促进探索性数据可视化: ggpubr在TCGA 基因组数据中的应用

Jerry Wang 2017年9月18日

1. 数据准备

TCGA 数据

下面的R代码将会安装RTCGA核心包以及clinical和mRNA基因表达数据包。

source("https://bioconductor.org/biocLite.R")
Install the main RTCGA package
biocLite("RTCGA")
Install the clinical and mRNA gene expression data packages
biocLite("RTCGA.clinical")
biocLite("RTCGA.mRNA")

查看对于每种癌症可提供的数据类型,用如下代码:

library(RTCGA)
infoTCGA()

##		Сс	hort		C1	inical				Methylation		-
##	ACC-counts		ACC	92		92		90	0	80	0	79
##	BLCA-counts		BLCA	412		412		10	112	412	0	408
##	BRCA-counts		BRCA				7 10		19	1097	526	1093
##	CESC-counts		CESC	307		307		95	50	307	0	304
##	CHOL-counts		CHOL	51		45		36	0	36	0	36
##	COAD-counts		COAD	460		458		51	69	457	153	457
##	COADREAD-counts	COAI	DREAD	631		629		16	104	622	222	623
##	DLBC-counts		DLBC	58		48		48	0	48	0	48
##	ESCA-counts		ESCA	185		185		84	51	185	0	184
##	FPPP-counts		FPPP	38		38		0	0	0	0	0
##	GBM-counts		GBM	613		595		77	0	420	540	160
##	GBMLGG-counts	GE	BMLGG			1110			52	936	567	676
##	HNSC-counts		HNSC	528		528		22	108	528	0	520
##	KICH-counts		KICH	113		113		66	0	66	0	66
##	KIPAN-counts	ŀ	KIPAN	973		941		83	0	892	88	889
##	KIRC-counts		KIRC	537		537		28	0	535	72	533
##	KIRP-counts		KIRP	323		291		89	0	291	16	290
##	LAML-counts		LAML	200		200		97	0	194	0	179
	LGG-counts		LGG	516		515		13	52	516	27	516
##	LIHC-counts		LIHC	377		377		70	0	377	0	371
##	LUAD-counts		LUAD	585		522		16	120	578	32	515
##	LUSC-counts		LUSC	504		504		01	0	503	154	501
##	MESO-counts		MES0	87		87		87	0	87	0	87
##	OV-counts		OV	602		591		86	0	594	574	304
##	PAAD-counts		PAAD	185		185		84	0	184	0	178
	PCPG-counts		PCPG	179		179		75	0	179	0	179
	PRAD-counts		PRAD	499		499		92	115	498	0	497
	READ-counts		READ	171		171		65	35	165	69	166
##	SARC-counts		SARC	261		261		57	0	261	0	259
##	SKCM-counts		SKCM	470		470		69	118	470	0	469
	STAD-counts		STAD	443		443		42	107	443	0	415
	STES-counts		STES	628		628		26	158	628	0	599
	TGCT-counts		TGCT	150		134		50	0	150	0	150
##	THCA-counts		THCA	503		503		99	98	503	0	501
	THYM-counts		THYM	124		124		23	0	124	0	120
	UCEC-counts		UCEC	560		548		40	106	547	54	545
	UCS-counts		UCS	57		57		56	0	57	0	57
	UVM-counts	· D	UVM	80		80		80	51	80	0	80
##	100					MAF ra						
	ACC-counts	0			16	90		0				
	BLCA-counts	0	40			130	39					
	BRCA-counts	0	107			977		0				
	CESC-counts	0	30			194		0				
	CHOL-counts	0			30	35		0				
	COADDEAD accounts	0	40			154	36					
	COADREAD-counts	0	54			223	48					
	DLBC-counts	0			33	48		0				
	ESCA-counts FPPP-counts	0	18	34 1. 23	20 0	185 0		0				
			4			290		0				
	GBM-counts	565 565	51				29 80					
	GBMLGG-counts		51 52			576 279	51					
	HNSC-counts	0			12 33	66	51					
	KICH-counts KIPAN-counts	0	87			644	79					
	KIRC-counts	0	87 51			417	45					
	KIRP-counts	0	29			161	28					
ਜਜ	MINI COURTS	U	25	, i .	ιυ	101	۷٥.	_				

```
0
## LAML-counts
                            188
                                   0 197
## LGG-counts
                      0
                            512
                                430 286
                                             516
## LIHC-counts
                      0
                            372
                                  63 198
                                             373
## LUAD-counts
                            513
                                 365 230
                                             542
## LUSC-counts
                      0
                            478
                                 328 178
                                               0
                      0
                                               0
## MESO-counts
                            87
                                  63
                                        0
                    570
## OV-counts
                            453
                                 426 316
                                             469
                      0
                                 123 150
## PAAD-counts
                            178
                                             184
## PCPG-counts
                      0
                           179
                                  80 179
                                               0
## PRAD-counts
                      0
                            494
                                 352 332
                                             498
## READ-counts
                      0
                           143
                                 131
                                      69
                                             122
## SARC-counts
                      0
                            259
                                 223 247
                                               0
                      0
## SKCM-counts
                            448
                                 353 343
                                             366
## STAD-counts
                      0
                           436
                                 357 289
                                             395
## STES-counts
                      0
                            620
                                 483 474
                                             395
## TGCT-counts
                      0
                           150
                                 118 149
                                               0
## THCA-counts
                      0
                            502
                                 222 402
                                             496
## THYM-counts
                      0
                           124
                                  90 123
                                               0
## UCEC-counts
                      0
                            538
                                440 248
                                               0
## UCS-counts
                      0
                             56
                                  48
                                      57
                                               0
## UVM-counts
                             80
                                  12
                                      80
```

基因表达数据

R函数 expressionsTCGA() (RTCGA 包内)可以轻松提取一种或者多种癌症中你感兴趣基因的表达值。

在接下来的代码中将会提取三个如下不同数据集中感兴趣的五个基因 GATA3, PTEN, XBP1, ESR1 和 MUC1 的 mRNA 表达值:

- Breast invasive carcinoma (BRCA),
- · Ovarian serous cystadenocarcinoma (OV) and
- Lung squamous cell carcinoma (LUSC)

```
## # A tibble: 1,305 x 7
##
               bcr_patient_barcode
                                                 GATA3
                                                             PTEN
                                                                        XBP1
                                     dataset
##
                             <chr>>
                                       <chr>
                                                 <db1>
                                                             <db1>
                                                                       <db1>
##
   1 TCGA-A1-A0SD-01A-11R-A115-07 BRCA. mRNA 2. 870500 1. 3613571
                                                                   2.983333
##
   2 TCGA-A1-A0SE-01A-11R-A084-07 BRCA. mRNA 2. 166250 0. 4283571
                                                                   2.550833
##
   3 TCGA-A1-A0SH-01A-11R-A084-07 BRCA. mRNA 1.323500 1.3056429
                                                                   3.020417
   4 TCGA-A1-A0SJ-01A-11R-A084-07 BRCA. mRNA
                                              1.841625
                                                        0.8096429
                                                                   3. 131333
   5 TCGA-A1-A0SK-01A-12R-A084-07 BRCA. mRNA -6.025250 0.2508571 -1.451750
##
   6 TCGA-A1-A0SM-01A-11R-A084-07 BRCA. mRNA 1.804500 1.3107857
                                                                   4.041083
##
   7 TCGA-A1-A0S0-01A-22R-A084-07 BRCA. mRNA -4. 879250 -0. 2369286 -0. 724750
  8 TCGA-A1-A0SP-01A-11R-A084-07 BRCA. mRNA -3.143250 -1.2432143 -1.193083
   9 TCGA-A2-A04N-01A-11R-A115-07 BRCA. mRNA 2.034000 1.2074286
## 10 TCGA-A2-A04P-01A-31R-A034-07 BRCA. mRNA -0.293125 0.2883571 -1.605083
## # ... with 1,295 more rows, and 2 more variables: ESR1 <dbl>, MUC1 <dbl>
```

```
nb_samples <- table(expr$dataset)
nb_samples</pre>
```

```
##
## BRCA. mRNA LUSC. mRNA OV. mRNA
## 590 154 561
```

我们可以减缓数据集的名字,即删除标签中的"mRNA"。完成这个过程可以使用R的基本函数 gsub()。同时简化病人的barcode列。

```
expr$dataset <- gsub(pattern = ".mRNA", replacement = "", expr$dataset)
expr$bcr_patient_barcode <- paste0(expr$dataset, c(1:590, 1:561, 1:154))
expr</pre>
```

```
## # A tibble: 1,305 x 7
                                                         XBP1
##
     bcr_patient_barcode dataset
                                    GATA3
                                                PTEN
                                                                    ESR1
                   <chr>
                          <chr>
                                    <dbl>
                                               <db1>
                                                         <db1>
                                                                   <db1>
##
## 1
                   BRCA1
                           BRCA 2.870500 1.3613571 2.983333 3.0842500
## 2
                   BRCA2
                           BRCA 2.166250 0.4283571 2.550833 2.3860000
## 3
                   BRCA3
                           BRCA 1. 323500 1. 3056429 3. 020417 0. 7912500
## 4
                   BRCA4 BRCA 1.841625 0.8096429 3.131333 2.4954167
## 5
                   BRCA5
                           BRCA -6. 025250 0. 2508571 -1. 451750 -4. 8606667
## 6
                   BRCA6
                           BRCA 1.804500 1.3107857 4.041083 2.7970000
## 7
                   BRCA7
                           BRCA -4.879250 -0.2369286 -0.724750 -4.4860833
## 8
                   BRCA8
                           BRCA -3. 143250 -1. 2432143 -1. 193083 -1. 6274167
## 9
                   BRCA9
                           BRCA 2.034000 1.2074286 2.278833 4.1155833
## 10
                  BRCA10
                           BRCA -0. 293125 0. 2883571 -1. 605083 0. 4731667
## # ... with 1,295 more rows, and 1 more variables: MUC1 <db1>
```

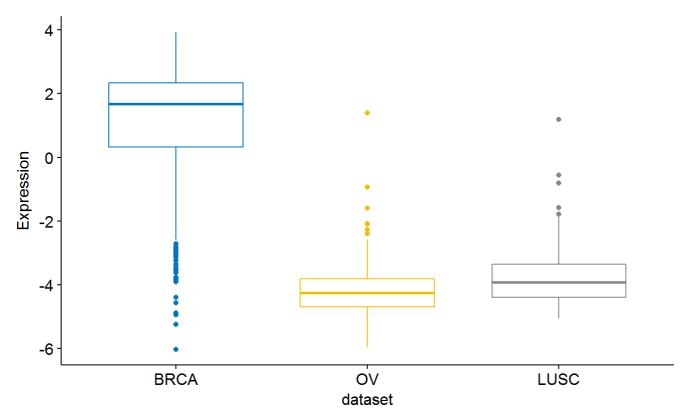
如果你执行安装RTCGA包有问题可以直接从本地文件中读取所需数据:

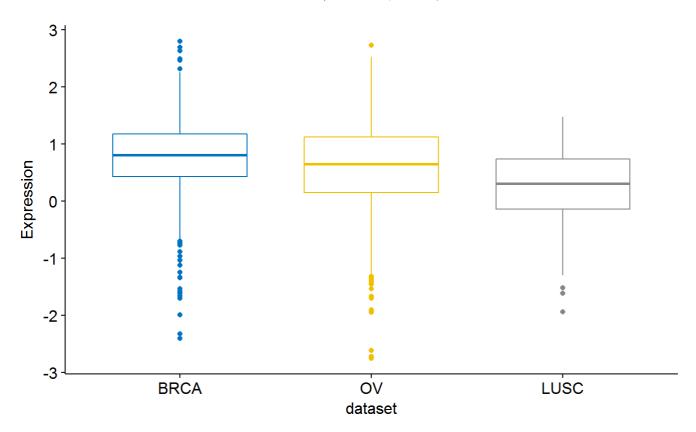
```
expr <- read.delim("expr_tcga.txt", stringsAsFactors = FALSE)
```

2. 箱线图

创建基因表达谱的箱线图,不同分组(数据集或者说癌症类型)使用不同的颜色:







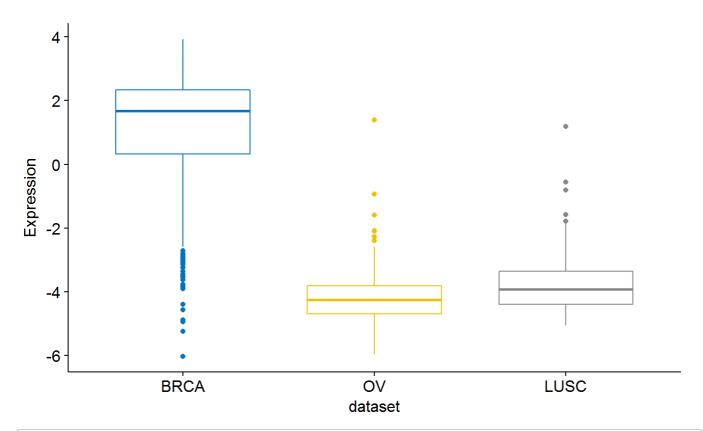
注意: 参数 palette 用来改变颜色调色板。允许使用的颜色调色板包括:

- "grey"使用灰度调色板。
- brewer palettes,例如: "RdBu", "Blues" 等等。可以使用函数 RColorBrewer::display.brewer.all()来 查看可用调色板。
- 自定义调色板: c("blue", "red") 或 c("#00AFBB", "#E7B800")。
- 来自 ggsci (https://cran.r-project.org/web/packages/ggsci/vignettes/ggsci.html)包的科学类杂志调色 板,例如: "npg", "aaas", "lancet", "jco", "ucscgb", "uchicago", "simpsons" 和 "rickandmorty"。

如果不想为每个基因都重复相同的R代码,可以创建一个plot list。

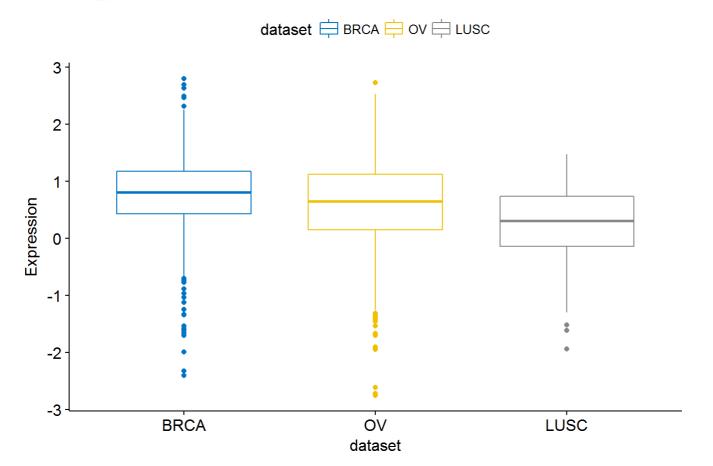
GATA3





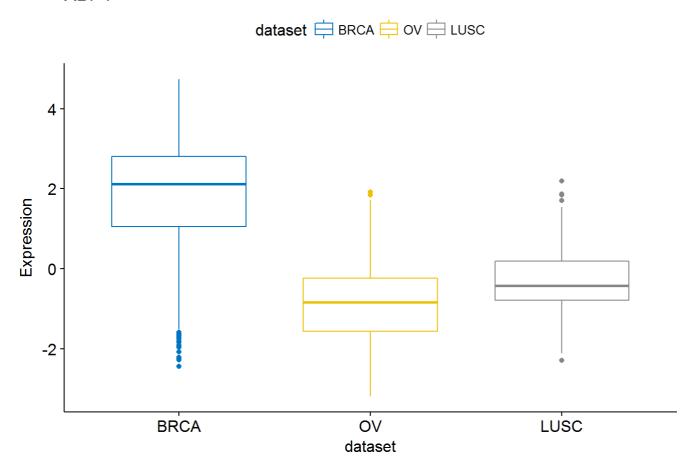
View PTEN
p\$PTEN

PTEN



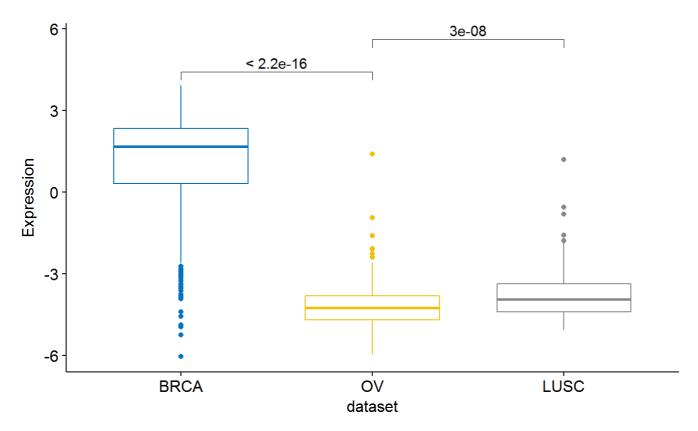
View XBP1
p\$XBP1

XBP1



当参数y包含多个变量(这里是多个基因名字),那么参数title, xlab 和 ylab 可以是与 y 等长的字符串向量。也可以是单个字符串,若如此该字符串将应用于所有的图片。

给箱线图添加p-values和显著性水平:



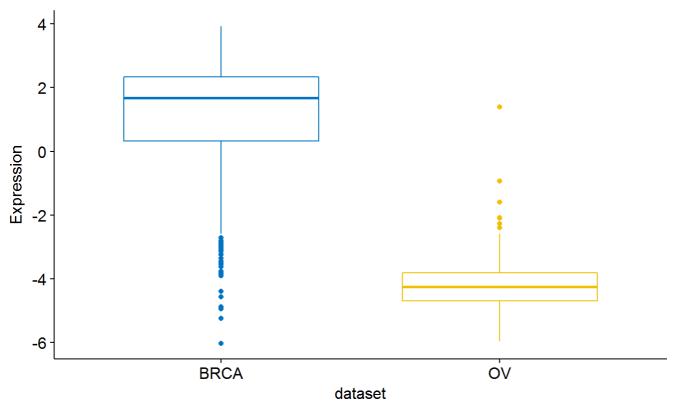
对于每一个基因, 你可以比较不同的组间的差异:

```
compare_means(c(GATA3, PTEN, XBP1) ~ dataset, data = expr)
```

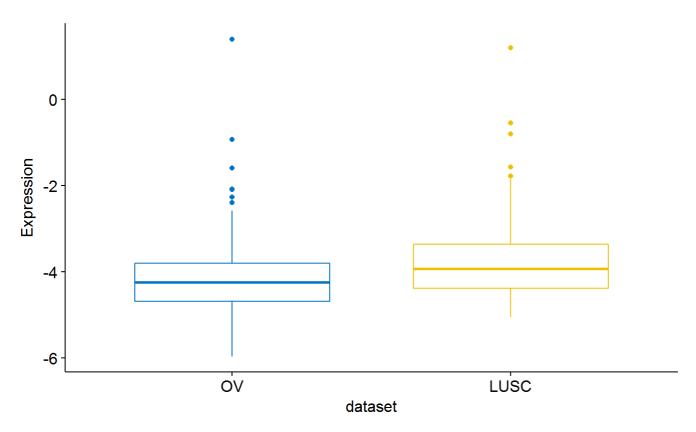
```
## # A tibble: 9 x 8
                                                p. adj p. format p. signif
       .y. group1 group2
            <chr>
                   <chr>
                                  <db1>
                                                <db1>
                                                         <chr>>
                                                                   <chr>>
     <fctr>
## 1 GATA3
              BRCA
                       OV 1.111768e-177 3.335304e-177
                                                       < 2e-16
## 2 GATA3
              BRCA
                     LUSC
                          6.684016e-73 1.336803e-72 < 2e-16
                           2.965702e-08 2.965702e-08 3.0e-08
## 3
     GATA3
                OV
                     LUSC
                                                                    ***
\#\# 4
      PTEN
             BRCA
                     0V 6.791940e-05 6.791940e-05 6.8e-05
                                                                   ***
## 5
      PTEN
              BRCA
                    LUSC 1.042830e-16 3.128489e-16 < 2e-16
                                                                    ***
      PTEN
                OV
                     LUSC 1.280576e-07 2.561153e-07 1.3e-07
## 6
## 7
      XBP1
              BRCA
                     0V 2.551228e-123 7.653685e-123 < 2e-16
## 8
      XBP1
              BRCA
                     LUSC 1.950162e-42 3.900324e-42 < 2e-16
                                                                    ***
## 9
                     LUSC 4.239570e-11 4.239570e-11 4.2e-11
      XBP1
                0V
                                                                    ***
## # ... with 1 more variables: method \langle chr \rangle
```

如果你想选择或者删除某些组(这里是癌症类型)来作图展示,那么可以使用参数 select 或者 remove:



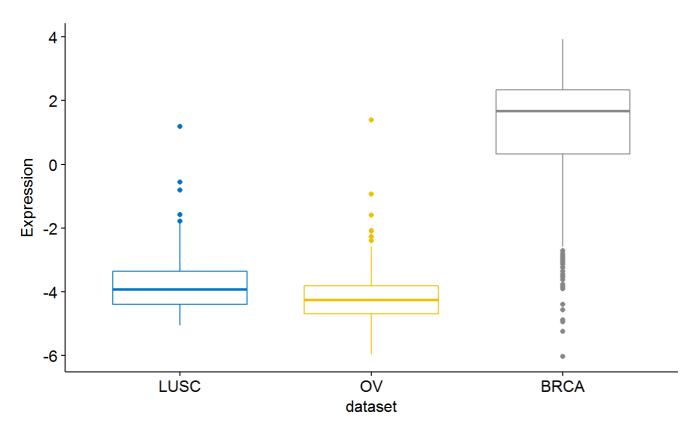






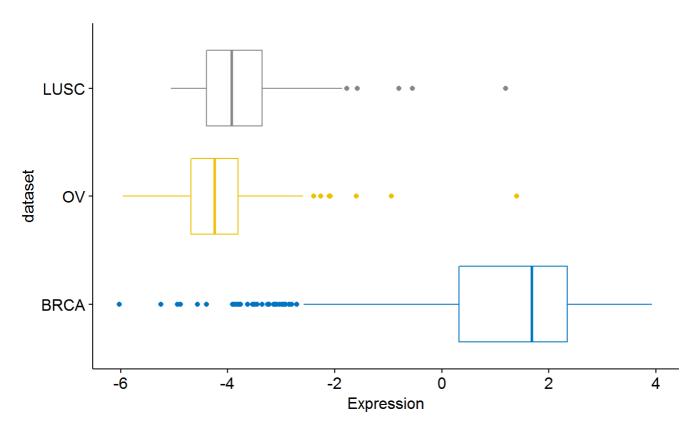
改变分组在x轴上的位置可以使用参数 order:

dataset 🖨 LUSC 🖨 OV 🖨 BRCA

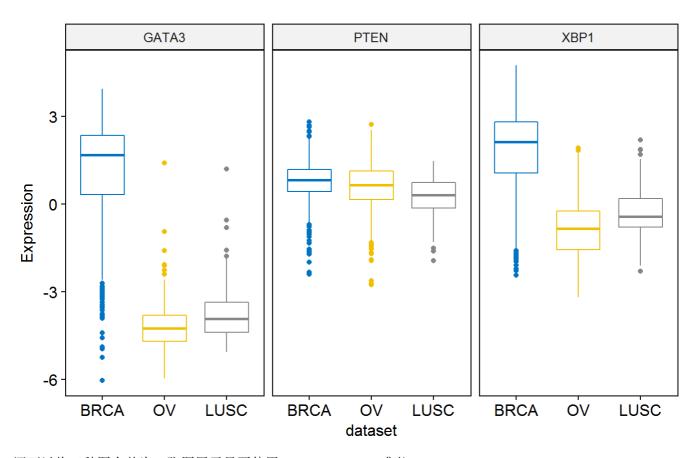


可以使用参数 rotate = TRUE 实现水平箱线图:

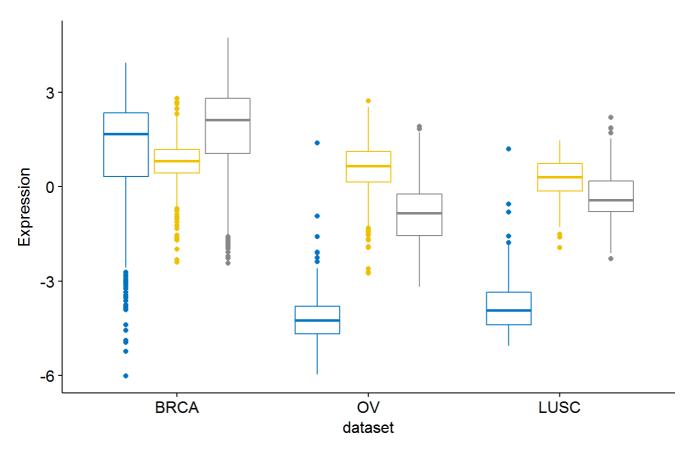




将三个基因的表达图放到一个作图区域可以使用参数 combine = TRUE:



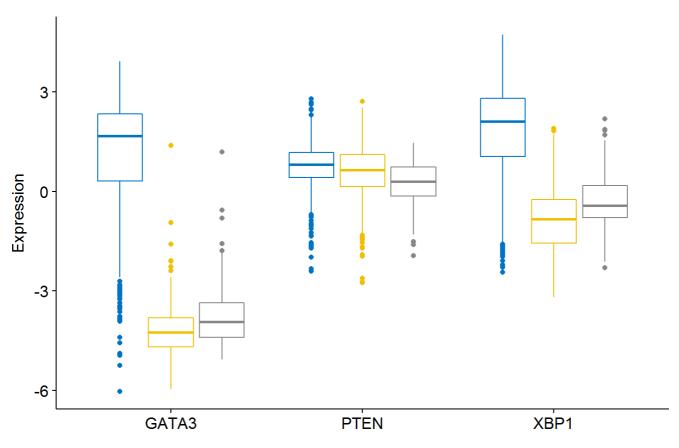
还可以将三种图合并为一张图展示只要使用 merge = TRUE 或者 merge = "asis":



上面的图很容易去比较每种癌症不同基因的表达水平。

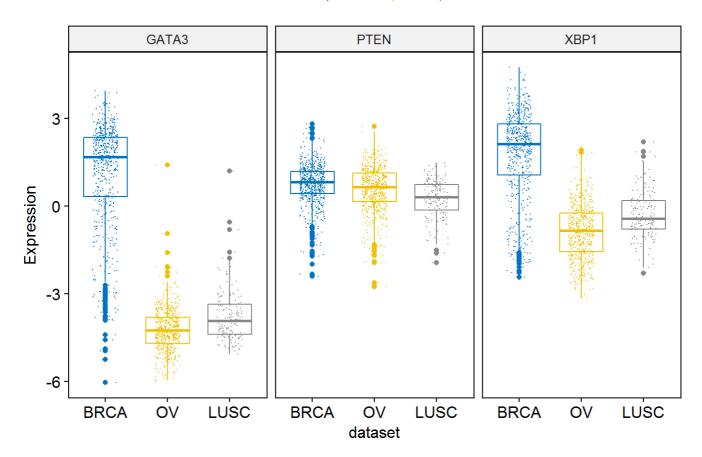
但是,你可能想把基因放到x轴方便去比较同一个基因在不同癌症中的表达水平。

在这种条件下,y变量(这里是基因)变成x轴标签,而x变量(这里是癌种)变成分组变量。如此做的话需要使用参数 merge = "flip"。



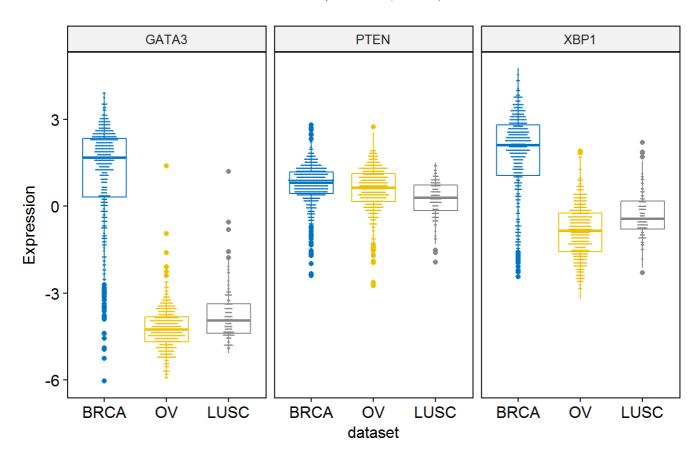
如果要在箱线图上添加打散的点。每一个点就是一个独立的观测值。可以添加 add = "jitter"。自定义添加元素的特性,指定参数 add. params。

```
ggboxplot(expr, x = "dataset",
    y = c("GATA3", "PTEN", "XBP1"),
    combine = TRUE,
    color = "dataset", palette = "jco",
    ylab = "Expression",
    add = "jitter",  # Add jittered points
    add.params = list(size = 0.1, jitter = 0.2) # Point size and the amount of jittering
)
```

你还可以在箱线图上添加 dotplot,并调整。

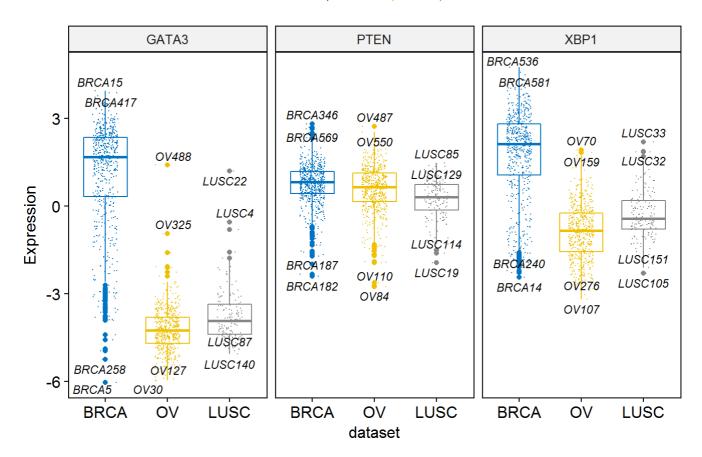
```
ggboxplot(expr, x = "dataset",
    y = c("GATA3", "PTEN", "XBP1"),
    combine = TRUE,
    color = "dataset", palette = "jco",
    ylab = "Expression",
    add = "dotplot",
    add.params = list(binwidth = 0.1, dotsize = 0.3)
)
```



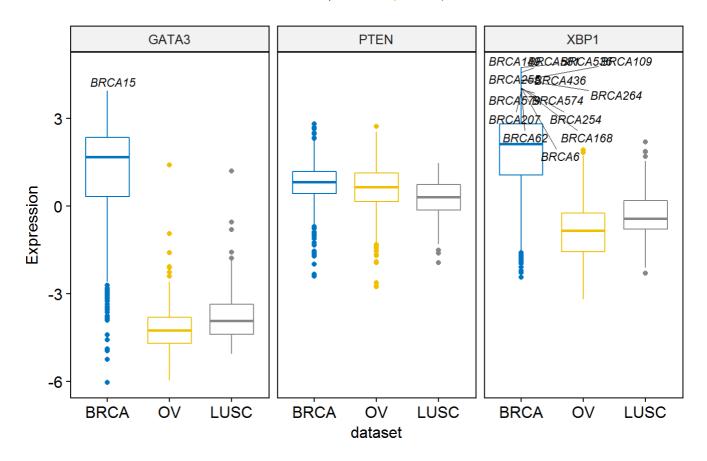
你可能想在箱线图上将前n最高或最低值的样品的名称显示出来。在这种情况下,您可以使用以下参数:

- label:包含点标签的列的名字
- label. select:可以有两种格式:
 - 一个字符串向量指定需要显示的标签名字
 - o 一个list包含一个或者以下多个组分的组合:
 - top. up 和 top. down:用来显示 top up/down 的点。例如: label.select = list(top.up = 10, top.down = 4)。
 - criteria:利用x和y变量值用来过滤满足条件的点。例如: label.select = list(criteria = "`y` > 3.9 & `y` < 5 & `x` %in% c('BRCA', 'OV')")。

```
ggboxplot(expr, x = "dataset",
          y = c ("GATA3", "PTEN", "XBP1"),
          combine = TRUE,
          color = "dataset", palette = "jco",
          ylab = "Expression",
          add = "jitter",
                                                         # Add jittered points
          add.params = list(size = 0.1, jitter = 0.2), # Point size and the amount of jitterin
g
          label = "bcr_patient_barcode",
                                                         # column containing point labels
          label.select = list(top.up = 2, top.down = 2), \# Select some labels to display
          font.label = list(size = 9, face = "italic"), # label font
          repel = TRUE
                                                         # Avoid label text overplotting
          )
```

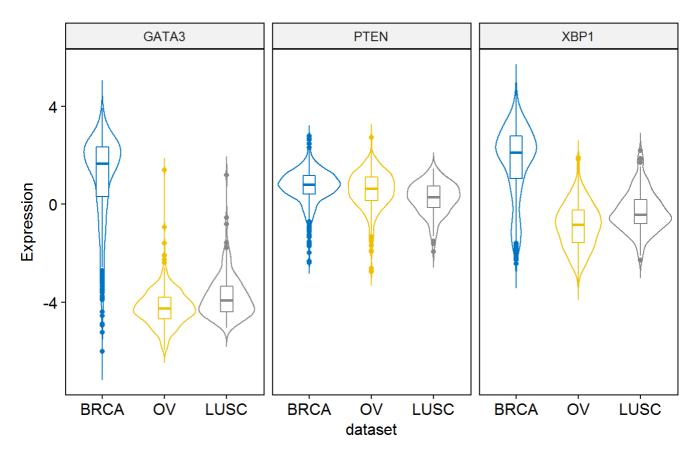


一个复杂的标签显示规则的例子如下:

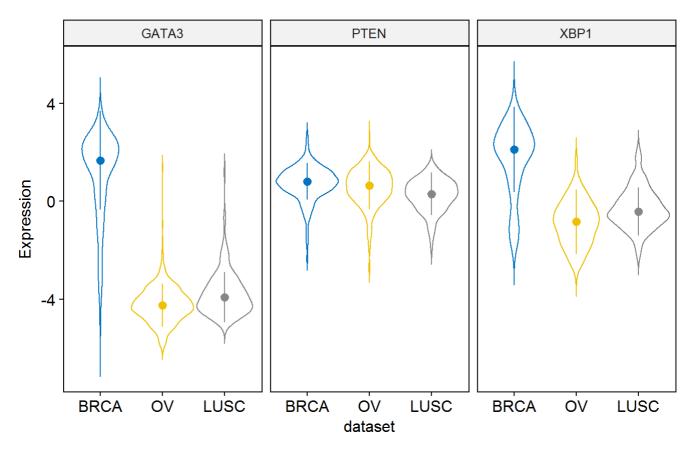


3. 小提琴图

接下来的R代码将作出小提琴图并且其内部是箱线图:



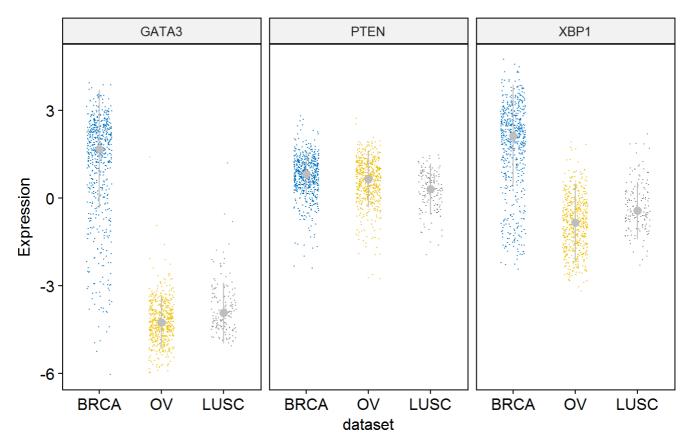
其内部不仅可以添加箱线图,也可以添加中位数+四分点范围(median + interquantile range):



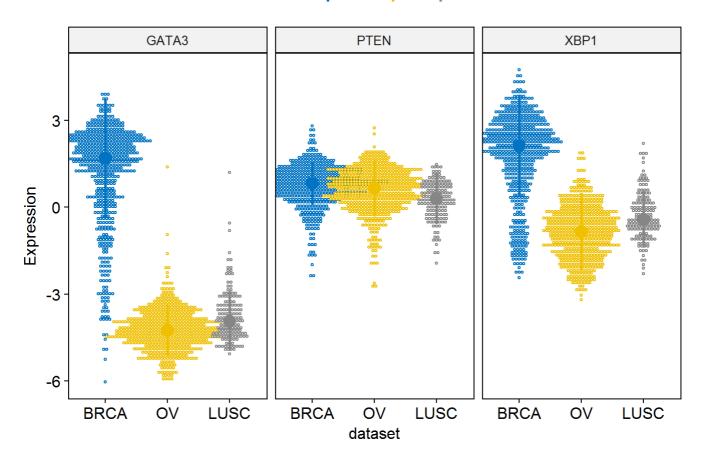
当使用函数 ggviolin() 时, add 参数合适的值可以是: "mean", "mean_se", "mean_sd", "mean_ci", "mean_range", "median", "median_iqr", "median_mad", "median_range".

也可以添加 "jitter" 点 与 "dotplot" 到小提琴图的内部。

4. 带状图与点图(Stripcharts and dot plots)



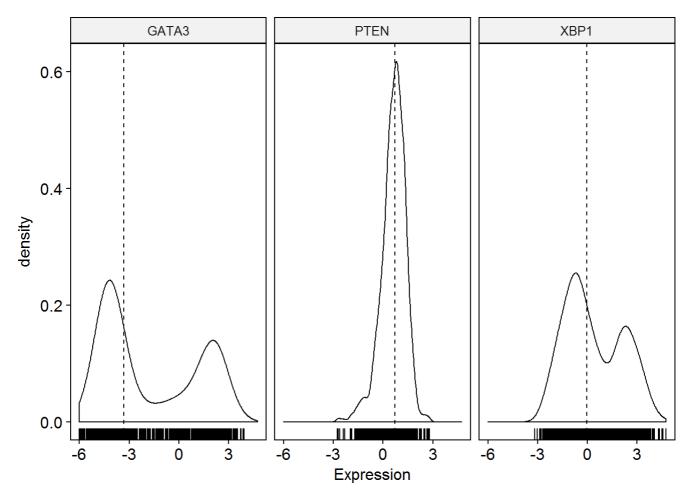
```
ggdotplot(expr, x = "dataset",
    y = c("GATA3", "PTEN", "XBP1"),
    combine = TRUE,
    color = "dataset", palette = "jco",
    fill = "white",
    binwidth = 0.1,
    ylab = "Expression",
    add = "median_iqr",
    add. params = list(size = 0.9))
```

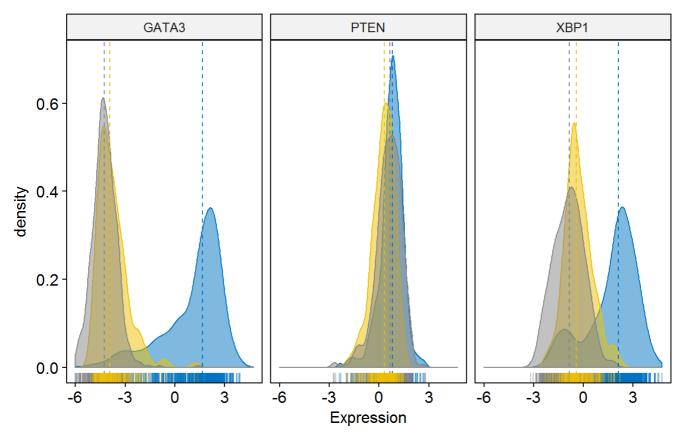


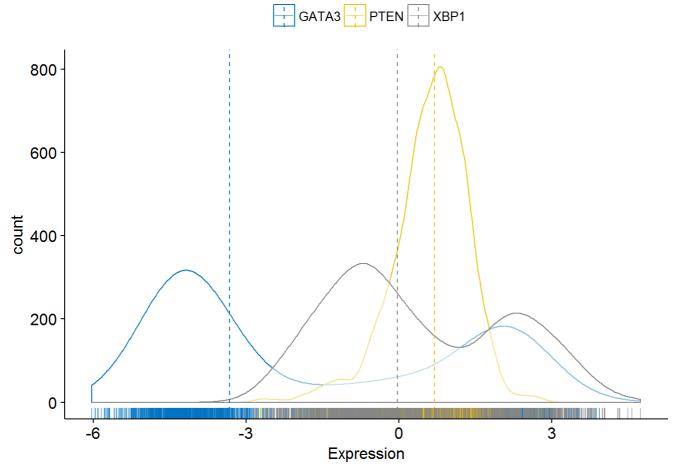
5. 密度图

用密度图显示数据分布可以使用 ggdensity() 函数。

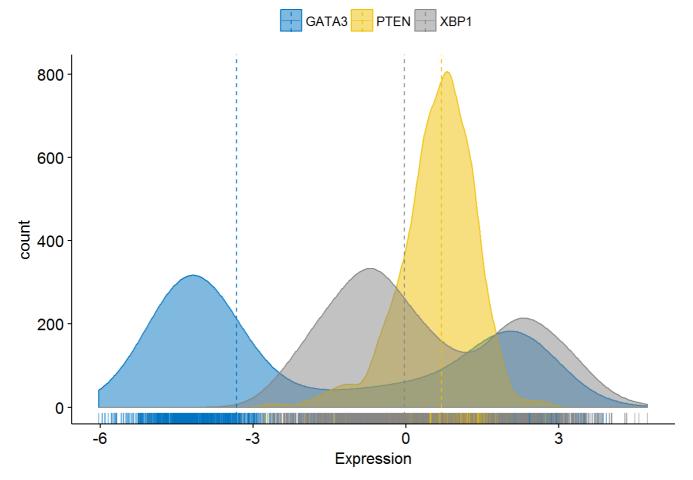
```
ggdensity(expr,
    x = c("GATA3", "PTEN", "XBP1"),
    y = "..density..",
    combine = TRUE,  # Combine the 3 plots
    xlab = "Expression",
    add = "median",  # Add median line.
    rug = TRUE  # Add marginal rug
)
```



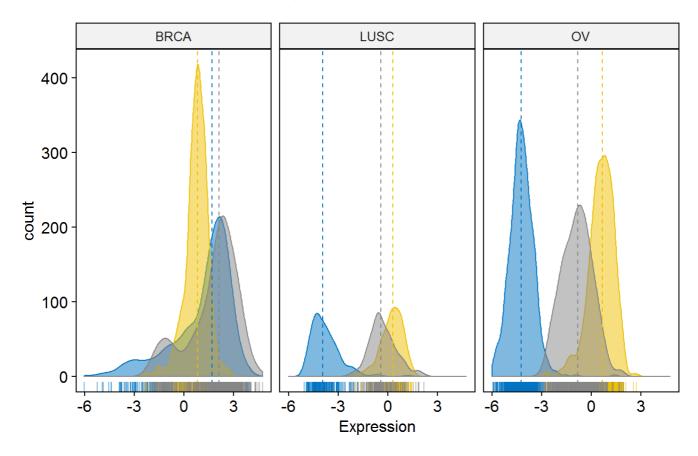




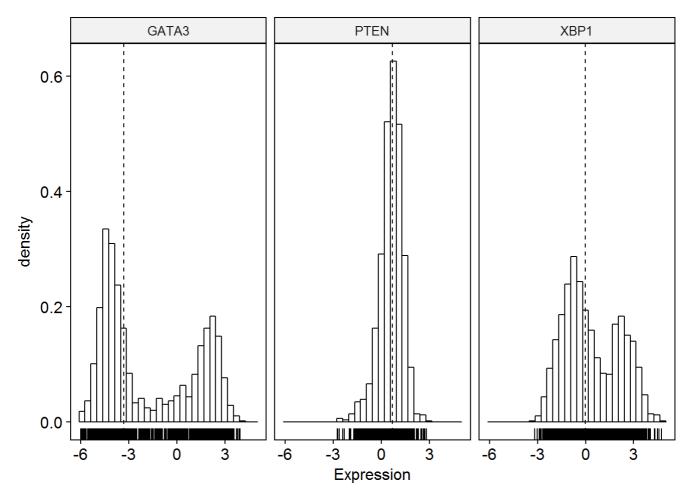
```
# color and fill by x variables
ggdensity(expr,
       x = c ("GATA3", "PTEN", "XBP1"),
       y = "...count...",
      color = ".x.", fill = ".x.",
                                         # color and fill by x variables
       merge = TRUE,
                                         # Merge the 3 plots
       xlab = "Expression",
       add = "median",
                                         # Add median line.
       rug = TRUE,
                                         # Add marginal rug
      palette = "jco"
                                         # Change color palette
)
```



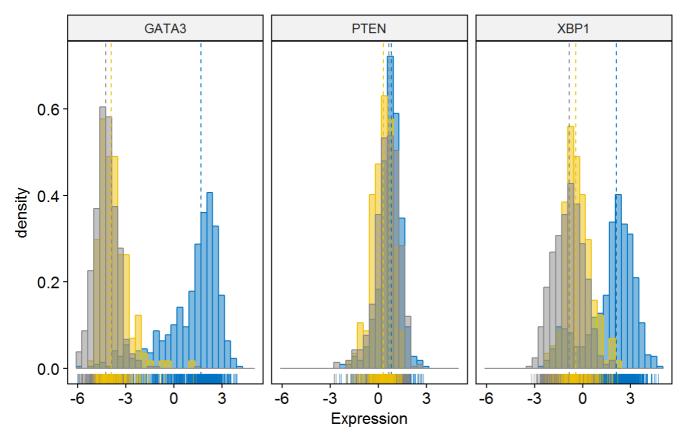
```
# Facet by "dataset"
ggdensity(expr,
      x = c ("GATA3", "PTEN", "XBP1"),
       y = "...count...",
      color = ".x.", fill = ".x.",
       facet.by = "dataset",
                                         # Split by "dataset" into multi-panel
       merge = TRUE,
                                         # Merge the 3 plots
       xlab = "Expression",
       add = "median",
                                         # Add median line.
       rug = TRUE,
                                         # Add marginal rug
       palette = "jco"
                                         # Change color palette
)
```

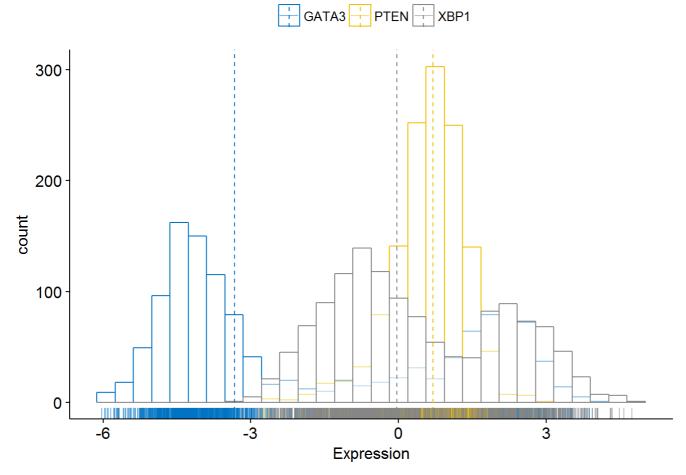


6. 直方图

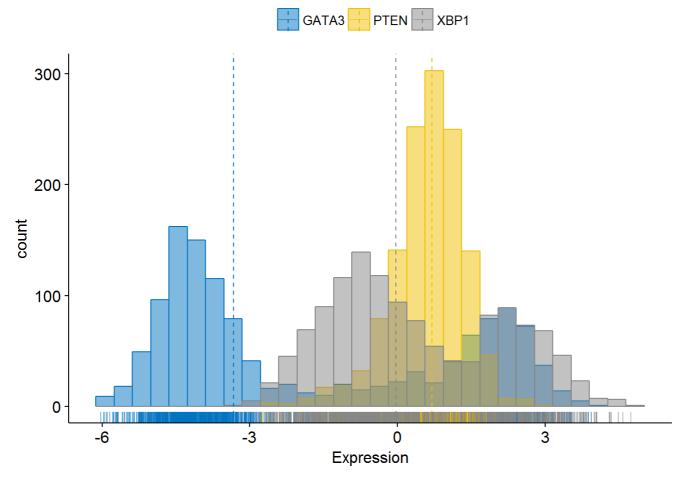


```
# Change color and fill by dataset
gghistogram(expr,
       x = c ("GATA3", "PTEN",
                               "XBP1"),
       y = "...density...",
                                         # Combine the 3 plots
       combine = TRUE,
       xlab = "Expression",
       add = "median",
                                         # Add median line.
       rug = TRUE,
                                         # Add marginal rug
       color = "dataset",
       fill = "dataset",
       palette = "jco"
)
```



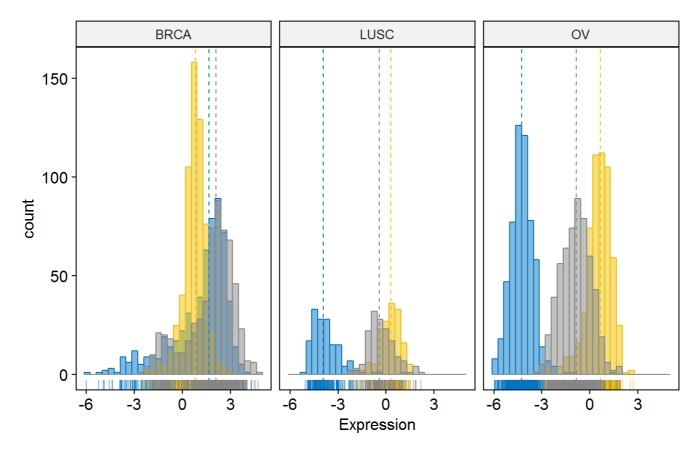


```
# color and fill by x variables
gghistogram(expr,
       x = c ("GATA3", "PTEN", "XBP1"),
       y = "..count..",
      color = ".x.", fill = ".x.",
                                         # color and fill by x variables
       merge = TRUE,
                                         # Merge the 3 plots
       xlab = "Expression",
                                         # Add median line.
       add = "median",
       rug = TRUE,
                                         # Add marginal rug
      palette = "jco"
                                         # Change color palette
)
```

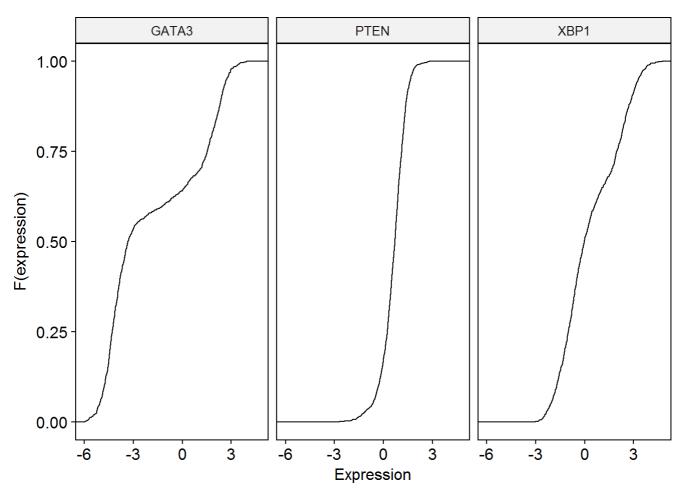


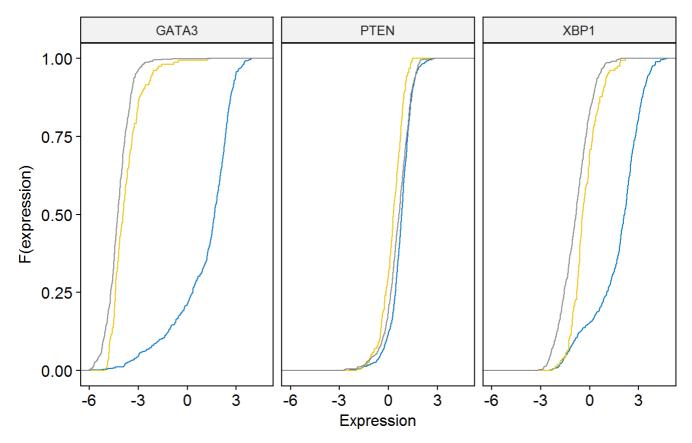
```
# Facet by "dataset"
gghistogram(expr,
      x = c ("GATA3", "PTEN", "XBP1"),
       y = "...count...",
      color = ".x.", fill = ".x.",
       facet.by = "dataset",
                                         # Split by "dataset" into multi-panel
       merge = TRUE,
                                         # Merge the 3 plots
       xlab = "Expression",
       add = "median",
                                         # Add median line.
       rug = TRUE,
                                         # Add marginal rug
       palette = "jco"
                                         # Change color palette
)
```

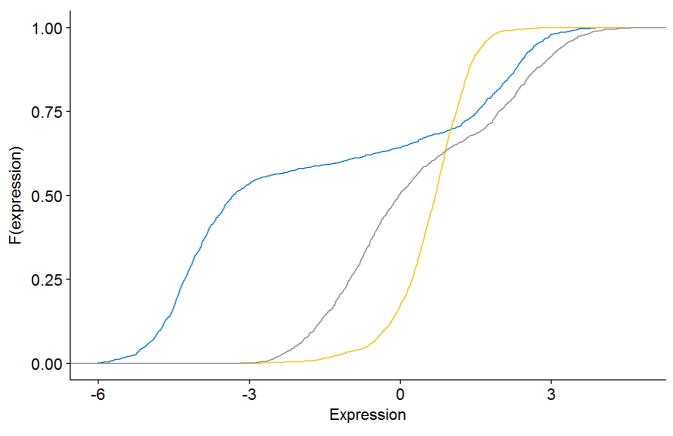


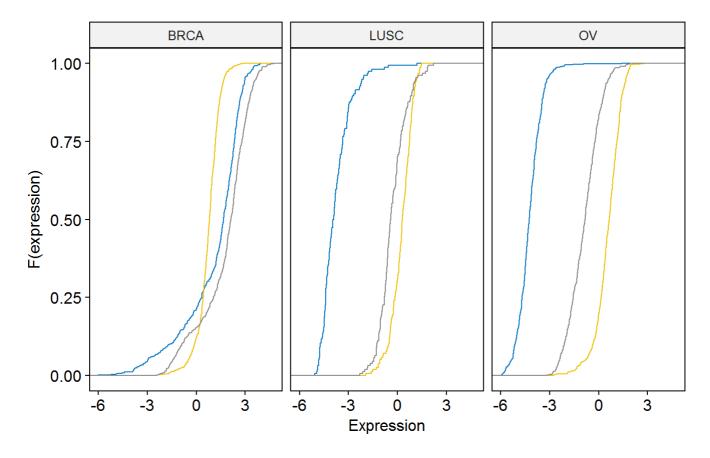


7.经验累积密度函数(Empirical cumulative density function)

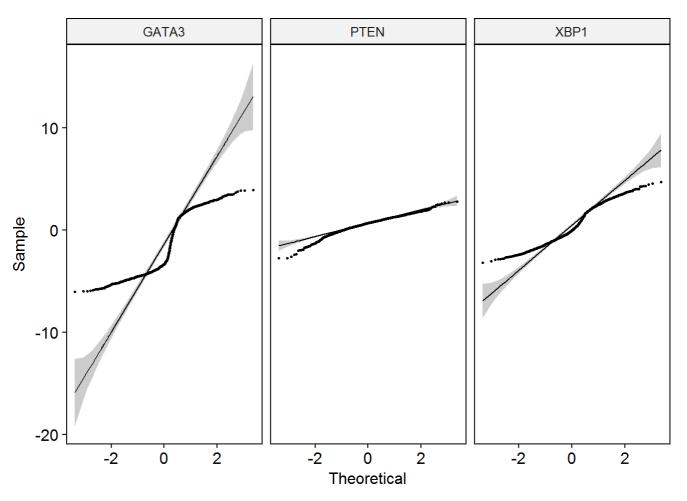


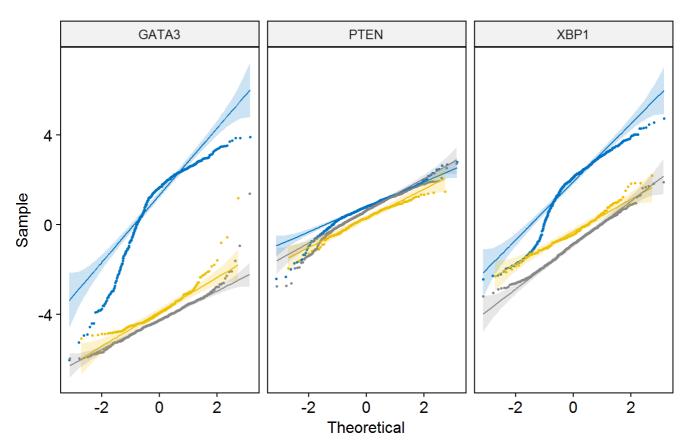


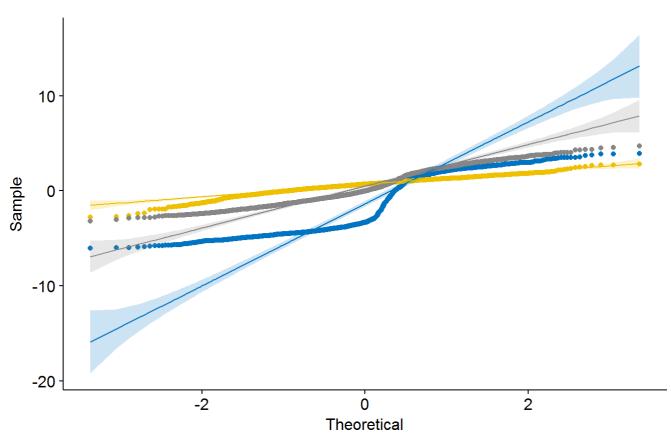


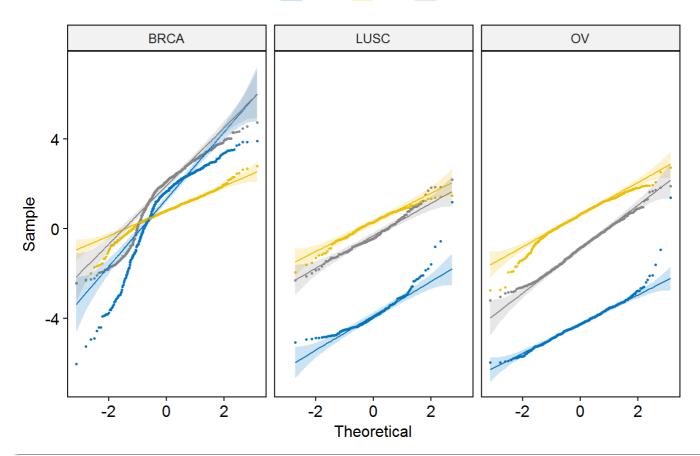


8. QQ图 (Quantile - Quantile plot)









参考: Facilitating Exploratory Data Visualization: Application to TCGA Genomic Data (http://www.sthda.com/english/articles/24-ggpubr-publication-ready-plots/77-facilitating-exploratory-data-visualization-application-to-tcga-genomic-data/)