Introduction to proteomics data analysis: robust summarization

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Contents

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1 Background

A study on the facultative pathogen Francisella tularensis was conceived by Ramond et al. (2015) [12]. F. tularensis enters the cells of its host by phagocytosis. The authors showed that F. tularensis is arginine deficient and imports arginine from the host cell via an arginine transporter, ArgP, in order to efficiently escape from the phagosome and reach the cytosolic compartment, where it can actively multiply. In their study, they compared the proteome of wild type F. tularensis (WT) to ArgP-gene deleted F. tularensis (knock-out, D8). For this exercise, we use a subset of the F. tularensis dataset where bacterial cultures were grown in biological quadruplicate and each biorep was run in technical triplicate on a nanoRSLC-Q Exactive PLUS instrument. The data were searched with MaxQuant version 1.4.1.2. and are available on the PRIDE repository: PXD001584.

2 Data

```r
library(tidyverse)
library(limma)
library(QFeatures)
library(msqrob2)
library(plotly)

peptidesFile <- "https://raw.githubusercontent.com/statOmics/MSqRobSumPaper/master/Francisella/data/maxq

ecols <- grep(
  "Intensity\\.",
  names(read.delim(peptidesFile))
  )

pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")

colnames(pe)
```

```
## CharacterList of length 1
## [["peptideRaw"]] Intensity.1WT_20_2h_n3_1 ... Intensity.3D8_20_2h_n5_3
```

The annotation can be derived from the file name.

```r
colData(pe)$genotype <- substr(colnames(pe[[1]]),12,13) %>%
                        as.factor
colData(pe)$biorep  <- paste(
  substr(colnames(pe[[1]]),12,13),
  substr(colnames(pe[[1]]),21,22),
  sep="_") %>% as.factor
```

We calculate how many non zero intensities we have per peptide and this is often useful for filtering.

```r
rowData(pe[["peptideRaw"]])$nNonZero <- rowSums(assay(pe[["peptideRaw"]]) > 0)
```

Because every biorep is assessed in technical triplicate, we will also calculate the number of biorepeats in which each peptide is observed.

```r
rowData(pe[["peptideRaw"]])$nNonZeroBiorep <- apply(
  assay(pe[["peptideRaw"]]),
  1,
  function(intensity)
    colData(pe)$biorep[intensity>0] %>%
    unique %>%
    length)
```

Peptides with zero intensities are missing peptides and should be represent with a `NA` value rather than `0`.

```
pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA
```

2.1 Data exploration

49% of all peptide intensities are missing.

3 Preprocessing

This section preforms preprocessing for the peptide data. This include

- log transformation,
- filtering and
- summarisation of the data.

3.1 Log transform the data

```
pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")
```

3.2 Filtering

1. Handling overlapping protein groups

In our approach a peptide can map to multiple proteins, as long as there is none of these proteins present in a smaller subgroup.

```
pe <- filterFeatures(pe,
  ~ Proteins %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins)
)
```

2. Remove reverse sequences (decoys) and contaminants

We now remove the contaminants and peptides that map to decoy sequences.

```
pe <- filterFeatures(pe, ~ Reverse != "+")
pe <- filterFeatures(pe, ~ Contaminant != "+")
```

3. Drop peptides that were only identified in a single biorepeat

Note, that in experiments without technical repeats we filter on the number of samples in which a peptide is picked up. Here, we will require that a peptide is picked up in at least two biorepeats.

```
pe <- filterFeatures(pe, ~ nNonZeroBiorep >= 2)
nrow(pe[["peptideLog"]])
```

```
## [1] 7542
```

We keep 7542 peptides upon filtering.

3.3 Normalize the data using median centering

We normalize the data by substracting the sample median from every intensity for peptide p in a sample i:

$$y_{ip}^{\text{norm}} = y_{ip} - \hat{\mu}_i$$

with $\hat{\mu}_i$ the median intensity over all observed peptides in sample i.

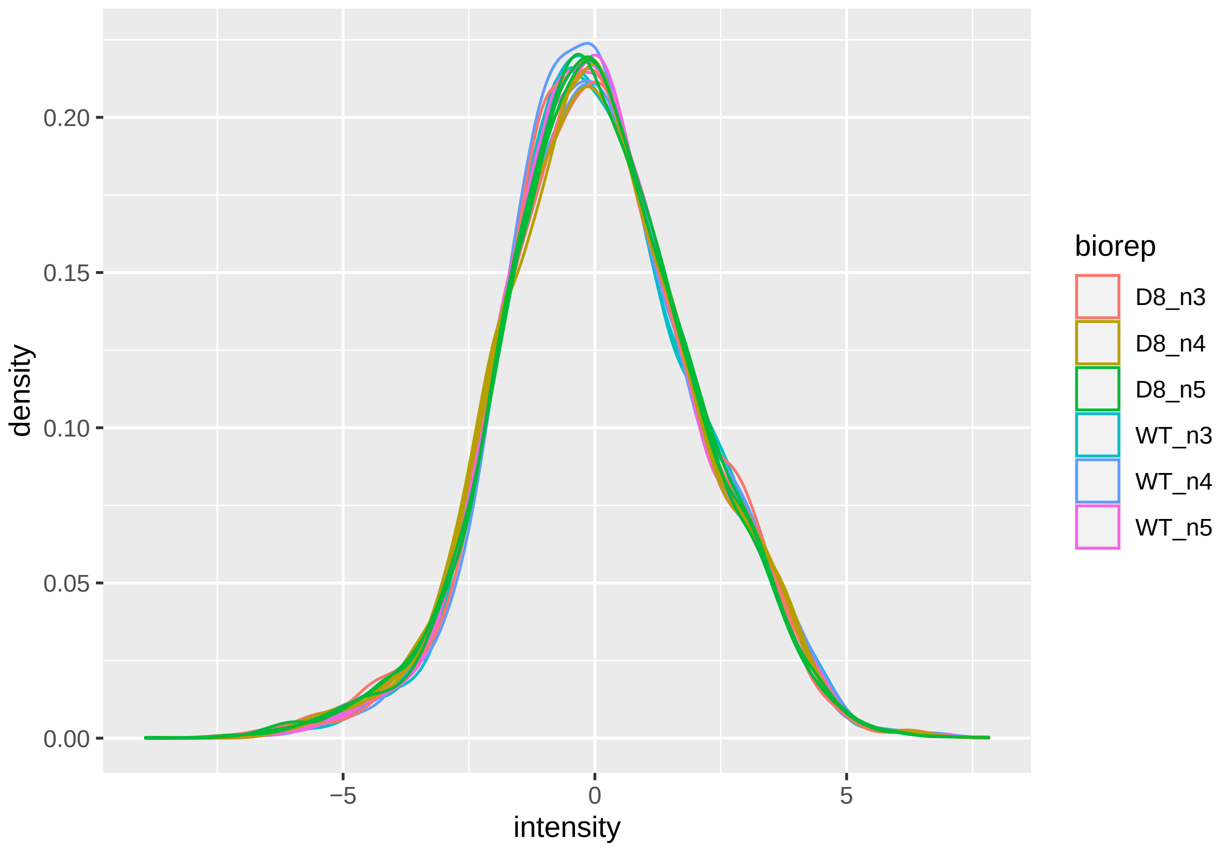
```
pe <- normalize(pe,
  i = "peptideLog",
  name = "peptideNorm",
  method = "center.median"
)
```

3.4 Explore normalized data

Upon the normalisation the density curves are nicely registered

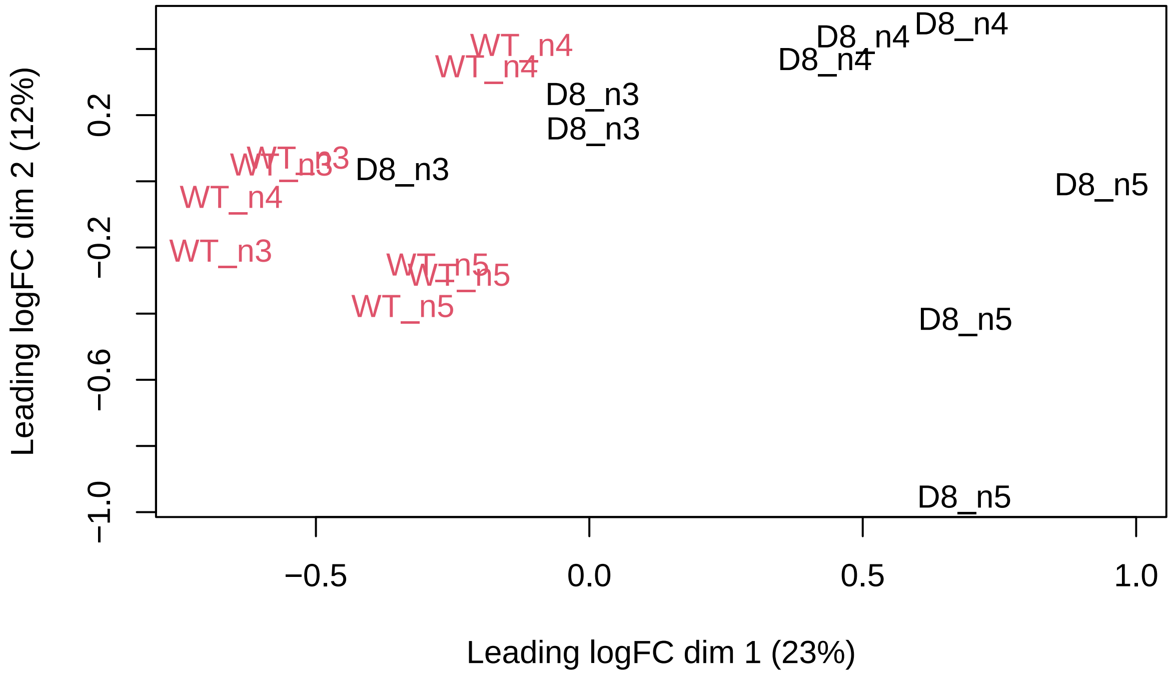
```
pe[["peptideNorm"]] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(biorep = colData(pe)[sample,"biorep"]) %>%
  ggplot(aes(x = intensity,group = sample,color = biorep)) +
    geom_density()
```

```
## Warning: Removed 40413 rows containing non-finite values (stat_density).
```



We can visualize our data using a Multi Dimensional Scaling plot, eg. as provided by the `limma` package.

```
pe[["peptideNorm"]] %>%
  assay %>%
  limma::plotMDS(col = as.numeric(colData(pe)$genotype),label=colData(pe)$biorep)
```



The first axis in the plot is showing the leading log fold changes (differences on the log scale) between the samples.

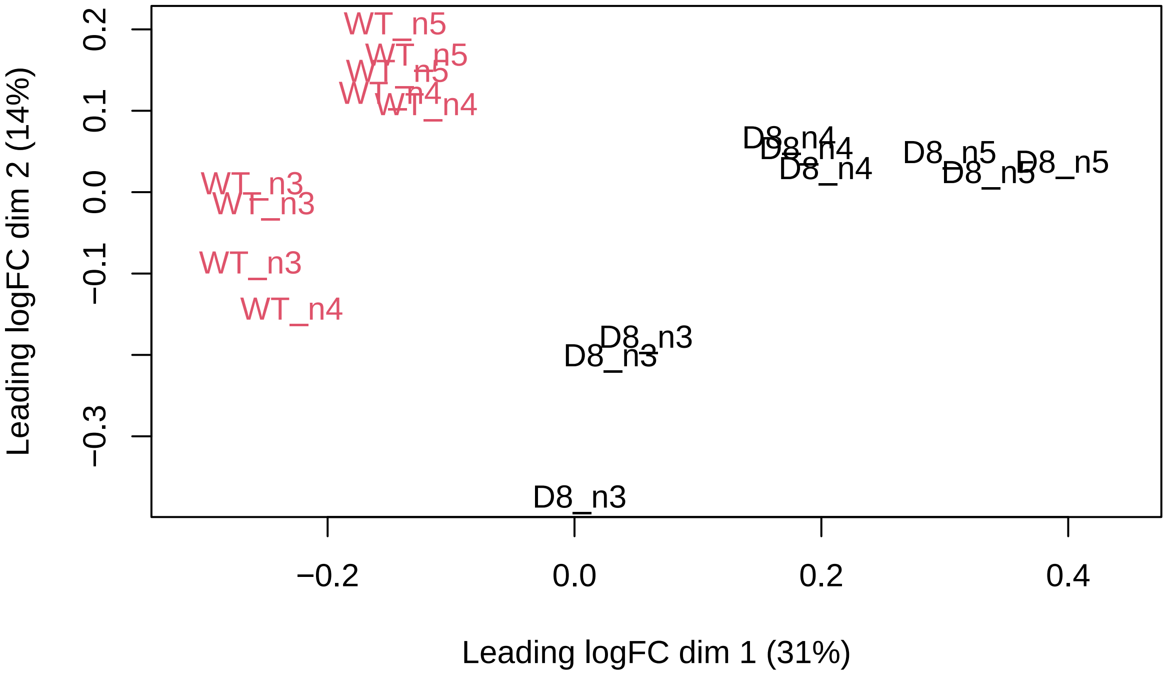
3.5 Summarization to protein level

- By default robust summarization is used: `fun = MsCoreUtils::robustSummary()`

```
pe <- aggregateFeatures(pe,
  i = "peptideNorm",
  fcol = "Proteins",
  na.rm = TRUE,
  name = "protein")
```

```
## Your quantitative and row data contain missing values. Please read the
## relevant section(s) in the aggregateFeatures manual page regarding the
## effects of missing values on data aggregation.
```

```
pe[["protein"]]%>%
  assay %>%
  limma::plotMDS(col = as.numeric(colData(pe)$genotype),label=colData(pe)$biorep)
```



Note that the samples upon robust summarisation show a clear separation according to the genotype in the first dimension of the MDS plot.

4 Data Analysis

4.1 Estimation

We model the protein level expression values using `msqrob`. By default `msqrob2` estimates the model parameters using robust regression.

We will model the data with a different group mean. The group is incoded in the variable `genotype` of the colData. We will also have to include a random effect for bio-repeat to address the pseudo-replication in the experiment. Indeed, the data from the same bio-repeat will be correlated!

We can specify this model by using a formula with the factor `genotype` as a fixed effect and as the factor `biorep` a random effect: `formula = ~genotype + (1|biorep)`.

In the current implementation of msqrob2, you can only work with mixed models if you set the ridge argument `ridge=TRUE`. The fixed effects are then estimated using ridge regression and random effects can be introduced in the model. Our implementation exploits the link between penalized ridge regression and mixed models. Indeed, by reformulating the fixed effects as random effects ridge regression can be implemented and the ridge penalty is estimated from the data.

Note, that ridge regression only works if two or more slope parameters have to be estimated for the fixed effects. Here, we only have a factor with two levels resulting in an encoding with only one slope parameter so the msqrob function will throw an error.

```
try(pe <- msqrob(object = pe, i = "protein", formula = ~ genotype + (1|biorep),ridge=TRUE,overwrite=TRU
```

```
## Error in msqrobLmer(y = assay(object[[i]]), formula = formula, data = colData(object),  :
##   The mean model must have more than two parameters for ridge regression.
##               if you really want to adopt ridge regression when your factor has only two levels
##               rerun the function with a formula where you drop the intercept. e.g. ~-1+condition
##
```

However, we can also encode ridge regression for the fixed effects directly by specifying the genotype also as a random effect. More information on the link between ridge regression, mixed models and random effect can be found in (Goeminne, Gevaert, and Clement 2016) and (Sticker et al. 2020)

```
pe <- msqrob(object = pe, i = "protein", formula = ~ (1|genotype) + (1|biorep), ridge=TRUE)
```

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```

4.2 Inference

First, we extract the parameter names of the model by looking at the first model. The models are stored in the row data of the assay under the default name msqrobModels.

```
getCoef(rowData(pe[["protein"]])$msqrobModels[[1]])
```

```
##            (Intercept) (Intercept)biorepD8_n3 (Intercept)biorepD8_n4
##           3.675785e-02          -2.694535e-01           5.394845e-02
## (Intercept)biorepD8_n5 (Intercept)biorepWT_n3 (Intercept)biorepWT_n4
##           8.643411e-02          -1.003137e-01          -3.294403e-03
## (Intercept)biorepWT_n5  (Intercept)genotypeD8  (Intercept)genotypeWT
##           2.326791e-01          -8.654787e-15           8.654787e-15
```

With our encoding we get an estimate for each genotype: (Intercept)genotypeD8 and (Intercept)genotypeWT.

Thus, we assess the contrast '(Intercept)genotypeD8 - (Intercept)genotypeWT = 0' with our statistical test. Note, that specifying this contrast will not work with the default makeContrast function due to the parameter names. So we therefore first

```
L <- makeContrast("genotypeD8 - genotypeWT=0", parameterNames = c("genotypeWT", "genotypeD8"))
```

Next, we change the rownames of the matrix L

```
rownames(L)<-paste0("(Intercept)",rownames(L))
L
```

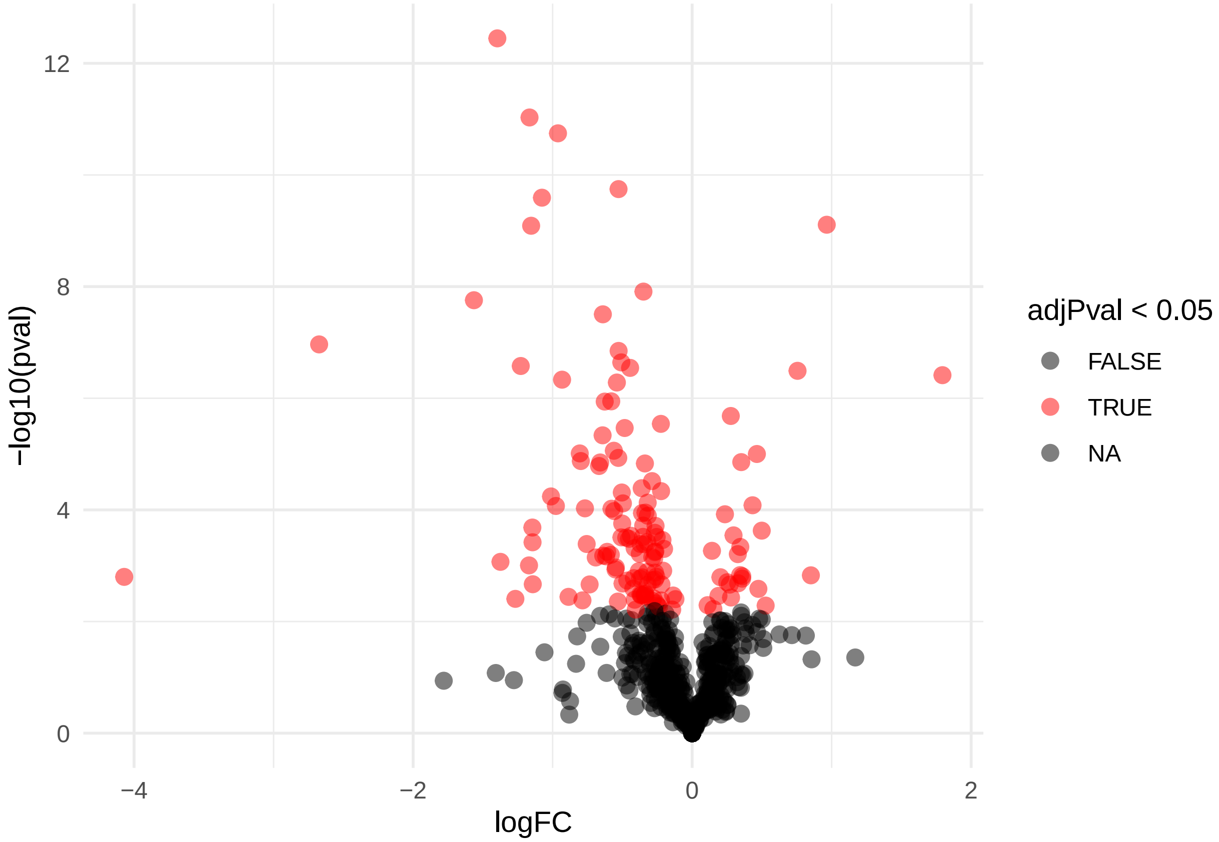
```
##                       genotypeD8 - genotypeWT
## (Intercept)genotypeWT                      -1
## (Intercept)genotypeD8                       1
```

```
pe <- hypothesisTest(object = pe, i = "protein", contrast = L)
```

4.3 Plots

4.3.1 Volcano-plot

```
volcano <- ggplot(rowData(pe[["protein"]])$`genotypeD8 - genotypeWT`,
                  aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) + theme_minimal()
volcano
```

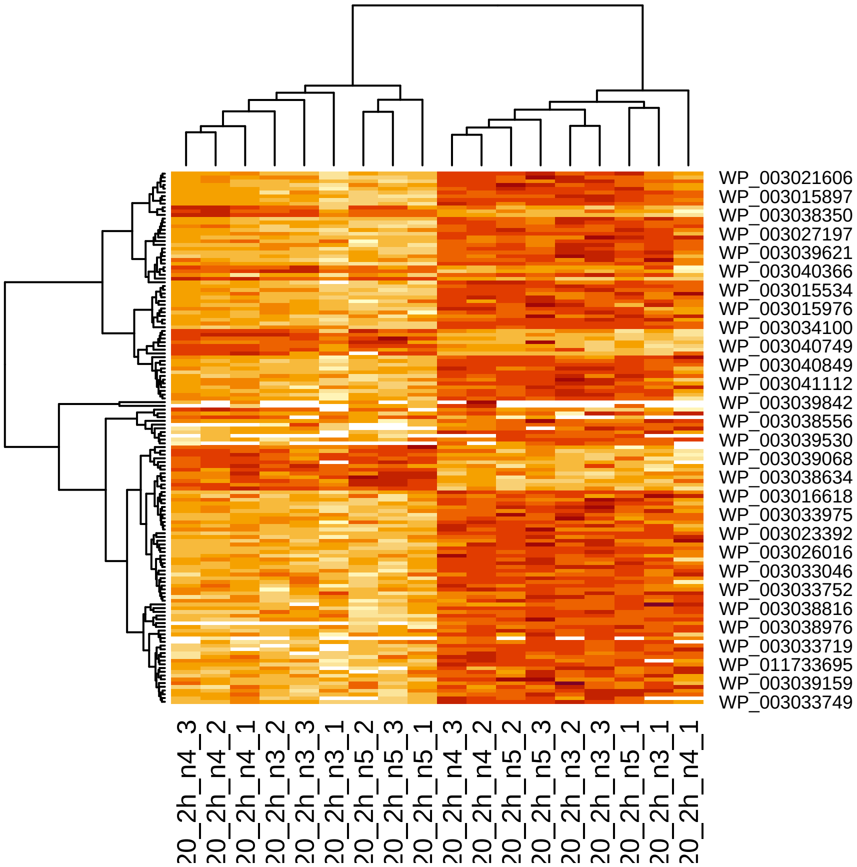


Note, that 142 proteins are found to be differentially abundant.

4.3.2 Heatmap

We first select the names of the proteins that were declared signficant.

```
sigNames <- rowData(pe[["protein"]])$`genotypeD8 - genotypeWT` %>%
  rownames_to_column("protein") %>%
  filter(adjPval<0.05) %>%
  pull(protein)
heatmap(assay(pe[["protein"]])[sigNames, ])
```



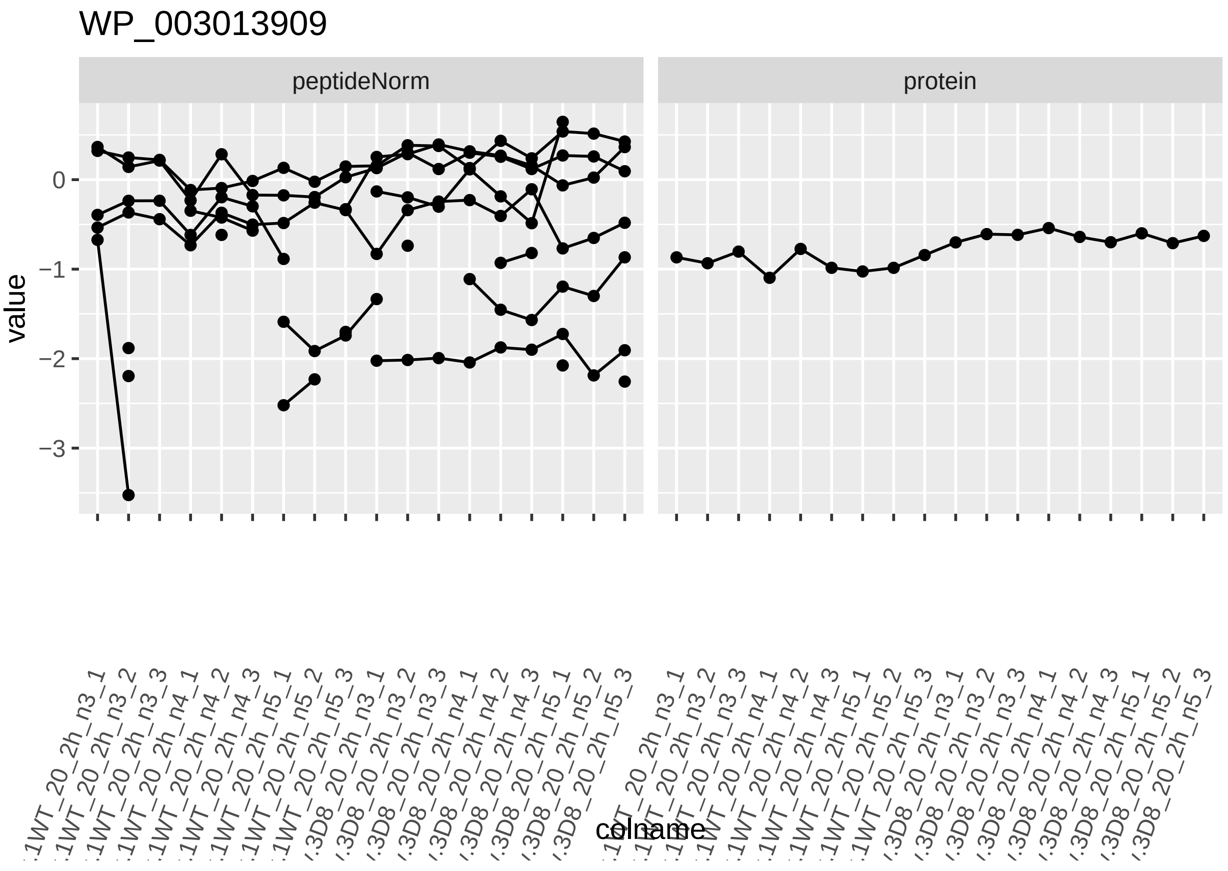
4.3.3 Detail plots

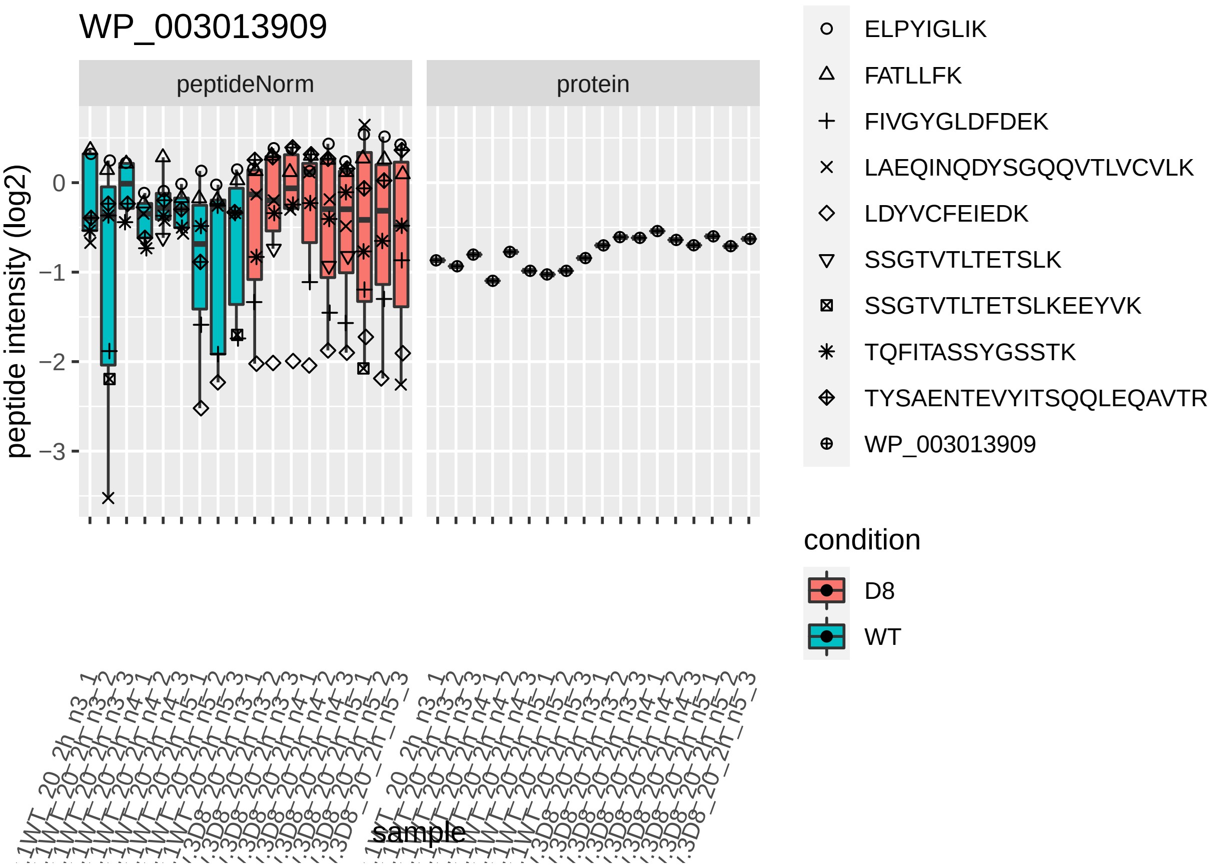
We will make detail plots for the first 10 DE proteins.

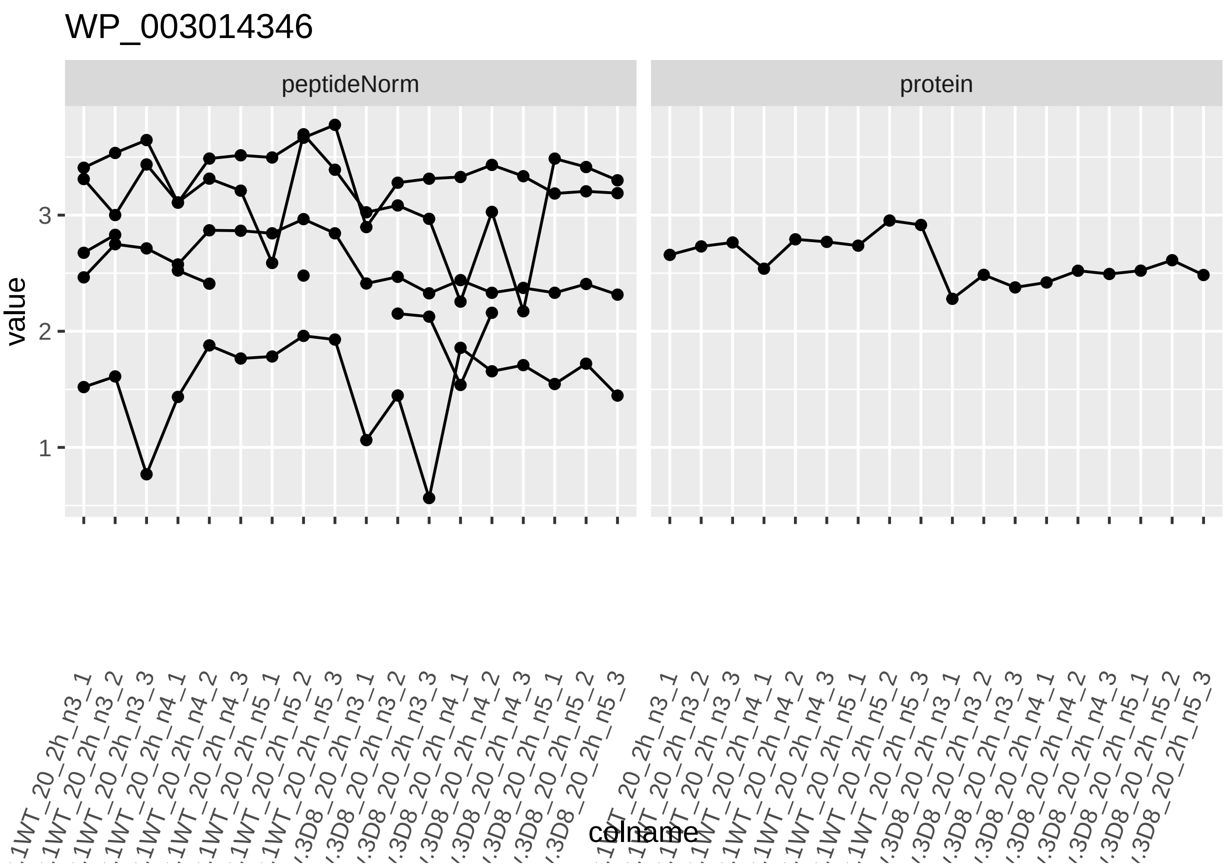
```
maxPlot <- 10
for (protName in sigNames[1:maxPlot])
{
pePlot <- pe[protName, , c("peptideNorm","protein")]
pePlotDf <- data.frame(longFormat(pePlot))
pePlotDf$assay <- factor(pePlotDf$assay,
                        levels = c("peptideNorm", "protein"))
pePlotDf$condition <- as.factor(colData(pePlot)[pePlotDf$colname, "genotype"])

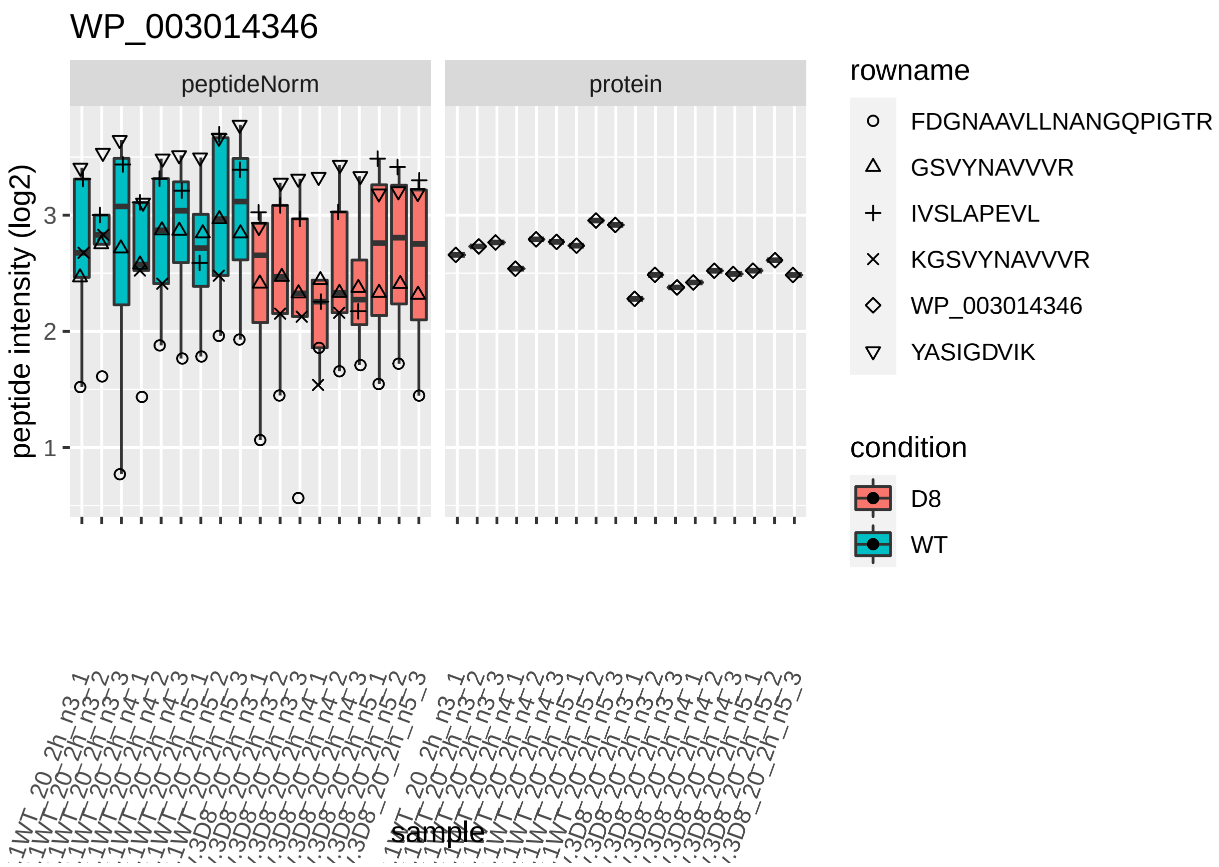
# plotting
p1 <- ggplot(data = pePlotDf,
       aes(x = colname, y = value, group = rowname)) +
    geom_line() +
    geom_point() +
    theme(axis.text.x = element_text(angle = 70, hjust = 1, vjust = 0.5)) +
    facet_grid(~assay) +
    ggtitle(protName)
print(p1)

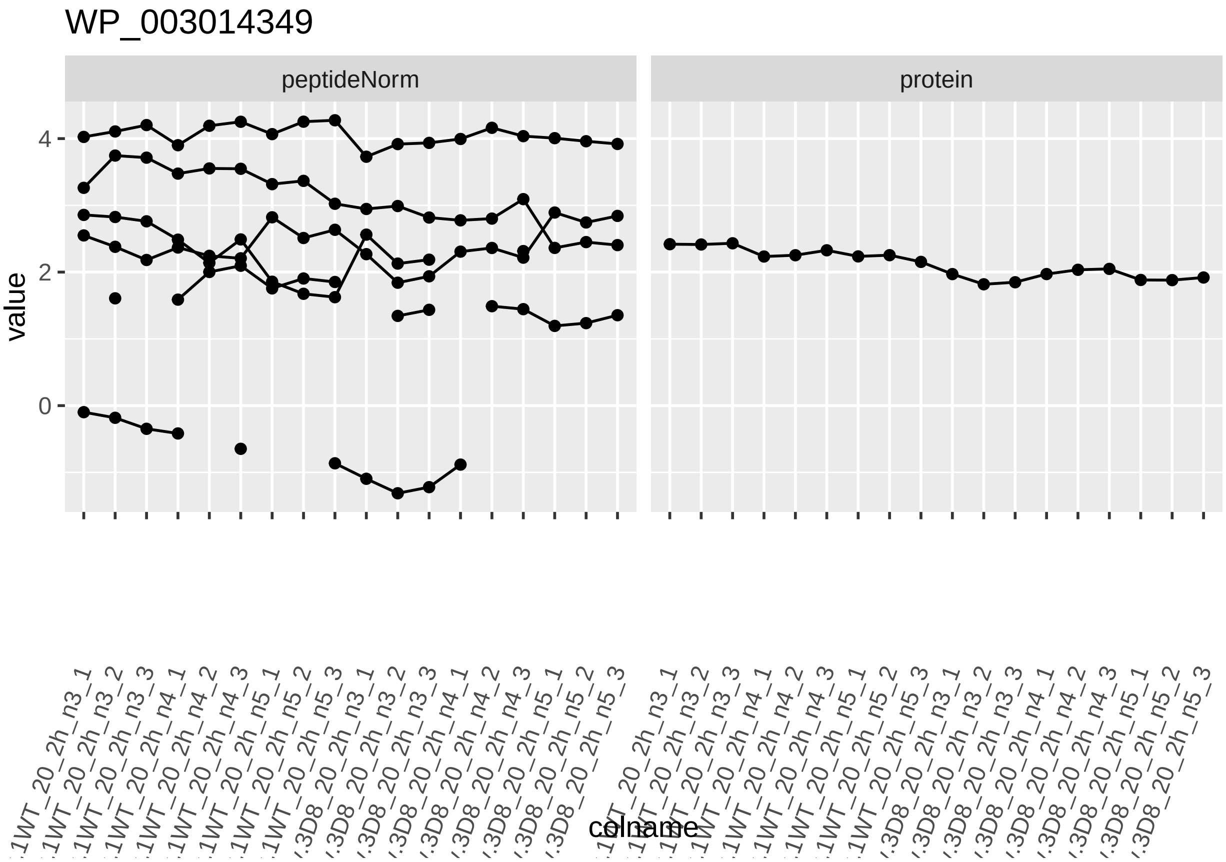
# plotting 2
p2 <- ggplot(pePlotDf, aes(x = colname, y = value, fill = condition)) +
  geom_boxplot(outlier.shape = NA) +
  geom_point(
    position = position_jitter(width = .1),
    aes(shape = rowname)) +
  scale_shape_manual(values = 1:nrow(pePlotDf)) +
  labs(title = protName, x = "sample", y = "peptide intensity (log2)") +
  theme(axis.text.x = element_text(angle = 70, hjust = 1, vjust = 0.5)) +
  facet_grid(~assay)
print(p2)
}
```

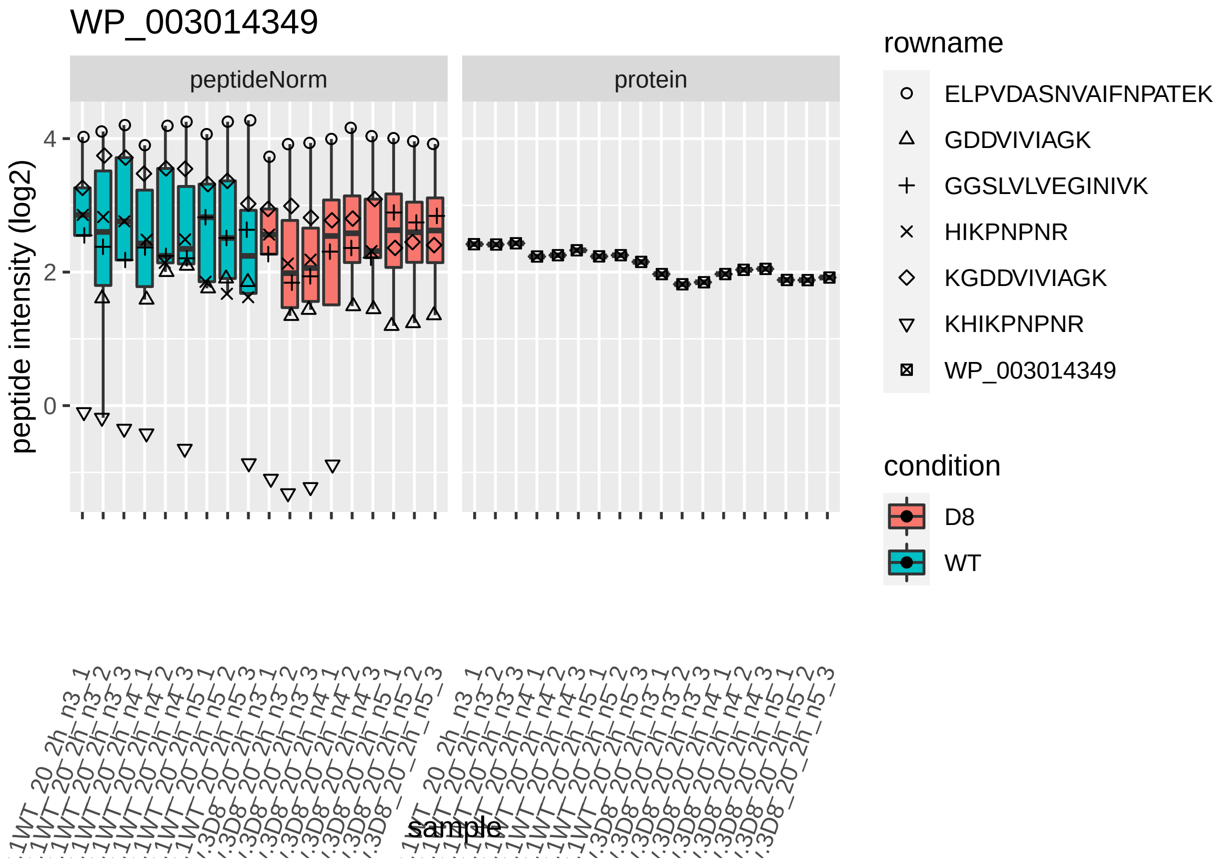


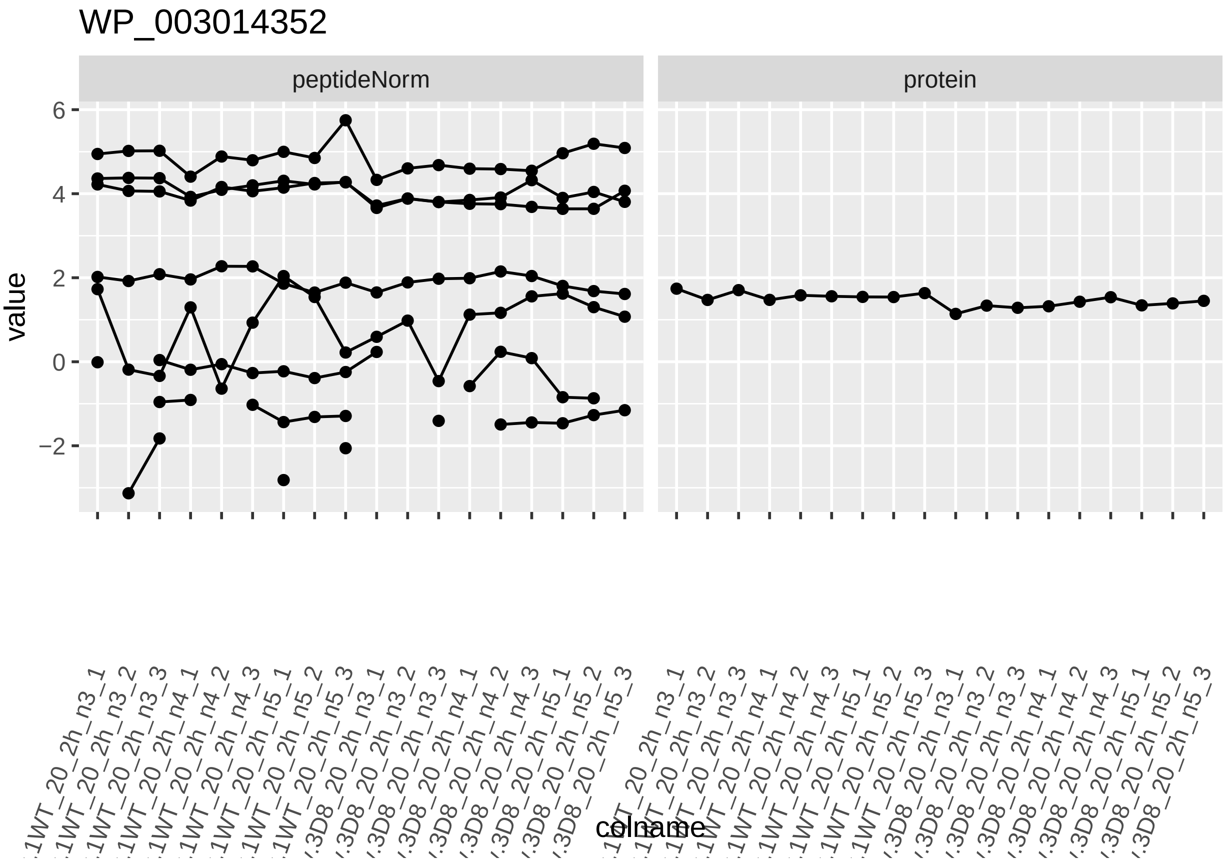

WP_003013909
peptideNorm
protein
peptide intensity (log2)
0
−1
−2
−3
sample
ELPYIGLIK
FATLLFK
FIVGYGLDFDEK
LAEQINQDYSGQQVTLVCVLK
LDYVCFEIEDK
SSGTVTLTETSLK
SSGTVTLTETSLKEEYVK
TQFITASSYGSSTK
TYSAENTEVYITSQQLEQAVTR
WP_003013909
condition
D8
WT

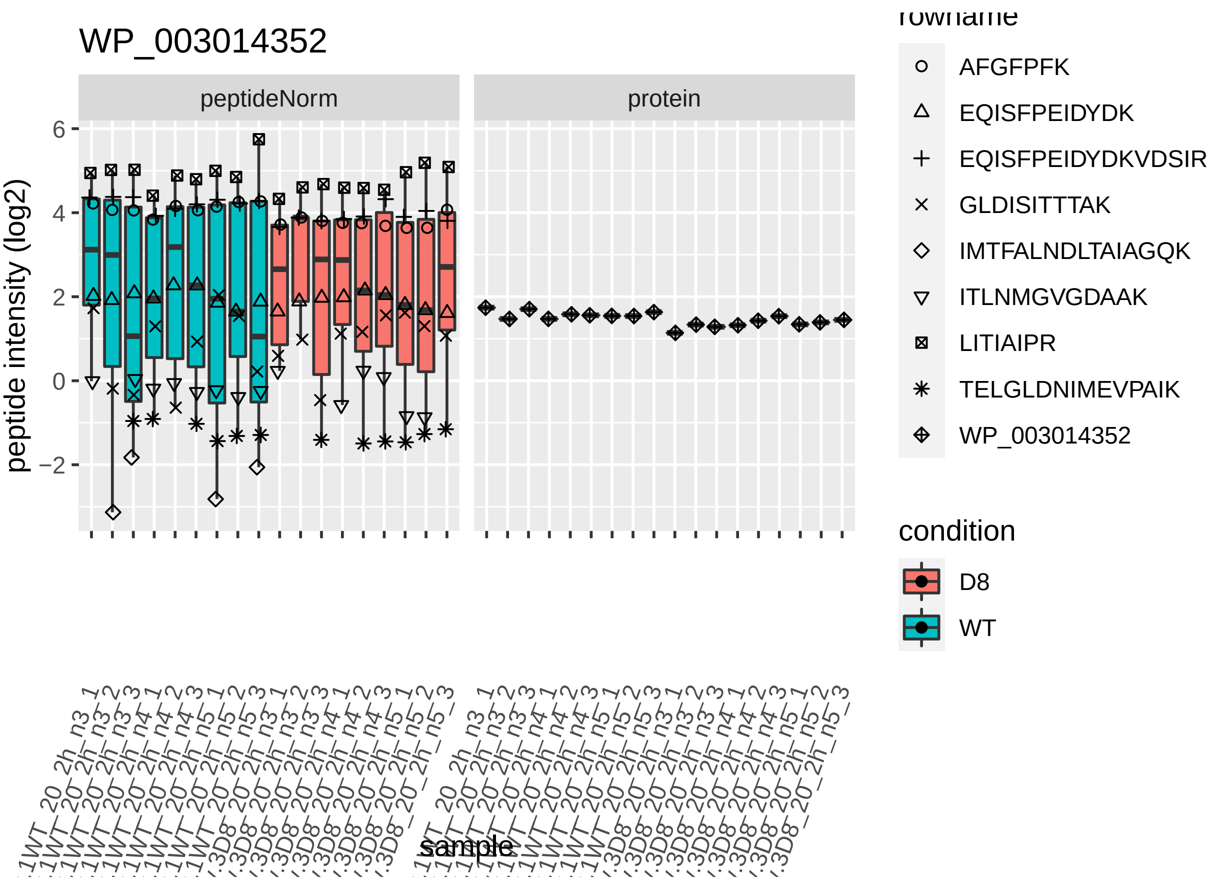

WP_003014346
peptideNorm
protein
value
3
2
1
colname
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3

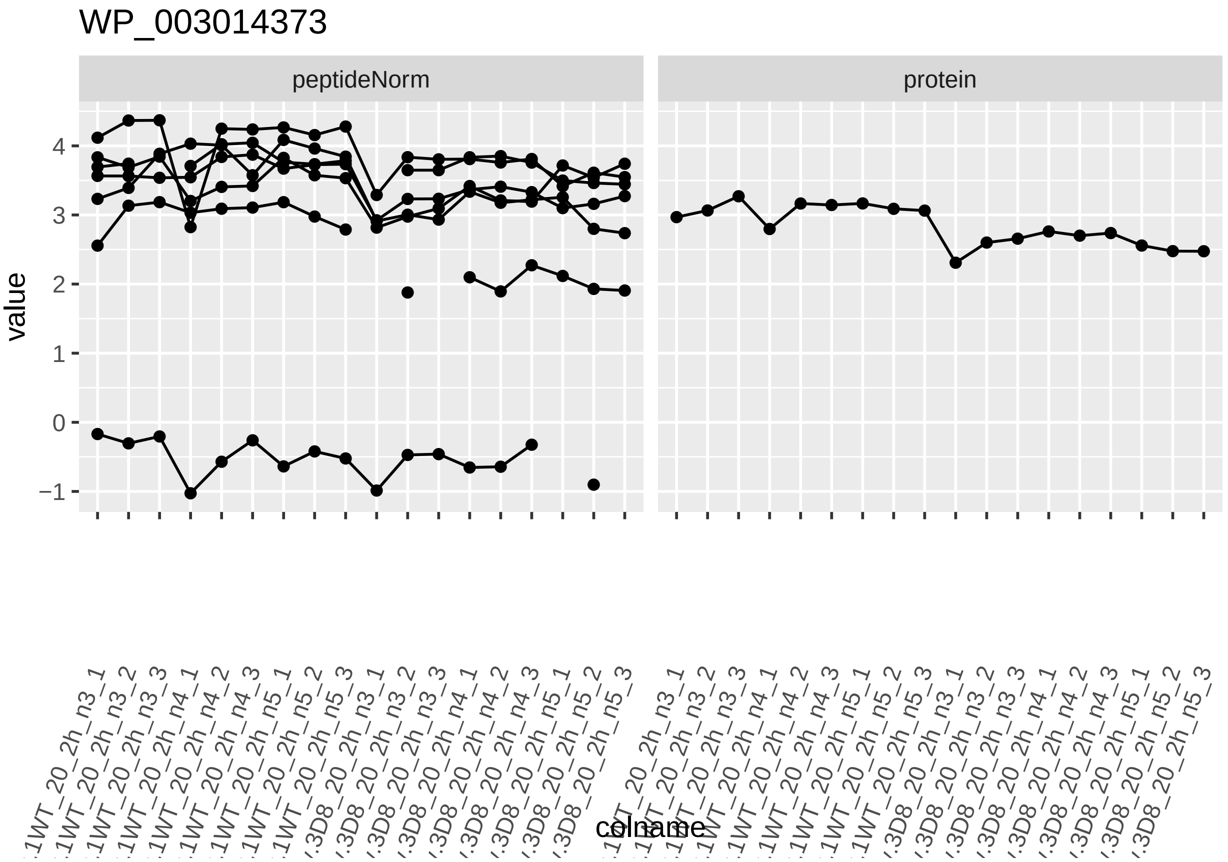

WP_003014346
peptideNorm
protein
peptide intensity (log2)
1
2
3
sample
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
rowname
○ FDGNAAVLLNANGQPIGTR
△ GSVYNAVVVR
+ IVSLAPEVL
× KGSVYNAVVVR
◇ WP_003014346
▽ YASIGDVIK
condition
🟥 D8
🟦 WT

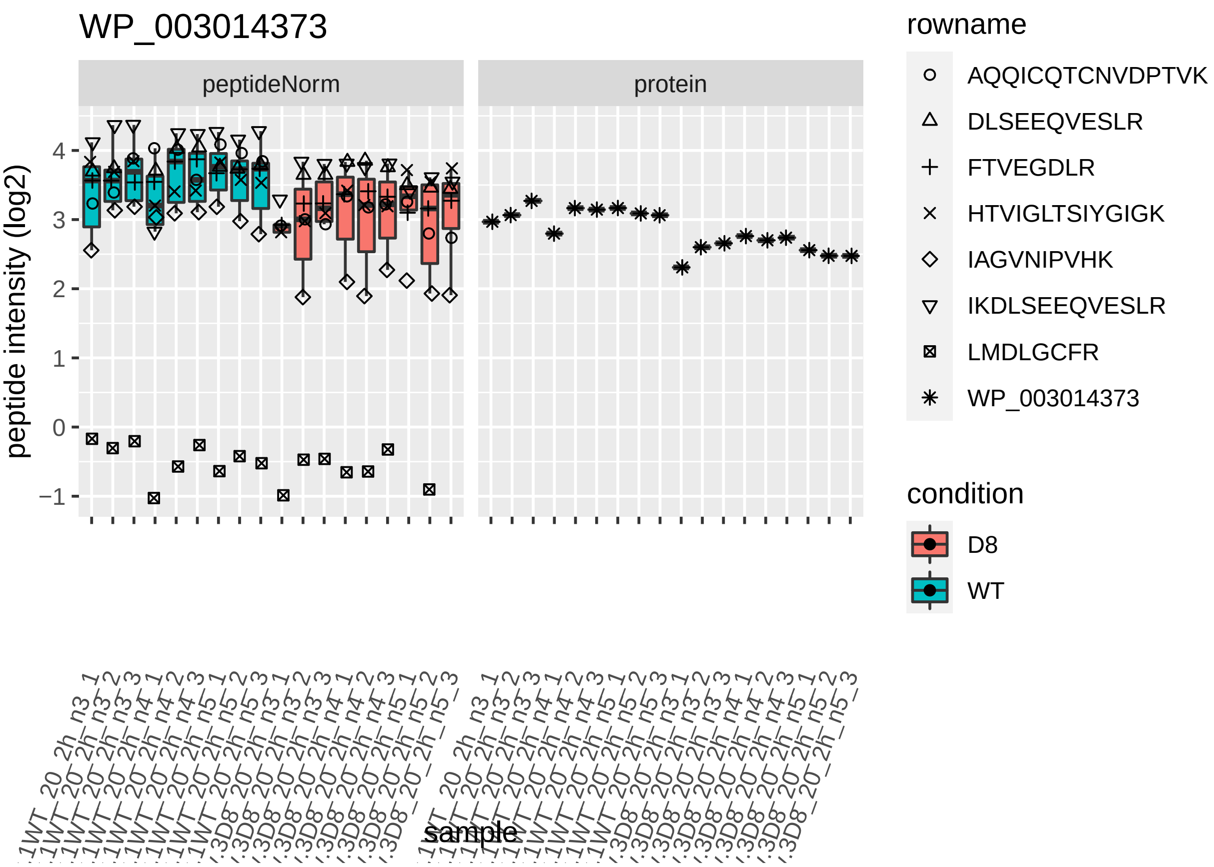

WP_003014349
peptideNorm
protein
value
4
2
0
colname
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
/.3D8_20_2h_n3_1
/.3D8_20_2h_n3_2
/.3D8_20_2h_n3_3
/.3D8_20_2h_n4_1
/.3D8_20_2h_n4_2
/.3D8_20_2h_n4_3
/.3D8_20_2h_n5_1
/.3D8_20_2h_n5_2
/.3D8_20_2h_n5_3

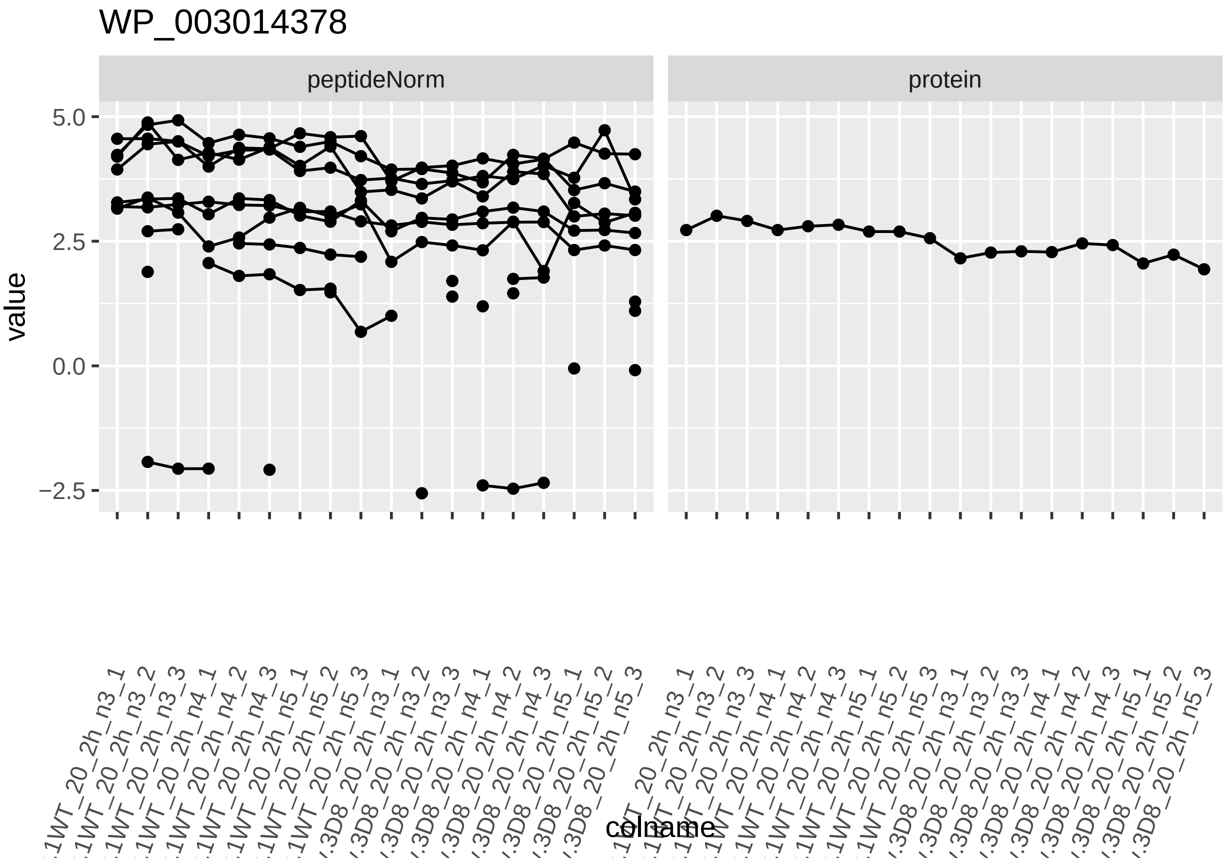

WP_003014349
peptideNorm
protein
peptide intensity (log2)
4
2
0
sample
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
rowname
○ ELPVDASNVAIFNPATEK
△ GDDVIVIAGK
+ GGSLVLVEGINIVK
× HIKPNPNR
◇ KGDDVIVIAGK
▽ KHIKPNPNR
⊠ WP_003014349
condition
🟥 D8
🟦 WT

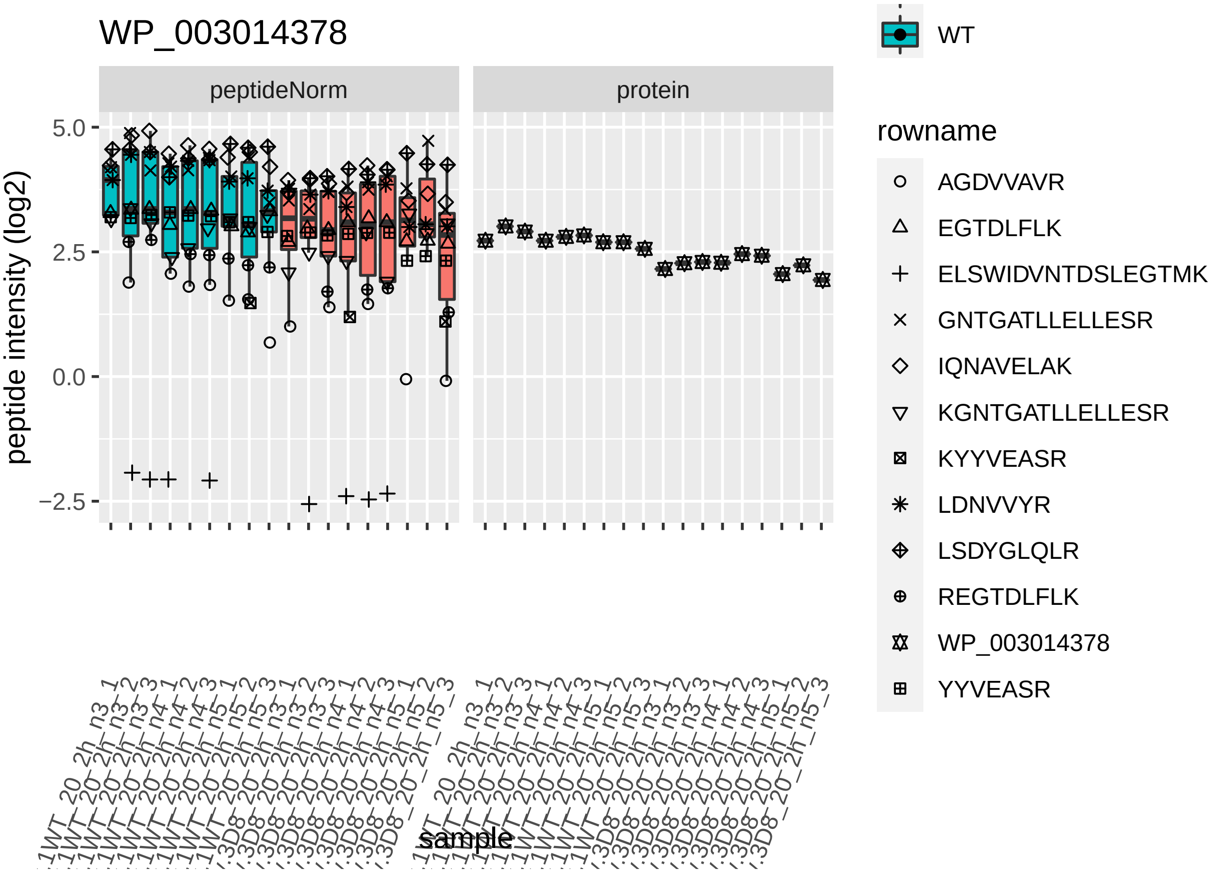

WP_003014352
peptideNorm
protein
value
6
4
2
0
−2
colname
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3

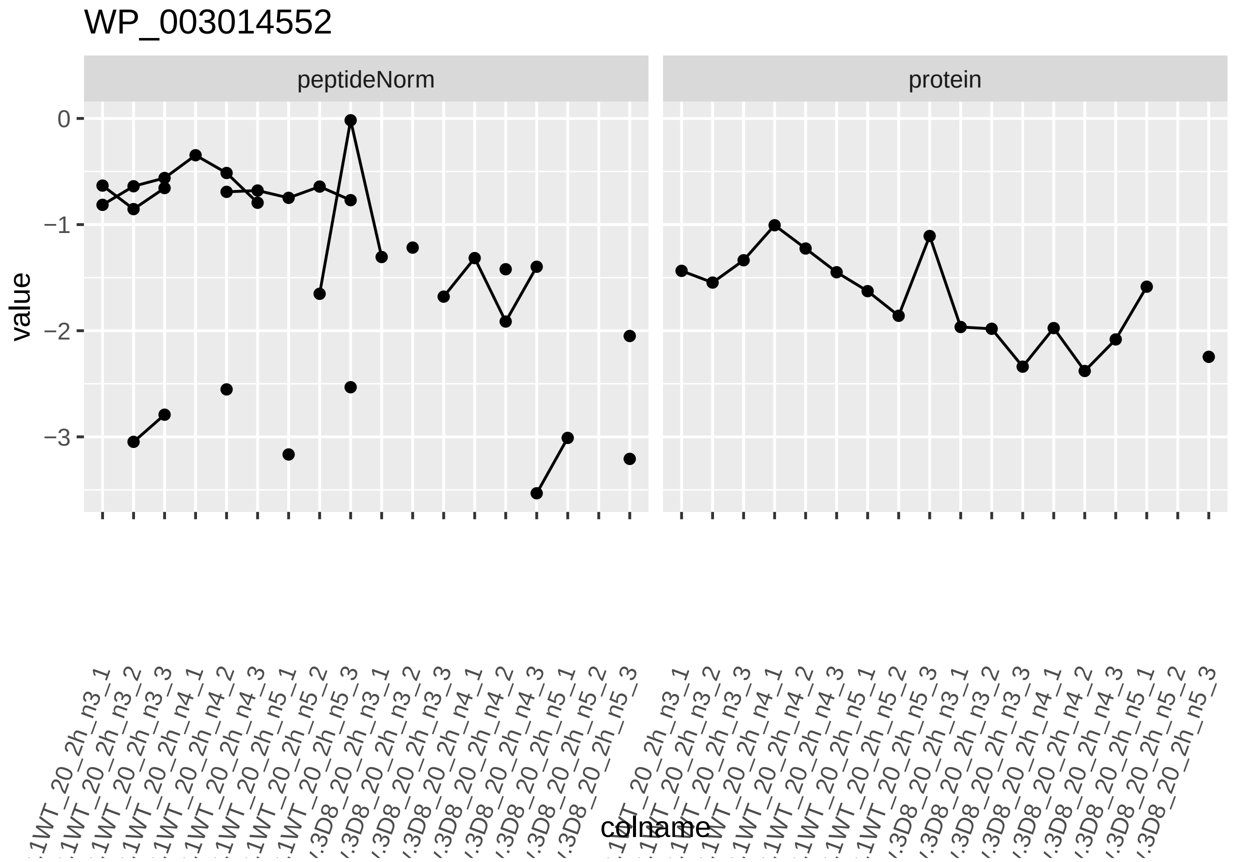

WP_003014352
peptideNorm
protein
peptide intensity (log2)
6
4
2
0
−2
sample
rowname
AFGFPFK
EQISFPEIDYDK
EQISFPEIDYDKVDSIR
GLDISITTTAK
IMTFALNDLTAIAGQK
ITLNMGVGDAAK
LITIAIPR
TELGLDNIMEVPAIK
WP_003014352
condition
D8
WT


WP_003014373
peptideNorm
protein
value
4
3
2
1
0
−1
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3
colname


WP_003014373
peptideNorm
protein
peptide intensity (log2)
4
3
2
1
0
−1
sample
rowname
○ AQQICQTCNVDPTVK
△ DLSEEQVESLR
+ FTVEGDLR
× HTVIGLTSIYGIGK
◇ IAGVNIPVHK
▽ IKDLSEEQVESLR
☒ LMDLGCFR
✳ WP_003014373
condition
🟥 D8
🟦 WT


WP_003014378
peptideNorm
protein
value
5.0
2.5
0.0
−2.5
colname
.1WT_20_2h_n3_1
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
/.3D8_20_2h_n3_1
/.3D8_20_2h_n3_2
/.3D8_20_2h_n3_3
/.3D8_20_2h_n4_1
/.3D8_20_2h_n4_2
/.3D8_20_2h_n4_3
/.3D8_20_2h_n5_1
/.3D8_20_2h_n5_2
/.3D8_20_2h_n5_3


WP_003014378
peptideNorm
protein
peptide intensity (log2)
5.0
2.5
0.0
−2.5
sample
WT
rowname
AGDVVAVR
EGTDLFLK
ELSWIDVNTDSLEGTMK
GNTGATLLELLESR
IQNAVELAK
KGNTGATLLELLESR
KYYVEASR
LDNVVYR
LSDYGLQLR
REGTDLFLK
WP_003014378
YYVEASR


WP_003014552
peptideNorm
protein
value
0
−1
−2
−3
colname
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
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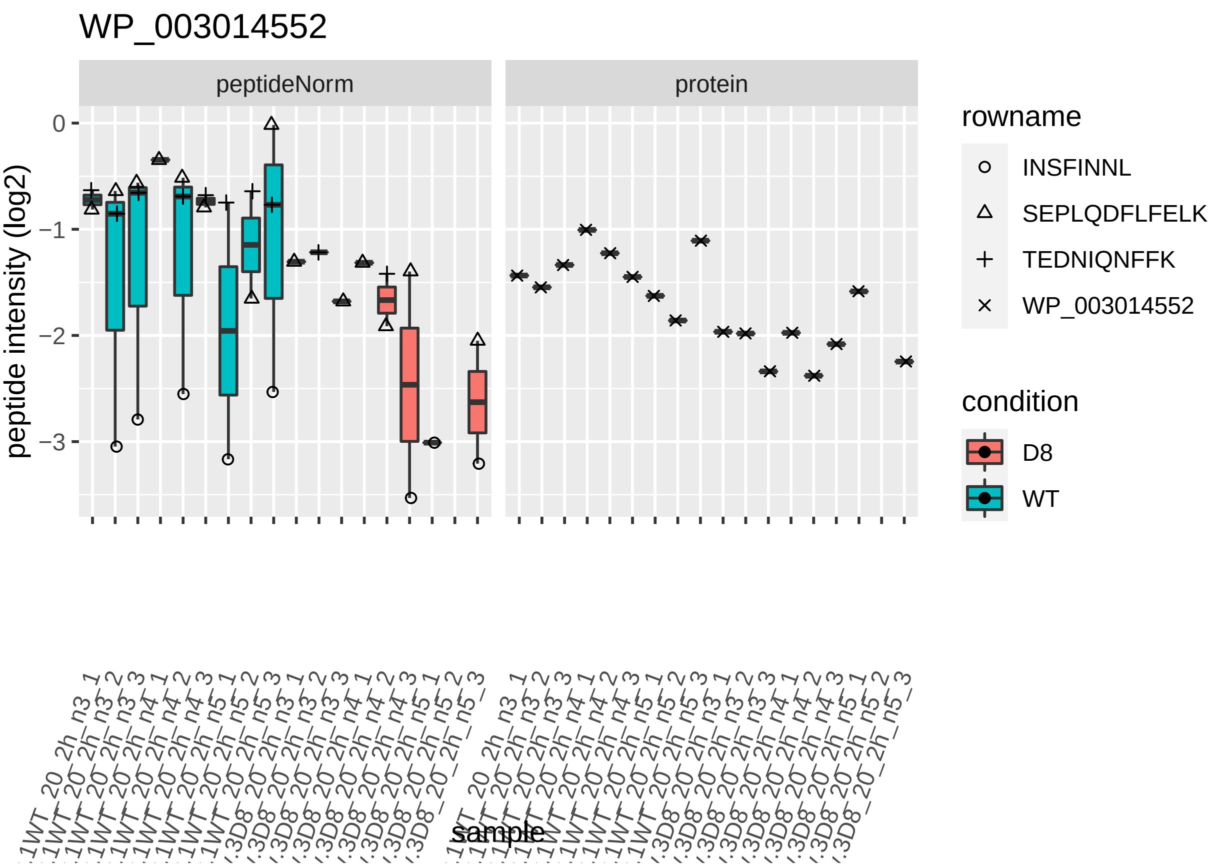
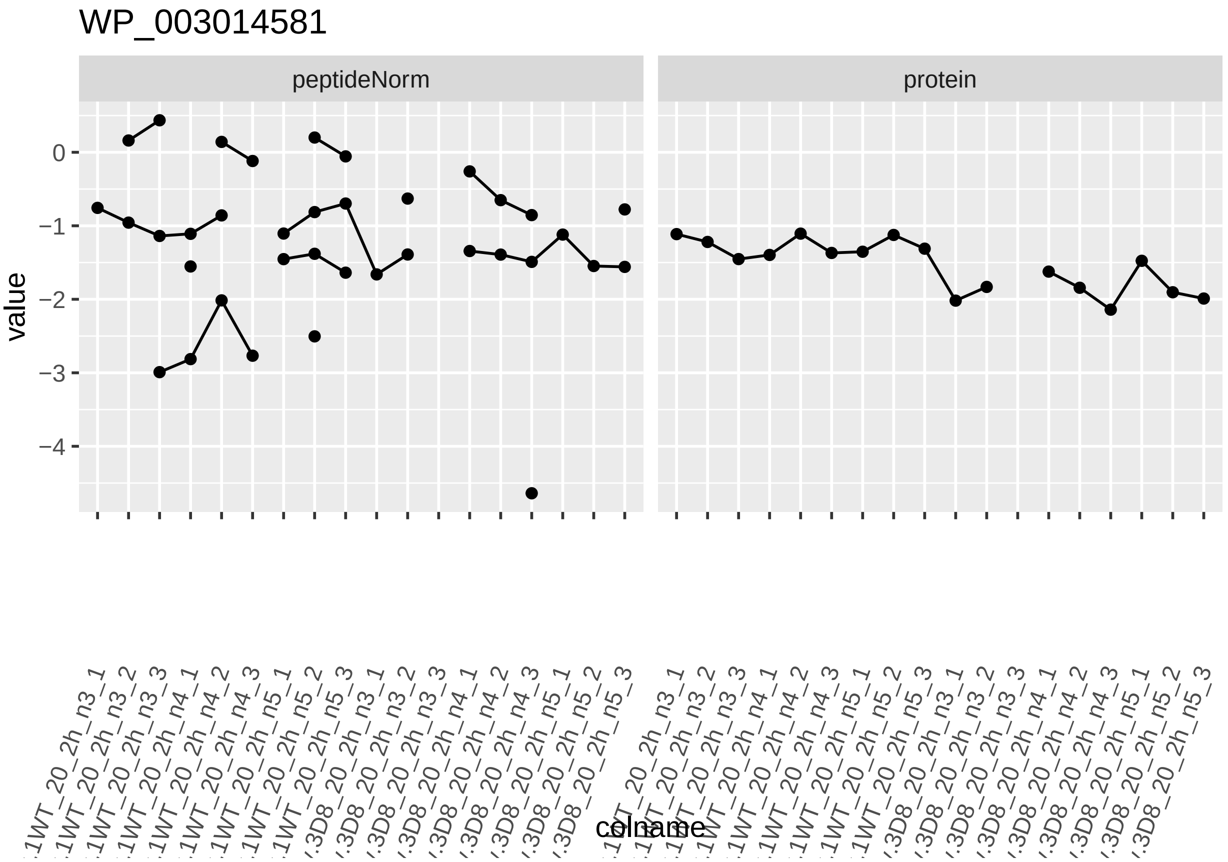
3D8_20_2h_n5_2
3D8_20_2h_n5_3


WP_003014552
peptideNorm
protein
peptide intensity (log2)
0
−1
−2
−3
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
sample
rowname
○ INSFINNL
△ SEPLQDFLFELK
+ TEDNIQNFFK
× WP_003014552
condition
🟥 D8
🟦 WT


WP_003014581
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protein
value
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−1
−2
−3
−4
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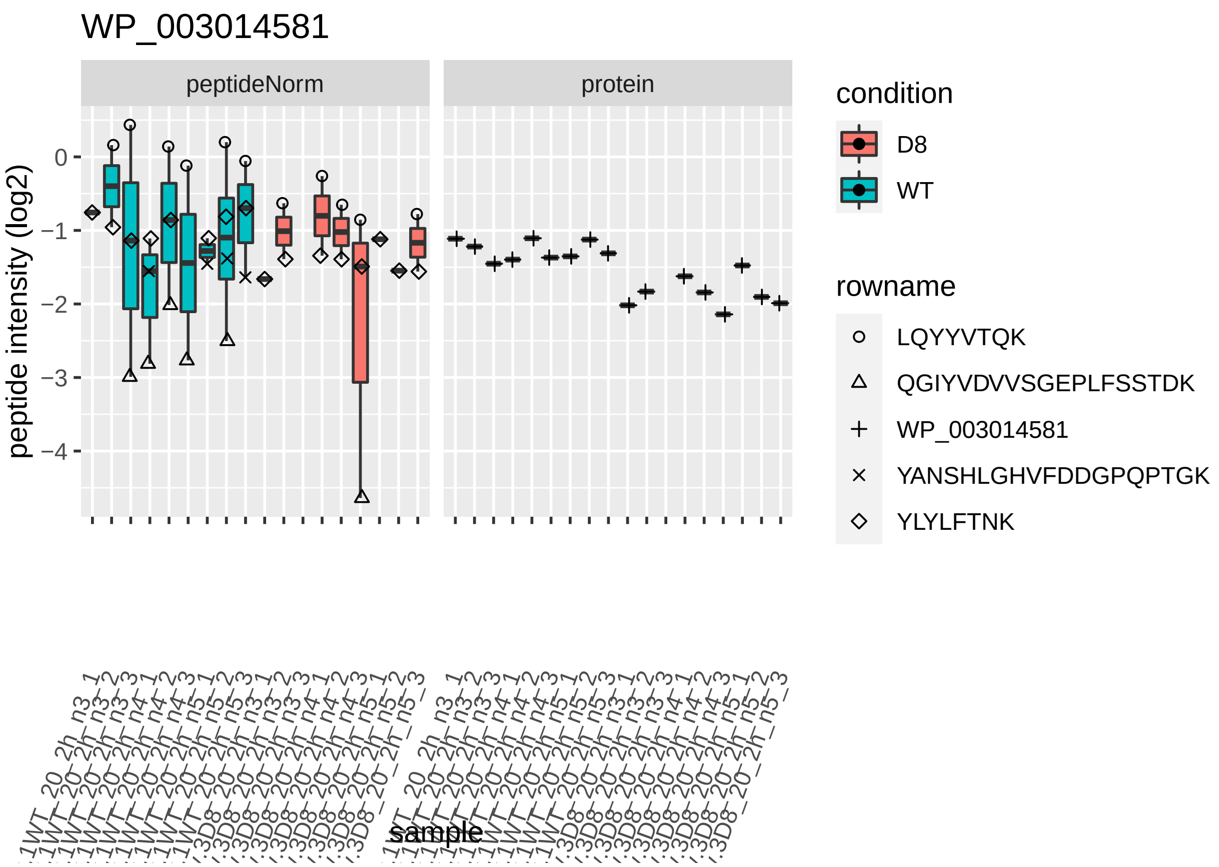
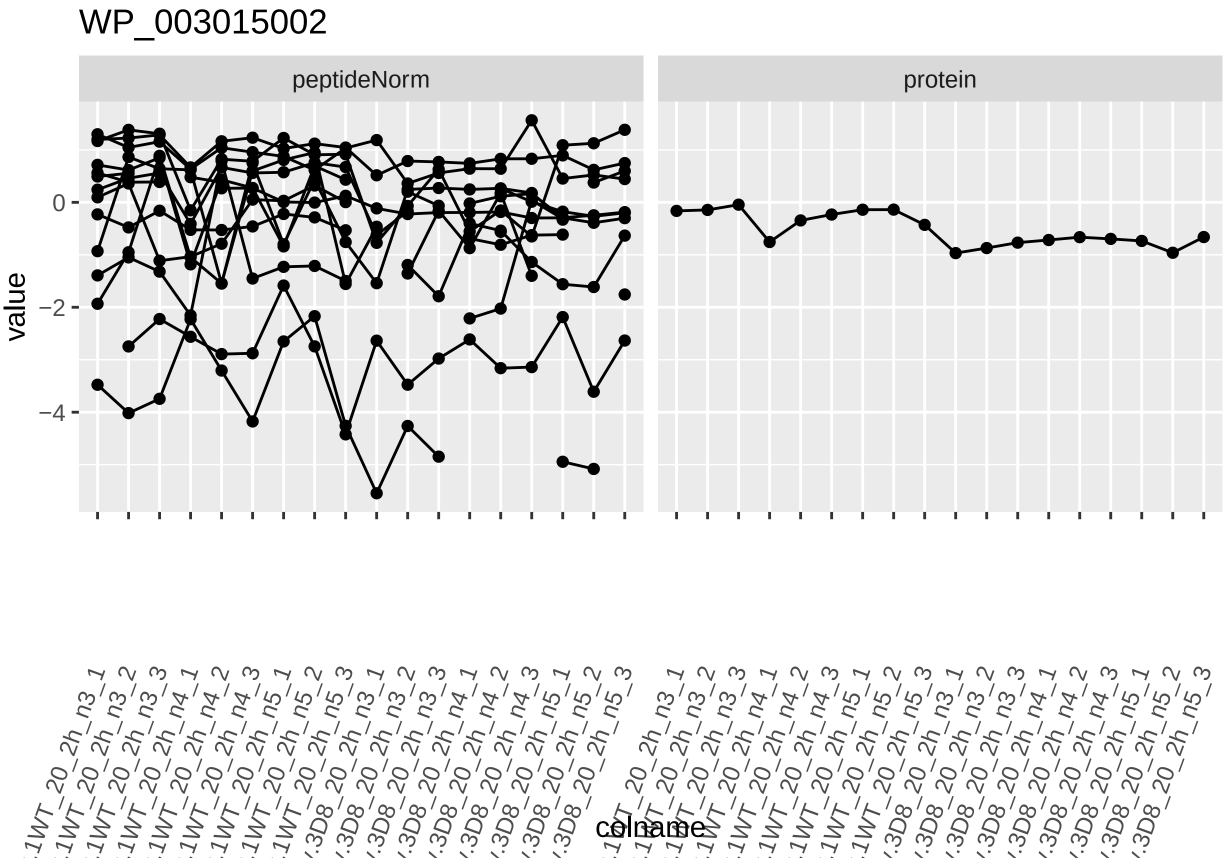
.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
.3D8_20_2h_n5_1
.3D8_20_2h_n5_2
.3D8_20_2h_n5_3
colname


WP_003014581
peptideNorm
protein
peptide intensity (log2)
0
−1
−2
−3
−4
sample
condition
D8
WT
rowname
LQYYVTQK
QGIYVDVVSGEPLFSSTDK
WP_003014581
YANSHLGHVFDDGPQPTGK
YLYLFTNK


WP_003015002
peptideNorm
protein
value
0
−2
−4
colname
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.1WT_20_2h_n3_2
.1WT_20_2h_n3_3
.1WT_20_2h_n4_1
.1WT_20_2h_n4_2
.1WT_20_2h_n4_3
.1WT_20_2h_n5_1
.1WT_20_2h_n5_2
.1WT_20_2h_n5_3
.3D8_20_2h_n3_1
.3D8_20_2h_n3_2
.3D8_20_2h_n3_3
.3D8_20_2h_n4_1
.3D8_20_2h_n4_2
.3D8_20_2h_n4_3
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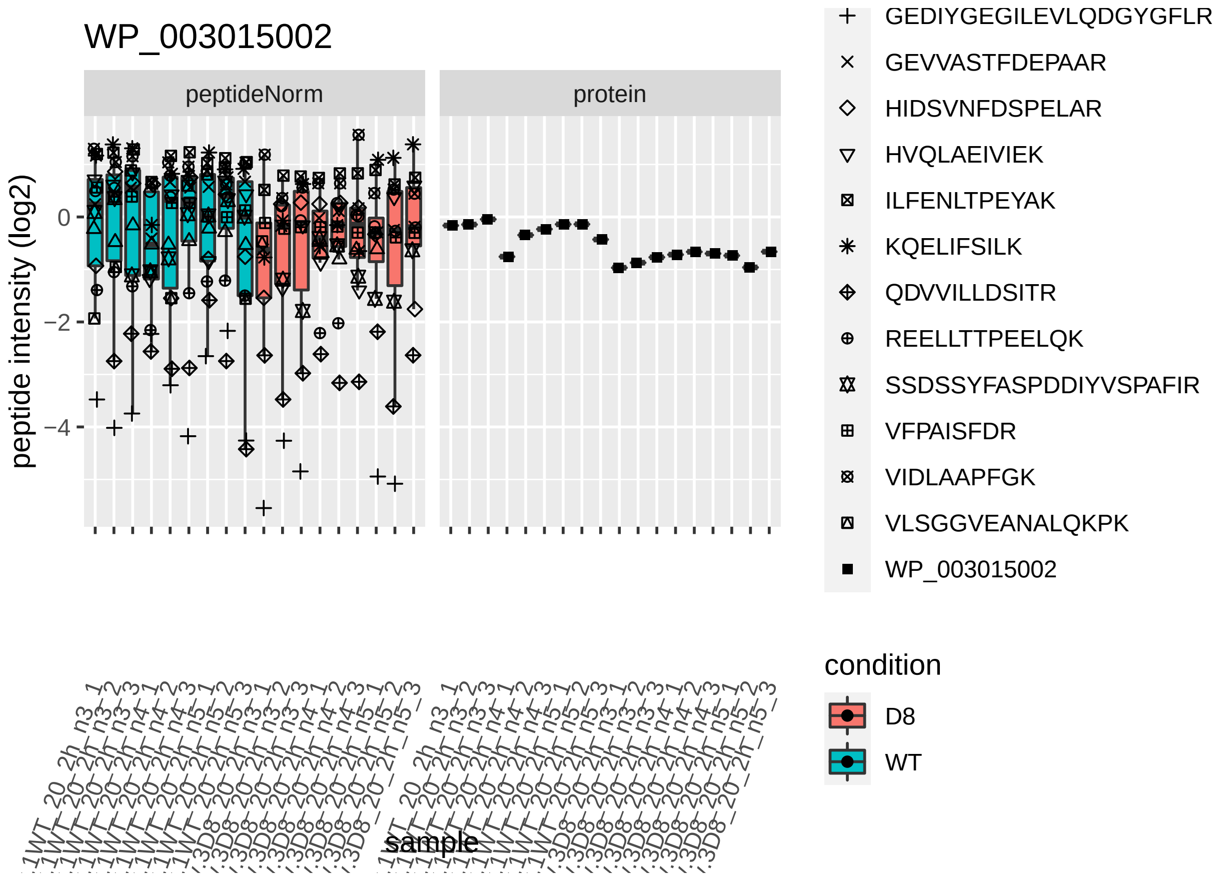
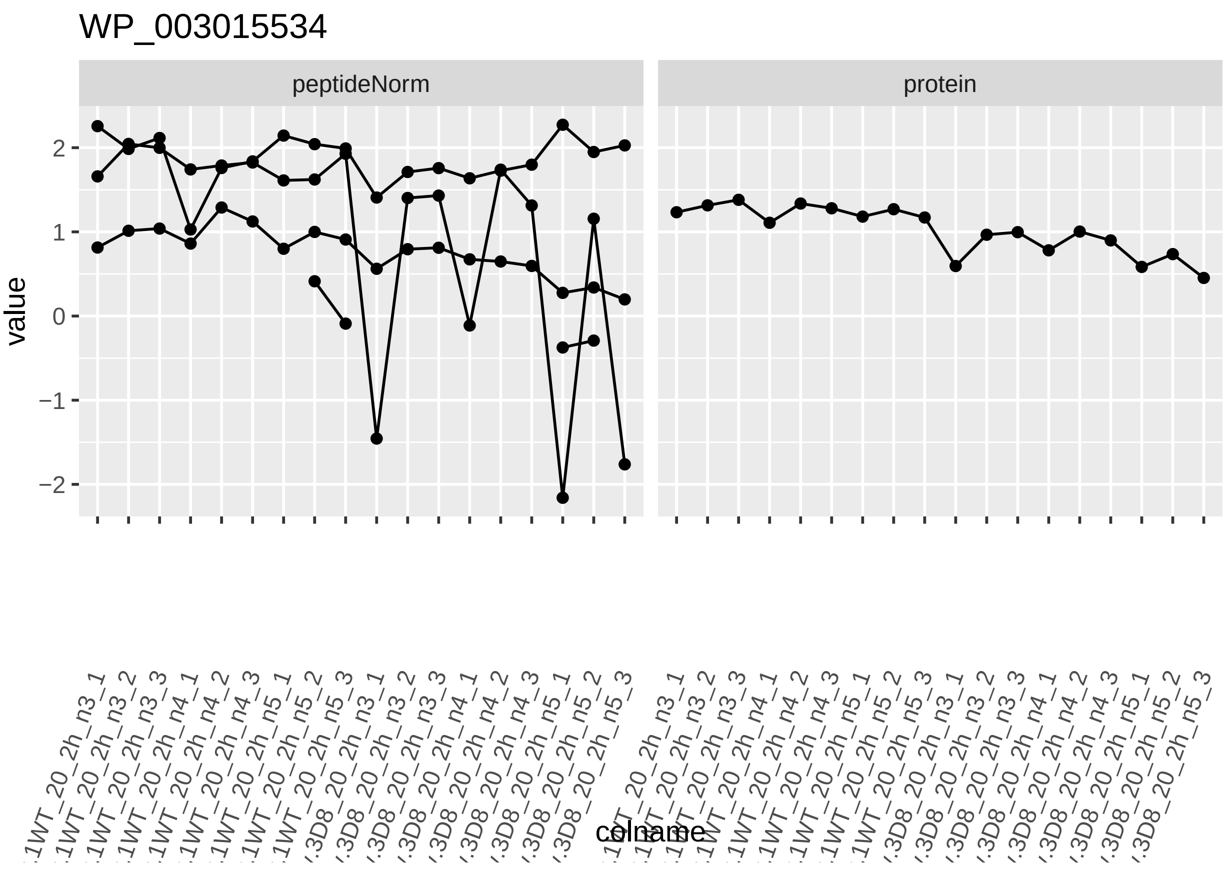
.3D8_20_2h_n5_3


WP_003015002
peptideNorm
protein
peptide intensity (log2)
0
−2
−4
sample
1WT_20_2h_n3_1
1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
3D8_20_2h_n5_1
3D8_20_2h_n5_2
3D8_20_2h_n5_3
GEDIYGEGILEVLQDGYGFLR
GEVVASTFDEPAAR
HIDSVNFDSPELAR
HVQLAEIVIEK
ILFENLTPEYAK
KQELIFSILK
QDVVILLDSITR
REELLTTPEELQK
SSDSSYFASPDDIYVSPAFIR
VFPAISFDR
VIDLAAPFGK
VLSGGVEANALQKPK
WP_003015002
condition
D8
WT


WP_003015534
peptideNorm
protein
value
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0
−1
−2
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1WT_20_2h_n3_2
1WT_20_2h_n3_3
1WT_20_2h_n4_1
1WT_20_2h_n4_2
1WT_20_2h_n4_3
1WT_20_2h_n5_1
1WT_20_2h_n5_2
1WT_20_2h_n5_3
3D8_20_2h_n3_1
3D8_20_2h_n3_2
3D8_20_2h_n3_3
3D8_20_2h_n4_1
3D8_20_2h_n4_2
3D8_20_2h_n4_3
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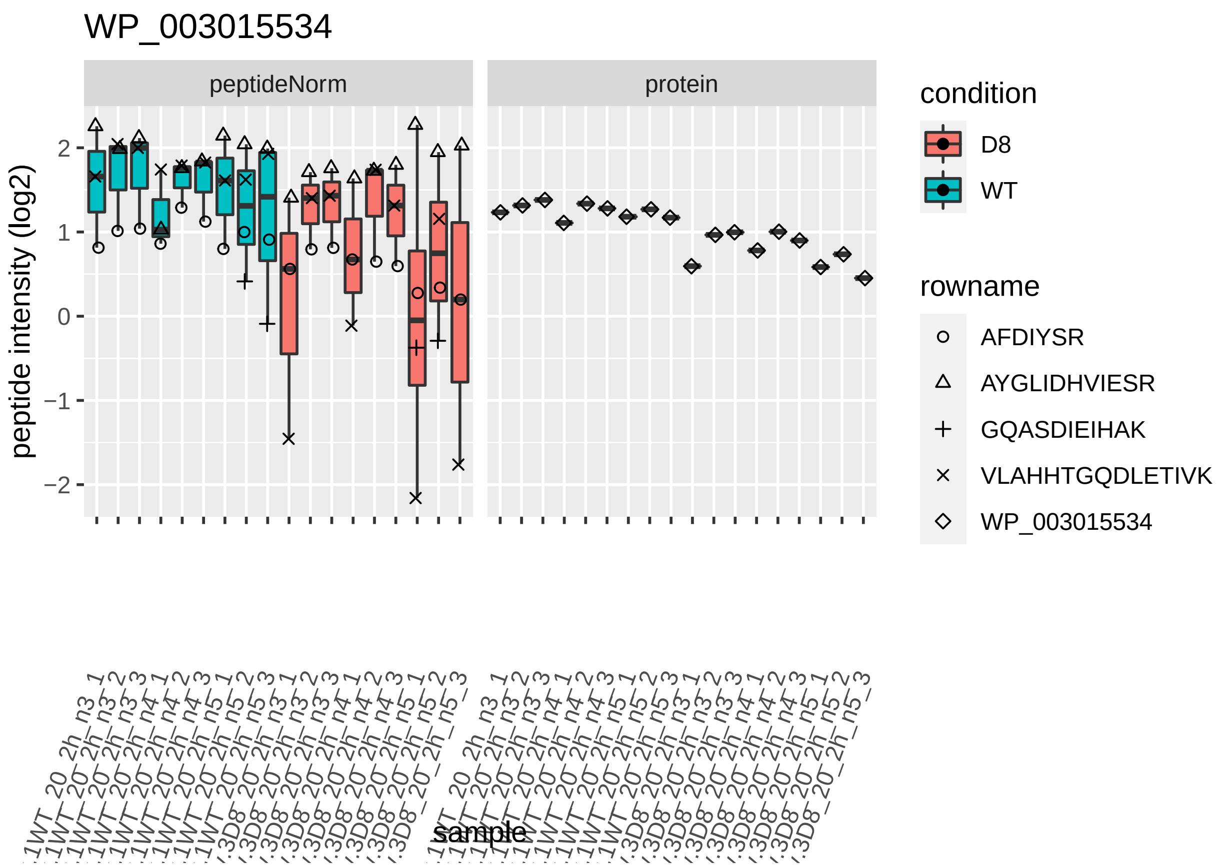
3D8_20_2h_n5_2
3D8_20_2h_n5_3
colname



Note, that the yeast protein is only covered by 3 peptides. Only one peptide is picked up in condition A. This peptide is also only once observed in spike-in condition B. This puts a considerable burden upon the inference and could be avoided by more stringent filtering.

5 Session Info

With respect to reproducibility, it is highly recommended to include a session info in your script so that readers of your output can see your particular setup of R.

```r
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## 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] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] plotly_4.10.0               msqrob2_1.2.0
##  [3] QFeatures_1.4.0             MultiAssayExperiment_1.20.0
##  [5] SummarizedExperiment_1.24.0 Biobase_2.54.0
##  [7] GenomicRanges_1.46.1        GenomeInfoDb_1.30.0
##  [9] IRanges_2.28.0              S4Vectors_0.32.3
## [11] BiocGenerics_0.40.0         MatrixGenerics_1.6.0
## [13] matrixStats_0.61.0          limma_3.50.0
## [15] forcats_0.5.1               stringr_1.4.0
## [17] dplyr_1.0.7                 purrr_0.3.4
## [19] readr_2.1.1                 tidyr_1.1.4
## [21] tibble_3.1.6                ggplot2_3.3.5
## [23] tidyverse_1.3.1
##
## loaded via a namespace (and not attached):
##  [1] nlme_3.1-153           ProtGenerics_1.26.0     bitops_1.0-7
##  [4] fs_1.5.2               lubridate_1.8.0         httr_1.4.2
##  [7] tools_4.1.2            backports_1.4.1         utf8_1.2.2
## [10] R6_2.5.1               DBI_1.1.2               lazyeval_0.2.2
## [13] colorspace_2.0-2       withr_2.4.3             tidyselect_1.1.1
## [16] compiler_4.1.2         cli_3.1.0               rvest_1.0.2
## [19] xml2_1.3.3             DelayedArray_0.20.0     labeling_0.4.2
## [22] scales_1.1.1           digest_0.6.29           minqa_1.2.4
## [25] rmarkdown_2.11         XVector_0.34.0          pkgconfig_2.0.3
## [28] htmltools_0.5.2        lme4_1.1-27.1           highr_0.9
## [31] dbplyr_2.1.1           fastmap_1.1.0           htmlwidgets_1.5.4
## [34] rlang_0.4.12           readxl_1.3.1            rstudioapi_0.13
## [37] farver_2.1.0           jquerylib_0.1.4         generics_0.1.1
## [40] jsonlite_1.7.3         BiocParallel_1.28.3     RCurl_1.98-1.5
## [43] magrittr_2.0.1         GenomeInfoDbData_1.2.7  Matrix_1.3-4
## [46] Rcpp_1.0.8             munsell_0.5.0           fansi_1.0.2
## [49] MsCoreUtils_1.6.0      lifecycle_1.0.1         stringi_1.7.6
## [52] yaml_2.2.1             MASS_7.3-54             zlibbioc_1.40.0
## [55] grid_4.1.2             parallel_4.1.2          crayon_1.4.2
## [58] lattice_0.20-45        splines_4.1.2           haven_2.4.3
## [61] hms_1.1.1              knitr_1.37              pillar_1.6.4
## [64] igraph_1.2.11          boot_1.3-28             codetools_0.2-18
## [67] reprex_2.0.1           glue_1.6.0              evaluate_0.14
## [70] data.table_1.14.2      modelr_0.1.8            nloptr_1.2.2.3
## [73] vctrs_0.3.8            tzdb_0.2.0              cellranger_1.1.0
## [76] gtable_0.3.0           clue_0.3-60             assertthat_0.2.1
## [79] xfun_0.29              broom_0.7.11            AnnotationFilter_1.18.0
## [82] viridisLite_0.4.0      cluster_2.1.2           ellipsis_0.3.2
```

References

Goeminne, L. J., K. Gevaert, and L. Clement. 2016. “Peptide-level Robust Ridge Regression Improves Estimation, Sensitivity, and Specificity in Data-dependent Quantitative Label-free Shotgun Proteomics.” *Mol Cell Proteomics* 15 (2): 657–68.

Sticker, A., L. Goeminne, L. Martens, and L. Clement. 2020. “Robust Summarization and Inference in

Proteome-wide Label-free Quantification." *Mol Cell Proteomics* 19 (7): 1209–19.