

# CGHcall: Calling aberrations for array CGH tumor profiles.

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## 1 Overview

CGHcall allows users to make an objective and effective classification of their aCGH data into copy number states (loss, normal, gain or amplification). This document provides an overview on the usage of the CGHcall package. For more detailed information on the algorithm and assumptions we refer to the article (van de Wiel et al., 2007) and its supplementary material. As example data we attached the first five samples of the Wilting dataset (Wilting et al., 2006). After filtering and selecting only the autosomal 4709 datapoints remained.

## 2 Example

In this section we will use CGHcall to call and visualize the aberrations in the dataset described above. First, we load the package and the data:

```
> library(CGHcall)
> data(Wilting)
> Wilting <- make_cghRaw(Wilting)
```

Next, we apply the `preprocess` function which:

- removes data with unknown or invalid position information.
- shrinks the data to `nchrom` chromosomes.
- removes data with more than `maxmiss` % missing values.
- imputes missing values using `impute.knn` from the package `impute` (Troyanskaya et al., 2001).

```
> cghdata <- preprocess(Wilting, maxmiss=30, nchrom=22)
```

Changing `impute.knn` parameter `k` from 10 to 4 due to small sample size.

To be able to compare profiles they need to be normalized. In this package we first provide very basic global median or mode normalization. This function also contains smoothing of outliers as implemented in the `DNAcopy` package (Venkatraman and Olshen, 2007). Furthermore, when the proportion of tumor cells is not 100% the ratios can be corrected. See the article and the supplementary material for more information on cellularity correction (van de Wiel et al., 2007).

```
> norm.cghdata <- normalize(cghdata, method="median", smoothOutliers=TRUE)
```

Applying median normalization ...  
Smoothing outliers ...

The next step is segmentation of the data. This package only provides a wrapper function that applies the `DNAcopy` algorithm (Venkatraman and Olshen, 2007). It provides extra functionality by allowing to undo splits differently for long and short segments, respectively. In the example below short segments are smaller than `clen=10` probes, and for such segments `undo.splits` is effective when segments are less than `undo.SD=3` (sd) apart. For long segments a less stringent criterion holds: `undo` when less than `undo.SD/relSDlong = 3/5` (sd) apart. If, for two consecutive segments, one is short and one is long, splits are undone in the same way as for two consecutive short segments. To save time we will limit our analysis to the first two samples from here on.

```
> norm.cghdata <- norm.cghdata[,1:2]
> seg.cghdata <- segmentData(norm.cghdata, method="DNAcopy",undo.splits="sdundo",undo..
+ clen=10, relSDlong=5)
```

```
Start data segmentation ..
Analyzing: Sample.1
Analyzing: Sample.2
```

Post-segmentation normalization allows to better set the zero level after segmentation.

```
> postseg.cghdata <- postsegnormalize(seg.cghdata)
```

Now that the data have been normalized and segments have been defined, we need to determine which segments should be classified as double losses, losses, normal, gains or amplifications. Cellularity correction is now provided WITHIN the calling step (as opposed to some earlier of CGHcall)

```
> tumor.prop <- c(0.75, 0.9)
> result <- CGHcall(postseg.cghdata,nclass=5,cellularity=tumor.prop)

[1] "Total number of segments present in the data: 90"
[1] "Number of segments used for fitting the model: 90"
```

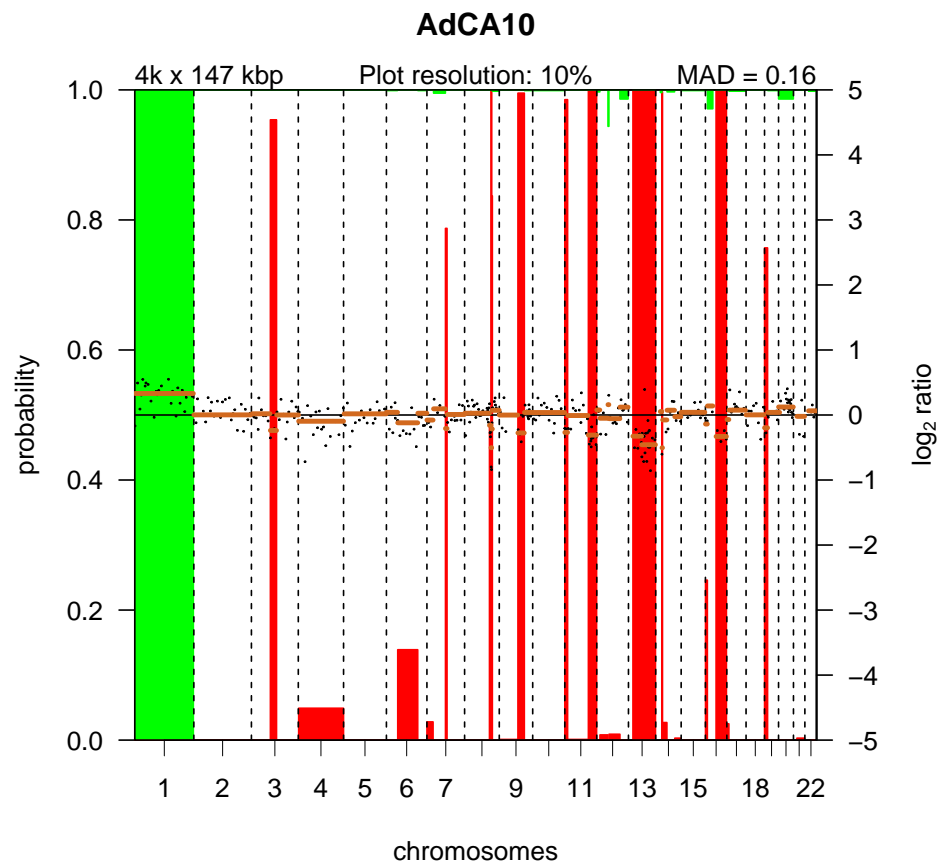
The result of CGHcall needs to be converted to a call object. This can be a large object for large arrays.

```
> result <- ExpandCGHcall(result,postseg.cghdata)
```

To visualize the results per profile we use the `plotProfile` function:

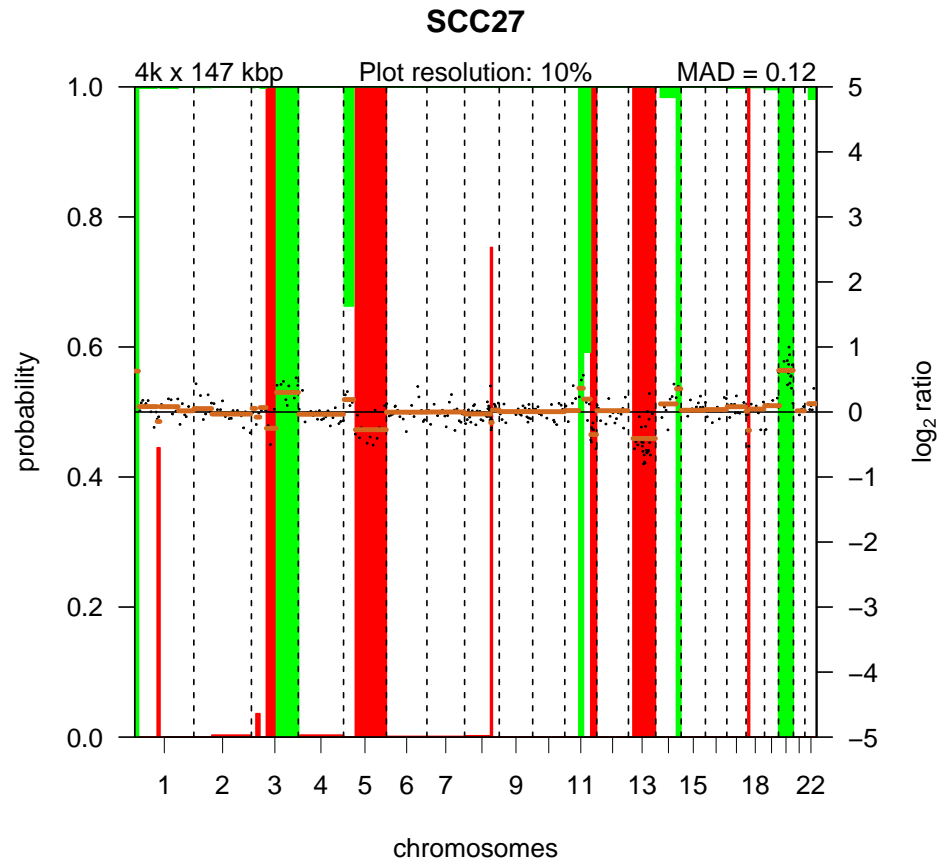
```
> plot(result[,1])
```

Plotting sample AdCA10



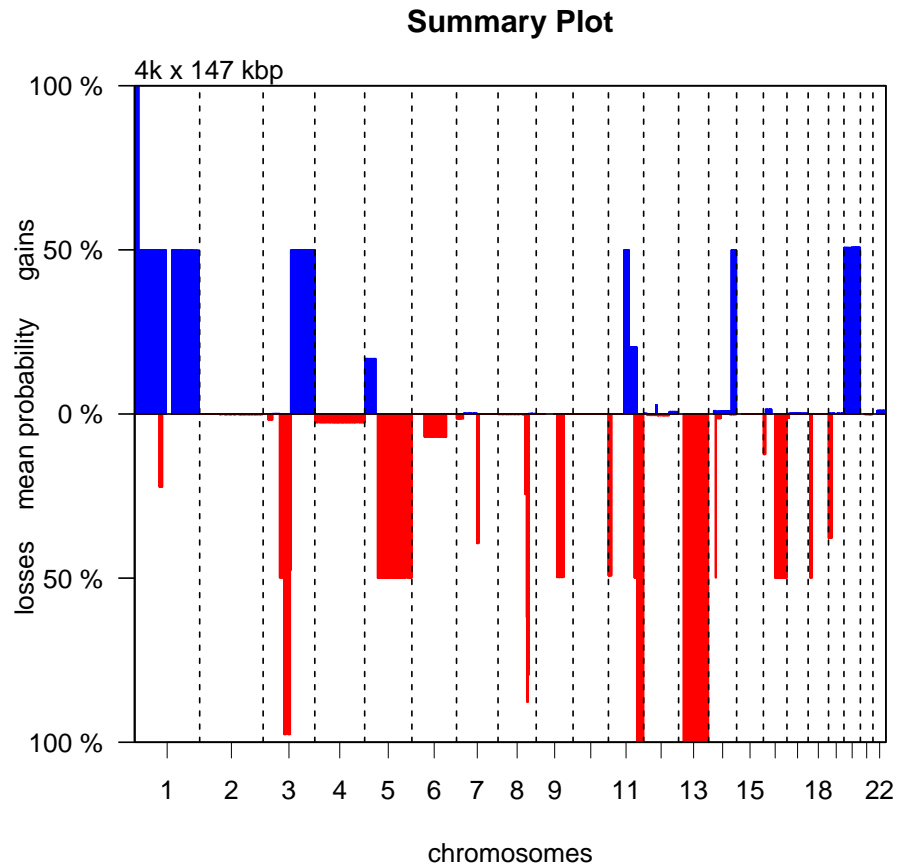
```
> plot(result[,2])
```

Plotting sample SCC27



Alternatively, we can create a summary plot of all the samples:

```
> summaryPlot(result)
```



Or a frequency plot::

```
> frequencyPlotCalls(result)
```



## References

- Troyanskaya, O., Cantor, M., Sherlock, G., Brown, P., Hastie, T., Tibshirani, R., Botstein, D., and Altman, R. B. (2001). Missing value estimation methods for DNA microarrays. *Bioinformatics*, 17:520–525.
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- Venkatraman, E. S. and Olshen, A. B. (2007). A faster circular binary segmentation algorithm for the analysis of array CGH data. *Bioinformatics*, 23:657–663.
- Wilting, S. M., Snijders, P. J. F., Meijer, G. A., Ylstra, B., van den Ijssel, P. R. L. A., Snijders, A. M., Albertson, D. G., Coffa, J., Schouten, J. P., van de Wiel, M. A., Meijer, C. J. L. M., and Steenbergen, R. D. M. (2006). Increased gene copy numbers at chromosome 20q are frequent in both squamous cell carcinomas and adenocarcinomas of the cervix. *J Pathol*, 209:220–230.