# Estimating copy number for Affymetrix 6.0 with the crlmm Package

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## 1 Estimating copy number

At present, software for copy number estimation is provided only for the Affymetrix 6.0 platform. This vignette estimates copy number for the HapMap samples.

### 1.1 Preprocess and genotype the samples

We preprocess and genotype the samples as described in the CRLMM vignette.

```
R> library(crlmm, lib.loc = "~/Rlibs/devel")
R> library(genomewidesnp6Crlmm)
```

Specify the complete path for the CEL files and a directory in which to store intermediate files:

```
R> celFiles <- list.celfiles("/thumper/ctsa/snpmicroarray/hapmap/raw/affy/1m",
     full.names = TRUE, pattern = ".CEL")
R> outdir <- "/thumper/ctsa/snpmicroarray/rs/data/hapmap/1m/affy"
```

Preprocess and genotype (for more info see the crlmm vignette).

```
R> if (!exists("crlmmResult")) {
     if (file.exists(file.path(outdir, "crlmmResult.rda"))) {
         load(file.path(outdir, "intensities.rda"))
         load(file.path(outdir, "crlmmResult.rda"))
     }
     else {
         crlmmResult <- crlmm(celFiles, save.it = TRUE,
             intensityFile = file.path(outdir,
                 "intensities.rda"))
         save(crlmmResult, file = file.path(outdir,
             "crlmmResult.rda"))
     }
 }
```

Quantile normalize the nonpolymorphic probes and save the results.

```
R> if (!exists("cnrmaResult")) {
     if (file.exists(file.path(outdir, "cnrmaResult.rda")))
         load(file.path(outdir, "cnrmaResult.rda"))
     else {
         cnrmaResult <- cnrma(celFiles)
         save(cnrmaResult, file = file.path(outdir,
             "cnrmaResult.rda"))
     }
 }
```

## 1.2 Copy number

We require 6 items for copy number estimation:

- quantile-normalized A intensities (I1 x J)
- quantile-normalized B intensities (I1 x J)
- quantile-normalized intensities from nonpolymorphic (NP) probes (I2 x J)
- genotype calls (I1 x J)
- confidence scores of the genotype calls (I1 x J)
- signal to noise ratio (SNR) of the samples (J)

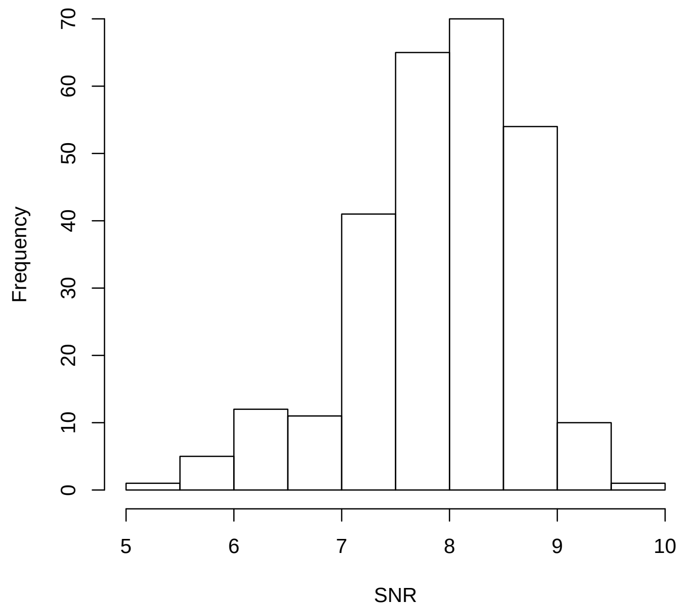
These items are extracted as follows:

```
R> A <- res$A
R> B <- res$B
R> calls <- crlmmResult$calls
R> conf <- crlmmResult$conf
R> SNR <- crlmmResult$SNR
R> NP <- cnrmaResult$NP
R> gc()

            used (Mb) gc trigger   (Mb)  max used   (Mb)
Ncells   3220451  172    4418719  236.0   4170209  222.8
Vcells 684978505 5226 1020629716 7786.8 722115390 5509.4
```

A histogram of the signal to noise ratio for the HapMap samples:

```
R> hist(SNR, xlab = "SNR", main = "")
```



We suggest excluding samples with a signal to noise ratio less than 5. As batch effects can be very large in the quantile-normalized intensitie, we suggest adjusting for date or chemistry plate. Ideally, one would have 70+ files in a given batch. For the HapMap date, we define batch by the chemistry plate.

```
R> sns <- colnames(calls)
R> sns[1]

[1] "NA06985_GW6_C.CEL"

R> plate <- substr(basename(sns), 13, 13)
R> table(plate)

plate
 A  C  Y
90 90 90
```

Intermediate files as well as the copy number estimates are stored in an environment created by the user. The intermediate files can be useful for creating SNP-level plots.

```
R> if (!exists("env")) {
     CHR <- 22
     if (!file.exists(file.path(outdir, paste("env_chr",
        CHR, ".rda", sep = "")))) {
        env <- new.env()
        computeCopynumber(chrom = CHR, A = A,
            B = B, calls = calls, conf = conf,
            NP = NP, plate = plate, envir = env,
            DF.PRIOR = 50, MIN.OBS = 1, SNR = SNR,
            SNRmin = 5)
        save(env, file = file.path(outdir, paste("env_chr",
            CHR, ".rda", sep = "")))
    }
    else {
        load(file.path(outdir, paste("env_chr",
            CHR, ".rda", sep = "")))
    }
}
```

A class representation would be useful for this sort of data (TODO). The oligoSnpSet class used below is a bit inefficient as the assay data elements are forced to be the same size. The advantage is that all of the assay data is bound to the meta-data.

```
R> require(oligoClasses)
R> data(snpProbes, package = "genomewidesnp6Crlmm")
R> data(cnProbes, package = "genomewidesnp6Crlmm")
R> position <- snpProbes[match(env[["snps"]], rownames(snpProbes)),
     "position"]
R> position.np <- cnProbes[match(rownames(env[["NP"]]),
     rownames(cnProbes)), "position"]
R> CA <- env[["CA"]]
R> CB <- env[["CB"]]
R> snpCT <- CA + CB
R> npCT <- env[["CT"]]
R> CT <- rbind(snpCT, npCT)
R> dimnames(CT) <- list(c(env[["snps"]], rownames(env[["NP"]])),
     env[["sns"]])
R> genotypes <- matrix(NA, nrow(CT), ncol(CT))
R> dimnames(genotypes) <- dimnames(CT)
R> genotypes[1:length(env[["snps"]]), ] <- env[["calls"]]
R> polymorphic <- c(rep(1, length(env[["snps"]])),
     rep(0, nrow(npCT)))
R> fD <- new("AnnotatedDataFrame", data = data.frame(chromosome = rep(CHR,
     nrow(CT)), polymorphic = polymorphic, position = c(position,
     position.np)), varMetadata = data.frame(labelDescription = c("chromosome",
     "polymorphic", "position")))
R> locusSet <- new("oligoSnpSet", copyNumber = CT,
     calls = genotypes, featureData = fD, phenoData = annotatedDataFrameFrom(CT,
         byrow = FALSE), annotation = "genomewidesnp6")
```

Note that an indicator for the polymorphic probes was created in the code chunk above. I use this indicator to smooth the estimates of copy number from the nonpolymorphic and polymorphic probes separately. The uncertainty estimates (work in progress) should reflect that the nonpolymorphic probes have more variance and the smoothing will take this into account. The above algorithm for estimating copy number is predicated on the assumption that most samples within a batch have copy number 2 at any given SNP. For common copy number variants, this assumption may not hold. An additional iteration using a bias correction can improve the estimates. Set the `bias.adj` argument to `TRUE`.

## 2 Suggested plots

**One sample at a time** Plot physical position versus copy number for the first sample. Recall that the copy number estimates were multiplied by 100 and stored as an integer.

```
R> require(ellipse)
R> par(las = 1)
R> plot(position(locusSet), copyNumber(locusSet)[,
     1]/100, pch = ".", cex = 2, xaxt = "n", col = "grey70",
     ylim = c(0, 6), ylab = "copy number", xlab = "physical position (Mb)",
     main = paste(sampleNames(locusSet)[1], ", CHR:",
         unique(chromosome(locusSet))))
R> axis(1, at = pretty(range(position(locusSet))),
     labels = pretty(range(position(locusSet)))/1e+06)
R> I <- fData(locusSet)$polymorphic == 0
R> points(position(locusSet)[I], copyNumber(locusSet)[I,
     1]/100, pch = ".", col = "blue", cex = 1)

R> require(SNPchip)
R> plotCytoband(22, new = FALSE, cytoband.ycoords = c(5.8,
     6), label.cytoband = FALSE)
```

In this example, the estimates of copy number for the nonpolymorphic probes appear correlated with the polymorphic probes – a good sign.

**One SNP at a time** This section needs to be cleaned up (TODO). The parameters needed for drawing prediction regions are plate-specific.

```
R> tau2A <- env[["tau2A"]]
R> tau2B <- env[["tau2B"]]
R> sig2A <- env[["sig2A"]]
R> sig2B <- env[["sig2B"]]
R> nuA <- env[["nuA"]]
R> nuB <- env[["nuB"]]
```

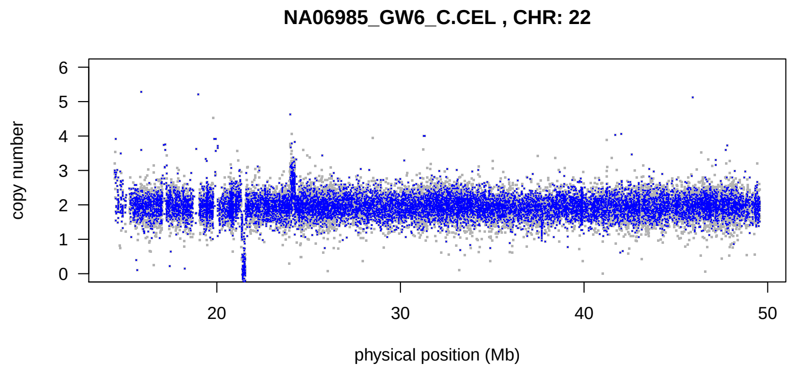


Figure 1: Total copy number (y-axis) for chromosome 22 plotted against physical position (x-axis) for one sample.

```
R> phiA <- env[["phiA"]]
R> phiB <- env[["phiB"]]
R> corr <- env[["corr"]]
R> corrA.BB <- env[["corrA.BB"]]
R> corrB.AA <- env[["corrB.AA"]]
R> A <- env[["A"]]
R> B <- env[["B"]]
```

Plot the prediction regions for total copy number 2 and 3 for the first plate. Plotting symbols are the genotype calls (1=AA, 2=AB, 3=BB); light grey points are from other plates. One could also add the prediction regions for 0-4 copies, but it gets crowded.

```
R> par(las = 1, pty = "s")
R> p <- 1
R> J <- grep(unique(plate)[p], plate)
R> ylim <- c(6.5, 13)
R> I <- which(phiA > 10 & phiB > 10)
R> i <- I[1]
R> log2(phiA[i, ])

[1] 9.071797 8.989059 8.876777

R> log2(phiB[i, ])

[1] 8.533698 8.419028 8.741665

R> plot(log2(A[i, ]), log2(B[i, ]), pch = as.character(calls[i,
     ]), col = "grey60", cex = 0.9, ylim = ylim,
     xlim = ylim, xlab = "A", ylab = "B")
R> points(log2(A[i, J]), log2(B[i, J]), col = "black",
     pch = as.character(calls[i, J]))
R> for (CT in 2) {
     if (CT == 2)
         ellipse.col <- "black"
     for (CA in 0:CT) {
         CB <- CT - CA
         A.scale <- sqrt(tau2A[i, p] * (CA == 0) +
             sig2A[i, p] * (CA > 0))
         B.scale <- sqrt(tau2B[i, p] * (CB == 0) +
             sig2B[i, p] * (CB > 0))
         scale <- c(A.scale, B.scale)
         if (CA == 0 & CB > 0)
             rho <- corrA.BB[i, p]
         if (CA > 0 & CB == 0)
             rho <- corrB.AA[i, p]
         if (CA > 0 & CB > 0)
             rho <- corr[i, p]
         lines(ellipse(x = rho, centre = c(log2(nuA[i,
             p] + CA * phiA[i, p]), log2(nuB[i,
             p] + CB * phiB[i, p])), scale = scale),
             col = ellipse.col, lwd = 2)
     }
 }
R> legend("topright", lwd = 3, col = "black", legend = "2 copies",
     bty = "n")
```

Look at the distribution of shifts in the predicted centers across the plates. The biggest shifts are for SNPs that have no observations in a subset of the plates – need more shrinkage here.

Now look at shifts for which we have at least 3 observations in each genotype cluster (TO DO).

## 3 Session information

```
R> sessionInfo()

R version 2.9.0 Under development (unstable) (2009-02-08 r47879)
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_

attached base packages:
[1] stats     graphics  grDevices utils     datasets
```

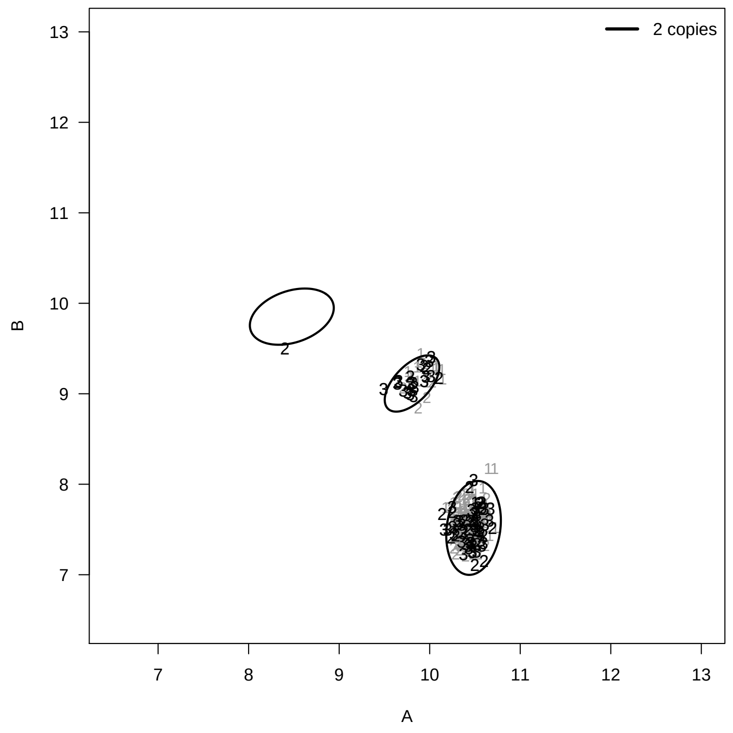


Figure 2: Prediction regions for copy number 2 for one SNP. The plate effects are negligible for this SNP and we only plot the prediction region for the first plate.

```
[6] methods   base

other attached packages:
[1] ellipse_0.3-5             oligoClasses_1.5.16
[3] Biobase_2.1.7             genomewidesnp6Crlmm_1.0.1
[5] crlmm_1.0.59

loaded via a namespace (and not attached):
 [1] affyio_1.11.3        annotate_1.21.3
 [3] AnnotationDbi_1.5.15 DBI_0.2-4
 [5] genefilter_1.23.2    mvtnorm_0.9-4
 [7] preprocessCore_1.5.3 RSQLite_0.7-1
 [9] splines_2.9.0        survival_2.34-1
[11] tools_2.9.0
```