MLExpResso

a tool for integrative analyses and visualization of gene expression and DNA methylation data

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MI^2

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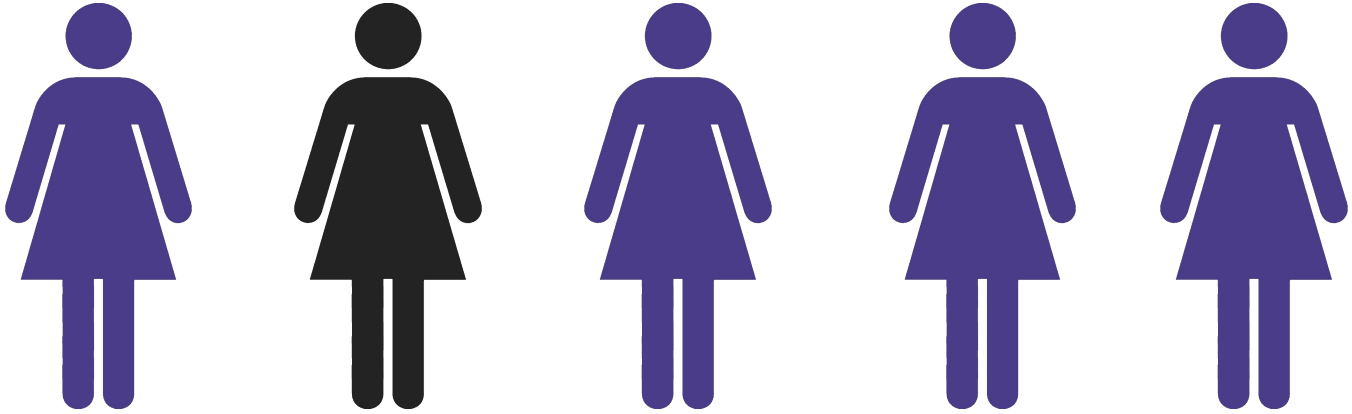
CANCER?

About

one in five

women diagnosed with breast cancer worldwide will have

HER2 positive breast cancer.



MLExpResso

1) Identification of differentially expressed genes
2) Identification of differentially methylated regions
3) Identification of regions with changes in expression and methylation
4) Visualization of identified regions

Expression of genes

100 samples x 730 genes

```
> BRCA_expression[1:8, 1:12]
```

	AANAT	AARSD1	AATF	AATK	ABCA5	ABCA6	ABCA8	ABCA9	ABCC3	ABI3	ABR	ACACA
TCGA-A1-A0SB-01A-11R-A144-07	9	2354	2870	317	1071	170	548	322	118	124	4960	5036
TCGA-A1-A0SD-01A-11R-A115-07	2	1846	5656	312	2107	735	1060	649	2786	823	6531	10428
TCGA-A1-A0SE-01A-11R-A084-07	11	3391	9522	736	1600	849	704	1176	2814	805	7302	20787
TCGA-A1-A0SF-01A-11R-A144-07	0	2169	4625	169	615	442	377	543	1925	500	5029	9562
TCGA-A1-A0SG-01A-11R-A144-07	1	2273	3473	92	249	547	417	413	1227	474	6982	8636
TCGA-A1-A0SH-01A-11R-A084-07	2	3113	7669	396	2337	1149	667	875	3910	929	9999	42456
TCGA-A1-A0SI-01A-11R-A144-07	1	1089	2870	97	556	647	1061	457	1572	635	5590	5298
TCGA-A1-A0SJ-01A-11R-A084-07	8	1857	11175	656	4443	1159	2648	922	2304	805	8935	11828

Methylation level of CpG probes

321 samples x 1464 CpG probes

```
> BRCA_methylation[1:8, 1:6]
```

	cg00021527	cg00031162	cg00032227	cg00050312	cg00053292	cg00063144
TCGA-A1-A0SD-01A-11D-A112-05	0.03781858	0.7910348	0.006391233	0.02356901	0.01806759	0.9192175
TCGA-A2-A04N-01A-11D-A112-05	0.01437552	0.7359370	0.008752293	0.02770303	0.01711573	0.9186985
TCGA-A2-A04P-01A-31D-A032-05	0.01360124	0.6967802	0.009442039	0.01402589	0.02012769	0.9186004
TCGA-A2-A04Q-01A-21D-A032-05	0.01525656	0.5341244	0.014674247	0.01510194	0.02155129	0.8985550
TCGA-A2-A04T-01A-21D-A032-05	0.01167384	0.7378100	0.012251559	0.01174021	0.02682894	0.9200222
TCGA-A2-A04U-01A-11D-A112-05	0.04266864	0.7428112	0.008710026	0.01303019	0.01765034	0.9525430
TCGA-A2-A04V-01A-21D-A032-05	0.02172694	0.5373882	0.011088346	0.01917646	0.01983831	0.9442921
TCGA-A2-A04W-01A-31D-A112-05	0.07874157	0.7497528	0.010024020	0.01340650	0.02791492	0.8860238

Identification of significant features - expression

LumA subtype vs other subtypes of breast cancer

```
> library(MLExpResso)
> res_expression <- calculate_test(
+   data = BRCA_expression,
+   condition = condition_expression,
+   test = "nbinom2"
+ )
> head(res_expression)
     id log2.fold          pval mean_LumA mean_other     mean
1 AURKB  2.303668 1.705520e-37  539.0426  2323.8868  1485.01
2  CBX2  2.777812 5.490481e-31  632.5106  4296.6038  2574.48
3 KPNA2  1.446017 3.398752e-26  11547.36   26427.38 19433.77
4  GSG2  1.407218 3.318054e-25  278.2128   629.3396   464.31
5 BIRC5  1.948513 9.512118e-24  1957.085   6658.358  4448.76
6 PRR11  1.967561 2.054430e-23   396.383   3479.981  2030.69
```

Identification of significant features - methylation

LumA subtype vs other subtypes of breast cancer

```
> BRCA_methylation_gen <- aggregate_probes(data = BRCA_methylation)
> head(BRCA_methylation_gen)[1:5, 1:5]
                                    AANAT     AARSD1       AATF       AATK        ABC1
TCGA-A1-A0SD-01A-11D-A112-05 0.7148533 0.8625816 0.24294092 0.7835302 0.01401806
TCGA-A2-A04N-01A-11D-A112-05 0.5850106 0.8355825 0.21367129 0.8466190 0.01275865
TCGA-A2-A04P-01A-31D-A032-05 0.4495537 0.8786166 0.03277413 0.3417919 0.01455092
TCGA-A2-A04Q-01A-21D-A032-05 0.7120650 0.8819490 0.03460160 0.7264985 0.01283647
TCGA-A2-A04T-01A-21D-A032-05 0.6010397 0.7739978 0.02501599 0.6276399 0.01278928
```

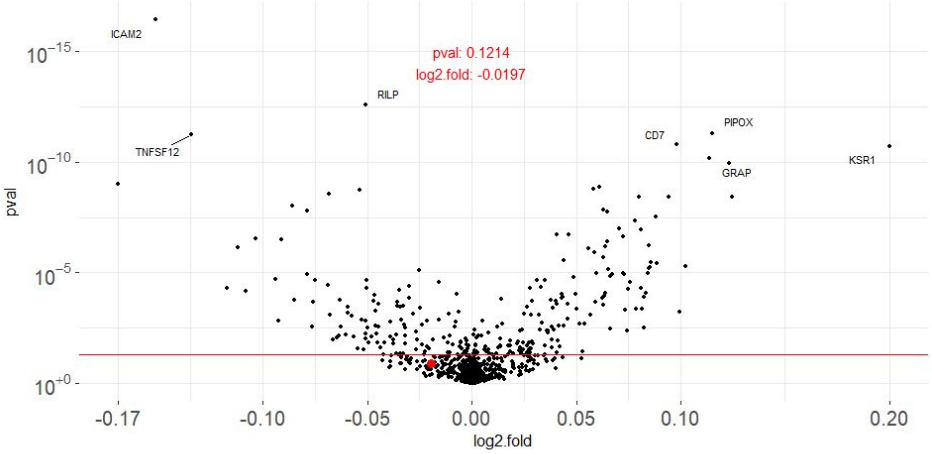
```
> res_methylation <- calculate_test(
+ data = BRCA_methylation_gen,
+ condition = condition_methylation,
+ test = "ttest"
+ )
> head(condition_methylation)
[1] "LumA"  "LumA"  "other" "other" "other" "other"       mean
1   ICAM2 -0.15151320 3.754116e-17 0.2547275  0.4062407 0.3330801
2    RILP -0.05073691 2.575168e-13 0.3079069  0.3586438 0.3341447
3   PIPOX  0.11505558 5.360053e-12 0.4242804  0.3092248 0.3647812
4 TNFSF12 -0.13412855 5.867083e-12 0.1791401  0.3132686 0.2485025
5     CD7  0.09822690 1.641919e-11 0.8635077  0.7652808 0.8127112
6    KSR1  0.19973400 2.054467e-11  0.658270   0.458536 0.5549808
```

Identification of significant features
plot_volcanoes()

CACNA1G

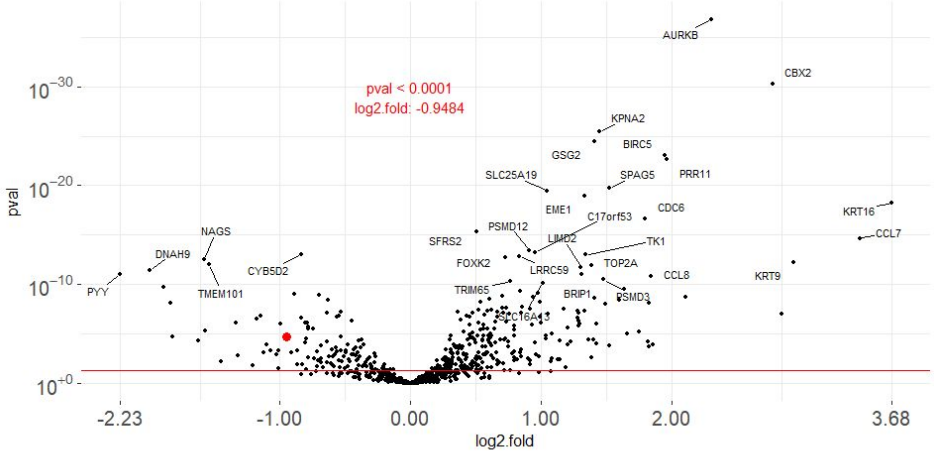
Methylation

	min	1st Q	med	mean	3rd Q	max	count
LumA	0.05	0.14	0.19	0.21	0.27	0.49	155
other	0.04	0.13	0.2	0.23	0.31	0.63	166



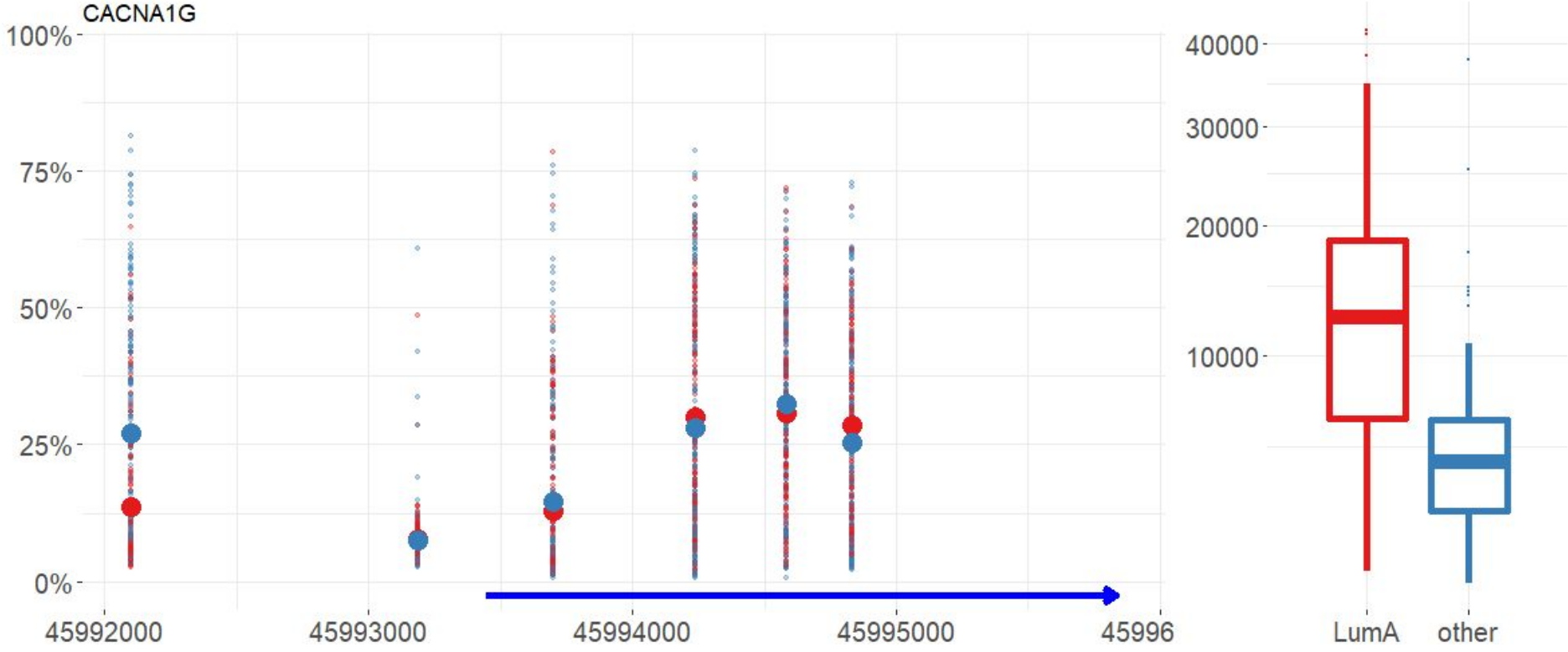
Expression (cpm)

	min	1st Q	med	mean	3rd Q	max	count
LumA	977	6351	12630	14300	18720	41720	47
other	752	2556	4360	6185	6314	37880	53



Integration of methylation and expression

plot_gene()



MLExpResso

Cheat Sheet

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Introduction

MLExpResso is an R package for integrative analyses and visualization of gene expression and DNA methylation data.

Key functions of this package are:

- identification of genes with affected expression – **calculate_test()** function,
- identification of DMR - differentially methylated regions - **calculate_test()** function,
- identification of regions with changes in expression and methylation - **calculate_comparition_table()** function,
- visualization of identified regions – **plot_gene()** and **plot_volcanoes()** functions.

The joint modeling and visualization of genes expression and methylation improve interpretability of identified signals.

MLExpRessoData

The methodology is supplemented with example applications to The Cancer Genome Atlas data.

MLExpRssoData is an R package which contains information from *The Cancer Genome Atlas (TCGA)* Data Portal. Data sets in this package are based on Bioconductor package **RTCGA**. In examples, we use both, methylation and expression data.

- **BRCA_exp** - It contains information about gene expression: read counts per-gene, computed for genes for 736 patients with breast cancer. Rows of this data set correspond to samples taken from patients. First column *SUBTYPE* corresponds to a subtype of BRCA cancer, next columns correspond to genes.
- **BRCA_met** - It contains information about methylation of CpG probes for patients with breast cancer. Rows of this data set correspond to patients, more precisely, to samples taken from patients. First column *SUBTYPE* corresponds to a subtype of BRCA cancer, next columns correspond to CpG probes. Values inside the table indicate the percentage methylation level of CpG probe for a specified sample.

For aggregation CpG probes to correspond genes we use the *Illumina human methylation* data set from **TxDb.Hsapiens.UCSC.hg18.knownGene** Bioconductor package.

Identification of genes with affected expression

MLExpResso::calculate_test(data, condition, test)

Function **calculate_test()** computes log folds, p-values and means for chosen test for both, methylation and expression data.

```
> BRCA_exp[1:3, 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

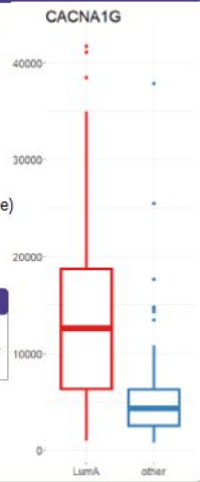
Example

```
library("MLExpResso")
library("MLExpRessoData")
exp <- BRCA_exp[ ,-1]
gr_exp <- BRCA_exp$SUBTYPE
gr_exp <- ifelse(gr_exp == 'LumA', 'LumA', 'other')
res_exp <- calculate_test(exp, gr_exp, 'lrt')
```

```
> head(res_exp)
     id log2.fold    pval mean_LumA mean_other  mean
1 AURKB        2.3 3.2e-32       539        2324  1485
2  CBX2        2.9 2.8e-26       633        4297  2574
3 KPNA2        1.4 8.6e-24     11547       26427 19434
4 PRR11        3.8 2.3e-22       396        3480  2031
5 BIRC5        2.0 2.0e-21      1957        6658  4449
6  GSG2        1.4 3.5e-21       278         629   464
```

Argument test allows using many different statistic tests for finding differences in expression. All available values are in the table below.

Value	Test
'ttest'	student's t-tets
'nbinom2'	negative binomial test
'lrt'	likelihood-ratio test
'qlf'	quasi-likelihood F-test



MLExpResso::plot_diff_boxplot(data, condition, gene)

Function plot_diff_boxplot() generates a boxplot of values from choosen data frame column with division in groups (two or more).

Example

```
plot_diff_boxplot(data = exp,
                  condition = gr_exp,
                  gene = 'CACNA1G')
```

Identification of DMR - differentially methylated regions

MLExpResso::calculate_test(data, condition, test)

Argument test allows using two different statistic tests for finding differences in methylation levels. All available values are in the table on the right.

Value	Test
'ttest'	student's t-test
'methyanalysis'	quasi-likelihood F-test

MLExpResso::aggregate_probes(data)

Function **aggregate_probes()** aggregates CpG probes to corresponding genes using, by default, *the Illumina human methylation* data.

```
> BRCA_met[1:3, 1:5]
```

	SUBTYPE	cg00021527	cg00031162	cg00032227	cg00050312
TCGA-A1-A0SD-01A-11D-A112-05	LumA	0.038	0.79	0.0064	0.024
TCGA-A2-A04N-01A-11D-A112-05	LumA	0.014	0.74	0.0088	0.028
TCGA-A2-A04P-01A-31D-A032-05	Basal	0.014	0.70	0.0094	0.014

Example

```
met <- aggregate_probes(BRCA_met)
gr_met <- BRCA_met$SUBTYPE
gr_met <- ifelse(gr_met == 'LumA', 'LumA', 'other')
res_met <- calculate_test(met, gr_met, 'ttest')
```

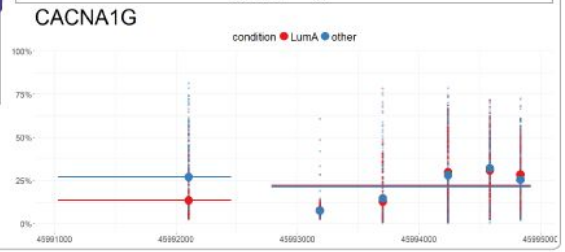
```
> head(res_met)
     id log2.fold    pval mean_LumA mean_other mean
1  ICAM2   -0.152 3.8e-17      0.25       0.41 0.33
2  RILP    -0.051 2.6e-13      0.31       0.36 0.33
3  PIPOX    0.115 5.4e-12      0.42       0.31 0.36
4 TNFSF12  -0.134 5.9e-12      0.18       0.31 0.25
5  CD7      0.098 1.6e-11      0.86       0.77 0.81
6  KSR1     0.200 2.1e-11      0.66       0.46 0.55
```

MLExpResso::plot_methylation_path(data, condition, gene)

Function plot_methylation_path() visualizes a chosen gene with marked CpG probes.
Y axis describes methylation level.
X axis describes a location of the probe on the chromosome.
Horizontal lines show the mean methylation level for each Island in a division to groups.
Groups are defined by colors. Large dots symbolize means of methylation level for CpG probes, small dots symbolize methylation levels for each observation.

Example

```
plot_methylation_path(data = BRCA_met ,
                      condition = gr_met,
                      gene = 'CACNA1G',
                      observ = T)
```



"MLGenSig: Machine Learning Methods for building the Integrated Genetic Signatures"

NCN Opus grant 2016/21/B/ST6/02176

GitHub https://github.com/geneticsMiNIng/MLGenSig

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