Identifying Copy Number Polymorphisms

Jacob Carey, Steven Cristiano, and Robert Scharpf

April 30, 2018

Contents

1 Introduction

Identify consensus start and stop coordinates of a copy number polymorphism

The collection of copy number variants (CNVs) identified in a study can be encapulated in a GRangesList, where each element is a GRanges of the CNVs identified for an individual. (For a study with 1000 subjects, the GRangesList object would have length 1000 if each individual had 1 or more CNVs.) For regions in which CNVs occur in more than 2 percent of study participants, the start and end boundaries of the CNVs may differ because of biological differences in the CNV size as well as due to technical noise of the assay and the uncertainty of the breakpoints identified by a segmentation of the genomic data. Among subjects with a CNV called at a given locus, the `consensusCNP` function identifies the largest region that is copy number variant in half of these subjects.

2 Simulating CNP data

Included in the CNPBayes package are objects of class `SnpArrayExperiment` and `GRangesList`. We begin by loading the necessary libraries and data.

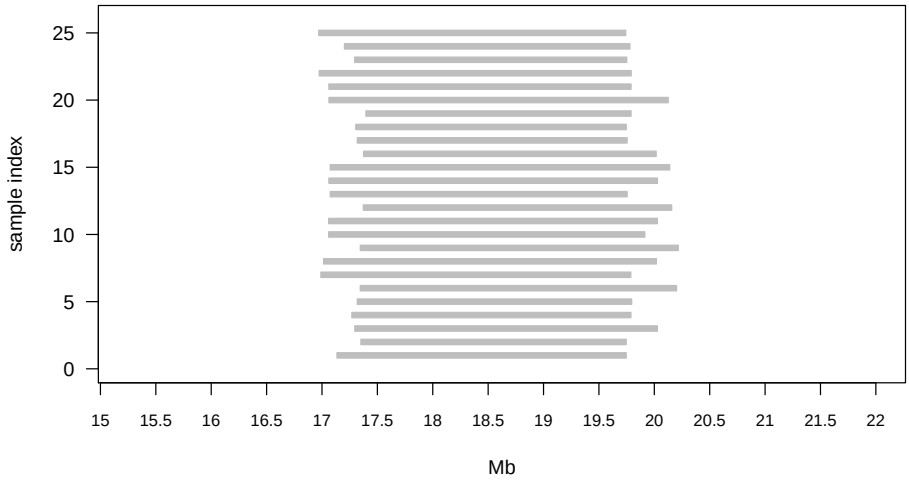
```
suppressMessages(library(CNPBayes))
suppressMessages(library(SummarizedExperiment))
se <- readRDS(system.file("extdata", "simulated_se.rds", package="CNPBayes"))
grl <- readRDS(system.file("extdata", "grl_deletions.rds", package="CNPBayes"))
```

The object `se` contains log R ratios and B Allele Frequencies, and the object `grl` is a `GRangesList` of simulated deletions.

3 Finding CNPs

After reading this saved data, we visualize the CNVs.

```
cnv.region <- consensusCNP(grl[1], max.width=5e6)
## unlist GRangesList...
## find consensus regions...
## .
## Dropping CNV regions failing min.width and max.width criteria. See ?consensusCNP to relax these settings.
i <- subjectHits(findOverlaps(cnv.region, rowRanges(se)))
## Loading required package: VanillaICE
## Welcome to VanillaICE version 1.42.0
xlim <- c(min(start(se)), max(end(se)))
par(las=1)
plot(0, xlim=xlim, ylim=c(0, 26), xlab="Mb", ylab="sample index", type="n",
     xaxt="n")
at <- pretty(xlim, n=10)
axis(1, at=at, labels=round(at/1e6, 1), cex.axis=0.8)
rect(start(grl), seq_along(grl)-0.2, end(grl), seq_along(grl)+0.2,
     col="gray", border="gray")
```

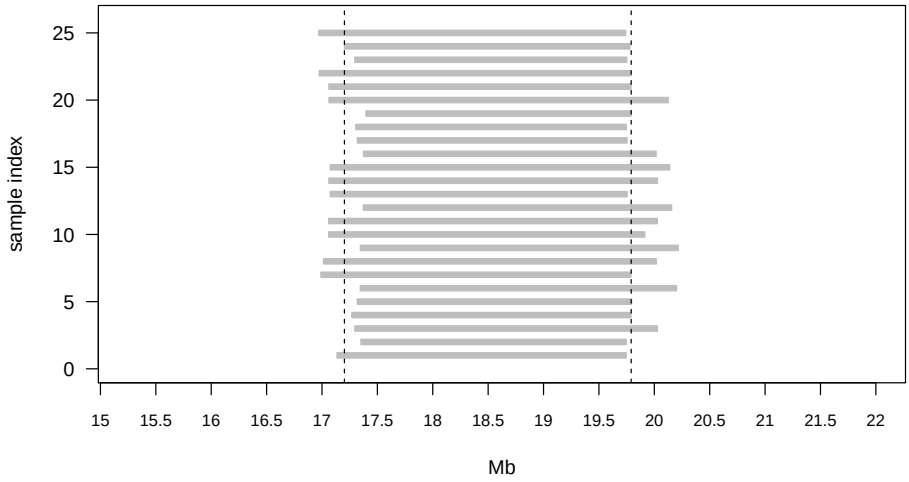


Before further analysis can be performed, the log R ratios in a CNV region must be summarized to a one dimensional object (Cardin et al. 2011). Within each CNP locus, log R ratios at each SNP are summarized by sample. Below are examples of using the median and the first principal component to create a one dimensional summary.

3.1 CNP Boundaries with Median Summary

To summarize samples within a CNV locus by the median log R ratios, we define the largest region that spans 50 percent of the samples using the function `consensusCNP`. Using log R ratios of SNPs contained in this region, the median is taken across samples. Because the deletions in this example are large ($>$ 2Mb), we specify a large value for `max.width` to avoid filtering these CNVs.

```
cnv.region <- consensusCNP(grl, max.width=5e6)
## unlist GRangesList...
## find consensus regions...
## .
## Dropping CNV regions failing min.width and max.width criteria. See ?consensusCNP to relax these settings.
i <- subjectHits(findOverlaps(cnv.region, rowRanges(se)))
med.summary <- matrixStats::colMedians(assays(se)[["cn"]][i, ], na.rm=TRUE)
par(las=1)
plot(0, xlim=xlim, ylim=c(0, 26), xlab="Mb", ylab="sample index", type="n",
     xaxt="n")
at <- pretty(xlim, n=10)
axis(1, at=at, labels=round(at/1e6, 1), cex.axis=0.8)
rect(start(grl), seq_along(grl)-0.2, end(grl), seq_along(grl)+0.2,
     col="gray", border="gray")
abline(v=c(start(cnv.region), end(cnv.region)), lty=2)
```

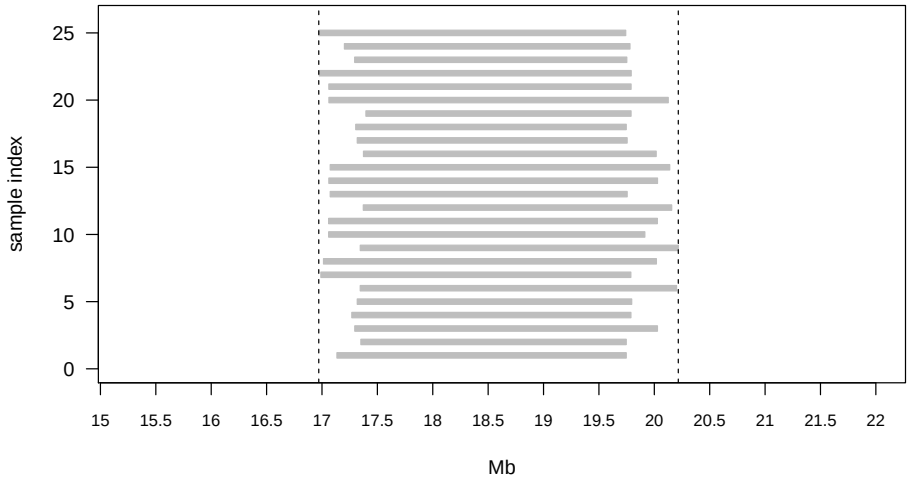


3.2 CNP Boundaries with PCA Summary

Another method for summarizing the log R ratios is by the first principal component on the markers for the entire region (Cardin et al. 2011). Disadvantages of this scale are interpretion of the copy number of the mixture components the potential for picking up batch effects rather than biological differences in copy number between groups of samples. The primary advantage of a principal component analysis is for non-germline events where the CNVs may be partially overlapping and do not share the same boundaries. We find that for germline copy number analyses, a median summary is adequate, retains an interpretable scale, and is less susceptible to batch effects than principal components.

```
cnv.region2 <- reduce(unlist(grl))
i.pc <- subjectHits(findOverlaps(cnv.region2, rowRanges(se)))
x <- assays(se)[["cn"]][i.pc, ]
nas <- rowSums(is.na(x))
na.index <- which(nas > 0)
x <- x[-na.index, , drop=FALSE]
pc.summary <- prcomp(t(x))$x[, 1]
meds.for.pc <- matrixStats::colMedians(x, na.rm=TRUE)
if(cor(pc.summary, meds.for.pc) < 0) pc.summary <- -1*pc.summary

par(las=1)
plot(0, xlim=xlim, ylim=c(0, 26), xlab="Mb", ylab="sample index", type="n",
     xaxt="n")
at <- pretty(xlim, n=10)
axis(1, at=at, labels=round(at/1e6, 1), cex.axis=0.8)
rect(start(grl), seq_along(grl)-0.2, end(grl), seq_along(grl)+0.2,
     col="gray", border="gray")
abline(v=c(start(cnv.region2),
           end(cnv.region2)), lty=2)
```

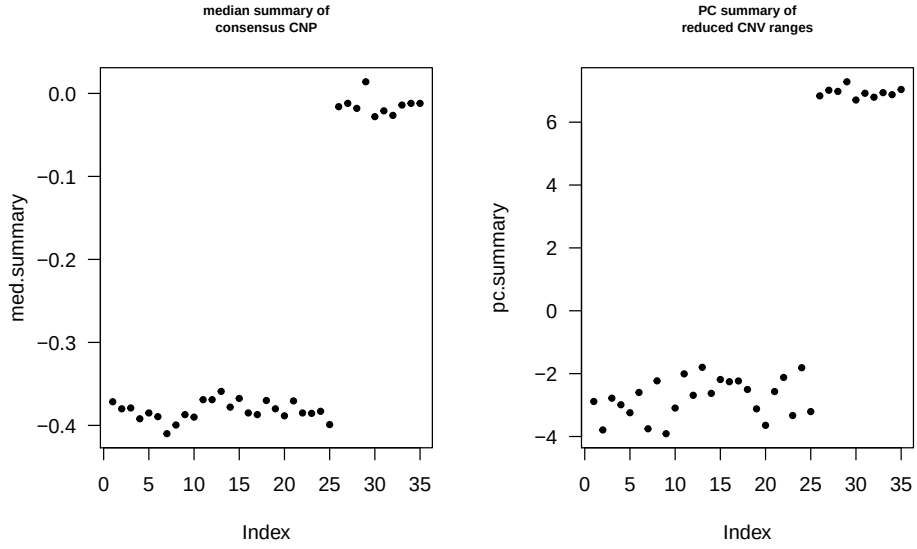


Note that boundaries of created by principal component summary method are wider than those in the above median summary method. In this example, the boundaries are not significantly different, but in samples with ragged starts and ends, the PCA method will provide greater coverage.

3.3 Summary Plots

Finally, we plot the one dimensional summaries.

```
par(mfrow=c(1,2), las=1)
plot(med.summary, main="median summary of\nconsensus CNP", cex.main=0.7, pch=20)
plot(pc.summary, main="PC summary of\nreduced CNV ranges", cex.main=0.7, pch=20)
```



3.4 Filtering

Loci with no duplications or deletions can be identified visually as a uni-modal normal distribution. A Shapiro-Wilk test test of normality (Shapiro and Wilk 1965) can be used to filter unimodal regions. To be conservative, we suggest retaining only those loci which have a p value < 0.1. In practice, we prefer Bayes factors or estimates of the marginal likelihood for model selection (see vignette `Overview.Rmd`).

```
shapiro.test(med.summary)
##
##  Shapiro-Wilk normality test
##
## data:  med.summary
## W = 0.63062, p-value = 3.652e-08
```

References

Cardin, Niall, Chris Holmes, Peter Donnelly, and Jonathan Marchini. 2011. "Bayesian Hierarchical Mixture Modeling to Assign Copy Number from a Targeted Cnv Array." *Genet. Epidemiol.* https://doi.org/10.1002/gepi.20604.

Shapiro, S. S., and M. B. Wilk. 1965. "An Analysis of Variance Test for Normality (Complete Samples)." *Biometrika* 52 (3-4). Oxford University Press (OUP):591–611. https://doi.org/10.1093/biomet/52.3-4.591.