RNAhybrid_heatmaps_fig2

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Setup

directory

```
setwd("D:/Krueger_Lab/Publications/miR181_paper/Figure2/RNAhybrid")

set.seed(123)
```

packages

```
source("D:/Krueger_Lab/Publications/miR181_paper_v21022023/Figure_theme/theme_paper.R")
library(BSgenome.Mmusculus.UCSC.mm10)
```

```
## Loading required package: BSgenome

## Loading required package: BiocGenerics

##
## Attaching package: 'BiocGenerics'

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

## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, 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

## Loading required package: stats4

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

##
```

```
## Attaching package: 'IRanges'

## The following object is masked from 'package:grDevices':
##
##     windows

## Loading required package: GenomeInfoDb

## Loading required package: GenomicRanges

## Loading required package: Biostrings

## Loading required package: XVector

##
## Attaching package: 'Biostrings'

## The following object is masked from 'package:base':
##
##     strsplit

## Loading required package: rtracklayer
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:Biostrings':
##
##     collapse, intersect, setdiff, setequal, union

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

## The following objects are masked from 'package:GenomicRanges':
##
##     intersect, setdiff, union

## The following object is masked from 'package:GenomeInfoDb':
##
##     intersect

## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union

## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union

## The following objects are masked from 'package:BiocGenerics':
##
##     combine, intersect, setdiff, union

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```r
library(ggplot2)
library(seqinr)
```

```
##
## Attaching package: 'seqinr'

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

## The following object is masked from 'package:Biostrings':
##
##     translate
```

```r
library(circlize)
```

```
## ========================================
## circlize version 0.4.15
## CRAN page: https://cran.r-project.org/package=circlize
## Github page: https://github.com/jokergoo/circlize
## Documentation: https://jokergoo.github.io/circlize_book/book/
##
## If you use it in published research, please cite:
## Gu, Z. circlize implements and enhances circular visualization
##   in R. Bioinformatics 2014.
##
## This message can be suppressed by:
##   suppressPackageStartupMessages(library(circlize))
## ========================================
```

```r
library(ComplexHeatmap)
```

```
## Loading required package: grid

##
## Attaching package: 'grid'

## The following object is masked from 'package:Biostrings':
##
##     pattern

## ========================================
## ComplexHeatmap version 2.15.2
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite either one:
## - Gu, Z. Complex Heatmap Visualization. iMeta 2022.
## - Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
##     genomic data. Bioinformatics 2016.
##
##
## The new InteractiveComplexHeatmap package can directly export static
## complex heatmaps into an interactive Shiny app with zero effort. Have a try!
##
## This message can be suppressed by:
##   suppressPackageStartupMessages(library(ComplexHeatmap))
## ========================================
```

Data

the files imported here are created with RNAhybrid with the “RNAhybrid fig 2” script

```
Personalized_Reader <- function(lambda){
 read.table(lambda, sep = ":") %>% select(V1, V5, V6, V7, V10, V11)}

#File lists
reslistA <- list.files(path = "D:/Krueger_Lab/Publications/miR181_paper_nongithub/Figure2/RNAhybrid/resu
reslistB <- list.files(path = "D:/Krueger_Lab/Publications/miR181_paper_nongithub/Figure2/RNAhybrid/resu


#import
myfilelistA <- lapply(reslistA, Personalized_Reader)
myfilelistB <- lapply(reslistB, Personalized_Reader)




resframeA <- bind_rows(myfilelistA)
resframeB <- bind_rows(myfilelistB)



#colnames
colnames(resframeA) <- c("rownumber", "mfs", "pvalue", "start_position", "binding_bases", "non_binding_
colnames(resframeB) <- c("rownumber", "mfs", "pvalue", "start_position", "binding_bases", "non_binding_

resframeA[is.na(resframeA$non_binding_bases),"non_binding_bases"] <- "                       "
resframeB[is.na(resframeB$non_binding_bases),"non_binding_bases"] <- "                       "



head(resframeA)
```

```
##   rownumber   mfs   pvalue start_position
## 1         1 -13.1 1.000000             87
## 2        10 -15.7 0.999882             93
## 3       100 -19.3 0.646155             36
## 4      1000 -21.9 0.197373              4
## 5     10000 -25.4 0.026603             54
## 6     10001 -18.1 0.883059             18
##                            binding_bases                       non_binding_bases
## 1               GAGUG  G  GUC CAA                      U        CU   G   CUUACAA
## 2                   G  GCUGUC                            UGA UG      GCAACUUACAA
## 3   AGU GGCUGUCG  ACU             UACAA  UG             CA
## 4              GUGG UG    UCGCAACU   CA             UGA    C              UA   A
## 5              UGAG GGCUG CG CAAC UUACA                    U     U             A
## 6              UGAGUGGC UGU CG                                        CAACUUACAA
```

```
head(resframeB)
```

```
##   rownumber   mfs   pvalue start_position                      binding_bases
## 1         1 -10.9 1.000000             84        UUGGG   GG   GUC U
## 2        10 -23.1 0.116906             85        UUGGG  UGGC GUCGUU CUU
## 3       100 -24.3 0.059292             24      UGGGUGG UG    UCG UUACU
## 4      1000 -21.3 0.302779              5         UGGGUGG UGU CGU ACU   CA
## 5     10000 -23.8 0.078879             53        UUGGG GGCUG CGUU AC UUACA
## 6     10001 -19.2 0.711209             18  UGGGUGGC UGU CGUU       ACUU
##                    non_binding_bases
## 1            U    CU    G UACUUACAA
## 2                   U      A   ACAA
## 3     U       C               UACAA
## 4        U       C       U   UA   A
## 5             U     U             A
## 6 U                            ACAA
```

```
# Ribo profiling data
RNA <- read.csv("D:/Krueger_Lab/Publications/miR181_paper/Figure3/RNA_masterframe.csv")
RPF <- read.csv("D:/Krueger_Lab/Publications/miR181_paper/Figure3/RPF_masterframe.csv")


# original bs data
mir181bs <- as.data.frame(readRDS("D:/Krueger_Lab/Publications/miR181_paper/Figure1/mir181_binding_sites
mir181bs$rownumber <- 1:length(mir181bs$seqnames)
```

colours

```
#colours
farbeneg <- "#b4b4b4"
farbe1 <- "#0073C2FF"
farbe2 <- "#EFC000FF"
farbe3 <- "#CD534CFF"
farbe4 <- "#7AA6DCFF"
farbe5 <- "#868686FF"
farbe6 <- "#003C67FF"
farbe7 <- "#8F7700FF"
farbe8 <- "#3B3B3BFF"
farbe9 <- "#A73030FF"
farbe10 <- "#4A6990FF"
farbe11 <- "#FF6F00FF"
farbe12 <- "#C71000FF"
farbe13 <- "#008EA0FF"
farbe14 <- "#8A4198FF"
farbe15 <- "#5A9599FF"
farbe16 <- "#FF6348FF"

RNApcol <- "#b56504"
RNAncol <- "#027d73"
RPFpcol <- "#c4c404"
RPFncol <- "#8d0391"
```

Process data (remove gaps)

Due to the loops in the mRNA there are additional spaces in the mirna. We only want the binding and non binding bases of hte mirna in te correct order. For that we will remove all gaps that origin in the mRNA loops.

```
#binding and non binding bases as characters in a list
Alistbb <- strsplit(resframeA$binding_bases,"")
Alistnb <- strsplit(resframeA$non_binding_bases,"")

Blistbb <- strsplit(resframeB$binding_bases,"")
Blistnb <- strsplit(resframeB$non_binding_bases,"")



#combine the two lists
Alist <- Map(cbind, Alistbb, Alistnb)
```

```
## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

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## length (arg 2)

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## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)
```

```
Alist <- lapply(Alist, as.data.frame)

Blist <- Map(cbind, Blistbb, Blistnb)
```

```
## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

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## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)
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## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)

## Warning in cbind(...): number of rows of result is not a multiple of vector
## length (arg 2)
```

```
Blist <- lapply(Blist, as.data.frame)




#remove all empty rows (mRNA loops)
Alist0 <- lapply(Alist, function(x){
  x[!(x[,1]== " " & x[,2] == " "),]
})

Blist0 <- lapply(Blist, function(x){
  x[!(x[,1]== " " & x[,2] == " "),]
})




#rewrite as characters
AlistF <- lapply(Alist0, function(x){
  paste(x[,1],  collapse = '')
})

BlistF <- lapply(Blist0, function(x){
  paste(x[,1],  collapse = '')
})








#Attach lists back onto original data.frame as new column
resframeA$binding_nospace <-unlist(AlistF)
head(resframeA$binding_nospace)
```

```
## [1] " GAGUGG  GUC CAA       " "   G  GCUGUC            "
## [3] "  AGUGGCUGUCG  ACUUACAA" "   GUGG UGUCGCAACU  CA "
## [5] "UGAG GGCUG CGCAACUUACA " "UGAGUGGCUGUCG          "
```

```
resframeB$binding_nospace <-unlist(BlistF)
head(resframeB$binding_nospace)
```

```
## [1] "UUGGG GG  GUC U         " "UUGGGUGGC GUCGUU CUU    "
## [3] " UGGGUGG UGUCGUUACU     " " UGGGUGG UGUCGU ACU  CA "
## [5] "UUGGG GGCUG CGUUACUUACA " " UGGGUGGCUGUCGUUACUU    "
```

Transform into Numbers

add 0s

replace all gaps with 0 and all letters with 1

```
#0
resframeA$binding_nospace <- chartr(" ", "0", resframeA$binding_nospace)
resframeB$binding_nospace <- chartr(" ", "0", resframeB$binding_nospace)




#1
resframeA$binding_nospace <- mgsub::mgsub(resframeA$binding_nospace, c("A", "U", "C", "G"), c(rep("1", 
resframeB$binding_nospace <- mgsub::mgsub(resframeB$binding_nospace, c("A", "U", "C", "G"), c(rep("1", 



head(resframeA)
```

```
##   rownumber   mfs   pvalue start_position
## 1         1 -13.1 1.000000             87
## 2        10 -15.7 0.999882             93
## 3       100 -19.3 0.646155             36
## 4      1000 -21.9 0.197373              4
## 5     10000 -25.4 0.026603             54
## 6     10001 -18.1 0.883059             18
##                             binding_bases                      non_binding_bases
## 1               GAGUG  G  GUC CAA                      U        CU   G   CUUACAA
## 2                   G  GCUGUC                            UGA UG      GCAACUUACAA
## 3   AGU GGCUGUCG  ACU             UACAA  UG            CA                       
## 4              GUGG UG    UCGCAACU   CA            UGA    C              UA   A
## 5              UGAG GGCUG CG CAAC UUACA                   U     U             A
## 6              UGAGUGGC UGU CG                                        CAACUUACAA
##           binding_nospace
## 1 01111110011101110000000
## 2 00010011111100000000000
## 3 00111111111110011111111
## 4 00011110111111111100110
## 5 11110111110111111111110
## 6 11111111111110000000000
```

```
head(resframeB)
```

```
##   rownumber   mfs   pvalue start_position                     binding_bases
## 1         1 -10.9 1.000000             84       UUGGG   GG   GUC U
## 2        10 -23.1 0.116906             85        UUGGG  UGGC GUCGUU CUU
## 3       100 -24.3 0.059292             24      UGGGUGG UG    UCG UUACU
## 4      1000 -21.3 0.302779              5         UGGGUGG UGU CGU ACU   CA
## 5     10000 -23.8 0.078879             53        UUGGG GGCUG CGUU AC UUACA
## 6     10001 -19.2 0.711209             18  UGGGUGGC UGU CGUU       ACUU
##                   non_binding_bases          binding_nospace
## 1            U    CU    G UACUUACAA 111110110011101000000000
## 2                   U      A   ACAA 111111111011111101110000
## 3     U       C               UACAA 011111110111111111100000
## 4        U       C       U   UA   A 011111110111111011100110
## 5             U     U             A 111110111110111111111110
## 6 U                            ACAA 011111111111111111110000
```

seperate into columns

for each base make 1 column so it can be added and also put into a heatmap

```
#for the heatmap with every binding site
heatframeA <- do.call(rbind.data.frame, strsplit(resframeA$binding_nospace,""))
heatframeA <- sapply( heatframeA, as.numeric )
colnames(heatframeA) <- c(23:1)
rownames(heatframeA) <- resframeA[,1]
head(heatframeA)
```

```
##       23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
## 1      0  1  1  1  1  1  1  0  0  1  1  1  0  1 1 1 0 0 0 0 0 0 0
## 10     0  0  0  1  0  0  1  1  1  1  1  1  0  0 0 0 0 0 0 0 0 0 0
## 100    0  0  1  1  1  1  1  1  1  1  1  1  1  0 0 1 1 1 1 1 1 1 1
## 1000   0  0  0  1  1  1  1  0  1  1  1  1  1  1 1 1 1 1 0 0 1 1 0
## 10000  1  1  1  1  0  1  1  1  1  1  0  1  1  1 1 1 1 1 1 1 1 1 0
## 10001  1  1  1  1  1  1  1  1  1  1  1  1  1  0 0 0 0 0 0 0 0 0 0
```

```
heatframeB <- do.call(rbind.data.frame, strsplit(resframeB$binding_nospace,""))
heatframeB <- sapply( heatframeB, as.numeric )
colnames(heatframeB) <- c(24:1)
rownames(heatframeB) <- resframeB[,1]
head(heatframeB)
```

```
##       24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
## 1      1  1  1  1  1  0  1  1  0  0  1  1  1  0  1 0 0 0 0 0 0 0 0 0
## 10     1  1  1  1  1  1  1  1  1  0  1  1  1  1  1 1 0 1 1 1 0 0 0 0
## 100    0  1  1  1  1  1  1  1  0  1  1  1  1  1  1 1 1 1 1 0 0 0 0 0
## 1000   0  1  1  1  1  1  1  1  0  1  1  1  1  1  1 0 1 1 1 0 0 1 1 0
## 10000  1  1  1  1  1  0  1  1  1  1  1  0  1  1  1 1 1 1 1 1 1 1 1 0
## 10001  0  1  1  1  1  1  1  1  1  1  1  1  1  1  1 1 1 1 1 1 0 0 0 0
```

```
#reverse column order
heatframeA <-heatframeA[,23:1]
heatframeB <- heatframeB[,24:1]
```

sum of columns

```
#sum for the small heatmap with the overall binding ratio for each base
framesumA <- colSums(heatframeA)
framesumB <- colSums(heatframeB)



framesum <- as.data.frame(rbind(framesumA,framesumB))
```

```
## Warning in rbind(...): number of columns of result is not a multiple of vector
## length (arg 1)
```

```
rownames(framesum) <- c("miR181a", "miR181b")
framesum
```

```
##            1    2    3    4    5    6     7    8    9   10    11    12    13
## miR181a 1205 4404 6851 6918 7960 8676  9500 7229 5796 7656 11289 12224 11736
## miR181b 1440 4912 7353 7449 8344 9403 10549 8757 9050 9925 11709 12121 11389
##            14    15    16    17    18    19    20    21    22   23   24
## miR181a 11082 10649 10034 11382 11281 11109 11391  9738  9347 6328 1205
## miR181b 10761 10522 10129 11123 11045 11178 12424 12436 11414 8803 6027
```

```
#scale for better comperativity
sframesum <- as.data.frame(t(scale(t(framesum))))
```

Heatmap

Colours

```
hmcols1 <- c("white", "black")
hmcols2 <- colorRamp2(c(-2, 2), c("white", "red"))
```

Heatmap of all the single reads

make heatmap without column clustering but with row clustering

```
HMA <- Heatmap(heatframeA, cluster_columns = F, col = hmcols1, row_km = 5, show_row_names = F, show_row
```

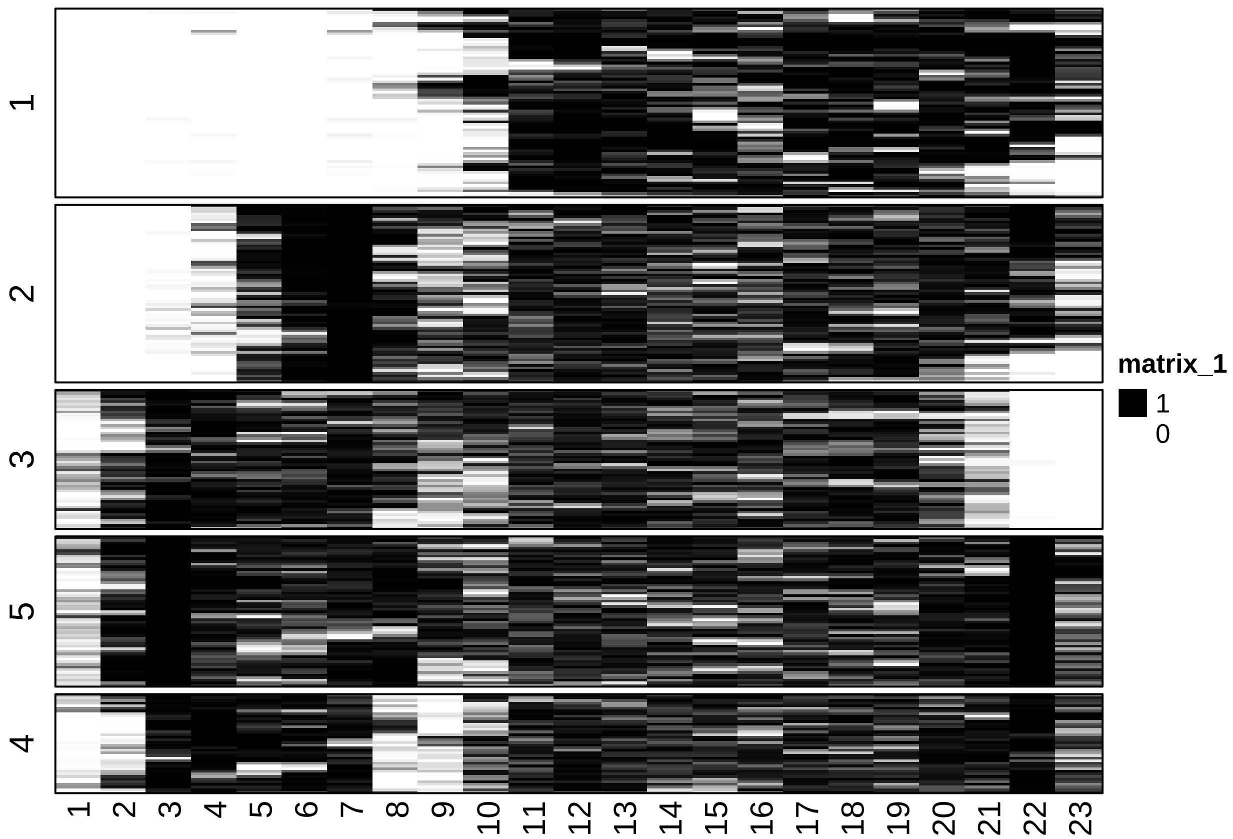
```
## `use_raster` is automatically set to TRUE for a matrix with more than
## 2000 rows. You can control `use_raster` argument by explicitly setting
## TRUE/FALSE to it.
##
## Set `ht_opt$message = FALSE` to turn off this message.
```

```
HMB <- Heatmap(heatframeB, cluster_columns = F, col = hmcols1, row_km = 5, show_row_names = F, show_row
```

```
## `use_raster` is automatically set to TRUE for a matrix with more than
## 2000 rows. You can control `use_raster` argument by explicitly setting
## TRUE/FALSE to it.
##
## Set `ht_opt$message = FALSE` to turn off this message.
```

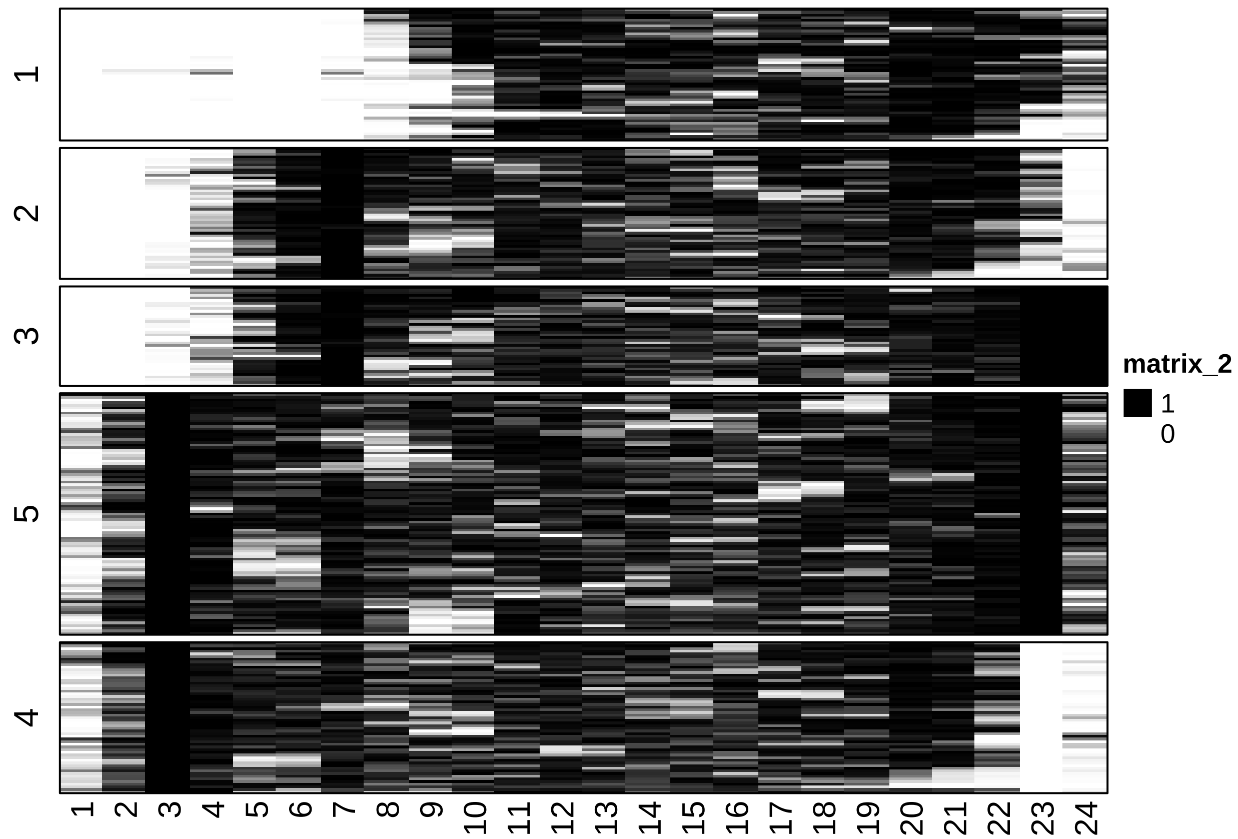
No B

```
HMA
```


1
2
3
5
4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
matrix_1
1
0

B

```
HMB
```



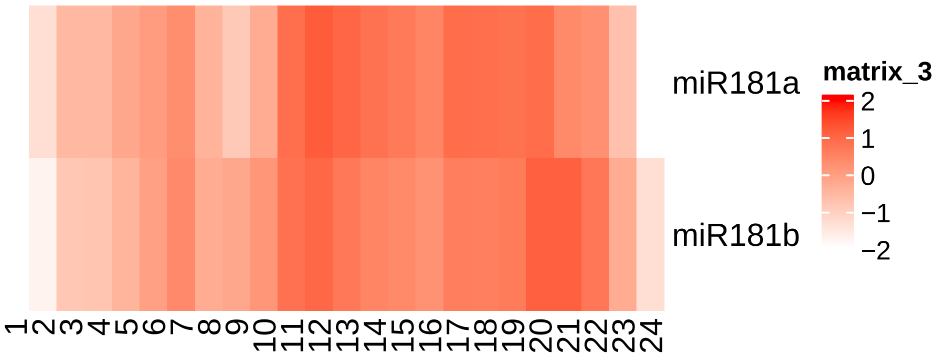
“Heatmap” of combined reads for mir__181a and b

No clustering, only sums

```
HMF <- Heatmap(sframesum, cluster_columns = F, cluster_rows = F, col = hmcols2)
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
HMF
```



cluster seperately

clustering

try to cluster seperately

```
#cluster by seed area
heat_ksA <- kmeans(heatframeA, centers = 5)
heat_k_namesA <- as.data.frame(heat_ksA$cluster)
#merge back with full data and adjust frame again
cframeA <- merge(heatframeA, heat_k_namesA, by=0)
rownames(cframeA) <- cframeA$Row.names
cframeA <- cframeA[,-1]

#order by clusters (will be needed for heatmap without clustering)
cframeA <- cframeA[order(cframeA$`heat_ksA$cluster`, decreasing = F),]
#remove cluster col
cframeAp <- cframeA[,-24]
head(cframeAp)
```

```
##       1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
## 10000 0 1 1 1 1 1 1 1 1  1  1  1  0  1  1  1  1  1  0  1  1  1  1
## 10003 0 0 1 1 1 1 0 0 0  1  1  1  1  1  0  0  1  1  1  1  1  1  0
## 10011 1 1 1 1 1 0 1 1 1  1  1  1  1  1  1  1  1  1  1  1  1  1  1
## 10012 0 1 1 1 0 0 1 1 1  1  1  1  1  1  1  0  0  1  1  1  1  1  0
## 10016 0 0 1 1 1 1 0 0 1  1  1  0  0  1  1  1  1  1  1  1  1  1  1
## 10017 1 1 1 1 1 1 1 0 0  0  1  1  1  1  1  1  0  0  1  0  1  1  1
```

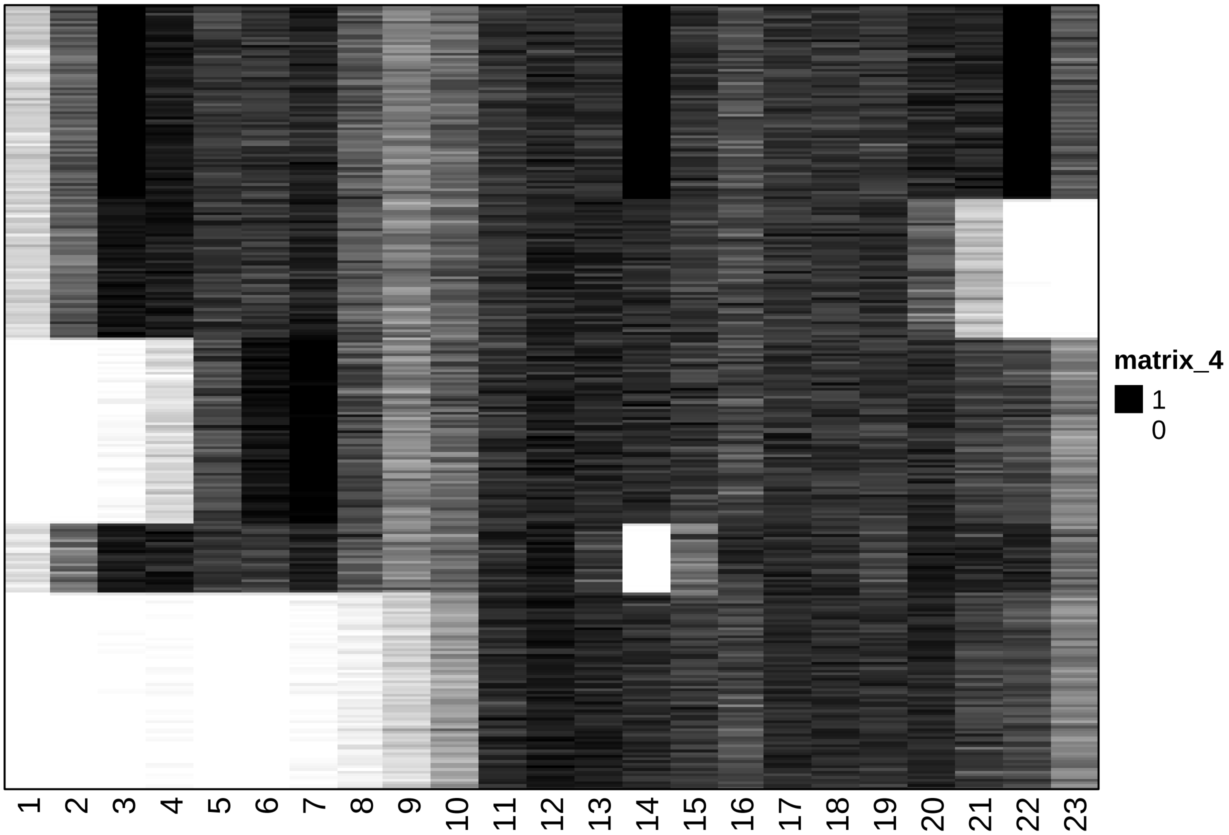
plot clustered

```
HMAsep <- Heatmap(cframeAp, cluster_columns = F, cluster_rows = F, col = hmcols1, show_row_names = F, sh
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
## `use_raster` is automatically set to TRUE for a matrix with more than
## 2000 rows. You can control `use_raster` argument by explicitly setting
## TRUE/FALSE to it.
##
## Set `ht_opt$message = FALSE` to turn off this message.
```

```
HMAsep
```



clustering by seed region

try to cluster seperately only by the binding bases

```
#cluster by seed area
heat_ksAseed <- kmeans(heatframeA[,1:8], centers = 5)
heat_k_namesAseed <- as.data.frame(heat_ksAseed$cluster)
#merge back with full data and adjust frame again
cframeAseed <- merge(heatframeA, heat_k_namesAseed, by=0)
rownames(cframeAseed) <- cframeAseed$Row.names
cframeAseed <- cframeAseed[,-1]

#order by clusters (will be needed for heatmap without clustering)
cframeAseed <- cframeAseed[order(cframeAseed$`heat_ksAseed$cluster`, decreasing = F),]
#remove cluster col
cframeAseedp <- cframeAseed[,-24]
head(cframeAseedp)
```

```
##       1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
## 100   1 1 1 1 1 1 1 1 0  0  1  1  1  1  1  1  1  1  1  1  1  0  0
## 1000  0 1 1 0 0 1 1 1 1  1  1  1  1  1  1  0  1  1  1  1  0  0  0
## 10000 0 1 1 1 1 1 1 1 1  1  1  1  0  1  1  1  1  1  0  1  1  1  1
## 10009 0 1 1 1 1 1 1 1 1  0  1  1  1  1  1  1  1  1  1  0  0  0  0
## 10010 0 1 1 1 1 1 1 1 1  1  1  1  1  0  0  1  0  0  0  0  0  0  0
## 10015 0 1 1 1 1 1 1 1 0  0  1  1  1  1  1  1  0  0  0  0  0  0  0
```

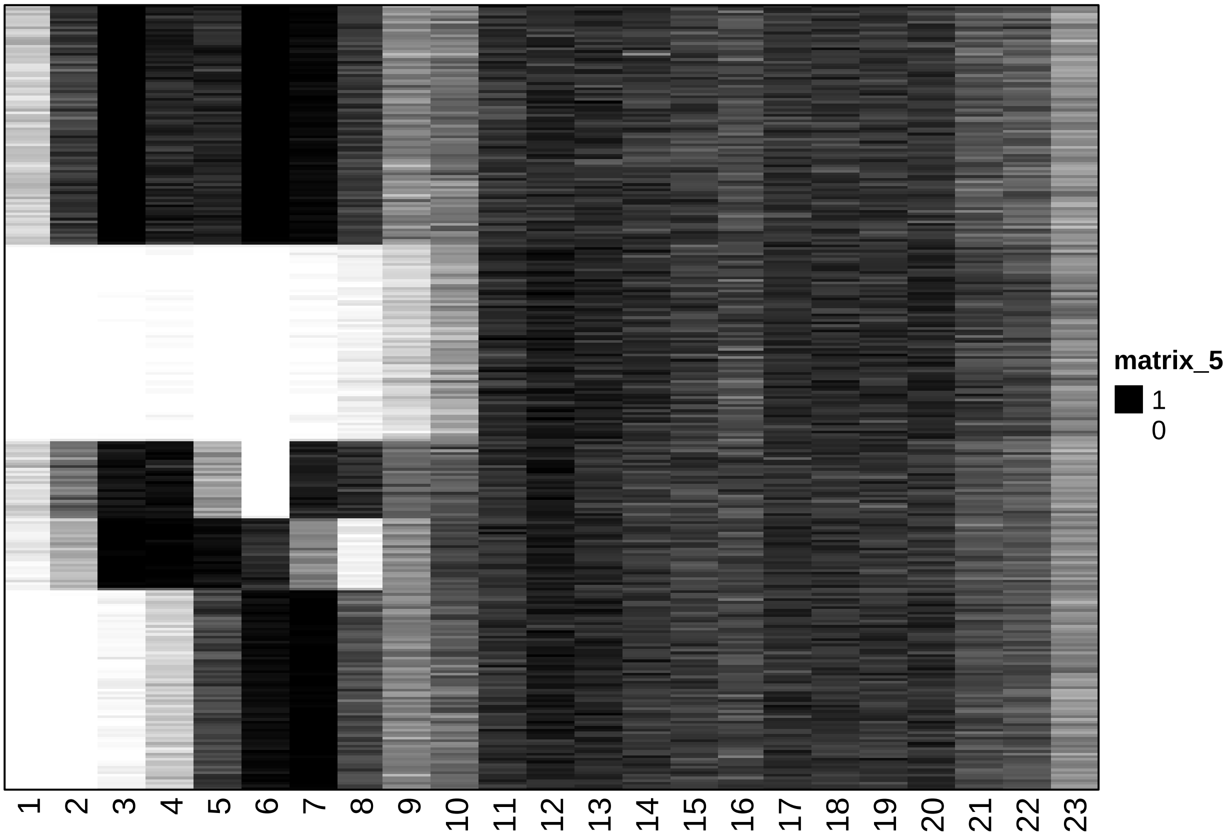
plot clustered by sed region

```
HMAseed <- Heatmap(cframeAseedp, cluster_columns = F, cluster_rows = F, col = hmcols1, show_row_names =
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
## `use_raster` is automatically set to TRUE for a matrix with more than
## 2000 rows. You can control `use_raster` argument by explicitly setting
## TRUE/FALSE to it.
##
## Set `ht_opt$message = FALSE` to turn off this message.
```

```
HMAseed
```



testcode

distframe <- dist(heatframeA) head(distframe) clustobj <- hclust(distframe)

plot(clustobj)

ECDF plots

merge back with original data for gene names

```
cframeA$rownumber <- as.numeric(rownames(cframeA))
```

```
bsseqHA <- left_join(mir181bs, cframeA, by="rownumber")

head(bsseqHA)
```

```
##   seqnames   start     end width strand scoreSum scoreMean scoreMax
## 1     chr1 6245651 6245657     7      +  9.52553  4.762765  6.00678
## 2     chr1 6248341 6248347     7      + 92.68921 23.172303 48.76900
## 3     chr1 6248857 6248863     7      + 14.07133  7.035665  7.04425
## 4     chr1 6248918 6248924     7      + 38.91451 12.971503 20.65080
## 5     chr1 7170481 7170487     7      + 66.92218 13.384436 25.84490
## 6     chr1 9899605 9899611     7      + 25.15963  6.289907  8.61019
##         geneType geneName             geneID region BS_ID          mir_IP
## 1 protein_coding   Rb1cc1 ENSMUSG00000025907    cds     5 mmu-miR-181a-5p
## 2 protein_coding   Rb1cc1 ENSMUSG00000025907    cds     8 mmu-miR-181a-5p
## 3 protein_coding   Rb1cc1 ENSMUSG00000025907    cds    10 mmu-miR-181a-5p
## 4 protein_coding   Rb1cc1 ENSMUSG00000025907    cds    11 mmu-miR-181a-5p
## 5 protein_coding   Pcmtd1 ENSMUSG00000051285   utr3    19 mmu-miR-181a-5p
## 6 protein_coding     Sgk3 ENSMUSG00000025915   utr3    23 mmu-miR-181a-5p
##   n_mir181 n_mir181a n_mir181b n_mir181c n_mir181d               set rownumber
## 1        1         1         0         0         0 ago_bs_mir181_chi         1
## 2        5         5         0         0         0 ago_bs_mir181_chi         2
## 3        6         6         0         0         0 ago_bs_mir181_chi         3
## 4        6         6         0         0         0 ago_bs_mir181_chi         4
## 5        4         4         0         0         0 ago_bs_mir181_chi         5
## 6        1         1         0         0         0 ago_bs_mir181_chi         6
##   1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 heat_ksA$cluster
## 1 0 0 0 0 0 0 0 1 1  1  0  1  1  1  0  0  1  1  1  1  1  1  0                5
## 2 0 0 0 0 0 0 0 0 1  1  1  1  1  1  1  1  1  1  1  1  1  1  0                5
## 3 0 0 0 0 1 1 1 0 1  1  1  1  1  1  1  1  1  0  1  1  1  1  1                3
## 4 0 1 1 1 1 1 1 0 0  1  1  1  1  1  1  1  0  0  1  1  1  1  1                1
## 5 1 1 1 1 1 1 1 1 0  0  1  1  1  0  0  0  0  0  0  0  0  0  0                2
## 6 0 1 1 1 1 1 0 1 1  1  0  1  1  0  1  1  1  0  0  1  1  1  1                4
```

sort RNA and RPF by cluster

```
#RNA
colnames(RNA)[16] <- "geneName"
RNA <- left_join(RNA, bsseqHA[!duplicated(bsseqHA$geneName),], by="geneName")
RNA[is.na(RNA$`heat_ksA$cluster`), "heat_ksA$cluster"] <- "Non-target"
head(RNA)
```

```
##   X                  Gene   baseMean log2FoldChange      lfcSE      stat
## 1 1  ENSMUSG00000104197.1 2009.89774       2.741830 0.10245760  26.76063
## 2 2 ENSMUSG00000004110.17 1028.70696      -2.019241 0.09914247 -20.36706
## 3 3 ENSMUSG00000027669.14  648.18701       1.905815 0.10065041  18.93499
## 4 4  ENSMUSG00000098206.1 4004.62856       2.148076 0.12889738  16.66501
## 5 5 ENSMUSG00000004552.16  516.71005      -2.252195 0.13648248 -16.50172
## 6 6  ENSMUSG00000069306.5   68.07176       2.763638 0.19108029  14.46323
##          pvalue          padj    WT_1411   WT_1601      WT_1710   KO_1411
## 1 9.288051e-158 1.183948e-153  487.52165  474.5874  405.8464624 3338.7077
## 2  3.277594e-92  2.088974e-88 1576.25174 1737.4574 1739.7225816  400.4560
## 3  5.872559e-80  2.495250e-76  248.88185  252.5740  252.2109307 1048.3637
## 4  2.354521e-62  7.503269e-59 1449.25030 1259.2746  985.7539896 5751.8330
## 5  3.566111e-61  9.091443e-58  872.62279 1026.4751  777.9463918  148.2821
## 6  2.068594e-47  4.394729e-44    2.04841    0.0000    0.8880667  123.7258
##     KO_1601   KO_1710    LFCandPADJSig      geneName seqnames     start
## 1 3428.5263 3924.1970   Significant up       Gm37632     <NA>        NA
## 2  357.0428  361.3113 Significant down       Cacna1e     chr1 154633673
## 3 1043.6635 1043.4281   Significant up          Gnb4     <NA>        NA
## 4 6644.9630 7936.6965   Significant up A430106G13Rik     <NA>        NA
## 5  112.9110  162.0230 Significant down          Ctse     chr1 131672503
## 6  146.4791  135.2892   Significant up      Hist1h4m     <NA>        NA
##         end width strand scoreSum scoreMean scoreMax       geneType
## 1        NA    NA   <NA>       NA        NA       NA           <NA>
## 2 154633679     7      - 10.09826   5.04913   8.4785 protein_coding
## 3        NA    NA   <NA>       NA        NA       NA           <NA>
## 4        NA    NA   <NA>       NA        NA       NA           <NA>
## 5 131672509     7      + 47.26199  11.81550  19.3220 protein_coding
## 6        NA    NA   <NA>       NA        NA       NA           <NA>
##               geneID region BS_ID          mir_IP n_mir181 n_mir181a n_mir181b
## 1               <NA>   <NA>  <NA>            <NA>       NA        NA        NA
## 2 ENSMUSG00000004110   utr5  <NA>            <NA>       NA        NA        NA
## 3               <NA>   <NA>  <NA>            <NA>       NA        NA        NA
## 4               <NA>   <NA>  <NA>            <NA>       NA        NA        NA
## 5 ENSMUSG00000004552    cds   382 mmu-miR-181a-5p        5         2         3
## 6               <NA>   <NA>  <NA>            <NA>       NA        NA        NA
##   n_mir181c n_mir181d               set rownumber  1  2  3  4  5  6  7  8  9 10
## 1        NA        NA              <NA>        NA NA NA NA NA NA NA NA NA NA NA
## 2        NA        NA   mir181_enriched      7405  0  0  1  1  0  0  1  1  1  1
## 3        NA        NA              <NA>        NA NA NA NA NA NA NA NA NA NA NA
## 4        NA        NA              <NA>        NA NA NA NA NA NA NA NA NA NA NA
## 5         0         0 ago_bs_mir181_chi       103  1  1  1  1  0  1  1  0  0  0
## 6        NA        NA              <NA>        NA NA NA NA NA NA NA NA NA NA NA
##   11 12 13 14 15 16 17 18 19 20 21 22 23 heat_ksA$cluster
## 1 NA NA NA NA NA NA NA NA NA NA NA NA NA       Non-target
## 2  1  1  1  1  1  0  1  0  1  1  1  0  0                2
## 3 NA NA NA NA NA NA NA NA NA NA NA NA NA       Non-target
## 4 NA NA NA NA NA NA NA NA NA NA NA NA NA       Non-target
## 5  1  1  1  1  0  1  1  1  0  1  1  0  0                2
## 6 NA NA NA NA NA NA NA NA NA NA NA NA NA       Non-target
```

```
table(RNA$`heat_ksA$cluster`)
```

```
## 
##          1          2          3          4          5 Non-target 
##        843        634        866        302        899       9757
```

```
#RPF
colnames(RPF)[16] <- "geneName"
RPF <- left_join(RPF, bsseqHA[!duplicated(bsseqHA$geneName),], by="geneName")
RPF[is.na(RPF$`heat_ksA$cluster`), "heat_ksA$cluster"] <- "Non-target"
head(RPF)
```

```
##   X                 Gene   baseMean log2FoldChange      lfcSE       stat
## 1 1  ENSMUSG00000000001.4 3788.57802     0.05462417 0.05957247  0.9169365
## 2 2 ENSMUSG00000000028.15 1086.36203    -0.12956555 0.07692175 -1.6843811
## 3 3 ENSMUSG00000000037.17   19.45454    -0.06042604 0.15784031 -0.3828302
## 4 4  ENSMUSG00000000056.7 1454.64304     0.05456272 0.08781406  0.6213438
## 5 5  ENSMUSG00000000078.7  850.27809     0.65975317 0.07971920  8.2759636
## 6 6 ENSMUSG00000000085.16  337.25837    -0.06342545 0.12383411 -0.5121808
##         pvalue         padj    WT_1411    WT_1601    WT_1710    KO_1411
## 1 3.591759e-01 6.850504e-01 3687.40632 3612.48756 3862.10415 3905.91865
## 2 9.210804e-02 3.386014e-01 1091.79093 1147.15325 1182.54053 1081.59050
## 3 7.018457e-01 8.852986e-01   13.71269   22.17027   29.07887   28.37993
## 4 5.343734e-01 8.038393e-01 1561.28714 1359.98786 1348.39777 1344.36759
## 5 1.274210e-16 2.314117e-14  649.06709  668.27536  622.50312 1054.26168
## 6 6.085245e-01 8.452822e-01  325.18653  326.21972  396.33417  351.07019
##      KO_1601     KO_1710   LFCandPADJSig geneName seqnames     start       end
## 1 3984.05486 3679.496580 Not significant    Gnai3     chr3 108118439 108118445
## 2 1030.01306  985.083926 Not significant    Cdc45     <NA>        NA        NA
## 3   17.83572    5.549769 Not significant    Scml2     chrX 161199514 161199520
## 4 1559.88270 1553.935207 Not significant     Narf    chr11 121237269 121237275
## 5 1022.58151 1084.979761  Significant up     Klf6    chr13   5867643   5867649
## 6  350.02608  274.713546 Not significant    Scmh1     <NA>        NA        NA
##   width strand  scoreSum scoreMean  scoreMax       geneType             geneID
## 1     7      -  17.16656   4.29164   6.24306 protein_coding ENSMUSG00000000001
## 2    NA   <NA>        NA        NA        NA           <NA>               <NA>
## 3     7      + 409.94460 136.64820 268.27800 protein_coding ENSMUSG00000000037
## 4     7      +  54.16402  10.83280  20.82290 protein_coding ENSMUSG00000000056
## 5     7      +  31.50993  10.50331  12.38550 protein_coding ENSMUSG00000000078
## 6    NA   <NA>        NA        NA        NA           <NA>               <NA>
##   region BS_ID          mir_IP n_mir181 n_mir181a n_mir181b n_mir181c n_mir181d
## 1    cds  5077 mmu-miR-181a-5p        2         2         0         0         0
## 2   <NA>  <NA>            <NA>       NA        NA        NA        NA        NA
## 3 intron  <NA>            <NA>       NA        NA        NA        NA        NA
## 4   utr5 16933 mmu-miR-181b-5p        1         0         1         0         0
## 5   utr3 19275 mmu-miR-181a-5p        3         3         0         0         0
## 6   <NA>  <NA>            <NA>       NA        NA        NA        NA        NA
##                  set rownumber  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16
## 1 ago_bs_mir181_chi      1261  0  0  0  0  1  1  1  1  0  0  1  1  1  1  1  0
## 2              <NA>        NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 3   mir181_enriched     12209  0  0  0  0  0  0  0  0  0  1  1  1  1  1  1  1
## 4 ago_bs_mir181_chi      4440  0  0  0  0  0  0  0  0  0  1  0  1  1  1  1  1
## 5 ago_bs_mir181_chi      4954  1  1  1  1  1  1  1  1  1  0  1  1  1  1  1  1
## 6              <NA>        NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
##   17 18 19 20 21 22 23 heat_ksA$cluster
## 1  1  1  1  1  0  0  0                3
## 2 NA NA NA NA NA NA NA       Non-target
## 3  1  1  1  1  1  0  0                5
## 4  1  1  1  1  1  1  1                5
## 5  0  0  1  1  1  1  1                1
## 6 NA NA NA NA NA NA NA       Non-target
```

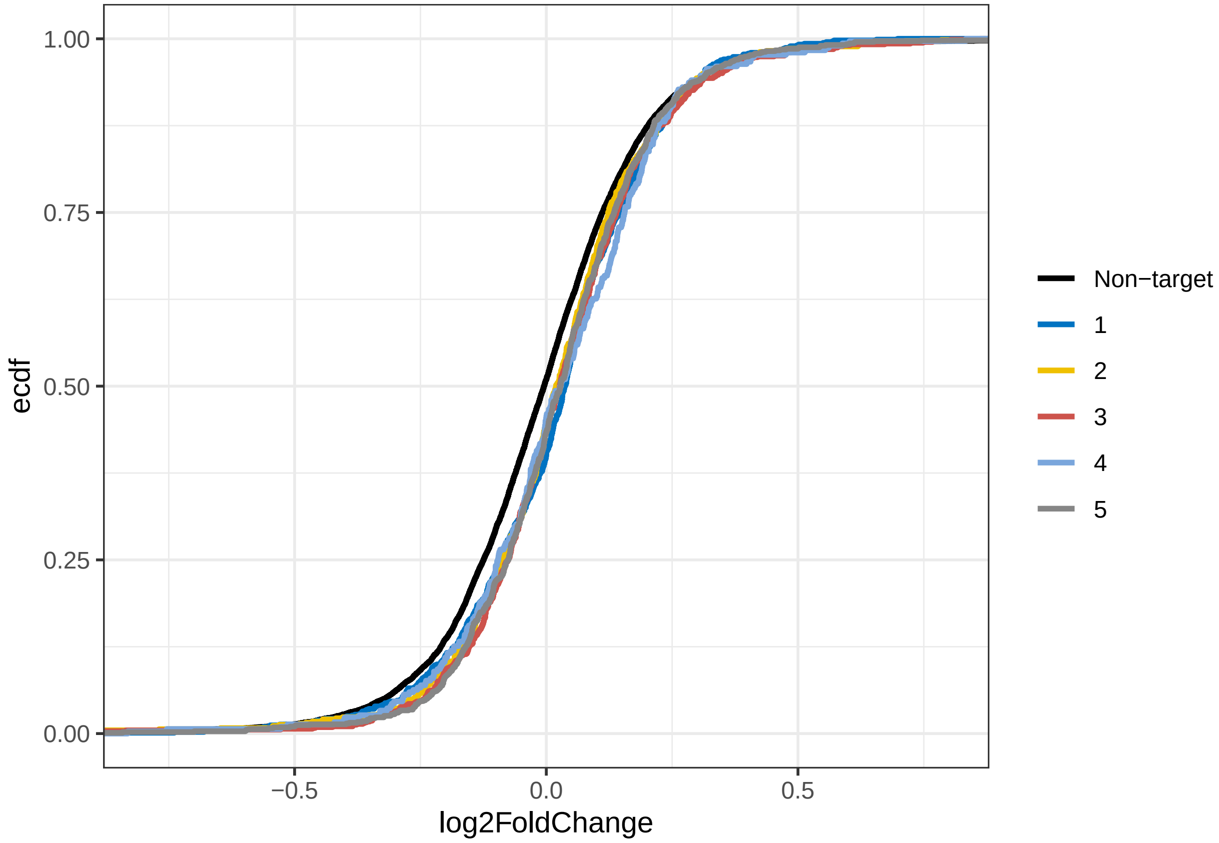
```
table(RPF$`heat_ksA$cluster`)
```

```
## 
##          1          2          3          4          5 Non-target 
##        837        629        858        296        885       7864 
```

plot ecdf

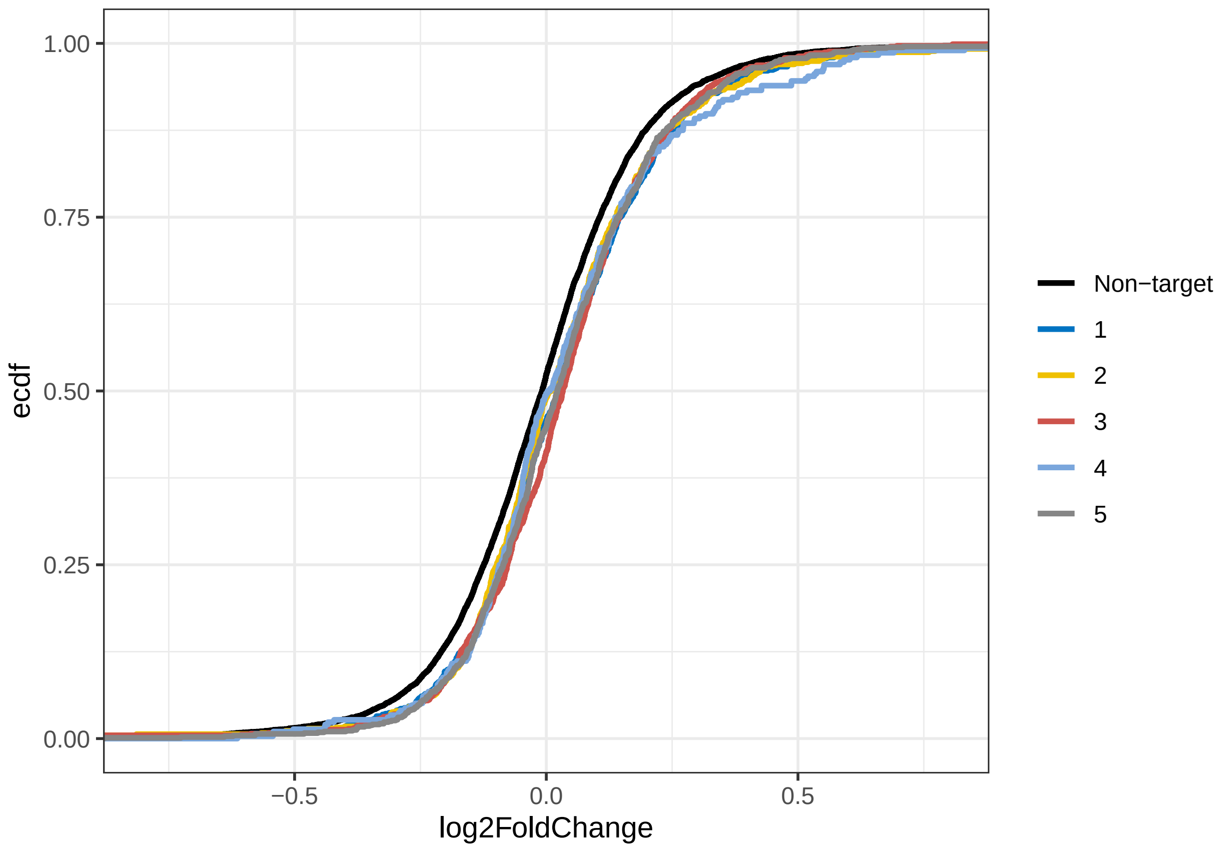
```
#RNA
RNAnumplot = ggplot(RNA, aes(log2FoldChange, colour=factor(`heat_ksA$cluster`,
                                                                 levels = c("Non-target", "1", "2", "3"
  stat_ecdf(geom = "step", linewidth=1) +
  coord_cartesian(xlim = c(-0.8, 0.8)) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4, farbe5)) +
  theme_bw() +
  theme(legend.title = element_blank())

RNAnumplot
```



```
#RPF
RPFnumplot = ggplot(RPF, aes(log2FoldChange, colour=factor(`heat_ksA$cluster`,
                                                                 levels = c("Non-target", "1", "2", "3"
  stat_ecdf(geom = "step", linewidth=1) +
  coord_cartesian(xlim = c(-0.8, 0.8)) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4, farbe5)) +
  theme_bw() +
  theme(legend.title = element_blank())

RPFnumplot
```



session info

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=German_Germany.utf8  LC_CTYPE=German_Germany.utf8
## [3] LC_MONETARY=German_Germany.utf8 LC_NUMERIC=C
## [5] LC_TIME=German_Germany.utf8
##
## attached base packages:
## [1] grid      stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] ComplexHeatmap_2.15.2              circlize_0.4.15
##  [3] seqinr_4.2-30                      ggplot2_3.4.2
##  [5] dplyr_1.1.1                        BSgenome.Mmusculus.UCSC.mm10_1.4.3
##  [7] BSgenome_1.66.3                    rtracklayer_1.58.0
##  [9] Biostrings_2.66.0                  XVector_0.38.0
## [11] GenomicRanges_1.50.2              GenomeInfoDb_1.34.9
## [13] IRanges_2.32.0                    S4Vectors_0.36.2
## [15] BiocGenerics_0.44.0
##
## loaded via a namespace (and not attached):
##  [1] MatrixGenerics_1.10.0       Biobase_2.58.0
##  [3] foreach_1.5.2               highr_0.10
##  [5] GenomeInfoDbData_1.2.9      Rsamtools_2.14.0
##  [7] yaml_2.3.7                  pillar_1.9.0
##  [9] lattice_0.20-45             glue_1.6.2
## [11] digest_0.6.31               RColorBrewer_1.1-3
## [13] colorspace_2.1-0            htmltools_0.5.4
## [15] Matrix_1.5-3                XML_3.99-0.14
## [17] pkgconfig_2.0.3             GetoptLong_1.0.5
## [19] magick_2.7.4                zlibbioc_1.44.0
## [21] scales_1.2.1                BiocParallel_1.32.6
## [23] tibble_3.2.1                farver_2.1.1
## [25] generics_0.1.3              withr_2.5.0
## [27] SummarizedExperiment_1.28.0 cli_3.6.0
## [29] magrittr_2.0.3              crayon_1.5.2
## [31] evaluate_0.20               fansi_1.0.4
## [33] doParallel_1.0.17           MASS_7.3-58.2
## [35] Cairo_1.6-0                 tools_4.2.3
## [37] GlobalOptions_0.1.2         BiocIO_1.8.0
## [39] lifecycle_1.0.3             matrixStats_0.63.0
## [41] mgsub_1.7.3                 munsell_0.5.0
## [43] cluster_2.1.4               DelayedArray_0.23.2
## [45] ade4_1.7-22                 compiler_4.2.3
## [47] rlang_1.1.0                 RCurl_1.98-1.12
## [49] iterators_1.0.14            rstudioapi_0.14
## [51] rjson_0.2.21                labeling_0.4.2
## [53] bitops_1.0-7                rmarkdown_2.21
## [55] restfulr_0.0.15             gtable_0.3.3
## [57] codetools_0.2-19            R6_2.5.1
## [59] GenomicAlignments_1.34.1    knitr_1.42
## [61] fastmap_1.1.1               utf8_1.2.3
## [63] clue_0.3-64                 shape_1.4.6
## [65] parallel_4.2.3              Rcpp_1.0.10
## [67] vctrs_0.6.1                 png_0.1-8
## [69] tidyselect_1.2.0            xfun_0.37
```