

Gene Expression Morphology

```
library(SummarizedExperiment)

## Loading required package: MatrixGenerics

## Loading required package: matrixStats

##
## Attaching package: 'MatrixGenerics'

## The following objects are masked from 'package:matrixStats':
##
##   colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##   colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##   colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##   colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##   colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##   colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##   colWeightedMeans, colWeightedMedians, colWeightedSds,
##   colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##   rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##   rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##   rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##   rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##   rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##   rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##   rowWeightedSds, rowWeightedVars

## Loading required package: GenomicRanges

## Loading required package: stats4

## Loading required package: BiocGenerics

## Loading required package: parallel

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:parallel':
##
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, parApply, parCapply, parLapply,
##   parLapplyLB, parRapply, parSapply, parSapplyLB
```

```

## The following objects are masked from 'package:stats':
##
##   IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##   anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##   dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##   grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##   order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##   rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##   union, unique, unsplit, which.max, which.min

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:base':
##
##   expand.grid

## Loading required package: IRanges

## Loading required package: GenomeInfoDb

## Loading required package: Biobase

## Welcome to Bioconductor
##
##   Vignettes contain introductory material; view with
##   'browseVignettes()'. To cite Bioconductor, see
##   'citation("Biobase")', and for packages 'citation("pkgname)".

##
## Attaching package: 'Biobase'

## The following object is masked from 'package:MatrixGenerics':
##
##   rowMedians

## The following objects are masked from 'package:matrixStats':
##
##   anyMissing, rowMedians

library(limma)

##
## Attaching package: 'limma'

## The following object is masked from 'package:BiocGenerics':
##
##   plotMA

```

```
library(glmnet)
```

```
## Loading required package: Matrix
```

```
##
```

```
## Attaching package: 'Matrix'
```

```
## The following object is masked from 'package:S4Vectors':
```

```
##
```

```
##     expand
```

```
## Loaded glmnet 4.0
```

```
library(pheatmap)
```

```
## Warning: package 'pheatmap' was built under R version 4.0.3
```

```
library(reshape2)
```

```
library(tidyverse)
```

```
## Warning: package 'tidyverse' was built under R version 4.0.3
```

```
## -- Attaching packages ----- tidyver
```

```
## v ggplot2 3.3.1    v purrr  0.3.4
```

```
## v tibble  3.0.1    v dplyr  1.0.0
```

```
## v tidyr   1.1.0    v stringr 1.4.0
```

```
## v readr   1.3.1    v forcats 0.5.0
```

```
## Warning: package 'purrr' was built under R version 4.0.3
```

```
## Warning: package 'stringr' was built under R version 4.0.3
```

```
## -- Conflicts ----- tidyver
```

```
## x dplyr::collapse() masks IRanges::collapse()
```

```
## x dplyr::combine() masks Biobase::combine(), BiocGenerics::combine()
```

```
## x dplyr::count() masks matrixStats::count()
```

```
## x dplyr::desc() masks IRanges::desc()
```

```
## x tidyr::expand() masks Matrix::expand(), S4Vectors::expand()
```

```
## x dplyr::filter() masks stats::filter()
```

```
## x dplyr::first() masks S4Vectors::first()
```

```
## x dplyr::lag() masks stats::lag()
```

```
## x tidyr::pack() masks Matrix::pack()
```

```
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
```

```
## x purrr::reduce() masks GenomicRanges::reduce(), IRanges::reduce()
```

```
## x dplyr::rename() masks S4Vectors::rename()
```

```
## x dplyr::slice() masks IRanges::slice()
```

```
## x tidyr::unpack() masks Matrix::unpack()
```

```
library(cowplot)
```

```
##
## *****

## Note: As of version 1.0.0, cowplot does not change the

## default ggplot2 theme anymore. To recover the previous

## behavior, execute:
## theme_set(theme_cowplot())

## *****
```

```
library(here)
```

```
## here() starts at /home/rstudio/promise
```

```
library(DESeq2)
```

```
library(clusterProfiler)
```

```
##

## clusterProfiler v3.18.1 For help: https://guangchuangyu.github.io/software/clusterProfiler
##
## If you use clusterProfiler in published research, please cite:
## Guangchuang Yu, Li-Gen Wang, Yanyan Han, Qing-Yu He. clusterProfiler: an R package for comparing bio

##
## Attaching package: 'clusterProfiler'

## The following object is masked from 'package:purrr':
##
## simplify

## The following object is masked from 'package:IRanges':
##
## slice

## The following object is masked from 'package:S4Vectors':
##
## rename

## The following object is masked from 'package:stats':
##
## filter
```

```
library(ReactomePA)
```

```
## ReactomePA v1.34.0 For help: https://guangchuangyu.github.io/ReactomePA
```

```
##
```

```
## If you use ReactomePA in published research, please cite:
```

```
## Guangchuang Yu, Qing-Yu He. ReactomePA: an R/Bioconductor package for reactome pathway analysis and v
```

```
library(enrichplot)
```

```
library(org.Hs.eg.db)
```

```
## Loading required package: AnnotationDbi
```

```
##
```

```
## Attaching package: 'AnnotationDbi'
```

```
## The following object is masked from 'package:clusterProfiler':
```

```
##
```

```
##      select
```

```
## The following object is masked from 'package:dplyr':
```

```
##
```

```
##      select
```

```
##
```

Define function namespace.

```
filter <- dplyr::filter
```

```
select <- dplyr::select
```

```
rename <- dplyr::rename
```

```
slice <- dplyr::slice
```

```
count <- dplyr::count
```

Set parameters for plotting.

```
theme_set(theme_cowplot())
```

Data loading

We load the gene expression data from an Rdata object from file.

```
load(here('data/processed/expression/promise_expr.rda'))
```

```
organoid_morphology <- read_delim(here::here("references/imaging/visual_classification_organoids.csv"),
```

```
  dplyr::select(line = organoid, morphology = visual_inspection_v2) %>%
```

```
  mutate(line = substr(line, 1, 4))
```

```

## Parsed with column specification:
## cols(
##   organoid = col_character(),
##   visual_inspection_morphology_2017 = col_character(),
##   visual_class_2_2017 = col_double(),
##   visual_inspection_v2 = col_character(),
##   visual_inspection_size_2017 = col_character(),
##   visual_class_1_2017 = col_double(),
##   visual_size_ranking_2018 = col_double(),
##   visual_cystic_ranking_2018 = col_double(),
##   clustering_jan = col_character()
## )

## annotate phenotype group
solid <- organoid_morphology %>%
  filter(morphology == "solid") %>%
  .$line
cystic <- organoid_morphology %>%
  filter(morphology == "cystic") %>%
  .$line

## organoid size
organoid_size_fit <- readRDS(here::here("data/processed/morphology/organoid_size.Rds")) %>% filter(!line %in% cystic)
mutate(line = as.character(line)) %>%
  dplyr::select(line, size = x, rep = replicate) %>%
  distinct() %>% arrange(line) %>%
  mutate(line = substr(line, 1, 4)) %>%
  mutate(rep = paste0("r", rep))

# adding metadata and filtering the promise_expr object
new_col <- colData(promise_expr) %>% as.data.frame() %>%
  left_join(., left_join(
    organoid_size_fit,
    organoid_morphology)) %>%
  dplyr::select(-(line:chip_name))

## Joining, by = "line"

## Joining, by = c("line", "rep")

colData(promise_expr) <- cbind(colData(promise_expr), new_col)

promise_expr_filtered <- promise_expr[promise_expr$line %in% (organoid_size_fit$line %>% unique()),]

## long data frame
promise_long <- assays(promise_expr)$expr %>%
  as_tibble(rownames = 'probe') %>%
  pivot_longer(values_to = 'expr', names_to = 'id', -probe) %>%
  left_join(as_tibble(rowData(promise_expr), rownames = 'probe')) %>%
  inner_join(as_tibble(colData(promise_expr), rownames = 'id')) %>%
  select(-chip_name)

## Joining, by = "probe"

```

```
## Joining, by = "id"
```

```
## exclude outlier
promise_long <- promise_long %>% filter(!line %in% c('D054', 'D055', 'D021'))

## adding phenotype information
promise_long <- promise_long %>%
  mutate(phenotype = ifelse(line %in% solid, 'solid',
                             ifelse(line %in% cystic, 'cystic', 'other'))) %>%
  filter(phenotype != 'other')
```

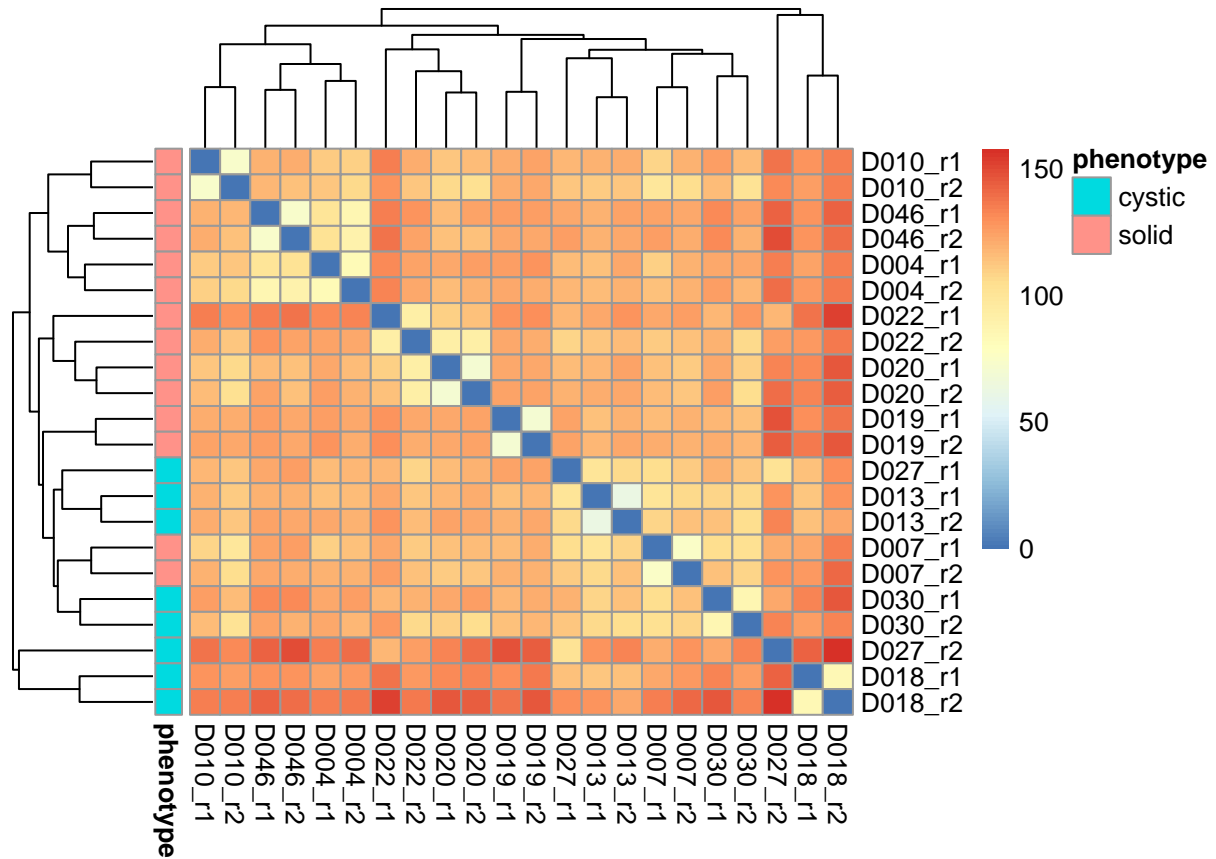
Quality control

We perform a clustering to check if the samples are grouped by line of origin.

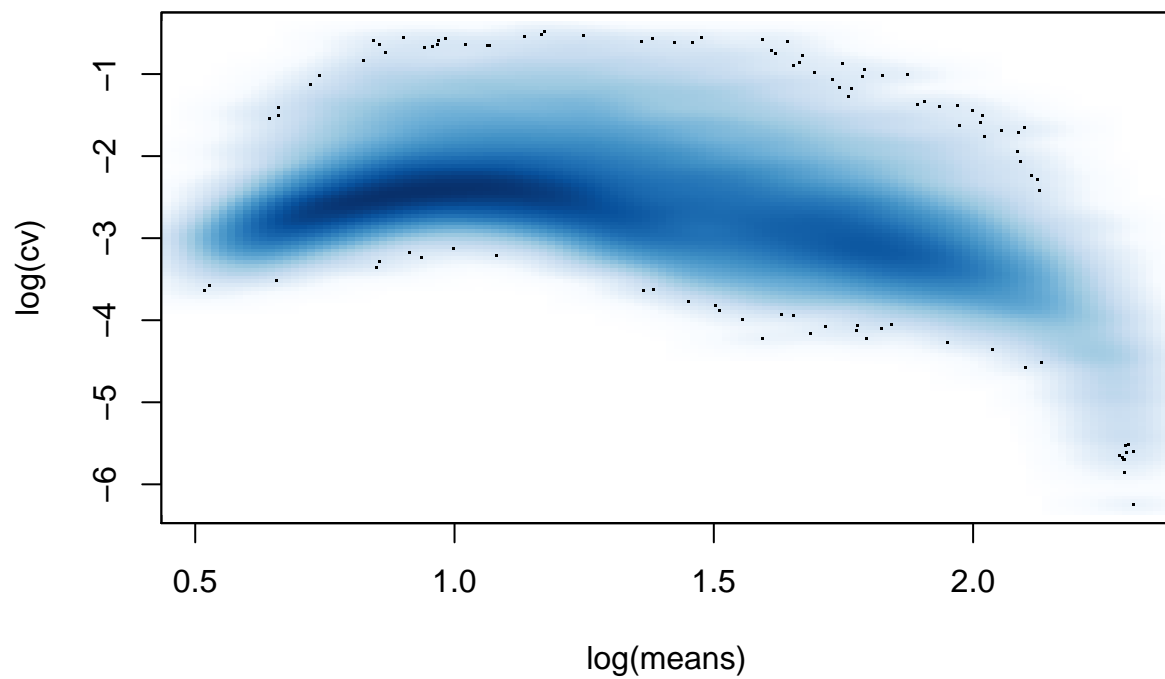
```
## make expr matrix
expr_mat <- assays(promise_expr_filtered)$expr

## generate heatmap annotation
anno <- promise_long %>%
  distinct(line, rep, phenotype) %>%
  unite(id, line, rep) %>%
  mutate_all(as.factor) %>%
  as.data.frame() %>% column_to_rownames('id')

## visualize distance matrix
pheatmap(as.matrix(dist(t(expr_mat))),
          annotation_row = anno)
```



```
## visualize most variable probes
pheatmap(expr_mat[order(apply(expr_mat, 1, sd), decreasing = T)[1:100],],
          annotation_col = anno)
```

```
hist_res <- hist(cv, 100, col="#e7efd8",
               freq = FALSE,
               main = "Histogram of coefficients of variance",
               xlab = "CV")

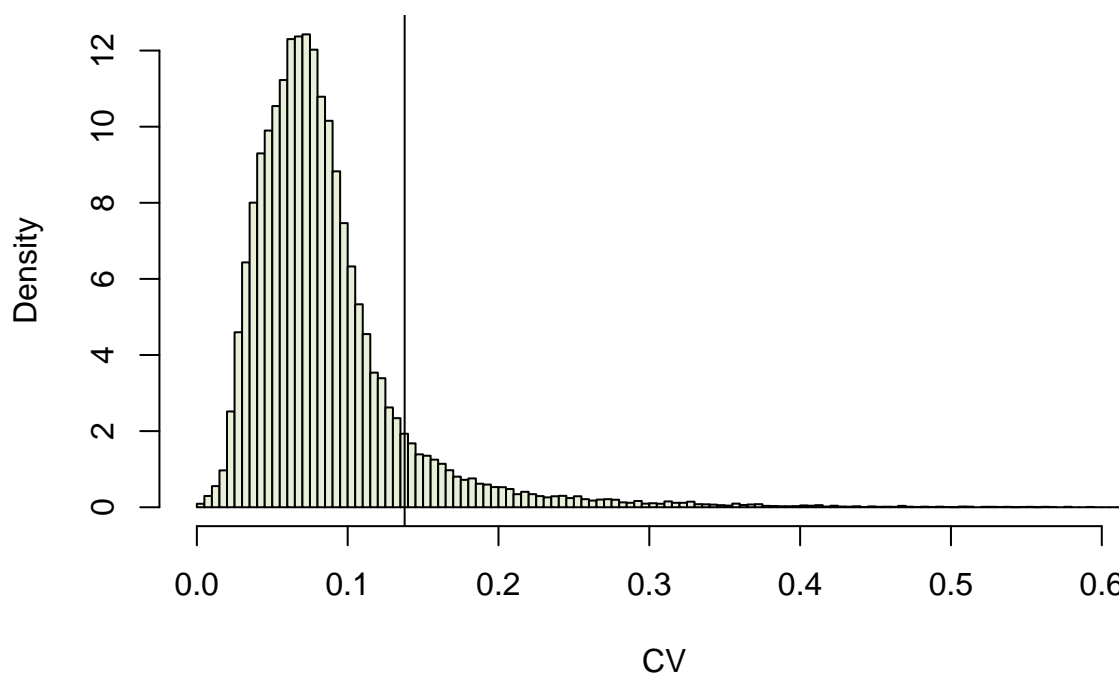
top_n = cv %>% length()
top_n = top_n * 0.10

keep_probes = cv %>% sort(decreasing = TRUE) %>% .[1:top_n]

thresh_n = min(keep_probes)

abline(v = thresh_n)
```

Histogram of coefficients of variance



Keeping the top 5% probes based on the coefficient of variance. This corresponds to this number of genes:

```
top_n
```

```
## [1] 5467.5
```

```
promise_expr_filtered = promise_expr_filtered[rowData(promise_expr_filtered) %>% rownames() %in% names(
```

```
pca_hco <- assays(promise_expr_filtered)$expr %>% t() %>% prcomp(., scale = TRUE)
summary(pca_hco)
```

```
## Importance of components:
```

```
##          PC1      PC2      PC3      PC4      PC5      PC6
## Standard deviation 29.8635 27.5841 23.3901 22.66317 21.31170 19.06585
## Proportion of Variance 0.1631 0.1392 0.1001 0.09395 0.08308 0.06649
## Cumulative Proportion 0.1631 0.3023 0.4024 0.49633 0.57941 0.64590
##          PC7      PC8      PC9     PC10     PC11     PC12
## Standard deviation 17.79842 17.13238 15.02831 13.22991 12.0828 11.70355
## Proportion of Variance 0.05794 0.05369 0.04131 0.03202 0.0267 0.02505
## Cumulative Proportion 0.70384 0.75753 0.79884 0.83086 0.8576 0.88262
##          PC13     PC14     PC15     PC16     PC17     PC18     PC19
## Standard deviation 9.96463 9.85098 9.56235 8.88428 8.18496 7.76289 7.50089
## Proportion of Variance 0.01816 0.01775 0.01673 0.01444 0.01225 0.01102 0.01029
## Cumulative Proportion 0.90078 0.91853 0.93526 0.94969 0.96195 0.97297 0.98326
##          PC20     PC21     PC22
```

```
## Standard deviation      7.2815 6.20355 2.605e-14
## Proportion of Variance 0.0097 0.00704 0.000e+00
## Cumulative Proportion  0.9930 1.00000 1.000e+00
```

```
#Build colnames
```

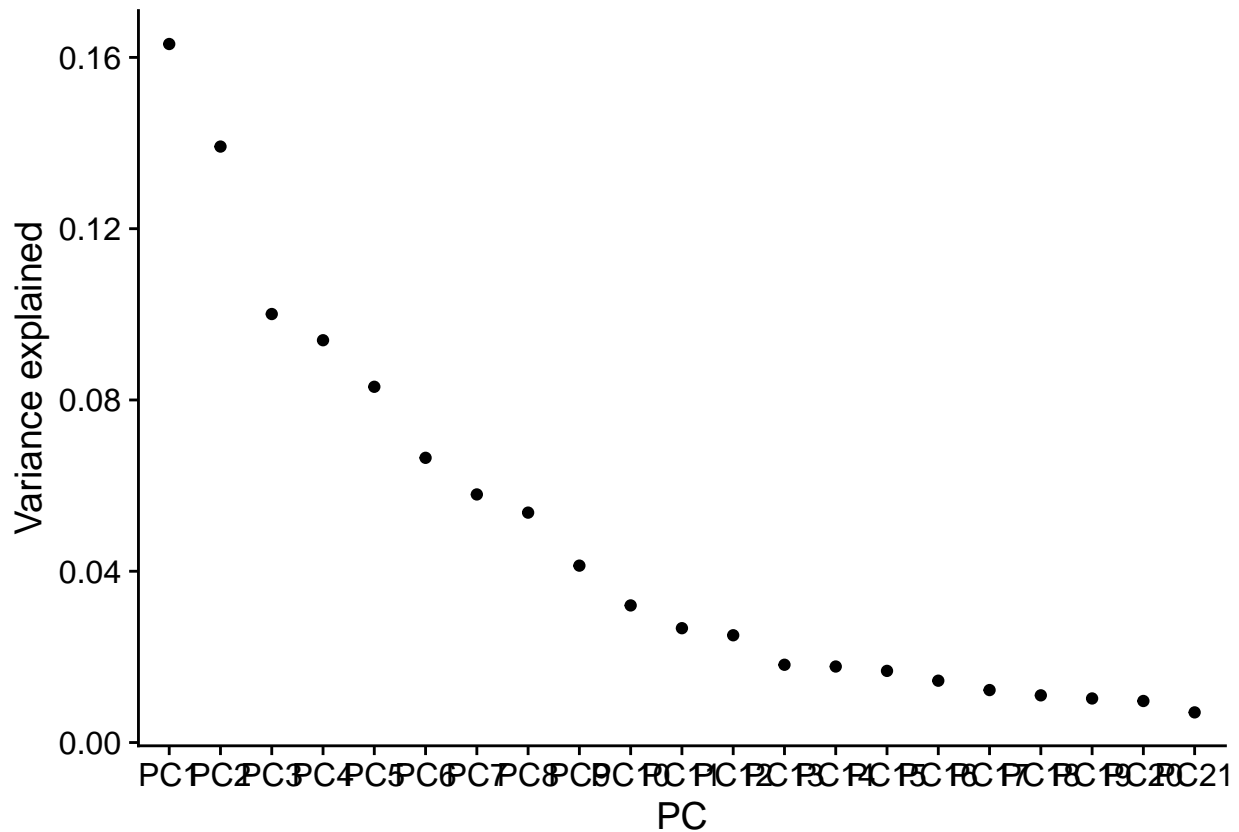
```
pca_percent <- paste(colnames(pca_hco$x), " ", "(",
                      as.character(round(pca_hco$sdev^2 / sum(pca_hco$sdev^2) * 100, 1)),
                      "%", ")", sep="")
```

```
#Format PCA
```

```
pca_hco$x_anno <- pca_hco$x %>%
  as.data.frame() %>%
  rownames_to_column("id") %>%
  separate(id, c("organoid", "line", "date", "array1", "array2"), sep="_") %>%
  unite(type, c("array1", "array2")) %>% mutate(type = substr(type, 1, nchar(type)-4))
```

```
## Warning: Expected 5 pieces. Missing pieces filled with 'NA' in 22 rows [1, 2, 3,
## 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

```
summary(pca_hco)$importance %>% as.data.frame() %>%
  rownames_to_column("metric") %>% gather(PC, value, -metric) %>%
  filter(metric == "Proportion of Variance") %>%
  mutate(PC = factor(PC, levels = .$PC)) %>%
  head(-1) %>%
  ggplot(aes(PC, value)) +
  geom_point() +
  labs(y = "Variance explained")
```



```
df = promise_long %>% mutate(symbol = ifelse(probe == '202410_x_at', 'IGF2', symbol)) %>%
  group_by(probe, line, symbol, phenotype) %>% summarise(expr = mean(expr))
```

```
plot_df = pca_hco$rotation %>% as.data.frame() %>% rownames_to_column("id") %>%
  gather(PC, value, -id) %>%
  nest(-PC) %>%
  mutate(head = purrr::map(data, ~ head(.x, 10)),
         tail = purrr::map(data, ~ tail(.x, 10)),
         headtail = purrr::map2(head, tail, ~ rbind(.x, .y))) %>%
  dplyr::select(PC, headtail) %>%
  unnest() %>%
  arrange(PC, value) %>%
  filter(PC %in% c("PC1", "PC2", "PC3")) %>%
  dplyr::select(-value, probe = id) %>%
  left_join(df)
```

```
plot_df %>%
  ggplot(aes(line, probe, fill = expr)) +
  geom_tile() +
  facet_wrap(~ PC)
```

```
pca_morph <- pca_hco$x_anno %>%
  left_join(organoid_morphology %>% rename(organoid = line)) %>%
  ggplot(aes(PC1, PC2, color = morphology)) +
  geom_point(size = 3) +
```

```

theme_classic() +
xlab(pca_percent[1]) +
ylab(pca_percent[2]) +
scale_color_manual(values = c("grey", "black")) +
theme(legend.position = "bottom") +
ggtitle("PCA on probe level - organoid morphology")

```

```
## Joining, by = "organoid"
```

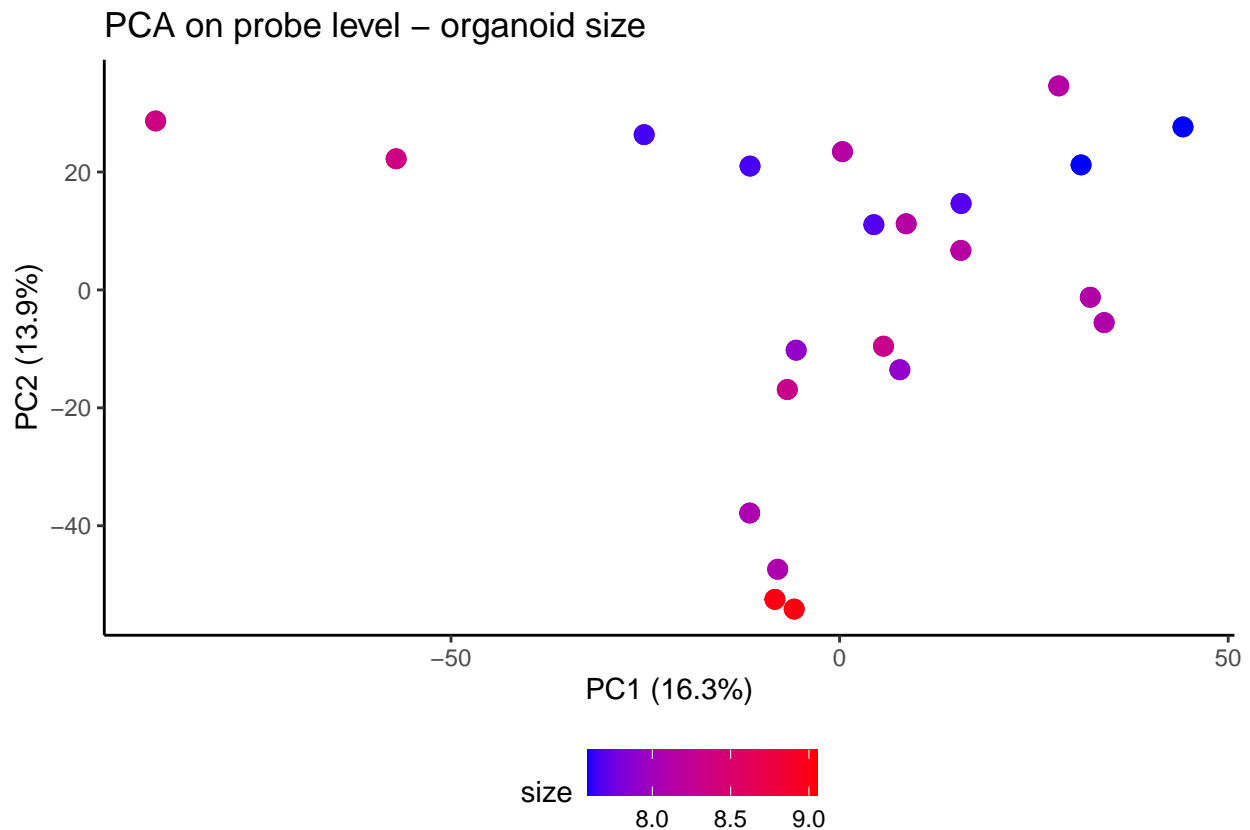
```

pca_size <- pca_hco$x_anno %>%
  left_join(organoid_size_fit %>% rename(organoid = line)) %>%
  ggplot(aes(PC1, PC2, color = size)) +
  geom_point(size = 3) +
  theme_classic() +
  xlab(pca_percent[1]) +
  ylab(pca_percent[2]) +
  theme(legend.position = "bottom") +
  #scale_color_viridis_c() +
  scale_color_gradient(low="blue", high="red") +
  #scale_colour_gradient2() +
  ggtitle("PCA on probe level - organoid size")

```

```
## Joining, by = "organoid"
```

```
pca_size
```

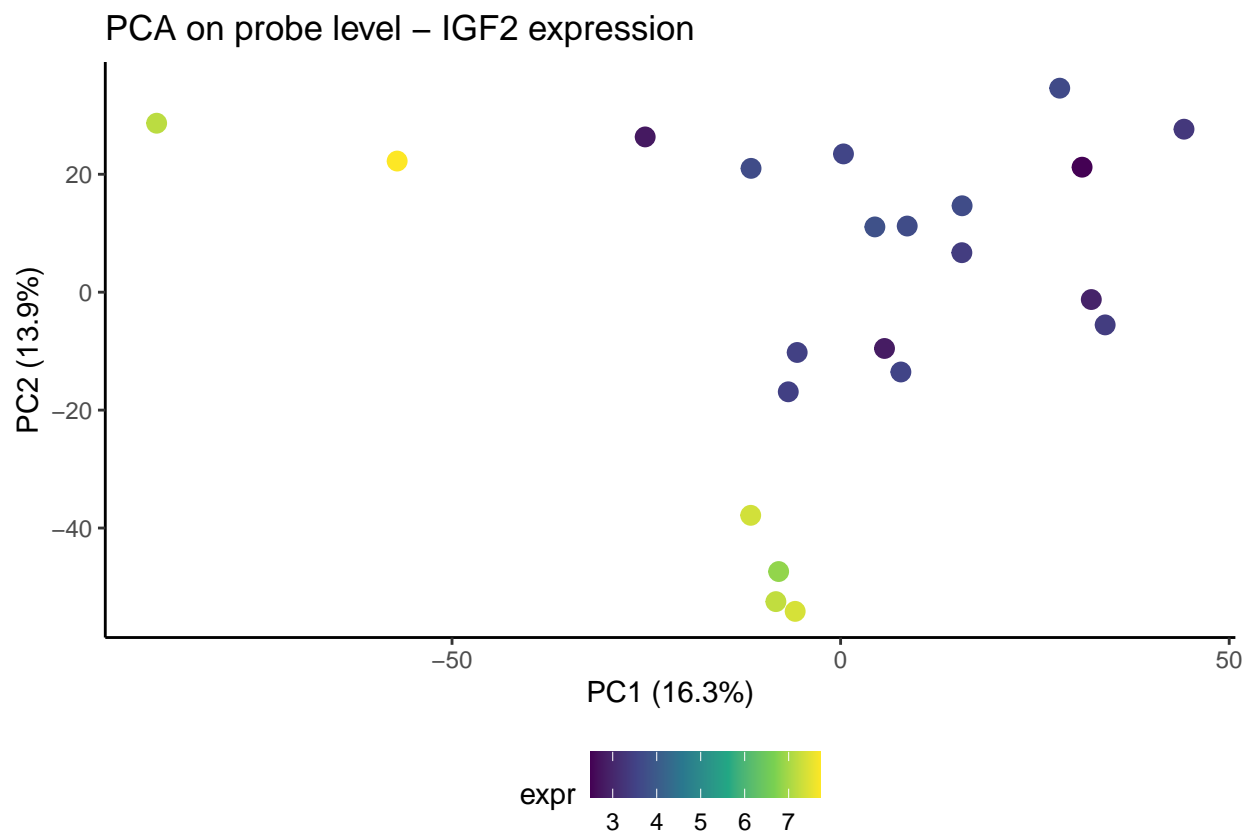


```
df = promise_long %>%
  mutate(symbol = ifelse(probe == '202410_x_at', 'IGF2', symbol)) %>%
  filter(symbol %in% c("IGF2")) %>%
  dplyr::select(organoid = line, expr, line = rep)

pca_igf <- pca_hco$x_anno %>%
  left_join(df) %>%
  ggplot(aes(PC1, PC2, color = expr)) +
  geom_point(size = 3) +
  theme_classic() +
  xlab(pca_percent[1]) +
  ylab(pca_percent[2]) +
  theme(legend.position = "bottom") +
  scale_color_viridis_c() +
  #scale_color_gradient(low="blue", high="red") +
  #scale_colour_gradient2() +
  ggtitle("PCA on probe level - IGF2 expression")
```

```
## Joining, by = c("organoid", "line")
```

```
pca_igf
```



```
promise_expr_filtered
```

```
## class: SummarizedExperiment
```

```
## dim: 5467 22
## metadata(0):
## assays(1): expr
## rownames(5467): 1405_i_at 1431_at ... 823_at 87100_at
## rowData names(3): ensg symbol entrez
## colnames(22): D013_r1 D018_r1 ... D027_r2 D030_r2
## colData names(5): line rep chip_name size morphology

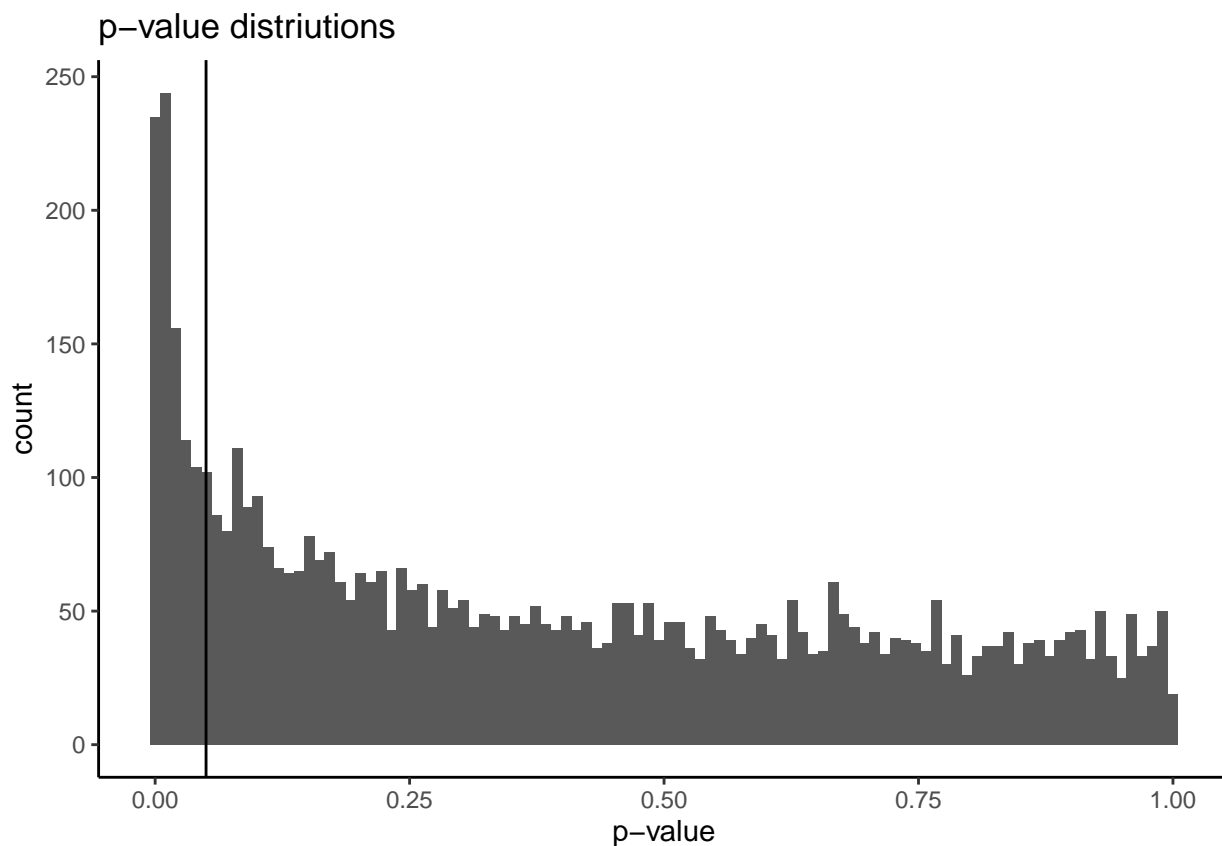
size_mat <- model.matrix(~size, colData(promise_expr_filtered))

size_fit <- limma::lmFit(assays(promise_expr_filtered)$expr, design = size_mat)
size_fit_bayes <- limma::eBayes(size_fit)

toptable_size = topTable(size_fit_bayes, number = Inf, coef = "size") %>% rownames_to_column("id") %>%
  left_join(rowData(promise_expr_filtered) %>% as.data.frame() %>% rownames_to_column("id"))

## Joining, by = "id"

toptable_size %>% ggplot(aes(P.Value)) +
  geom_histogram(bins = 100) +
  theme_classic() +
  geom_vline(xintercept = 0.05) +
  xlab("p-value") +
  ggtitle("p-value distributions")
```




```
#scale_y_log10()
```

```
topatable_size %>%  
  mutate(symbol = ifelse(id == '202410_x_at', 'IGF2', symbol)) %>%  
  filter(adj.P.Val <= 0.05) %>%  
  arrange(desc(t))
```

	id	logFC	AveExpr	t	P.Value	adj.P.Val
## 1	1558982_at	1.2509978	2.840901	5.539030	9.243174e-06	0.0150907349
## 2	1555579_s_at	2.8129566	3.202352	5.469689	1.104133e-05	0.0150907349
## 3	224646_x_at	3.5024184	4.707762	5.121655	2.706593e-05	0.0173865682
## 4	224997_x_at	2.2593491	3.901914	5.100029	2.862248e-05	0.0173865682
## 5	202409_at	4.8668738	4.921226	4.990974	3.795656e-05	0.0181632702
## 6	224348_s_at	1.9213001	3.413336	4.858079	5.356483e-05	0.0181632702
## 7	203329_at	1.7829919	3.443832	4.843898	5.557175e-05	0.0181632702
## 8	211163_s_at	2.6320054	3.541634	4.777876	6.595394e-05	0.0181632702
## 9	210881_s_at	3.4245120	3.897909	4.756351	6.974259e-05	0.0181632702
## 10	238751_at	1.0607588	2.762081	4.734748	7.376428e-05	0.0181632702
## 11	242979_at	1.7021497	3.731296	4.685273	8.387098e-05	0.0181632702
## 12	214203_s_at	1.1224794	3.710596	4.673373	8.650191e-05	0.0181632702
## 13	239002_at	1.4012657	4.284654	4.672495	8.669932e-05	0.0181632702
## 14	207194_s_at	1.5223992	3.195445	4.667579	8.781279e-05	0.0181632702
## 15	237737_at	3.2705961	3.620004	4.561052	1.157793e-04	0.0200301294
## 16	202410_x_at	3.0757784	4.432061	4.556213	1.172424e-04	0.0200301294
## 17	1559462_at	3.3655915	3.230372	4.534749	1.239579e-04	0.0204225367
## 18	221210_s_at	1.2188760	4.262146	4.514200	1.307461e-04	0.0204225367
## 19	1556348_at	1.2080576	2.679526	4.501514	1.351211e-04	0.0205196388
## 20	234867_at	0.9388959	2.547859	4.477102	1.439543e-04	0.0212702249
## 21	207433_at	1.0996136	2.688327	4.165810	3.222660e-04	0.0409727467
## 22	215606_s_at	0.9994194	4.057820	4.151251	3.346082e-04	0.0415750649
## 23	228008_at	1.1779810	4.859478	4.100351	3.815522e-04	0.0421258825
## 24	223537_s_at	1.0485515	3.143601	4.099573	3.823185e-04	0.0421258825
## 25	221029_s_at	1.6981563	3.320153	4.030909	4.562668e-04	0.0480865727
## 26	1558560_s_at	0.8666646	2.460096	4.008288	4.835976e-04	0.0489597823
## 27	204759_at	-1.2631926	4.714396	-4.022555	4.661768e-04	0.0480865727
## 28	234994_at	-2.6270939	5.094970	-4.027003	4.608744e-04	0.0480865727
## 29	1553756_at	-0.9627007	3.589697	-4.096585	3.852742e-04	0.0421258825
## 30	243671_at	-1.2492294	4.244554	-4.097635	3.842330e-04	0.0421258825
## 31	219630_at	-2.0949527	5.295127	-4.108676	3.734512e-04	0.0421258825
## 32	1558626_at	-1.0820527	3.614877	-4.138912	3.454342e-04	0.0419664196
## 33	227174_at	-3.6299421	5.284416	-4.258612	2.535460e-04	0.0330032433
## 34	200897_s_at	-1.5996625	5.305043	-4.280363	2.396723e-04	0.0319582557
## 35	229659_s_at	-1.9843584	4.918529	-4.292414	2.323131e-04	0.0317513868
## 36	1556173_a_at	-1.3401579	4.243401	-4.318930	2.169014e-04	0.0304051307
## 37	1553589_a_at	-2.1820951	5.203255	-4.346130	2.021482e-04	0.0290827356
## 38	200907_s_at	-1.7250754	4.658721	-4.523865	1.275083e-04	0.0204225367
## 39	227318_at	-2.7680087	3.697641	-4.588479	1.078249e-04	0.0196492932
## 40	228274_at	-1.1761612	4.540411	-4.600141	1.046101e-04	0.0196492932
## 41	235144_at	-2.8157003	4.663474	-4.619573	9.946514e-05	0.0194205687
## 42	236741_at	-2.6580248	4.351694	-4.659372	8.970336e-05	0.0181632702
## 43	244780_at	-2.1263221	4.291621	-4.712679	7.811253e-05	0.0181632702
## 44	1557776_at	-1.3564647	3.614313	-4.728099	7.504820e-05	0.0181632702
## 45	228067_at	-1.5478420	4.888431	-4.837117	5.655779e-05	0.0181632702

## 46	226147_s_at	-3.7015594	5.973357	-4.859248	5.340269e-05	0.0181632702
## 47	227341_at	-1.5619333	5.132031	-4.939200	4.340528e-05	0.0181632702
## 48	202158_s_at	-1.4682600	4.962011	-4.950563	4.214572e-05	0.0181632702
## 49	53991_at	-1.4258926	3.783513	-4.973971	3.966569e-05	0.0181632702
## 50	219572_at	-2.4660736	4.304208	-5.144843	2.549138e-05	0.0173865682
## 51	226560_at	-2.1657911	5.639952	-5.219547	2.101892e-05	0.0173865682
## 52	238567_at	-2.2427698	5.174345	-5.270518	1.842946e-05	0.0173865682
## 53	243718_at	-1.5715053	3.942846	-6.855898	3.434413e-07	0.0009387967
## 54	212531_at	-2.4852526	7.339031	-6.942484	2.784298e-07	0.0009387967
##	B		ensg	symbol	entrez	
## 1	3.546707104		<NA>	<NA>	<NA>	
## 2	3.385894821	ENSG00000173482		PTPRM	5797	
## 3	2.573062547	ENSG00000130600		H19	283120	
## 4	2.522290912	ENSG00000130600		H19	283120	
## 5	2.265893787		<NA>	<NA>	<NA>	
## 6	1.952759568		<NA>	<NA>	<NA>	
## 7	1.919308376	ENSG00000173482		PTPRM	5797	
## 8	1.763510863	ENSG00000173535	TNFRSF10C		8794	
## 9	1.712696650	ENSG00000207805	MIR483		619552	
## 10	1.661688262		<NA>	<NA>	<NA>	
## 11	1.544847627	ENSG00000169047	IRS1		3667	
## 12	1.516739894	ENSG00000100033	PRODH		5625	
## 13	1.514665523	ENSG00000066279	ASPM		259266	
## 14	1.503052343	ENSG00000105371	ICAM4		3386	
## 15	1.251414948		<NA>	<NA>	<NA>	
## 16	1.239984344		<NA>	IGF2	<NA>	
## 17	1.189284721		<NA>	<NA>	<NA>	
## 18	1.140754085	ENSG00000135838	NPL		80896	
## 19	1.110793101	ENSG00000119285	HEATR1		55127	
## 20	1.053149872	ENSG00000211818	TRAV39		28642	
## 21	0.319736105	ENSG00000136634	IL10		3586	
## 22	0.285550064	ENSG00000082805	ERC1		23085	
## 23	0.166149087	ENSG00000116017	ARID3A		1820	
## 24	0.164324656		<NA>	<NA>	<NA>	
## 25	0.003568326		<NA>	<NA>	<NA>	
## 26	-0.049304596	ENSG00000117475	BLZF1		8548	
## 27	-0.015961362	ENSG00000136161	RCBTB2		1102	
## 28	-0.005564374	ENSG00000164484	TMEM200A		114801	
## 29	0.157321762	ENSG00000237009	GLIS3-AS1		84850	
## 30	0.159782629		<NA>	<NA>	<NA>	
## 31	0.185664252	ENSG00000162366	PDZK1IP1		10158	
## 32	0.256587993		<NA>	<NA>	<NA>	
## 33	0.537940745	ENSG00000166415	WDR72		256764	
## 34	0.589149566	ENSG00000129116	PALLD		23022	
## 35	0.617531188		<NA>	<NA>	<NA>	
## 36	0.680002975		<NA>	<NA>	<NA>	
## 37	0.744116144	ENSG00000162366	PDZK1IP1		10158	
## 38	1.163579502	ENSG00000129116	PALLD		23022	
## 39	1.316201306	ENSG00000261115	TMEM178B		100507421	
## 40	1.343751304	ENSG00000139410	SDSL		113675	
## 41	1.389653701	ENSG00000165105	RASEF		158158	
## 42	1.483667045	ENSG00000166415	WDR72		256764	
## 43	1.609572990	ENSG00000163082	SGPP2		130367	
## 44	1.645987418		<NA>	<NA>	<NA>	

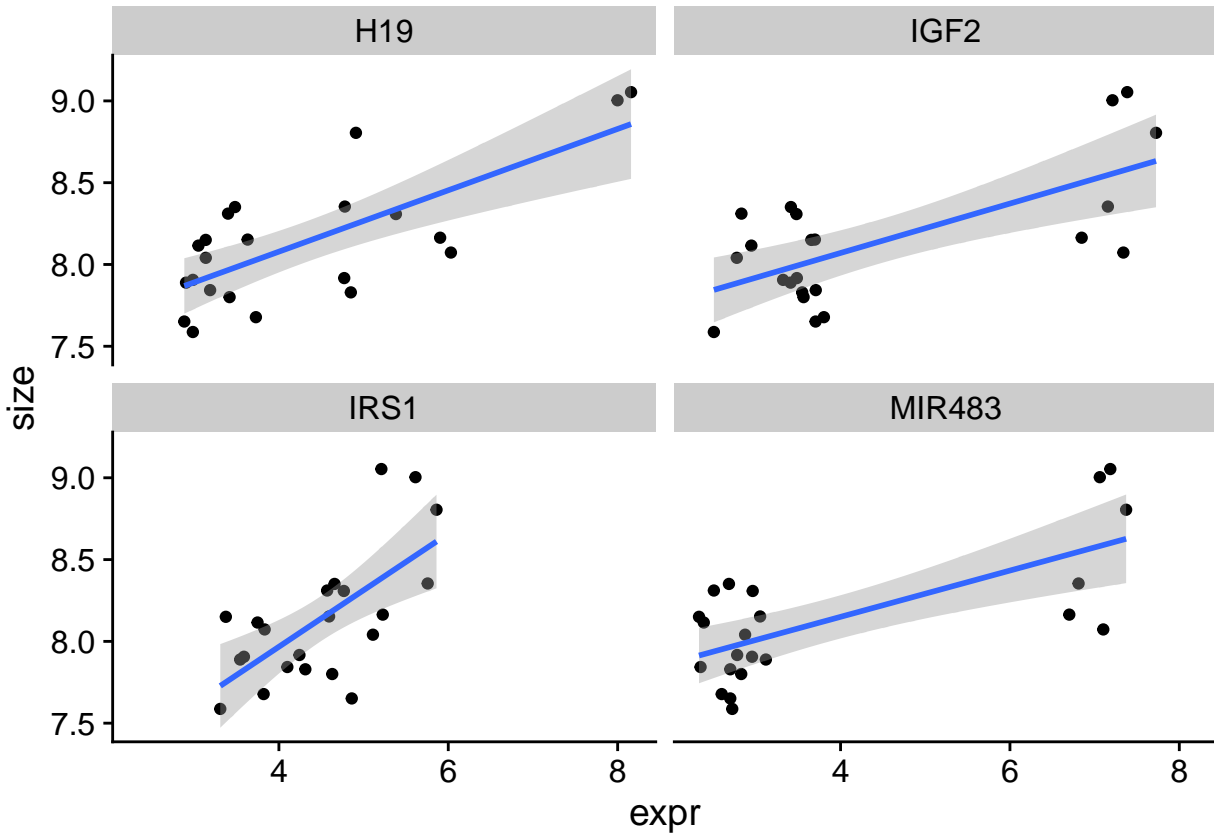
```
## 45 1.903312415 ENSG00000196872 KIAA1211L 343990
## 46 1.955516403 ENSG00000162896 PIGR 5284
## 47 2.143978122 <NA> <NA> <NA>
## 48 2.170745953 ENSG00000048740 CELF2 10659
## 49 2.225867236 ENSG00000146966 DENND2A 27147
## 50 2.627473619 ENSG00000081803 CADPS2 93664
## 51 2.802541550 ENSG00000163082 SGPP2 130367
## 52 2.921785884 ENSG00000163082 SGPP2 130367
## 53 6.496288873 <NA> <NA> <NA>
## 54 6.681960166 ENSG00000148346 LCN2 3934
```

```
promise_long %>%
  mutate(symbol = ifelse(probe == '202410_x_at', 'IGF2', symbol)) %>%
  filter(symbol %in% c("H19", "MIR483", "IRS1", "IGF2")) %>% arrange(expr) %>% arrange(line) %>%
  group_by(line, rep, symbol) %>%
  summarise(expr = mean(expr)) %>%
  left_join(organoid_size_fit) %>%
  arrange(symbol) %>%
  ggplot(aes(expr, size)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~ symbol, ncol = 2)
```

```
## 'summarise()' regrouping output by 'line', 'rep' (override with '.groups' argument)
```

```
## Joining, by = c("line", "rep")
```

```
## 'geom_smooth()' using formula 'y ~ x'
```



```
## gene ranks for enrichment, best T-value for each gene
ranks <- top3_res %>%
  filter(!tp53, !sex) %>%
  arrange(desc(abs(t)))
ranks <- setNames(ranks$t, ranks$entrez)

## get pathway annotation
pathways <- fgsea::reactomePathways(names(ranks))

## perform GSEA
enr <- fgsea::fgsea(pathways, ranks, nperm = 1e5, maxSize=500)
enr <- as_tibble(enr) %>% arrange(pval)

## genes for interesting terms
eg <- enr %>% filter(pathway == 'IGF1R signaling cascade') %>% pull(leadingEdge) %>% unlist()

## a function to draw barcode plots
custom_barcode_plot <- function(stat_vector, sig_genes, term){
  ## generate barcode plot
  bc_plot <- plotEnrichment(sig_genes, stat_vector)

  ## remove unwanted layers
  bc_plot$layers <- list()

  ## add barcode at the bottom
```

```

lowest_pos <- min(bc_plot$data[,2])
dash_length <- abs(purrr::reduce(range(bc_plot$data[,2]), '-')*0.1)
middle <- which.min(abs(sort(stat_vector, decreasing=T)))

bc_plot_custom <- bc_plot + geom_segment(aes(x=x, xend=x), y=lowest_pos,
                                         yend=lowest_pos-dash_length) +
  geom_line(colour='#4daf4a') +
  geom_hline(yintercept=lowest_pos, colour='#cccccc') +
  geom_hline(yintercept=0, colour='#cccccc') + xlab('') +
  theme_classic() +
  geom_tile(data=tibble(rank=1:length(stat_vector),
                        y=lowest_pos-(1.25*dash_length)),
            aes(x=rank, y=y, fill=rank),
            width=1,
            height=0.5*dash_length) +
  scale_fill_gradient2(low = '#b2182b', high='#2166ac',
                       mid='#f7f7f7', midpoint = middle) +
  scale_x_continuous(expand = c(0, 0)) +
  scale_y_continuous(expand = c(0, 0)) +
  theme(panel.grid=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x = element_blank(),
        legend.position = 'none') +
  ggtitle(term) +
  ylab('Enrichment score')

return(bc_plot_custom)
}

signature_genes <- pathways['IGF1R signaling cascade'] %>% unlist()

custom_barcode_plot(ranks, signature_genes,
                    'IGF1R signaling')

```

Organoid morphology

```
promise_expr_filtered
```

```

## class: SummarizedExperiment
## dim: 5467 22
## metadata(0):
## assays(1): expr
## rownames(5467): 1405_i_at 1431_at ... 823_at 87100_at
## rowData names(3): ensg symbol entrez
## colnames(22): D013_r1 D018_r1 ... D027_r2 D030_r2
## colData names(5): line rep chip_name size morphology

```

```

morph_mat <- model.matrix(~morphology, colData(promise_expr_filtered))

morph_fit <- limma::lmFit(assays(promise_expr_filtered)$expr, design = morph_mat)

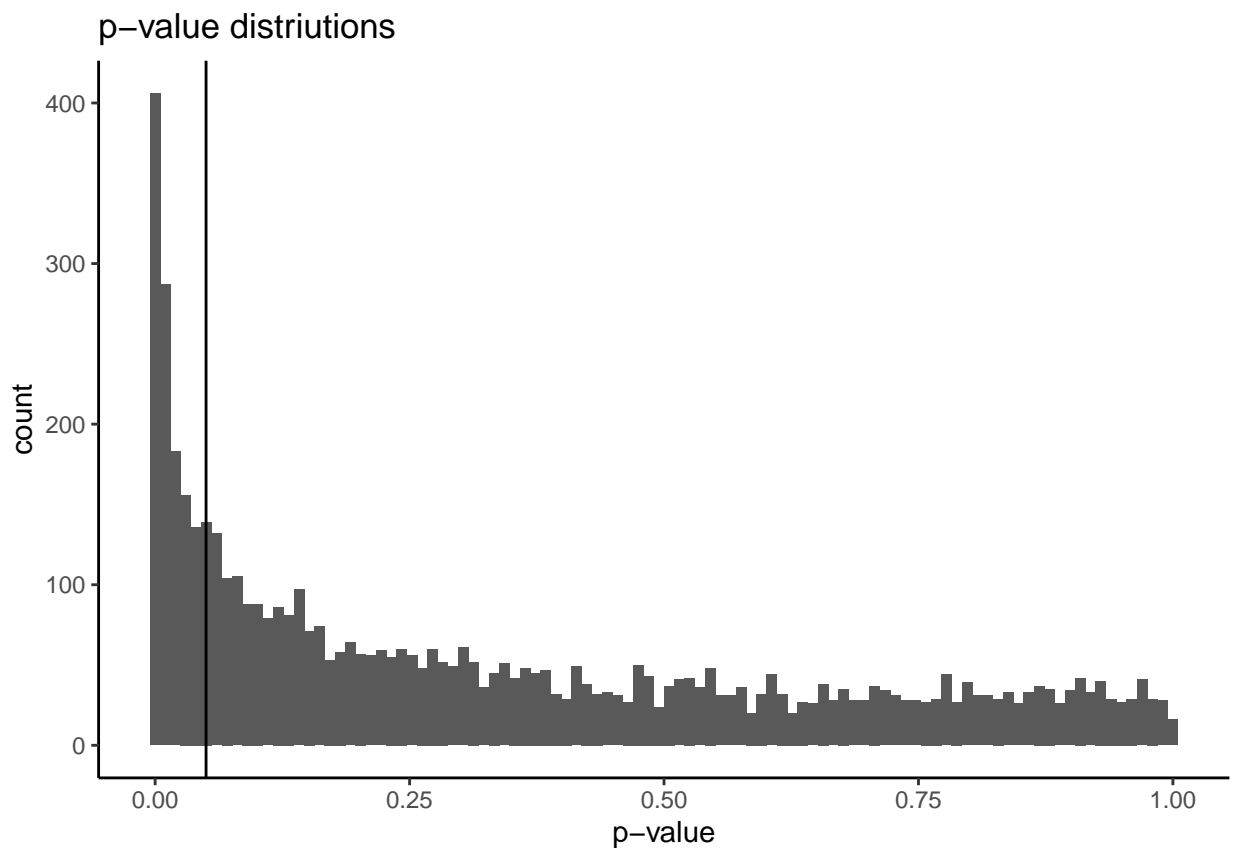
```

```
morph_fit_bayes <- limma::eBayes(morph_fit)

toptable_morph <- topTable(morph_fit_bayes, number = Inf, coef = "morphologysolid") %>% rownames_to_column()
  left_join(rowData(promise_expr_filtered) %>% as.data.frame() %>% rownames_to_column("id"))
```

```
## Joining, by = "id"
```

```
toptable_morph %>% ggplot(aes(P.Value)) +
  geom_histogram(bins = 100) +
  theme_classic() +
  geom_vline(xintercept = 0.05) +
  xlab("p-value") +
  ggtitle("p-value distributions")
```



```
#scale_y_log10()
```

```
toptable_morph %>%
  filter(adj.P.Val <= 0.05) %>%
  arrange(desc(t))
```

```
##           id      logFC AveExpr      t      P.Value      adj.P.Val
## 1  218948_at  1.3364259  4.773437  7.335437  1.138037e-07  0.0002715919
## 2  205471_s_at  1.9618024  5.666268  7.173988  1.667782e-07  0.0002715919
```

## 3	234307_s_at	1.3809464	4.443650	6.997911	2.539755e-07	0.0002776968
## 4	201601_x_at	2.8502536	6.728519	6.347024	1.241935e-06	0.0008784154
## 5	208121_s_at	3.6204283	6.210329	6.333131	1.285408e-06	0.0008784154
## 6	205472_s_at	1.7656487	4.878046	6.262560	1.531378e-06	0.0009302273
## 7	214022_s_at	2.6739632	6.633202	6.133349	2.112996e-06	0.0011551748
## 8	205983_at	2.3106159	6.894900	5.964612	3.225403e-06	0.0015054650
## 9	228915_at	1.7901572	6.304750	5.882497	3.966442e-06	0.0016680416
## 10	239332_at	1.6166107	5.196017	5.788847	5.025274e-06	0.0019623694
## 11	225627_s_at	1.4358055	4.917180	5.683400	6.565290e-06	0.0022432777
## 12	232069_at	1.0232628	3.591341	5.508901	1.023806e-05	0.0027985747
## 13	216565_x_at	1.7427828	6.176039	5.315160	1.680936e-05	0.0037026940
## 14	229376_at	1.3374760	5.493887	5.106677	2.872934e-05	0.0046923084
## 15	201641_at	1.0819557	3.937014	5.022365	3.570340e-05	0.0050048838
## 16	207401_at	1.0844420	4.065702	4.898641	4.913692e-05	0.0062472448
## 17	207529_at	3.3924349	5.744326	4.778824	6.697135e-05	0.0070968932
## 18	210164_at	2.2312823	4.573007	4.735402	7.492918e-05	0.0074748117
## 19	220394_at	2.1259842	3.767126	4.717605	7.845839e-05	0.0074748117
## 20	220432_s_at	1.3065862	4.706551	4.666625	8.951632e-05	0.0081564288
## 21	206067_s_at	1.9896958	3.401899	4.651820	9.301048e-05	0.0083145637
## 22	224370_s_at	1.0171527	2.636462	4.646523	9.429357e-05	0.0083145637
## 23	207681_at	1.0428742	3.928094	4.605068	1.049660e-04	0.0088284520
## 24	243927_x_at	1.0354244	3.692917	4.523835	1.295056e-04	0.0101063393
## 25	231119_at	0.9282093	4.086619	4.445811	1.584493e-04	0.0109650922
## 26	227702_at	1.7490532	4.736435	4.403062	1.769558e-04	0.0120927140
## 27	243508_at	0.9104274	3.402069	4.392319	1.819358e-04	0.0121532072
## 28	1564854_at	0.7336247	3.442836	4.286799	2.389040e-04	0.0143526200
## 29	201315_x_at	1.5218847	6.855321	4.256067	2.586104e-04	0.0149133476
## 30	228656_at	1.1508111	5.398742	4.221273	2.828749e-04	0.0154647735
## 31	206224_at	2.1394697	4.781214	4.210694	2.906913e-04	0.0157347440
## 32	203413_at	1.7440913	4.178978	4.147573	3.419865e-04	0.0175660762
## 33	221031_s_at	1.2725841	4.673703	4.145514	3.438028e-04	0.0175660762
## 34	205805_s_at	0.8772004	3.621705	4.114341	3.725002e-04	0.0188561000
## 35	228010_at	1.5406401	4.445808	4.108407	3.782263e-04	0.0189703048
## 36	243136_at	0.8124101	3.663700	4.092747	3.937582e-04	0.0195697812
## 37	231513_at	1.1838564	5.044723	4.083307	4.034237e-04	0.0196921201
## 38	238063_at	1.1743546	3.941293	4.042924	4.474953e-04	0.0207700301
## 39	1559051_s_at	0.9536481	3.802508	4.023797	4.699992e-04	0.0210613590
## 40	206286_s_at	1.5053137	6.172871	3.997144	5.032329e-04	0.0220093953
## 41	220622_at	1.2474449	3.987566	3.977886	5.286809e-04	0.0225804578
## 42	202549_at	0.7675697	3.681212	3.964922	5.465235e-04	0.0228079690
## 43	211685_s_at	1.1835280	4.122501	3.959368	5.543485e-04	0.0229592671
## 44	207814_at	2.4481028	5.637473	3.925575	6.043957e-04	0.0239428447
## 45	206765_at	1.3231036	5.395785	3.919166	6.143788e-04	0.0239428447
## 46	236365_at	0.9816982	3.622657	3.918848	6.148784e-04	0.0239428447
## 47	242346_x_at	0.8530758	3.537003	3.914409	6.218921e-04	0.0239428447
## 48	221038_at	0.6682957	3.199024	3.905791	6.357378e-04	0.0241359632
## 49	212203_x_at	1.6526175	7.546933	3.899534	6.459789e-04	0.0241691634
## 50	228212_at	0.8994244	3.476083	3.878868	6.809755e-04	0.0243037554
## 51	234973_at	1.2008265	5.307719	3.878121	6.822739e-04	0.0243037554
## 52	212850_s_at	1.0542362	5.491128	3.874244	6.890584e-04	0.0243037554
## 53	1553602_at	1.4953563	4.471338	3.863971	7.073543e-04	0.0247891423
## 54	200952_s_at	1.2477851	4.097446	3.841640	7.487921e-04	0.0252892343
## 55	207412_x_at	0.8721873	4.050903	3.841332	7.493792e-04	0.0252892343
## 56	214708_at	0.9205460	4.977594	3.829302	7.727009e-04	0.0256600408

## 57	227613_at	1.1941412	3.041108	3.827524	7.762083e-04	0.0256600408
## 58	227609_at	1.2060959	4.977669	3.823828	7.835485e-04	0.0256600408
## 59	204201_s_at	1.1212488	4.472284	3.821339	7.885288e-04	0.0256600408
## 60	206268_at	1.7771134	4.477295	3.818463	7.943239e-04	0.0256956744
## 61	226731_at	0.9121799	3.622344	3.811413	8.087065e-04	0.0256965565
## 62	227339_at	1.0483155	5.544980	3.806994	8.178527e-04	0.0256965565
## 63	230720_at	1.4238145	3.847073	3.782561	8.702827e-04	0.0265800864
## 64	232060_at	1.1423556	3.474807	3.762129	9.166540e-04	0.0278408189
## 65	220144_s_at	0.9948208	5.203082	3.753001	9.381450e-04	0.0280264423
## 66	214811_at	0.6980263	2.997076	3.738998	9.720791e-04	0.0286527230
## 67	242947_at	0.9139364	3.649712	3.730449	9.933859e-04	0.0287346060
## 68	228461_at	0.7428157	3.441151	3.722167	1.014465e-03	0.0288858424
## 69	231250_at	1.2849328	5.572062	3.708097	1.051284e-03	0.0294736823
## 70	206994_at	1.3999591	5.251029	3.697675	1.079400e-03	0.0296536569
## 71	222731_at	1.2485605	5.044754	3.692256	1.094308e-03	0.0299129092
## 72	238356_at	0.8948840	2.818922	3.667847	1.163998e-03	0.0311306189
## 73	1569582_at	0.9963615	3.139193	3.665359	1.171341e-03	0.0311306189
## 74	228208_x_at	1.0657903	4.410445	3.598844	1.385364e-03	0.0347421298
## 75	228985_at	0.8131602	3.282180	3.583983	1.438171e-03	0.0352577533
## 76	233949_s_at	1.0719686	3.630417	3.581374	1.447641e-03	0.0353314948
## 77	219580_s_at	1.0784137	5.209052	3.543796	1.591010e-03	0.0371711726
## 78	244783_at	0.6477985	3.031865	3.529234	1.650217e-03	0.0378662615
## 79	221022_s_at	0.7174429	3.838991	3.516304	1.704581e-03	0.0384041033
## 80	229205_at	0.9416162	2.983549	3.498882	1.780607e-03	0.0397329640
## 81	1557129_a_at	0.8576067	4.540707	3.481417	1.860129e-03	0.0408406591
## 82	227629_at	0.7995510	3.976164	3.470125	1.913375e-03	0.0415095971
## 83	228718_at	0.7679749	3.548084	3.467109	1.927845e-03	0.0416582063
## 84	222283_at	0.9469372	4.057091	3.450424	2.009840e-03	0.0425883606
## 85	205815_at	2.2980122	4.424197	3.444233	2.041122e-03	0.0428856891
## 86	244396_at	0.7701699	3.723121	3.436612	2.080277e-03	0.0430790672
## 87	215629_s_at	1.0268823	4.452029	3.420780	2.163969e-03	0.0444752515
## 88	48031_r_at	1.0490097	4.010787	3.416007	2.189840e-03	0.0446710997
## 89	1553970_s_at	1.5071638	4.470662	3.413729	2.202287e-03	0.0447580097
## 90	204582_s_at	0.6408333	3.410277	3.404042	2.256007e-03	0.0451779913
## 91	212915_at	1.2176545	5.798631	3.400711	2.274774e-03	0.0453875439
## 92	202687_s_at	0.9288759	5.271313	3.395211	2.306086e-03	0.0456788783
## 93	228391_at	0.8241467	4.370898	3.391236	2.328984e-03	0.0459659028
## 94	87100_at	0.7427808	3.611479	3.379650	2.396972e-03	0.0471375748
## 95	1556667_at	0.7298015	4.070283	3.375344	2.422727e-03	0.0474732907
## 96	243848_at	0.6074908	3.297696	3.369507	2.458073e-03	0.0479938679
## 97	215242_at	0.7626842	2.954091	3.365379	2.483373e-03	0.0483153111
## 98	206628_at	1.0372093	5.482407	3.351853	2.568042e-03	0.0489180603
## 99	235063_at	0.7113764	3.961861	3.338242	2.656068e-03	0.0497185303
## 100	244190_at	0.8437254	3.111433	3.330835	2.705188e-03	0.0499604629
## 101	221558_s_at	1.2448623	5.129557	3.327144	2.729998e-03	0.0499604629
## 102	229695_at	-0.6842914	2.433780	-3.326784	2.732427e-03	0.0499604629
## 103	207691_x_at	-0.6336564	2.725576	-3.327467	2.727820e-03	0.0499604629
## 104	210675_s_at	-1.6714251	4.587578	-3.328022	2.724076e-03	0.0499604629
## 105	1558515_at	-0.8135245	3.821246	-3.329392	2.714866e-03	0.0499604629
## 106	237515_at	-1.2823101	3.456712	-3.336942	2.664629e-03	0.0497185303
## 107	219431_at	-0.8270405	3.729414	-3.340538	2.641018e-03	0.0496166534
## 108	229497_at	-0.6994218	2.961449	-3.341590	2.634148e-03	0.0496166534
## 109	243829_at	-0.8407309	3.693063	-3.345178	2.610849e-03	0.0493893218
## 110	1558105_a_at	-0.6786860	3.761529	-3.348811	2.587470e-03	0.0491170077


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## 111 232063_x_at -0.5992631 3.088316 -3.354350 2.552206e-03 0.0487863907
## 112 212503_s_at -0.9604262 4.185261 -3.354461 2.551503e-03 0.0487863907
## 113 1554242_a_at -1.2673210 3.713849 -3.355178 2.546970e-03 0.0487863907
## 114 210613_s_at -0.6885088 3.502220 -3.356848 2.536453e-03 0.0487863907
## 115 241198_s_at -0.7076917 3.704568 -3.363612 2.494274e-03 0.0483553120
## 116 228365_at -0.6023124 2.295865 -3.396399 2.299287e-03 0.0456788783
## 117 237690_at -0.8584257 2.588820 -3.405552 2.247549e-03 0.0451740887
## 118 1558103_a_at -1.0577656 2.531211 -3.408758 2.229698e-03 0.0449806691
## 119 221078_s_at -0.5903859 2.879933 -3.410087 2.222340e-03 0.0449806691
## 120 1556029_s_at -0.9878196 2.995782 -3.416388 2.187764e-03 0.0446710997
## 121 239384_at -0.6632297 3.336025 -3.429847 2.115650e-03 0.0436462624
## 122 243021_at -0.6159794 2.902872 -3.439023 2.067812e-03 0.0429837487
## 123 239577_at -0.6463301 3.420616 -3.439101 2.067412e-03 0.0429837487
## 124 212092_at -1.3438382 4.113784 -3.443001 2.047405e-03 0.0428856891
## 125 218764_at -1.1903313 4.046345 -3.444076 2.041923e-03 0.0428856891
## 126 231697_s_at -1.0441091 3.789298 -3.454394 1.990027e-03 0.0423325995
## 127 1554670_at -0.6360038 2.941416 -3.459378 1.965426e-03 0.0419726033
## 128 221729_at -0.6025994 2.612174 -3.460050 1.962132e-03 0.0419726033
## 129 206336_at -0.7250692 2.543627 -3.464660 1.939672e-03 0.0417487758
## 130 207798_s_at -0.6065185 3.141027 -3.470241 1.912819e-03 0.0415095971
## 131 227949_at -0.8218680 3.253786 -3.473116 1.899126e-03 0.0415095971
## 132 219872_at -0.8442559 2.679131 -3.490299 1.819263e-03 0.0401044840
## 133 219298_at -1.4393199 3.845035 -3.493010 1.806965e-03 0.0399946540
## 134 212094_at -2.3976295 4.341562 -3.496552 1.791019e-03 0.0398028584
## 135 1555526_a_at -0.8770414 3.908158 -3.511251 1.726300e-03 0.0386790304
## 136 238712_at -0.7191555 2.740336 -3.515737 1.707005e-03 0.0384041033
## 137 236244_at -0.7526855 3.283379 -3.520647 1.686128e-03 0.0382492260
## 138 204932_at -1.3782765 3.640851 -3.522592 1.677928e-03 0.0382218129
## 139 209387_s_at -1.1086679 6.531063 -3.527985 1.655394e-03 0.0378662615
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## 143 223596_at -0.6591527 2.910089 -3.545077 1.585904e-03 0.0371711726
## 144 231192_at -1.0802637 2.257748 -3.551170 1.561824e-03 0.0368038539
## 145 240390_at -0.5863723 2.865857 -3.554985 1.546931e-03 0.0366107068
## 146 227231_at -0.9806173 3.180427 -3.556642 1.540508e-03 0.0366107068
## 147 241885_at -0.7779340 2.528195 -3.557586 1.536858e-03 0.0366107068
## 148 208086_s_at -0.6793964 3.372401 -3.563385 1.514628e-03 0.0363178485
## 149 205229_s_at -1.2229388 4.506456 -3.570377 1.488239e-03 0.0358422947
## 150 204672_s_at -0.5844230 2.737482 -3.570451 1.487961e-03 0.0358422947
## 151 215072_x_at -0.6849456 3.157829 -3.572940 1.478681e-03 0.0358422947
## 152 206099_at -0.9477459 3.237666 -3.590691 1.414097e-03 0.0348237300
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## 159 219957_at -0.7506244 3.689158 -3.623244 1.302747e-03 0.0332151564
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## 165 208498_s_at -1.1399407 2.947067 -3.660969 1.184410e-03 0.0311306189
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## 184 201116_s_at -1.6197168 4.238082 -3.790430 8.530471e-04 0.0262000482
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## 186 215172_at -1.4503731 2.381159 -3.799055 8.345391e-04 0.0258481172
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## 188 204933_s_at -1.7229224 4.165553 -3.808312 8.151147e-04 0.0256965565
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## 190 1552946_at -0.8934449 2.782929 -3.811960 8.075821e-04 0.0256965565
## 191 1554703_at -0.7758642 3.421001 -3.822994 7.852140e-04 0.0256600408
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## 198 1569003_at -0.9807810 4.042904 -3.878575 6.814843e-04 0.0243037554
## 199 213939_s_at -0.8148065 3.427499 -3.879473 6.799240e-04 0.0243037554
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## 209 209755_at -1.2779127 3.525579 -3.927058 6.021096e-04 0.0239428447
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## 213 204955_at -1.8319159 4.411220 -3.989188 5.135963e-04 0.0221089044
## 214 203476_at -1.2966252 4.823536 -3.992810 5.088533e-04 0.0220785794
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## 244    1560703_at -0.8412417 2.900308 -4.359745 1.979045e-04 0.0130123117
## 245    224367_at -2.0565908 4.581792 -4.391573 1.822870e-04 0.0121532072
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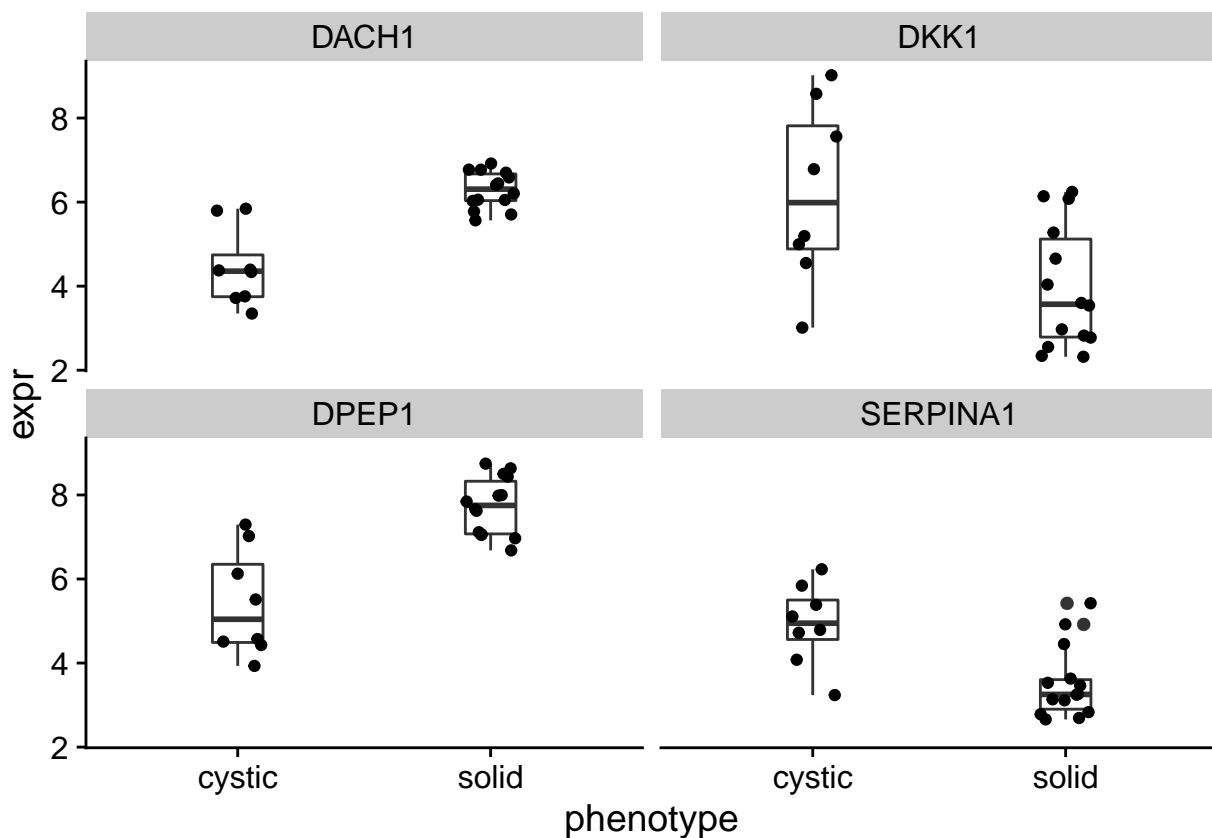
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```
## 297 6.226093685 <NA> <NA> <NA>
## 298 7.155543813 ENSG00000172137 CALB2 794
## 299 7.338399651 ENSG00000156535 CD109 135228
```

```
promise_long %>%
  filter(symbol %in% c("DACH1", "SERPINA1", "DKK1", "DPEP1")) %>% arrange(expr) %>% arrange(line) %>%
  group_by(line, rep, symbol, phenotype) %>%
  summarise(expr = mean(expr)) %>%
  left_join(organoid_size_fit) %>%
  ggplot(aes(phenotype, expr)) +
  ggrastr::geom_boxplot_jitter(width = .2) +
  geom_jitter(width = .1) +
  #geom_boxplot() +
  #geom_smooth(method = "lm") +
  facet_wrap(~ symbol, ncol = 2)
```

```
## 'summarise()' regrouping output by 'line', 'rep', 'symbol' (override with '.groups' argument)
```

```
## Joining, by = c("line", "rep")
```



```
# I only feed the top 5% most variable genes into the GO enrichment
df = toptable_morph %>% drop_na() %>%
  group_by(entrez) %>%
  summarise(t = mean(t)) %>%
  arrange(desc(t))
```

```

## 'summarise()' ungrouping output (override with '.groups' argument)

ranks = setNames(df$t, as.character(df$entrez))
#ranks = setNames(as.character(df$t), df$entrez)

## GO term enrichment (biological process)
gse_go <- gseGO(
  geneList = ranks,
  OrgDb = org.Hs.eg.db,
  ont = 'BP',
  nPerm = 1e5,
  minGSSize = 10,
  maxGSSize = 500,
  pvalueCutoff = 0.2
)

## preparing geneSet collections...

## GSEA analysis...

## Warning in .GSEA(geneList = geneList, exponent = exponent, minGSSize =
## minGSSize, : We do not recommend using nPerm parameter incurrent and future
## releases

## Warning in fgsea(pathways = geneSets, stats = geneList, nperm = nPerm, minSize
## = minGSSize, : You are trying to run fgseaSimple. It is recommended to use
## fgseaMultilevel. To run fgseaMultilevel, you need to remove the nperm argument
## in the fgsea function call.

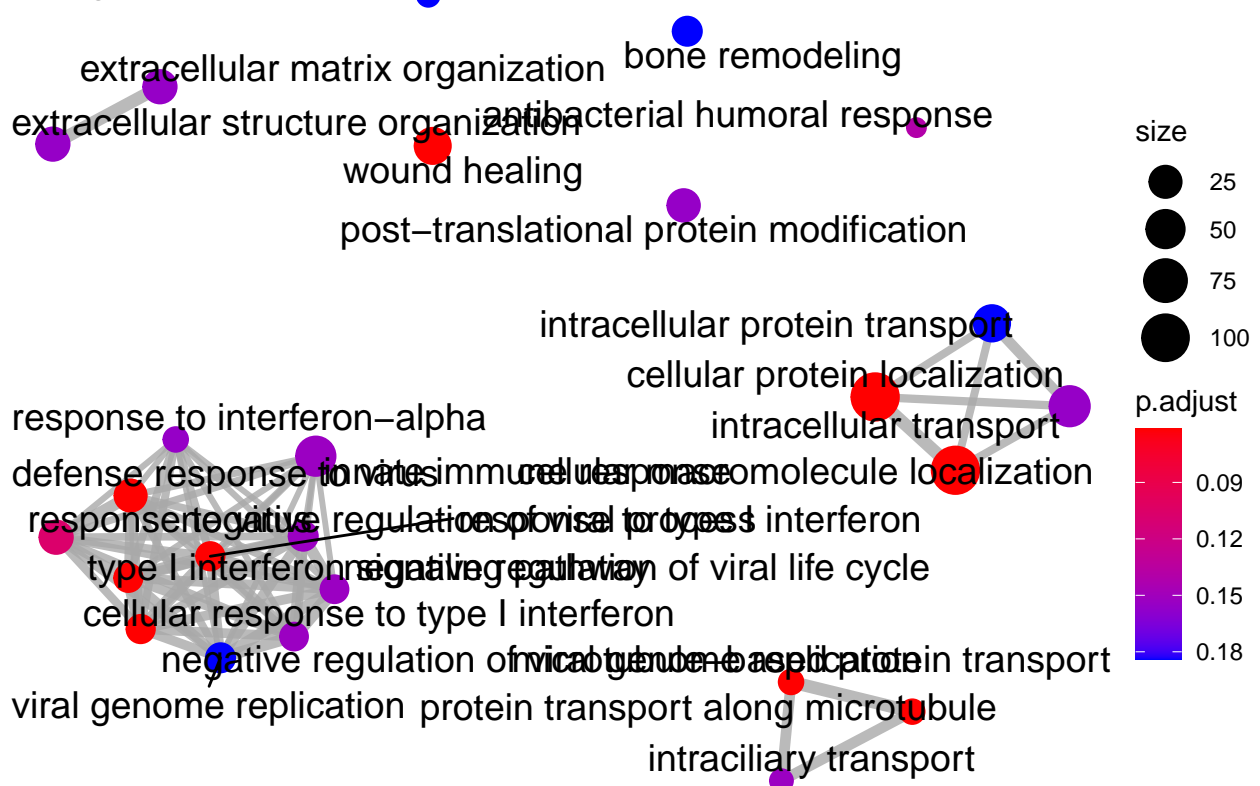
## leading edge analysis...

## done...

go <- pairwise_termsim(gse_go)
emapplot(go)

```

collagen biosynthetic process



```
## Reactome enrichment analysis
```

```
gse_reactome <- gsePathway(
  geneList = ranks,
  organism = 'human',
  nPerm = 1e5,
  minGSSize = 10,
  maxGSSize = 500,
  pvalueCutoff = 0.2
)

reactome <- pairwise_termsim(gse_reactome)
emapplot(reactome)
```

```
## parse signatures and make list
```

```
intestinal_sig <- readxl::read_excel(here('data/external/expression/merloz-suarez_sigantures.xls'),
  sheet = 1, skip = 4) %>% .[,1:4] %>%
  gather(signature, symbol) %>% drop_na() %>%
  mutate(symbol = gsub('\\\\*', '', symbol))
```

```
## New names:
## * '' -> ...5
```

```
## run gsea with clusterprofiler
```

```
df = toptable_morph %>% drop_na() %>%
  group_by(symbol) %>%
```

```

summarise(t = mean(t)) %>%
  arrange(desc(t))

## 'summarise()' ungrouping output (override with '.groups' argument)

ranks_symbol = setNames(df$t, as.character(df$symbol))

gse_sig <- GSEA(
  geneList = ranks_symbol,
  TERM2GENE = intestinal_sig,
  nPerm = 1e5,
  minGSSize = 1,
  maxGSSize = 1000,
  pvalueCutoff = 1
)

## preparing geneSet collections...

## GSEA analysis...

## Warning in .GSEA(geneList = geneList, exponent = exponent, minGSSize =
## minGSSize, : We do not recommend using nPerm parameter incurent and future
## releases

## Warning in fgsea(pathways = geneSets, stats = geneList, nperm = nPerm, minSize
## = minGSSize, : You are trying to run fgseaSimple. It is recommended to use
## fgseaMultilevel. To run fgseaMultilevel, you need to remove the nperm argument
## in the fgsea function call.

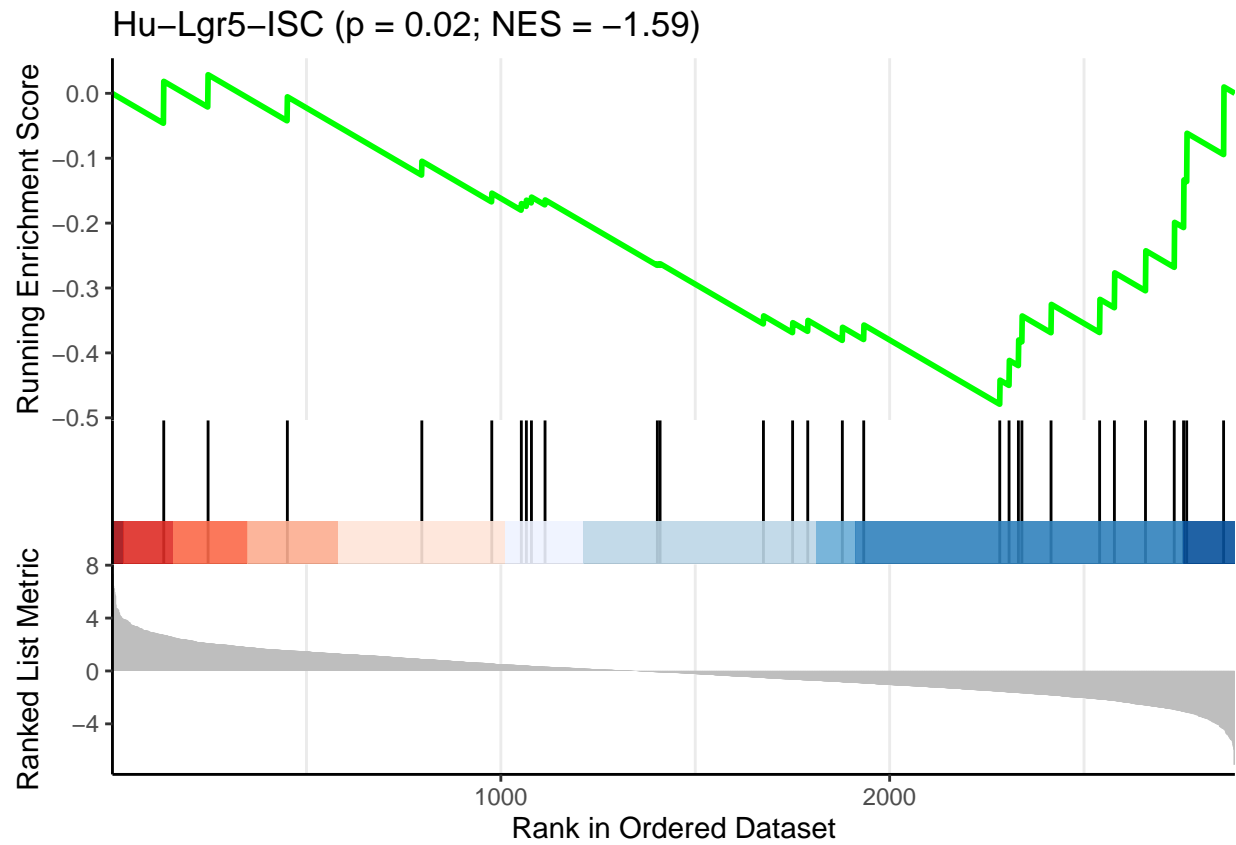
## leading edge analysis...

## done...

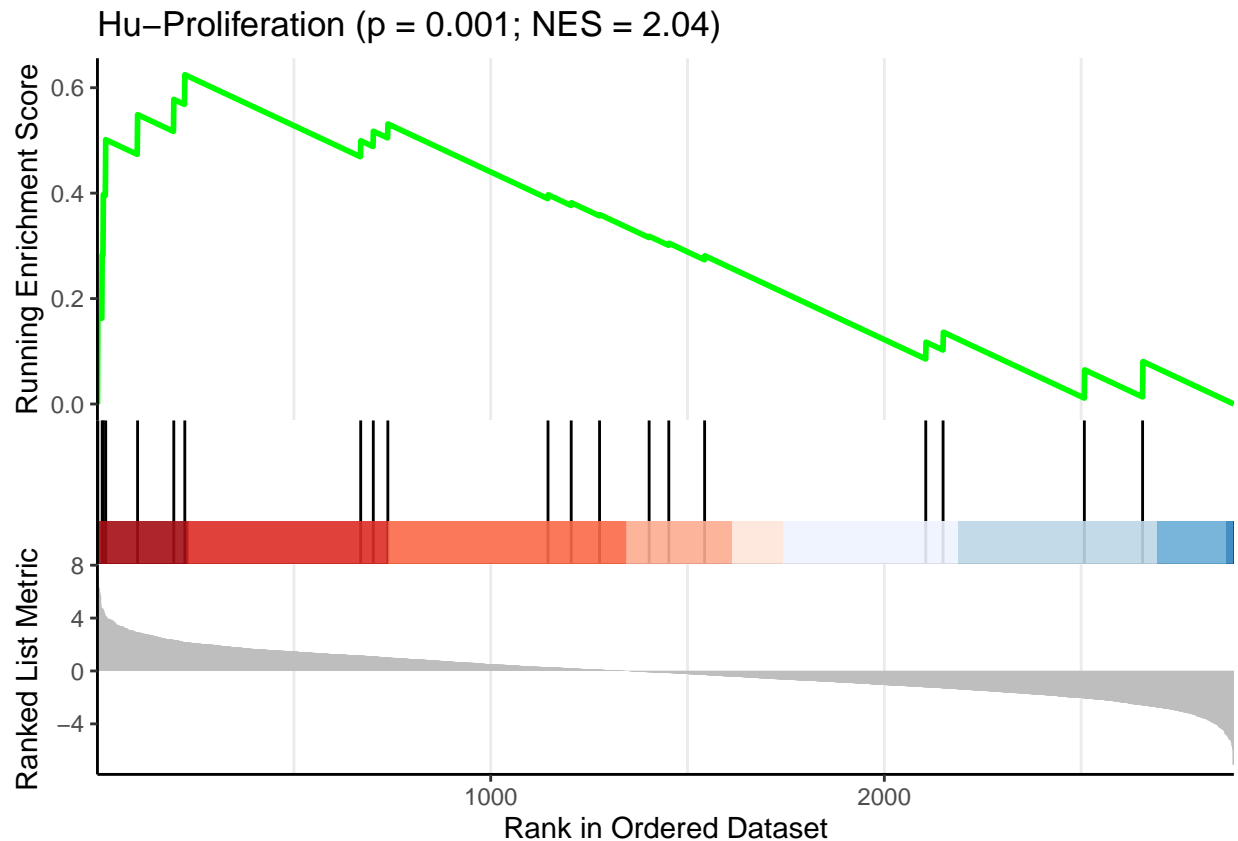
## output as tibble
gse_sig_tbl <- as_tibble(gse_sig)

## lgr5 signature
gseaplot2(
  gse_sig, geneSetID = gse_sig$ID[2],
  title = paste0(gse_sig$ID[2],
    ' (p = ', round(gse_sig_tbl$pvalue[2], 3),
    '; NES = ', round(gse_sig_tbl$NES[2], 2), ')')
)

```



```
## proliferation
gseaplot2(
  gse_sig, geneSetID = gse_sig$ID[1],
  title = paste0(gse_sig$ID[1],
    ' (p = ', round(gse_sig_tbl$pvalue[1], 3),
    '; NES = ', round(gse_sig_tbl$NES[1], 2), ')')
)
```



Organoid size and morphology

```
## model matrix
mm <- model.matrix(~phenotype, data = sample_anno)

## set technical replicate plots
blocks <- as.factor(sample_anno$line)
## compute within-block correlations
corfit <- duplicateCorrelation(expr_mat, mm, block = blocks)

## fit model and compute model coefficients
fit_phenotype <- lmFit(expr_mat, mm,
                      block = blocks,
                      cor = corfit$consensus.correlation)
fit_phenotype <- eBayes(fit_phenotype)

## extract results and annotate gene symbols
probe_to_symbol <- promise_expr %>% distinct(probe, symbol) %>%
  group_by(probe) %>% slice(1) %>% ungroup()
phenotype_res <- topTable(fit_phenotype, coef = 'phenotypesolid', n = Inf) %>%
  as_tibble(rownames = 'probe') %>%
  left_join(probe_to_symbol)
```

other

```
volc_data <- top3_res %>%
  mutate(group = ifelse(symbol %in% c('IGF2', 'MIR483', 'H19', 'INS-IGF2'), 'igf2', 'none')) %>%
  mutate(label = ifelse((group != 'none') & (adj.P.Val < 0.01), symbol, ''))

co05 <- volc_data %>% filter(adj.P.Val > 0.05) %>% pull(P.Value) %>%
  head(1) %>% log10() %>% '*'(-1)

ggplot() +
  ggrastr::geom_point_rast(data=subset(volc_data, group == 'none'),
    aes(logFC, -log10(P.Value)), colour='#dddddd') +
  geom_point(data=subset(volc_data, group == 'igf2'),
    aes(logFC, -log10(P.Value)), colour='#4285f4') +
  geom_hline(yintercept = co05, linetype='dashed') +
  ggrepel::geom_text_repel(data=subset(volc_data, group == 'igf2'),
    aes(logFC, -log10(P.Value), label=label)) +
  ylab('P-value [-log10]') + xlab('Fold change [log2]') +
  scale_y_continuous(expand=c(0,0))
```

Organoid size and gene expression

```
sessionInfo()
```

```
## R version 4.0.0 (2020-04-24)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.2 LTS
##
## Matrix products: default
## BLAS/LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-p0.3.8.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=C
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel stats4 stats      graphics grDevices utils      datasets
## [8] methods  base
##
## other attached packages:
##  [1] org.Hs.eg.db_3.12.0      AnnotationDbi_1.52.0
##  [3] enrichplot_1.10.2       ReactomePA_1.34.0
##  [5] clusterProfiler_3.18.1  DESeq2_1.30.1
##  [7] here_0.1                 cowplot_1.0.0
##  [9] forcats_0.5.0           stringr_1.4.0
## [11] dplyr_1.0.0             purrr_0.3.4
```



```

## [13] readr_1.3.1          tidyr_1.1.0
## [15] tibble_3.0.1         ggplot2_3.3.1
## [17] tidyverse_1.3.0      reshape2_1.4.4
## [19] pheatmap_1.0.12      glmnet_4.0
## [21] Matrix_1.2-18        limma_3.46.0
## [23] SummarizedExperiment_1.20.0 Biobase_2.50.0
## [25] GenomicRanges_1.42.0  GenomeInfoDb_1.26.7
## [27] IRanges_2.24.1       S4Vectors_0.28.1
## [29] BiocGenerics_0.36.1   MatrixGenerics_1.2.1
## [31] matrixStats_0.59.0
##
## loaded via a namespace (and not attached):
## [1] readxl_1.3.1          shadowtext_0.0.7      backports_1.1.7
## [4] fastmatch_1.1-0      plyr_1.8.6            igrph_1.2.5
## [7] splines_4.0.0        BiocParallel_1.24.1   digest_0.6.25
## [10] foreach_1.5.0        htmltools_0.4.0       GOsemSim_2.16.1
## [13] viridis_0.5.1        GO.db_3.12.1          fansi_0.4.1
## [16] checkmate_2.0.0      magrittr_1.5          memoise_1.1.0
## [19] annotate_1.68.0      graphlayouts_0.7.0    modelr_0.1.8
## [22] colorspace_1.4-1     rappdirs_0.3.1        blob_1.2.1
## [25] rvest_0.3.5          ggrepel_0.8.2         haven_2.3.1
## [28] xfun_0.14            crayon_1.3.4          RCurl_1.98-1.2
## [31] jsonlite_1.6.1       graph_1.68.0          scatterpie_0.1.4
## [34] genefilter_1.72.1    survival_3.1-12       iterators_1.0.12
## [37] glue_1.4.1           polyclip_1.10-0       gtable_0.3.0
## [40] zlibbioc_1.36.0      XVector_0.30.0        DelayedArray_0.16.3
## [43] graphite_1.36.0      shape_1.4.4           scales_1.1.1
## [46] DOSE_3.16.0          DBI_1.1.0             Rcpp_1.0.4.6
## [49] viridisLite_0.3.0    xtable_1.8-4          reactome.db_1.74.0
## [52] bit_1.1-15.2         httr_1.4.1            fgsea_1.16.0
## [55] RColorBrewer_1.1-2   ellipsis_0.3.1        pkgconfig_2.0.3
## [58] XML_3.99-0.3         farver_2.0.3          dbplyr_1.4.4
## [61] locfit_1.5-9.4       labeling_0.3           tidyselect_1.1.0
## [64] rlang_0.4.6          munsell_0.5.0         cellranger_1.1.0
## [67] tools_4.0.0          downloader_0.4         cli_2.0.2
## [70] generics_0.0.2       RSQLite_2.2.0         broom_0.5.6
## [73] evaluate_0.14        yaml_2.2.1            knitr_1.28
## [76] bit64_0.9-7          fs_1.4.1              tidygraph_1.2.0
## [79] ggraph_2.0.3         nlme_3.1-147          ggrastr_0.2.3
## [82] DO.db_2.9            xml2_1.3.2            compiler_4.0.0
## [85] rstudioapi_0.11      beeswarm_0.2.3        reprex_0.3.0
## [88] tweenr_1.0.1         geneplotter_1.68.0    stringi_1.4.6
## [91] lattice_0.20-41      vctrs_0.3.1           pillar_1.4.4
## [94] lifecycle_0.2.0      BiocManager_1.30.10   data.table_1.12.8
## [97] bitops_1.0-6         qvalue_2.22.0         R6_2.4.1
## [100] KernSmooth_2.23-16   gridExtra_2.3         vipor_0.4.5
## [103] codetools_0.2-16     MASS_7.3-51.5         assertthat_0.2.1
## [106] rprojroot_1.3-2      withr_2.2.0           GenomeInfoDbData_1.2.4
## [109] mgcv_1.8-31          hms_0.5.3             grid_4.0.0
## [112] rmarkdown_2.2        rvcheck_0.1.8         Cairo_1.5-12
## [115] ggforce_0.3.1        lubridate_1.7.8       ggbeeswarm_0.6.0

```