

# RNAhybrid\_heatmaps\_fig2-25 elite set

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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

```

```
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
```

```
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))
## =====
```

```
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/res")
reslistB <- list.files(path = "D:/Krueger_Lab/Publications/miR181_paper_nongithub/Figure2/RNAhybrid/res")

#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_bases")
colnames(resframeB) <- c("rownumber", "mfs", "pvalue", "start_position", "binding_bases", "non_binding_bases")

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   binding_bases
## 1    12529 -17.9 0.911034         10      UGAG GC   GUC CA
## 2    12530 -21.5 0.243804         59      UGAG   U   CUGUCGC CU  UACA
## 3    12531 -19.9 0.517782         57      GAGUGG CUGU G   ACU  UACAA
## 4    12532 -27.7 0.006767         29              AGU GGCUGUCG ACUUAC
## 5    12533 -19.0 0.713812         41      GAGUGGCU   GCA   ACU   UACAA
## 6    12534 -16.1 0.999191         41      UGAG GG  UGUCG  ACUU   ACAA
##
##               non_binding_bases
## 1              UG  U      G  ACUUACAA
## 2              GG      AA      A
## 3      U              C  CA
## 4              UG              CA      AA
## 5  U              GUC
## 6      U  C      CA
```

```
head(resframeB)
```

```
##   rownumber   mfs   pvalue start_position   binding_bases
## 1    12529 -17.9 0.911034         10      UGAG GC   GUC CA
```

```
## 2      12530 -21.5 0.243804      59  UGAG      U  CUGUCGC  CU  UACA
## 3      12531 -19.9 0.517782      57      GAGUGG CUGU  G   ACU  UACAA
## 4      12532 -27.7 0.006767      29      AGU  GGCUGUCG  ACUUAC
## 5      12533 -19.0 0.713812      41  GAGUGGCU   GCA   ACU   UACAA
## 6      12534 -16.1 0.999191      41   UGAG GG  UGUCG   ACUU   ACAA

##              non_binding_bases
## 1              UG  U      G  ACUUACAA
## 2              GG      AA      A
## 3      U              C  CA
## 4              UG              CA      AA
## 5 U              GUC
## 6      U  C      CA

# 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.rds"))
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 <- "#008EAOFF"
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 the mirna in the correct order. For that we will remove all gaps that originate 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)
Alist <- lapply(Alist, as.data.frame)

Blist <- Map(cbind, Blistbb, Blistnb)
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] "UGAG GC GUC CA      " "UGAGU CUGUCGC CUUACA "
## [3] " GAGUGGCUGU G ACUUACAA" " AGUGGCUGUCG ACUUAC "
## [5] " GAGUGGCU GCAACUUACAA" "UGAG GG UGUCG ACUUACAA"

```

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

```
## [1] "UGAG GC GUC CA      " "UGAGU CUGUCGC CUUACA "
## [3] " GAGUGGCUGU G ACUUACAA" " AGUGGCUGUCG ACUUAC "
## [5] " GAGUGGCU GCAACUUACAA" "UGAG GG UGUCG ACUUACAA"
```

## 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", 4), rep("0", 16)))
resframeB$binding_nospace <- mgsub::mgsub(resframeB$binding_nospace, c("A", "U", "C", "G"), c(rep("1", 4), rep("0", 16)))
```

```
head(resframeA)
```

```
##   rownumber   mfs   pvalue start_position binding_bases
## 1      12529 -17.9 0.911034          10      UGAG GC GUC CA
## 2      12530 -21.5 0.243804          59      UGAG   U CUGUCGC CU UACA
## 3      12531 -19.9 0.517782          57      GAGUGG CUGU G ACU UACAA
## 4      12532 -27.7 0.006767          29      AGU GGCUGUCG ACUUAC
## 5      12533 -19.0 0.713812          41      GAGUGGCU GCA ACU UACAA
## 6      12534 -16.1 0.999191          41      UGAG GG UGUCG ACUU ACAA
##                                     non_binding_bases binding_nospace
## 1                UG U      G ACUUACAA 111100110111011000000000
## 2                GG      AA      A 11111001111111001111110
## 3      U                C CA      01111111111010011111111
## 4      UG                CA      AA 00111111111110011111100
## 5 U      GUC                01111111100011111111111
## 6      U C      CA      11110110111110011111111
```

```
head(resframeB)
```

```
##   rownumber   mfs   pvalue start_position binding_bases
## 1      12529 -17.9 0.911034          10      UGAG GC GUC CA
## 2      12530 -21.5 0.243804          59      UGAG   U CUGUCGC CU UACA
## 3      12531 -19.9 0.517782          57      GAGUGG CUGU G ACU UACAA
## 4      12532 -27.7 0.006767          29      AGU GGCUGUCG ACUUAC
## 5      12533 -19.0 0.713812          41      GAGUGGCU GCA ACU UACAA
## 6      12534 -16.1 0.999191          41      UGAG GG UGUCG ACUU ACAA
##                                     non_binding_bases binding_nospace
## 1                UG U      G ACUUACAA 111100110111011000000000
## 2                GG      AA      A 11111001111111001111110
## 3      U                C CA      01111111111010011111111
## 4      UG                CA      AA 00111111111110011111100
```

```
## 5 U      GUC      0111111110001111111111
## 6      U C      CA  1111011011111001111111
```

## 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
## 12529 1 1 1 1 0 0 1 1 0 1 1 1 0 1 1 0 0 0 0 0 0 0
## 12530 1 1 1 1 1 0 0 1 1 1 1 1 1 1 0 0 1 1 1 1 1 0
## 12531 0 1 1 1 1 1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1
## 12532 0 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 0 0
## 12533 0 1 1 1 1 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 1 1
## 12534 1 1 1 1 0 1 1 0 1 1 1 1 1 0 0 1 1 1 1 1 1 1
```

```
heatframeB <- do.call(rbind.data.frame, strsplit(resframeB$binding_nospace,""))
heatframeB <- sapply( heatframeB, as.numeric )
colnames(heatframeB) <- c(23:1)
rownames(heatframeB) <- resframeB[,1]
head(heatframeB)
```

```
##      23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
## 12529 1 1 1 1 0 0 1 1 0 1 1 1 0 1 1 0 0 0 0 0 0 0
## 12530 1 1 1 1 1 0 0 1 1 1 1 1 1 1 0 0 1 1 1 1 1 0
## 12531 0 1 1 1 1 1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1
## 12532 0 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 0 0
## 12533 0 1 1 1 1 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 1 1
## 12534 1 1 1 1 0 1 1 0 1 1 1 1 1 0 0 1 1 1 1 1 1 1
```

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

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

```
##      1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
## miR181a 107 445 702 697 778 855 922 690 572 737 1095 1146 1120 1064 1015 974
## miR181b 107 445 702 697 778 855 922 690 572 737 1095 1146 1120 1064 1015 974
##      17 18 19 20 21 22 23
```



```
## miR181a 1092 1077 1071 1068 909 885 609
## miR181b 1092 1077 1071 1068 909 885 609
#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"))
```

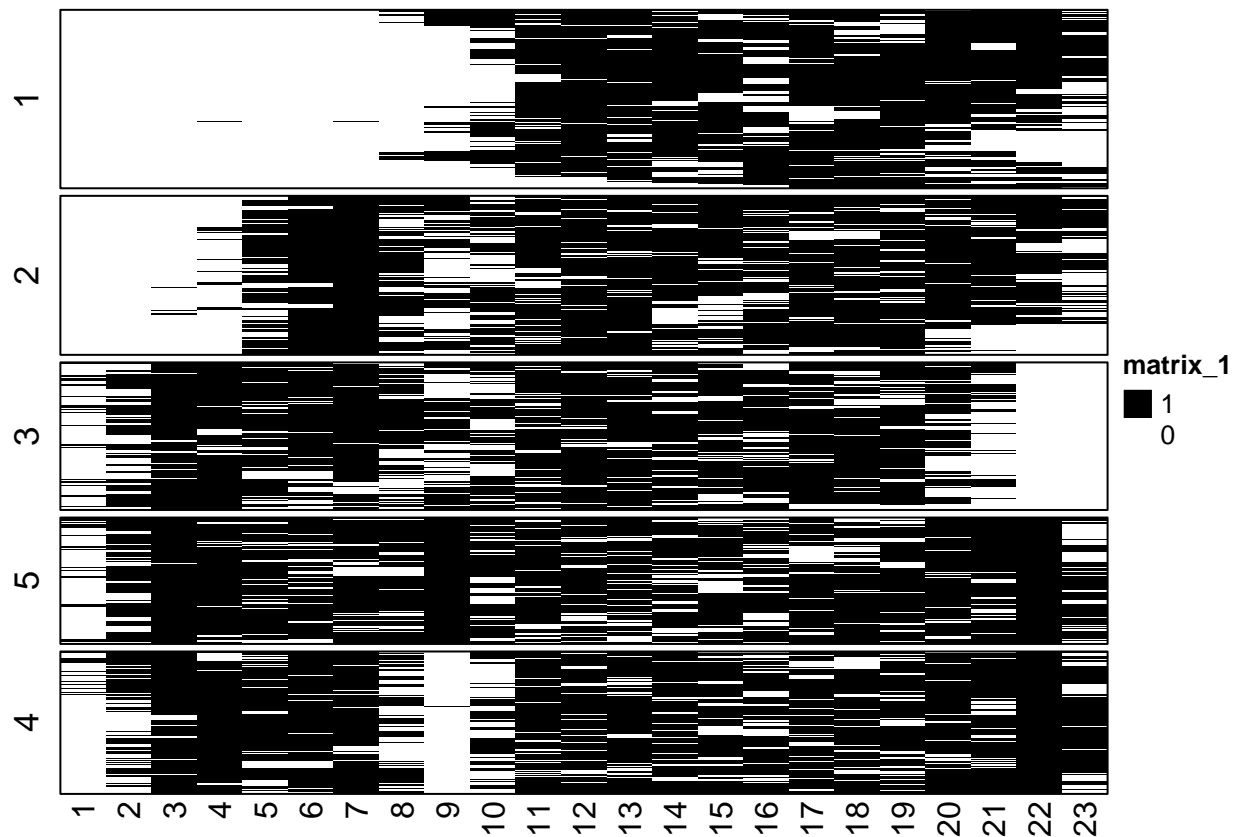
## Heatmap of all the single reads

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

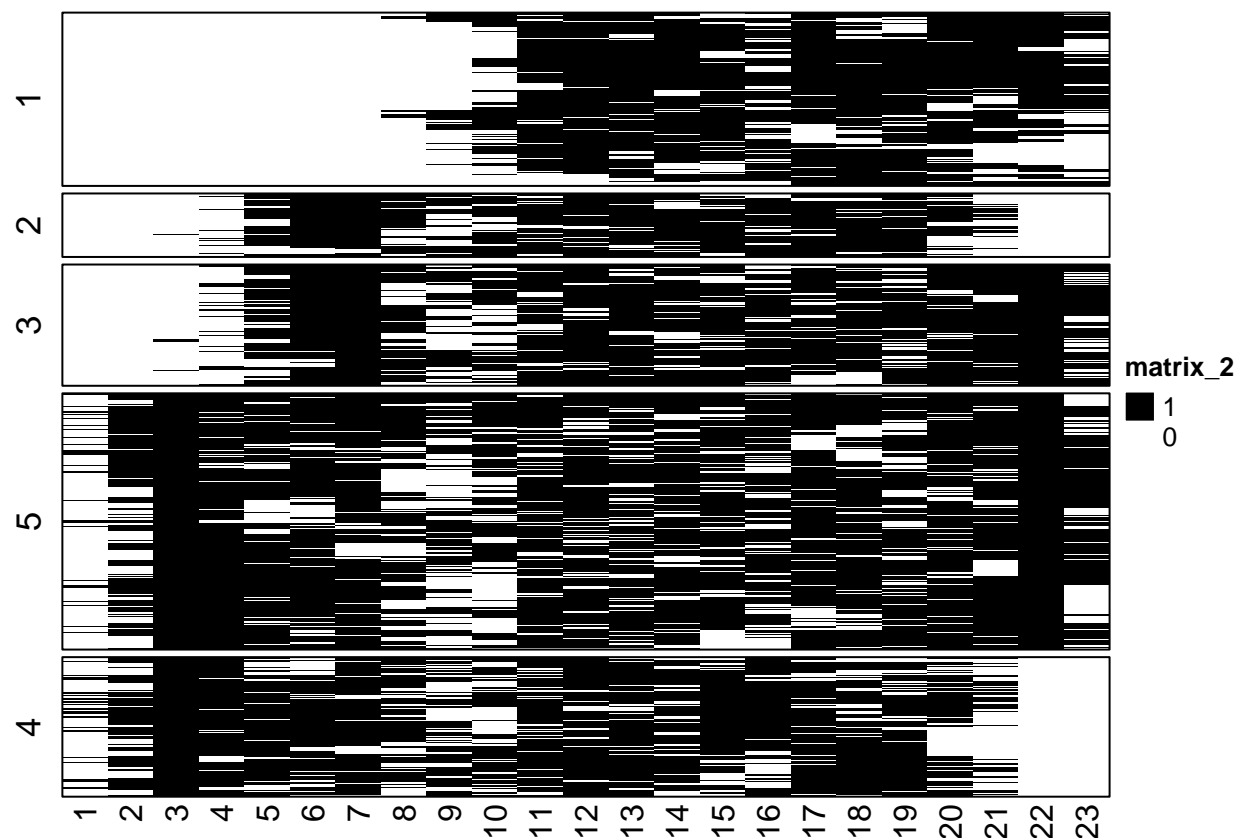
No B

HMA



B

HMB



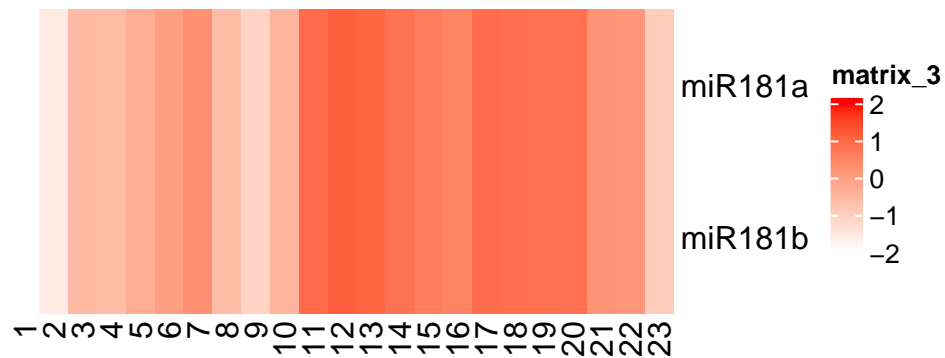
## “Heatmap” of combined reads for mir\_181a and b

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

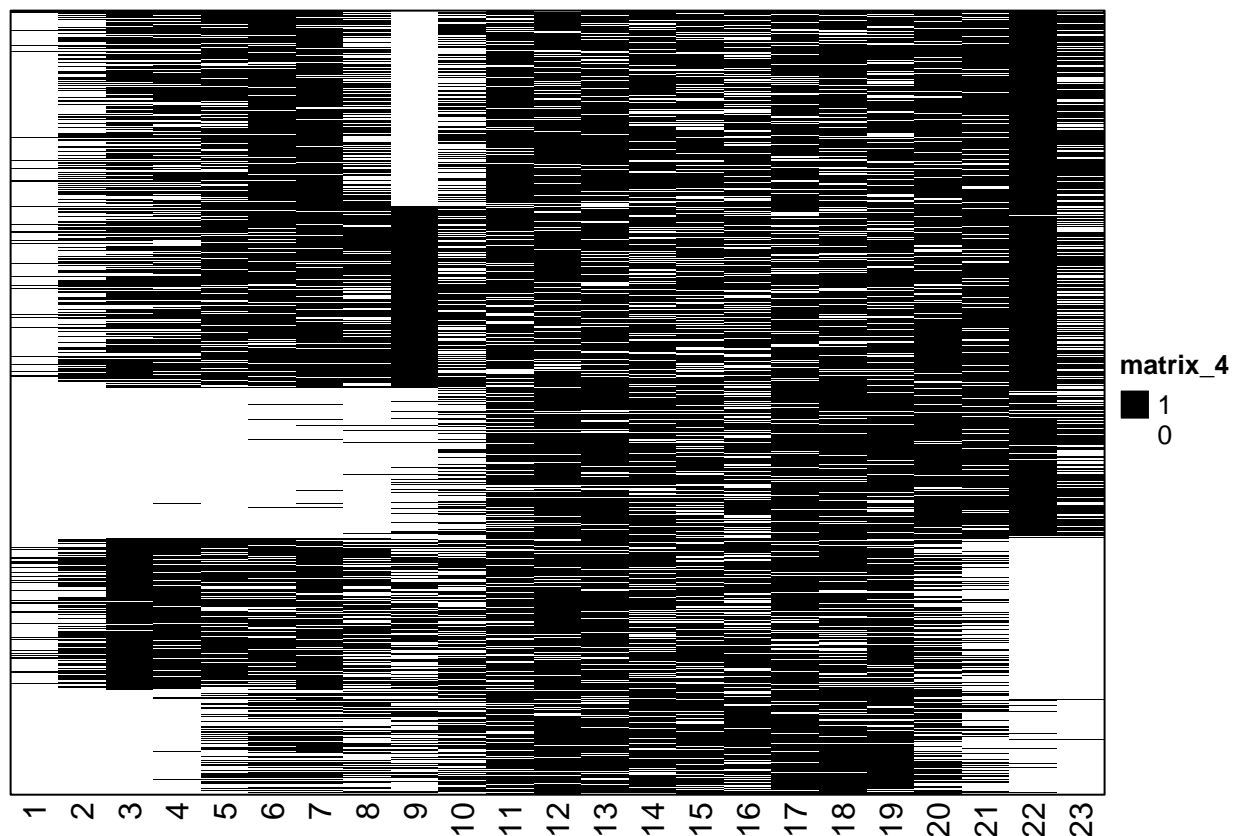
```
#cluster by seed area
heat_ksA <- kmeans(heatframeA, centers = 5)
heat_k_namesA <- as.data.frame(heat_ksA$cluster)
#merge back with full data and adjust frame again
cframeA <- merge(heatframeA, heat_k_namesA, by=0)
rownames(cframeA) <- cframeA$Row.names
cframeA <- cframeA[,-1]

#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
## 12530 0 1 1 1 1 1 1 0 0  1  1  1  1  1  1  0  0  1  1  1  1  1
## 12531 1 1 1 1 1 1 1 1 0  0  1  0  1  1  1  1  1  1  1  1  1  0
## 12534 1 1 1 1 1 1 1 1 0  0  1  1  1  1  1  0  1  1  0  1  1  1
## 12539 1 1 1 1 1 1 1 0 0  1  1  1  1  1  1  1  1  1  1  1  1  1
## 12540 0 0 0 0 1 1 1 0 0  1  0  1  1  0  0  1  0  1  1  1  1  1
## 12542 0 1 1 1 1 1 1 1 0  1  1  1  1  1  0  1  1  1  1  0  1  1
```

### plot clustered seperately

```
HMAsep <- Heatmap(cframeAp, cluster_columns = F, cluster_rows = F, col = hmcols1, show_row_names = F, s
## Warning: The input is a data frame-like object, convert it to a matrix.
HMAsep
```



## clustering by seed region

try to cluster separately 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)
```

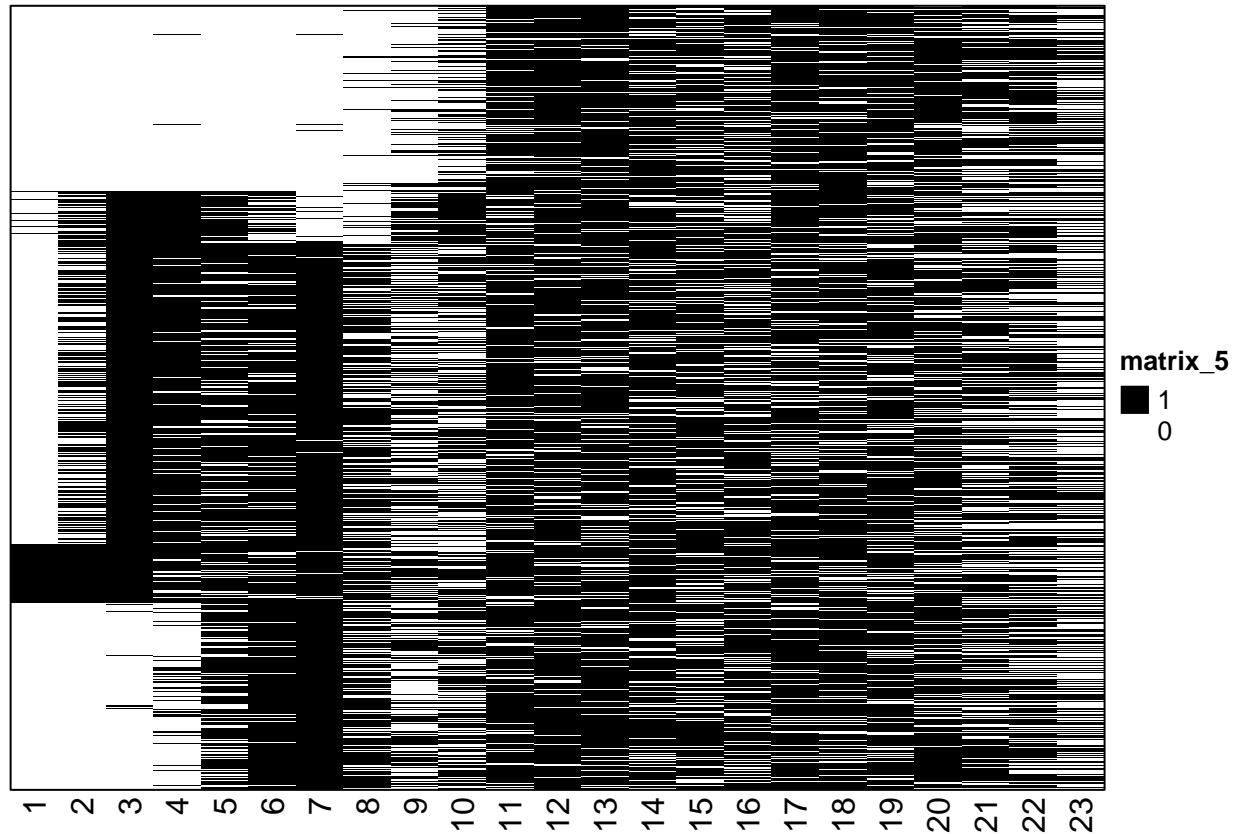
```
##      1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
## 12529 0 0 0 0 0 0 0 0 1 1 0 1 1 1 0 1 1 0 0 1 1 1 1
## 12547 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 0
## 12551 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 1 1 1 1 1 1 1
## 12559 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1
## 12563 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 1 1 1 1 1 1 1 1
## 12569 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 1 1 0 1 1 1 1 1
```

## plot clustered by seed region

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

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

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 Pcmt1 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 WT KO
## 1 1 1 0 0 0 ago_bs_mir181_chi 1 1
## 2 5 5 0 0 0 ago_bs_mir181_chi 1 1
## 3 6 6 0 0 0 ago_bs_mir181_chi 1 0
## 4 6 6 0 0 0 ago_bs_mir181_chi 1 1
## 5 4 4 0 0 0 ago_bs_mir181_chi 1 1
## 6 1 1 0 0 0 ago_bs_mir181_chi NA NA
## geneID.2 geneName.1 region.1 counts.bs.1_KO counts.bs.2_KO
## 1 ENSMUSG00000025907 Rb1cc1 cds 4 3
## 2 ENSMUSG00000025907 Rb1cc1 cds 28 32
## 3 ENSMUSG00000025907 Rb1cc1 cds 13 11
## 4 ENSMUSG00000025907 Rb1cc1 cds 15 15
## 5 ENSMUSG00000051285 Pcmt1 utr3 12 22
## 6 <NA> <NA> <NA> NA NA
## counts.bs.3_KO counts.bs.4_WT counts.bs.5_WT counts.bs.6_WT
## 1 3 3 10 3
## 2 27 46 41 20
## 3 4 22 13 12
## 4 10 33 20 18
## 5 14 16 20 9
## 6 NA NA NA NA
## geneID.1 counts.bg.1_KO counts.bg.2_KO counts.bg.3_KO
## 1 ENSMUSG00000025907 1609 1973 1250
## 2 ENSMUSG00000025907 1609 1973 1250
## 3 ENSMUSG00000025907 1609 1973 1250
## 4 ENSMUSG00000025907 1609 1973 1250
## 5 ENSMUSG00000051285 1355 1706 1064
## 6 <NA> NA NA NA
## counts.bg.4_WT counts.bg.5_WT counts.bg.6_WT resBs.baseMean
## 1 2638 2231 1352 92.10645
## 2 2638 2231 1352 281.53271
## 3 2638 2231 1352 145.51107
## 4 2638 2231 1352 186.74162
## 5 1654 1348 755 151.36245
## 6 NA NA NA NA
## resBs.log2FoldChange resBs.lfcSE resBs.stat resBs.pvalue resBs.padj
## 1 -0.1093039 0.5923673 0.03419066 0.8533018 0.9652601
## 2 0.2749428 0.2351157 1.35874137 0.2437557 0.6729889
## 3 -0.1805519 0.3623758 0.25017050 0.6169550 0.8961239
## 4 -0.2606282 0.3062717 0.73169661 0.3923338 0.7868678
## 5 0.1466485 0.3122905 0.22052922 0.6386370 0.9013566
## 6 NA NA NA NA NA

```

```
##   resBg.baseMean resBg.log2FoldChange resBg.lfcSE resBg.stat resBg.pvalue
## 1              NA                  NA          NA          NA          NA
## 2              NA                  NA          NA          NA          NA
## 3              NA                  NA          NA          NA          NA
## 4              NA                  NA          NA          NA          NA
## 5              NA                  NA          NA          NA          NA
## 6              NA                  NA          NA          NA          NA
##   resBg.padj tpm.counts.bg.1_KO tpm.counts.bg.2_KO tpm.counts.bg.3_KO
## 1          NA          133.7259          117.9980          129.8669
## 2          NA          133.7259          117.9980          129.8669
## 3          NA          133.7259          117.9980          129.8669
## 4          NA          133.7259          117.9980          129.8669
## 5          NA          248.6210          225.2505          244.0445
## 6          NA              NA              NA              NA
##   tpm.counts.bg.4_WT tpm.counts.bg.5_WT tpm.counts.bg.6_WT
## 1          139.8635          146.2855          163.5360
## 2          139.8635          146.2855          163.5360
## 3          139.8635          146.2855          163.5360
## 4          139.8635          146.2855          163.5360
## 5          193.5994          195.1330          201.6149
## 6              NA              NA              NA
##   BS_ID.1 tpm_support_KO tpm_support_WT tpm_supported down
## 1 ENSMUSG00000025907.bs5              3              3      TRUE FALSE
## 2 ENSMUSG00000025907.bs8              3              3      TRUE FALSE
## 3 ENSMUSG00000025907.bs10             3              3      TRUE FALSE
## 4 ENSMUSG00000025907.bs11             3              3      TRUE FALSE
## 5 ENSMUSG00000051285.bs4              3              3      TRUE FALSE
## 6      <NA>              NA              NA      NA      NA
## rownumber  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22
## 1          1 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 2          2 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 3          3 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 4          4 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 5          5 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 6          6 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
##   23 heat_ksA$cluster
## 1 NA              NA
## 2 NA              NA
## 3 NA              NA
## 4 NA              NA
## 5 NA              NA
## 6 NA              NA
```

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 ENSMUSG000000069306.5 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 WT KO      geneID.2 geneName.1
## 1      NA      NA      <NA> NA NA      <NA>      <NA>
## 2      NA      NA      mir181_enriched NA NA      <NA>      <NA>
## 3      NA      NA      <NA> NA NA      <NA>      <NA>
## 4      NA      NA      <NA> NA NA      <NA>      <NA>
## 5      0      0 ago_bs_mir181_chi 1 0 ENSMUSG00000004552      Ctse
## 6      NA      NA      <NA> NA NA      <NA>      <NA>
##      region.1 counts.bs.1_KO counts.bs.2_KO counts.bs.3_KO counts.bs.4_WT
## 1      <NA>      NA      NA      NA      NA
## 2      <NA>      NA      NA      NA      NA
## 3      <NA>      NA      NA      NA      NA
## 4      <NA>      NA      NA      NA      NA
## 5      cds      0      0      1      15
## 6      <NA>      NA      NA      NA      NA
##      counts.bs.5_WT counts.bs.6_WT      geneID.1 counts.bg.1_KO
## 1      NA      NA      <NA>      NA
## 2      NA      NA      <NA>      NA
## 3      NA      NA      <NA>      NA
## 4      NA      NA      <NA>      NA
## 5      9      6 ENSMUSG00000004552      431
## 6      NA      NA      <NA>      NA
##      counts.bg.2_KO counts.bg.3_KO counts.bg.4_WT counts.bg.5_WT counts.bg.6_WT
## 1      NA      NA      NA      NA      NA

```



```

## 2      NA      NA      NA      NA      NA
## 3      NA      NA      NA      NA      NA
## 4      NA      NA      NA      NA      NA
## 5      735      417      1560      1199      558
## 6      NA      NA      NA      NA      NA
##      resBs.baseMean resBs.log2FoldChange resBs.lfcSE resBs.stat resBs.pvalue
## 1      NA      NA      NA      NA      NA
## 2      NA      NA      NA      NA      NA
## 3      NA      NA      NA      NA      NA
## 4      NA      NA      NA      NA      NA
## 5      57.03841      -3.535302      1.220067      13.8548      0.000197492
## 6      NA      NA      NA      NA      NA
##      resBs.padj resBg.baseMean resBg.log2FoldChange resBg.lfcSE resBg.stat
## 1      NA      NA      NA      NA      NA
## 2      NA      NA      NA      NA      NA
## 3      NA      NA      NA      NA      NA
## 4      NA      NA      NA      NA      NA
## 5      0.009740614      NA      NA      NA      NA
## 6      NA      NA      NA      NA      NA
##      resBg.pvalue resBg.padj tpm.counts.bg.1_KO tpm.counts.bg.2_KO
## 1      NA      NA      NA      NA
## 2      NA      NA      NA      NA
## 3      NA      NA      NA      NA
## 4      NA      NA      NA      NA
## 5      NA      NA      165.9967      203.7031
## 6      NA      NA      NA      NA
##      tpm.counts.bg.3_KO tpm.counts.bg.4_WT tpm.counts.bg.5_WT tpm.counts.bg.6_WT
## 1      NA      NA      NA      NA
## 2      NA      NA      NA      NA
## 3      NA      NA      NA      NA
## 4      NA      NA      NA      NA
## 5      200.7646      383.2804      364.3204      312.776
## 6      NA      NA      NA      NA
##      BS_ID.1 tpm_support_KO tpm_support_WT tpm_supported down
## 1      <NA>      NA      NA      NA      NA
## 2      <NA>      NA      NA      NA      NA
## 3      <NA>      NA      NA      NA      NA
## 4      <NA>      NA      NA      NA      NA
## 5      ENSMUSG00000004552.bs4      3      3      TRUE TRUE
## 6      <NA>      NA      NA      NA      NA
##      rownumber 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
## 1      NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 2      7405 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 3      NA NA NA NA NA NA NA NA 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 NA NA NA NA NA NA NA NA
## 5      103 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 6      NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
##      23 heat_ksA$cluster
## 1 NA      Non-target
## 2 NA      Non-target
## 3 NA      Non-target
## 4 NA      Non-target
## 5 NA      Non-target
## 6 NA      Non-target

```

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

```
##
##          1          2          3          4          5 Non-target
##         19          9         20         14         11        13228
```

```
#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 ENSMUSG000000000001.4 3788.57802      0.05462417 0.05957247 0.9169365
## 2 2 ENSMUSG000000000028.15 1086.36203     -0.12956555 0.07692175 -1.6843811
## 3 3 ENSMUSG000000000037.17  19.45454     -0.06042604 0.15784031 -0.3828302
## 4 4 ENSMUSG000000000056.7 1454.64304      0.05456272 0.08781406 0.6213438
## 5 5 ENSMUSG000000000078.7  850.27809      0.65975317 0.07971920 8.2759636
## 6 6 ENSMUSG000000000085.16 337.25837     -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 ENSMUSG000000000001
## 2     NA <NA>      NA      NA      NA      <NA>      <NA>
## 3      7      + 409.94460 136.64820 268.27800 protein_coding ENSMUSG000000000037
## 4      7      +  54.16402 10.83280 20.82290 protein_coding ENSMUSG000000000056
## 5      7      +  31.50993 10.50331 12.38550 protein_coding ENSMUSG000000000078
## 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 WT KO      geneID.2 geneName.1 region.1 counts.bs.1_KO
## 1 ago_bs_mir181_chi 1 0 ENSMUSG000000000001      Gnai3      cds      2
## 2      <NA> NA NA      <NA>      <NA>      <NA>      NA
## 3 mir181_enriched NA NA      <NA>      <NA>      <NA>      NA
## 4 ago_bs_mir181_chi 1 0 ENSMUSG000000000056      Narf      utr5      9
## 5 ago_bs_mir181_chi 1 1 ENSMUSG000000000078      Klf6      utr3      4
## 6      <NA> NA NA      <NA>      <NA>      <NA>      NA
##      counts.bs.2_KO counts.bs.3_KO counts.bs.4_WT counts.bs.5_WT counts.bs.6_WT
```

## 1	5	1	9	4	0
## 2	NA	NA	NA	NA	NA
## 3	NA	NA	NA	NA	NA
## 4	10	3	33	24	17
## 5	1	4	11	7	5
## 6	NA	NA	NA	NA	NA
##	geneID.1	counts.bg.1_KO	counts.bg.2_KO	counts.bg.3_KO	
## 1	ENSMUSG00000000001	781	932	606	
## 2	<NA>	NA	NA	NA	
## 3	<NA>	NA	NA	NA	
## 4	ENSMUSG00000000056	747	973	503	
## 5	ENSMUSG00000000078	319	419	249	
## 6	<NA>	NA	NA	NA	
##	counts.bg.4_WT	counts.bg.5_WT	counts.bg.6_WT	resBs.baseMean	
## 1	1137	948	500	47.01432	
## 2	NA	NA	NA	NA	
## 3	NA	NA	NA	NA	
## 4	1110	1035	543	128.71190	
## 5	607	466	244	48.53992	
## 6	NA	NA	NA	NA	
##	resBs.log2FoldChange	resBs.lfcSE	resBs.stat	resBs.pvalue	resBs.padj
## 1	-0.3611064	0.6582148	0.3064341	0.5798766299	0.88338480
## 2	NA	NA	NA	NA	NA
## 3	NA	NA	NA	NA	NA
## 4	-1.2675269	0.3692805	13.1695749	0.0002845313	0.01292422
## 5	-0.7513615	0.5789425	1.7903402	0.1808847223	0.60039272
## 6	NA	NA	NA	NA	NA
##	resBg.baseMean	resBg.log2FoldChange	resBg.lfcSE	resBg.stat	resBg.pvalue
## 1	NA	NA	NA	NA	NA
## 2	NA	NA	NA	NA	NA
## 3	NA	NA	NA	NA	NA
## 4	783.0873	-0.1165693	0.09866149	-1.181507	0.23740125
## 5	363.8138	-0.2226921	0.10871231	-2.048453	0.04051564
## 6	NA	NA	NA	NA	NA
##	resBg.padj	tpm.counts.bg.1_KO	tpm.counts.bg.2_KO	tpm.counts.bg.3_KO	
## 1	NA	292.31323	251.01601	283.52995	
## 2	NA	NA	NA	NA	
## 3	NA	NA	NA	NA	
## 4	0.4626108	189.76592	177.86829	159.73294	
## 5	0.1364387	88.69694	83.83397	86.54584	
## 6	NA	NA	NA	NA	
##	tpm.counts.bg.4_WT	tpm.counts.bg.5_WT	tpm.counts.bg.6_WT		
## 1	271.4737	279.9290	272.36080		
## 2	NA	NA	NA		
## 3	NA	NA	NA		
## 4	179.8832	207.4341	200.75881		
## 5	107.6655	102.2224	98.73814		
## 6	NA	NA	NA		
##	BS_ID.1	tpm_support_KO	tpm_support_WT	tpm_supported	down
## 1	ENSMUSG00000000001.bs2	3	3	TRUE	FALSE
## 2	<NA>	NA	NA	NA	NA
## 3	<NA>	NA	NA	NA	NA
## 4	ENSMUSG00000000056.bs1	3	3	TRUE	TRUE
## 5	ENSMUSG00000000078.bs1	3	3	TRUE	FALSE

```
## 6          <NA>          NA          NA          NA          NA
##  rownumber  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22
## 1      1261 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 2      NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 3     12209 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 4      4440 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 5      4954 NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 6      NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
## 23 heat_ksA$cluster
## 1 NA      Non-target
## 2 NA      Non-target
## 3 NA      Non-target
## 4 NA      Non-target
## 5 NA      Non-target
## 6 NA      Non-target
```

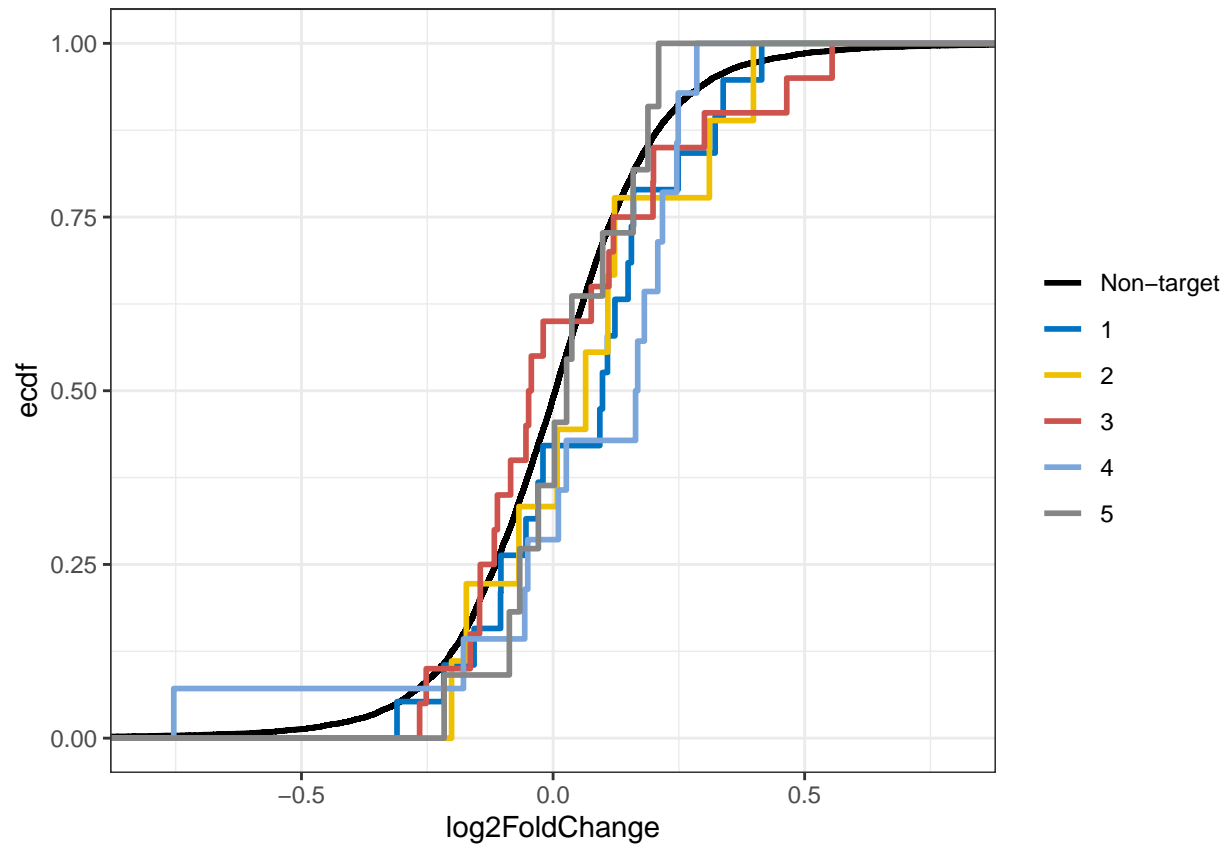
```
table(RPF$`heat_ksA$cluster`)
```

```
##
##      1      2      3      4      5 Non-target
##     19      9     20     14     10     11297
```

## 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()))

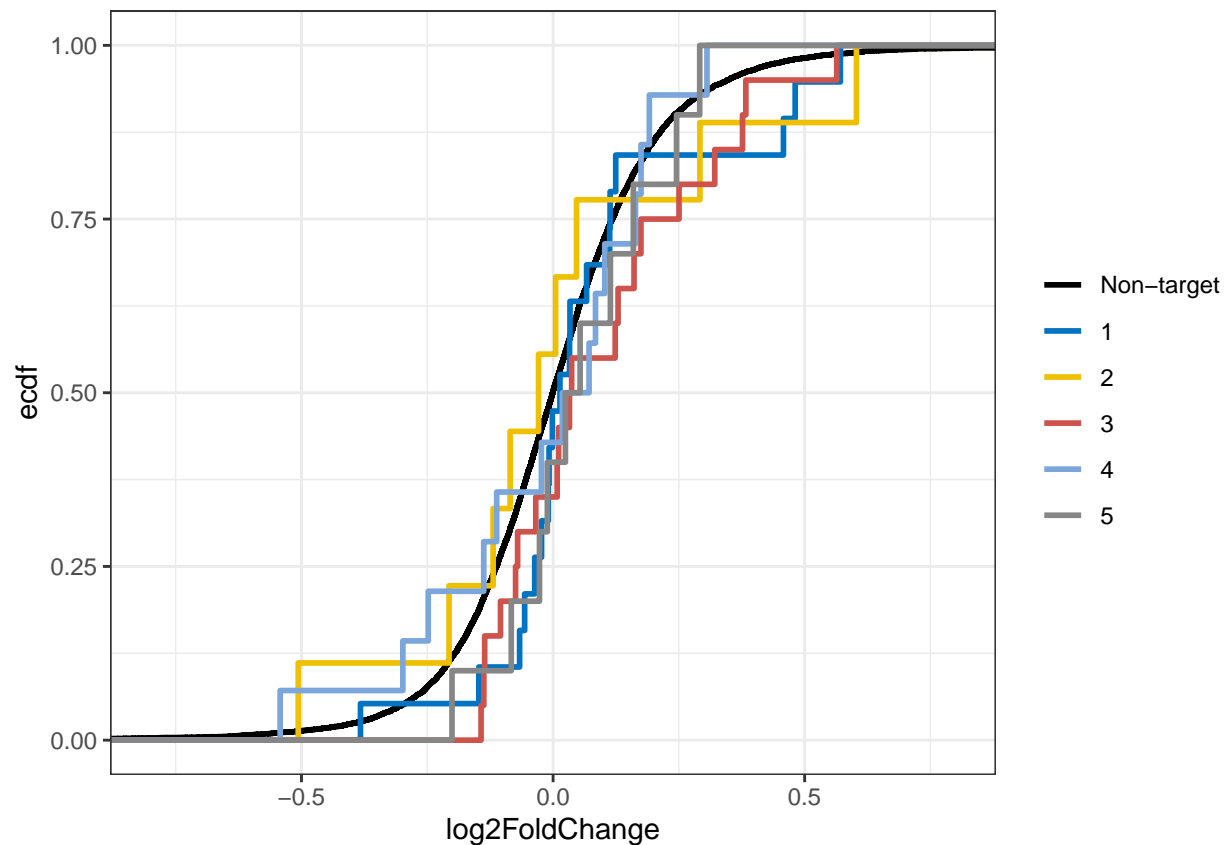
RNAnumplot
```



```
#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 19045)
##
## 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

```