Prepare empirical-JTK_CYCLE analysis

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General directory setting

```
wd <- here::here()
shared <- fs::path_dir(wd), "shared")</pre>
```

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"))
```

```
parent_out_dir <- fs::path("analysis", "phase_analysis_ejtk")</pre>
```

Input data preparation

RNA

```
### Setting some parameters
dir_input <- fs::path(parent_out_dir)
dir_output <- fs::path(parent_out_dir, "rna_exon")
path_out <- function(...) fs::path(wd, dir_output, ...)
fs::dir_create(path_out())

### Load read count data
tbl_input <-
    fs::path(wd, "data_preproc", "readcount", "count_rna_exon", "count_by_gene.csv") %>%
    readr::read_csv(show_col_types = FALSE) %>%
    dplyr::select(Geneid, dplyr::matches("^zt\\d+_[12]_rna$")) %>%
    dplyr::select(1:3, 10:13, 4:9)
dplyr::glimpse(tbl_input)
```

```
$ zt21_1_rna <dbl> 39, 109, 1, 88, 620, 105, 620, 7, 5832, 73, 0, 399, 1086, 1...
$ zt21_2_rna <dbl> 23, 109, 7, 87, 606, 84, 619, 8, 6158, 63, 0, 369, 1072, 16...
### Load scale factor
sf default rna <-
 readRDS(fs::path(wd, "analysis", "deseq2_rna", "sf_default_rna.rds")) %>%
  \{.[c(1:2,9:12,3:8)]\}
sf default rna
 zt0_1_rna zt0_2_rna zt3_1_rna zt3_2_rna zt6_1_rna zt6_2_rna zt12_1_rna
 0.8852572 0.9861809 0.9919602 0.9850716 0.9549625
                                                      0.9323711 1.1518050
zt12_2_rna zt18_1_rna zt18_2_rna zt21_1_rna zt21_2_rna
1.1281778 1.0642214 1.0457061 0.9602838 0.9825095
### Check and normalize read count
if(any(colnames(tbl_input[,-1]) != names(sf_default_rna))) stop()
tbl input[,-1] <- purrr::modify2(tbl input[,-1], sf default rna, <math>\sim .x / .y)
### Filter AGI
AGI filtered rna <-
  readRDS(fs::path(wd, "analysis", "deseq2_rna", "AGI_filtered_rna.rds"))
str(AGI_filtered_rna)
 chr [1:23981] "AT1G01010" "AT1G01020" "AT1G03987" "AT1G01030" "AT1G01040" ...
tbl_input <- dplyr::filter(tbl_input, Geneid %in% AGI_filtered_rna)
dplyr::glimpse(tbl input)
Rows: 23,981
Columns: 13
            <chr> "AT1G01010", "AT1G01020", "AT1G03987", "AT1G01030", "AT1G01...
$ Geneid
$ zt0 1 rna <dbl> 22.592304, 91.498831, 7.907306, 188.645738, 639.362202, 98....
$ zt0_2_rna <dbl> 30.420381, 83.149041, 7.098089, 207.872603, 641.870039, 112...
$ zt3_1_rna <dbl> 31.251255, 72.583560, 9.072945, 114.923971, 492.963347, 89....
$ zt6_1_rna <dbl> 33.509168, 69.112660, 3.141485, 97.386020, 475.411326, 76.4...
$ zt6_2_rna <dbl> 23.595754, 84.730209, 6.435206, 86.875277, 470.842551, 67.5...
$ zt12 1 rna <dbl> 38.200910, 70.324402, 0.000000, 47.751137, 333.389759, 59.0...
$ zt12_2_rna <dbl> 38.1145605, 58.5014185, 0.8863851, 54.9558780, 351.0085109,...
$ zt18_1_rna <dbl> 32.887894, 85.508523, 3.758616, 113.698146, 693.464728, 122...
$ zt18_2_rna <dbl> 38.251667, 84.153667, 1.912583, 113.798709, 701.918090, 128...
$ zt21_1_rna <dbl> 40.612994, 113.508111, 1.041359, 91.639576, 645.642465, 109...
$ zt21_2_rna <dbl> 23.409443, 110.940404, 7.124613, 88.548763, 616.787934, 85....
### Save eJTK input data
header line <-
 paste(c("#", paste0("ZT", rep(c(0, 3, 6, 12, 18, 21), each=2))), collapse = "\t")
header_line
```

[1] "#\tZT0\tZT0\tZT3\tZT3\tZT6\tZT6\tZT12\tZT12\tZT18\tZT18\tZT21\tZT21"

```
dplyr::rowwise(tbl_input) %>%
  dplyr::group_split() %>%
  purrr::map_chr(~ paste(as.character(.x), collapse = "\t")) %>%
```

```
{c(header_line, .)} %>%
readr::write_lines(path_out("data.txt"))
readr::read_lines(path_out("data.txt"), n_max = 10) %>% cat(sep = "\n")
```

```
ZTO ZTO ZT3 ZT3 ZT6 ZT6 ZT12
                                   ZT12
                                           ZT18
                                                   ZT18
                                                           ZT21
                                                                   ZT21
AT1G01010
              22.5923039558941
                                        30.4203809915218
                                                                31.251255141716 27.4091747292632
33.5091683231262
                    23.5957542817426
                                        38.2009098308248
                                                             38.1145605272843
                                                                                 32.8878936064003
38.2516670289166
                   40.612993756216 23.409443041413
              91.498831021371 83.1490413768263
                                                         72.5835603291468
                                                                                 81.2123695681872
AT1G01020
69.1126596664478
                    84.7302085571664
                                        70.3244021885638
                                                                                 85.5085233766407
                                                             58.5014184837386
84.1536674636164
                   113.508110754552
                                       110.94040397887
AT1G03987
              7.90730638456292
                                    7.09808889802175
                                                           9.07294504114335
                                                                                  5.0757730980117
                   6.43520571320251
3.14148453029308
                                          0.886385128541494 3.75861641216003
                                                                                 1.91258335144583
                                      0
1.04135881426195
                   7.12461309956048
AT1G01030
             188.645738031715
                                   207.872603442066
                                                          114.923970521149
                                                                                 122.833708971883
97.3860204390855
                     86.8752771282339
                                           47.751137288531 54.9558779695726
                                                                                 113.698146467841
113.798709411027
                   91.6395756550515
                                       88.5487628088231
AT1G01040
              639.362201951802
                                        641.87003892111 492.963347235455
                                                                                 540.062257628445
475.411325584353
                   470.842551349317
                                       333.389758523562
                                                             351.008510902432
                                                                                 693.464728043526
701.918089980619
                   645.642464842409
                                       616.787934047664
AT1G03993
               98.2765222081392
                                        112.555409668631
                                                                89.721345406862 101.515461960234
76.4427902371317
                    67.5696599886264
                                        59.0377697385474
                                                             60.2741887408216
                                                                                 122.155033395201
128.14308454687 109.342675497505 85.4953571947258
AT1G01050
             436.031466348755
                                   432.983422779327
                                                          273.196456238872
                                                                                 288.303911967065
265.979023564814
                     270.278639954506
                                           328.180543546631
                                                                 314.66672063223 503.654599229444
529.785588350494
                   645.642464842409
                                       630.01935837542
AT1G03997
             9.03692158235763
                                    5.07006349858697
                                                           3.02431501371445
                                                                                 2.03030923920468
                    5.36267142766876
2.09432302019539
                                       1.73640499231022
                                                             0.886385128541494
                                                                                 3.75861641216003
1.91258335144583
                   7.28951169983365
                                       8.14241497092626
               12495.803318005 12716.7332671558
                                                         908.302609118906
                                                                                 970.487816339837
AT1G01060
55.4995600351778 69.7147285596939
                                       3.47280998462044
                                                           1.77277025708299
                                                                                 455.732239974404
473.364379482842
                   6073.20460477569
                                       6267.62392387049
```

mORF

```
### Setting some parameters
dir_input <- fs::path(parent_out_dir)
dir_output <- fs::path(parent_out_dir, "ribo_ccds_psite")
path_out <- function(...) fs::path(wd, dir_output, ...)
fs::dir_create(path_out())

### Load read count data
tbl_input <-
fs::path(wd, "data_preproc", "readcount", "count_ribo_central_cds_psite", "count_by_gene.csv") %>%
    readr::read_csv(show_col_types = FALSE) %>%
    dplyr::select(Geneid, dplyr::matches("^zt\\d+_[12]_ribo$")) %>%
    dplyr::select(1:3, 10:13, 4:9)
dplyr::glimpse(tbl_input)
```

```
$ zt18_1_ribo <dbl> 65, 55, 63, 175, 1233, 122, 173, 1265, 5103, 2707, 74, 158...
$ zt18_2_ribo <dbl> 45, 50, 57, 177, 1276, 132, 196, 1229, 5235, 2936, 59, 166...
$ zt21_1_ribo <dbl> 31, 46, 43, 123, 887, 1618, 177, 1226, 5083, 2595, 60, 160...
$ zt21_2_ribo <dbl> 41, 56, 74, 139, 1111, 2120, 235, 1431, 5686, 3742, 80, 17...
```

```
### Load scale factor
sf_default_ribo <-
    readRDS(fs::path(wd, "analysis", "deseq2_ribo", "sf_default_ribo.rds")) %>%
    {.[c(1:2,9:12,3:8)]}
sf_default_ribo
```

```
zt0_1_ribo zt0_2_ribo zt3_1_ribo zt3_2_ribo zt6_1_ribo zt6_2_ribo
1.3815980 0.8917084 1.0702754 0.7875683 0.7440773 0.8097870
zt12_1_ribo zt12_2_ribo zt18_1_ribo zt18_2_ribo zt21_1_ribo zt21_2_ribo
1.3827508 0.9011186 1.1266239 1.1130669 0.9709120 1.1715257
```

```
### Check and normalize read count
if(any(colnames(tbl_input[,-1]) != names(sf_default_ribo))) stop()
tbl_input[,-1] <- purrr::modify2(tbl_input[,-1], sf_default_ribo, ~ .x / .y)

### Filter AGI
AGI_filtered_ribo <-
    readRDS(fs::path(wd, "analysis", "deseq2_ribo", "AGI_filtered_ribo.rds"))
str(AGI_filtered_ribo)</pre>
```

```
chr [1:21274] "AT1G01010" "AT1G01020" "AT1G01030" "AT1G01040" "AT1G01050" ...
```

```
tbl_input <- dplyr::filter(tbl_input, Geneid %in% AGI_filtered_ribo)
dplyr::glimpse(tbl_input)</pre>
```

```
Rows: 21,274
Columns: 13
              <chr> "AT1G01010", "AT1G01020", "AT1G01030", "AT1G01040", "AT1G0...
$ Geneid
$ zt0_1_ribo <dbl> 42.704172, 53.561165, 90.474940, 98.436735, 812.826862, 30...
$ zt0_2_ribo <dbl> 34.764726, 62.800796, 68.408009, 61.679353, 669.501339, 26...
$ zt3_1_ribo <dbl> 44.848271, 40.176576, 51.388644, 149.494237, 386.816338, 1...
$ zt3 2 ribo <dbl> 31.743279, 50.789247, 57.137903, 68.565483, 396.156126, 12...
$ zt6 1 ribo <dbl> 25.534980, 24.191034, 26.878926, 206.967733, 421.999143, 1...
$ zt6_2_ribo <dbl> 34.576992, 43.221240, 46.925918, 111.140332, 324.776749, 2...
$ zt12_1_ribo <dbl> 59.302081, 25.311864, 33.990217, 241.547502, 445.488805, 0...
$ zt12_2_ribo <dbl> 62.144984, 35.511420, 55.486593, 85.449353, 348.455804, 2....
$ zt18_1_ribo <dbl> 57.694496, 48.818420, 55.919281, 155.331336, 1094.420210, ...
$ zt18_2_ribo <dbl> 40.42884, 44.92093, 51.20986, 159.02009, 1146.38214, 118.5...
$ zt21_1_ribo <dbl> 31.928743, 47.378135, 44.288257, 126.685014, 913.574041, 1...
$ zt21_2_ribo <dbl> 34.997097, 47.800913, 63.165493, 118.648696, 948.335979, 1...
```

```
### Save eJTK input data
header_line <-
paste(c("#", paste0("ZT", rep(c(0, 3, 6, 12, 18, 21), each=2))), collapse = "\t")
header_line</pre>
```

[1] "#\tZT0\tZT0\tZT3\tZT3\tZT6\tZT6\tZT12\tZT12\tZT18\tZT18\tZT21\tZT21"

```
dplyr::rowwise(tbl_input) %>%
  dplyr::group_split() %>%
  purrr::map_chr(~ paste(as.character(.x), collapse = "\t")) %>%
  {c(header_line, .)} %>%
  readr::write_lines(path_out("data.txt"))
readr::read_lines(path_out("data.txt"), n_max = 10) %>% cat(sep = "\n")
```

```
ZTO ZTO ZT3 ZT3 ZT6 ZT6 ZT12
                                    ZT12
                                            ZT18
                                                    ZT18
                                                            7T21
                                                                    ZT21
AT1G01010
             42.7041717476715
                                    34.7647261362627
                                                          44.8482710326735
                                                                                31.7432793623567
25.5349799892894
                    34.5769923102144
                                                            62.1449841296481
                                                                                57.6944960786652
                                        59.3020812425734
40.4288371482759
                    31.9287432760018
                                        34.997097341495
AT1G01020
             53.5611645648761
                                   62.8007956009906
                                                          40.1765761334367
                                                                                50.7892469797708
                                                           35.5114195026561
                    43.221240387768 25.3118639450008
                                                                                48.8184197588705
24.1910336740636
44.920930164751 47.3781351837446
                                  47.800913442042
AT1G01030
              90.4749401433718
                                       68.4080094939362
                                                               51.388643891605
                                                                                57.1379028522421
26.8789263045151
                   46.9259181352909
                                        33.9902172975725
                                                            55.4865929729001
                                                                                55.9192808147063
                    44.288256802196 63.1654927626984
51.2098603878161
AT1G01040
             98.4367348759886
                                   61.6793528224015
                                                          149.494236775578
                                                                                68.5654834226906
206.967732544766
                    111.140332425689
                                        241.547501646579
                                                            85.4493531782661
                                                                                155.331335596406
159.020092783219
                    126.685013643491
                                        118.648695865069
AT1G01050
             812.826862248053
                                   669.501338817704
                                                          386.816337656809
                                                                                396.156126442212
421.999142980887
                    324.776749199514
                                       445.488805432014
                                                            348.455803869813
                                                                                1094.42021023068
1146.38213780445
                    913.574041477858
                                        948.335979180512
AT1G01060
             3081.21456152267
                                   2642.11918635596
                                                          156.968948614357
                                                                                129.512579798415
16.1273557827091
                                        0 2.219463718916 108.288131101495
                    22.2280664851378
                                                                                118.591255634943
1666.47440711519
                    1809.6060088773
AT1G01070
             281.558013726173
                                   289.332236875993
                                                          98.1055928839732
                                                                                91.4206445635874
                    43.221240387768 66.5340423697164
56.4457452394817
                                                           38.8406150810301
                                                                                153.556120332447
176.090046245824
                    182.302824511365
                                        200.593118908569
                                                                                1376.38859315179
AT1G01080
              1111.75606448175
                                       1185.3650169687 1638.83057065228
1939.31453287077
                    1712.79601193812
                                       1800.03512454592
                                                            1337.22689064689
                                                                                1122.82365445402
1104.15646344958
                    1262.73029859285
                                        1221.48405599218
             4432.54826750407
                                  4752.67449566068
                                                          4125.10659602611
                                                                                4019.96889844886
AT1G01090
3496.94831221742
                   3628.11440740749
                                        2629.54106582923
                                                            2459.16580055893
                                                                                4529.46174599121
4703.22138824943
                   5235,28393780378
                                        4853.49988984734
```

uORF

```
### Setting some parameters
dir_input <- fs::path(parent_out_dir)
dir_output <- fs::path(parent_out_dir, "ribo_uorf_psite")
path_out <- function(...) fs::path(wd, dir_output, ...)
fs::dir_create(path_out())

### Load read count data
tbl_input <-
    fs::path(wd, "data_preproc", "readcount", "count_ribo_uorf_psite", "count_by_gene.csv") %>%
    readr::read_csv(show_col_types = FALSE) %>%
    dplyr::select(Geneid, dplyr::matches("^zt\\d+_[12]_ribo$")) %>%
    dplyr::select(1:3, 10:13, 4:9)
dplyr::glimpse(tbl_input)
```

```
$ zt6_2_ribo <dbl> 11, 5, 15, 16, 13, 3, 7, 28, 1, 10, 17, 0, 0, 28, 32, 15, ...
$ zt12_1_ribo <dbl> 0, 3, 25, 9, 16, 2, 11, 35, 4, 12, 32, 2, 1, 51, 48, 8, 21...
$ zt12_2_ribo <dbl> 3, 5, 14, 10, 10, 4, 4, 30, 2, 15, 12, 1, 0, 31, 36, 9, 8,...
$ zt18_1_ribo <dbl> 31, 21, 10, 9, 13, 1, 22, 25, 2, 37, 12, 3, 1, 31, 43, 19,...
$ zt18_2_ribo <dbl> 29, 15, 6, 11, 7, 0, 20, 22, 6, 39, 20, 0, 0, 21, 42, 7, 1...
$ zt21_1_ribo <dbl> 432, 8, 5, 19, 11, 2, 14, 25, 2, 37, 13, 2, 1, 23, 33, 12,...
$ zt21_2_ribo <dbl> 580, 9, 7, 20, 7, 0, 13, 24, 1, 46, 25, 1, 1, 27, 24, 12, ...
### Load scale factor
sf default ribo <-
  readRDS(fs::path(wd, "analysis", "deseq2_ribo", "sf_default_ribo.rds")) %>%
  \{.[c(1:2,9:12,3:8)]\}
sf_default_ribo
 zt0_1_ribo zt0_2_ribo zt3_1_ribo zt3_2_ribo zt6_1_ribo zt6_2_ribo
                         1.0702754
                                     0.7875683
                                                 0.7440773
  1.3815980
            0.8917084
                                                               0.8097870
zt12 1 ribo zt12 2 ribo zt18 1 ribo zt18 2 ribo zt21 1 ribo zt21 2 ribo
  1.3827508
              0.9011186
                          1.1266239
                                      1.1130669
                                                  0.9709120
                                                               1.1715257
### Check and normalize read count
if(any(colnames(tbl_input[,-1]) != names(sf_default_ribo))) stop()
tbl_input[,-1] <- purrr::modify2(tbl_input[,-1], sf_default_ribo, ~ .x / .y)
### Filter AGI
AGI_filtered_ribo <-
  readRDS(fs::path(wd, "analysis", "deseq2_ribo", "AGI_filtered_ribo.rds"))
str(AGI_filtered_ribo)
 chr [1:21274] "AT1G01010" "AT1G01020" "AT1G01030" "AT1G01040" "AT1G01050" ...
tbl_input <- dplyr::filter(tbl_input, Geneid %in% AGI_filtered_ribo)
dplyr::glimpse(tbl_input)
Rows: 4,753
Columns: 13
              <chr> "AT1G01060", "AT1G01210", "AT1G01230", "AT1G01240", "AT1G0...
$ Geneid
$ zt0 1 ribo <dbl> 838.1598455, 10.8569928, 8.6855943, 19.5425871, 8.6855943,...
$ zt0 2 ribo <dbl> 1019.391486, 7.850099, 3.364328, 23.550298, 12.335871, 1.1...
$ zt3_1_ribo <dbl> 94.368237, 6.540373, 19.621119, 29.898847, 10.277729, 4.67...
$ zt3_2_ribo <dbl> 79.993064, 5.078925, 21.585430, 27.934086, 10.157849, 2.53...
$ zt6_1_ribo <dbl> 9.407624, 6.719732, 24.191034, 32.254712, 18.815248, 2.687...
$ zt6_2_ribo <dbl> 13.583818, 6.174463, 18.523389, 19.758281, 16.053604, 3.70...
$ zt12_1_ribo <dbl> 0.0000000, 2.1695883, 18.0799028, 6.5087650, 11.5711378, 1...
$ zt12_2_ribo <dbl> 3.329196, 5.548659, 15.536246, 11.097319, 11.097319, 4.438...
$ zt18_1_ribo <dbl> 27.5158366, 18.6397603, 8.8760763, 7.9884687, 11.5388992, ...
$ zt18 2 ribo <dbl> 26.0541395, 13.4762790, 5.3905116, 9.8826046, 6.2889302, 0...
$ zt21_1_ribo <dbl> 444.942487, 8.239676, 5.149797, 19.569230, 11.329554, 2.05...
$ zt21_2_ribo <dbl> 495.0808892, 7.6822897, 5.9751142, 17.0717548, 5.9751142, ...
### Save eJTK input data
header_line <-
```

```
### Save eJTK input data
header_line <-
paste(c("#", paste0("ZT", rep(c(0, 3, 6, 12, 18, 21), each=2))), collapse = "\t")
header_line</pre>
```

```
dplyr::rowwise(tbl_input) %>%
  dplyr::group_split() %>%
  purrr::map_chr(~ paste(as.character(.x), collapse = "\t")) %>%
  {c(header_line, .)} %>%
  readr::write_lines(path_out("data.txt"))
readr::read_lines(path_out("data.txt"), n_max = 10) %>% cat(sep = "\n")
```

```
ZTO ZTO ZT3 ZT3 ZT6 ZT6 ZT12
                                 ZT12
                                         ZT18
                                                ZT18
                                                        7T21
                                                                ZT21
             838.159845488197
AT1G01060
                                   1019.39148573751
                                                         94.3682369645838
                                                                              79.993063993139
9.40762420658029
                   13.5838184075842
                                      0
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```

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
        /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
[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:
              graphics grDevices datasets utils
                                                      methods
                                                                base
[1] stats
other attached packages:
[1] ggplot2_3.4.2 magrittr_2.0.3
loaded via a namespace (and not attached):
 [1] compiler_4.2.1
                         pillar_1.9.0
                                             BiocManager_1.30.18
```

[4] tools_4.2.1 [7] jsonlite_1.8.4 [10] tibble_3.2.1 [13] rlang_1.1.0 [16] parallel_4.2.1 [19] fastmap_1.1.0 [22] knitr_1.42 [25] vctrs_0.6.1 [28] rprojroot_2.0.3 [31] glue_1.6.2 [34] fansi_1.0.3	bit_4.0.5 evaluate_0.20 gtable_0.3.1 cli_3.6.0 yaml_2.3.6 withr_2.5.0 generics_0.1.3 hms_1.1.3 grid_4.2.1 here_1.0.1 vroom_1.6.0	digest_0.6.31 lifecycle_1.0.3 pkgconfig_2.0.3 rstudioapi_0.14 xfun_0.40 dplyr_1.1.1 fs_1.5.2 bit64_4.0.5 tidyselect_1.2.0 R6_2.5.1 rmarkdown_2.24
[34] fansi_1.0.3 [37] purrr_1.0.1 [40] scales_1.2.1 [43] renv_1.0.3 [46] crayon_1.5.2	vroom_1.6.0 tzdb_0.3.0 htmltools_0.5.3 utf8_1.2.2	<pre>rmarkdown_2.24 readr_2.1.4 colorspace_2.0-3 munsell_0.5.0</pre>