mir181 binding sites - union of mir181 enriched binding sites and Ago binding sites targeted by mir181

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Contents

1 Libraries and settings

```
# ----------------------------------------
# libraries
# ----------------------------------------
library(tidyverse)
library(GenomicRanges)

# ----------------------------------------
# settings
# ----------------------------------------
out <- "/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/Figure1/mir181_
```

2 What was done?

mir181 binding sites are defined as the union of - AGO binding sites that contain at least 2 chimirc mir181 crosslinks (from the IP_WT chimeric reads or the IP_mir181_WT chimeric reads) in a window from 10nt before till 10nt after a the AGO binding site - binding sites defined on enriched mir181 data (IP_mir181_WT)

- the two subgroups are plotted as a venn diagram (figure 1 XX)
- this is compared to the differntially regulated AGO binding sites from the mir181 KO condition (TODO)
- the genetype and gene region of the mir 181 binding sites (union) are ploted (Figure2XX)

3 Files

```
# ----------------------------------------
# mir181 enriched binding sites
# ----------------------------------------
mir181_enriched <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_

# ----------------------------------------
# chimeric reads
# ----------------------------------------

chimeric_reads <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_pa

# ----------------------------------------
# AGO binding sites
# ----------------------------------------
ago_bs <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/Met
```

4 mir181 binding sites

4.1 Get AGO binding sites with chimeric mir181

```
# use region of bs +-10nt for overlaps
ago_bs_10 <- ago_bs + 10

# use chimeric reads from both mir181 enriched and non-enriched data
chimeric_reads <- c(makeGRangesFromDataFrame(chimeric_reads$IP_WT, keep.extra.columns = T), makeGRangesF

# find overlaps of mirt and AGO bs
idx <- findOverlaps(ago_bs_10, chimeric_reads )

# make a data frame from the ago bs
names(ago_bs)<- 1:NROW(ago_bs)
ago_bs <- as.data.frame(ago_bs)
ago_bs$BS_ID <- rownames(ago_bs)

# add mir info to ago bs
ago_bs_mir181_chi <- cbind(ago_bs[queryHits(idx),], mir_IP = chimeric_reads [subjectHits(idx),]$Name)

ago_bs_mir181_chi <- ago_bs_mir181_chi[grepl(ago_bs_mir181_chi$mir_IP,
                                     pattern = "miR-181"),]

# count chimerics
mir181_chi <- ago_bs_mir181_chi %>% group_by(BS_ID) %>%
  summarize(n_mir181 = sum(grepl(mir_IP,pattern = "miR-181")),
            n_mir181a = sum(grepl(mir_IP,pattern = "miR-181a")),
            n_mir181b = sum(grepl(mir_IP,pattern = "miR-181b")),
            n_mir181c = sum(grepl(mir_IP,pattern = "miR-181c")),
            n_mir181d = sum(grepl(mir_IP,pattern = "miR-181d")),
            .groups = "keep") %>% subset (n_mir181 >0)

ago_bs_mir181_chi <- ago_bs_mir181_chi %>%
  subset(!duplicated(ago_bs_mir181_chi$BS_ID)) %>%
```

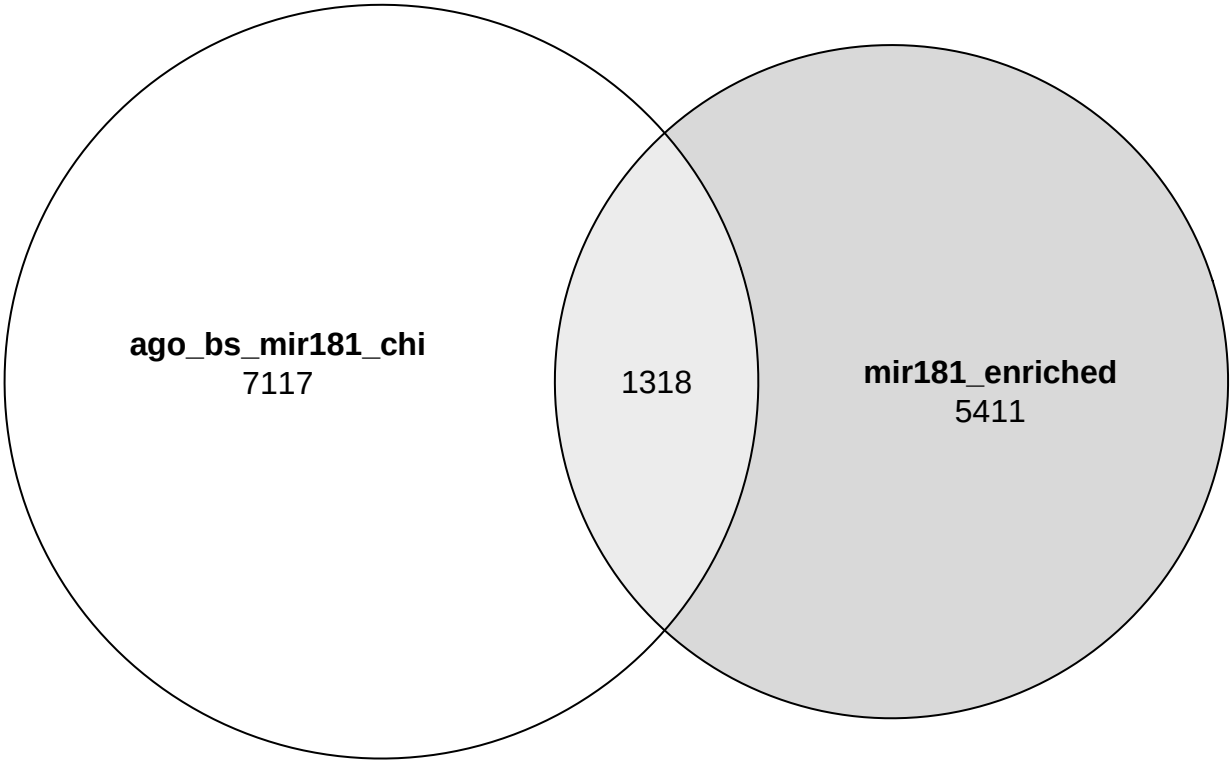
```
  left_join(., mir181_chi, by ="BS_ID") %>% makeGRangesFromDataFrame(keep.extra.columns = T)
```

4.2 Combine AGO binding sites with chimeric mir181 with mir181 enriched binding sites

```
only_ago_bs_mir181_chi <- subsetByOverlaps(ago_bs_mir181_chi, mir181_enriched, type = "any", invert = T)
only_ago_bs_mir181_chi$set <- "ago_bs_mir181_chi"

only_mir181_enriched <- subsetByOverlaps(mir181_enriched, ago_bs_mir181_chi,  type = "any", invert = T)
only_mir181_enriched$set <- "mir181_enriched"

both_mir181_enriched_chi <- subsetByOverlaps(ago_bs_mir181_chi, mir181_enriched, type = "any")
both_mir181_enriched_chi$set <- "ago_bs_mir181_chi&mir181_enriched"


mir181_bs <- c(only_ago_bs_mir181_chi, only_mir181_enriched, both_mir181_enriched_chi)
```

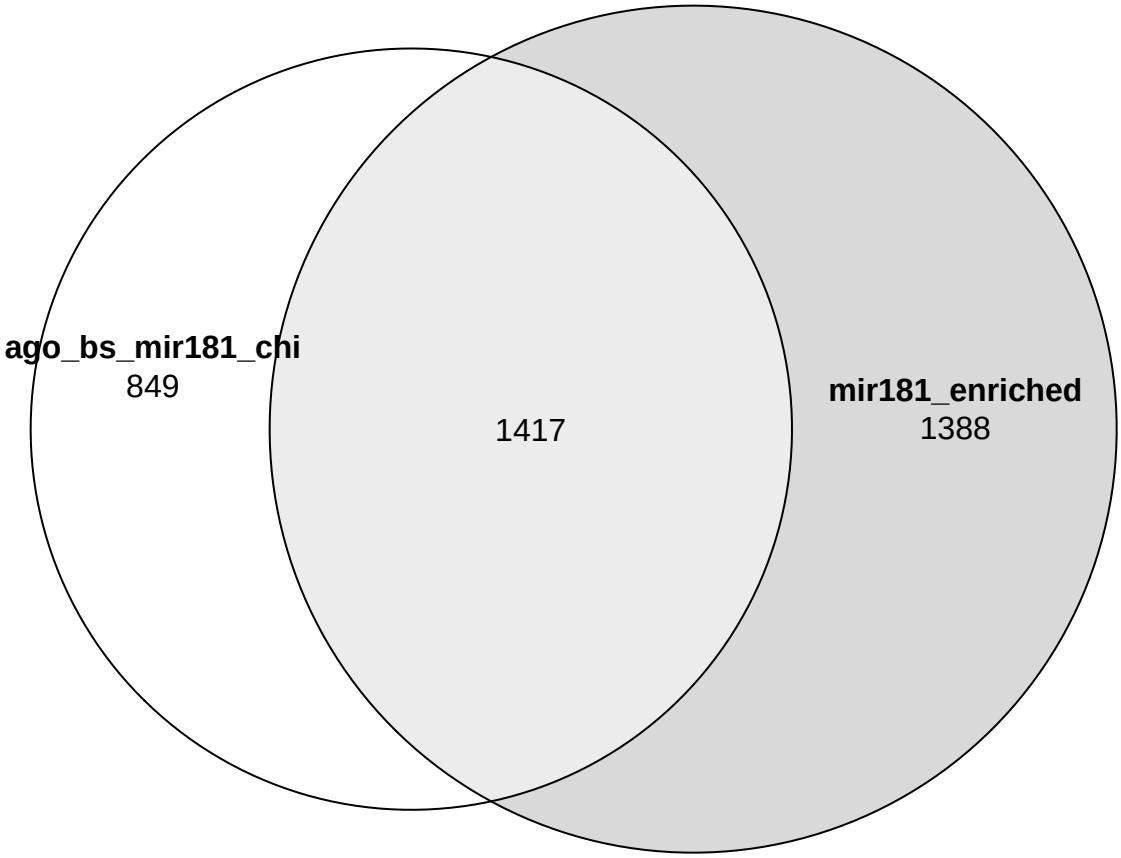
4.2.1 Venn binding sites from both sets

```
venn_df <- data.frame(ago_bs_mir181_chi =
                        ((mir181_bs$set == "ago_bs_mir181_chi") | (mir181_bs$set == "ago_bs_mir181_chi&m
                      mir181_enriched =
                        ((mir181_bs$set == "mir181_enriched") | (mir181_bs$set == "ago_bs_mir181_chi&mi

venn <- eulerr::euler(venn_df)
plot(venn, quantities = T)
```



4.2.2 Venn bound genes from both sets

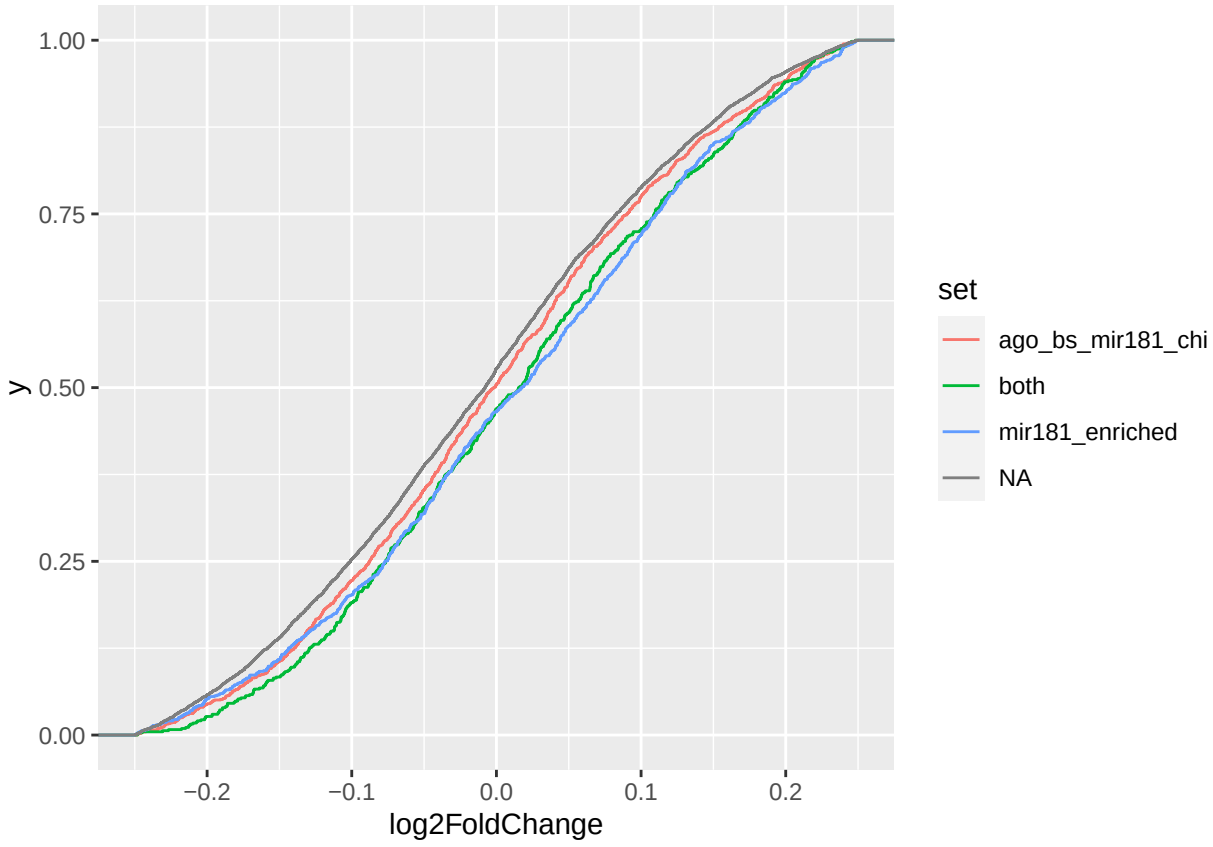
```
bound_genes <- unique(mir181_bs$geneID)
venn_df <- data.frame(ago_bs_mir181_chi =
                        (bound_genes %in% c(mir181_bs[mir181_bs$set == "ago_bs_mir181_chi"]$geneID,
                                            mir181_bs[mir181_bs$set == "ago_bs_mir181_chi&mir181_enrich
                      mir181_enriched =
                        (bound_genes %in% c(mir181_bs[mir181_bs$set == "mir181_enriched"]$geneID,
                                            mir181_bs[mir181_bs$set == "ago_bs_mir181_chi&mir181_enrich

venn <- eulerr::euler(venn_df)
plot(venn, quantities = T)
```



4.2.3 Ribofootprint both sets

```
# TODO need to be other script later
rpf <- read.csv("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/files_RNAseq_RiboP/RPF_maste
rpf <- mutate(rpf, Gene = substr(Gene,1,18))

rpf <- rpf %>% mutate(., set =
                        case_when(Gene %in% both_mir181_enriched_chi$geneID ~ "both",
                                  (Gene %in% ago_bs_mir181_chi$geneID) & !(Gene %in% both_mir181_enrich
                                  (Gene %in% mir181_enriched$geneID) & !(Gene %in% both_mir181_enriched
                                  ))

ggplot(rpf, aes(x = log2FoldChange, color = set))+
```

```
  stat_ecdf()+
  xlim(-0.25, 0.25)
```



5 Save output

```
saveRDS(mir181_bs, paste0(out, "mir181_bs.rds"))
```

6 Session Info

```
sessionInfo()
```

```
## R version 4.2.2 (2022-10-31)
## 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] stats4    stats     graphics  grDevices utils     datasets  methods
```

```
## [8] base
##
## other attached packages:
##  [1] GenomicRanges_1.50.2 GenomeInfoDb_1.34.7  IRanges_2.32.0
##  [4] S4Vectors_0.36.1     BiocGenerics_0.44.0  forcats_0.5.2
##  [7] stringr_1.5.0        dplyr_1.0.10         purrr_1.0.1
## [10] readr_2.1.3          tidyr_1.3.0          tibble_3.1.8
## [13] ggplot2_3.4.0        tidyverse_1.3.2      knitr_1.42
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.10            lubridate_1.9.1        assertthat_0.2.1
##  [4] digest_0.6.31          utf8_1.2.2             R6_2.5.1
##  [7] cellranger_1.1.0       backports_1.4.1        reprex_2.0.2
## [10] evaluate_0.20          highr_0.10             httr_1.4.4
## [13] pillar_1.8.1           zlibbioc_1.44.0        rlang_1.0.6
## [16] googlesheets4_1.0.1    readxl_1.4.1           rstudioapi_0.14
## [19] rmarkdown_2.20         labeling_0.4.2         googledrive_2.0.0
## [22] polyclip_1.10-4        RCurl_1.98-1.9         munsell_0.5.0
## [25] broom_1.0.3            polylabelr_0.2.0       eulerr_7.0.0
## [28] compiler_4.2.2         modelr_0.1.10          xfun_0.36
## [31] pkgconfig_2.0.3        htmltools_0.5.4        tidyselect_1.2.0
## [34] GenomeInfoDbData_1.2.9 fansi_1.0.4            crayon_1.5.2
## [37] tzdb_0.3.0             dbplyr_2.3.0           withr_2.5.0
## [40] bitops_1.0-7           grid_4.2.2             jsonlite_1.8.4
## [43] gtable_0.3.1           lifecycle_1.0.3        DBI_1.1.3
## [46] magrittr_2.0.3         scales_1.2.1           cli_3.6.0
## [49] stringi_1.7.12         farver_2.1.1           XVector_0.38.0
## [52] fs_1.6.0               xml2_1.3.3             ellipsis_0.3.2
## [55] generics_0.1.3         vctrs_0.5.2            tools_4.2.2
## [58] glue_1.6.2             hms_1.1.2              fastmap_1.1.0
## [61] yaml_2.3.7             timechange_0.2.0       colorspace_2.1-0
## [64] gargle_1.2.1           rvest_1.0.3            haven_2.5.1
```