The *wateRmelon* Package

Chloe CY Wong, Ruth Pidsley and Leonard C Schalkwyk

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1 About the package

The *wateRmelon* package is designed to make it convenient to use the data quality metrics and normalization methods from our paper [1] as part of existing pipelines or work flows, and so as much as possible we have implemented S4 methods for *MethyLumiSet* objects (*methylumi* package), *MethylSet* and *RGChannelSet* objects (*minfi* package) and *exprmethy450* objects (*IMA* package).

In addition to our own functions, the package also contains functions by Matthieu Defrance [2] and Nizar Touleimat [3] and Andrew Teschendorff [4] as well as a wrapper for the SWAN method [5].

2 Installation

Because it is designed to work with several Bioconductor packages it unavoidably has many dependencies from CRAN as well as Bioconductor. In principle install.packages() installs dependencies automatically, but if there are problems you can install them by hand using the following commands:

```
> install.packages('ROCR', 'matrixStats')
> source("http://bioconductor.org/biocLite.R")
> biocLite( 'limma', 'minfi',
+    'IlluminaHumanMethylation450kmanifest',
+    'methylumi', 'lumi')
```

Installing the latest package from a local copy (assuming it is in the current working directory of your R session):

```
> install.packages('wateRmelon_0.9.9.tar.gz', repos=NULL, type='source')
```

3 Trying it out

The package contains a small subset of 450K array data which can be used to explore functions quickly; the `melon` data set for example is a *MethyLumiSet* with 12 samples but only 3363 features:

```
> library('wateRmelon')
> # load in melon dataset
> data (melon)
> # display dimensions of data matrix
> dim(melon)

Features  Samples
    3363       12

> # quality filter using default thresholds
> melon.pf<-pfilter(melon)

0 samples having 1 % of sites with a detection p-value greater than 0.05 were removed
Samples removed:
72 sites were removed as beadcount <3 in 5 % of samples
40 sites having 1 % of samples with a detection p-value greater than 0.05 were remove

> # preprocess using our best method
> melon.dasen.pf <- dasen(melon.pf)
```

4 Our performance metrics

We have taken advantage of known DNA methylation patterns associated with genomic imprinting and X-chromosome inactivation (XCI), in addition to the performance of SNP genotyping assays present on the array, to derive three independent metrics which we use to test alternative schemes of correction and normalization. These metrics also have potential utility as quality scores for datasets. All of them are expressed in such a way that lower values indicate better performance (i.e. better predicted ability to detect real methylation differences between samples).

4.1 Genomic imprinting

This is based on the hemi-methylation of genomic imprinting differentially methylated regions (DMRs), and is a standard-error-like measure of dispersion(SE).

```
> # calculate iDMR metrics on QC'd betas
> dmrse_row(melon.pf)

[1] 0.005428861

> # calculate iDMR metrics on QC'd and preprocessed betas
> dmrse_row(melon.dasen.pf)

[1] 0.002086381

> # slightly lower (better) standard errors
```

4.2 SNP genotypes

A very simple genotype calling by one-dimensional K-means clustering is performed on each SNP, and for those SNPs where there are three genotypes represented the squared deviations are summed for each genotype (similar to a standard deviation for each of allele A homozygote, AB heterozygote and allele B homozygote). By default these are further divided by the square root of the number of samples to get a standard error-like statistic.

```
> # calculate SNP metrics on QC'd betas
> genki(melon.pf)

NULL
[1] 8.129585e-05 2.020173e-04 7.819409e-05

> # calculate SNP metrics on QC'd and preprocessed betas
> genki(melon.dasen.pf)

NULL
[1] 0.0000507474 0.0001255203 0.0000452997

> # slightly lower (better) standard errors
```

4.3 X-chromosome inactivation

This is based on the male-female difference in DNA methylation, almost all of which is due to hypermethylation of the inactive X in females. This difference is thus a good predictor of X-chromosome location for probes, and this can be used for a Receiver Operating Characteristic (ROC) curve analysis. We report 1-(area under curve) (AUC) so that smaller values

indicate better performance, just as in our other two metrics. This requires the samples to be of known sex (and not all the same) and chromosome assignments for all probes. It takes more time to calculate than the other two metrics. Note that the roc and the auk are both sea birds.

```
> # calculate X-chromosome metrics on QC'd betas
> seabi(melon.pf, sex=pData(melon.pf)$sex, X=fData(melon.pf)$CHR=='X')

[1] 0.2597014

> # calculate X-chromosome metrics on QC'd and preprocessed betas
> seabi(melon.dasen.pf,
+    sex=pData(melon.dasen.pf)$sex,
+    X=fData(melon.dasen.pf)$CHR=='X'
+ )

[1] 0.1010268
```

5 Suggested analysis workflow

5.1 Load and tidy data

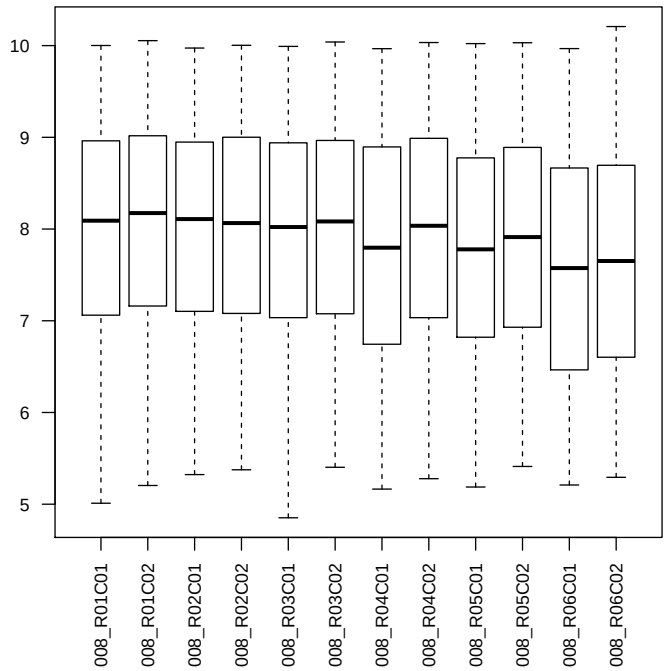
You can use a variety of methods to load your data, either from GenomeStudio final report text files or from iDAT files. *methylumi* and *IMA* can read text files, we recommend *methylumi* because the `exprmethy450` object only stores betas and not raw intensities. *methylumi* and *minfi* can both read iDAT files, and produce objects that can be used by our functions. Neither contains the full annotation that comes inside the final report text file. If you use the GenomeStudio file we recommend saving the unnormalized, uncorrected version of the data. We also recommend keeping the barcode names (SentrixID_RnnCnn) as the column headers or in a separate dataframe.

```
> library(methylumi)
> melon <- methyLumiR('finalreport.txt')
```

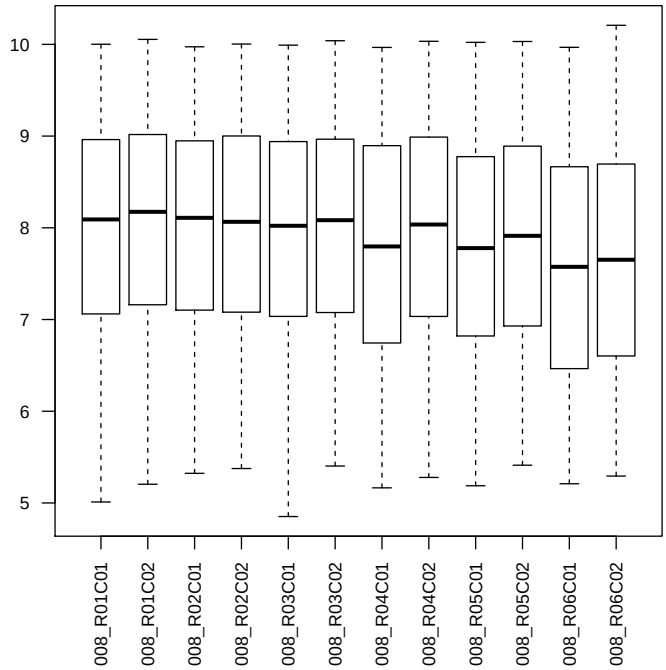
Normalization only works well at cleaning up minor distributional differences between samples. Failed or otherwise atypical samples should be filtered out beforehand. Also if you have different tissues or similar drastic divides in your data it may not be optimal to normalize everything together.

Visualization of raw intensities is a good way of identifying grossly atypical (failed) samples.

```
> boxplot(log(methylated(melon)), las=2, cex.axis=0.8 )
```




```
> boxplot(log(unmethylated(melon)), las=2, cex.axis=0.8 )
```



Filtering by detection p-value provides a straightforward approach for removing both failed samples and probes. The `pfilter` function conveniently discards samples with more than (by default) 1% of probes above the .05 detection p-value threshold, and probes with any samples with beadcount under 3 or more than 1% above the p-value threshold.

```
> melon.pf <- pfilter(melon)

0 samples having 1 % of sites with a detection p-value greater than 0.05 were removed
Samples removed:
72 sites were removed as beadcount <3 in 5 % of samples
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```

It has come to our attention that data read in using the various packages and input methods will give subtly variable data output as they calculate detection p-value and beta values differently, and do/don?t give information about beadcount. The pfilter function does not correct for this, but simply uses the detection p-value and bead count provided by each package.

5.2 Normalize and calculate betas

In our analysis[1] we tested 15 preprocessing and normalization methods. This involved processing 10 data sets 15 different ways and calculating three metrics of performance from each one. You can do the same thing with your data if you like, but our recommended method `dasen` will work well for most data sets. If you suspect varying dye bias (if you have arrays scanned on different instruments, for example), you might want to try `nanes`.

```
> melon.dasen.pf <- dasen(melon.pf)
```

5.3 Example workflow: method for MethyLumiSet

```
> data(melon)
> # load in melon dataset
> melon.pf<-pfilter(melon)

0 samples having 1 % of sites with a detection p-value greater than 0.05 were removed
Samples removed:
72 sites were removed as beadcount <3 in 5 % of samples
40 sites having 1 % of samples with a detection p-value greater than 0.05 were remove

> # perform QC on raw data matrix using default thresholds
> melon.dasen.pf<-dasen(melon.pf)
> # preprocess using our best method
> sex  <- pData(melon.dasen.pf)$sex
> # extract phenotypic information for test
> bet<-betas(melon.dasen.pf)
> # extract processed beta values
> melon.sextest<-sextest(bet,sex)
> # run t-test to idenitify sex difference
```

5.4 Further analysis

We can't offer much help with the actual analysis, which will be different for every experiment. In general though, you need to get your experimental variables into the same order as your arrays and apply some kind of statistical test to each row of the table of betas. In the workflow shown here, the array barcodes are preserved and can be retrieved with `sampleNames` or `colnames`. It's convenient to read in a samplesheet, for example in csv format, with the barcodes as row names.

6 References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

[2] Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

[3] Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341)

[4]Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S. A Beta-Mixture Quantile Normalisation method for correcting probe design bias in Illumina Infinium 450k DNA methylation data. Bioinformatics. 2012 Nov 21.

[5] Maksimovic J, Gordon L, Oshlack A: SWAN: Subset quantile Within-Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. Genome biology 2012, 13(6):R44