

Analysis of data from aCGH experiments using parallel computing and ff objects

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1 Overview:

ADaCGH2 is a package for the analysis of CGH data. The main features of ADaCGH2 are:

- Parallelization of (several of) the main segmentation/calling algorithms currently available, allow efficient usage of computing clusters.
- Data access data using the *ff* package (<http://cran.r-project.org/web/packages/ff/index.html>), making it possible to analyze data from very large projects.

- Parallelization and *ff* are used simultaneously. WaviCGH Carro et al. (2010) (<http://wavi.bioinfo.cnio.es>), a web-server application for the analysis and visualization of array-CGH data that uses ADaCGH2, constitutes a clear demonstration of the usage of *ff* on a computing cluster with shared storage over NFS.

ADaCGH2 is a major re-write of our former package ADaCGH Díaz-Uriarte and Rueda (2007). We have improved the parallelization and, specially, changed completely the data handling routines. Thanks to the usage of the *ff* package, ADaCGH2 can now analyze data sets of more than four million probes in machines with no more than 2 GB of RAM.

2 A commented example

For both interactive and non-interactive executions we will often execute the following in sequence:

1. Convert the original data to *ff* objects
2. Initialize the computing cluster
3. Carry out segmentation and calling
4. Plot the results

We cover each in turn in the remaining of this section.

```
> library(ADaCGH2)
```

2.1 Choosing a working directory

This package reads and writes quite a few files to the hard drive. The easiest way to organize your work is to create a separated directory for each project. All plot files and *ff* data will be stored there. At the end of this example, we will remove this directory.

(Just in case, I check for its existence first.)

```
> if(!file.exists("ADaCGH2_vignette_tmp_dir"))
+   dir.create("ADaCGH2_vignette_tmp_dir")
> originalDir <- getwd()
> setwd("ADaCGH2_vignette_tmp_dir")
```

2.2 Convert original data to ff objects

The first two steps need not be carried out in that sequence. Conversion of original data does not use the computing cluster, but can be done after initialization of the cluster. The purpose of this step is to write down the ff files to disk, so they are available for the segmentation and plotting functions. (See below, section 4 for examples of using objects from Limma and snapCGH).

To allow the conversion to be carried out in data from previous sessions, the conversion takes as input the name of an RData.

```
> fname <- list.files(path = system.file("data", package = "ADaCGH2"),
+                    full.names = TRUE, pattern = "inputEx1")
> tableChromArray <- inputDataToADaCGHData(filename = fname)
```

Calling gc before returning to track memory usage

```
          used (Mb) gc trigger (Mb) max used (Mb)
Ncells 1613184 86.2   2564037 137.0 1967602 105.1
Vcells 1651609 12.7   2685683  20.5 2194211  16.8
```

Files saved in current directory

/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc/ADaCGH2_vignette_tmp_dir with
chromData.RData, posData.RData, cghData.RData, probeNames.RData

```
> tableChromArray
```

	Index	ArrayNum	ArrayName	ChromNum	ChromName	posInit	posEnd
1	1	1	L.1	1	1	1	616
2	2	2	L.2	1	1	1	616
3	3	3	m4	1	1	1	616
4	4	1	L.1	2	2	617	882
5	5	2	L.2	2	2	617	882
6	6	3	m4	2	2	617	882
7	7	1	L.1	3	3	883	1113
8	8	2	L.2	3	3	883	1113
9	9	3	m4	3	3	883	1113
10	10	1	L.1	4	4	1114	1234
11	11	2	L.2	4	4	1114	1234
12	12	3	m4	4	4	1114	1234
13	13	1	L.1	5	5	1235	1425
14	14	2	L.2	5	5	1235	1425
15	15	3	m4	5	5	1235	1425
16	16	1	L.1	6	6	1426	1624
17	17	2	L.2	6	6	1426	1624

18	18	3	m4	6	6	1426	1624
19	19	1	L.1	7	7	1625	1825
20	20	2	L.2	7	7	1625	1825
21	21	3	m4	7	7	1625	1825
22	22	1	L.1	8	8	1826	1965
23	23	2	L.2	8	8	1826	1965
24	24	3	m4	8	8	1826	1965
25	25	1	L.1	9	9	1966	2286
26	26	2	L.2	9	9	1966	2286
27	27	3	m4	9	9	1966	2286
28	28	1	L.1	10	10	2287	2519
29	29	2	L.2	10	10	2287	2519
30	30	3	m4	10	10	2287	2519
31	31	1	L.1	11	11	2520	2880
32	32	2	L.2	11	11	2520	2880
33	33	3	m4	11	11	2520	2880
34	34	1	L.1	12	12	2881	3084
35	35	2	L.2	12	12	2881	3084
36	36	3	m4	12	12	2881	3084
37	37	1	L.1	13	13	3085	3172
38	38	2	L.2	13	13	3085	3172
39	39	3	m4	13	13	3085	3172
40	40	1	L.1	14	14	3173	3323
41	41	2	L.2	14	14	3173	3323
42	42	3	m4	14	14	3173	3323
43	43	1	L.1	15	15	3324	3446
44	44	2	L.2	15	15	3324	3446
45	45	3	m4	15	15	3324	3446
46	46	1	L.1	16	16	3447	3601
47	47	2	L.2	16	16	3447	3601
48	48	3	m4	16	16	3447	3601
49	49	1	L.1	17	17	3602	3837
50	50	2	L.2	17	17	3602	3837
51	51	3	m4	17	17	3602	3837
52	52	1	L.1	18	18	3838	3898
53	53	2	L.2	18	18	3838	3898
54	54	3	m4	18	18	3838	3898
55	55	1	L.1	19	19	3899	4141
56	56	2	L.2	19	19	3899	4141
57	57	3	m4	19	19	3899	4141
58	58	1	L.1	20	20	4142	4241
59	59	2	L.2	20	20	4142	4241
60	60	3	m4	20	20	4142	4241
61	61	1	L.1	21	21	4242	4284

62	62	2	L.2	21	21	4242	4284
63	63	3	m4	21	21	4242	4284
64	64	1	L.1	22	22	4285	4368
65	65	2	L.2	22	22	4285	4368
66	66	3	m4	22	22	4285	4368

The first command is used in this example to find the complete path of the example data set. The actual call to the function is the second expression. The return object is not used for anything further and can be deleted (or not saved).

With large data sets that can be the single step that consumes the most RAM, since we need to load the original data into R. Even if we remove the original data and call `gc()`, R might not return all of the memory to the operating system, and this might be inconvenient in multiuser environments and/or long running processes. Thus, it is possible to execute the above in a separate R process that is spawned exclusively just for the conversion. For instance, we could use the `multicore` package and do:

```
> require(multicore)
> parallel(inputDataToADaCGHData(filename = fname), silent = FALSE)
> tableChromArray <- collect()[[1]]
> if(inherits(tableChromArray, "try-error")) {
+   stop("ERROR in input data conversion")
+ }
>
```

That way, the `ff` are produced and stored locally in the hard drive, but the R process where the original data was loaded (and the conversion to `ff` carried out) dies after the conversion, freeing back the memory to the operating system.

2.3 Initialize computing cluster

Initialization is done with the function `snowfallInit`, a function which uses the package `snowfall` to initialize the cluster and set up the slave nodes. In the example we will use a socket cluster, but MPI can also be used (our servers use LAM-MPI). In this example we will use 2 nodes.

```
> snowfallInit(universeSize = 2, typecluster = "SOCK")
```

```
R Version: R version 3.0.0 (2013-04-03)
```

```
Library ADaCGH2 loaded.
```

2.4 Carry out segmentation and calling

Segmentation and calling are carried out with the `pSegment` functions. Here we show just one such example. Many more are available from the help.

```
> help(pSegment)

> hs_mad.out <- pSegmentHaarSeg("cghData.RData",
+                               "chromData.RData", merging = "MAD")
```

We can take a quick look at the output. We first open the `ff` objects (the output is a list of `ff` objects) and then call `summary` on the smoothed list

```
> lapply(hs_mad.out, open)
```

```
$outSmoothed
[1] TRUE
```

```
$outState
[1] TRUE
```

```
> summary(hs_mad.out[[1]][,])
```

	L.1	L.2	m4
Min.	:-1.49400	Min. :-1.360000	Min. :-1.542250
1st Qu.:	-0.07997	1st Qu.:-0.010616	1st Qu.:-0.063417
Median :	0.01077	Median : 0.008625	Median :-0.016141
Mean :	0.00569	Mean : 0.005947	Mean : 0.002951
3rd Qu.:	0.07764	3rd Qu.: 0.013814	3rd Qu.: 0.098133
Max. :	0.79718	Max. : 0.632500	Max. : 1.342250

2.5 Plot the results

Plotting produces PNG files for easier sharing over the Internet. The plotting function takes as main arguments the names of RData files. We first write to disk the (`ff`) object with the results, and then call the plotting function:

```
> save(hs_mad.out, file = "hs_mad.out.RData", compress = FALSE)
> pChromPlot(outRDataName = "hs_mad.out.RData",
+            cghRDataName = "cghData.RData",
+            chromRDataName = "chromData.RData",
+            posRDataName = "posData.RData",
+            probenamesRDataName = "probeNames.RData",
+            imgheight = 350)
```

3 Using CGHregions

The CGHregions package van de Wiel (2009) is a BioConductor package that implements a well known method van de Wiel and van Wieringen (2007) for dimension reduction for aCGH data (see a review of common regions issues and methods in Rueda and Diaz-uriarte (2010)).

The CGHregions function accepts different type of input, among others a data frame. The function outputToCGHregions produces that data frame, ready to be used as input to CGHregions (for the next example, you will need to have the *CGHregions* package installed).

```
> forcghr <- outputToCGHregions(hs_mad.out)
> if(require(CGHregions)) {
+   regions1 <- CGHregions(forcghr)
+   regions1
+ }

[1] 1 0 7
[1] 2 0 7
[1] "Tuning on small data set finished...started with entire data set"
[1] 2.000000000 0.004996431 21.000000000 1.000000000
[1] "Average error threshold is satisfied for any distance value c"
[1] "c = 3, nr of regions: 22"
[1] "Finished with entire data set."
cghRegions (storageMode: lockedEnvironment)
assayData: 22 features, 3 samples
  element names: regions
protocolData: none
phenoData: none
featureData
  featureNames: 1 2 ... 22 (22 total)
  fvarLabels: Chromosome Start ... AveDist (5 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

>
```

Please note that outputToCGHregions does NOT check if the calls are something that can be meaningfully passed to CGHregions. In particular, you probably do NOT want to use this function when pSegment has been called using merging = "none".

4 Input data from Limma and snapCGH

Many aCGH studies use pre-processing steps similar to those of gene expression data. The `MAList` object, from *Limma* and `SegList` object, from *snapCGH*, are commonly used to store aCGH information. The following examples illustrate the usage of the function `inputDataToADaCGHData` to convert `MAList` and `SrgList` data into a format suitable for *ADaCGH2*.

We will start with objects produced by *snapCGH*. The following code is copied from the *snapCGH* vignette (pp. 2 and 3). Please check the original vignette for details. In summary, a set of array files are read, the data are normalized and, finally, averaged over clones. *snapCGH* uses *limma* for the initial import of data and, next, with the `read.clonesinfo` function adds additional information such as chromosome and position. The MA object created is of class `MAList`, but with added information (compared to a basic, original, *limma* `MAList` object). `MA2` is of type `SegList`.

```
> datadir <- system.file("testdata", package = "snapCGH")
> targets <- readTargets("targets.txt", path = datadir)
> RG1 <- read.maimages(targets$FileName, path = datadir, source = "genepix")

Read /home/biocbuild/bbs-2.12-bioc/R/library/snapCGH/testdata/10Mbslide28.gpr
Read /home/biocbuild/bbs-2.12-bioc/R/library/snapCGH/testdata/10Mbslide29.gpr

> RG1 <- read.clonesinfo("cloneinfo.txt", RG1, path = datadir) ## snapCGH-specific
> RG1$printer <- getLayout(RG1$genes)
> types <- readSpotTypes("SpotTypes.txt", path = datadir)
> RG1$genes$Status <- controlStatus(types, RG1)

Matching patterns for: ID Name Chr
Found 21 CTD-
Found 9 CTA-
Found 405 Chrom8
Found 66 Chrom3
Found 75 Chrom1
Setting attributes: values Color

> RG1$design <- c(-1, -1)
> RG2 <- backgroundCorrect(RG1, method = "minimum") ## class RGList
> MA <- normalizeWithinArrays(RG2, method = "median") ## class MAList
> class(MA)

[1] "MAList"
attr(,"package")
[1] "limma"

> MA2 <- processCGH(MA, method.of.averaging = mean, ID = "ID") ## class SegList
```



```

Averaging duplicated clones
Processing chromosome 1
Processing chromosome 2
Processing chromosome 3
Processing chromosome 4
Processing chromosome 5
Processing chromosome 6
Processing chromosome 7
Processing chromosome 8
Processing chromosome 9
Processing chromosome 10
Processing chromosome 11
Processing chromosome 12
Processing chromosome 13
Processing chromosome 14
Processing chromosome 15
Processing chromosome 16
Processing chromosome 17
Processing chromosome 18
Processing chromosome 19
Processing chromosome 20
Processing chromosome 21
Processing chromosome 22

```

```

> class(MA2)

[1] "SegList"
attr(,"package")
[1] "snapCGH"

>
>

```

All the information (intensity ratios and location) is available in the MA and MA2 objects. We can directly convert them to *ADaCGH2* objects (we set `na.omit = TRUE` as the data contain missing values). The first call process the MAList and the second the SegList.

```

> tmp <- inputDataToADaCGHData(MAList = MA, na.omit = TRUE)

```

We have identical MidPos!!!

Calling gc before returning to track memory usage

```

          used (Mb) gc trigger (Mb) max used (Mb)
Ncells 1744015 93.2   2732238 146.0 2203710 117.7
Vcells 2293829 17.6   3609273  27.6 3608762  27.6

```

Files saved in current directory

```

/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc/ADaCGH2_vignette_tmp_dir with
chromData.RData, posData.RData, cghData.RData, probeNames.RData

```

```

> tmp <- inputDataToADaCGHData(MAList = MA2, na.omit = TRUE,
+                               minNumPerChrom = 4)

```

Calling gc before returning to track memory usage

```

          used (Mb) gc trigger (Mb) max used (Mb)
Ncells 1744482 93.2   2732238 146.0 2203710 117.7
Vcells 2294916 17.6   3609273  27.6 3608762  27.6

```

Files saved in current directory

```

/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc/ADaCGH2_vignette_tmp_dir with
chromData.RData, posData.RData, cghData.RData, probeNames.RData

```

```

>
>

```

We need to change the argument to `minNumPerChrom` because, after the data processing step in `processCGH`, chromosome 21 has only four observations.

The original `MAList` as produced directly from *limma* do not have chromosome and position information. That is what the `read.clonesinfo` function from *snapCGH* did. To allow using objects directly from *limma* and incorporating position information, we will use an approach to directly mimicks that in *snapCGH*. If you use and `MAList` you can also provide a `cloneinfo` argument; this can be either the full path to a file with the format required by `read.clonesinfo` or, else, the name of an object with (at least) three columns, names `ID`, `Chr`, and `Position`.

We copy from the *limma* vignette (section 3.2, p.8), changing the names of objects by appending “.limma”.

```

> targets.limma <- readTargets("targets.txt", path = datadir)
> RG.limma <- read.maimages(targets.limma, path = datadir, source="genepix")

```

```

Read /home/biocbuild/bbs-2.12-bioc/R/library/snapCGH/testdata/10Mbslide28.gpr
Read /home/biocbuild/bbs-2.12-bioc/R/library/snapCGH/testdata/10Mbslide29.gpr

```

```

> RG.limma <- backgroundCorrect(RG.limma, method="normexp", offset=50)

```

```

Array 1 corrected
Array 2 corrected
Array 1 corrected
Array 2 corrected

```

```

> MA.limma <- normalizeWithinArrays(RG.limma)
>

```

We can add the chromosomal and position information in two different ways. First, as in `read.clonesinfo` or, else, we can provide the name of a file (with the same format as required by `read.clonesinfo`). Note that `fclone` is a path (and, thus, a character vector).

```

> fclone <- list.files(path = system.file("testdata", package = "snapCGH"),
+                      full.names = TRUE, pattern = "cloneinfo.txt")
> fclone

```

```

[1] "/home/biocbuild/bbs-2.12-bioc/R/library/snapCGH/testdata/cloneinfo.txt"

```

```

> tmp <- inputDataToADaCGHData(MAList = MA.limma,
+                              cloneinfo = fclone,
+                              na.omit = TRUE)

```

Assuming `cloneinfo` is a file (possibly with full path)
 We have identical `MidPos!!!`

Calling `gc` before returning to track memory usage

	used (Mb)	gc trigger	(Mb)	max used	(Mb)
Ncells	1745538	93.3	2732238	146.0	2203710
Vcells	2431156	18.6	3869736	29.6	3609089

Files saved in current directory

`/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc/ADaCGH2_vignette_tmp_dir` with
`chromData.RData`, `posData.RData`, `cghData.RData`, `probeNames.RData`

```

>

```

Alternatively, we can provide the name of an object with the additional information. For illustrative purposes, we can use here the columns of the `MA` object.

```

> acloneinfo <- MA$genes
> tmp <- inputDataToADaCGHData(MAList = MA.limma,
+                              cloneinfo = acloneinfo,
+                              na.omit = TRUE)

```

```
Assuming cloneinfo is an R data frame
We have identical MidPos!!!
```

```
Calling gc before returning to track memory usage
```

```
          used (Mb) gc trigger  (Mb) max used  (Mb)
Ncells 1745494 93.3    2732238 146.0  2203710 117.7
Vcells 2470296 18.9    3869736  29.6  3609089  27.6
```

```
Files saved in current directory
/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc/ADaCGH2_vignette_tmp_dir with
chromData.RData, posData.RData, cghData.RData, probeNames.RData
```

```
>
>
```

5 Clean up actions

These are not strictly necessary, but we will explicitly stop the cluster and remove the directory we created. To make sure there are no file permission problems, we also 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).

```
> sfStop()
> load("chromData.RData")
> load("posData.RData")
> load("cghData.RData")
> delete(cghData); rm(cghData)

[1] TRUE

> delete(posData); rm(posData)

[1] TRUE

> delete(chromData); rm(chromData)

[1] TRUE

> lapply(hs_mad.out, delete)

$outSmoothed
[1] TRUE
```

```
$outState
[1] TRUE

> rm(hs_mad.out)
> setwd(originalDir)
> print(getwd())

[1] "/tmp/RtmpURLVGG/Rbuild7f6e63837c1c/ADaCGH2/inst/doc"

> Sys.sleep(3)

> unlink("ADaCGH2_vignette_tmp_dir", recursive = TRUE)
> print(dir())

[1] "ADaCGH2.Rnw" "ADaCGH2.bib" "ADaCGH2.tex"

> Sys.sleep(3)
```

References

- Carro, A., Rico, D., Rueda, O. M., Díaz-Uriarte, R., and Pisano, D. G. (2010). waviCGH: a web application for the analysis and visualization of genomic copy number alterations. *Nucleic acids research*, 38 Suppl:W182–7.
- Díaz-Uriarte, R. and Rueda, O. M. (2007). ADaCGH: A parallelized web-based application and R package for the analysis of aCGH data. *PLoS one*, 2(1):e737.
- Rueda, O. M. and Diaz-uriarte, R. (2010). Finding Recurrent Copy Number Alteration Regions : A Review of Methods. *Current Bioinformatics*, 5:1–17.
- van de Wiel, M. a. and van Wieringen, W. N. (2007). CGHregions: Dimension Reduction for Array CGH Data with Minimal Information Loss. *Cancer informatics*, 3(0):55–63.
- van de Wiel, S. V. . M. (2009). *CGHregions: Dimension Reduction for Array CGH Data with Minimal Information Loss*. R package version 1.7.1.