CGHcall: Calling aberrations for array CGH tumor profiles.

Sjoerd Vosse and Mark van de Wiel

October 1, 2012

Department of Epidemiology & Biostatistics
VU University Medical Center

`mark.vdwiel@vumc.nl`

Contents

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 segements, 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)

EM algorithm started ...
[1] "Total number of segments present in the data: 90"
[1] "Number of segments used for fitting the model: 90"
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 550293 29.4     899071 48.1   792607 42.4
Vcells 908682  7.0    1598044 12.2  1598039 12.2
Calling iteration 1 :
[1] "optim results"
[1] "time: 22"
[1] "minimum: 3748.69878446397"
     j       rl       mudl       musl         mun      mug     mudg      mua
[1,] 2 3732.566 -0.7730131 -0.2898698 0.01374418 0.347191 0.593527 1.057513
          sddl       sdsl         sdn       sdg      sddg       sda
[1,] 0.1935916 0.08495295 0.06150202 0.1134993 0.1140091 0.1183082
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 551452 29.5     899071 48.1   899071 48.1
Vcells 910767  7.0    1598044 12.2  1598039 12.2
Calling iteration 2 :
[1] "optim results"
[1] "time: 17"
```

```
[1] "minimum: 3742.64242208813"
     j       rl       mudl       musl       mun      mug      mudg      mua
[1,] 2 3731.237 -0.7801531 -0.2854698 0.0173235 0.346793 0.5928465 1.056921
          sddl       sdsl        sdn        sdg       sddg       sda
[1,] 0.3283833 0.08301401 0.05550779 0.09518613 0.09570998 0.1552889
EM algorithm done ...
Computing posterior probabilities for all segments ...
Total time: 1 minutes
```

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)
```

```
Adjusting segmented data for cellularity ...
Cellularity sample 1 :  0.75
Cellularity sample 2 :  0.9
Adjusting normalized data for cellularity ...
Cellularity sample 1 :  0.75
Cellularity sample 2 :  0.9
[1] 1
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 553401 29.6     899071 48.1   899071 48.1
Vcells 947586  7.3    1598044 12.2  1598039 12.2
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 553411 29.6     899071 48.1   899071 48.1
Vcells 965347  7.4    1757946 13.5  1598039 12.2
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 553410 29.6     899071 48.1   899071 48.1
Vcells 965346  7.4    1757946 13.5  1598039 12.2
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 553437 29.6     899071 48.1   899071 48.1
Vcells 993765  7.6    1757946 13.5  1598039 12.2
         used (Mb) gc trigger (Mb) max used (Mb)
Ncells 553475 29.6     899071 48.1   899071 48.1
Vcells 997341  7.7    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  553483 29.6     899071 48.1   899071 48.1
Vcells 1000897  7.7    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
```

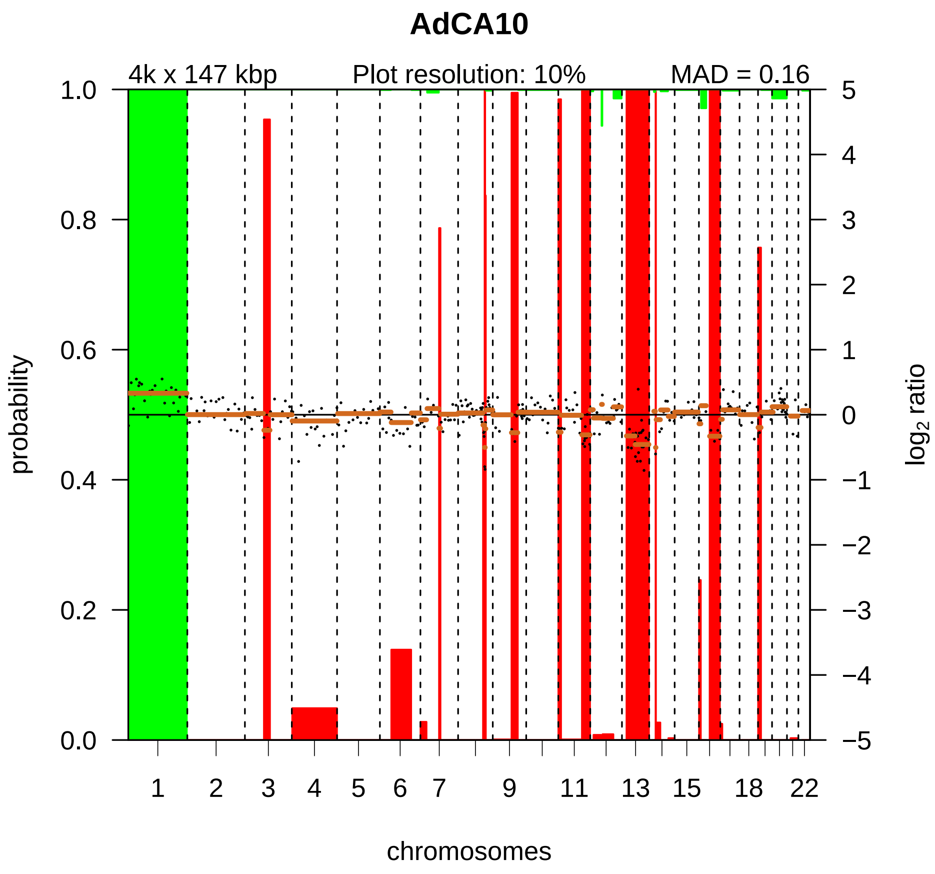
```
Ncells  553491 29.6     899071 48.1   899071 48.1
Vcells 1004453  7.7    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  553499 29.6     899071 48.1   899071 48.1
Vcells 1008009  7.7    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  553507 29.6     899071 48.1   899071 48.1
Vcells 1011565  7.8    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  553511 29.6     899071 48.1   899071 48.1
Vcells 1015120  7.8    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  553538 29.6     899071 48.1   899071 48.1
Vcells 1036473  8.0    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554418 29.7     899071 48.1   899071 48.1
Vcells 1045851  8.0    1757946 13.5  1598039 12.2
[1] 2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554422 29.7     899071 48.1   899071 48.1
Vcells 1063612  8.2    1757946 13.5  1598039 12.2
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554423 29.7     899071 48.1   899071 48.1
Vcells 1063613  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554422 29.7     899071 48.1   899071 48.1
Vcells 1063612  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554426 29.7     899071 48.1   899071 48.1
Vcells 1067165  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554422 29.7     899071 48.1   899071 48.1
Vcells 1063612  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554430 29.7     899071 48.1   899071 48.1
Vcells 1067168  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554438 29.7     899071 48.1   899071 48.1
Vcells 1070724  8.2    1757946 13.5  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
```

```
Ncells  554446 29.7     899071 48.1   899071 48.1
Vcells 1074280  8.2    1925843 14.7  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554454 29.7     899071 48.1   899071 48.1
Vcells 1077836  8.3    1925843 14.7  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554458 29.7     899071 48.1   899071 48.1
Vcells 1081391  8.3    1925843 14.7  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  554485 29.7     899071 48.1   899071 48.1
Vcells 1102744  8.5    1925843 14.7  1747068 13.4
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells  559544 29.9     984024 52.6   899071 48.1
Vcells 1082920  8.3    1925843 14.7  1925843 14.7
FINISHED!
Total time: 0 minutes
```

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

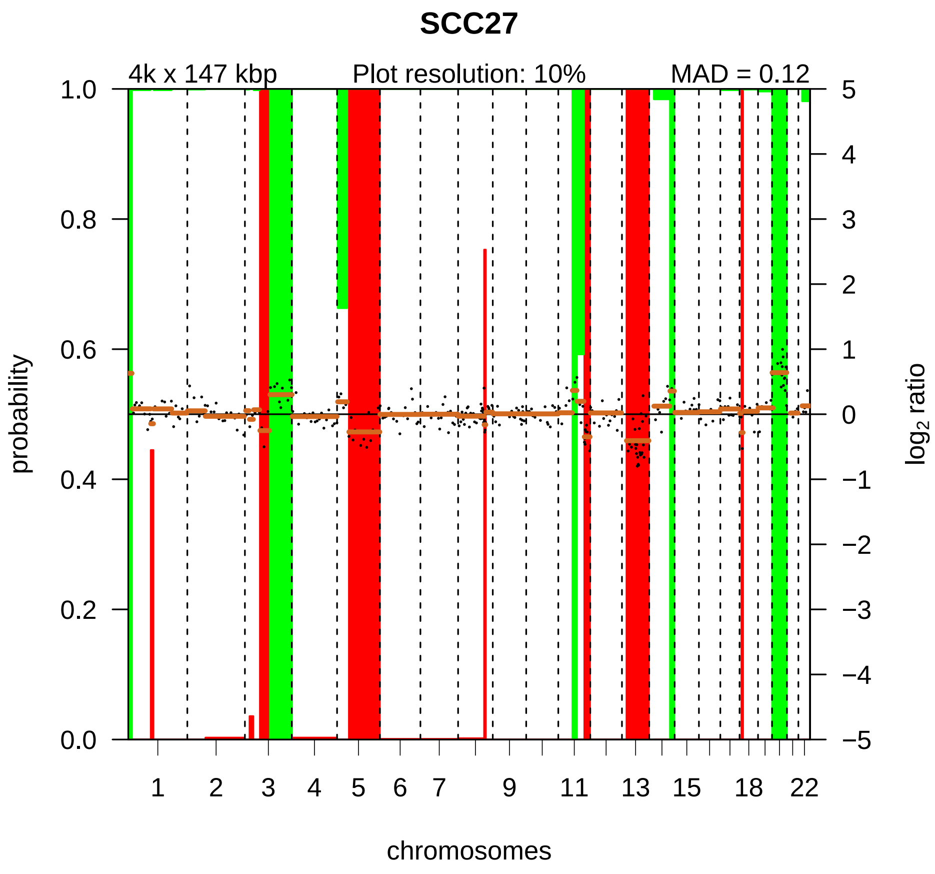
```
> plot(result[,1])
```

```
Plotting sample AdCA10
```



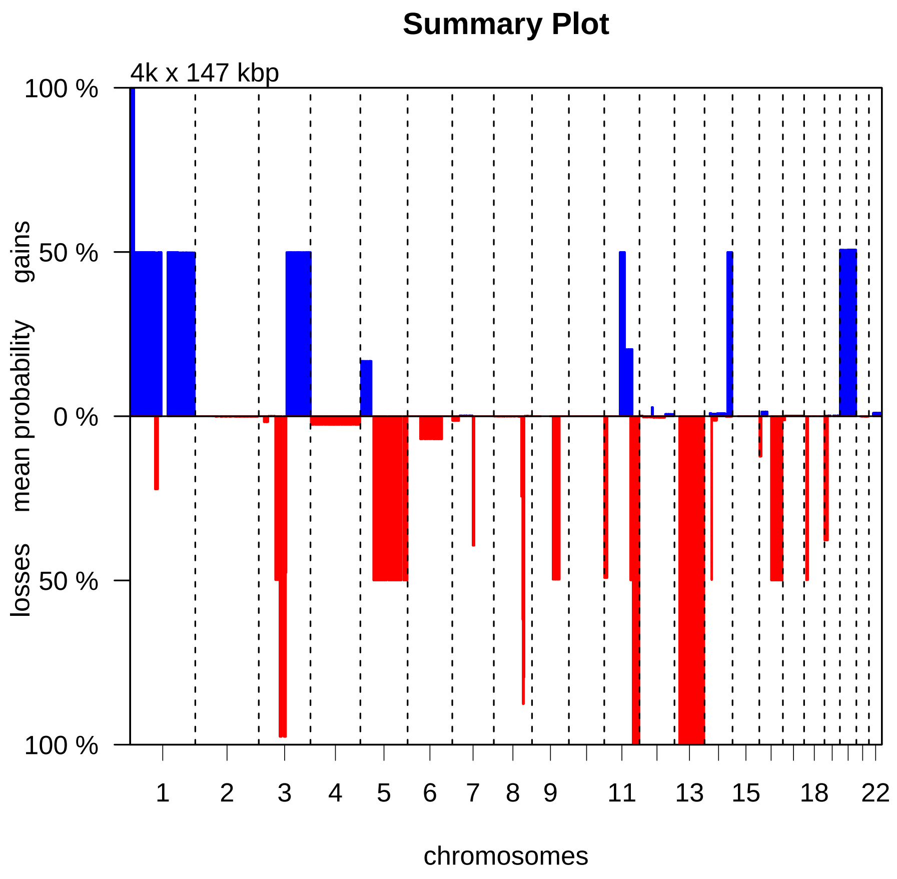
```
> plot(result[,2])

Plotting sample SCC27
```



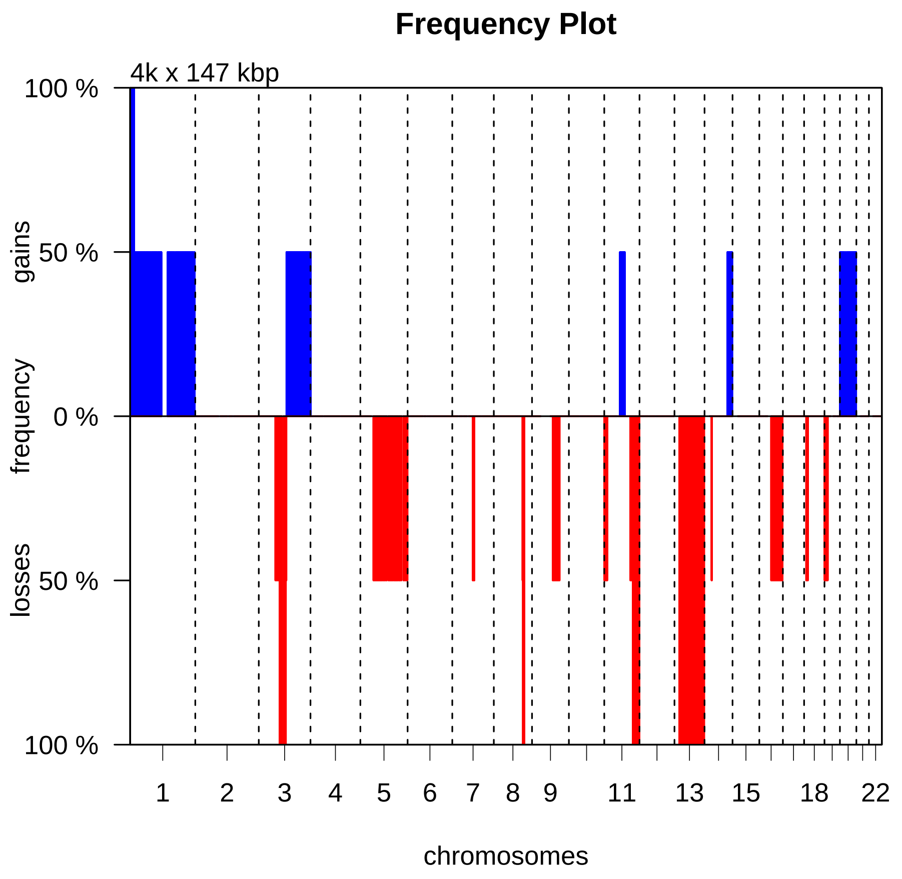
Alternatively, we can create a summary plot of all the samples:

```
> 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.

van de Wiel, M. A., Kim, K. I., Vosse, S. J., van Wieringen, W. N., Wilting, S. M., and Ylstra, B. (2007). CGHcall: calling aberrations for array CGH tumor profiles. *Bioinformatics*, 23:892–894.

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.