Analysis of data from aCGH experiments using parallel computing and ff objects: long list of examples

Ramón Díaz-Uriarte¹, Daniel Rico², and Oscar M. Rueda³

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1. Department of Biochemistry, Universidad Autonoma de Madrid Instituto de Investigaciones Biomedicas "Alberto Sols" (UAM-CSIC), Madrid (SPAIN). 2. Structural Computational Biology Group. Spanish National Cancer Center (CNIO), Madrid (SPAIN).

3. Cancer Research UK Cambridge Research Institute Cambridge, UK rdiaz02@gmail.com, drico@cnio.es, Oscar.Rueda@cancer.org.uk

Contents

1	This vignette	1
2	Creating objects	1
3	The examples 3.1 RAM objects and forking	5
4	Clean up actions	11

1 This vignette

We provide here example calls of all segmentation methods, with different options for methods, as well as different options for type of input object and clustering. This is provided here as both extended help and as a simple way of checking that all the functions can be run and yield the same results regardless of type of input and clustering.

2 Creating objects

We must ensure that we can run this vignette as stand alone. Thus, we load the package and create all necessary objects. This repeats work done in the main vignette.

We first try to move to the "/tmp" directory, if it exists. If it does not, the code will be executed in your current directory.

- > try(setwd("~/tmp"))
- > library(ADaCGH2)
- > ## loading in-RAM objects
- > data(inputEx)
- > summary(inputEx)

```
ΙD
                   chromosome
                                    position
                                                          L.1
Hs.101850:
                         :1.00
             1
                 Min.
                                 Min.
                                        :1.18e+06
                                                            :-1.078
                                                     1st Qu.:-0.226
Hs.1019 :
                 1st Qu.:1.00
                                 1st Qu.:3.60e+07
Hs.105460:
                 Median :2.00
                                 Median :7.08e+07
                                                     Median :-0.016
Hs.105656: 1
                 Mean
                         :2.28
                                 Mean
                                        :9.26e+07
                                                     Mean
                                                            :-0.035
                 3rd Qu.:3.00
                                                     3rd Qu.: 0.160
Hs.105941:
             1
                                 3rd Qu.:1.50e+08
Hs.106674:
                 Max.
                        :5.00
                                        :2.44e+08
                                                     Max.
                                                            : 0.883
 (Other) :494
                                                     NA's
                                                            :5
     L.2
                                                           L3
                        m4
                                          m5
Min.
        :-0.795
                          :-0.187
                                    Min.
                                            :-4.67
                                                     Min.
                                                            :-13.27
                  Min.
 1st Qu.:-0.139
                  1st Qu.: 1.979
                                    1st Qu.:-0.02
                                                     1st Qu.:
                                                               3.63
Median :-0.006
                  Median : 2.281
                                    Median: 0.44
                                                     Median :
                                                               3.92
Mean
        : 0.008
                  Mean
                         : 3.450
                                    Mean
                                           : 1.60
                                                     Mean
                                                               1.98
                  3rd Qu.: 5.824
3rd Qu.: 0.134
                                    3rd Qu.: 3.04
                                                     3rd Qu.: 4.11
        : 1.076
                  Max.
                         : 6.604
                                    Max.
                                            : 9.60
                                                     Max.
                                                            : 6.37
Max.
NA's
                                    NA's
                                            :41
                                                     NA's
        :15
                                                            :9
       m6
Min.
        :-0.77
1st Qu.:-0.23
Median :-0.04
Mean
        :-0.04
3rd Qu.: 0.16
Max.
        : 0.78
NA's
        :203
> head(inputEx)
                              ID chromosome position
                                                        L.1
                                                               L.2
                                                                       m4
                                                                             m5
                      Hs.212680
1*1180411*Hs.212680
                                          1
                                              1180411
                                                         NA
                                                             0.038 6.226 3.226
1*1188041.5*Hs.129780 Hs.129780
                                              1188042
                                                         NA 0.028 6.174 3.174
1*1194444*Hs.42806
                       Hs.42806
                                          1
                                              1194444
                                                         NA 0.042 6.174 3.174
1*1332537*Hs.76239
                       Hs.76239
                                          1
                                             1332537
                                                         NA
                                                            0.285 5.624 2.624
                                                         NA 0.058 5.851 2.851
1*2362211*Hs.40500
                       Hs.40500
                                          1
                                             2362211
1*2372287*Hs.449936
                      Hs.449936
                                             2372287 0.294 -0.006 5.685 2.685
                         L3 m6
1*1180411*Hs.212680
                      6.038 NA
1*1188041.5*Hs.129780 6.028 NA
1*1194444*Hs.42806
                      6.042 NA
1*1332537*Hs.76239
                         NA NA
1*2362211*Hs.40500
                         NA NA
1*2372287*Hs.449936
                         NA NA
> cgh.dat <- inputEx[, -c(1, 2, 3)]</pre>
> chrom.dat <- as.integer(inputEx[, 2])</pre>
> pos.dat <- inputEx[, 3]</pre>
> ## choosing working dir for cluster
> originalDir <- getwd()</pre>
> if(!file.exists("ADaCGH2_vignette_tmp_dir"))
    dir.create("ADaCGH2_vignette_tmp_dir")
> setwd("ADaCGH2_vignette_tmp_dir")
> ## creating ff objects
> fnameRdata <- list.files(path = system.file("data", package = "ADaCGH2"),</pre>
                       full.names = TRUE, pattern = "inputEx.RData")
```

```
> inputToADaCGH(ff.or.RAM = "ff",
                         RDatafilename = fnameRdata)
   ... done reading; starting checks
      ... checking identical MidPos
      ... checking need to reorder inputData, data.frame version
   ... done with checks; starting writing
   ... done writing/saving probeNames
   ... done writing/saving chromData
   ... done writing/saving posData
   ... done writing/saving cghData
 Calling gc at end
          used (Mb) gc trigger (Mb) max used
Ncells 1565205 83.6
                       2403845 128.4 1967602 105.1
Vcells 1528075 11.7
                       2481603 19.0 2153025 16.5
Files saved in current directory
/home/ramon/tmp/ADaCGH2_vignette_tmp_dir
with names :
chromData.RData, posData.RData, cghData.RData, probeNames.RData.
> ## initializing cluster
> number.of.nodes <- detectCores()</pre>
> cl2 <- parallel::makeCluster(number.of.nodes, "PSOCK")</pre>
> parallel::clusterSetRNGStream(cl2)
> parallel::setDefaultCluster(cl2)
> parallel::clusterEvalQ(NULL, library("ADaCGH2"))
[[1]]
 [1] "ADaCGH2"
                     "ffbase"
                                    "ff"
                                                    "bit"
                                                                    "tools"
                                                                   "aCGH"
 [6] "snapCGH"
                     "DNAcopy"
                                    "limma"
                                                    "waveslim"
[11] "multtest"
                     "survival"
                                    "splines"
                                                    "cluster"
                                                                    "tilingArray"
[16] "pixmap"
                     "Biobase"
                                    "BiocGenerics" "parallel"
                                                                   "methods"
[21] "stats"
                     "graphics"
                                    "grDevices"
                                                    "utils"
                                                                   "datasets"
[26] "base"
[[2]]
 [1] "ADaCGH2"
                     "ffbase"
                                    "ff"
                                                    "bit"
                                                                   "tools"
                                                                    "aCGH"
[6] "snapCGH"
                     "DNAcopy"
                                    "limma"
                                                    "waveslim"
[11] "multtest"
                     "survival"
                                    "splines"
                                                    "cluster"
                                                                    "tilingArray"
                                                                   "methods"
[16] "pixmap"
                     "Biobase"
                                    "BiocGenerics" "parallel"
[21] "stats"
                                                    "utils"
                                                                   "datasets"
                     "graphics"
                                    "grDevices"
[26] "base"
```

```
[[3]]
                     "ffbase"
 [1] "ADaCGH2"
                                     "ff"
                                                                     "tools"
                                                     "bit"
 [6] "snapCGH"
                     "DNAcopy"
                                     "limma"
                                                     "waveslim"
                                                                    "aCGH"
                     "survival"
                                     "splines"
[11] "multtest"
                                                     "cluster"
                                                                     "tilingArray"
[16] "pixmap"
                     "Biobase"
                                     "BiocGenerics" "parallel"
                                                                     "methods"
[21] "stats"
                     "graphics"
                                     "grDevices"
                                                     "utils"
                                                                    "datasets"
[26] "base"
[[4]]
 [1] "ADaCGH2"
                     "ffbase"
                                     "ff"
                                                     "bit"
                                                                     "tools"
[6] "snapCGH"
                     "DNAcopy"
                                     "limma"
                                                     "waveslim"
                                                                     "aCGH"
                                     "splines"
                                                     "cluster"
[11] "multtest"
                     "survival"
                                                                     "tilingArray"
                     "Biobase"
                                     "BiocGenerics" "parallel"
                                                                    "methods"
[16] "pixmap"
[21] "stats"
                     "graphics"
                                     "grDevices"
                                                     "utils"
                                                                     "datasets"
[26] "base"
> wdir <- getwd()</pre>
> parallel::clusterExport(NULL, "wdir")
> parallel::clusterEvalQ(NULL, setwd(wdir))
[[1]]
[1] "/home/ramon/tmp/ADaCGH2_vignette_tmp_dir"
[[2]]
[1] "/home/ramon/tmp/ADaCGH2_vignette_tmp_dir"
[[3]]
[1] "/home/ramon/tmp/ADaCGH2_vignette_tmp_dir"
[[4]]
[1] "/home/ramon/tmp/ADaCGH2_vignette_tmp_dir"
3
    The examples
```

3.1 RAM objects and forking

```
chrom.dat,
                                            pos.dat,
                                           merging = "MAD")
> biohmm.mad.bic.RAM.fork <- pSegmentBioHMM(cgh.dat,
                                                chrom.dat,
                                                pos.dat,
                                                merging = "MAD",
                                                aic.or.bic = "BIC")
> cghseg.merge.RAM.fork <- pSegmentCGHseg(cgh.dat,</pre>
                                              chrom.dat,
                                              merging = "mergeLevels")
> cghseg.mad.RAM.fork <- pSegmentCGHseg(cgh.dat,</pre>
                                            chrom.dat,
                                            merging = "MAD")
> cghseg.none.RAM.fork <- pSegmentCGHseg(cgh.dat,
                                             chrom.dat,
                                            merging = "none")
> waves.merge.RAM.fork <- pSegmentWavelets(cgh.dat,
                              chrom.dat, merging = "mergeLevels")
> waves.mad.RAM.fork <- pSegmentWavelets(cgh.dat,
                                             chrom.dat, merging = "MAD")
> waves.none.RAM.fork <- pSegmentWavelets(cgh.dat,
                                              chrom.dat, merging = "none")
```

3.2 ff objects and cluster

Compared to the section 3.1, the main differences are that we explicitly set the typeParall argument to "cluster" (the default is "fork") and the change in the names of the input data (which now refer to the names of the RData objects that contain the ff objects).

```
> cbs.ff.cluster <- pSegmentDNAcopy("cghData.RData", "chromData.RData",
                                    typeParall = "cluster")
> cbs.mad.ff.cluster <- pSegmentDNAcopy("cghData.RData", "chromData.RData",
                                        merging = "MAD",
                                        typeParall = "cluster")
> cbs.none.ff.cluster <- pSegmentDNAcopy("cghData.RData", "chromData.RData",
                                         merging = "none",
                                         typeParall = "cluster")
> hmm.ff.cluster <- pSegmentHMM("cghData.RData", "chromData.RData",
                                merging = "mergeLevels",
                                typeParall = "cluster")
> hmm.mad.ff.cluster <- pSegmentHMM("cghData.RData", "chromData.RData",
                                    merging = "MAD",
                                    typeParall = "cluster")
> hs.ml.ff.cluster <- pSegmentHaarSeg("cghData.RData", "chromData.RData",
                               merging = "mergeLevels",
                                      typeParall = "cluster")
> hs.mad.ff.cluster <- pSegmentHaarSeg("cghData.RData", "chromData.RData",
                               merging = "MAD", typeParall = "cluster")
> glad.ff.cluster <- pSegmentGLAD("cghData.RData", "chromData.RData",
                                  typeParall = "cluster")
```

```
> biohmm.ff.cluster <- pSegmentBioHMM("cghData.RData",
                                   "chromData.RData",
                                   "posData.RData",
                                   merging = "mergeLevels",
                                       typeParall = "cluster")
> biohmm.mad.ff.cluster <- pSegmentBioHMM("cghData.RData",
                                           "chromData.RData",
                                           "posData.RData",
                                           merging = "MAD",
                                           typeParall = "cluster")
> biohmm.mad.bic.ff.cluster <- pSegmentBioHMM("cghData.RData",
                                               "chromData.RData",
                                               "posData.RData",
                                               merging = "MAD",
                                               aic.or.bic = "BIC",
                                               typeParall = "cluster")
> cghseg.merge.ff.cluster <- pSegmentCGHseg("cghData.RData",
                                             "chromData.RData",
                                             merging = "mergeLevels",
                                             typeParall = "cluster")
> cghseg.mad.ff.cluster <- pSegmentCGHseg("cghData.RData",
                                           "chromData.RData",
                                           merging = "MAD",
                                           typeParall = "cluster")
> cghseg.none.ff.cluster <- pSegmentCGHseg("cghData.RData",
                                            "chromData.RData",
                                            merging = "none",
                                            typeParall = "cluster")
> waves.merge.ff.cluster <- pSegmentWavelets("cghData.RData",
                                              "chromData.RData",
                                              merging = "mergeLevels",
                                              typeParall = "cluster")
> waves.mad.ff.cluster <- pSegmentWavelets("cghData.RData",
                                            "chromData.RData",
                                            merging = "MAD",
                                            typeParall = "cluster")
> waves.none.ff.cluster <- pSegmentWavelets("cghData.RData",
                                             "chromData.RData",
                                             merging = "none",
+
                                             typeParall = "cluster")
```

3.3 ff objects and forking

The main difference with section 3.2 is the argument typeParall; we did not need to pass it explicitly (since the default is fork), but we will do for clarity.

```
> cbs.none.ff.fork <- pSegmentDNAcopy("cghData.RData", "chromData.RData",
                                       merging = "none", typeParall = "fork")
> hmm.ff.fork <- pSegmentHMM("cghData.RData", "chromData.RData",</pre>
                             merging = "mergeLevels", typeParall = "fork")
> hmm.mad.ff.fork <- pSegmentHMM("cghData.RData", "chromData.RData",
                                  merging = "MAD", typeParall = "fork")
> hs.ml.ff.fork <- pSegmentHaarSeg("cghData.RData", "chromData.RData",</pre>
                                merging = "mergeLevels", typeParall = "fork")
> hs.mad.ff.fork <- pSegmentHaarSeg("cghData.RData", "chromData.RData",
                                merging = "MAD", typeParall = "fork")
> glad.ff.fork <- pSegmentGLAD("cghData.RData", "chromData.RData",
                                typeParall = "fork")
> biohmm.ff.fork <- pSegmentBioHMM("cghData.RData",
                                   "chromData.RData",
                                   "posData.RData",
                                   merging = "mergeLevels",
                                    typeParall = "fork")
> biohmm.mad.ff.fork <- pSegmentBioHMM("cghData.RData",</pre>
                                           "chromData.RData",
                                           "posData.RData",
                                           merging = "MAD",
                                        typeParall = "fork")
> biohmm.mad.bic.ff.fork <- pSegmentBioHMM("cghData.RData",
                                               "chromData.RData",
                                               "posData.RData",
                                               merging = "MAD",
                                               aic.or.bic = "BIC",
                                            typeParall = "fork")
> cghseg.merge.ff.fork <- pSegmentCGHseg("cghData.RData",
                                             "chromData.RData",
                                             merging = "mergeLevels",
                                          typeParall = "fork")
> cghseg.mad.ff.fork <- pSegmentCGHseg("cghData.RData",
                                           "chromData.RData",
                                           merging = "MAD", typeParall = "fork")
> cghseg.none.ff.fork <- pSegmentCGHseg("cghData.RData",
                                            "chromData.RData",
                                            merging = "none", typeParall = "fork")
> waves.merge.ff.fork <- pSegmentWavelets("cghData.RData",
                                           "chromData.RData",
                                           merging = "mergeLevels",
                                           typeParall = "fork")
> waves.mad.ff.fork <- pSegmentWavelets("cghData.RData",
                                            "chromData.RData",
                                         merging = "MAD",
                                         typeParall = "fork")
> waves.none.ff.fork <- pSegmentWavelets("cghData.RData",
                                          "chromData.RData",
                                          merging = "none",
                                          typeParall = "fork")
```

3.4 Comparing output

Here we verify that using different input and clustering methods does not change the results. Before carrying out the comparisons, however, we open the ff objects gently.

First, we will open the objects created above (same objects as were also created in the main vignette, in section "Carrying out segmentation and calling"). Instead of inserting many calls to each individual object, we open all available objects that match ff.cluster. To do that quickly we store the names of the objects

```
> ff.cluster.obj <- ls(pattern = "*.ff.cluster")

pages with the string "TRUE")
> tmpout <-
+ capture.output(
+ lapply(ff.cluster.obj, function(x) lapply(get(x), open))
+ )

We repeat that operation with the output from section 3.3:
> ff.fork.obj <- ls(pattern = "*.ff.fork")
> tmpout <-
+ capture.output(
+ lapply(ff.fork.obj, function(x) lapply(get(x), open))
+ )
>
```

And we create the list of results from the RAM and forking runs (no need for special opening here, since these are not ff objects)

```
> RAM.fork.obj <- ls(pattern = "*.RAM.fork")</pre>
```

We can now compare the output. We want to compare the output from three different methods, so we need to run three comparisons (this is what we did explicitly in the help for pSegment). Since this is a very repetitive operation, we define a small utility function that will return TRUE if both components (outSmoothed and outState) of all three objects are identical. (Since the function will take as input not an actual object, but a name, we use get inside the function.)

We use all.equal to compare the output from the smoothing, to allow for possible numerical fuzz (that could result from differences in storage). When comparing the assigned state, however, we check for exact identity.

```
> identical3 <- function(x, y, z) {</pre>
    comp1 <- all.equal(get(x)$outSmoothed[ , ], get(y)$outSmoothed[ , ])</pre>
    comp2 <- all.equal(get(y)$outSmoothed[ , ], get(z)$outSmoothed[ , ])</pre>
    comp3 <- identical(get(x)$outState[ , ], get(y)$outState[ , ])</pre>
    comp4 <- identical(get(y)$outState[ , ], get(z)$outState[ , ])</pre>
    if (!all(isTRUE(comp1), isTRUE(comp2), comp3, comp4)) {
      cat(paste("Comparing ", x, y, z, "\n",
             "not equal: some info from comparisons. \n",
                 "\n comp1 = ", paste(comp1, sep = " ", collapse = "\n
                                                                              "),
                 "\n comp2 = ", paste(comp2, sep = " ", collapse = "\n
                                                                              "),
                 "\n comp3 = ", paste(comp3, sep = " ", collapse = "\n
                                                                              "),
                 "\n comp4 = ", paste(comp4, sep = " ", collapse = "\n
                                                                              "),
```

```
"\n\n"))
      return (FALSE)
   } else {
      TRUE
   }
+ }
  You should expect most (though not necessarily all) the comparisons to yield a TRUE.
In some cases, however, different runs of the same method might not yield the same results
(e.g., CBS, HMM, etc). If you get non-identical results, you can try running those methods
a few times, to check for differences.
> mapply(identical3, RAM.fork.obj,
             ff.fork.obj, ff.cluster.obj)
Comparing biohmm.mad.bic.RAM.fork biohmm.ff.fork biohmm.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 0.974
   Component 2: Mean relative difference: 1.06
   Component 3: Mean relative difference: 0.03742
   Component 4: Mean relative difference: 0.05093
   Component 5: Mean relative difference: 0.007367
   Component 6: Mean relative difference: 0.9384
comp2 = TRUE
 comp3 = FALSE
comp4 = TRUE
Comparing biohmm.mad.RAM.fork biohmm.mad.bic.ff.fork biohmm.mad.bic.ff.cluster
not equal: some info from comparisons.
comp1 = Component 3: Mean relative difference: 0.07866
comp2 = TRUE
comp3 = TRUE
comp4 = TRUE
Comparing biohmm.RAM.fork biohmm.mad.ff.fork biohmm.mad.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 5.226
   Component 2: Mean relative difference: 4.876
   Component 3: Mean relative difference: 0.0308
   Component 4: Mean relative difference: 0.05044
   Component 5: Mean relative difference: 0.007393
   Component 6: Mean relative difference: 1.897
comp2 = TRUE
 comp3 = FALSE
comp4 = TRUE
```

Comparing cbs.mad.RAM.fork cbs.ff.fork cbs.ff.cluster not equal: some info from comparisons.

```
comp1 = Component 1: Mean relative difference: 0.974
   Component 2: Mean relative difference: 1.06
   Component 3: Mean relative difference: 0.02329
   Component 4: Mean relative difference: 0.02681
   Component 5: Mean relative difference: 0.008088
   Component 6: Mean relative difference: 0.9384
comp2 = TRUE
 comp3 = FALSE
comp4 = TRUE
Comparing cbs.none.RAM.fork cbs.mad.ff.fork cbs.mad.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 1.15
   Component 3: Mean relative difference: 0.07285
comp2 = Component 1: Mean relative difference: 0.8297
   Component 4: Mean relative difference: 0.0334
comp3 = FALSE
comp4 = TRUE
Comparing cbs.RAM.fork cbs.none.ff.fork cbs.none.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 5.226
   Component 2: Mean relative difference: 4.876
   Component 3: Mean relative difference: 0.02322
   Component 4: Mean relative difference: 0.02384
   Component 5: Mean relative difference: 0.008117
   Component 6: Mean relative difference: 1.897
 comp2 = Component 4: Mean relative difference: 0.0334
 comp3 = FALSE
 comp4 = FALSE
Comparing hmm.mad.RAM.fork hmm.ff.fork hmm.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 0.3577
   Component 2: Mean relative difference: 1.005
   Component 3: Mean relative difference: 0.01367
   Component 4: Mean relative difference: 0.04048
   Component 5: Mean relative difference: 0.0087
   Component 6: Mean relative difference: 0.9926
 comp2 = Component 3: Mean relative difference: 0.008796
 comp3 = FALSE
 comp4 = TRUE
Comparing hmm.RAM.fork hmm.mad.ff.fork hmm.mad.ff.cluster
not equal: some info from comparisons.
comp1 = Component 1: Mean relative difference: 0.3488
   Component 2: Mean relative difference: 3.519
   Component 3: Mean relative difference: 0.01368
```

```
Component 4: Mean relative difference: 0.04033
    Component 5: Mean relative difference: 0.008706
    Component 6: Mean relative difference: 2.252
         Component 6: Mean relative difference: 0.1534
 comp3 =
         FALSE
 comp4 =
          TRUE
biohmm.mad.bic.RAM.fork
                            biohmm.mad.RAM.fork
                                                         biohmm.RAM.fork
                  FALSE
                                           FALSE
                                                                   FALSE
       cbs.mad.RAM.fork
                              cbs.none.RAM.fork
                                                            cbs.RAM.fork
                  FALSE
                                           FALSE
                                                                    FALSE
                          cghseg.merge.RAM.fork
    cghseg.mad.RAM.fork
                                                    cghseg.none.RAM.fork
                   TRUE
                                            TRUE
                                                                     TRUE
          glad.RAM.fork
                               hmm.mad.RAM.fork
                                                            hmm.RAM.fork
                   TRUE
                                           FALSE
                                                                    FALSE
        hs.mad.RAM.fork
                                 hs.ml.RAM.fork
                                                      waves.mad.RAM.fork
                   TRUE
                                            TRUE
                                                                     TRUE
   waves.merge.RAM.fork
                            waves.none.RAM.fork
                   TRUE
                                            TRUE
```

(Of course, we depend on the lists of names of objects having the output from the same method and option in the same position, which is the case in these examples).

4 Clean up actions

>

These are not strictly necessary, but we will explicitly stop the cluster. In this vignette, we will not execute the code below to remove the directory we created or the objects, in case you want to check them out or play around with them, but the code is below.

To make sure there are no file permission problems, we add code below to explicitly delete some of the "ff" files and objects (and we wait a few seconds to allow pending I/O operations to happen before we delete the directory).

```
> parallel::stopCluster(c12)
> ## This is the code to remove all the files we created
> ## and the temporary directory.
> ## We are not executing it!
> load("chromData.RData")
> load("posData.RData")
> load("cghData.RData")
> delete(cghData); rm(cghData)
> delete(posData); rm(posData)
> delete(chromData); rm(chromData)
> tmpout <-
    capture.output(
      lapply(ff.fork.obj, function(x) {
        lapply(get(x), delete)}))
> rm(list = ff.fork.obj)
> tmpout <-
```

```
+ capture.output(
+ lapply(ff.cluster.obj, function(x) {
+ lapply(get(x), delete)}))
> rm(list = ff.cluster.obj)
> setwd(originalDir)
> print(getwd())
> Sys.sleep(3)
> unlink("ADaCGH2_vignette_tmp_dir", recursive = TRUE)
> Sys.sleep(3)
```