

Time to Delivery Analysis on Follow-Up qPCR Data

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1 Goal

Analyze data from qPCR experiments.

2 Prerequisites

Install necessary packages from bioconductor repository. Run this code only once to install packages.

```
pkgs_needed = c("ggplot2", "magrittr", "readr", "dplyr",
                "readxl", "stringr", "tidyr", "car",
                "interactions", "ggthemes", "cowplot",
                "MASS", "boot")
letsinstall = setdiff(pkgs_needed, installed.packages())
if (length(letsinstall) > 0) {
  source("http://bioconductor.org/biocLite.R")
  biocLite(letsinstall)
}
```

Load packages.

```
library("magrittr")
library("ggplot2")
library("readr")
library("dplyr")
library("readxl")
library("stringr")
library("tidyr")
library("MASS")
library("interactions")
library("ggthemes")
library("cowplot")
library("car")
library("boot")
theme_set(theme_few())
scale_colour_discrete = function(...) scale_colour_few()
if(params$treatment == "H1N1") {
  color_condition = "#FAA43A"
} else {
  color_condition = "#5DA5DA"
}
params$treatment
## [1] "H1N1"
```

3 Import Data

Read, merge, and tidy sample tables.

```
sample_info_filename = "MSS Case_Control Matches (Masked)_new_matched.xlsx"
data_filename = "PTB Raw qPCR data.xlsx"
```

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```
new_matched = read_excel(sample_info_filename)
new_matched %<>% mutate(time_to_delivery_calc =
  gestage_enroll - gestage_delivery)
qpcr_data = read_excel(data_filename)
qpcr_data$SampleID %<>% as.character
qpcr_data %<>% dplyr::left_join(new_matched,by = "SampleID",
  suffix = c("", "2"))
qpcr_data %<>% dplyr::select(sample_id = SampleID,
  ptb_case,
  time_to_delivery = time_to_delivery_calc,
  gestage_delivery,
  RGS13_baseline,
  IFNL_baseline,
  IFNL_flu)
qpcr_data %<>% gather(RGS13_baseline, IFNL_baseline, IFNL_flu,
  key = "key", value = "expr")
qpcr_data %<>% mutate(
  treatment = if_else(condition = str_detect(qpcr_data$key, "baseline"),
    true = "mock",
    false = "H1N1"))
qpcr_data %<>% mutate(
  gene_name = sapply(strsplit(qpcr_data$key,split = "-"),
    function(str) str[[1]]))
qpcr_data %<>% dplyr::select(-key)
qpcr_data$treatment = factor(qpcr_data$treatment,levels = c("mock","H1N1"))
qpcr_data %<>% rename(ptb = ptb_case)
qpcr_data
## # A tibble: 165 x 7
##   sample_id ptb   time_to_delivery gestage_delivery   expr treatment
##   <chr>     <chr>           <dbl>           <dbl> <dbl> <fct>
## 1 1.1      cont~           -6.57           37.6 0.160  mock
## 2 1.2      case            -5.57           21.3 0.0876 mock
## 3 1.3      cont~          -27.4           42.4 0.00572 mock
## 4 2.1      case           -18.3           33.6 0.0540 mock
## 5 2.2      cont~          -21.9           37.4 0.00703 mock
## 6 2.3      cont~          -24.3           39.3 0.00144 mock
## 7 3.2      cont~          -19.4           38   0.00470 mock
## 8 3.3      case           -14.7           33.4 0.0167 mock
## 9 4.3      cont~          -22.1           40   0.0529 mock
## 10 5.1     cont~          -21.9           40.9 0.0104 mock
## # ... with 155 more rows, and 1 more variable: gene_name <chr>
```

4 Fit Model

4.1 RGS13

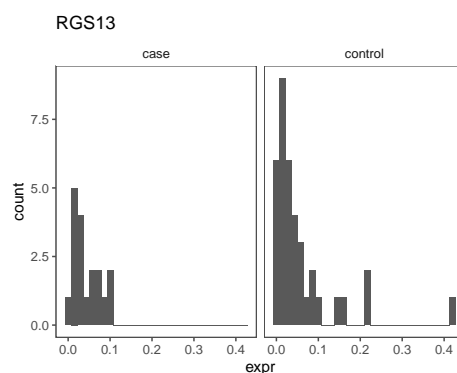
Subset to RGS13.

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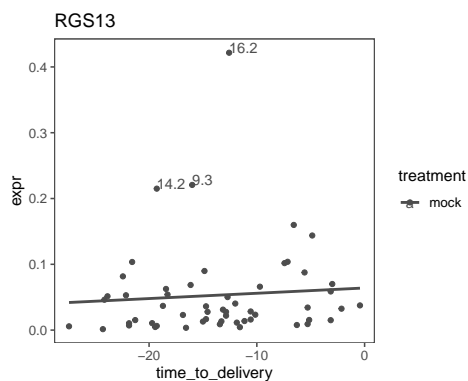
```
qpcr_data_RGS13 = qpcr_data %>% dplyr::filter(gene_name == "RGS13")
table(qpcr_data_RGS13$gene_name)
##
## RGS13
## 55
```

Plotting to see possible outliers.

```
ggplot(qpcr_data_RGS13, aes(expr)) +
  geom_histogram(bins = 30) +
  facet_wrap(~ptb) +
  ggtitle("RGS13")
```



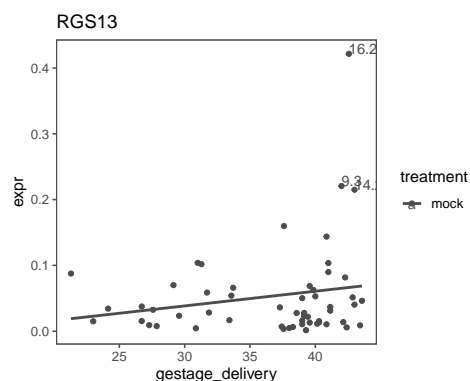
```
ggplot(qpcr_data_RGS13, aes(x = time_to_delivery, y = expr,
  color = treatment)) +
  geom_smooth(method = lm, se = FALSE) +
  geom_point() +
  geom_text(aes(label = ifelse(expr > quantile(expr, probs = 0.95),
    sample_id, "")), hjust = 0, vjust = 0) +
  ggtitle("RGS13")
```



```
ggplot(qpcr_data_RGS13, aes(x = gestage_delivery, y = expr,
  color = treatment)) +
  geom_smooth(method = lm, se = FALSE) +
  geom_point() +
  geom_text(aes(label = ifelse(expr > quantile(expr, probs = 0.95),
    sample_id, "")), hjust = 0, vjust = 0) +
```

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```
ggtitle("RGS13")
```



Fit model for RGS13.

```
lm_fit = lm(expr ~ time_to_delivery + gestage_delivery, qpcr_data_RGS13)
summary(lm_fit)
##
## Call:
## lm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_RGS13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.08642 -0.02985 -0.01733  0.01426  0.32746
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.104550   0.064954  -1.610   0.11354
## time_to_delivery  0.004714   0.001974   2.388   0.02061 *
## gestage_delivery  0.006059   0.002214   2.736   0.00848 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.06689 on 52 degrees of freedom
## Multiple R-squared:  0.131, Adjusted R-squared:  0.09754
## F-statistic: 3.918 on 2 and 52 DF, p-value: 0.026
```

Fit robust model using rank-based estimates of regression coefficients.

```
lm_fit = rlm(expr ~ time_to_delivery + gestage_delivery, qpcr_data_RGS13)
summary(lm_fit)
##
## Call: rlm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_RGS13)
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.04962 -0.02148 -0.01265  0.02540  0.36320
##
## Coefficients:
##              Value Std. Error t value
## (Intercept)   -0.0210  0.0383   -0.5479
```

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```
## time_to_delivery 0.0025 0.0012 2.1358
## gestage_delivery 0.0026 0.0013 1.9913
##
## Residual standard error: 0.03594 on 52 degrees of freedom
```

Remove potential outlier and refit model to see if we still obtain a similar result.

```
qpcr_data_RGS13 %<>% dplyr::filter(sample_id != "16.2")
lm_fit = lm(expr ~ time_to_delivery + gestage_delivery, qpcr_data_RGS13)
summary(lm_fit)
##
## Call:
## lm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_RGS13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.06230 -0.02892 -0.01508  0.01854  0.16243
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.041939   0.047497  -0.883   0.3814
## time_to_delivery  0.002920   0.001441   2.026   0.0480 *
## gestage_delivery  0.003507   0.001631   2.150   0.0363 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04805 on 51 degrees of freedom
## Multiple R-squared:  0.09024, Adjusted R-squared:  0.05456
## F-statistic: 2.529 on 2 and 51 DF, p-value: 0.08966
```

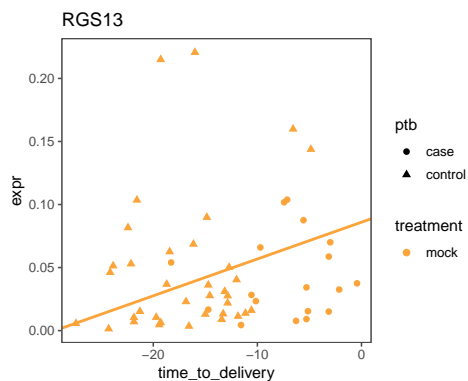
Plot of fitted intercept and slope with `gestage_delivery` fixed at its mean value.

```
coefs = coef(lm_fit)
gmne = ggplot(qpcr_data_RGS13,
              aes(x = time_to_delivery, y = expr,
                  shape = ptb, color = treatment)) +
  geom_point(size = 2) +
  scale_colour_manual(values = color_condition)

mean_gestage = mean(qpcr_data_RGS13$gestage_delivery)

gmne + geom_abline(intercept = coefs["(Intercept)"] +
                  mean_gestage*coefs["gestage_delivery"],
                  slope = coefs["time_to_delivery"],
                  color = color_condition, size = 1) + ggtitle("RGS13")
```

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```
ggsave(filename = "time_to_delivery_RGS13.png",  
        width = 4,height = 3,dpi = 600)
```

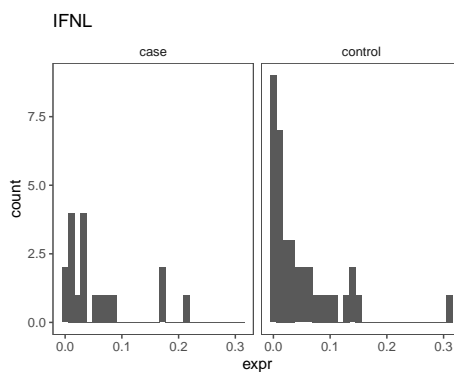
4.2 IFNL

Subset to IFNL.

```
qpcr_data_IFNL = qpcr_data %>%  
  dplyr::filter(gene_name == "IFNL") %>%  
  dplyr::filter(treatment == params$treatment)
```

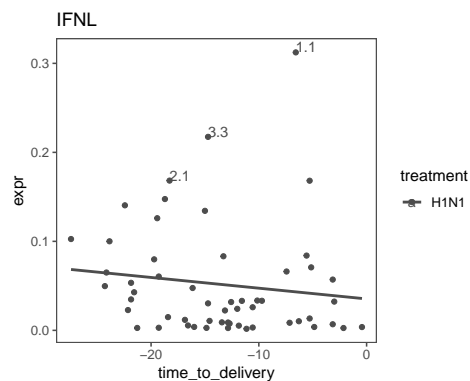
Plotting to see possible outliers.

```
ggplot(qpcr_data_IFNL,aes(expr)) +  
  geom_histogram(bins = 30) +  
  facet_wrap(~ptb) +  
  ggtitle("IFNL")
```

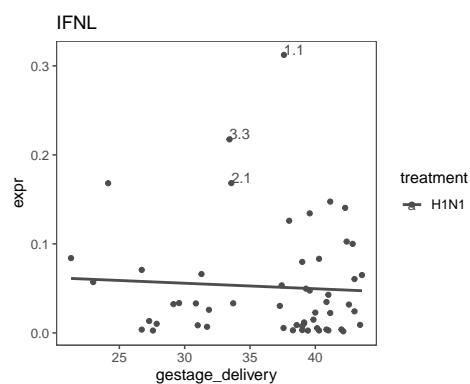


```
ggplot(qpcr_data_IFNL,aes(x = time_to_delivery, y = expr,  
                          color = treatment)) +  
  geom_smooth(method = lm, se = FALSE) +  
  geom_point() +  
  geom_text(aes(label = ifelse(expr > quantile(expr, probs = 0.95),  
                              sample_id,"")), hjust = 0, vjust = 0) +  
  ggtitle("IFNL")
```

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```
ggplot(qpcr_data_IFNL, aes(x = gestage_delivery, y = expr,
                           color = treatment)) +
  geom_smooth(method = lm, se = FALSE) +
  geom_point() +
  geom_text(aes(label = ifelse(expr > quantile(expr, probs = 0.95),
                               sample_id, "")), hjust = 0, vjust = 0) +
  ggtitle("IFNL")
```



Fit model for IFNL.

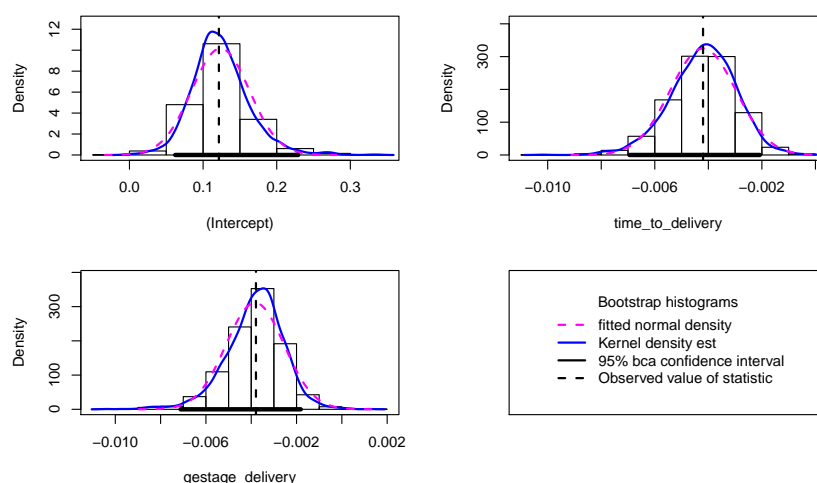
```
lm_fit = lm(expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_IFNL)
summary(lm_fit)
##
## Call:
## lm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_IFNL)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.06255 -0.03630 -0.02286  0.01274  0.28765
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.128679   0.059892   2.149   0.0363 *
## time_to_delivery -0.003371   0.001820  -1.852   0.0697 .
## gestage_delivery -0.003355   0.002042  -1.643   0.1063
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


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```
##
## Residual standard error: 0.06168 on 52 degrees of freedom
## Multiple R-squared:  0.06513,    Adjusted R-squared:  0.02917
## F-statistic: 1.811 on 2 and 52 DF,  p-value: 0.1736
```

Fit robust linear model fit and bootstrap resampling for confidence intervals and p -values.

```
lm_fit_robust = rlm(expr ~ time_to_delivery + gestage_delivery,
                    data = qpcr_data_IFNL, maxit = 200)
summary(lm_fit_robust)
##
## Call: rlm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_IFNL,
##          maxit = 200)
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.055348 -0.023425 -0.007549  0.022293  0.305845
##
## Coefficients:
##              Value Std. Error t value
## (Intercept)    0.1215  0.0367     3.3067
## time_to_delivery -0.0042  0.0011    -3.7603
## gestage_delivery -0.0038  0.0013    -3.0283
##
## Residual standard error: 0.03476 on 52 degrees of freedom
# bootstrap confidence interval and p-value
set.seed(0xdada)
lm_fit_robust_boot = Boot(lm_fit_robust, R = 1999)
summary(lm_fit_robust_boot)
##
## Number of bootstrap replications R = 1999
##              original   bootBias   bootSE   bootMed
## (Intercept)    0.1214549  2.2265e-03 0.0392497 0.1203258
## time_to_delivery -0.0041976 -6.5577e-07 0.0012283 -0.0041381
## gestage_delivery -0.0037916 -3.9577e-05 0.0012824 -0.0037249
hist(lm_fit_robust_boot, legend="separate")
```



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```
Confint(lm_fit_robust_boot, level = 0.95, type = "perc")
## Bootstrap percent confidence intervals
##
##              Estimate      2.5 %      97.5 %
## (Intercept)    0.121454935  0.054894128  0.211460343
## time_to_delivery -0.004197606 -0.006825338 -0.001967332
## gestage_delivery -0.003791648 -0.006666368 -0.001533704
Confint(lm_fit_robust_boot, level = 0.95, type = "bca")
## Bootstrap bca confidence intervals
##
##              Estimate      2.5 %      97.5 %
## (Intercept)    0.121454935  0.062133949  0.229138615
## time_to_delivery -0.004197606 -0.006944031 -0.002076893
## gestage_delivery -0.003791648 -0.007120584 -0.001813251
# p-value for stimulation x time to delivery interaction term
R = 1999
t_obsv = lm_fit_robust_boot$t0["time_to_delivery"]
t_boot = lm_fit_robust_boot$t[, "time_to_delivery"] - t_obsv
pvalue = (1 + sum(abs(t_boot) > abs(t_obsv))) / (R + 1)
pvalue
## [1] 0.002
```

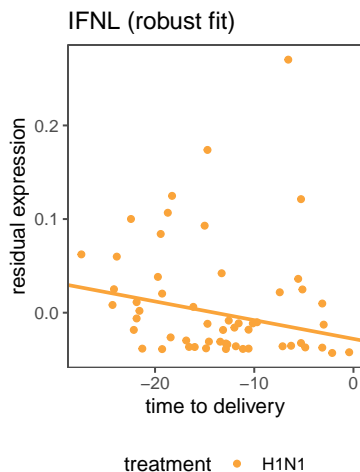
Refit linear model after removing possible outlier.

```
qpcr_data_IFNL_with_outlier = qpcr_data_IFNL
lm_fit = lm(expr ~ time_to_delivery + gestage_delivery,
             data = qpcr_data_IFNL %>%
               dplyr::filter(sample_id != "1.1"))
summary(lm_fit)
##
## Call:
## lm(formula = expr ~ time_to_delivery + gestage_delivery, data = qpcr_data_IFNL %>%
##   dplyr::filter(sample_id != "1.1"))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.06625 -0.02991 -0.01006  0.01867  0.14859
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.163117   0.045133   3.614 0.000689 ***
## time_to_delivery -0.005487   0.001401  -3.917 0.000268 ***
## gestage_delivery -0.005236   0.001555  -3.367 0.001452 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04616 on 51 degrees of freedom
## Multiple R-squared:  0.2372, Adjusted R-squared:  0.2073
## F-statistic: 7.93 on 2 and 51 DF, p-value: 0.001003
```

Residual plots.

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```
tb_subset = qpcr_data_IFNL
rlm_delivery = rlm(expr ~ gestage_delivery, data = tb_subset)
tb_subset %<>% mutate(residual_expr = rlm_delivery$residuals)
rlm_delivery = rlm(residual_expr ~ time_to_delivery, data = tb_subset)
a = rlm_delivery$coefficients[[1]]
b = rlm_delivery$coefficients[[2]]
tb_subset %<>% mutate(y_hat = a + b*tb_subset$time_to_delivery)
gmine_with_outlier = ggplot(tb_subset, aes(time_to_delivery, residual_expr,
                                             color = treatment)) +
  geom_abline(intercept = a, slope = b, color = color_condition, size = 1) +
  geom_point() +
  scale_colour_manual(values = color_condition) +
  ggtitle("IFNL (robust fit)") +
  xlab("time to delivery") +
  ylab("residual expression") +
  theme(legend.position = "bottom")
gmine_with_outlier
```



```
ggsave(gmine_with_outlier,
  filename = paste0("robust_fit_IFNL_", params$treatment, ".png"),
  width = 3, height = 4, dpi = 600)
```

Session Info

```
sessionInfo()
## R version 3.5.1 (2018-07-02)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS 10.15.1
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
##
```

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```
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices utils      datasets  methods   base
##
## other attached packages:
## [1] boot_1.3-22      car_3.0-3        carData_3.0-2
## [4] cowplot_1.0.0    ggthemes_4.1.1   interactions_1.1.1
## [7] MASS_7.3-51.4    tidyr_1.0.0      stringr_1.4.0
## [10] readxl_1.3.1     dplyr_0.8.3      readr_1.3.1
## [13] ggplot2_3.2.1    magrittr_1.5      BiocStyle_2.10.0
##
## loaded via a namespace (and not attached):
## [1] tidyselect_0.2.5 xfun_0.6          purrr_0.3.3
## [4] pander_0.6.3      haven_2.1.0       colorspace_1.4-1
## [7] vctrs_0.2.0       generics_0.0.2    htmltools_0.3.6
## [10] yaml_2.2.0        utf8_1.1.4        rlang_0.4.1
## [13] pillar_1.4.2      foreign_0.8-71     glue_1.3.1
## [16] withr_2.1.2       jtools_2.0.1      lifecycle_0.1.0
## [19] munsell_0.5.0     gtable_0.3.0      cellranger_1.1.0
## [22] zip_2.0.2          evaluate_0.13     labeling_0.3
## [25] forcats_0.4.0     knitr_1.22        rio_0.5.16
## [28] curl_4.2           fansi_0.4.0       Rcpp_1.0.3
## [31] scales_1.0.0      backports_1.1.5    BiocManager_1.30.4
## [34] abind_1.4-5        hms_0.5.2         digest_0.6.22
## [37] openxlsx_4.1.0.1  stringi_1.4.3     bookdown_0.9
## [40] grid_3.5.1         cli_1.1.0          tools_3.5.1
## [43] lazyeval_0.2.2    tibble_2.1.3      crayon_1.3.4
## [46] pkgconfig_2.0.3    zeallot_0.1.0     ellipsis_0.3.0
## [49] data.table_1.12.6 assertthat_0.2.1   rmarkdown_1.12
## [52] R6_2.4.1           compiler_3.5.1
```