minfi methylation pipeline

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`minfi` - Analyze Illumina Infinium DNA methylation arrays

- Reads Illumina's 450k array raw data (IDAT files) into R
- Performs QC and normalization
- Identifies differential methylation positions (DMP)

```
source("https://bioconductor.org/biocLite.R")
biocLite("minfi")
biocLite("minfiData")
```

```
library(minfi)
library(minfiData)
```

https://bioconductor.org/packages/release/bioc/html/minfi.html

Methylation data

```
baseDir <- system.file("extdata", package = "minfiData")
list.files(baseDir)
```

```
## [1] "5723646052"      "5723646053"      "SampleSheet.csv"
```

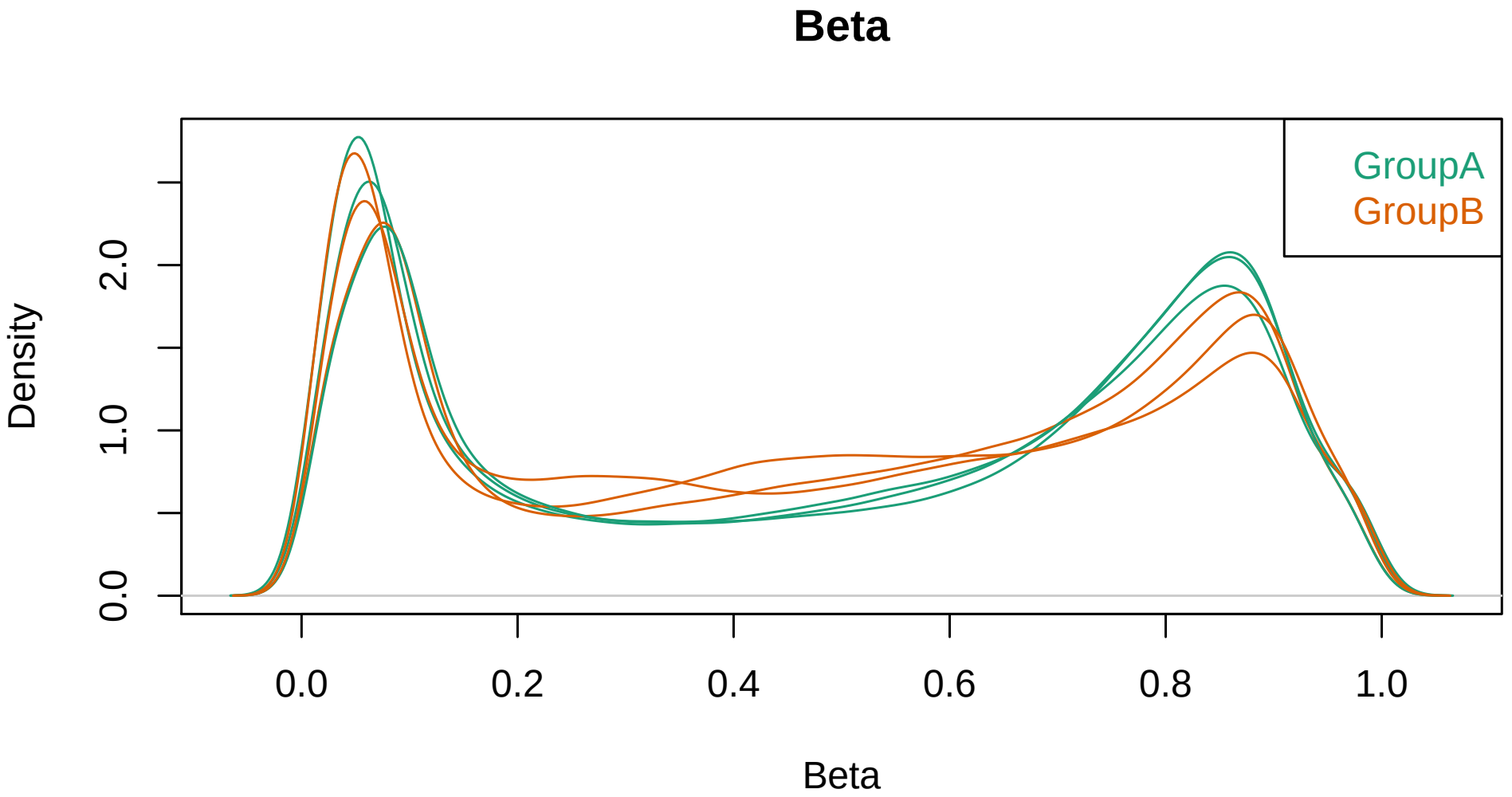
```
targets <- read.metharray.sheet(baseDir)
```

```
## [1] "/Users/mdozmorov/Library/R/3.4/library/minfiData/extda
```

```
RGset <- read.metharray.exp(targets = targets)
pd <- pData(RGset) ## phenotypic data
```

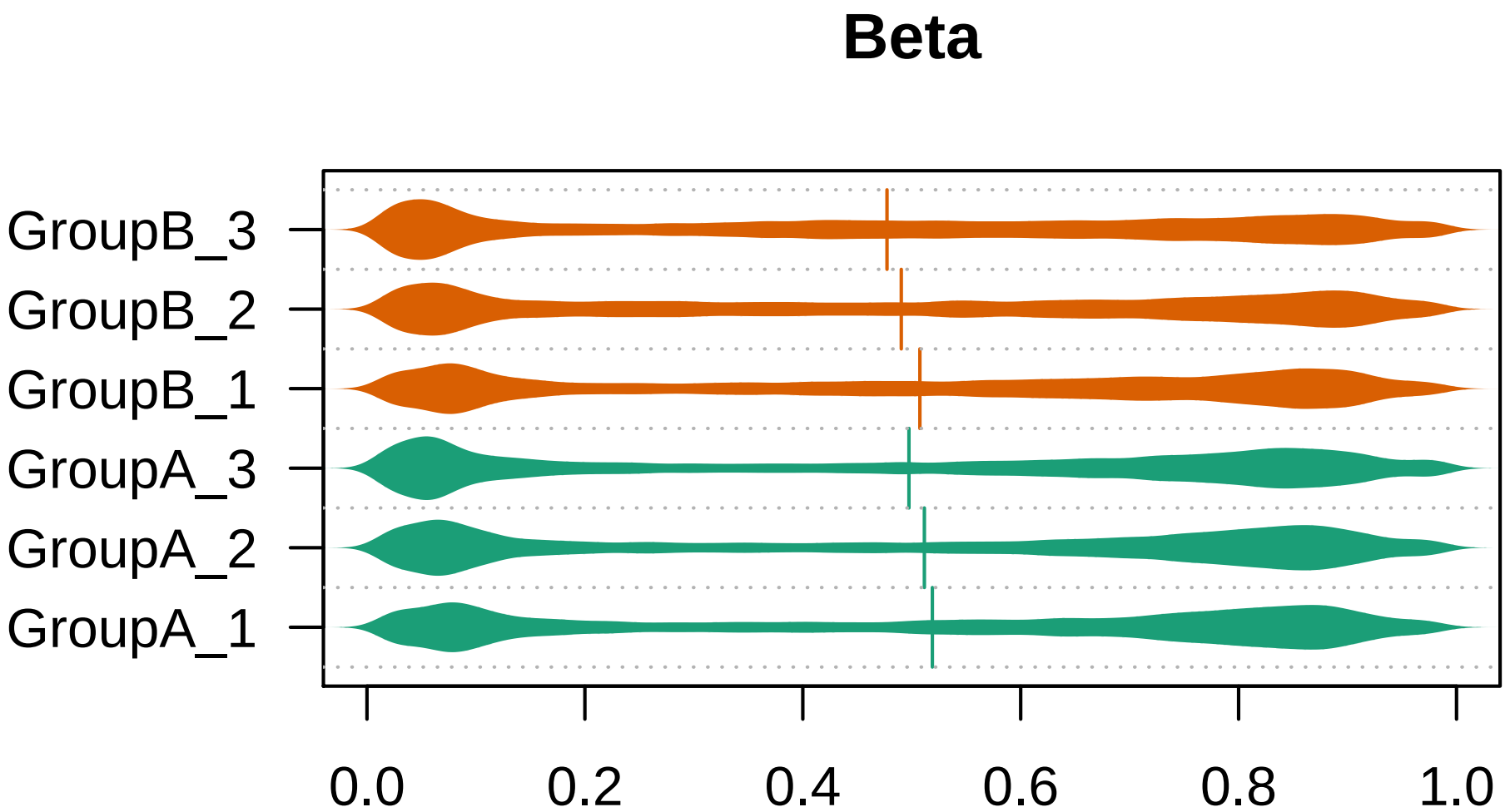
QC: Beta values are expected to cluster around 0/1.

```
densityPlot(RGset, sampGroups = pd$Sample_Group, main = "Beta"
```



QC: Beta values are expected to cluster around 0/1.

```
par(oma=c(2,10,1,1))
densityBeanPlot(RGset, sampGroups = pd$Sample_Group, sampNames
```



Normalization

Different methods for normalization have been proposed and still being developed

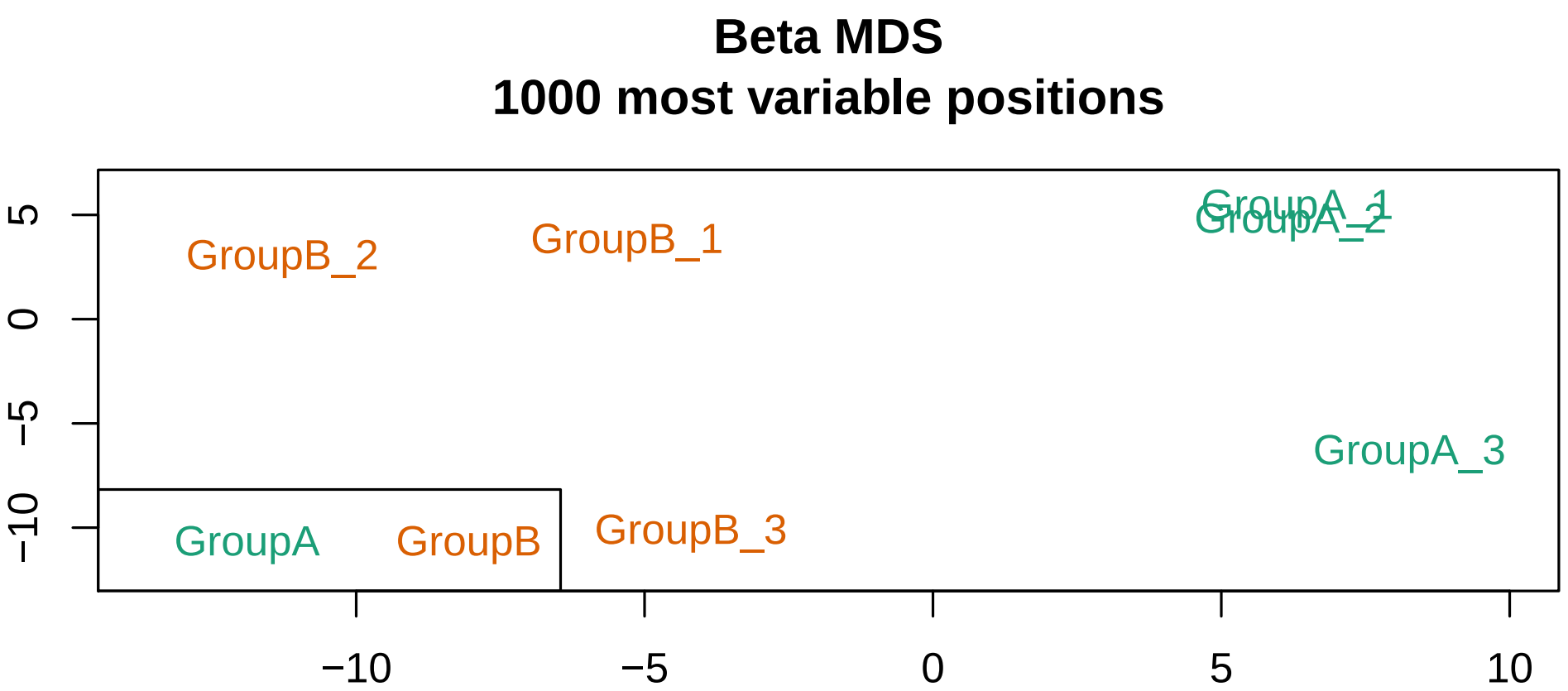
- Dye-bias adjustment
- Probe type I and II adjustment

```
MSet.norm <- preprocessIllumina(RGset, bg.correct = TRUE,
                                normalize = "controls",
                                reference = 2)
```

Yousefi P. et. al. "**Considerations for normalization of DNA methylation data by Illumina 450K BeadChip assay in population studies**" Epigenetics 2013 http://www.tandfonline.com/doi/abs/10.4161/epi.26037

Multi-dimensional scaling (MDS) plot

```
mdsPlot(MSet.norm, numPositions = 1000, sampGroups = pd$Sample
```



Similar to PCA, useful to identify outlier samples.

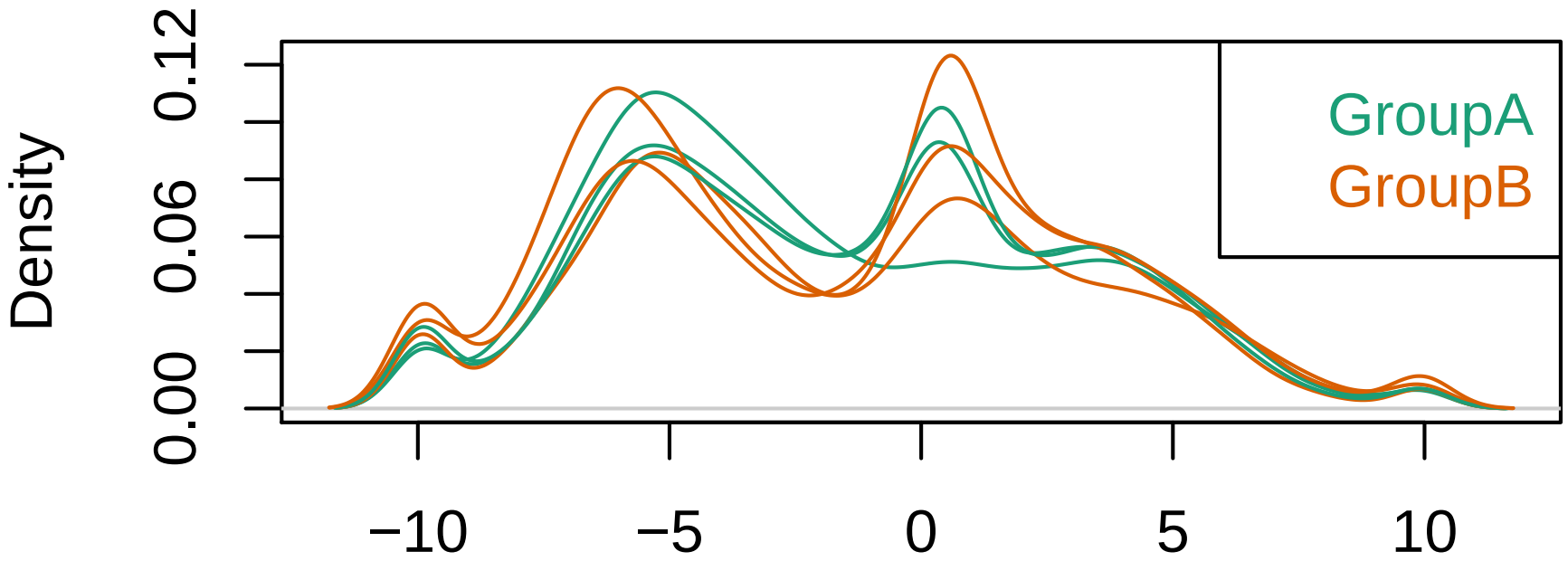
Getting M-values

```
# A small subset to speed up the demo:
mset <- MSet.norm[1:20000,]
# Getting the M values:
M <- getM(mset, type = "beta", betaThreshold = 0.001)
```

QC: M values should show the level of methylation centered around 0

Beta values ≤ 0.001, or ≥ 0.999 are truncated to avoid numerical issues.

```
# Look at the density distribution
par(oma=c(2,10,1,1))
densityPlot(M, sampGroups = pd$Sample_Group, sampNames = pd$Sa
```



Differentially methylated positions

```
dmp <- dmpFinder(M, pheno=pd$Sample_Group, type="categorical")
head(dmp)
```

```
##                intercept         f         pval         qval
## cg10805483 -9.964341 1706.1212 2.053224e-06 0.02639720
## cg20386875 -5.434480 1445.1107 2.859882e-06 0.02639720
## cg07155336 -5.799521  550.9746 1.952772e-05 0.05148498
## cg13059719 -2.505878  549.6611 1.962059e-05 0.05148498
## cg08343042 -3.565042  506.2230 2.310839e-05 0.05148498
## cg23098069  1.532107  497.6219 2.390872e-05 0.05148498
```

Rows ordered by p-value.

Plotting methylation levels

```
cpgs <- rownames(dmp)[1:4]
par(mfrow=c(2,2))
plotCpg(mset, cpg=cpgs, pheno=pd$Sample_Group)
```