Vignette Title

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Contents

Package (Abstract?)

A basic task of the Package ... is the detection of differentially expressed and methylated genes. The package ... uses the negative binomial test from `DESeq` package.

Standard Workflow

In this vignette we will work with the data sets containing information about gene expression and methylation for patients with breast cancer. We will analyze differences between methylation and expression for patients with different subtypes of BRCA cancer.

Function test_diff

The main function of the package is `test_diff`. It allows to find differences between genes methylation or expression, taking into account additional information about samples.

Methylation

Methylation is a process by which methyl groups are added to the DNA molecule. It can change the activity of a DNA without changing the sequence. DNA methylation typically acts to repress gene transcription. But there exists a situations in which adding the methyl groups intensifies it. DNA methylation is associated with a lots of key processes including genomic imprinting, repression of transposable elements, aging and carcinogenesis. In our work we want to bind methylation process and carcinogenesis.

In this section, we will work with the methylation level data from TCGA database.
Data set `BRCA_methylation_chr17` contains information about methylation of CpG islands located on 17th chromosome for patienst with breast cancer.

```
load("BRCA_methylation_chr17.rda")
head(BRCA_methylation_chr17)[1:5,1:4]
```

```
##                               SUBTYPE cg00021527 cg00031162  cg00032227
## TCGA-A1-A0SD-01A-11D-A112-05    LumA 0.03781858  0.7910348 0.006391233
## TCGA-A2-A04N-01A-11D-A112-05    LumA 0.01437552  0.7359370 0.008752293
## TCGA-A2-A04P-01A-31D-A032-05   Basal 0.01360124  0.6967802 0.009442039
## TCGA-A2-A04Q-01A-21D-A032-05   Basal 0.01525656  0.5341244 0.014674247
## TCGA-A2-A04T-01A-21D-A032-05   Basal 0.01167384  0.7378100 0.012251559
```

In this analysis we would like to find genes with different methylation. At first we need to use function `map_to_gene`, which generates new data frame with CpG islands mapped to genes.

```
library(MetExpR)
BRCA_methylation_chr17_gen <- map_to_gene(BRCA_methylation_chr17[,-1])
head(BRCA_methylation_chr17_gen)[1:5,1:4]
```

```
##                                  AANAT    AARSD1       AATF      AATK
## TCGA-A1-A0SD-01A-11D-A112-05 0.7148533 0.8625816 0.24294092 0.7835302
## TCGA-A2-A04N-01A-11D-A112-05 0.5850106 0.8355825 0.21367129 0.8466190
## TCGA-A2-A04P-01A-31D-A032-05 0.4495537 0.8786166 0.03277413 0.3417919
## TCGA-A2-A04Q-01A-21D-A032-05 0.7120650 0.8819490 0.03460160 0.7264985
## TCGA-A2-A04T-01A-21D-A032-05 0.6010397 0.7739978 0.02501599 0.6276399
```

Function `test_diff` allows us to test for differences between the base means for two or more conditions.

In this case we have two conditions, connected with subtypes of breast cancer.

Before we go to the testing, we need to define condition values for each sample. We would like to test for differences between `LumA` subtype and `other` subtypes of breast cancer, so we create vector, which each element corresponds to a sample. Our division to this two group relies on numbers of occurences each subtype. The `LumA` subtype is the most common, in case of breast cancer.

```
condition <- ifelse(BRCA_methylation_chr17$SUBTYPE=="LumA","LumA", "other")
head(condition,8)
```

```
## [1] "LumA"  "LumA"  "other" "other" "other" "other" "LumA"  "other"
```

T-test

One of the tools for testing differences between values is t-test. The null hypothesis, we have consider is that, means in two groups are equal.

To use it in `test_diff` function, we set value of parameter `test` on "ttest".

```
test.mety <- test_diff(BRCA_methylation_chr17_gen, condition, test="ttest")
```

As a result we obtain a data frame with columns corresponds to: id of gene, mean, logarithm of fold change, p-value for t-test, adjusted p-value (BH method). For more information about customizing this function see the help page for `test_diff`.

```
head(test.mety)
```

```
##              id      mean    log.fold         pval         padj
## ICAM2     ICAM2 0.3330801 -0.15151320 3.754116e-17 3.063359e-14
## RILP       RILP 0.3341447 -0.05073691 2.575168e-13 1.050668e-10
## PIPOX     PIPOX 0.3647812  0.11505558 5.360053e-12 1.196885e-09
## TNFSF12 TNFSF12 0.2485025 -0.13412855 5.867083e-12 1.196885e-09
## CD7         CD7 0.8127112  0.09822690 1.641919e-11 2.679612e-09
```

```
## KSR1       KSR1 0.5549808  0.19973400 2.054467e-11 2.794075e-09
```

Expression

Gene expression is the process by which information from a gene is used in the synthesis of proteins. The process of gene expression is used by all known life.

In this section we will use data set `BRCA_mRNAseq_chr17`, which contains information about gene expression. This data set contains per-gene read counts computed for genes from 17th chromosome for 100 patients with breast cancer.

```
load("BRCA_mRNAseq_chr17.rda")
```

```
BRCA_mRNAseq_chr17[1:5,1:5]
```

```
##                               SUBTYPE AANAT AARSD1 AATF AATK
## TCGA-A1-A0SB-01A-11R-A144-07  Normal     9   2354 2870  317
## TCGA-A1-A0SD-01A-11R-A115-07    LumA     2   1846 5656  312
## TCGA-A1-A0SE-01A-11R-A084-07    LumA    11   3391 9522  736
## TCGA-A1-A0SF-01A-11R-A144-07    LumA     0   2169 4625  169
## TCGA-A1-A0SG-01A-11R-A144-07    LumA     1   2273 3473   92
```

Nbinom test

Negative binomial distribution test is an another tool for finding differences between the base means of data having two or more conditions.

As in the t-test w need also a description of the samples, which we keep in a vector, whose elements correspond to different gorups.

In our example we will test for differential expression between groups with LumA breast cancer subtype and other subtypes of that cancer. Again we will use vector `conditions`, which consist of two values corresponds to subtype of breast cancer: LumA and other.

```
condition<-ifelse(BRCA_mRNAseq_chr17$SUBTYPE=="LumA","LumA","other")
head(condition,8)
```

```
## [1] "other" "LumA"  "LumA"  "LumA"  "LumA"  "LumA"  "other" "LumA"
```

For using negative binomial test, in function `test_diff` we set value “nbinom” for parameter `test`. Evaluation for nbinorm may take a few minutes.

```
test.expr <- test_diff(BRCA_mRNAseq_chr17[,-1], condition, test="nbinom")
```

As a result we obtain the following data frame:

```
head(test.expr)
```

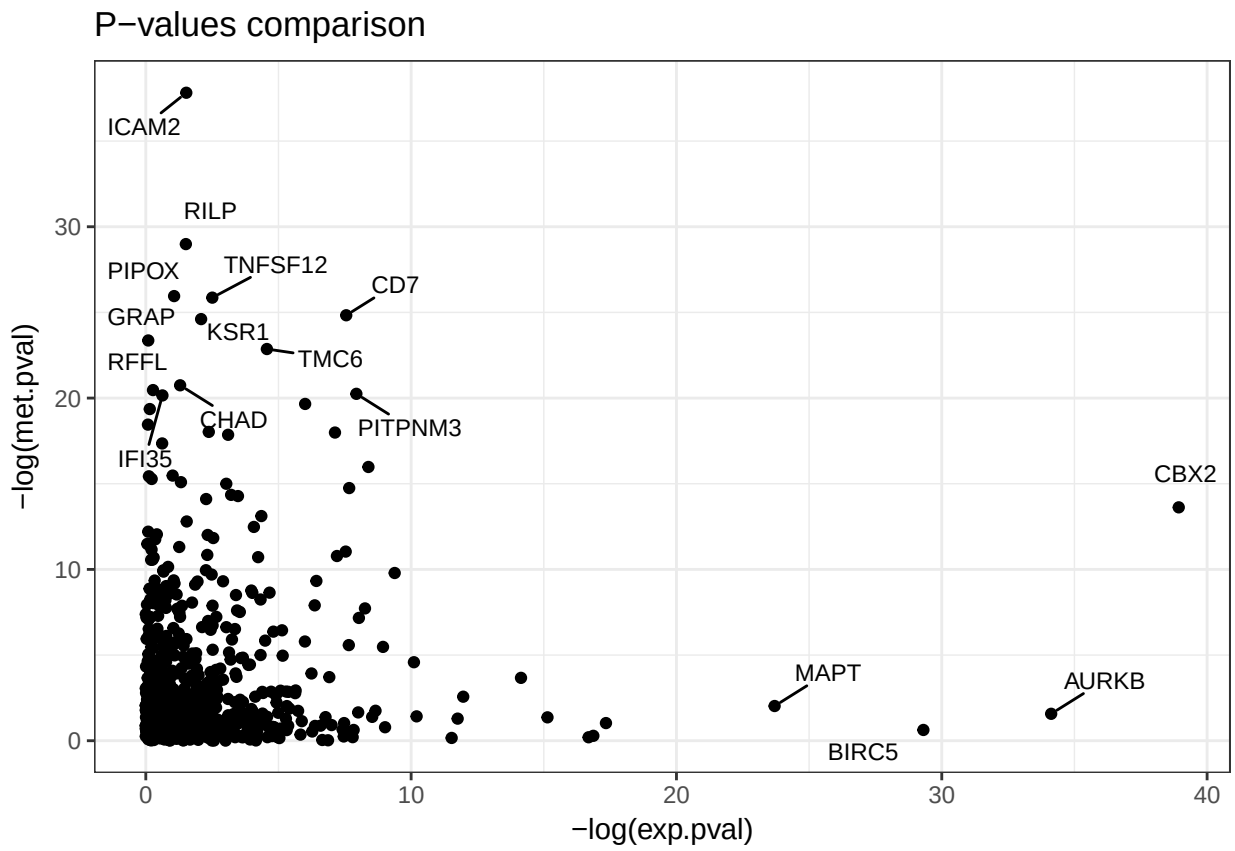
```
##       id        mean    log.fold      pval      padj
## 1  AANAT    3.455436 -0.40216187 0.9453634 0.9920531
## 2 AARSD1 2779.448414  0.07427334 0.6627296 0.8992428
## 3   AATF 6750.269650 -0.13313947 0.6864598 0.9111193
## 4   AATK  352.805108 -0.02272566 0.8051408 0.9585881
## 5  ABCA5 1933.257431  0.07031881 0.5837682 0.8489059
## 6  ABCA6  689.547294  0.44680041 0.1289441 0.3857753
```

Visualization

log-log p-value

Firstly, we want to visualise the p-values for expression and methylation from negative binomial test and t-test respectively.

```
p_values_plot(test.expr, test.mety)
```

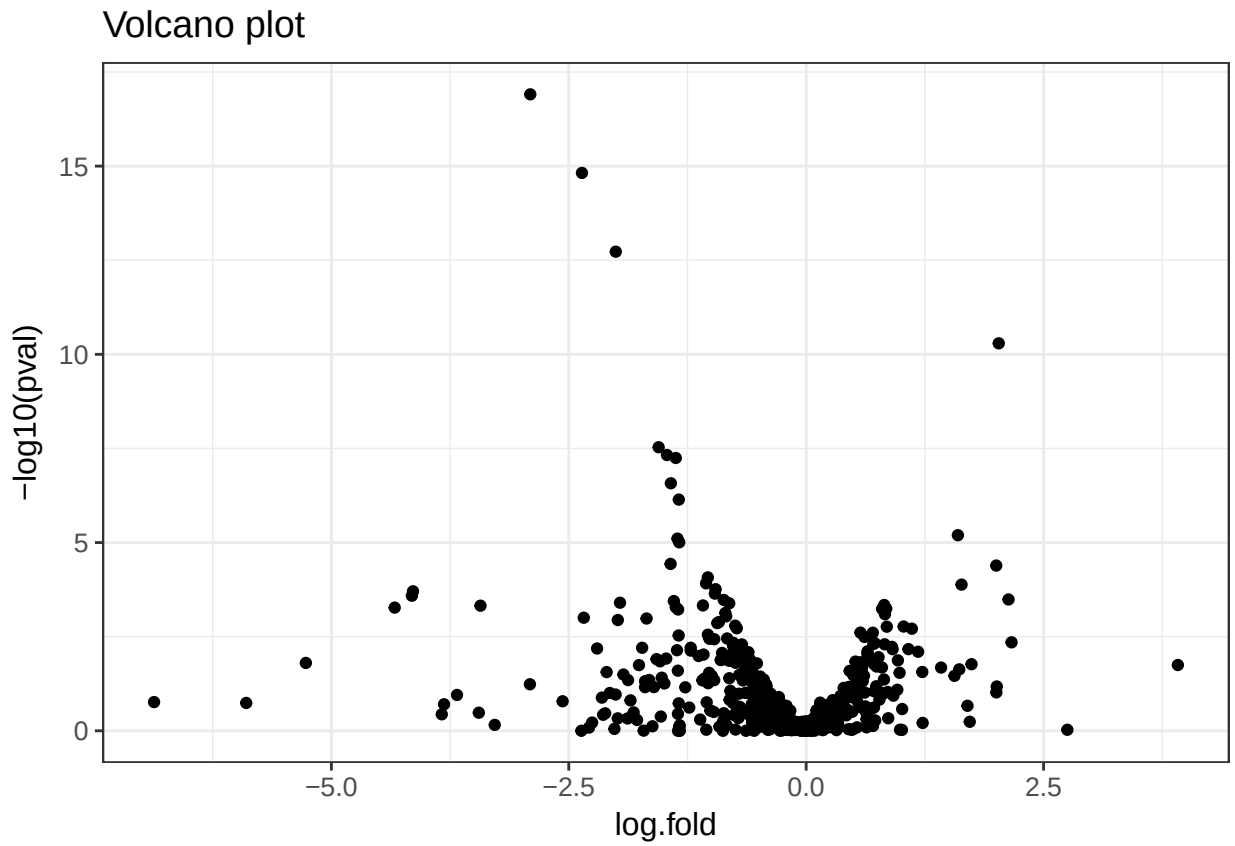


The marked values are the genes with p-values, from methylation or expression, lower than 0.05.

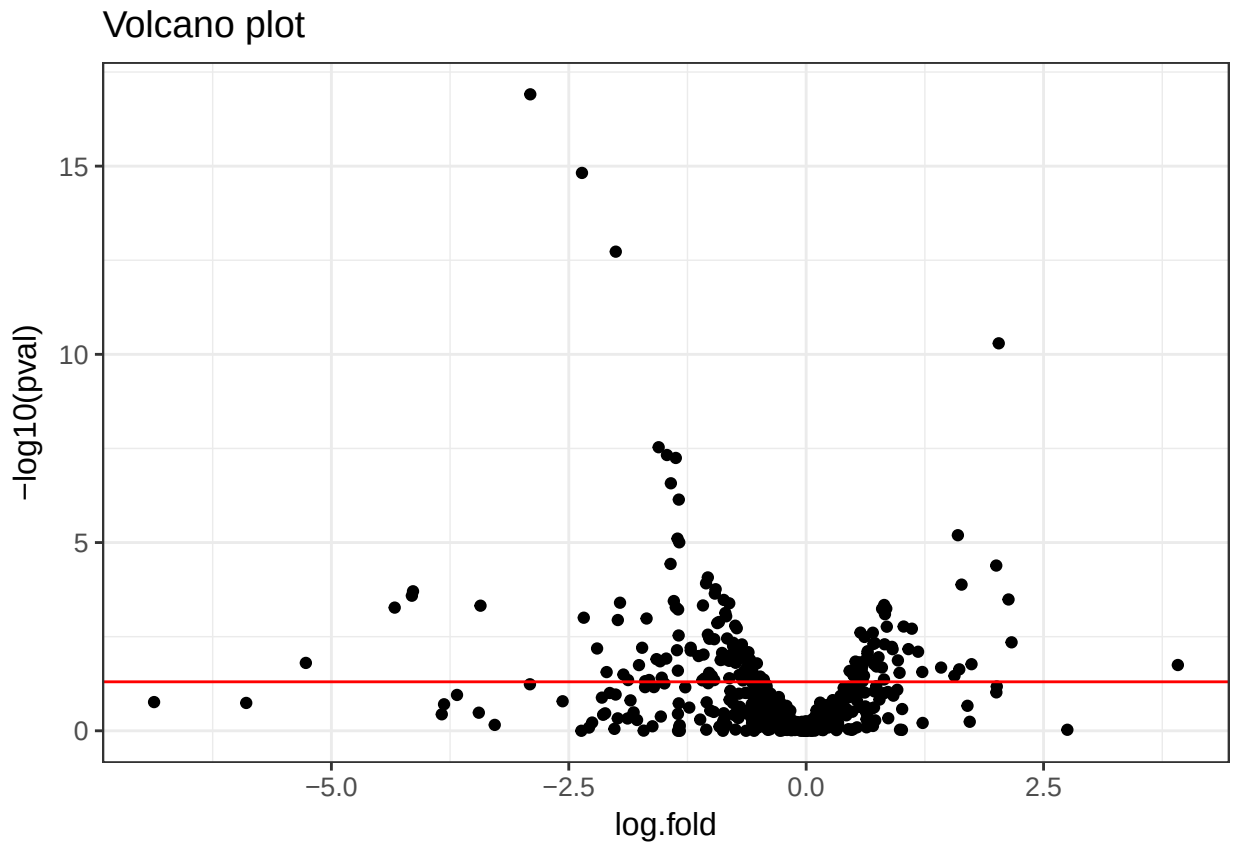
Volcano plot

For identify changes in our data sets we use a volcano plot, some type of scatter-plot. In our package it plots logarithm of p-value versus logarithm of fold-change on the y and x axes, respectively.

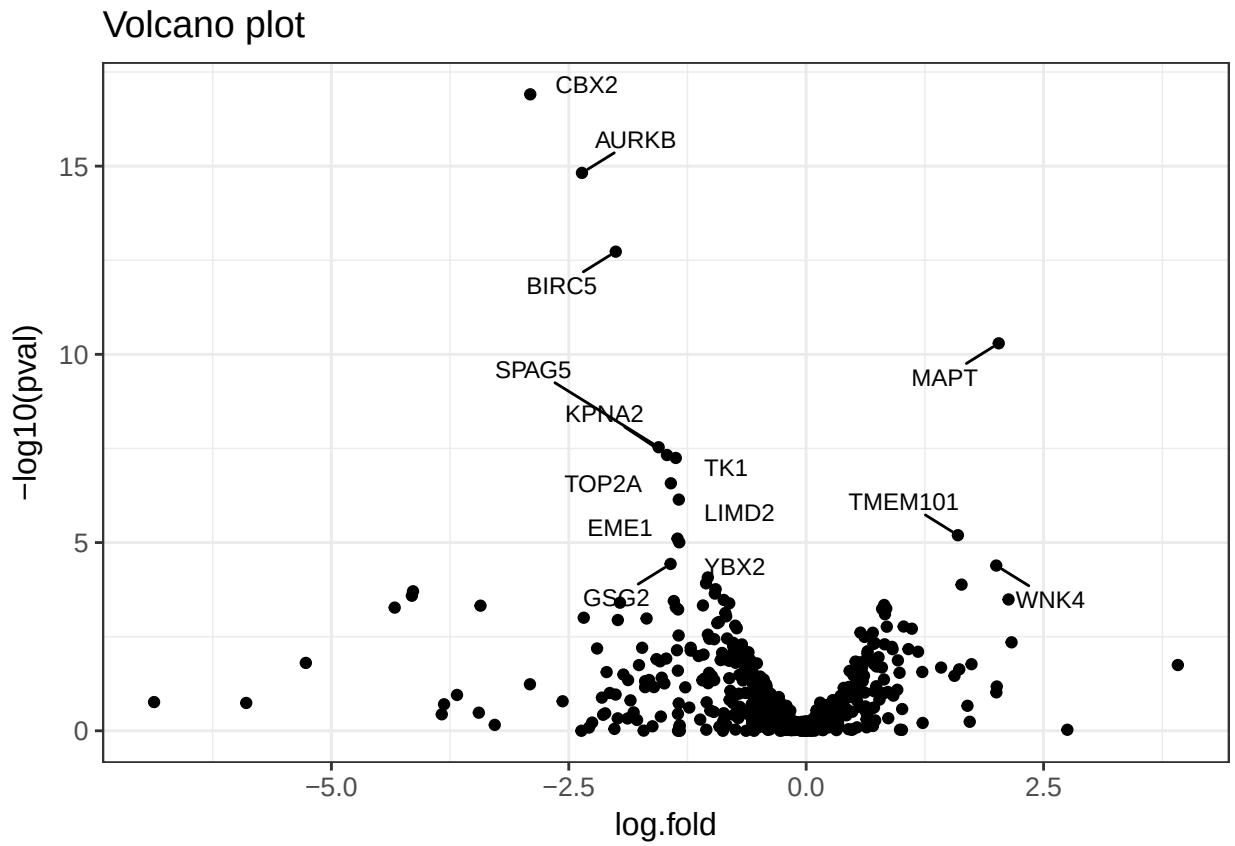
```
volcano_plot(test.expr)
```




```
volcano_plot(test.expr, line = 0.05)
```




```
volcano_plot(test.expr, names = 0.00005)
```



Function `volcano_plot` has parameters that allows to better analyze the results: `line` and `names`. The `line` parameter allows to set the horizontal line on plot on selected value. The `names` parameter signs those genes for which p-value are smaller than given value.

Methylation and expression for one gene.

In the end we want to present the distribution of methylation and expression for choosen gene `BRCA1`.

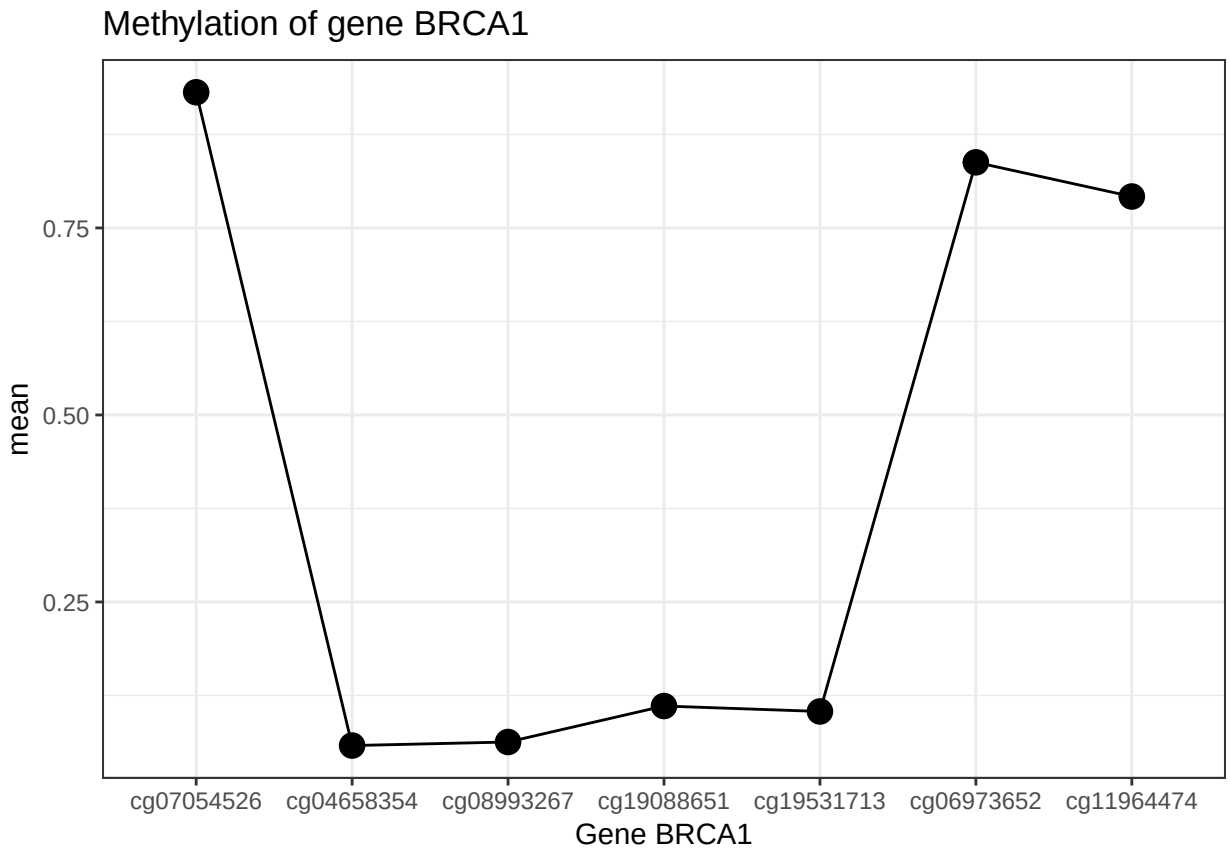
```
#library(easyGgplot2)
BRCA1_gene <- CpG_mean(BRCA_methylation_chr17, "BRCA1")
head(BRCA1_gene)
```

```
##             Name Symbol CPG_ISLAND_LOCATIONS       mean
## 7047  cg07054526  BRCA1 17:38525979-38526990 0.93138624
## 4712  cg04658354  BRCA1 17:38530194-38531162 0.05802403
## 8929  cg08993267  BRCA1 17:38530194-38531162 0.06280625
## 19075 cg19088651  BRCA1 17:38530194-38531162 0.11090400
## 19527 cg19531713  BRCA1 17:38530194-38531162 0.10361921
## 6961  cg06973652  BRCA1 17:38531525-38532730 0.83772148
```

```
#p1 <-genereg_vs_met(BRCA_methylation_chr17, "BRCA1")
#p2 <-boxplot_gene_expr(BRCA_mRNAseq_chr17,"BRCA1")
#ggplot2.multiplot(p1,p2)
```

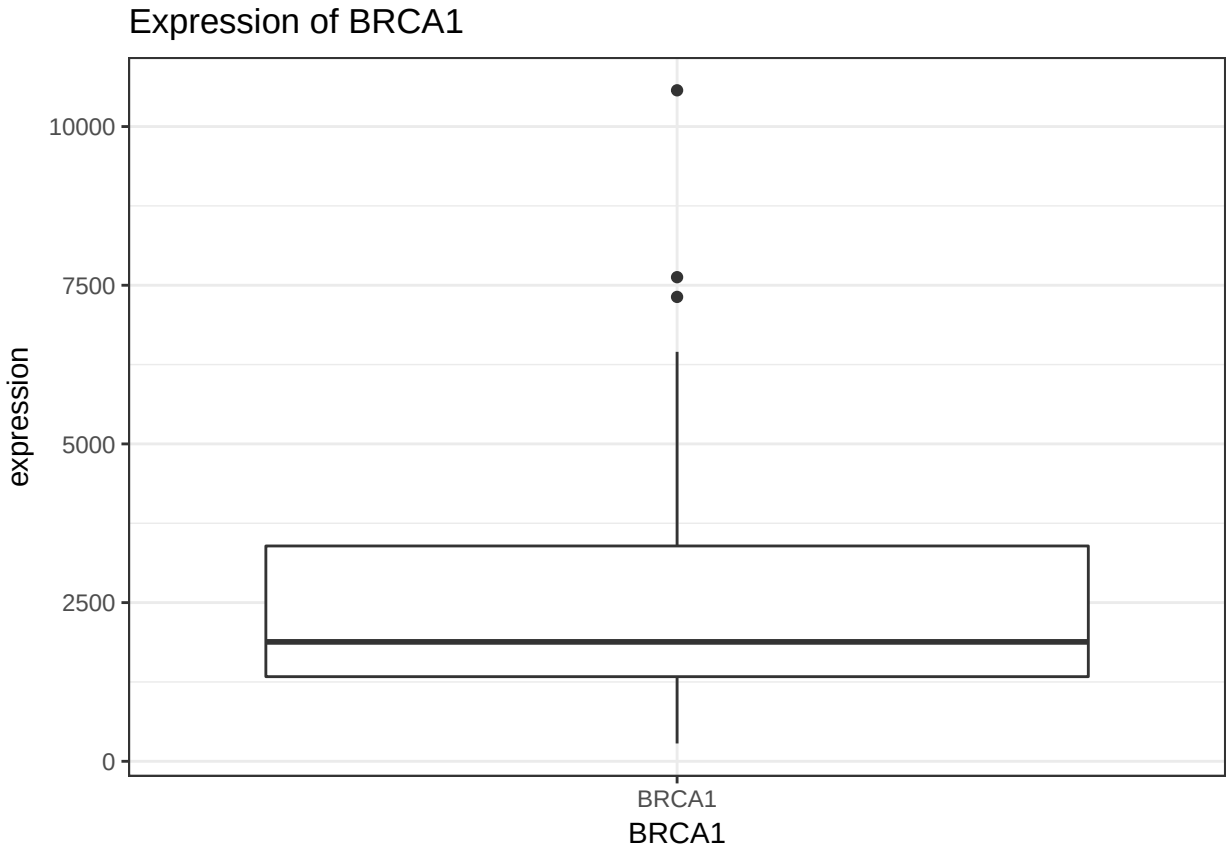
First we visualise the mean value of methylation for each CpG island on this gene using function `genereg_vs_met`.

```
genereg_vs_met(BRCA_methylation_chr17, "BRCA1")
```



With function `boxplot_gene_expr`, we consider the distribution of expression from 100 probes.

```
boxplot_gene_expr(BRCA_mRNAseq_chr17,"BRCA1")
```


Expression of BRCA1
10000
7500
5000
2500
0
expression
BRCA1
BRCA1