Using crlmm for copy number estimation and genotype calling with Illumina platforms

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Abstract

This vignette illustrates the steps necessary for obtaining marker-level estimates of allele-specific copy number from the raw Illumina IDAT files. Hidden markov models or segmentation algorithms can be applied to the marker-level estimates. Examples of both are illustrated.

1 About this vignette

Allele-specific copy number estimation in the crlmm package is available for several Illumina platforms. As described in the `copynumber` vignette, copy number estimation in crlmm works best when there are a sufficient number of samples such that AA, AB, and BB genotypes are observed at most loci. For small studies (e.g., fewer than 50 samples), there will be a large number of SNPs that are monomorphic. For monomorphic SNPs, the estimation problem becomes more difficult and alternative strategies that estimate the relative total copy number may be preferable. In addition to installing crlmm, one must also install the appropriate annotation package for the Illumina platform. In the following code, we list the platforms for which annotation packages are currently available. Next we create a directory where output files will be stored and indicate the directory that contains the IDAT files that will be used in our analysis.

```
> library(ff)
> pkgs <- annotationPackages()
> pkgs[grep("Crlmm", pkgs)]
```

```
 [1] "genomewidesnp6Crlmm"    "genomewidesnp5Crlmm"
 [3] "human370v1cCrlmm"       "human370quadv3cCrlmm"
 [5] "human550v3bCrlmm"       "human650v3aCrlmm"
 [7] "human610quadv1bCrlmm"   "human660quadv1aCrlmm"
 [9] "human1mduov3bCrlmm"     "humanomni1quadv1bCrlmm"
```

```
> if (getRversion() < "2.13.0") {
     rpath <- getRversion()
 } else rpath <- "trunk"
> outdir <- paste("/thumper/ctsa/snpmicroarray/rs/ProcessedData/crlmm/",
     rpath, "/illumina_vignette", sep = "")
> dir.create(outdir, recursive = TRUE, showWarnings = FALSE)
> datadir <- "/thumper/ctsa/snpmicroarray/illumina/IDATS/370k"

> ldPath(outdir)
> ocProbesets(150000)
> ocSamples(200)
```

This vignette was created using Illumina IDAT files that are located in a specific directory on my computer (`pathToCels`). Long computations are saved in the output directory `outdir`. Towards this end, we make repeated use of the `checkExists` – an ad-hoc approach for storing long computations. This function simply

checks that a variable is in the workspace. If not, the variable is loaded from the indicated path. If the file (with '.rda' extension) does not exist in this path, the function indicated by the .FUN argument is executed. Alternatively, if `.load.it` is `FALSE` the function will be executed regardless of whether the file exists. Users should see the weaver package for a more 'correct' approach for cacheing long computations. The following code chunk checks that that the variables specific to the directory structure on my computer exist. Users should modify the `outdir` and `datadir` variables as appropriate.

```
> if (!file.exists(outdir)) stop("Please specify valid directory for storing output")
> if (!file.exists(datadir)) stop("Please specify the correct path to the CEL files")
```

2 Preprocessing Illumina IDAT files, genotyping, and copy number estimation

To perform copy number analysis on the Illumina platform, several steps are required. The first step is to read in the IDAT files and create a container for storing the red and green intensities. These intensities are quantile normalized in the function `crlmmIllumina`, and then genotyped using the crlmm algorithm. Details on the crlmm genotyping algorithm are described elsewhere. We will make use of the normalized intensities when we estimate copy number. The object returned by the `genotype.Illumina` function is an instance of the `CNSet` class, a container for storing the genotype calls, genotype confidence scores, the normalized intensities for the A and B channels, and summary statistics on the batches. The batch variable can be the date on which the array was scanned or the 96 well microarray chemistry plate on which the samples were processed. Both date and chemistry plate are useful surrogates for experimental factors that effect subgroups of the probe intensities. (Quantile normalization does not remove batch effects.) The genotype confidence scores are saved as an integer, and can be converted back to a $[0, 1]$ probability scale by the transformation $round(-1000 * log2(1 - p))$.

```
> samplesheet = read.csv(file.path(datadir, "HumanHap370Duo_Sample_Map.csv"),
     header = TRUE, as.is = TRUE)
> samplesheet <- samplesheet[-c(28:46, 61:75, 78:79), ]
> arrayNames <- file.path(datadir, unique(samplesheet[,
     "SentrixPosition"]))
> grnfiles = all(file.exists(paste(arrayNames, "_Grn.idat",
     sep = "")))
> redfiles = all(file.exists(paste(arrayNames, "_Red.idat",
     sep = "")))
> load.it <- TRUE
> if (!load.it) {
     rmFiles <- list.files(outdir, pattern = ".ff", full.names = TRUE)
     unlink(rmFiles)
 }
> plate <- samplesheet$Plate
> table(plate)

plate
WG1000442-DNA WG1000443-DNA WG1000444-DNA WG1000445-DNA WG1000446-DNA
            3             3             3             3             3
WG1000447-DNA WG1000449-DNA WG1000450-DNA WG1000451-DNA WG1000452-DNA
            4             4             3             2             4
WG1000453-DNA WG1000454-DNA WG1000456-DNA WG1000462-DNA WG1000464-DNA
            3             4             1             2             1

> batch <- as.factor(rep("1", nrow(samplesheet)))
> container <- checkExists("container", .path = outdir,
     .FUN = crlmm:::genotype.Illumina, sampleSheet = samplesheet,
```

```
    path = dirname(arrayNames[1]), arrayInfoColNames = list(barcode = NULL,
        position = "SentrixPosition"), cdfName = "human370v1c",
    batch = batch, .load.it = load.it)
> GT.CONF.THR <- 0.8
> cnSet <- checkExists("cnSet", .path = outdir, .FUN = crlmmCopynumber,
    object = container, .load.it = "FALSE", GT.CONF.THR = GT.CONF.THR)
```

The `cnSet` returned by the `crlmmCopynumber` function contains all of the parameters used to compute allele-specific copy number. In order to reduce I/O and speed computation, the allele-specific copy number estimates are not written to file nor saved in the `CNSet` object. Rather, allele-specific estimates are computed on the fly by the functions `CA`, `CB`, or `totalCopynumber`. The following codechunks provide a few examples.

Copy number for polymorphic markers on chromosomes 1 - 22.

```
> snp.index <- which(isSnp(cnSet) & !is.na(chromosome(cnSet)) &
    chromosome(cnSet) < 22)
> ca <- CA(cnSet, i = snp.index, j = 1:5)
> cb <- CB(cnSet, i = snp.index, j = 1:5)
> ct <- ca + cb
```

Alternatively, total copy number can be obtained by

```
> ct2 <- totalCopynumber(cnSet, i = snp.index, j = 1:5)
> stopifnot(all.equal(ct, ct2))
```

Missing values can arise at polymorphic when the confidence score of the genotype calls are below the threshold indicated by the threshold `GT.CONF.THR` in `crlmmCopynumber`. See `?crlmmCopynumber` for additional details. In the following codechunk, we compute the number of samples that had confidence scores below 0.8 at loci for which $\widehat{C}A$ and $\widehat{C}B$ are missing.

```
> missing.copynumber <- which(rowSums(is.na(ct)) > 0)
> if (length(missing.copynumber) > 0) {
    invisible(open(snpCallProbability(cnSet)))
    gt.confidence <- i2p(snpCallProbability(cnSet)[snp.index,
        ])
    n.below.threshold <- rowSums(gt.confidence < 0.95)
    unique(n.below.threshold[missing.copynumber])
 }
```

At nonpolymorphic loci, either the `CA` or `totalCopynumber` functions can be used to obtain estimates of total copy number.

```
> marker.index <- which(!isSnp(cnSet) & chromosome(cnSet) <
    23)
> ct <- CA(cnSet, i = marker.index, j = 1:5)
> stopifnot(all(CB(cnSet, i = marker.index, j = 1:5) ==
    0))
> ct2 <- totalCopynumber(cnSet, i = marker.index, j = 1:5)
> stopifnot(all.equal(ct, ct2))
```

Nonpolymorphic markers on chromosome X:

```
> npx.index <- which(chromosome(cnSet) == 23 & !isSnp(cnSet))
> M <- sample(which(cnSet$gender == 1), 5)
> F <- sample(which(cnSet$gender == 2), 5)
> cn.M <- CA(cnSet, i = npx.index, j = M)
> cn.F <- CA(cnSet, i = npx.index, j = F)
> boxplot(data.frame(cbind(cn.M, cn.F)), pch = ".", col = "grey60",
    outline = FALSE)
```

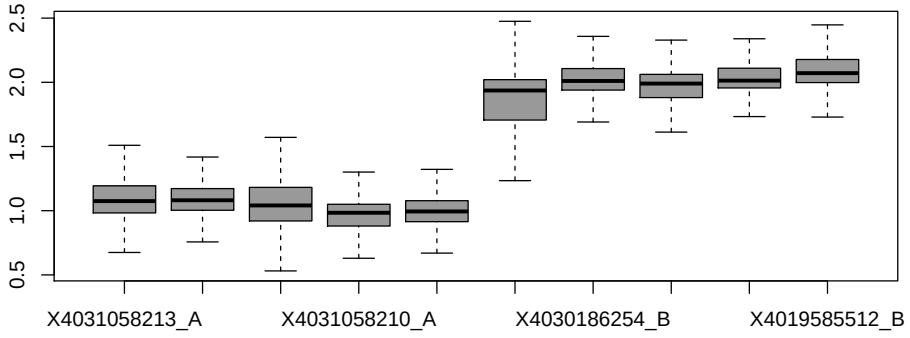


Figure 1: Copy number estimates for nonpolymorphic loci on chromosome X (5 men, 5 women). crlmm assumes that the median copy number across samples at a given marker on X is 1 for men and 2 for women.

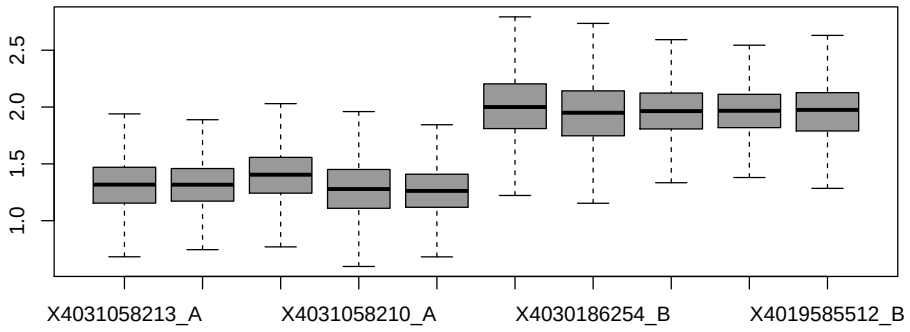


Figure 2: Copy number estimates for polymorphic markers on chromosome X.

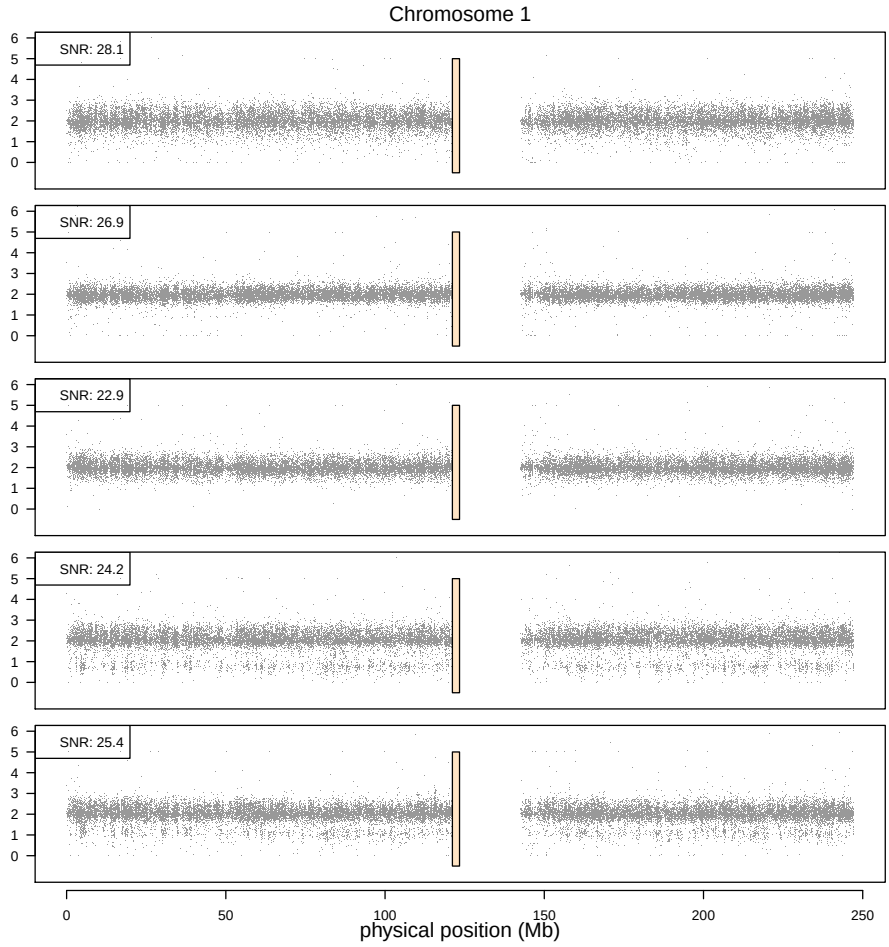
```
> X.markers <- which(isSnp(cnSet) & chromosome(cnSet) ==
    23)
> ca.M <- CA(cnSet, i = X.markers, j = M)
> cb.M <- CB(cnSet, i = X.markers, j = M)
> ca.F <- CA(cnSet, i = X.markers, j = F)
> cb.F <- CB(cnSet, i = X.markers, j = F)
> cn.M <- ca.M + cb.M
> cn.F <- ca.F + cb.F
> boxplot(data.frame(cbind(cn.M, cn.F)), pch = ".", outline = FALSE,
    col = "grey60")
> cn2 <- totalCopynumber(cnSet, i = X.markers, j = c(M,
    F))
> stopifnot(all.equal(cbind(cn.M, cn.F), cn2))
```

Often it is of interest to smooth the total copy number estimates (the sum of the allele-specific copy number) as a function of the physical position. The following code chunk can be used to create an instance of `CopyNumberSet` that contains the CA + CB at polymorphic markers and 'CA' at nonpolymorphic markers.

```
> marker.index <- which(chromosome(cnSet) <= 22)
> sample.index <- 1:5
> invisible(open(cnSet))
> copynumberSet <- as(cnSet[marker.index, 1:5], "CopyNumberSet")
> invisible(close(cnSet))
> copynumberSet <- copynumberSet[order(chromosome(copynumberSet),
     position(copynumberSet)), ]
> indices <- split(1:nrow(copynumberSet), chromosome(copynumberSet))
> dup.index <- unlist(sapply(indices, function(i, position) i[duplicated(position[i])],
     position = position(copynumberSet)))
> if (length(dup.index) > 0) copynumberSet <- copynumberSet[-dup.index,
     ]
> missing.index <- which(rowSums(is.na(copyNumber(copynumberSet))) ==
     ncol(copynumberSet))
> if (length(missing.index) > 0) {
     table(chromosome(copynumberSet)[missing.index])
     copynumberSet <- copynumberSet[-missing.index, ]
 }
```

We suggest plotting the total copy number as a function of the physical position to evaluate whether a wave-correction is needed.

```
> require(SNPchip)
> data(chromosomeAnnotation)
> chrAnn <- chromosomeAnnotation[1, ]
> layout(matrix(1:5, 5, 1), heights = c(1, 1, 1, 1, 1))
> par(mar = c(0.5, 0.5, 0.5, 0.5), las = 1, oma = c(4,
     4, 4, 4))
> i <- which(chromosome(copynumberSet) == 1)
> x <- position(copynumberSet)[i]
> for (j in 1:5) {
     plot(x, copyNumber(copynumberSet)[i, j], pch = ".",
         cex = 0.6, col = "grey60", xaxt = "n", ylim = c(-1,
             6), ylab = "total copy number", yaxt = "n",
         xlim = c(0, max(x)))
     legend("topleft", legend = paste("SNR:", round(copynumberSet$SNR[j],
         1)))
     axis(2, at = 0:6, labels = 0:6)
     if (j == 5) {
         mtext("physical position (Mb)", 1, outer = TRUE,
             line = 2)
         mtext("Chromosome 1", 3, line = 0, outer = TRUE)
     }
     xx <- c(chrAnn[1:2], chrAnn[2:1])
     yy <- c(-0.5, -0.5, 5, 5)
     polygon(xx, yy, col = "bisque")
 }
> axis(1, pretty(x, n = 8), labels = pretty(x, n = 8)/1e+06,
     outer = TRUE, line = 0)
```



3 Smoothing marker-level estimates of total copy number

In this section, we show how the total copy number can be smoothed using either the hidden Markov model implemented in the R package VanillaICE or circular binary segmentation implemented in the R packageDNAcopy.

Smoothing via a hidden Markov model.

```
> if (require(VanillaICE)) {
      hmmOpts <- hmm.setup(copynumberSet, c("hom-del",
          "hem-del", "normal", "amp"), copynumberStates = 0:3,
          normalIndex = 3, log.initialP = rep(log(1/4),
              4))
      timing <- system.time(fit.cn <- hmm(copynumberSet,
          hmmOpts, TAUP = 1e+10, verbose = FALSE))
      hmm.df <- as.data.frame(fit.cn)
      print(hmm.df[1:5, c(2:4, 7:9)])
 }

      start       end     width state numMarkers LLR
1 142649580 247189073 104539494     3      12845   0
2      8856  94730234  94721379     3      12244   0
```

```
3     41894  90576572  90534679     3      11704   0
4      7764  52495853  52488090     3       6467   0
5     73747  46384240  46310494     3       5883   0
```

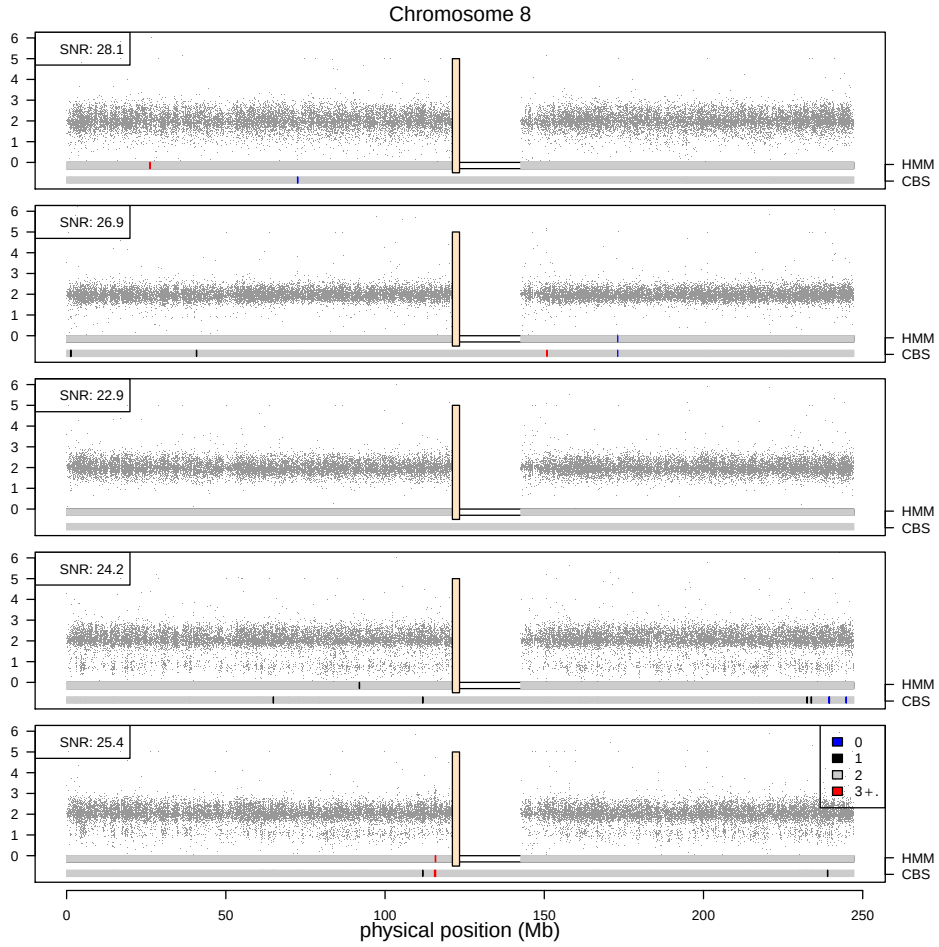
Smoothing via segmentation.

```
> library("DNAcopy")
> CNA.object <- CNA(genomdat = copyNumber(copynumberSet),
      chrom = chromosome(copynumberSet), maploc = position(copynumberSet),
      data.type = "logratio", sampleid = sampleNames(copynumberSet))
> smu.object <- smooth.CNA(CNA.object)
> if (!load.it) unlink(file.path(outdir, "cbs.segments.rda"))
> cbs.segments <- checkExists("cbs.segments", .path = outdir,
      .FUN = segment, x = smu.object, .load.it = load.it)
> cbs.segments <- cbind(cbs.segments$output, cbs.segments$segRows)
> cbs.segments$call <- rep(3, nrow(cbs.segments))
> cbs.segments$call[cbs.segments$seg.mean > 2.5] <- 4
> cbs.segments$call[cbs.segments$seg.mean < 1.25 & cbs.segments$seg.mean >
      0.75] <- 2
> cbs.segments$call[cbs.segments$seg.mean < 0.75] <- 1
> cbs.ir <- RangedData(IRanges(cbs.segments$loc.start,
      cbs.segments$loc.end), chrom = cbs.segments$chrom,
      numMarkers = cbs.segments$num.mark, seg.mean = cbs.segments$seg.mean,
      cnCall = cbs.segments$call, sampleId = substr(cbs.segments$ID,
          2, 13))
```

Visualizing inferred CNV. In the following code chunk, we plot the total copy number versus physical position for chromosome 1 and overlay the copy number predictions from the HMM and a CNV call from the segmentation obtained by thresholding the segment means. We restrict our focus to regions that have 3 or more markers.

```
> require(SNPchip)
> CHR <- 1
> cnSet2 <- copynumberSet
> fit.cn2 <- fit.cn[fit.cn$numMarkers >= 3, ]
> cbs.ir2 <- cbs.ir[cbs.ir$numMarkers >= 3, ]
> copynumberSet2 <- copynumberSet[which(chromosome(copynumberSet) ==
      CHR), ]
> layout(matrix(1:5, 5, 1), heights = c(1, 1, 1, 1, 1))
> par(mar = c(0.5, 0.5, 0.5, 0.5), las = 1, oma = c(4,
      4, 4, 4))
> for (j in 1:5) {
      fit.cn3 <- fit.cn2[fit.cn2$chrom == CHR & fit.cn2$sampleId ==
          sampleNames(copynumberSet2)[j], ]
      cbs.ir3 <- cbs.ir2[cbs.ir2$chrom == CHR & cbs.ir2$sampleId ==
          sampleNames(copynumberSet2)[j], ]
      plot(position(copynumberSet2), copyNumber(copynumberSet2)[,
          j], pch = ".", cex = 0.6, col = "grey60", xaxt = "n",
          ylim = c(-1, 6), ylab = "total copy number",
          yaxt = "n", xlim = c(0, max(position(copynumberSet2))))
      legend("topleft", legend = paste("SNR:", round(copynumberSet2$SNR[j],
          1)))
      axis(2, at = 0:6, labels = 0:6)
      if (j == 5) {
```

```
        mtext("physical position (Mb)", 1, outer = TRUE,
            line = 2)
        mtext("Chromosome 8", 3, line = 0, outer = TRUE)
        at <- pretty(position(copynumberSet2))
    }
    w <- width(fit.cn3)
    fit.cn3 <- fit.cn3[order(w, decreasing = TRUE), ]
    col <- c("blue", "black", "grey80", "red")
    x <- c(min(start(fit.cn3)), max(end(fit.cn3)))
    xx <- c(x, rev(x))
    y <- rep(c(-0.3, 0), each = 2)
    polygon(xx, y, col = "white")
    for (i in seq_along(w)) {
        x <- c(start(fit.cn3)[i], end(fit.cn3)[i])
        xx <- c(x, rev(x))
        statecolor <- col[fit.cn3$state[i]]
        polygon(xx, y, col = statecolor, border = statecolor)
    }
    w <- width(cbs.ir3)
    cbs.ir3 <- cbs.ir3[order(w, decreasing = TRUE), ]
    y <- rep(c(-1, -0.7), each = 2)
    for (i in seq_along(w)) {
        x <- c(start(cbs.ir3)[i], end(cbs.ir3)[i])
        xx <- c(x, rev(x))
        statecolor <- col[cbs.ir3$cnCall[i]]
        polygon(xx, y, col = statecolor, border = statecolor)
    }
    axis(4, at = c(-0.9, -0.1), c("CBS", "HMM"), cex = 0.8)
    if (j == 5) {
        legend("topright", fill = col, legend = c(0,
            1, 2, expression(3 + .)))
        labels <- at/1e+06
        axis(1, at = at, labels = labels, outer = T,
            line = 0)
    }
    xx <- c(chrAnn[1:2], chrAnn[2:1])
    yy <- c(-0.5, -0.5, 5, 5)
    polygon(xx, yy, col = "bisque")
}
```



TODO: Samples 4 and 5 have a sparse band of CN-estimates near 1 that is uniformly distributed across chromosome 1. This suggests markers that are either not well fit by the linear model, or a problem with the genotyping. Need to check.

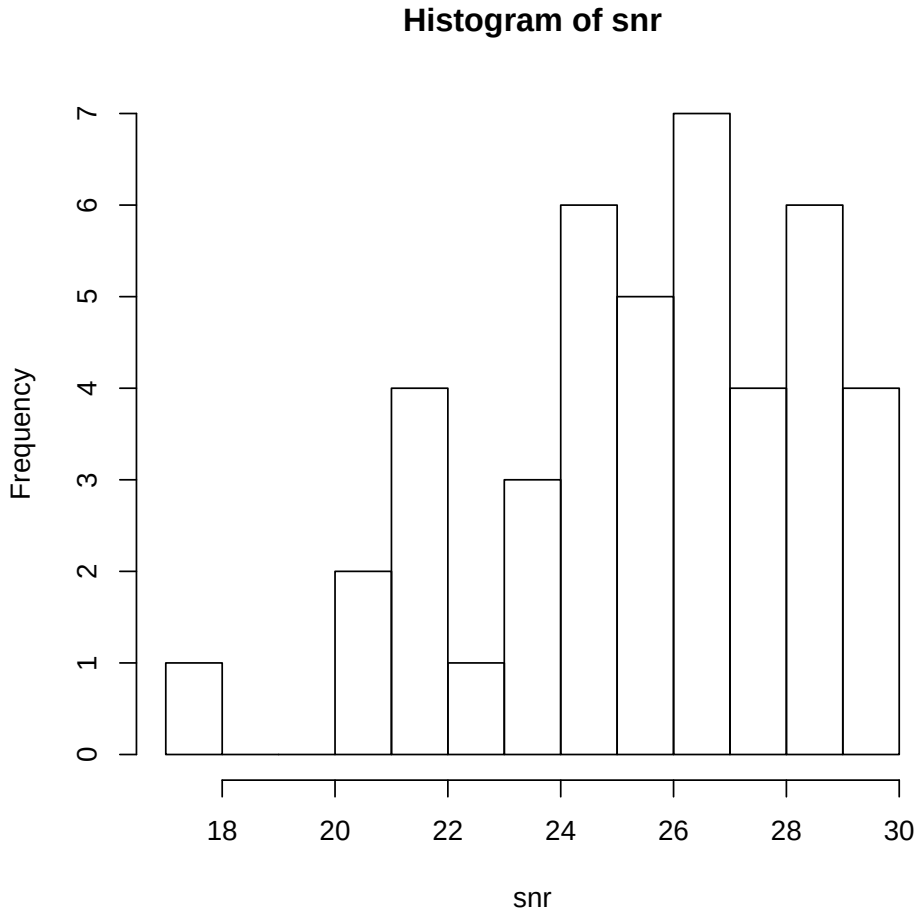
```
> fit.cn <- fit.cn2
> cbs.ir <- cbs.ir2
> nm.hmm <- sapply(split(fit.cn$numMarkers, fit.cn$sampleId),
    sum)
> stopifnot(length(unique(nm.hmm)) == 1)
> nm.cbs <- sapply(split(cbs.ir$numMarkers, cbs.ir$sampleId),
    sum)
> stopifnot(length(unique(nm.cbs)) == 1)
> stopifnot(all.equal(nm.cbs, nm.hmm))
> hmm.states <- rep(fit.cn$state, fit.cn$numMarkers)
> sample.id <- rep(fit.cn$sampleId, fit.cn$numMarkers)
> hmm.states <- split(hmm.states, sample.id)
> cbs.states <- rep(cbs.ir$cnCall, cbs.ir$numMarkers)
> sample.id <- rep(cbs.ir$sampleId, cbs.ir$numMarkers)
> cbs.states <- split(cbs.states, sample.id)
> tabs <- vector("list", length(cbs.states))
> for (i in seq_along(cbs.states)) tabs[[i]] <- table(cbs.states[[i]],
    hmm.states[[i]])
> names(tabs) <- names(cbs.states)
```

Plots of the signal to noise ratio of the sample – an overall measure of how well the genotype clusters separate – can be useful for identifying samples of lower quality. (Note that this statistic tends to be much higher for Illumina than for the Affymetrix platforms.)

```
> open(cnSet$SNR)
```

```
[1] FALSE
```

```
> snr <- cnSet$SNR[, ]
> hist(snr, breaks = 15)
```



We plot the total copy number estimates versus physical position the two samples with the lowest (top) and highest (bottom) signal to noise ratios.

```
> low.snr <- which(snr == min(snr))
> high.snr <- which(snr == max(snr))
> marker.index <- which(chromosome(cnSet) == 1)
> x <- position(cnSet)[marker.index]
> cn.total <- totalCopynumber(cnSet, i = marker.index,
      j = c(low.snr, high.snr))
> snrs <- round(snr[c(low.snr, high.snr)], 2)
> par(mfrow = c(2, 1), las = 1, mar = c(0.5, 4, 0.5, 0.5),
      oma = c(4, 1, 1, 1))
> for (j in 1:2) {
```

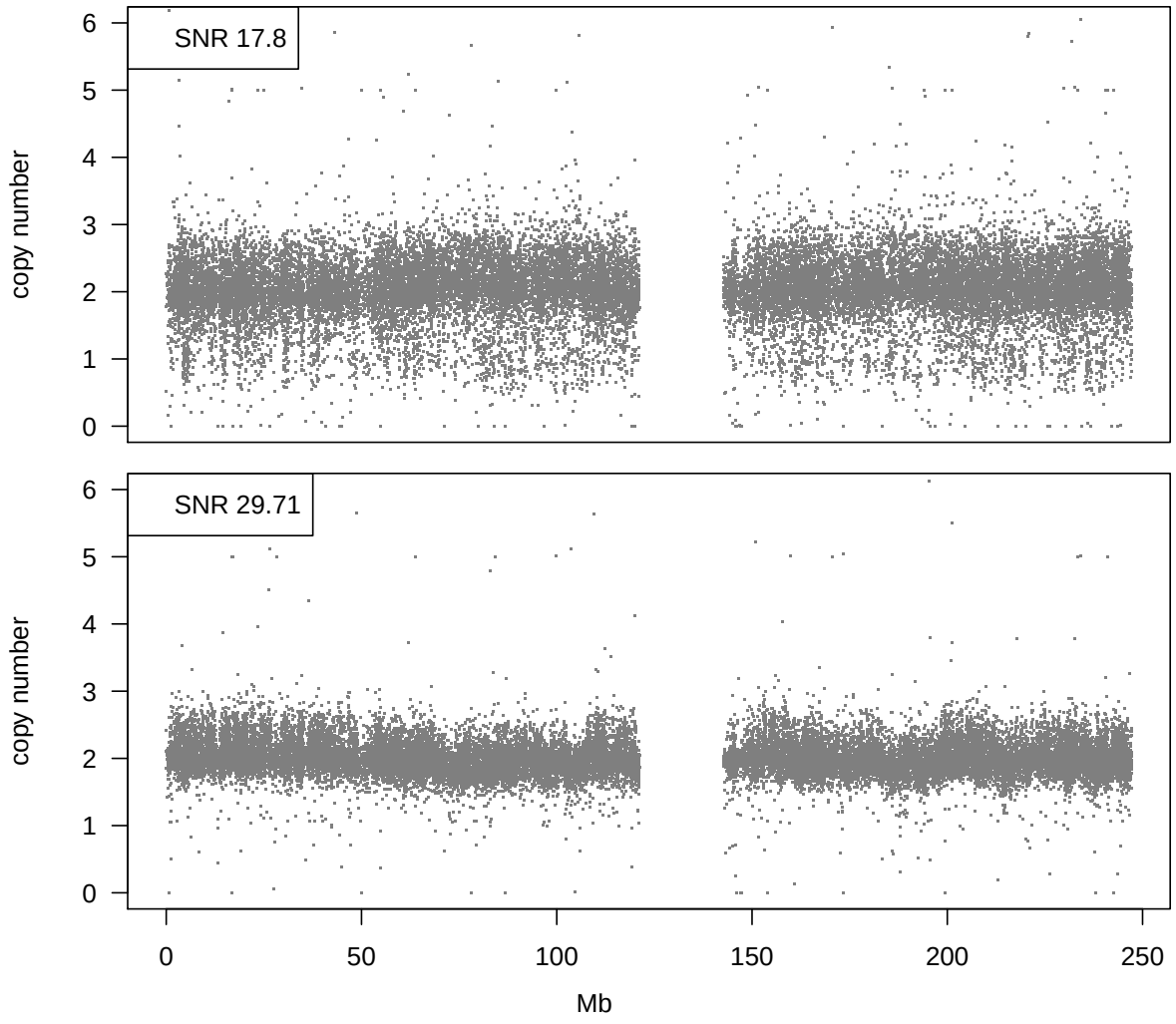


Figure 3: Copy number estimates for samples with low signal to noise ratio (top) and high signal to noise ratio (bottom). The waves in the scatterplots are often correlated with GC-content and can be removed by scatterplot smoothers or any of the published wave-correction tools for genotyping arrays.

```
    cn <- cn.total[, j]
    plot(x, cn, pch = ".", ylab = "copy number", xaxt = "n",
        ylim = c(0, 6), col = "grey50")
    legend("topleft", legend = paste("SNR", snrs[j]))
}
> axis(1, at = pretty(x), labels = pretty(x/1e+06))
> mtext("Mb", 1, outer = TRUE, line = 2)
```

4 Session information

```
> toLatex(sessionInfo())
```

- R version 2.13.0 Under development (unstable) (2010-11-13 r53582), `x86_64-unknown-linux-gnu`

- Locale: LC_CTYPE=en_US.iso885915, LC_NUMERIC=C, LC_TIME=en_US.iso885915, LC_COLLATE=en_US.iso885915, LC_MONETARY=C, LC_MESSAGES=en_US.iso885915, LC_PAPER=en_US.iso885915, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.iso885915, LC_IDENTIFICATION=C
- Base packages: base, datasets, graphics, grDevices, methods, stats, tools, utils
- Other packages: Biobase 2.11.6, bit 1.1-4, crlmm 1.9.6, DNAcopy 1.23.8, ff 2.1-4, IRanges 1.7.37, oligoClasses 1.13.5, SNPchip 1.13.0, VanillaICE 1.13.1
- Loaded via a namespace (and not attached): affyio 1.19.2, annotate 1.29.2, AnnotationDbi 1.13.2, Biostrings 2.19.0, DBI 0.2-5, ellipse 0.3-5, genefilter 1.33.0, mvtnorm 0.9-92, preprocessCore 1.13.1, RSQLite 0.9-3, splines 2.13.0, survival 2.36-1, xtable 1.5-6