irCLIP-RNP dataset to compare noUV vs UVC samples from two gel sections ranging from 30-70kDa and 70-350kDa

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This is the pipeline used to analyze the HNRNPC irCLIP-RNP datasets of 4 noUV and 4 UVC samples. We have subjected to MS two gel sections ranging from 30 to 70kDa (named "free RNA ligation zone") and from 70 to 350KDa (named "whole RNP zone"). The experiment was performed in 4 independent replicates in HEK293T cells.

1. Prepare the dataset

```
# Load the libraries
library(formatR)
library(DEP2)
library(tidyverse)
library(ggpubr)
library(Clipper)
library(viridis)
library(patchwork)
library(hrbrthemes)
library(igraph)
library(ggraph)
library(colormap)
library(UpSetR)
library(ggplot2)
library(arcdiagram)
library(pheatmap)
library(grid)
library(DESeq2)
library(corrplot)
library(psych)
library(paletteer)
library(data.table)
```

In the first step, we prepared the dataset to create a SummarizedExperiment object starting from the proteinGroups.txt output file from MaxQuant.

```
# Load data
data <- read.csv("~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC
    /0_Data/proteinGroups.txt",
    sep = "\t")

# Remove RPL proteins
data <- data[-grep("RPL", data$Gene.names), ]

# Generate unique names and ids
unique_pg <- make_unique(data, name = "Gene.names", ids = "Protein.IDs")
unique_pg <- unique_pg %>%
```

```
    arrange(name)

# Get the LFQ columns
ecols <- grep("LFQ.intensity.", colnames(unique_pg))

# Keep isoform with higher LFQ intensity
iso <- grep("\\.\\d+$", unique_pg$name)
rbp <- gsub("\\.1", "", c(unique_pg$name[iso]))

# Find original row name of the isoform with higher
# intensity
find_max_value <- function(rbp) {
    filtered_df <- unique_pg[unique_pg$name %like% rbp, grep("LFQ.intensity.",
        colnames(unique_pg))]
    filtered_df$rowSums <- rowSums(filtered_df[, grep("LFQ.intensity.",
        colnames(filtered_df))])
    max_value <- which.max(filtered_df$rowSums)
    rownames <- rownames(filtered_df)[-max_value]
    return(rownames)
}
max_iso <- c(unlist(lapply(rbp, find_max_value)))

# Remove low intensity isoforms
unique_pg <- unique_pg[!(rownames(unique_pg) %in% max_iso), ]

head(unique_pg, n = 2)
```

```
##       Protein.IDs Majority.protein.IDs Peptide.counts..all.
## 1 Q8NE71-2;Q8NE71      Q8NE71-2;Q8NE71               10;10
## 2 Q9UG63;Q9UG63-2      Q9UG63;Q9UG63-2                 3;3
##   Peptide.counts..razor.unique. Peptide.counts..unique.
## 1                         10;10                   10;10
## 2                           3;3                     3;3
##                            Protein.names Gene.names
## 1 ATP-binding cassette sub-family F member 1      ABCF1
## 2 ATP-binding cassette sub-family F member 2      ABCF2
##

                          Fasta.headers
## 1 sp|Q8NE71-2|ABCF1_HUMAN Isoform 2 of ATP-binding cassette sub-family F member 1 OS=Homo
    sapiens OX=9606 GN=ABCF1;sp|Q8NE71|ABCF1_HUMAN ATP-binding cassette sub-family F member 1 OS=
    Homo sapiens OX=9606 GN=ABCF1 PE=1 SV=2
## 2 sp|Q9UG63|ABCF2_HUMAN ATP-binding cassette sub-family F member 2 OS=Homo sapiens OX=9606 GN=
    ABCF2 PE=1 SV=2;sp|Q9UG63-2|ABCF2_HUMAN Isoform 2 of ATP-binding cassette sub-family F member
     2 OS=Homo sapiens OX=9606 GN=ABCF2
##   Number.of.proteins Peptides Razor...unique.peptides Unique.peptides
## 1                  2       10                      10               10
## 2                  2        3                       3                3
##   Peptides.BZ1A Peptides.BZ1B Peptides.BZ2A Peptides.BZ2B Peptides.BZ3A
## 1             0             1             0             3             0
## 2             0             0             0             1             0
##   Peptides.BZ3B Peptides.BZ4A Peptides.BZ4B Peptides.BZ5A Peptides.BZ5B
## 1             8             0             5             0             2
## 2             0             0             0             0             2
##   Peptides.BZ6A Peptides.BZ6B Peptides.BZ7A Peptides.BZ7B Peptides.BZ8A
## 1             0             3             0             9             0
## 2             0             0             0             0             0
##   Peptides.BZ8B Razor...unique.peptides.BZ1A Razor...unique.peptides.BZ1B
## 1             8                            0                            1
## 2             0                            0                            0
##   Razor...unique.peptides.BZ2A Razor...unique.peptides.BZ2B
## 1                            0                            3
## 2                            0                            1
##   Razor...unique.peptides.BZ3A Razor...unique.peptides.BZ3B
## 1                            0                            8
## 2                            0                            0
```

```
##   Razor...unique.peptides.BZ4A Razor...unique.peptides.BZ4B
## 1                            0                            5
## 2                            0                            0
##   Razor...unique.peptides.BZ5A Razor...unique.peptides.BZ5B
## 1                            0                            2
## 2                            0                            2
##   Razor...unique.peptides.BZ6A Razor...unique.peptides.BZ6B
## 1                            0                            3
## 2                            0                            0
##   Razor...unique.peptides.BZ7A Razor...unique.peptides.BZ7B
## 1                            0                            9
## 2                            0                            0
##   Razor...unique.peptides.BZ8A Razor...unique.peptides.BZ8B
## 1                            0                            8
## 2                            0                            0
##   Unique.peptides.BZ1A Unique.peptides.BZ1B Unique.peptides.BZ2A
## 1                    0                    1                    0
## 2                    0                    0                    0
##   Unique.peptides.BZ2B Unique.peptides.BZ3A Unique.peptides.BZ3B
## 1                    3                    0                    8
## 2                    1                    0                    0
##   Unique.peptides.BZ4A Unique.peptides.BZ4B Unique.peptides.BZ5A
## 1                    0                    5                    0
## 2                    0                    0                    0
##   Unique.peptides.BZ5B Unique.peptides.BZ6A Unique.peptides.BZ6B
## 1                    2                    0                    3
## 2                    2                    0                    0
##   Unique.peptides.BZ7A Unique.peptides.BZ7B Unique.peptides.BZ8A
## 1                    0                    9                    0
## 2                    0                    0                    0
##   Unique.peptides.BZ8B Sequence.coverage....
## 1                    8                 16.0
## 2                    0                  5.9
##   Unique...razor.sequence.coverage.... Unique.sequence.coverage....
## 1                                 16.0                          16.0
## 2                                  5.9                           5.9
##   Mol..weight..kDa. Sequence.length Sequence.lengths    Q.value    Score
## 1            91.679              807          807;845 0.0000000 24.5060
## 2            71.289              623          623;634 0.0071429  2.5769
##   Sequence.coverage.BZ1A.... Sequence.coverage.BZ1B....
## 1                          0                        1.9
## 2                          0                        0.0
##   Sequence.coverage.BZ2A.... Sequence.coverage.BZ2B....
## 1                          0                        5.3
## 2                          0                        2.1
##   Sequence.coverage.BZ3A.... Sequence.coverage.BZ3B....
## 1                          0                       14.3
## 2                          0                        0.0
##   Sequence.coverage.BZ4A.... Sequence.coverage.BZ4B....
## 1                          0                        7.8
## 2                          0                        0.0
##   Sequence.coverage.BZ5A.... Sequence.coverage.BZ5B....
## 1                          0                        4.1
## 2                          0                        3.9
##   Sequence.coverage.BZ6A.... Sequence.coverage.BZ6B....
## 1                          0                        4.2
## 2                          0                        0.0
##   Sequence.coverage.BZ7A.... Sequence.coverage.BZ7B....
## 1                          0                       14.6
## 2                          0                        0.0
##   Sequence.coverage.BZ8A.... Sequence.coverage.BZ8B.... Intensity
## 1                          0                       12.5  27370000
## 2                          0                        0.0   1016500
##   Intensity.BZ1A Intensity.BZ1B Intensity.BZ2A Intensity.BZ2B Intensity.BZ3A
## 1              0        1004900              0        1409000              0
## 2              0              0              0         229450              0
##   Intensity.BZ3B Intensity.BZ4A Intensity.BZ4B Intensity.BZ5A Intensity.BZ5B
## 1        6035900              0        1245700              0        1840700
```

```
## 2                  0                  0                  0                  0            787080
##     Intensity.BZ6A  Intensity.BZ6B  Intensity.BZ7A  Intensity.BZ7B  Intensity.BZ8A
## 1                  0            2046100                  0            8748400                  0
## 2                  0                  0                  0                  0                  0
##     Intensity.BZ8B LFQ.intensity.BZ1A LFQ.intensity.BZ1B LFQ.intensity.BZ2A
## 1            5039200                          0                 840440                          0
## 2                  0                          0                      0                          0
##    LFQ.intensity.BZ2B LFQ.intensity.BZ3A LFQ.intensity.BZ3B LFQ.intensity.BZ4A
## 1              1381200                          0            3266700                          0
## 2                    0                          0                  0                          0
##    LFQ.intensity.BZ4B LFQ.intensity.BZ5A LFQ.intensity.BZ5B LFQ.intensity.BZ6A
## 1               901280                          0             584820                          0
## 2                    0                          0                  0                          0
##    LFQ.intensity.BZ6B LFQ.intensity.BZ7A LFQ.intensity.BZ7B LFQ.intensity.BZ8A
## 1               527290                          0            1768200                          0
## 2                    0                          0                  0                          0
##    LFQ.intensity.BZ8B MS.MS.count.BZ1A MS.MS.count.BZ1B MS.MS.count.BZ2A
## 1              2179100                  0                  6                  0
## 2                    0                  0                  0                  0
##    MS.MS.count.BZ2B MS.MS.count.BZ3A MS.MS.count.BZ3B MS.MS.count.BZ4A
## 1                  5                  0                 12                  0
## 2                  1                  0                  0                  0
##    MS.MS.count.BZ4B MS.MS.count.BZ5A MS.MS.count.BZ5B MS.MS.count.BZ6A
## 1                  6                  0                  3                  0
## 2                  0                  0                  3                  0
##    MS.MS.count.BZ6B MS.MS.count.BZ7A MS.MS.count.BZ7B MS.MS.count.BZ8A
## 1                  5                  0                 16                  0
## 2                  0                  0                  0                  0
##    MS.MS.count.BZ8B MS.MS.count
## 1                 15          68
## 2                  0           4
##
                                                      Peptide.sequences
## 1 AANAAENDFSVSQAEMSSR;FAALDNEEEDKEEEIIK;IGFFNQQYAEQLR;ILAGLGFDPEMQNRPTQK;LQGQLEQGDDTAAER;
   MEETPTEYLQR;NQDEESQEAPELLK;RLQGQLEQGDDTAAER;STLLLLLTGK;TFFEELAVEDK
## 2
                               ETTEVDLLTK;ILHGLGFTPAMQR;IPPPVIMVQNVSFK
##    Only.identified.by.site  Reverse  Potential.contaminant   id
## 1                                                          389
## 2                                                          477
##                                              Peptide.IDs
## 1  19;813;1337;1380;1888;2034;2286;2554;2864;2966
## 2                                           783;1389;1437
##                                           Peptide.is.razor
## 1  True;True;True;True;True;True;True;True;True;True
## 2                                           True;True;True
##                                           Mod..peptide.IDs
## 1  21;888;1465;1512;1513;2068;2233;2589;2875;3223;3337
## 2                                              851;1522;1574
##

                                                                                     Evidence.IDs
## 1
    146;147;148;149;150;7675;7676;15398;15399;16197;16198;16199;16200;16201;16202;16203;16204;22272;22273;22274;2

## 2

                                                                                 7419;16250;16602
##



                                          MS.MS.IDs
## 1
    289;290;291;292;293;18629;18630;38974;38975;38976;38977;41026;41027;41028;41029;41030;41031;41032;41033;41034

## 2
```

```
                    18012;41139;42139;42140
##                                                    Best.MS.MS
## 1 289;18629;38974;41030;56092;57905;68534;76633;90419;92665
## 2                                          18012;41139;42140
##   Oxidation..M..site.IDs Oxidation..M..site.positions Taxonomy.IDs   name
## 1                    314                         406       -1;-1 ABCF1
## 2                                                      -1;-1 ABCF2
##          ID
## 1 Q8NE71-2
## 2   Q9UG63
```

2. Create a SummarizedExperiment

We used the following design to create a SummarizedExperiment.

```
#Load design matrix
design <- read.delim("~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_
    4UVC/0_Data/Design_matrix.txt")
design
```

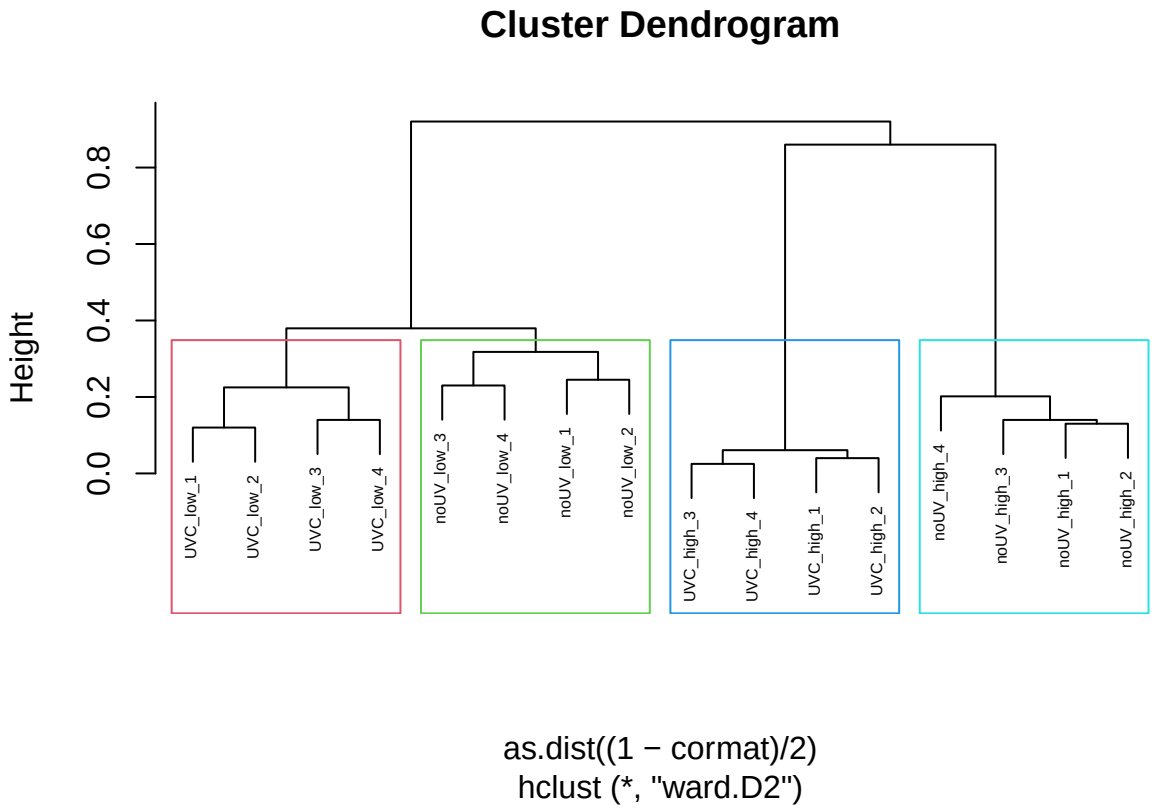
```
##                 label condition replicate
## 1  LFQ.intensity.BZ1A  noUV_low         1
## 2  LFQ.intensity.BZ1B noUV_high         1
## 3  LFQ.intensity.BZ2A  noUV_low         2
## 4  LFQ.intensity.BZ2B noUV_high         2
## 5  LFQ.intensity.BZ3A  noUV_low         3
## 6  LFQ.intensity.BZ3B noUV_high         3
## 7  LFQ.intensity.BZ4A  noUV_low         4
## 8  LFQ.intensity.BZ4B noUV_high         4
## 9  LFQ.intensity.BZ5A    UVC_low         1
## 10 LFQ.intensity.BZ5B   UVC_high         1
## 11 LFQ.intensity.BZ6A    UVC_low         2
## 12 LFQ.intensity.BZ6B   UVC_high         2
## 13 LFQ.intensity.BZ7A    UVC_low         3
## 14 LFQ.intensity.BZ7B   UVC_high         3
## 15 LFQ.intensity.BZ8A    UVC_low         4
## 16 LFQ.intensity.BZ8B   UVC_high         4
```

```
set.seed(3)
se <- make_se(unique_pg, columns = ecols, expdesign = design)

#Filter and impute
filt <- filter_se(se,thr = 0, filter_formula = ~ Reverse != '+' & Potential.contaminant !="+" &
    Peptides > 1 & Unique.peptides > 0)
write.table(as.data.frame(filt@assays@data@listData), file = "~/Documents/Postdoc/PD_Projects/3_
    irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC/3_DEP/HNRNPC_4noUV_4UVC_LFQ_intensity_raw.txt",
     row.names = TRUE, sep = "\t", quote = F)
norm <- normalize_vsn(filt)
se_imp <- DEP2::impute(norm,fun = "QRILC")
```

3. Correlation analysis

We first performed a correlation analysis to compare noUV and UVC samples from the two gel sections.

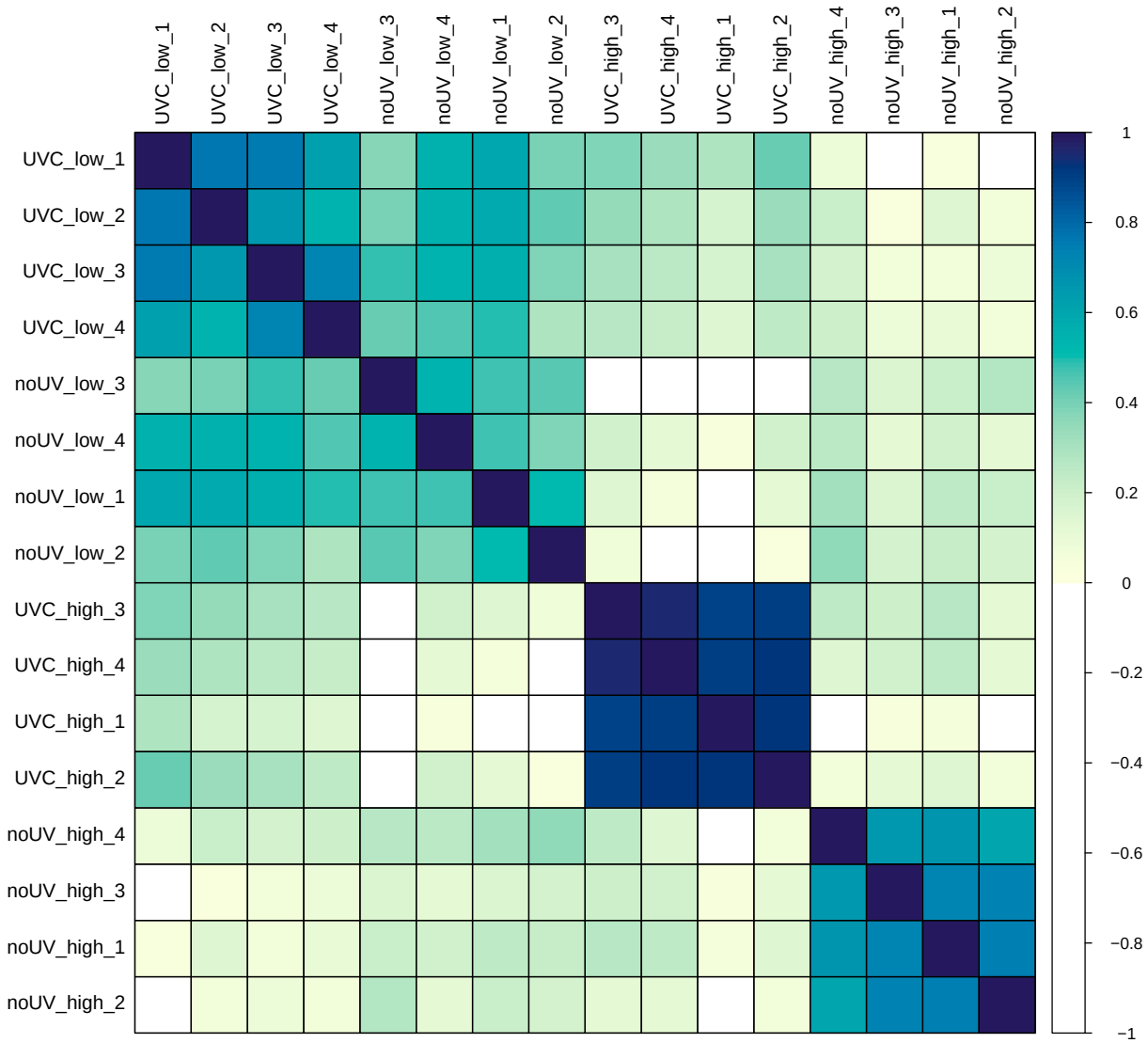
```
#Prepare matrix for clustering
se_log2 <- assay(se_imp)
se_log2 <- as.matrix(se_log2)

#Calculate the correlation and correlation test matrix
cormat <- round(cor(se_log2),2)

#Calculate the clustering
hc <- hclust(as.dist((1-cormat)/2), method = "ward.D2")
sub_grp <- cutree(hc, k = 4)

#Plot dendogram
plot(hclust(as.dist((1-cormat)/2), method = "ward.D2"), cex=0.5)
rect.hclust(hc, k = 4, border = 2:5)
```




```
#Save dendogram as pdf
pdf("~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC/2_
    Correlation/Dendo.pdf")
plot(hclust(as.dist((1-cormat)/2), method = "ward.D2"), cex=0.5)
rect.hclust(hc, k = 4, border = 2:5)
dev.off()
```

```
#Reorder according to clustering results
```

```
hc1 <- as.dendrogram(hc)
ord.hc1 <- order.dendrogram(hc1)
cormat.ord <- cormat[ord.hc1,ord.hc1]

#Determine the color
my.breaks <- c(seq(-1, 1, by=0.01))
my.colors <- rev(c(paletteer_c("grDevices::YlGnBu", length(my.breaks)/2), rep("#FFFFFF", length(
    my.breaks)/2)))

#Generate correlogram
corrplot(cormat.ord, method = "color", col = my.colors, number.font = 1, number.cex = 1 , order =
     "original",
    #addCoef.col = "black",
    addgrid.col = "black",
    tl.col = "black", diag = TRUE)
```



```
#Save the correlation heatmap as pdf
pdf("~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC/2_
    Correlation/Correlogram.pdf", height = 10, width = 10)
corrplot(cormat.ord, method = "color", col = my.colors, number.font = 1, number.cex = 1 , order =
     "original",
    addgrid.col = "black", tl.col = "black", diag = TRUE)
dev.off()
```

4. Perform enrichment analysis using DEP

To perform the enrichment analysis, we tested every condition against the UVC samples coming from the highest gel section using the DEP2 R package (PMID: 37624922).

```
#DEP analysis function
DEP_analysis <- function(se, a) {
  diff <- test_diff(se, type = "control", control = a, fdr.type = "BH")
  dep <- add_rejections(diff, alpha = 0.1, lfc = 0)
  results <- get_results(dep)
  return(list(se = se, diff = diff, dep = dep,
              results = results))
}

#Run DEP analysis
se_DE <- DEP_analysis(se =  se_imp,a = "UVC_high")

head(se_DE$results)
```

```
##      name        ID noUV_high_vs_UVC_high_p.val noUV_low_vs_UVC_high_p.val
## 1   ABCF1 Q8NE71-2                   0.63838066                1.399774e-04
## 2   ACTA1    P68133                   0.72471269                2.764722e-03
## 3   ACTG1    P63261                   0.55352530                1.591473e-03
## 4    ADAR P55265-5                   0.01298609                7.900736e-05
## 5 ATP1A1 P05023-3                   0.36385612                1.629904e-04
## 6  CDC40    O60508                   0.99263796                7.261130e-04
##   UVC_low_vs_UVC_high_p.val noUV_high_vs_UVC_high_p.adj
## 1             0.0032931663                      0.7990
## 2             0.0060297335                      0.8280
## 3             0.0069772667                      0.7380
## 4             0.0005115172                      0.0433
## 5             0.1945082174                      0.5720
## 6             0.0015461881                      1.0000
##   noUV_low_vs_UVC_high_p.adj UVC_low_vs_UVC_high_p.adj
## 1                   0.000265                   0.00732
## 2                   0.003760                   0.01220
## 3                   0.002320                   0.01320
## 4                   0.000165                   0.00213
## 5                   0.000300                   0.22100
## 6                   0.001130                   0.00424
##   noUV_high_vs_UVC_high_significant noUV_low_vs_UVC_high_significant
## 1                             FALSE                             TRUE
## 2                             FALSE                             TRUE
## 3                             FALSE                             TRUE
## 4                              TRUE                             TRUE
## 5                             FALSE                             TRUE
## 6                             FALSE                             TRUE
##   UVC_low_vs_UVC_high_significant significant noUV_high_vs_UVC_high_ratio
## 1                            TRUE        TRUE                     0.74300
## 2                            TRUE        TRUE                     0.43200
## 3                            TRUE        TRUE                     0.44600
```

```
## 4                              TRUE         TRUE                  −3.35000
## 5                             FALSE         TRUE                   1.16000
## 6                              TRUE         TRUE                  −0.00927
##   noUV_low_vs_UVC_high_ratio UVC_low_vs_UVC_high_ratio noUV_high_centered
## 1                      −7.75                    −5.37               3.840
## 2                       4.28                     3.83              −1.700
## 3                       2.81                     2.29              −0.939
## 4                      −6.33                    −5.21               0.371
## 5                      −6.09                    −1.68               2.810
## 6                      −4.14                    −3.78               1.970
##   noUV_low_centered UVC_high_centered UVC_low_centered
## 1            −4.66               3.09          −2.2800
## 2             2.14              −2.13           1.6900
## 3             1.42              −1.39           0.9020
## 4            −2.60               3.72          −1.4900
## 5            −4.44               1.65          −0.0247
## 6            −2.16               1.98          −1.8000
```

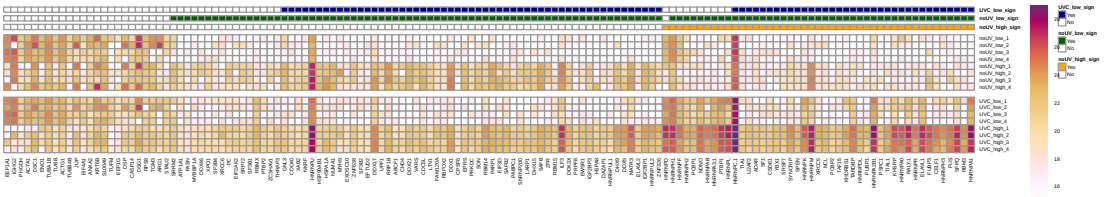
```
#Save imputed LFQ intensities
write.table(as.data.frame(se_DE$dep@assays@data@listData), file = "~/Documents/Postdoc/PD_
    Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC/3_DEP/HNRNPC_4noUV_4UVC_LFQ_
    intensity.txt", row.names = TRUE, sep = "\t", quote = F)

write.table(se_DE$results, file = "~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_
    control/0_4noUV_4UVC/3_DEP/HNRNPC_4noUV_4UVC_res_LFQ_intensity.txt", row.names = FALSE, sep =
    "\t", quote = F)
```

5. Heatmap of detected proteins

We generated a heatmap using the imputed intensities of all the proteins that were detected through irCLIP-RNP in at least one condition. We provided also an annotation regarding the significance against noUV "low" (free RNA ligation zone) and "high" (whole RNP zone) gel sections as well as UVC "low" gel section (FDR < 0.05 and FC > 3).

```
#Create heatmap of imputed intensities
table.hm <- se_log2[,c(1,3,5,7,2,4,6,8,9,11,13,15,10,12,14,16)]

#Color breaks
my.breaks <- c(seq(15, 29, by=0.1))
my.colors <- c(colorRampPalette(colors = rev(c("#6c2c73","#aa0663", "#c93a56", "#db664e", "#
    e4904f", "#e5b961", "#eec67b", "#f7d394", "#ffe0ae", "#ffe0cd", "#ffe7ec", "#fff3fd", "#
    fdfdfd")))(length(my.breaks)))

#Annotation about significance
se_DE$results$noUV_low_sign <- ifelse(se_DE$results$noUV_low_vs_UVC_high_p.adj < 0.05 & se_DE$
    results$noUV_low_vs_UVC_high_ratio < -log2(3), 1, 0)
se_DE$results$noUV_high_sign <- ifelse(se_DE$results$noUV_high_vs_UVC_high_p.adj < 0.05 & se_DE$
    results$noUV_high_vs_UVC_high_ratio < -log2(3), 1, 0)
se_DE$results$UVC_low_sign <- ifelse(se_DE$results$UVC_low_vs_UVC_high_p.adj < 0.05 & se_DE$
    results$UVC_low_vs_UVC_high_ratio < -log2(3), 1, 0)

annotation <- se_DE$results[,c(13,14,15,20,21,22)]
rownames(annotation) <- se_DE$results$name
annotation[annotation == 1] <- "Yes"
annotation[annotation == 0] <- "No"
annotation$noUV_low_sign <- as.factor(annotation$noUV_low_sign)
annotation$noUV_high_sign <- as.factor(annotation$noUV_high_sign)
annotation$UVC_low_sign <- as.factor(annotation$UVC_low_sign)
annotation$avglogfc <- rowMeans(annotation[1:3])
```

```
annotation <- annotation %>% arrange(noUV_high_sign, UVC_low_sign, noUV_low_sign, -1*avglogfc)
table.hm <- table.hm[match(rownames(annotation), rownames(table.hm)),]

write.table(annotation, file = "~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_
    control/0_4noUV_4UVC/3_DEP/HNRNPC_4noUV_4UVC_res_sign_prot.txt", row.names = TRUE, sep = "\t"
    , quote = F)

ann_colors = list(noUV_low_sign = c("Yes"="darkgreen", "No"="white"), noUV_high_sign = c("Yes"="
    orange", "No"="white"), UVC_low_sign = c("Yes"="darkblue", "No"="white"))

#Make the heatmap
pheatmap(
  mat                = t(table.hm),
  annotation_col = annotation[c(5,4,6)],
  annotation_colors = ann_colors,
  color = my.colors,
  breaks = my.breaks,
  cellwidth = 4,
  cellheight = 4,
  show_colnames      = TRUE,
  show_rownames      = TRUE,
  drop_levels        = TRUE,
  fontsize           = 3,
  cluster_rows       = FALSE,
  cluster_cols       = FALSE,
  scale              = "none",
  angle_col = 90,
  gaps_row = c(8)
)
```



```
#Save the heatmap
pheatmap(
  mat                = t(table.hm),
  annotation_col = annotation[c(5,4,6)],
  annotation_colors = ann_colors,
  color = my.colors,
  breaks = my.breaks,
  cellwidth = 4,
  cellheight = 4,
  show_colnames      = TRUE,
  show_rownames      = TRUE,
  drop_levels        = TRUE,
  fontsize           = 3,
  cluster_rows       = FALSE,
  cluster_cols       = FALSE,
  scale              = "none",
  angle_col = 90,
  gaps_row = c(8),
```

```
  filename = "~/Documents/Postdoc/PD_Projects/3_irCLIP-RNP/MS/irCLIP-RNP_control/0_4noUV_4UVC/3_
    DEP/all_prot_heatmap_LFQ.pdf"
)
```

All the visualizations were saved as pdf and modified in illustrator.

```
sessionInfo()
```

```
## R version 4.2.1 (2022-06-23)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/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] grid      stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] data.table_1.15.2          paletteer_1.6.0
##  [3] psych_2.4.3                corrplot_0.92
##  [5] DESeq2_1.38.3              pheatmap_1.0.12
##  [7] arcdiagram_0.1.12          UpSetR_1.4.0
##  [9] colormap_0.1.4             ggraph_2.2.1
## [11] igraph_2.0.3               hrbrthemes_0.8.7
## [13] patchwork_1.2.0            viridis_0.6.5
## [15] viridisLite_0.4.2          Clipper_0.0.0.9000
## [17] ggpubr_0.6.0               lubridate_1.9.3
## [19] forcats_1.0.0              stringr_1.5.1
## [21] dplyr_1.1.4                purrr_1.0.2
## [23] readr_2.1.5                tidyr_1.3.1
## [25] tibble_3.2.1               ggplot2_3.5.0
## [27] tidyverse_2.0.0            DEP2_0.4.8.24
## [29] R6_2.5.1                   limma_3.54.2
## [31] MSnbase_2.24.2             ProtGenerics_1.30.0
## [33] mzR_2.32.0                 Rcpp_1.0.12
## [35] MsCoreUtils_1.10.0         SummarizedExperiment_1.28.0
## [37] Biobase_2.58.0             GenomicRanges_1.50.2
## [39] GenomeInfoDb_1.34.9        IRanges_2.32.0
## [41] S4Vectors_0.36.2           BiocGenerics_0.44.0
## [43] MatrixGenerics_1.10.0      matrixStats_1.2.0
## [45] formatR_1.14
##
## loaded via a namespace (and not attached):
##   [1] missForest_1.5              bit64_4.0.5
##   [3] knitr_1.45                  DelayedArray_0.24.0
##   [5] KEGGREST_1.38.0             RCurl_1.98-1.14
##   [7] AnnotationFilter_1.22.0     doParallel_1.0.17
##   [9] generics_0.1.3              preprocessCore_1.60.2
##  [11] RSQLite_2.3.5               proxy_0.4-27
##  [13] bit_4.0.5                   tzdb_0.4.0
##  [15] httpuv_1.6.14               assertthat_0.2.1
##  [17] TCseq_1.22.6                xfun_0.42
##  [19] hms_1.1.3                   evaluate_0.23
##  [21] promises_1.2.1              fansi_1.0.6
##  [23] DBI_1.2.2                   geneplotter_1.76.0
##  [25] ellipsis_0.3.2              RSpectra_0.16-1
##  [27] QFeatures_1.8.0             backports_1.4.1
```

```
##  [29] fontLiberation_0.1.0          V8_4.4.2
##  [31] prismatic_1.1.1               annotate_1.76.0
##  [33] fontBitstreamVera_0.1.1       vctrs_0.6.5
##  [35] imputeLCMD_2.1                abind_1.4-5
##  [37] cachem_1.0.8                  withr_3.0.0
##  [39] ggforce_0.4.2                 itertools_0.1-3
##  [41] GenomicAlignments_1.34.1      fdrtool_1.2.17
##  [43] MultiAssayExperiment_1.24.0   mnormt_2.1.1
##  [45] cluster_2.1.6                 lazyeval_0.2.2
##  [47] crayon_1.5.2                  crul_1.4.0
##  [49] glmnet_4.1-8                  edgeR_3.40.2
##  [51] pkgconfig_2.0.3               tweenr_2.0.3
##  [53] nlme_3.1-164                  rlang_1.1.3
##  [55] lifecycle_1.0.4               sandwich_3.1-0
##  [57] downloader_0.4                fontquiver_0.2.1
##  [59] httpcode_0.3.0                affyio_1.68.0
##  [61] extrafontdb_1.0               randomForest_4.7-1.1
##  [63] polyclip_1.10-6               rngtools_1.5.2
##  [65] Matrix_1.6-5                  carData_3.0-5
##  [67] zoo_1.8-12                    GlobalOptions_0.1.2
##  [69] png_0.1-8                     rjson_0.2.21
##  [71] bitops_1.0-7                  Biostrings_2.66.0
##  [73] blob_1.2.4                    doRNG_1.8.6
##  [75] shape_1.4.6.1                 rstatix_0.7.2
##  [77] tmvtnorm_1.6                  ggsignif_0.6.4
##  [79] scales_1.3.0                  memoise_2.0.1
##  [81] magrittr_2.0.3                plyr_1.8.9
##  [83] zlibbioc_1.44.0               compiler_4.2.1
##  [85] RColorBrewer_1.1-3            pcaMethods_1.90.0
##  [87] clue_0.3-65                   Rsamtools_2.14.0
##  [89] cli_3.6.2                     affy_1.76.0
##  [91] XVector_0.38.0                MASS_7.3-60.0.1
##  [93] tidyselect_1.2.1              vsn_3.66.0
##  [95] stringi_1.8.3                 highr_0.10
##  [97] yaml_2.3.8                    norm_1.0-11.1
##  [99] askpass_1.2.0                 locfit_1.5-9.9
## [101] MALDIquant_1.22.2             ggrepel_0.9.5
## [103] tools_4.2.1                   timechange_0.3.0
## [105] parallel_4.2.1                circlize_0.4.16
## [107] rstudioapi_0.15.0             foreach_1.5.2
## [109] gridExtra_2.3                 farver_2.1.1
## [111] mzID_1.36.0                   Rtsne_0.17
## [113] digest_0.6.35                 BiocManager_1.30.22
## [115] shiny_1.8.0                   gfonts_0.2.0
## [117] car_3.1-2                     broom_1.0.5
## [119] later_1.3.2                   ncdf4_1.22
## [121] httr_1.4.7                    gdtools_0.3.5
## [123] AnnotationDbi_1.60.2          ComplexHeatmap_2.14.0
## [125] colorspace_2.1-0              XML_3.99-0.16.1
## [127] reticulate_1.35.0             umap_0.2.10.0
## [129] splines_4.2.1                 rematch2_2.1.2
## [131] graphlayouts_1.1.1            gmm_1.8
## [133] systemfonts_1.0.5             xtable_1.8-4
## [135] jsonlite_1.8.8                tidygraph_1.3.1
## [137] pillar_1.9.0                  htmltools_0.5.7
## [139] mime_0.12                     glue_1.7.0
## [141] fastmap_1.1.1                 BiocParallel_1.32.6
## [143] class_7.3-22                  codetools_0.2-19
## [145] mvtnorm_1.2-4                 utf8_1.2.4
## [147] lattice_0.22-5                curl_5.2.1
## [149] openssl_2.1.1                 Rttf2pt1_1.3.12
## [151] survival_3.5-8                rmarkdown_2.26
## [153] munsell_0.5.0                 e1071_1.7-14
## [155] GetoptLong_1.0.5              GenomeInfoDbData_1.2.9
## [157] iterators_1.0.14              impute_1.72.3
## [159] reshape2_1.4.4                gtable_0.3.4
## [161] extrafont_0.19
```