

# Detection of de novo copy number alterations in case-parent trios using the R package **MinimumDistance**

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## Abstract

For the analysis of case-parent trio genotyping arrays, copy number variants (CNV) appearing in the offspring that differ from the parental copy numbers are often of interest (de novo CNV). This package defines a statistic, referred to as the minimum distance, that can be useful for identifying de novo copy number alterations in the offspring. We smooth the minimum distance using the circular binary segmentation algorithm implemented in the Bioconductor package **DNAcopy**. Trio copy number states are inferred from the maximum a posteriori probability of the segmented data, incorporating information from the log R ratios and B allele frequencies. As both log R ratios and B allele frequencies can be estimated from Illumina and Affymetrix arrays, this package supports de novo copy number inference in both platforms.

## 1 Introduction

There are numerous R packages available from Bioconductor for smoothing copy number alterations. For example, the biocview **CopyNumberVariants** in the 2.9 release of Bioconductor lists 27 packages. For the analysis of germline diseases, hidden Markov models have emerged as a popular tool as inference regarding the latent copy number state incorporates information from both the estimates of copy number (or relative copy number) and the allelic frequencies, such as the B allele frequency [4] or genotype calls [1, 7, 5]. For the analysis of somatic cell diseases such as cancer, algorithms that segment the genome into regions of constant copy number (referred to here as segmentation algorithms) may be more preferable for the detection of copy number aberrations (CNA) as a mixture of cells with different copy numbers give rise to mosaic (non-integer) copy numbers. Examples include circular binary segmentation implemented in the R package **DNAcopy** and the **GLAD**, both of which were originally developed for array CGH platforms [3, 2, 6]. One disadvantage of segmentation algorithms is that inference regarding duplication and deletions is not directly available.

More recently, HMMs and segmentation algorithms have been developed for inferring common regions of copy number alterations in multiple samples. However, relatively few algorithms are available for inferring copy number alterations, especially de novo copy number alterations, in family-based study designs involving case-parent trios. Instead, a common strategy has been a two-step approach of identifying copy number alterations in the individual samples and then comparing the results across samples to infer whether an alteration observed in the offspring is de novo. A disadvantage of the two-step approach is that unadjusted sources of technical variation, such as waves, can contribute to false positives. To our knowledge, the joint HMM implemented in the PennCNV software is the only software to date that provides direct inference regarding de novo alterations in case parent study designs.

This package develops an alternative framework for inferring regions of de novo copy number alterations in case-parent trios. Like PennCNV, inference regarding de novo alterations integrates information from both the log R ratios and B allele frequencies. Differences in the two approaches are most apparent in non-HapMap experimental datasets in which technical and experimental sources of variation appear to contribute to a large number of false positives.

This vignette describes the analysis pipeline from preprocessed and normalized estimates of copy number and allele frequencies to inference regarding de novo copy number alterations. The workflow is illustrated using publicly available HapMap trios assayed on the Illumina 610quad array. Section 2 outlines two approaches for processing the data: Section 2.1 describes a pipeline in which a list of files are provided as input

and the output is a `RangedDataCBS` object of genomic intervals and the inference for the trio copy number state; Section 2.2 provides a more detailed workflow in which intermediate data structures are created to facilitate visualization of the low-level copy number summaries along with inference from the `MinimumDistance` algorithm. Details regarding the intermediate data structures and visualization are described in Sections 2.2 and ??, respectively. Where possible, we have adopted standard data structures for encapsulating the low-level data (`eSet` extensions) and the segmented data (`RangedData` extensions).

## 2 Detection of de novo copy number

There are two options for processing. Section provides a simple pipeline for reading a list of files containing log R ratios and B allele frequencies and returning the segmented data with the trio copy number calls. The second option in Section provides a more detailed workflow of de novo copy number analysis with intermediate data structures useful for storing the log R ratios and B allele frequencies in a manner than can be easily accessed for subsequent plotting. The latter can be particularly useful for visual inspection of the trio copy number calls.

### 2.1 Simple Usage: generating ranged data with posterior calls

Here, we illustrate a simple pipeline for reading in BeadStudio files and returning a list of genomic ranges. There are 3 BeadStudio files provided with the `MinimumDistance` package – one for the father (“F.txt”), mother (“M.txt”), and offspring (“O.txt”). In order to keep the size of the `MinimumDistance` package small, these files contain data for only 1000 markers.

```
R> library(MinimumDistance)
R> path <- system.file("extdata", package="MinimumDistance")
R> fnames <- list.files(path, pattern=".txt")
R> fnames
```

```
[1] "F.txt" "M.txt" "O1.txt" "O.txt"
```

Before reading the data, we create a `data.frame` indicating the filenames for the father, mother, and offspring. In the following codechunk, we have a single file for the father, mother, and offspring (one trio). For processing multiple trios, the arguments to `data.frame` can be vectors where the *i*th element in each vector are the files for one trio.

```
R> pedigreeInfo <- data.frame(F="F.txt", M="M.txt", O="O.txt")
```

Note that the sample identifiers for father and mother can be duplicated, but the offspring sample identifiers must be unique. We encapsulate this information more formally in an S4 class called *Pedigree* and use the *Pedigree* constructor to generate an instance of the class:

```
R> ped <- Pedigree(pedigreeInfo)
R> ped
```

```
Pedigree
trios:
      F      M      O
1 F.txt M.txt O.txt
```

```
pedigreeIndex:
  individualId memberId index.in.pedigree
1          F.txt         F              1
2          M.txt         M              1
3          O.txt         O              1
```

The function `callDenovoSegments` reads the BeadStudio files using information contained in the `ped` object, computes the minimum distance (see Section 2.2), segments the minimum distance using circular binary segmentation, and calls the trio copy number state using the maximum posterior probability.

```
R> map.segs <- callDenovoSegments(path=path, pedigreeData=ped,
                                cdfname="human610quadv1b",
                                chromosome=1, segmentParents=FALSE)
```

```
|
|
|
|=====| 100%
```

```
R> head(map.segs)
```

```
RangedDataCBS with 1 row and 10 value columns across 1 space
  space      ranges |      id      chrom num.mark
<factor>    <IRanges> | <character> <integer> <integer>
1         1 [10004, 4892064] |      0.txt         1      1000
  seg.mean start.index end.index mindist.mad lik.state
<numeric>  <numeric> <numeric>  <numeric> <numeric>
1   -0.0138         1      1000   0.1725746  6556.195
  argmax  lik.norm
<integer> <numeric>
1       61  6556.195
```

The object returned by `callDenovoSegments` is an object of class `RangedDataHMM`. The state symbols are the integer copy number inferred for the father, mother and offspring with an offset of 1. For example, the symbol for state '321' would indicate two DNA copies in father, 1 copy in the mother, and 0 copies in the offspring. In the above example, de novo copy number alterations were not detected and the state '333' indicates that the father, mother, and offspring appear to be diploid for the interval beginning at 10004 bp and ending at 4892064.

## 2.2 Pipeline using intermediate data structures for storing log R ratios and B allele frequencies

This section describes a pipeline that permits the user to manipulate and plot intermediate data structures.

### Instantiating a `TrioSetList` object

A key design consideration for data structures that capture information on the low-level copy number and allele frequencies is that the 'unit' of our analysis is a trio. A common query is to access the low level statistical summaries for a set of markers for all members in a trio. By organizing statistical summaries in arrays with dimension `feature x trios x family member` such queries are straightforward. As the number of trios in a typical genome-wide association study may exceed 1000, it may be impractical to store the low-level summaries in a single array. To make the size of the arrays more manageable and to facilitate parallelization (not yet implemented), we organize the low-level summaries by chromosome using the `TrioSetList` class. The `TrioSetList` class has a slot for storing an object of class `Pedigree` (discussed previously), as well as slots for storing sample-level covariates on the offspring (`phenoData`), mother (`motherPhenoData`), and the father (`fatherPhenoData`). Often technical, experimental, and phenotypic covariates for the samples in an experiment are stored in a single tab-delimited file in which columns are covariates and rows are samples. Such a spreadsheet can be read into R as a `data.frame`. Supplying such a `data.frame` to the argument `sample.sheet` in the `TrioSetList` constructor function will build `AnnotatedDataFrame` objects for father, mother, and offspring, extracting the appropriate rows (samples) from the `data.frame`. For example, in the following codechunk we load a `data.frame` called 'sample.sheet' and pass the `sample.sheet` object to the `TrioSetList` constructor. In addition, we load a matrix of log R ratios and a matrix of B allele frequencies.

(The matrices for the log R ratios and B allele frequencies contain only 25 markers for each chromosome as the intent is to illustrate the steps required for initializing a data structure of the `TrioSetList` class.) Using the information stored in the `Pedigree` class, the matrices are transformed to 3-dimensional arrays and stored in the `assayDataList` slot of the `TrioSetList` class. Accessors `lrr` and `baf` are available for extracting the list of arrays for the log R ratios and B allele frequencies.

```
R> library(human610quadv1bCrlmm)
R> path <- system.file("extdata", package="MinimumDistance")
R> load(file.path(path, "pedigreeInfo.rda"))
R> load(file.path(path, "sample.sheet.rda"))
R> load(file.path(path, "logRratio.rda"))
R> load(file.path(path, "baf.rda"))
R> nms <- paste("NA", substr(sample.sheet$Sample.Name, 6, 10), sep="")
R> trioSetList <- TrioSetList(lrr=logRratio, ## must provide row.names
                             baf=baf,
                             pedigree=Pedigree(pedigreeInfo),
                             sample.sheet=sample.sheet,
                             row.names=nms,
                             cdfname="human610quadv1bCrlmm")

R> show(trioSetList)
```

TrioSetList of length 22

Accessors for sample-level covariates:

```
R> motherPhenoData(trioSetList)
```

```
An object of class "AnnotatedDataFrame"
 sampleNames: NA06985 NA11882
 varLabels: Sample.Name ID ... SentrrixPosition (7 total)
 varMetadata: labelDescription
```

```
R> fatherPhenoData(trioSetList)
```

```
An object of class "AnnotatedDataFrame"
 sampleNames: NA06993 NA11881
 varLabels: Sample.Name ID ... SentrrixPosition (7 total)
 varMetadata: labelDescription
```

```
R> offspringPhenoData(trioSetList)
```

```
An object of class "AnnotatedDataFrame"
 sampleNames: NA06991 NA10859
 varLabels: Sample.Name ID ... SentrrixPosition (7 total)
 varMetadata: labelDescription
```

**Segmentation and posterior calls.** To keep the size of the `MinimumDistance` package small, the `trioSetList` object created in the previous section contained only 25 markers for each of the 22 autosomes. While useful for illustrating construction of the `TrioSetList` class, there are too few markers included in the example to illustrate the smoothing and posterior calling algorithm for detecting de novo copy number events. To demonstrate these steps, we load a `TrioSetList` object containing several thousand markers for chromosomes 7 and 22 for 2 HapMap trios:

```
R> data(trioSetListExample)
```

For a given trio, the signed minimum absolute difference of the offspring and parental  $\log_2$  R ratios ( $\mathbf{r}$ ) is defined as

$$\mathbf{d} \equiv (\mathbf{r}_O - \mathbf{r}_M) \times \mathbb{I}_{[|\mathbf{r}_O - \mathbf{r}_F| > |\mathbf{r}_O - \mathbf{r}_M|]} + (\mathbf{r}_O - \mathbf{r}_F) \times \mathbb{I}_{[|\mathbf{r}_O - \mathbf{r}_F| \leq |\mathbf{r}_O - \mathbf{r}_M|]}. \quad (1)$$

Computation of  $\mathbf{d}$  by the function `calculateMindist` is vectorized in R:

```
R> data(trioSetListExample)
R> md <- calculateMindist(lrr(trioSetList))
```

The circular binary segmentation algorithm implemented in the R package `DNAcopy` can be used to smooth the minimum distance estimates. The method `segment2` is a wrapper to the `CNA.smooth` and `segment` functions defined in `DNAcopy`. Additional arguments to the `segment` function can be passed through the `...` argument in `segment2`, allowing users to modify the segmentation from the default values in `DNAcopy`.

```
R> md.segs <- segment2(object=trioSetList, md=md)
```

The object returned by the `segment2` method is an object of class `RangedDataCBS`.

```
R> head(md.segs)
```

RangedDataCBS with 6 rows and 6 value columns across 1 space

	space	ranges	chrom	num.mark
	<factor>	<IRanges>	<integer>	<numeric>
1	1	[ 37124, 158812247]	7	34075
2	1	[ 37124, 61668154]	7	14923
3	1	[61673005, 62336389]	7	64
4	1	[62342453, 158812247]	7	18985
5	1	[14456412, 16871770]	22	445
6	1	[16872032, 16872749]	22	2

	id	start.index	end.index	seg.mean
	<character>	<integer>	<integer>	<numeric>
1	NA12878	1	34080	-0.0041
2	NA12864	1	14969	-0.0173
3	NA12864	14970	15033	0.2125
4	NA12864	15034	34080	-0.0197
5	NA12878	1	445	0.0063
6	NA12878	446	447	0.5924

Useful accessors for `RangedDataCBS` objects include

```
R> sampleNames(md.segs)[1:4]
```

```
[1] "NA12878" "NA12864" "NA12864" "NA12864"
```

```
R> coverage2(md.segs)[1:4]
```

```
[1] 34075 14923 64 18985
```

```
R> chromosome(md.segs)[1:4]
```

```
[1] 7 7 7 7
```

```
R> mean(md.segs)[1:4]
```

```
[1] -0.0041 -0.0173 0.2125 -0.0197
```

An *ad-hoc* approach to increase the chance that a given segment obtained from the segmentation of the minimum distance is homogeneous with respect to the offspring copy number is to edit the start and stop position of segments with a nonzero mean minimum distance. The approach currently implemented is to define a new start and stop position if a breakpoint of the segmented log R ratios for the offspring occurs in the interval. To illustrate, the following diagram uses vertical dashes (|) to denote breakpoints:

```
1 ...--|-----|---...    ## minimum distance segment (before editing)
2 ...----|-----|-----...  ## segmenation of log R ratios for offspring

->

3 ...--|-|-----|---|---...    ## after editing
```

In the above illustration, posterior calls are provided for the 3 segments indicated in line 3 instead of the single segment in line 1. Two additional steps are therefore required: (1) segmentation of the offspring log R ratios and (2) editing of the minimum distance breakpoints when appropriate. The following codechunk, segments the log R ratios for all chromosomes in the `trioSetList` object. For purposes of visualization, we also segment the parental log R ratios:

```
R> lrr.segs <- segment2(trioSetList, segmentParents=TRUE)
```

Minimum distance segments with a mean absolute value greater than 1 median absolute deviation from zero are edited using the `narrow`:

```
R> mads.md <- mad2(md, byrow=FALSE)
R> md.segs2 <- narrow(md.segs, lrr.segs, thr=0.75, mad.minimumdistance=mads.md)
```

To each range (segment) the maximum posterior probability and the posterior probability of the normal range are computed using the function `computeBayesFactor`.

```
R> map.segs <- computeBayesFactor(object=trioSetList, ranges=md.segs2)
R> show(map.segs)
```

The ratio of posterior probabilities can be used to rank genomic ranges by the evidence for a de novo copy number alteration. Note that physically adjacent genomic ranges in a sample that are assigned the same copy number state will be collapsed into a single range by the `computeBayesFactor` function.

## 2.3 Visualizations

The R package `VanillaICE` contains methods for visualizing *RangedData* objects that build on the infrastructure in the `lattice` package. In the following codechunk, we construct a trellis object for the log R ratios, minimum distance, and B allele frequencies.

```
R> trioSet <- stack(trioSetList)
R> rd.denovoDel <- map.segs[state(map.segs) == 332, ]
R> ##stop()
R> library("VanillaICE")
R> mindist(trioSet) <- do.call("rbind", md)
R> frame <- 100e3
R> ##trace(MinimumDistance:::xypanelTrioBaf, browser)
R> figs <- MinimumDistance:::xyplotTrioLrrBaf(rd=rd.denovoDel,
                                             object=trioSet,
                                             frame=frame,
                                             ylab="log R ratio and BAFs",
                                             xlab="physical position",
                                             panel=MinimumDistance:::xypanelTrioBaf,
```

```
scales=list(x="free", y=list(alternating=1)),
lrr.segments=lrr.segs,
md.segments=md.segs,
layout=c(1, 4),
ylim=c(-3, 1.5))
```

The final graphic is displayed in Figure 1.

```
R> print(figs[[2]])
```

### 3 Session information

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

- R Under development (unstable) (2012-02-05 r58286), 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=en\_US.iso885915, LC\_MESSAGES=en\_US.iso885915, LC\_PAPER=C, 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 2.15.3, BiocGenerics 0.1.4, BiocInstaller 1.3.7, bit 1.1-8, codetools 0.2-8, ff 2.2-5, foreach 1.3.2, human610quadv1bCrlmm 1.0.2, IRanges 1.13.19, iterators 1.0.5, MinimumDistance 0.2.15, oligoClasses 1.17.21, VanillaICE 1.17.13
- Loaded via a namespace (and not attached): affyio 1.23.1, annotate 1.33.2, AnnotationDbi 1.17.15, Biostrings 2.23.6, compiler 2.15.0, crlmm 1.13.8, DBI 0.2-5, DNACopy 1.29.0, ellipse 0.3-5, genefilter 1.37.0, grid 2.15.0, lattice 0.20-0, msm 1.1, mvtnorm 0.9-9992, preprocessCore 1.17.1, RSQLite 0.11.1, splines 2.15.0, survival 2.36-10, xtable 1.6-0, zlibbioc 1.1.1

### References

- [1] Stefano Colella, Christopher Yau, Jennifer M Taylor, Ghazala Mirza, Helen Butler, Penny Clouston, Anne S Bassett, Anneke Seller, Christopher C Holmes, and Jiannis Ragoussis. QuantiSNP: an Objective Bayes Hidden-Markov Model to detect and accurately map copy number variation using SNP genotyping data. *Nucleic Acids Res*, 35(6):2013–2025, 2007.
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- [3] Adam B Olshen, E. S. Venkatraman, Robert Lucito, and Michael Wigler. Circular binary segmentation for the analysis of array-based DNA copy number data. *Biostatistics*, 5(4):557–72, Oct 2004.
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- [5] Robert B Scharpf, Giovanni Parmigiani, Jonathan Pevsner, and Ingo Ruczinski. Hidden Markov models for the assessment of chromosomal alterations using high-throughput SNP arrays. *Annals of Applied Statistics*, 2(2):687–713, 2008.

- [6] E. S. Venkatraman and Adam B Olshen. A faster circular binary segmentation algorithm for the analysis of array cgh data. *Bioinformatics*, 23(6):657–663, Mar 2007.
- [7] Kai Wang, Zhen Chen, Mahlet G Tadesse, Joseph Glessner, Struan F A Grant, Hakon Hakonarson, Maja Bucan, and Mingyao Li. Modeling genetic inheritance of copy number variations. *Nucleic Acids Res*, 36(21):e138, Dec 2008.



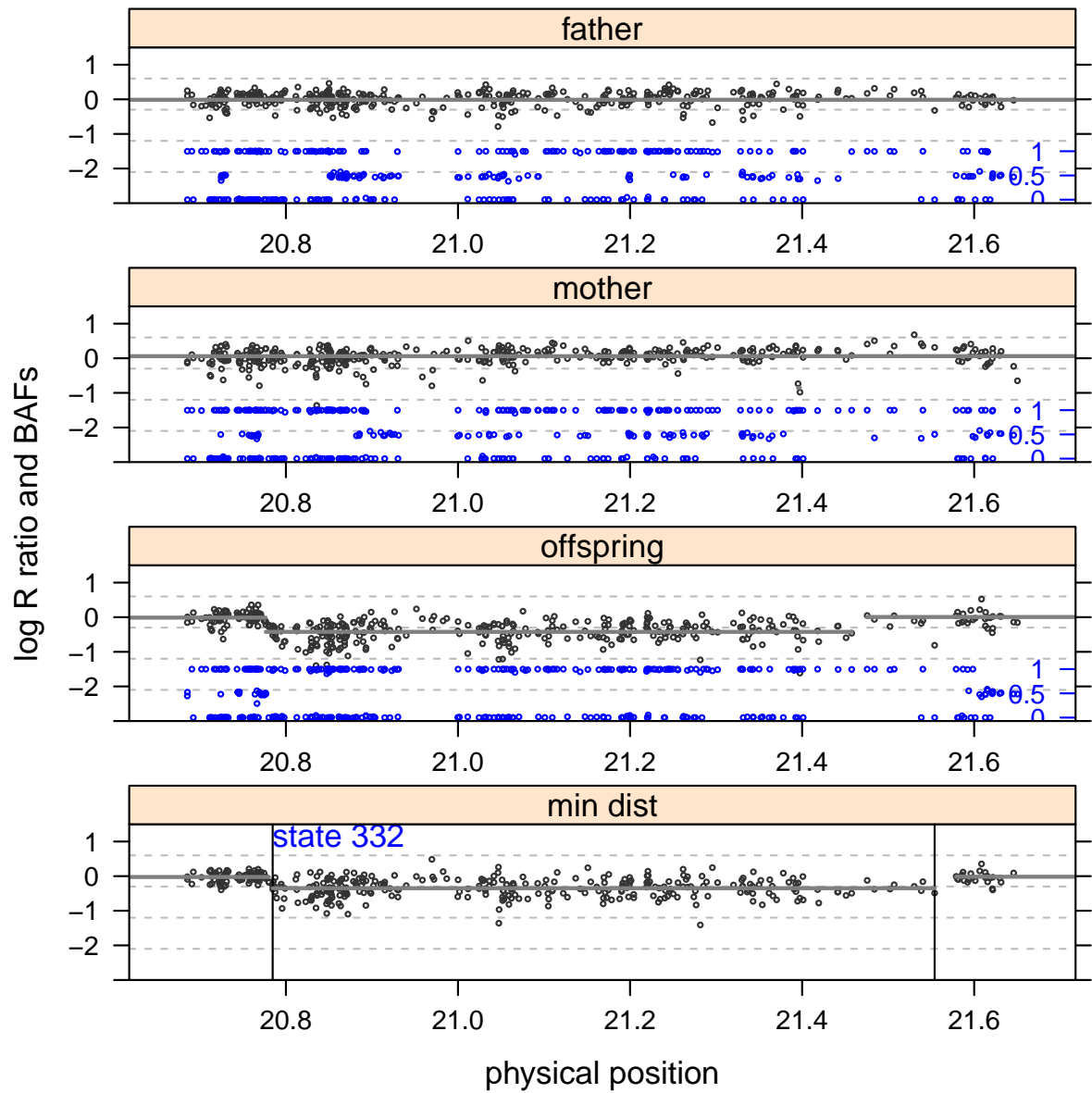


Figure 1: The left panels display the log R ratios for the father (top left), mother, and offspring, as well as the minimum distance (bottom left). The right panels display the B allele frequencies for the father, mother, and offspring. The orange vertical lines indicate the start and stop positions of an inferred de novo hemizygous deletion in the offspring (state '332').