

# Figure 1B

Toshihiro Arae

## General directory setting

```
wd <- here::here()
shared <- fs::path(fs::path_dir(wd), "shared")
```

## Loading packages

```
library(magrittr)
library(ggplot2)
```

## Load common R scripts

```
#source(fs::path(wd, "script_r", "MISC.R"))
#source(fs::path(here::here(), "script_r", "MISC_PALETTE.R"))
```

## Load script

```
source(fs::path(wd, "script_r", "MISC_FIG.R"))
readLines(fs::path(wd, "script_r", "MISC_FIG.R")) %>% cat(sep = "\n")

library(magrittr)
library(ggplot2)

COL_PALETTE <-
  viridis::inferno(6, begin = .1, end = .9) %>%
  rev() %>%
  setNames(nm = c("ZT0", "ZT3", "ZT6", "ZT12", "ZT18", "ZT21"))

LABEL_PALETTE <-
  COL_PALETTE %>%
  prismatic::clr_darken(shift = .15) %>%
  setNames(names(COL_PALETTE))

label_number_si <-
  purrr::partial(scales::label_number, scale_cut = scales::cut_short_scale())

ggsave_single <- function(..., width = 86, height = 230, dpi = 300) {
  f <- purrr::partial(ggsave, width = width, height = height, dpi = dpi, units = "mm")
  f(...)
}

ggsave_double <- function(..., width = 178, height = 230, dpi = 300) {
  f <- purrr::partial(ggsave, width = width, height = height, dpi = dpi, units = "mm")
  f(...)
}

#' Utility functions for making secondary y-axis
#' @param y1 numeric vector
#' @param y2 numeric vector
#' @name util_2nd_axis
```

```

#' @examples
#' make_scale_y1_to_y2(1:5, 6:10)(1:10)
#' make_scale_y2_to_y1(1:5, 6:10)(1:10)
#'
#' iris_ <- dplyr::select(iris, x = Sepal.Length, y1 = Petal.Length, y2 = Petal.Width)
#' gp1 <-
#'   iris_ %>%
#'   ggplot() +
#'   geom_point(aes(x, y1), color = "#CD3700") +
#'   geom_point(aes(x, y2), color = "#473C8B")
#'
#' to_y1 <- with(iris_, {make_scale_y2_to_y1(y1, y2)})
#' to_y2 <- with(iris_, {make_scale_y1_to_y2(y1, y2)})
#' gp2 <-
#'   iris_ %>%
#'   ggplot() +
#'   geom_point(aes(x, y1), color = "#CD3700") +
#'   geom_point(aes(x, y = to_y1(y2)), color = "#473C8B") +
#'   scale_y_continuous(sec.axis = sec_axis(trans = to_y2, name = "y2"))
#' patchwork::wrap_plots(gp1, gp2)
#'
NULL

#' Create transformation function of range(y1) to range(y2)
#' @rdname util_2nd_axis
#' @export
#'
make_scale_y1_to_y2 <- function(y1, y2) {
  function(n) {
    scales::rescale.numeric(
      n,
      to = range(y2, na.rm = TRUE, finite = TRUE),
      from = range(y1, na.rm = TRUE, finite = TRUE)
    )
  }
}

#' Create transformation function of range(y2) to range(y1)
#' @rdname util_2nd_axis
#' @export
#'
make_scale_y2_to_y1 <- function(y1, y2) {
  function(n) {
    scales::rescale.numeric(
      n,
      to = range(y1, na.rm = TRUE, finite = TRUE),
      from = range(y2, na.rm = TRUE, finite = TRUE)
    )
  }
}

#' Create transformation function of range(y2) to range(y1)
#' @rdname util_2nd_axis
#' @export
#'
make_scale_y2_to_y1_se <- function(y1, y2) {
  to <- range(y1, na.rm = TRUE, finite = TRUE)
  from <- range(y2, na.rm = TRUE, finite = TRUE)
  function(n) n / (diff(from) / diff(to))
}

```

```
dir_output <- fs::path("analysis", "fig", "fig01B")
path_out <- function(...) fs::path(wd, dir_output, ...)
fs::dir_create(path_out())
```

## Load input data

```
fastqc_dirs <-
  c("fastq_cat", "fastq_qf", "fastq_cl_adaptor",
    "fastq_cl_umi", "fastq_rm_ncRNA", "fastq_rm_marker",
    "mapped_by_star", "umi_dedup",
    "bam_filtered_len") %>%
  {fs::path(wd, "report_fastqc", .)}

li_fastqc_data_list <-
  fastqc_dirs %>%
  purrr::map(~ ngsReports::FastqcDataList(fs::dir_ls(.x, regexp = ".zip$")))

filter_fastqc <- function(fastqc_li) {
  # matches to RNA-seq
  zip_fpath_pattern <- "^(^zt\\d{1,2}_2_fastqc.zip$)|(_al.sort_fastqc.zip$)"
  fastqc_li %>%
    purrr::set_names(fs::path_file) %>%
    {.[stringr::str_which(names(.), zip_fpath_pattern, negate = TRUE)]}
}

extract_summary <- function(fastqc_li) purrr::map(fastqc_li, ~ .x@Summary)
extract_basic_stat <- function(fastqc_li) purrr::map(fastqc_li, ~ .x@Basic_Statistics)
extract_per_seq_gc <- function(fastqc_li) {
  purrr::map(fastqc_li, ~ dplyr::mutate(.x@Per_sequence_GC_content, qcfile = .x@path))
}
extract_seq_len_distribution <- function(fastqc_li) {
  purrr::map(fastqc_li, ~ dplyr::mutate(.x@Sequence_Length_Distribution, qcfile = .x@path))
}
```

## Plotting

```
theme_fig01B <- function(base_size = 10, base_line_size = 1/22) {
  list(
    theme_linedraw(
      base_size = base_size,
      base_line_size = base_line_size
    )
  )
}
```

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```
tbl_plot <-
  li_fastqc_data_list %>%
  {purrr::set_names(., nm = fastqc_dirs)} %>%
  purrr::map(filter_fastqc) %>%
  purrr::map(~ extract_seq_len_distribution(.x) %>% dplyr::bind_rows()) %>%
  purrr::imap(~ dplyr::mutate(.x, dir = .y)) %>%
  dplyr::bind_rows() %>%
  dplyr::mutate(mean = (Lower + Upper) / 2)

tbl_plot <-
  tbl_plot %>%
  dplyr::mutate(dir = forcats::fct_inorder(fs::path_file(dir))) %>%
```

```

dplyr::mutate(file = stringr::str_extract(qcfile, "zt\\d+_\\d") %>%
  stringr::str_to_upper()) %>%
dplyr::mutate(file = forcats::fct_relevel(
  file, paste0(paste0("ZT", rep(c(0, 3, 6, 12, 18, 21), each = 2)), c("_1", "_2"))
)) %>%
dplyr::mutate(cond = stringr::str_extract(file, "ZT\\d+")) %>%
dplyr::mutate(cond = forcats::fct_relevel(
  cond, paste0("ZT", c("0", "3", "6", "12", "18", "21"))
)) %>%
dplyr::mutate(rep = stringr::str_match(file, "(\\d)$")[,2])

gp <-
tbl_plot %>%
dplyr::filter(dir == "umi_dedup") %>%
ggplot(aes(mean, Count), group = file) +
geom_line(aes(color = cond, linetype = rep), key_glyph = "timeseries") +
theme_fig01B(base_size = 10, base_line_size = 1) +
scale_linetype_manual(values = c("1" = "solid", "2" = "dashed")) +
scale_color_manual(values = alpha(COL_PALETTE, .8)) +
scale_x_continuous("Read length (nt)", limits = c(20, 45)) +
scale_y_continuous("Read counts", expand = expansion(mult = c(0, .1)),
  labels = label_number_si()) +
guides(
  linetype = guide_legend(title = "repeats", ncol = 2),
  color = guide_legend(title = "samples", nrow = 2, byrow = TRUE)
) +
theme(legend.position = "bottom", legend.direction = "vertical")
ggsave_single(gp, filename = path_out("fig01B_type2_s.png"), height = 90)

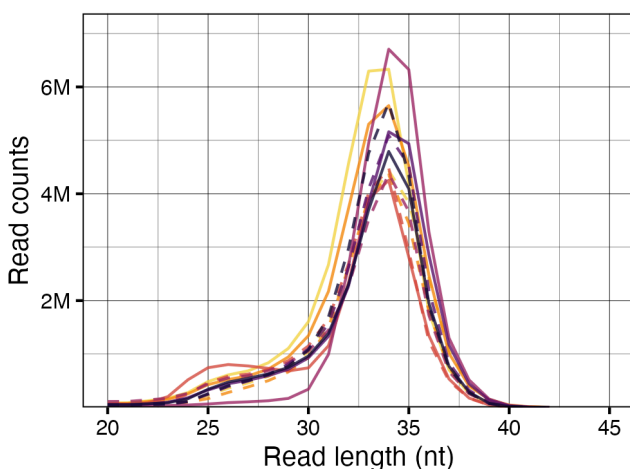
```

Warning: Removed 135 rows containing missing values (`()`).

```
ggsave_single(gp, filename = path_out("fig01B_type2_s.svg"), height = 90)
```

Warning: Removed 135 rows containing missing values (`()`).

```
knitr::include_graphics(path_out("fig01B_type2_s.png"))
```



samples

ZT0 ZT3 ZT6 ZT12 ZT18 ZT21

repeats

1 2

# Sessioninfo

```
sessionInfo()
```

R version 4.2.1 (2022-06-23)

Platform: aarch64-apple-darwin20 (64-bit)

Running under: macOS Ventura 13.1

Matrix products: default

BLAS: /Library/Frameworks/R.framework/Versions/4.2-arm64/Resources/lib/libRblas.0.dylib

LAPACK: /Library/Frameworks/R.framework/Versions/4.2-arm64/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] stats graphics grDevices datasets utils methods base

other attached packages:

[1] ggplot2\_3.4.2 magrittr\_2.0.3

loaded via a namespace (and not attached):

[1] Rcpp_1.0.11	svglite_2.1.0	lattice_0.20-45
[4] lubridate_1.9.2	here_1.0.1	tidyr_1.3.0
[7] png_0.1-7	zoo_1.8-11	ngsReports_1.12.4
[10] Biostrings_2.64.1	rprojroot_2.0.3	digest_0.6.31
[13] utf8_1.2.2	R6_2.5.1	GenomeInfoDb_1.32.4
[16] stats4_4.2.1	evaluate_0.20	httr_1.4.5
[19] pillar_1.9.0	zlibbioc_1.42.0	rlang_1.1.0
[22] lazyeval_0.2.2	rstudioapi_0.14	data.table_1.14.4
[25] S4Vectors_0.34.0	DT_0.26	rmarkdown_2.24
[28] labeling_0.4.2	textshaping_0.3.6	stringr_1.5.0
[31] pander_0.6.5	htmlwidgets_1.5.4	RCurl_1.98-1.9
[34] munsell_0.5.0	compiler_4.2.1	xfun_0.40
[37] systemfonts_1.0.4	pkgconfig_2.0.3	BiocGenerics_0.42.0
[40] htmltools_0.5.3	tidyselect_1.2.0	tibble_3.2.1
[43] gridExtra_2.3	GenomeInfoDbData_1.2.8	IRanges_2.30.1
[46] fansi_1.0.3	viridisLite_0.4.1	crayon_1.5.2
[49] dplyr_1.1.1	withr_2.5.0	prismatic_1.1.1
[52] MASS_7.3-57	bitops_1.0-7	grid_4.2.1
[55] DBI_1.1.3	jsonlite_1.8.4	gtable_0.3.1
[58] lifecycle_1.0.3	scales_1.2.1	stringi_1.7.12
[61] cli_3.6.0	farver_2.1.1	XVector_0.36.0
[64] renv_1.0.3	viridis_0.6.2	fs_1.5.2
[67] ragg_1.2.5	ggdendro_0.1.23	generics_0.1.3
[70] vctrs_0.6.1	tools_4.2.1	forcats_1.0.0
[73] glue_1.6.2	purrr_1.0.1	fastmap_1.1.0
[76] yaml_2.3.6	timechange_0.1.1	colorspace_2.0-3
[79] BiocManager_1.30.18	plotly_4.10.0	knitr_1.42