

# An Introduction to the Oligo Package

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## 1 Introduction

The `oligo` package is designed to support all microarray designs provided by Affymetrix and NimbleGen: expression, tiling, SNP and exon arrays. With the increase in the density of the current technologies, `oligo` uses the resources offered by the `BufferedMatrix` packages to handle the feature-level information. As of now, chip-specific packages are built via `makePlatformDesign` and transitioning to the `pdInfoBuilder` package, which creates the data packages for the Affymetrix SNP arrays.

## 2 Analyzing Affymetrix SNP Arrays

Genotyping can be performed using `oligo` and you will need:

- `oligo` and its dependencies;
- Chip specific data package, eg. `pd.mapping50k.xba240`: package that contains the array specifications and SNP annotation.
- CEL files.

Figure 1 shows the general workflow for genotyping using the `oligo` package.

We will start by loading the `oligo` package and importing the CEL files available on the `sampleDataAffy100K`. The intensity matrix will be a *BufferedMatrix* object and this will require the use of temporary files in order to reduce the RAM usage. Although the temporary files can be stored anywhere, a better approach will be to use a local directory rather than using a directory on the network. The `tmpdir` in the `read.celfiles()` sets the directory where the temporary files are going to be stored.

```
R> library(oligo)
R> library(hapmap100kxba)
R> pathCelFiles <- system.file("celFiles", package = "hapmap100kxba")
R> fullFilenames <- list.celfiles(path = pathCelFiles,
  full.names = TRUE)
```

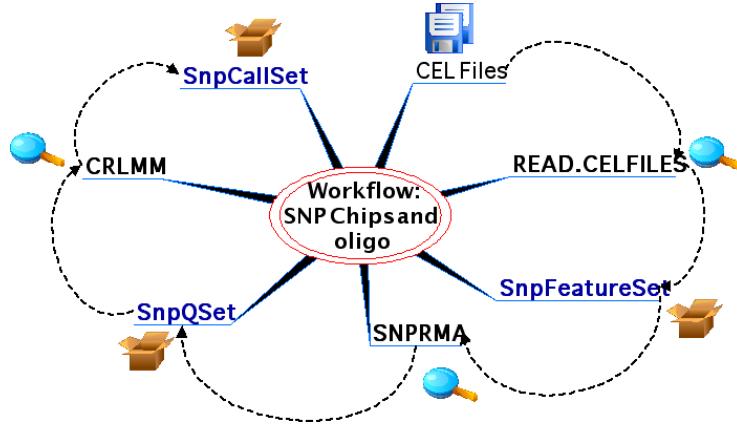


Figure 1: Genotyping workflow using the oligo package.

```
R> temporaryDir <- tempdir()
R> preProcessedData <- justSNPRMA(fullFileNames,
  tmpdir = temporaryDir)
```

#### Calculating Expression

The SNPRMA approach described in [?] is implemented in the `justSNPRMA` methods (see Figure 2), which preprocess the CEL files in a more effective way in terms of memory management. It returns a *SnpQSet* object without creating a *SnpFeatureSet* object. The *SnpQSet* object contains the summarized data.

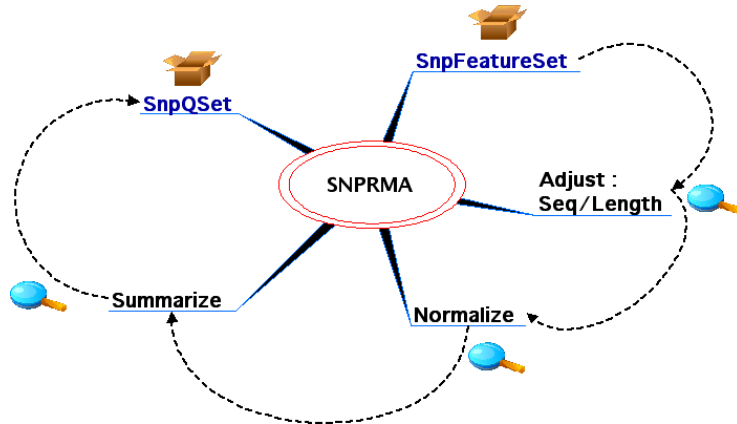


Figure 2: SNPRMA overview

For each SNP there are four numbers  $(\theta_{A-}, \theta_{B-}, \theta_{A+}, \theta_{B+})$ , which are proportional to the log-intensities in each of these combinations of allele and strand

(-: antisense; +: sense). They are represented by four matrices: **antisense-ThetaA**, **antisenseThetaB**, **senseThetaA** and **senseThetaB**, which are the components of the *SnpQSet* object. One can extract these objects using accessors of the same name.

Average intensities and log-ratios are defined as across allele and within strand, ie:

$$A_s = \frac{\theta_{A,s} + \theta_{B,s}}{2} \quad (1)$$

$$M_s = \theta_{A,s} - \theta_{B,s}, \quad (2)$$

where  $s$  defines the strand (antisense or sense). These quantities can be obtained via **getA()** and **getM()** methods, which return high-dimensional arrays with dimensions corresponding to SNP's, samples and strands, respectively.

```
R> theA <- getA(preProcessedData)
R> theM <- getM(preProcessedData)
R> dim(theA)
```

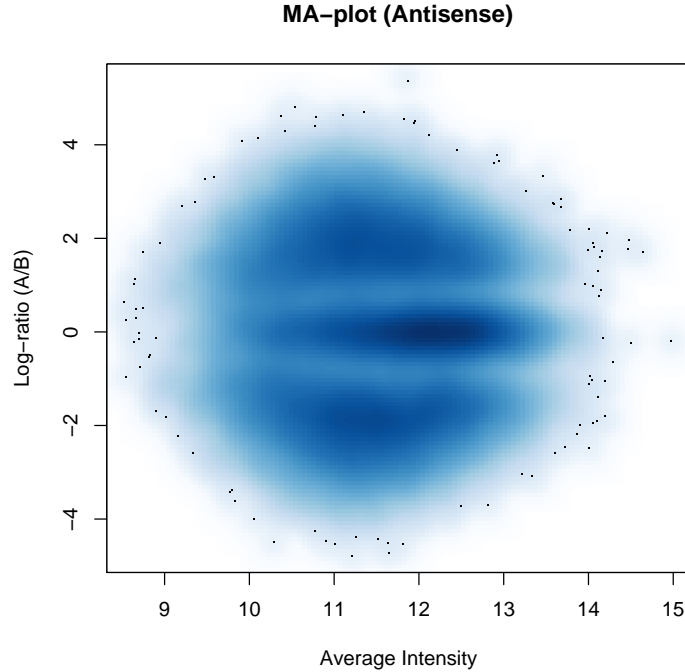
```
[1] 58960      3      2
```

```
R> str(theM)
```

```
num [1:58960, 1:3, 1:2] -2.309 -3.231 -2.616 -0.126 -1.406 ...
- attr(*, "dimnames")=List of 3
..$ : chr [1:58960] "SNP_A-1507972" "SNP_A-1510136" "SNP_A-1511055" "SNP_A-1518245" ...
..$ : chr [1:3] "CEU_NA06985_XBA.CEL.gz" "CEU_NA06991_XBA.CEL.gz" "CEU_NA06993_XBA.CEL.gz"
..$ : chr [1:2] "antisense" "sense"
```

These measures can be used, for example, to create an MA-plot and are later used for genotyping. The example below generates an MA-plot for the first sample using only the antisense strand data:

```
R> library(geneplotter)
R> smoothScatter(theA[, 1, 1], theM[, 1, 1], main = "MA-plot (Antisense)",
  xlab = "Average Intensity", ylab = "Log-ratio (A/B)")
```



The CRLMM algorithm [?] can be applied on a *SnpQSet* object in order to produce genotype calls. It involves running a mixture of regressions via EM algorithm to adjust for average intensity and fragment length in the log-ratio scale. These adjustments may take long time to run, depending on the combination of number of samples and computer resources available. To save time in subsequent analyses, we must specify the name of the file that will store the results obtained with the EM algorithm using the `correctionFile` argument. If the file passed to `correctionFile` does not exist, it is created, otherwise it is loaded. Figure 3 presents a diagram of the CRLMM algorithm:

**A word of warning:** the `crlmm()` method searches for a variable `gender` in the *phenoData* slot of the *SnpQSet* object. If it fails to find that variable, it will try estimate the gender from the data. If there is not enough discrimination power to estimate the gender, the following error message will be returned:

**empty cluster: try a better set of initial centers**

Increasing the sample size is one of the possible solutions, although the preferred one is to have `gender` already defined in the *phenoData* slot.

The *phenoData* slot includes covariates about the samples. Genotyping and copy number analyses often make use of gender information in order to provide more precise inferences. The code below exemplifies the creation of the *phenoData* object.

```
R> aboutSamples <- data.frame(gender = c("female",
    "female", "male"))
```

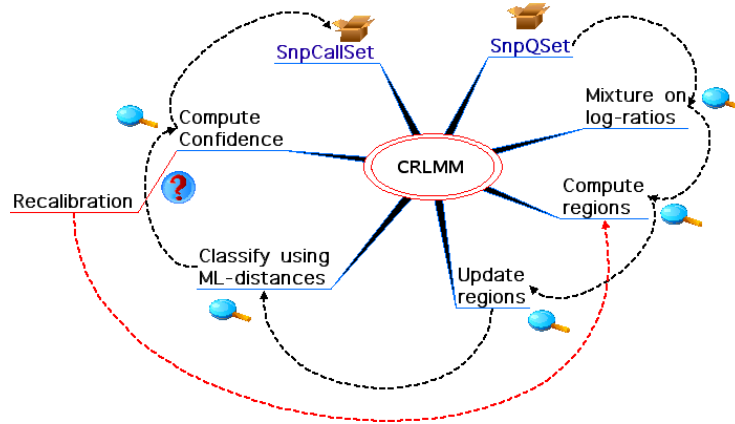


Figure 3: CRLMM Algorithm diagram

```
R> rownames(aboutSamples) <- sampleNames(preProcessedData)
R> aboutVars <- data.frame(labelDescription = "male/female")
R> rownames(aboutVars) <- "gender"
R> phenoData(preProcessedData) <- new("AnnotatedDataFrame",
  data = aboutSamples, varMetadata = aboutVars)

R> crlmmOut <- crlmm(preProcessedData, correctionFile = "exampleCorrection.rda",
  verbose = FALSE)
```

```
File with correction information - from EM - was found.
Loading...
Done.
Updating centers and scales.Done.
Computing confidence for calls on 3 arraysDone
Computing confidence for calls on 3 arraysDone
```

The `crlmmOut` object above belongs to the *SnpCallSet* class and contains the genotype calls and confidence measures associated to the calls, represented respectively by the `calls` and `callsConfidence` matrices. These matrices can be accessed using the methods of the same name as demonstrated below:

```
R> calls(crlmmOut)[1:5, 1:2]

                CEU_NA06985_XBA.CEL.gz CEU_NA06991_XBA.CEL.gz
SNP_A-1507972                3                3
SNP_A-1510136                3                3
SNP_A-1511055                3                3
SNP_A-1518245                2                3
SNP_A-1641749                3                3

R> callsConfidence(crlmmOut)[1:5, 1:2]
```

	CEU_NA06985_XBA.CEL.gz	CEU_NA06991_XBA.CEL.gz
SNP_A-1507972	335.0993	276.6474
SNP_A-1510136	553.5865	642.2731
SNP_A-1511055	336.5706	361.2075
SNP_A-1518245	147.7133	356.1431
SNP_A-1641749	183.8331	170.5362

The genotype calls are represented by 1 (AA), 2 (AB) and 3 (BB). The confidence is the log-likelihood ratio of the two most likely calls.

## 2.1 Exploring the Annotation Package

The user who is willing to make deeper investigation using the annotations provided for each SNP array can use SQL queries to access more other information that might not be directly exposed.

The example below demonstrates how to see the available tables, fields and extract chromosome, physical location and cytoband for the first five SNP's (probes querying specific SNP's have names starting with the string "SNP").

```
R> annot <- annotation(preProcessedData)
R> require(annot, character.only = TRUE)
R> conn <- oligo::db(get(annot))
R> dbListTables(conn)
```

```
[1] "featureSet"    "mmfeature"     "pm_mm"
[4] "pmfeature"     "qcmfeature"    "qcpm_qcmm"
[7] "qcpmfeature"   "sequence"      "sqlite_stat1"
[10] "table_info"
```

```
R> dbListFields(conn, "featureSet")
```

```
[1] "fsetid"        "man_fsetid"    "affy_snp_id"
[4] "dbsnp_rs_id"   "chrom"         "physical_pos"
[7] "strand"        "cytoband"      "allele_a"
[10] "allele_b"      "gene_assoc"    "fragment_length"
```

```
R> sql <- "SELECT man_fsetid, chrom, physical_pos FROM featureSet WHERE man_fsetid LIKE 'SNP'"
R> dbGetQuery(conn, sql)
```

	man_fsetid	chrom	physical_pos
1	SNP_A-1650338	2	168433267
2	SNP_A-1716667	19	40749462
3	SNP_A-1712945	19	53411226
4	SNP_A-1711654	21	31501701
5	SNP_A-1717655	1	15312743

### 3 Details

This document was written using:

```
R> sessionInfo()
```

```
R version 2.6.0 Under development (unstable) (2007-06-05 r41831)
x86_64-unknown-linux-gnu
```

```
locale:
```

```
LC_CTYPE=en_US.UTF-8;LC_NUMERIC=C;LC_TIME=en_US.UTF-8;LC_COLLATE=en_US.UTF-8;LC_MONETARY=en_
```

```
attached base packages:
```

```
[1] "splines"  "tools"    "stats"    "graphics"
[5] "grDevices" "utils"    "datasets" "methods"
[9] "base"
```

```
other attached packages:
```

```
      geneplotter      lattice
      "1.15.1"        "0.15-8"
      annotate        AnnotationDbi
      "1.15.1"        "0.0.77"
pd.mapping50k.xba240 hapmap100kxba
      "0.2.5"          "1.0"
      oligo           preprocessCore
      "1.1.1"          "0.99.7"
BufferedMatrixMethods BufferedMatrix
      "1.1.2"          "1.1.1"
      RSQLite         DBI
      "0.5-4"          "0.2-3"
      affyio          Biobase
      "1.5.2"          "1.15.13"
```

```
loaded via a namespace (and not attached):
```

```
      grid    KernSmooth RColorBrewer
      "2.6.0"   "2.22-20"    "0.2-3"
```