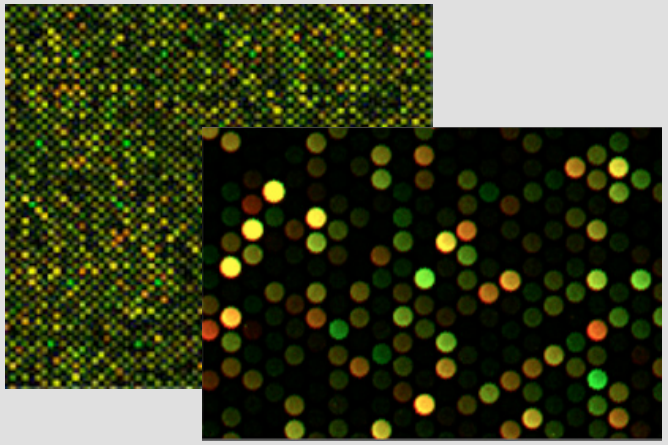
Agilent dual-color hybridization

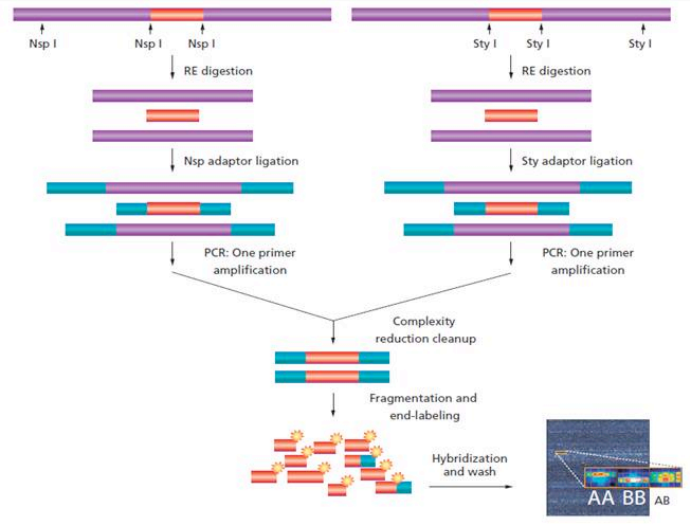
Competitive hybridization between two DNAs

Sample Vs. Reference



$$Log_2\left(\frac{sample}{reference}\right) = LRR$$

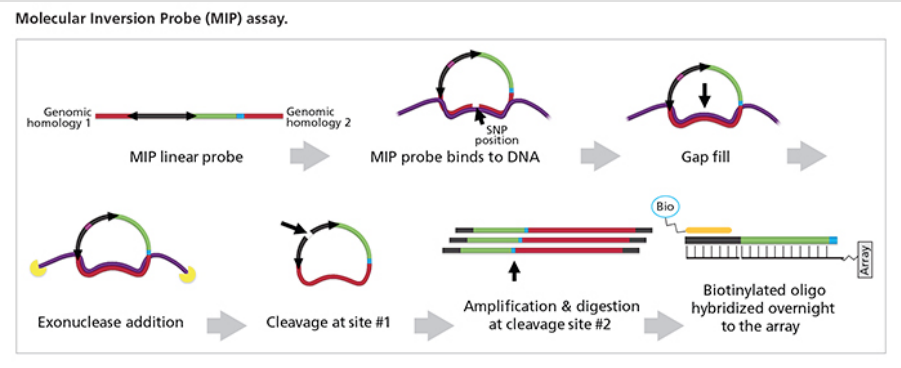
sample	>	reference	loss
sample	=	reference	neutral
sample	<	reference	gain



Affymetrix technologies

SNP5 -> SNP6 -> cytoScanHD (~2.7e6 probes)

- Single-color hybridization
- CN + SNP probes
- 'virtual' reference (hapmap270)
- Average spacing: 880bp (range: 384 – 1737)



OncoScan, dedicated to FFPE samples
~230K probes exclusively SNP, new technology
- Average spacing: 2.8Kb

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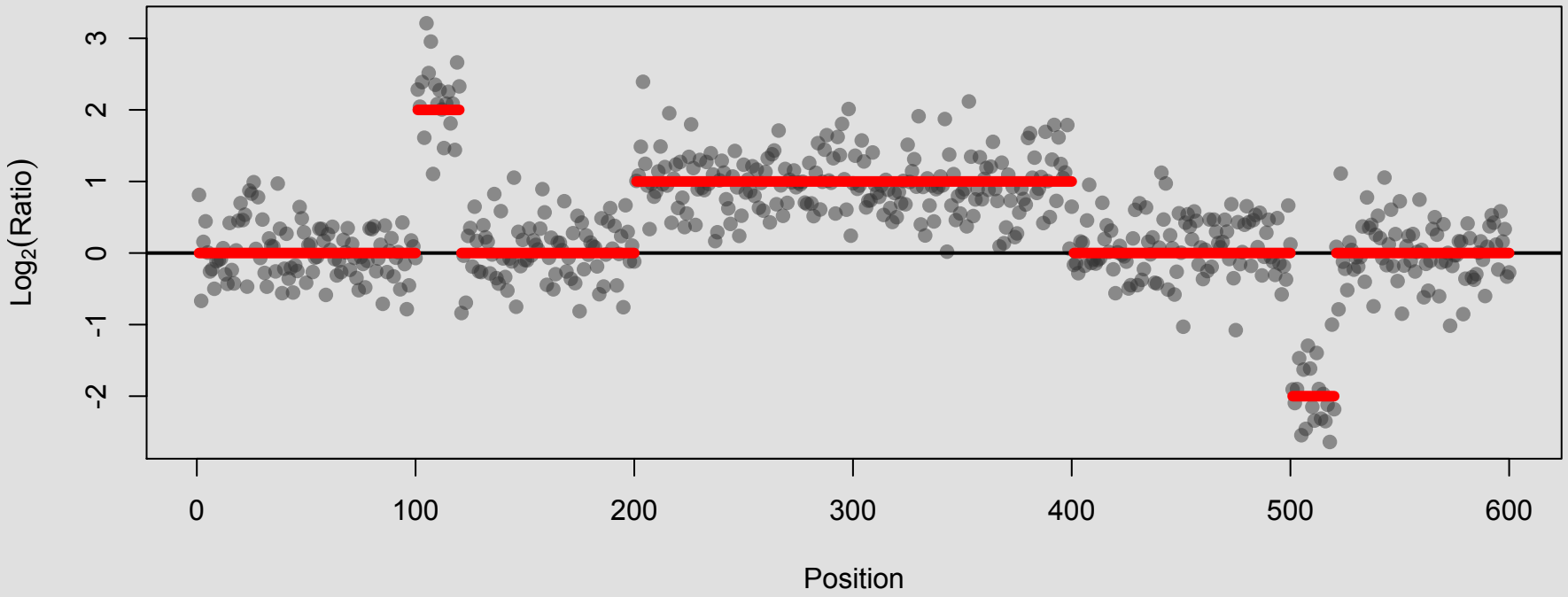
The main idea:

- **taking into account the physical relation between markers (probes).**
- **Summarizing the signal over all the related points.**



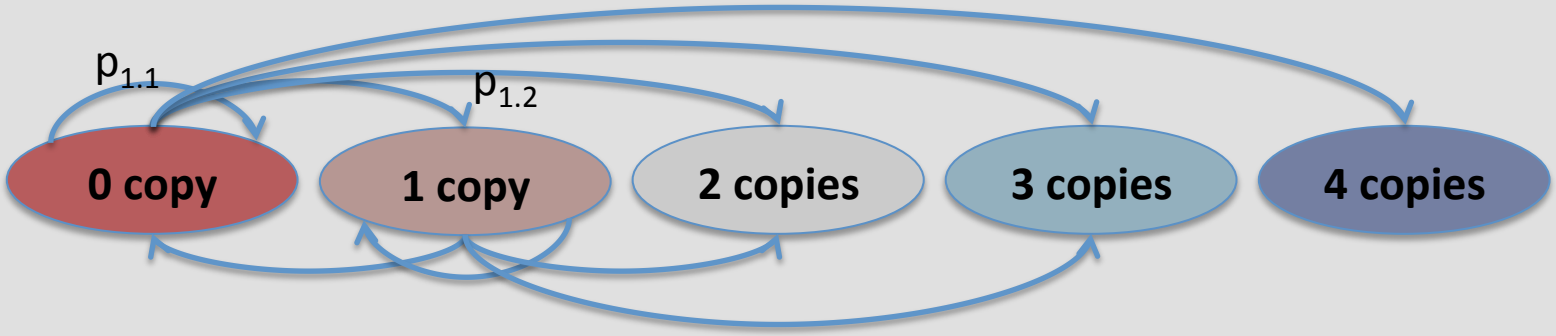
Issues:

- **How to segment efficiently ?** ← **A matter of algorithm**
- **How to estimate gains & losses ?** ← **A matter of neutral 2-copies line … ?**

Segmentation algorithms

The Hidden Markov Model (HMM)

Rational: contiguous states might be somehow linked due to their contiguous positions



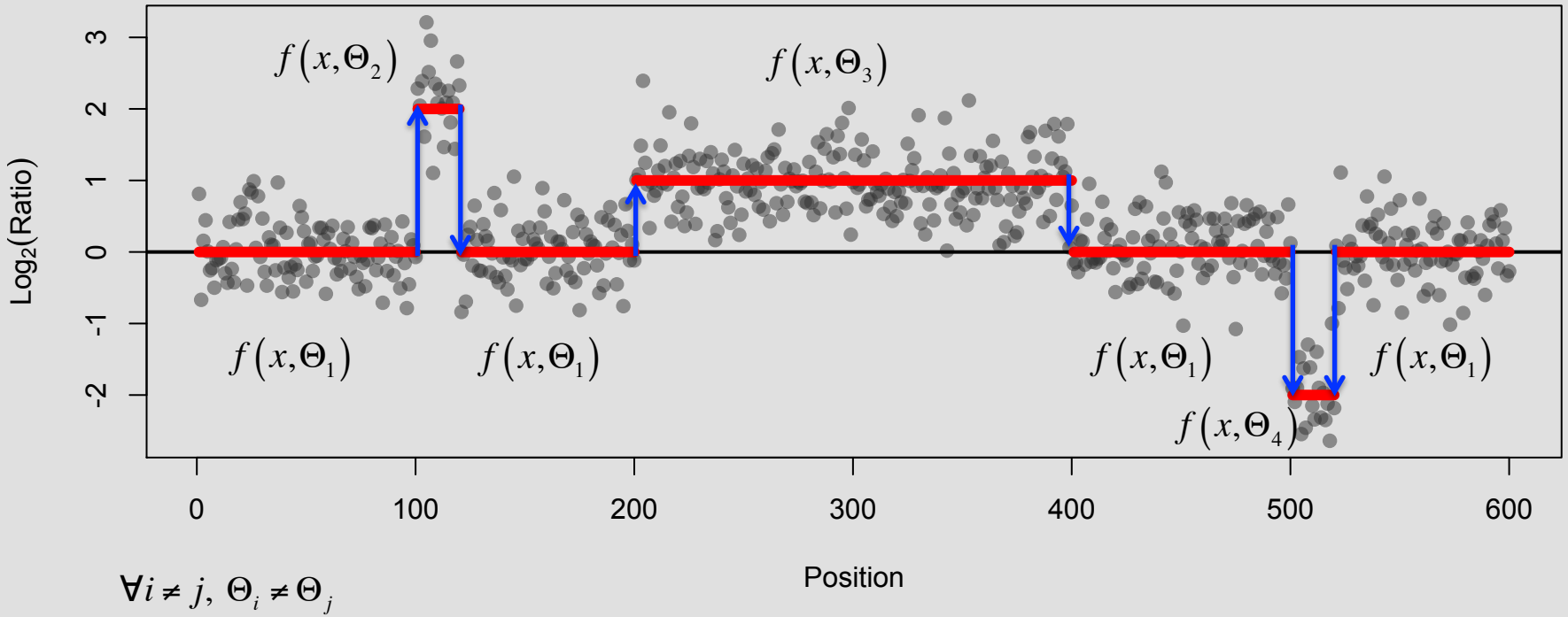
Transition matrix $P(S_i \mid S_{i-1})$

Forward/Backward, Viterbi algorithm

Segmentation algorithms

The circular binary segmentation (CBS)

Rational: there exist a sequence of *k* breakpoints $t_1,\ldots,t_k$ such that the LRR mean (and possibly the variance) is the same between two changes, and different from one to another.



$\forall i \neq j,\ \Theta_i \neq \Theta_j$

breakpoints

Can be computed using recursive or dynamic programming

Several solutions in R (do not reinvent the wheel…)

BioHMM: a heterogeneous hidden Markov model for segmenting array CGH data.
Marioni *et al.* Bioinformatics 2006

A faster circular binary segmentation algorithm for the analysis of array CGH data.
Venkatraman E S and Olshen Adam B. Bioinformatics 2007

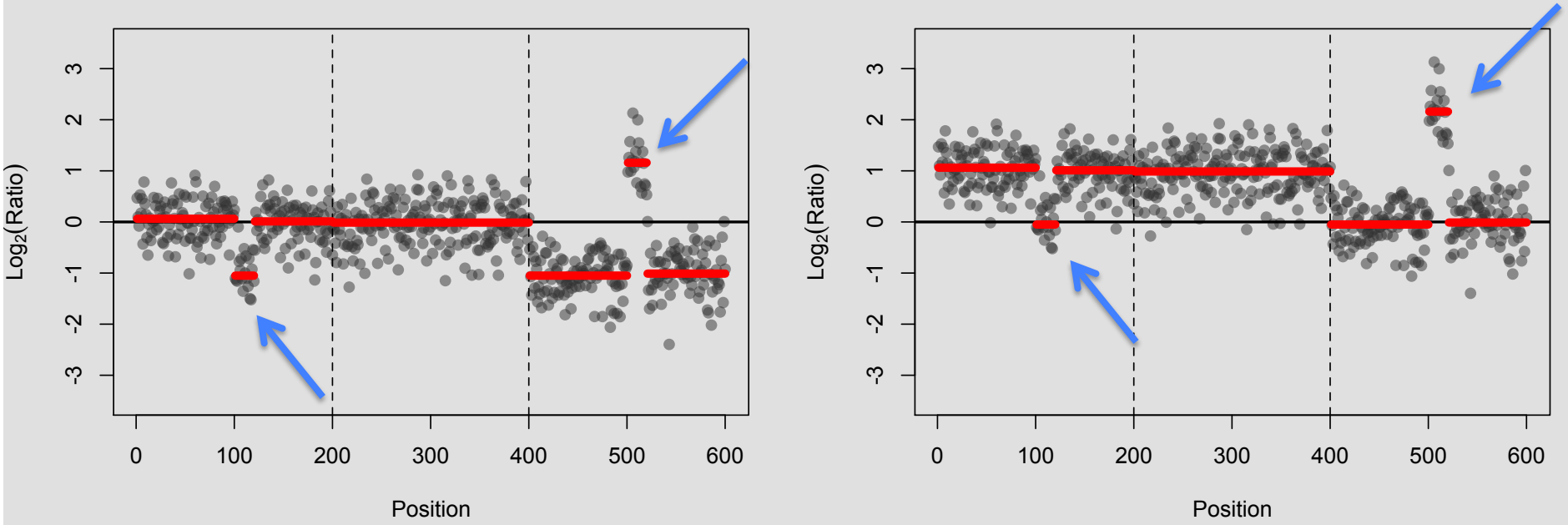
Method comparisons:

A comparison study: applying segmentation to array CGH data for downstream analyses.
Willenbrock H and Fridlyand J, Bioinformatics 2005.

Comparative analysis of algorithms for identifying amplifications and deletions in array CGH data
Lai *et al.* Bioinformatics 2005

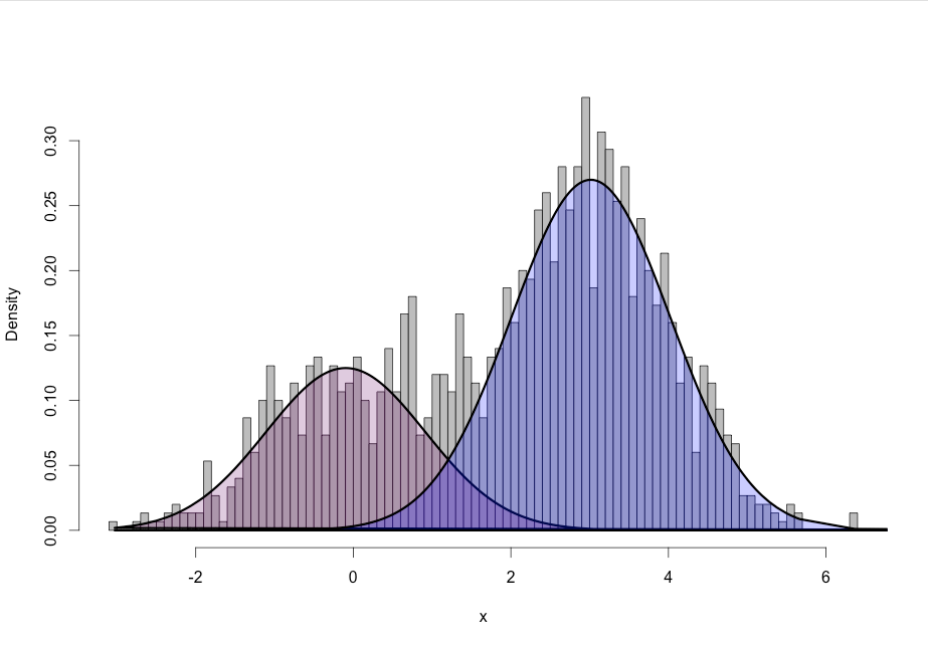
The centralization problem

Depending on the centralization choice, alterations can be different, and so the decision.



Which one is correct ?

Centralizing using the Expectation-Maximization (EM) algorithm



$$g(x,\Theta) = \sum_{k=1}^{K} \pi_k f_k(x,\theta_k)$$

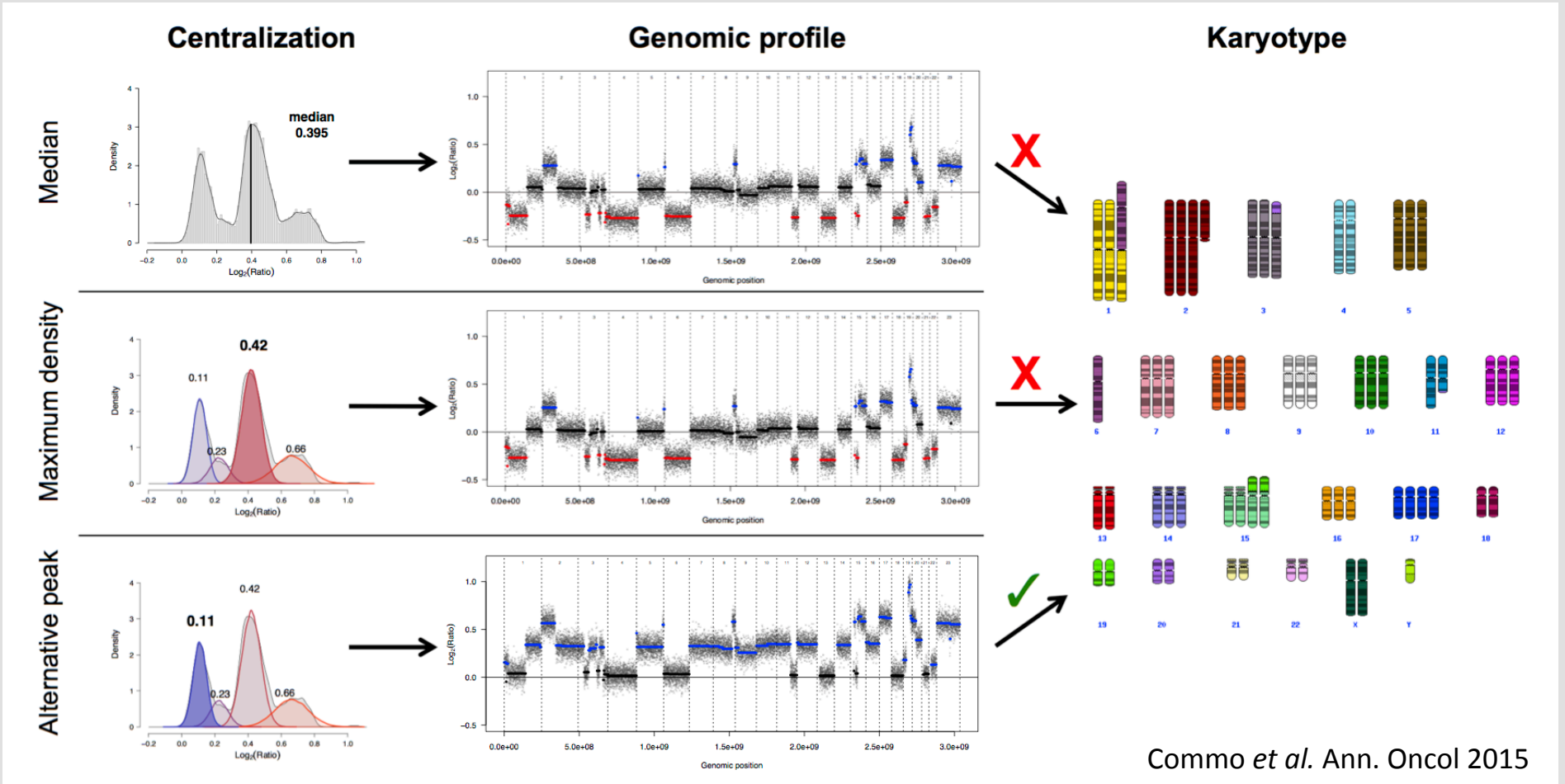
Where K is the number of classes,
π_k the proportion of class k,
θ_k the set of parameters for the density f_k

Do not reinvent the wheel (again…), the R package *mclust* does the job for you.

This approach was suggested in Chen *et al.* (Bioinformatics, 2008), where they choose the highest density peak, with a .95 tolerance, for centralizing the LRRs.

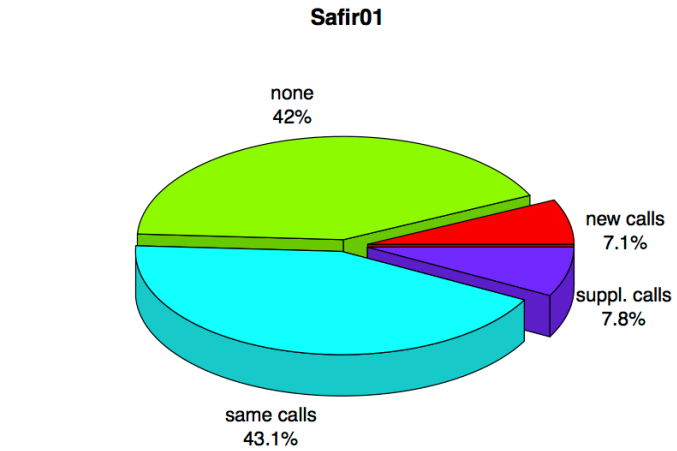
Real cases: the NCI-60 cell lines, aCGH profiles compared to skygrams

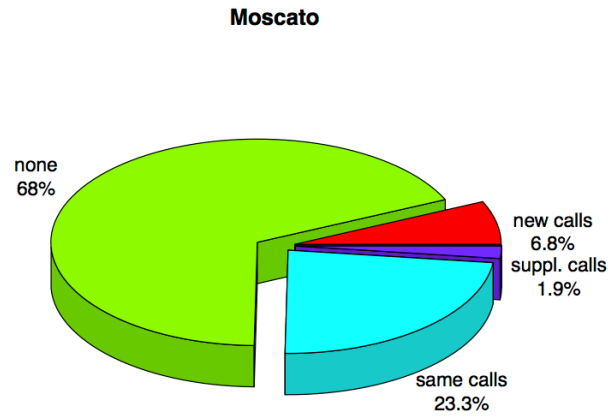
"NCI and NCBI's SKY/M-FISH and CGH Database (2001), http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi"

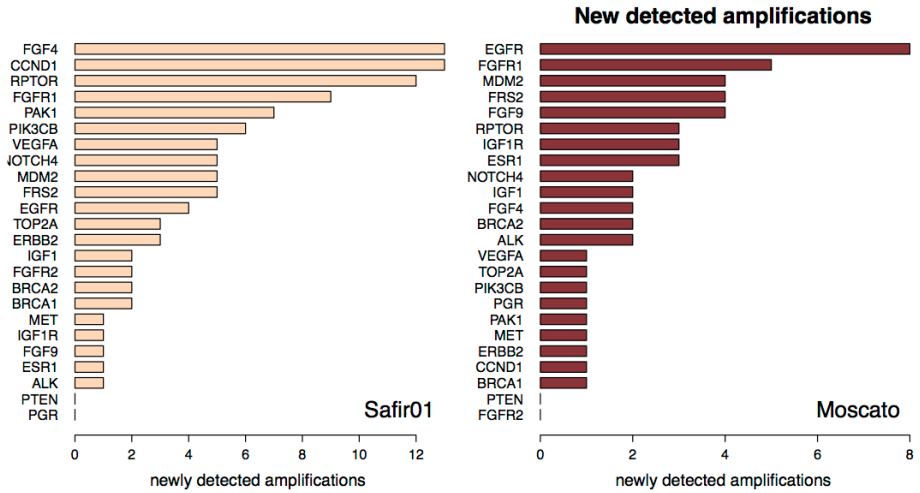


Commo *et al.* Ann. Oncol 2015

Impact on decisions for a therapeutic orientation





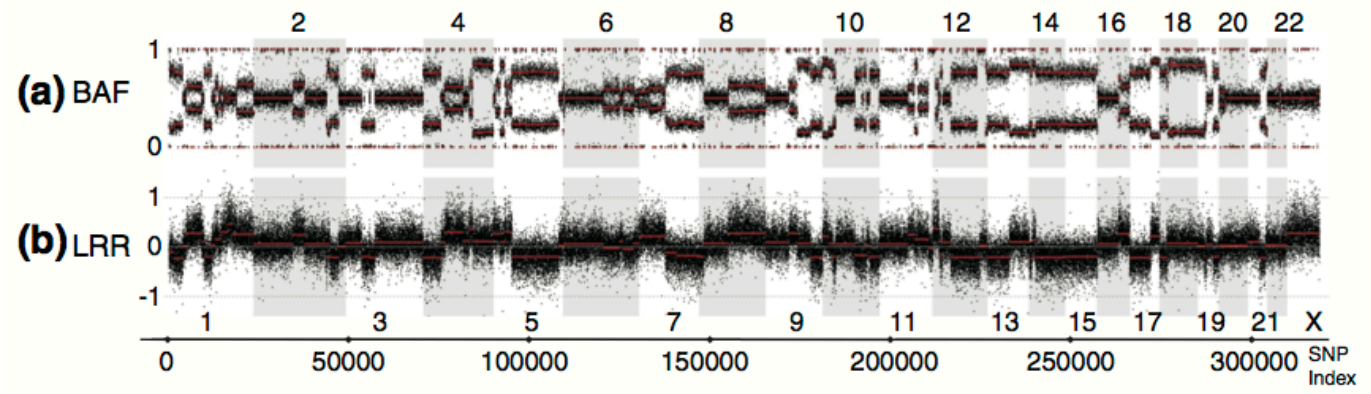


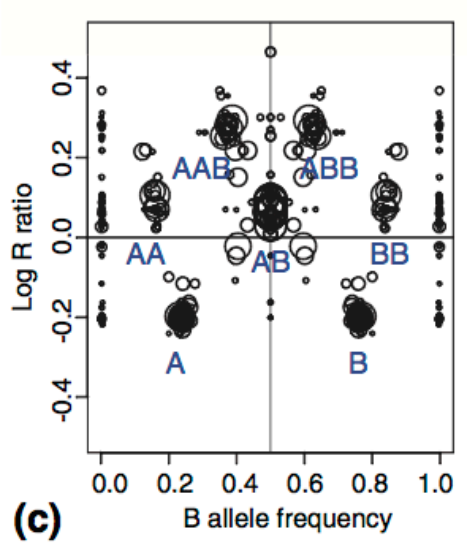
	Safir01	Moscato
FGF4	13	2
RPTOR	12	3
CCND1	13	1
FGFR1	9	5
EGFR	4	8
FRS2	5	4
MDM2	5	4
PAK1	7	1
NOTCH4	5	2
PIK3CB	6	1
VEGFA	5	1
FGF9	1	4
BRCA2	2	2
ERBB2	3	1
ESR1	1	3
IGF1	2	2
IGF1R	1	3
TOP2A	3	1
ALK	1	2
BRCA1	2	1
FGFR2	2	0
MET	1	1
PGR	0	1
PTEN	0	0

Commo *et al.* Ann. Oncol 2015

Alternatives

Genome Alteration Print (GAP)





The whole-genome single-nucleotide polymorphism (SNP) array profile and genome alteration print (GAP). The whole-genome profile of genomic rearrangements in the BLC_B1_T45 sample measured by 300K Illumina SNP-array and corresponding GAP. (a) Allelic imbalances are represented by B-allele frequency (BAF). (b) Copy-number variation profile is represented by log R ratio (LRR), centered at zero. (c) The GAP of the sample is a combined sideview projection of segmented LRR and BAF. Each region of the genome is represented by two symmetric circles in the case of allelic imbalance and by one circle centered at BAF = 0.5 in the case of a balanced genotype. Attribution of copy numbers and genotypes corresponds to a near-diploid model of rearrangements.
Popova et al. Genome Biology 2009 10:R128 doi:10.1186/gb-2009-10-11-r128

Other methods: Absolute, ASCAT, SOMATICS, PICNIC

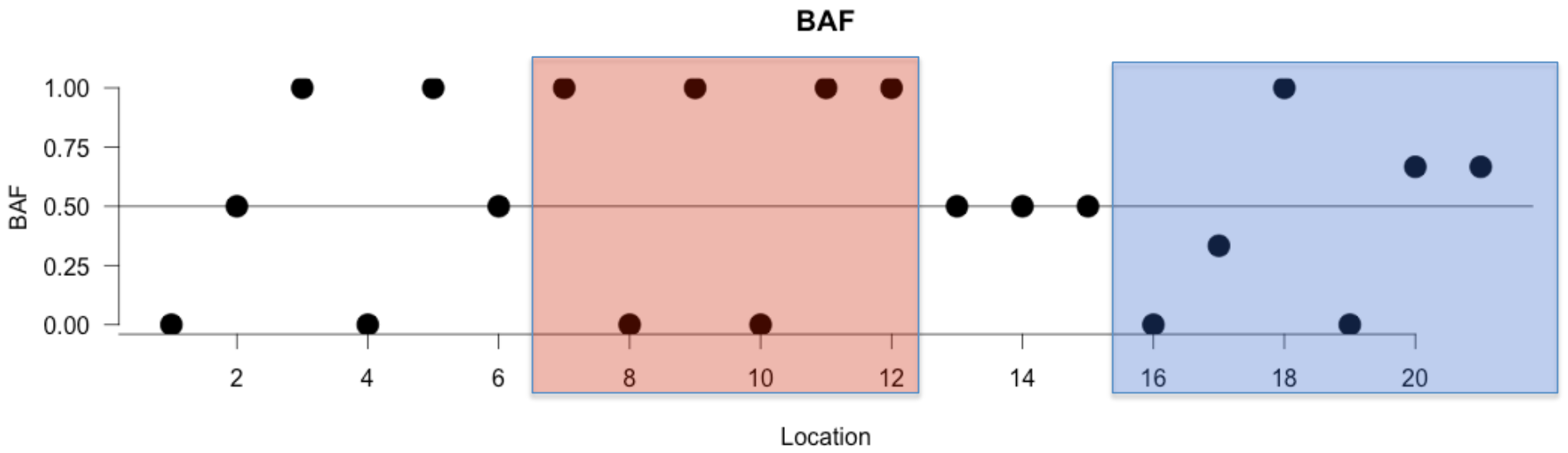
Interpreting loss of heterozygosity (LOH)

$$BAF = \frac{B}{A+B}$$

Lost Gain

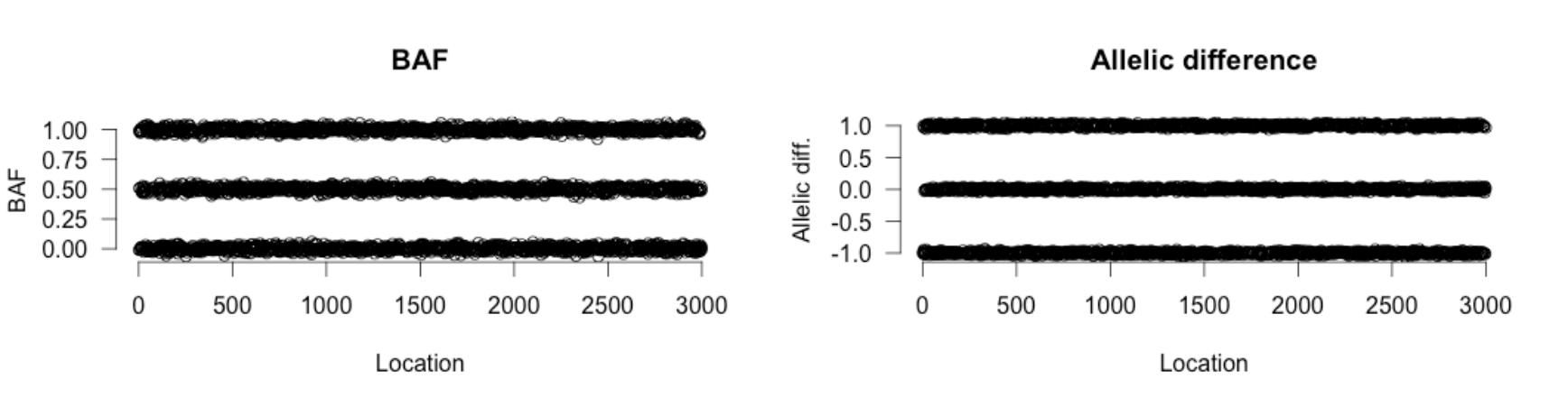
Mother: AABABBBABABBBAAAABABB
Father: ABBABAABBABBAAA

Genotype: AA AB BB AA BB BA B A B A B B BA AB AB AAA AAB BBB AAA BBA BBA

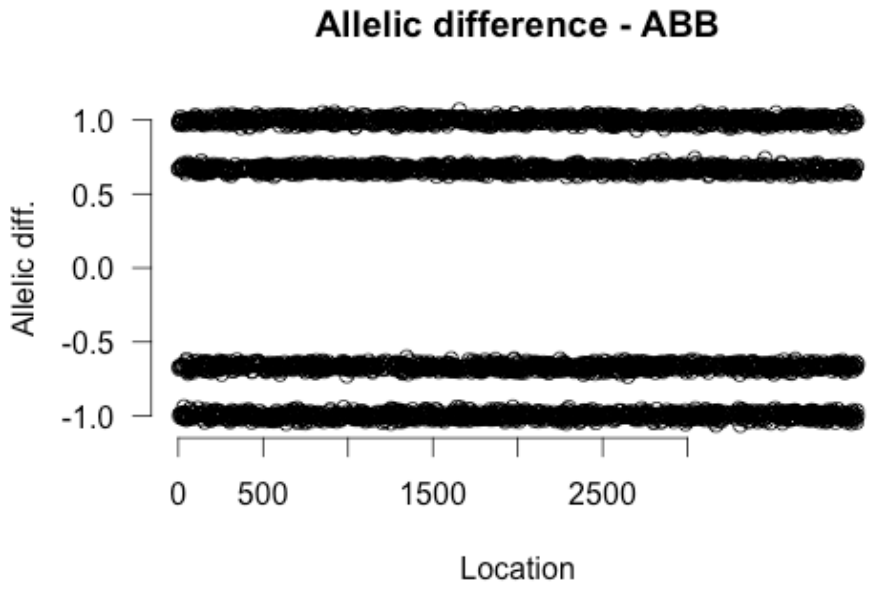


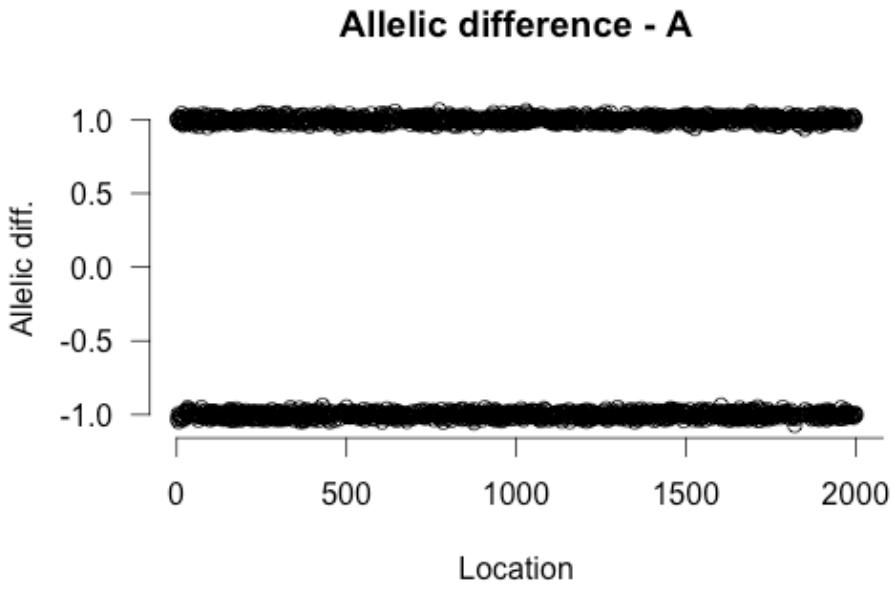
$$BAF = \frac{B}{A+B} \; ; \; Allelic.Difference = \frac{A-B}{A+B}$$

snp	1	2	3	4	5	6	7	8	9	10
Genotype A	a	b	a	b	b	a	a	b	b	a
Genotype B	a	b	b	b	a	a	a	a	b	b
BAF = B/(A+B)	0	1	0.5	1	0	0	0	0	1	0.5
Allelic diff = (A-B)/(A+B)	1	-1	0	-1	0	1	1	0	-1	0



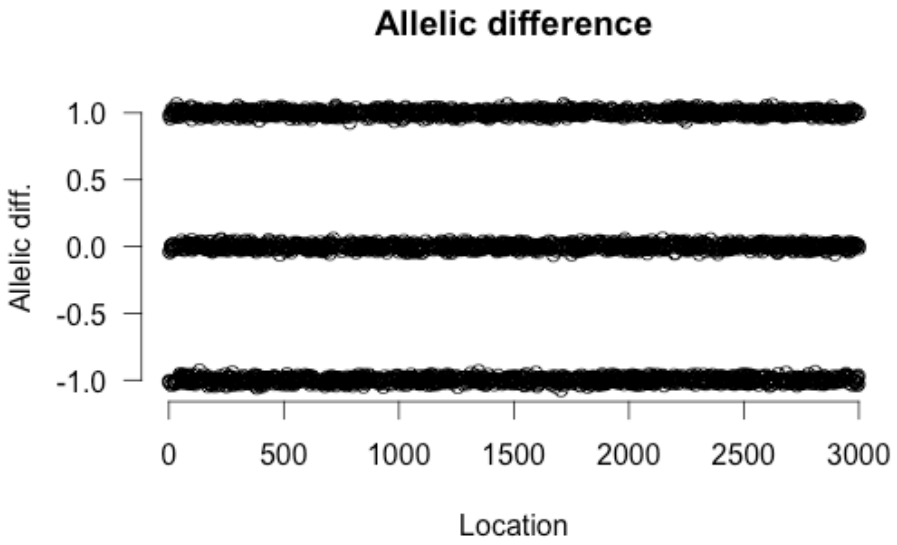
Probe location	1	2	3	4	5	6	7	8	9	10
Genotype A	a	b	a	b	b	a	a	b	b	a
Genotype B	a	b	b	b	a	a	a	a	b	b
Genotype B	a	b	b	b	a	a	a	a	b	b
Allelic diff = (A-B)/(A+B)	1	-1	-2/3	-1	2/3	1	1	2/3	-1	-2/3

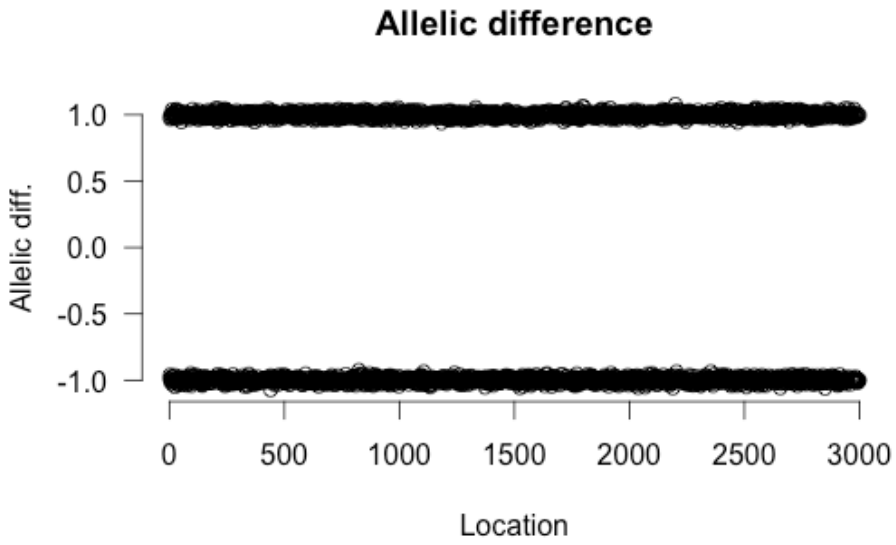




The problem is…

Since signals are adjusted such that *AA = 1* and *BB = -1*, and so *0 is AB…*



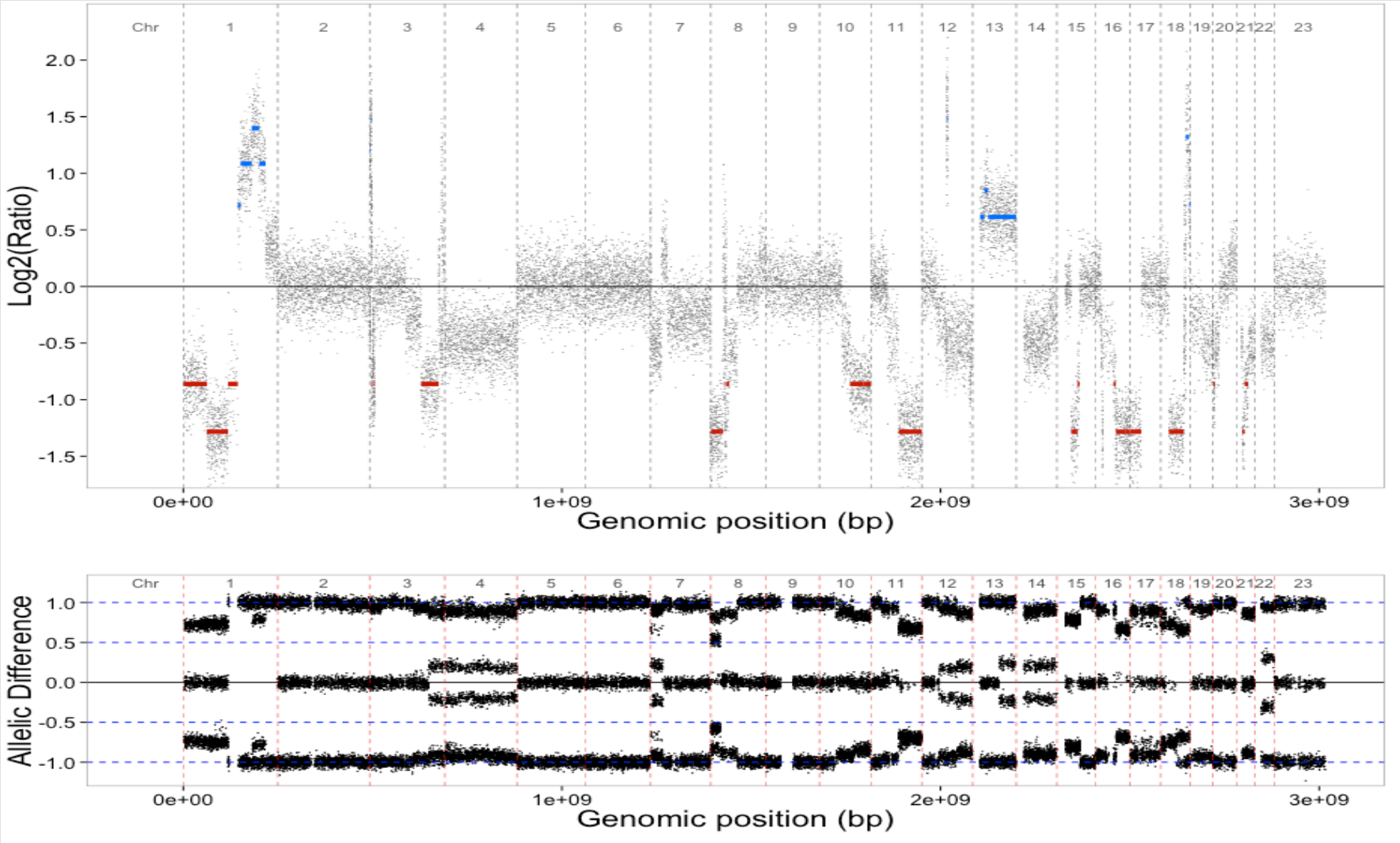


Can be any balanced genotype: AB or AABB

Can be any genotype A or AA or AAA

In case of mosaicism, things can be even more complicated…

A real case…


Chr
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
Log2(Ratio)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
0e+00
1e+09
2e+09
3e+09
Genomic position (bp)
Allelic Difference
1.0
0.5
0.0
-0.5
-1.0
0e+00
1e+09
2e+09
3e+09
Genomic position (bp)

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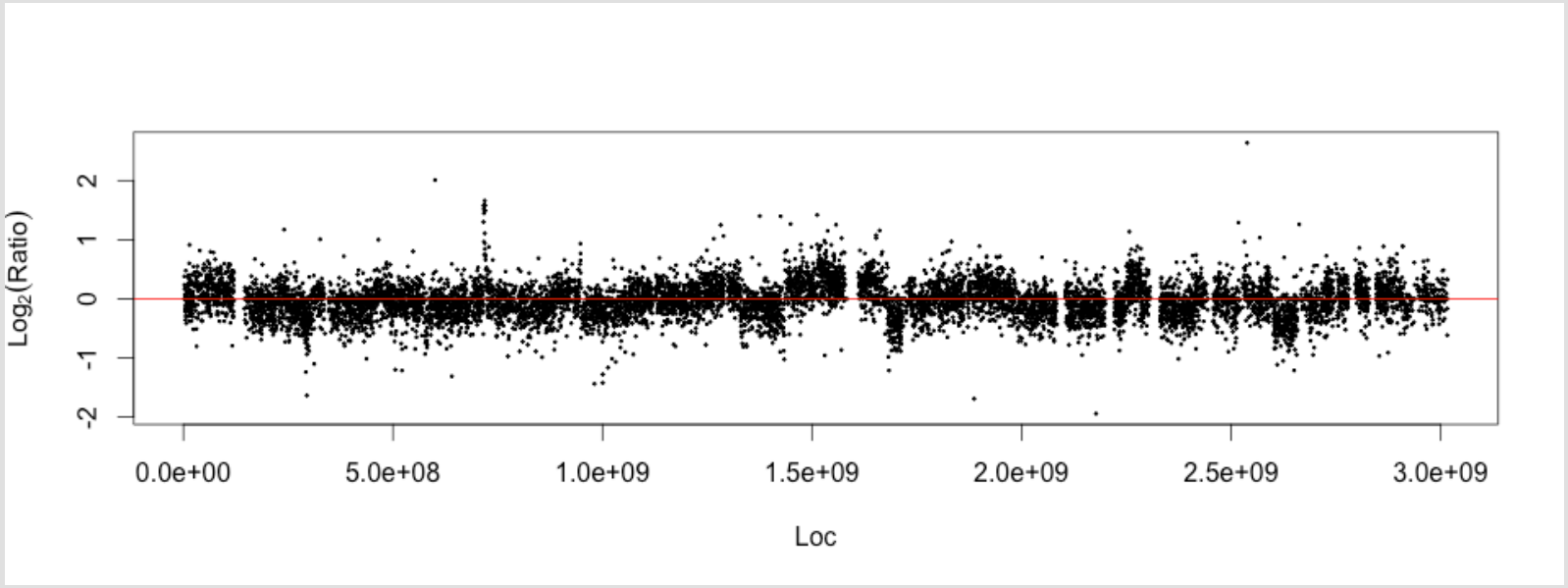
```
####################
# Using DNAcopy
####################

> require(DNAcopy)
> op <- par(no.readonly = TRUE)

# Loading supp. data
> path <- "/Users/fredcommo/Documents/myProjects/IFSBM/data"
> load(file.path(path, "hg19.rda"))
> load(file.path(path, "geneDB.rda"))

# Reading file
> filePath <- file.path(path, "Affy_cytoScan.cyhd.CN5.CNCHP_short.txt.gz")
> foo <- readLines(filePath, n=750)
> idx <- grep("ProbeSet", foo)
> cnSet <- read.delim(filePath, skip=idx-1, stringsAsFactors=FALSE)
> dim(cnSet)
> head(cnSet)

  ProbeSetName Chromosome Position CNState Log2Ratio SmoothSignal LOH Allele.Difference
1      C-7SARK          1   849467       2  0.059410     1.859233   0                NA
2      C-6HYCN          1   874571       2 -0.209187     1.855083   0                NA
3      C-7SEBI          1   874841       2 -0.391675     1.852993   0                NA
4      S-3WRNV          1   882803      -1  0.000000     2.000000   0          -0.47663
5      C-7SBEJ          1   884724       2 -0.058026     1.854296   0                NA
6      C-5YZUW          1   884761       2  0.128548     1.850769   0                NA
```

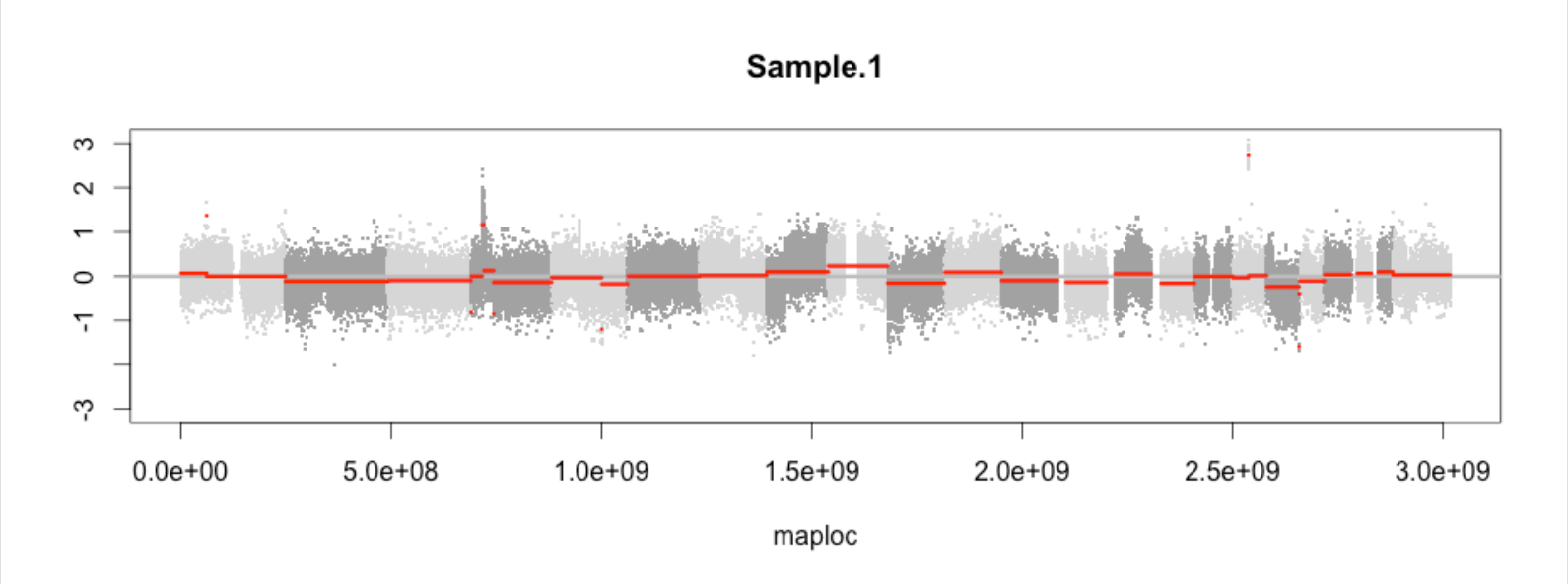
```
# Filtering SNP probes
> cnSet <- cnSet[grep("^S", cnSet$ProbeSet),]
> dim(cnSet)
> head(cnSet)

 ProbeSetName Chromosome Position CNState Log2Ratio SmoothSignal LOH Allele.Difference
4       S-3WRNV          1   882803      -1  0.000000     2.000000   0         -0.476630
9       S-4GXBG          1   888659       2 -0.296734     1.856312   0          0.604524
23      S-4LYTY          1   918573       2  0.440532     1.888353   0          0.258405
24      S-4HQZX          1   920733       2  0.092134     1.891228   0          1.078725
46      S-4KCPC          1  1039857       2 -0.184646     1.958233   0         -0.706869
47      S-3UOZS          1  1041366       2 -0.071947     1.960523   0         -0.014035
```

```
# Checking Chr names
> table(cnSet$Chromosome)

 1    10    11    12    13    14    15    16    17    18    19     2    20    21    22     3
14913  9329 10249  8950  7328  7017  6624  5488  4664  5467  2904 16500  4255  2667  2280 13735
    4     5     6     7     8     9     X
12947 12383 13733 12208 10245  8060  5748


> cnSet$Chromosome[cnSet$Chromosome=="X"] <- 23
> cnSet$Chromosome <- as.numeric(cnSet$Chromosome)
> table(cnSet$Chromosome)

 1     2     3     4     5     6     7     8     9    10    11    12    13    14    15    16
14913 16500 13735 12947 12383 13733 12208 10245  8060  9329 10249  8950  7328  7017  6624  5488
   17    18    19    20    21    22    23
 4664  5467  2904  4255  2667  2280  5748

> cnSet <- cnSet[order(cnSet$Chromosome, cnSet$Position),]
```

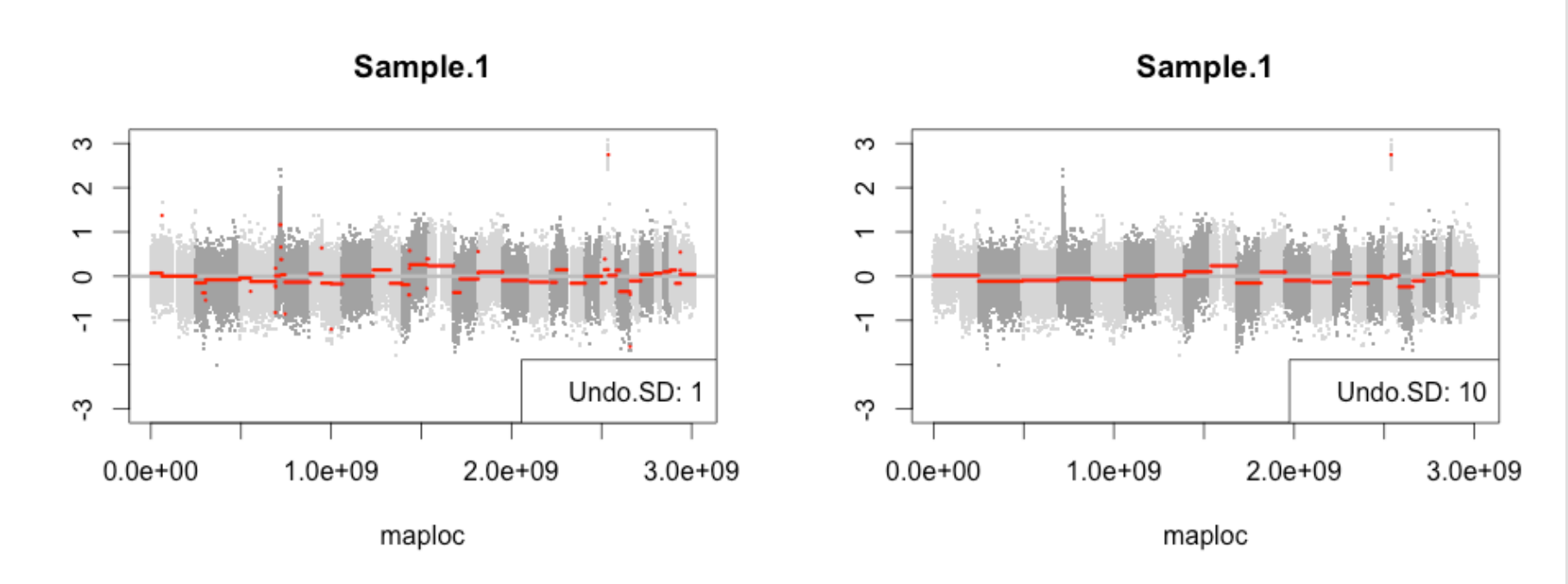
```
# Calculating genomic positions
> locs <- lapply(1:24, function(chr){
    l <- cnSet$Position[cnSet$Chromosome==chr]
    return(l + hg19$cumlen[chr])
 })
> locs <- do.call(c, locs)
> s <- sample(1:nrow(cnSet), 1e4)

# A quick preview
> plot(locs[s], cnSet$Log2Ratio[s], cex=.2, xlab="Loc", ylab=expression(Log[2]
(Ratio)))
> abline(h = 0, col="red”)
```



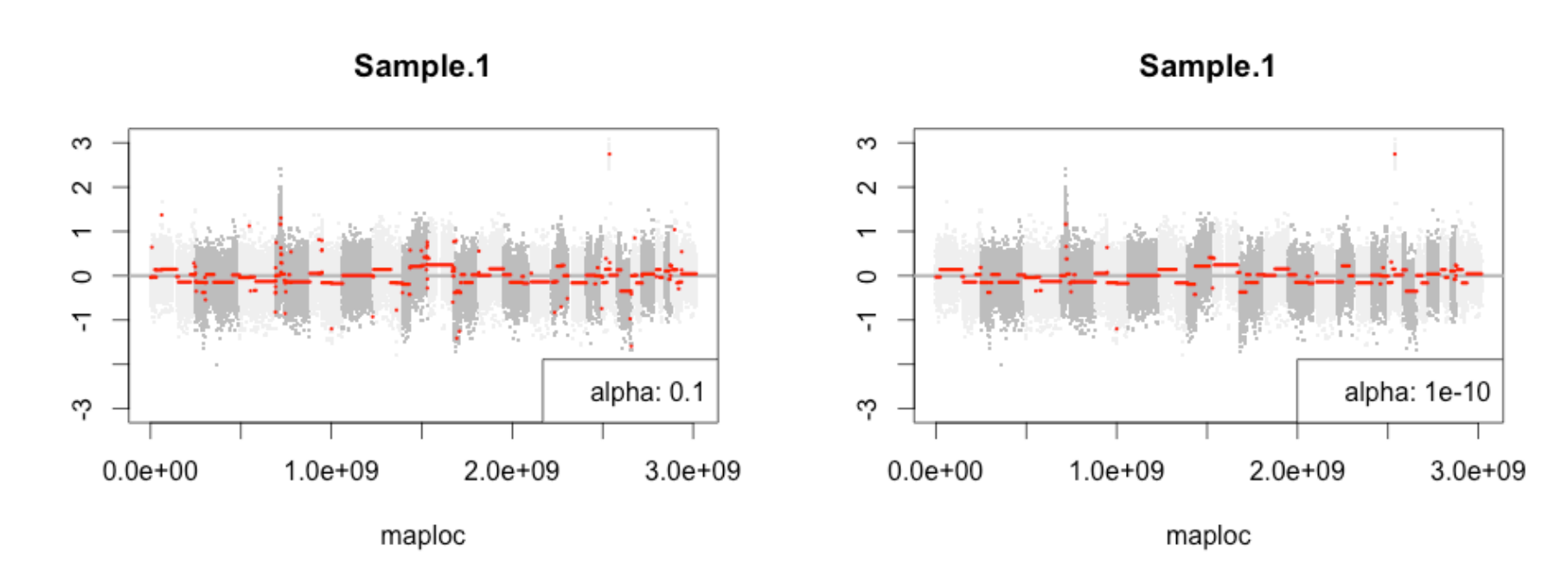
```
# Segmenting using DNAcopy and the default params
    # Constructing a DNAcopy object
> LR <- cnSet$Log2Ratio
> Chr <- cnSet$Chromosome
> ptcols <- c("grey65", "grey85")
> cnaObj <- CNA(LR, Chr, locs, presorted = TRUE)
> cnaObj <- smooth.CNA(cnaObj)
> segObj <- segment(cnaObj, undo.splits = "sdundo")

    # Plotting
> ptcols <- c("grey65", "grey85")
> plot(segObj, xmaploc=TRUE, pt.cols=ptcols)
```



```
# The Undo.SD effect
> par(mfrow=c(1, 2))
> for(s in c(1, 10)){
    segObj <- segment(cnaObj, undo.splits = "sdundo", undo.SD = s)
    plot(segObj, xmaploc=TRUE, pt.cols=ptcols)
    legend("bottomright", legend=sprintf("Undo.SD: %s", s))
}
> par(op)
```

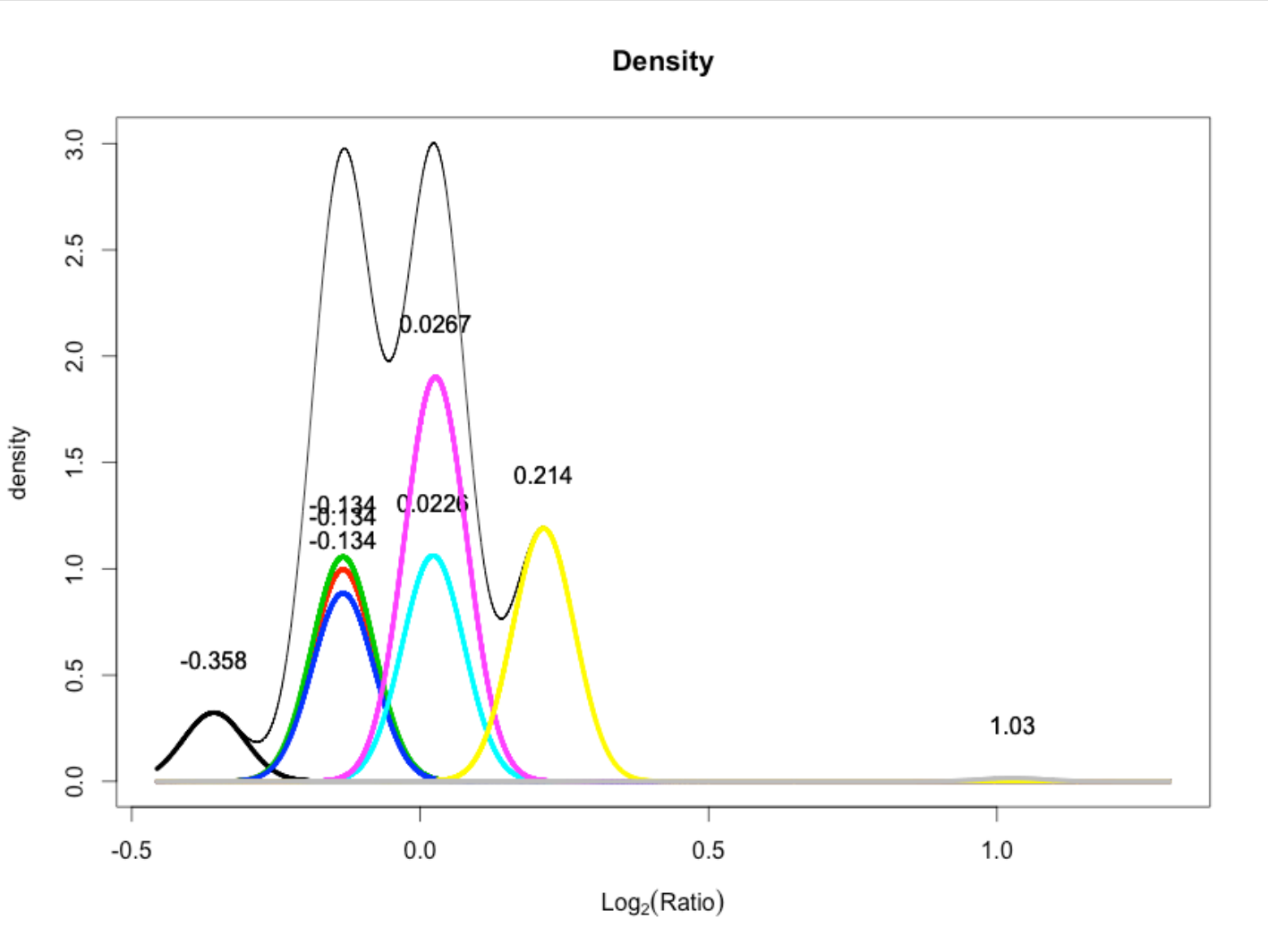


```
# The alpha tolerance effect
> par(mfrow=c(1, 2))
> for(a in c(1e-1, 1e-10)){
    segObj <- segment(cnaObj, undo.splits = "sdundo", undo.SD = .5, alpha = a)
    plot(segObj, xmaploc=TRUE, pt.cols=c("grey75", "grey95"))
    legend("bottomright", legend=sprintf("alpha: %s", a))
}
> par(op)
```

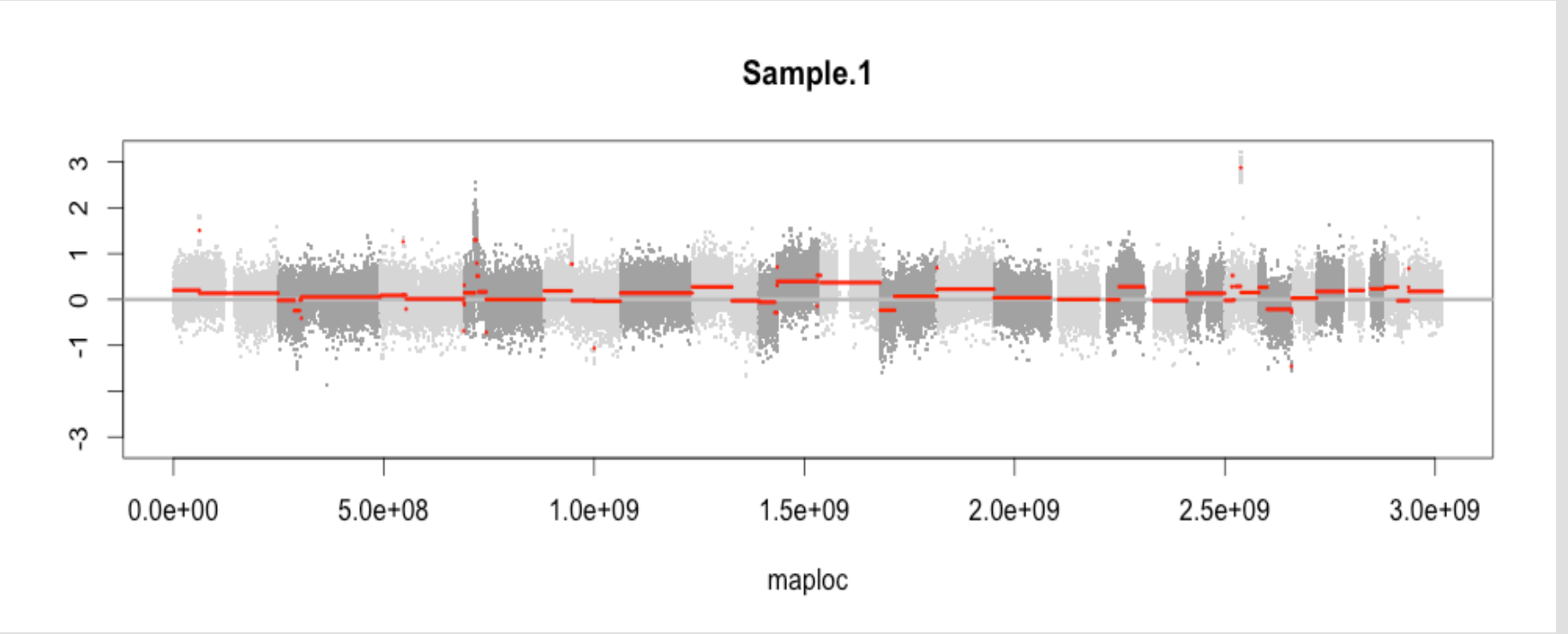


```
# Centering the profile
> require(mclust)
> rLR <- runmed(LR, k=101)
> rLR <- sort(rLR)
> idx <- seq(1, length(rLR), len=25e3)
> model <- Mclust(rLR[idx])
> means <- model$parameters$mean
> props <- model$parameters$pro
> s2 <- model$parameters$variance$sigmasq
> if(length(s2)==1) s2 <- rep(s2, length(means))

> plot(model, what="density", xlab=expression(Log[2](Ratio)))
> for(ii in 1:length(means)){
    m <- means[ii]; s <- sqrt(s2[ii]); p <- props[ii]
    d <- dnorm(rLR, m, s)*p
    lines(rLR, d, col=ii, lwd=4)
    text(m, max(d)+.25, labels=format(m, digits=3))
}
```

Expectation-Maximization modeling



```
# Final profile
> choice <- -0.134
> LR <- LR - choice

> cnaObj <- CNA(LR, Chr, locs, presorted = TRUE)
> cnaObj <- smooth.CNA(cnaObj)
> segObj <- segment(cnaObj, undo.splits = "sdundo", undo.SD = 1, alpha = 1e-2)
> plot(segObj, xmaploc=TRUE, pt.cols=ptcols)
```



```
# Getting the gene list within a specifi segment
> st <- segObj$output
> chr <- 17
> sgt <- st[st$chrom==chr,]
> subdb <- geneDB[geneDB$chr==chr,]
> s <- sgt$loc.start[6]
> e <- sgt$loc.end[6]

> idx <- which(s <= subdb$genomStart & subdb$genomEnd<=e)
> subdb[idx, c("symbol", "fullName")]
```

	symbol	fullName
4560	CDK12	cyclin-dependent kinase 12
8489	**ERBB2**	**v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2**
9428	FBXL20	F-box and leucine-rich repeat protein 20
11279	GRB7	growth factor receptor-bound protein 7
13682	IKZF3	IKAROS family zinc finger 3 (Aiolos)
15386	TCAP	titin-cap
16967	MED1	mediator complex subunit 1
17252	MIEN1	migration and invasion enhancer 1
18670	MIR4728	microRNA 4728
20625	NEUROD2	neuronal differentiation 2
23462	PNMT	phenylethanolamine N-methyltransferase
23572	PGAP3	post-GPI attachment to proteins 3
24598	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B
35517	STARD3	StAR-related lipid transfer (START) domain containing 3

Any question ?