elt2_promoter_microscopy

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Install Packages

Install the necessary packages. Do this once the first time analysis is performed. Uncomment and execute the following code chunk.

```
# install.packages("tidyverse")
# install.packages("readxl")
# install.packages("ggpubr")
# install.packages("mratios")
```

Load Package Libraries

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --
## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.6     v dplyr   1.0.8
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.1
## Warning: package 'tidyr' was built under R version 4.1.2
## Warning: package 'readr' was built under R version 4.1.2
## Warning: package 'dplyr' was built under R version 4.1.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## Warning: package 'readxl' was built under R version 4.1.2
```

Load in quantification data

Strains: - JM149 = elt-2 promoter reporter, caIs71[elt-2p::GFP::HIS-2B::unc-54 3'UTR + rol-6(su1006)] - JM259 = elt-2 promoter reporter + elt-7 delection, elt-7(tm840) V; caIs71[elt-2p::GFP::HIS-2B::unc-54 3'UTR + rol-6(su1006)]

Each worm should have the following measurements:

- One intestine fluorescence measurement
- Four background measurements

The input data should have the following columns (without quotes):

- "treatment": The type of RNAi treatment
- "life_stage": The worm developmental stage
- "worm": the worm number imaged

- "measurement_type": `background` or `GFP`
- "measurement_num": the measurement number from ImageJ
- "area": region of interest area
- "mean": mean gray pixel intensity
- "min": minimum pixel intensity
- "max": maximum pixel intensity
- "intDen": product of area and sum of the values of the pixels in the selection
- "rawIntDen": sum of the values of the pixels in the selection
- "experiment": experiment name including rep number
- "strain": strain or reporter gene name

More info here: https://imagej.nih.gov/ij/docs/guide/146-30.html

```
sheets <- c("Rep1_ELT-2_RNAi",  "Rep1_L4440_RNAi", "Rep2_ELT-2_RNAi",  "Rep2_L4440_RNAi", "Rep3_ELT-2_R

image_df_JM259 <- data.frame()
for(sheet in sheets) {
 toappend <- read_excel("../01_input/JM259_Reps123_elt-2_Promoter_ImageJ_Analysis.xlsx", sheet = sheet)
 toappend <- toappend %>% mutate(experiment = sheet)
 toappend$worm <- as.character(toappend$worm)
 image_df_JM259 <- bind_rows(image_df_JM259, toappend)
}
image_df_JM259$strain <- "JM259"

image_df_JM149 <- data.frame()
for(sheet in sheets) {
 toappend <- read_excel("../01_input/JM149_Reps123_elt-2_Promoter_ImageJ_Analysis.xlsx", sheet = sheet)
 toappend <- toappend %>% mutate(experiment = sheet)
 toappend$worm <- as.character(toappend$worm)
 image_df_JM149 <- bind_rows(image_df_JM149, toappend)
}

image_df_JM149$strain <- "JM149"


image_df<- image_df_JM149 %>% bind_rows(image_df_JM259)
image_df <- image_df %>% filter(life_stage == "L1")
head(image_df)
```

```
##   treatment life_stage worm measurement_type measurement_num  area      mean
## 1     ELT-2         L1    1       background               1 0.472 0.0180000
## 2     ELT-2         L1    1       background               2 0.472 0.0006901
## 3     ELT-2         L1    1       background               3 0.472 0.0050000
## 4     ELT-2         L1    1       background               4 0.472 0.0170000
## 5     ELT-2         L1    1              GFP               5 8.007 2.5880000
## 6     ELT-2         L1    2       background               6 0.472 0.0200000
##   min max     intDen rawIntDen      experiment strain
## 1   0   1  0.0090000        79 Rep1_ELT-2_RNAi  JM149
## 2   0   1  0.0003255         3 Rep1_ELT-2_RNAi  JM149
## 3   0   1  0.0020000        21 Rep1_ELT-2_RNAi  JM149
## 4   0   1  0.0080000        73 Rep1_ELT-2_RNAi  JM149
## 5   0  42 20.7240000    190993 Rep1_ELT-2_RNAi  JM149
## 6   0   1  0.0090000        86 Rep1_ELT-2_RNAi  JM149
```

```
colnames(image_df)
```

```
##  [1] "treatment"        "life_stage"       "worm"             "measurement_type"
##  [5] "measurement_num"  "area"             "mean"             "min"
##  [9] "max"              "intDen"           "rawIntDen"        "experiment"
## [13] "strain"
```

```
# Set the factor levels for ordering in downstream plotting
image_df$treatment <- factor(image_df$treatment, levels = c("L4440", "ELT-2"))
# Give each worm a unique ID
image_df <- image_df %>%
  mutate(ID = paste(strain, experiment, life_stage, worm, sep = "_"))
```

Calculate corrected total cell fluorescence (CTCF)

CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings)
More information here: https://theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescence-using-imagej.html

```
# Take the mean of the background measurements
background_df<- image_df %>%
  group_by(strain, treatment, worm, measurement_type, life_stage, experiment, ID) %>%
  summarize(Mean_Background = mean(mean)) %>%
  filter(measurement_type == "background") %>%
  ungroup() %>%
  dplyr::select(ID, Mean_Background)
```

```
## `summarise()` has grouped output by 'strain', 'treatment', 'worm',
## 'measurement_type', 'life_stage', 'experiment'. You can override using the
## `.groups` argument.
```

```
background_df
```

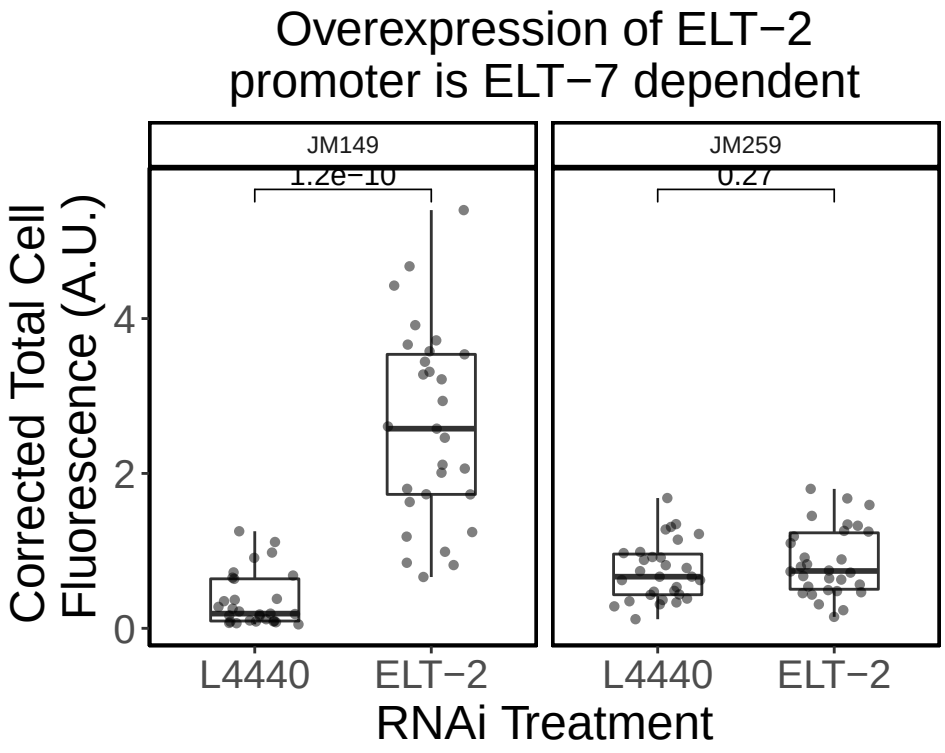
```
## # A tibble: 118 x 2
##    ID                          Mean_Background
##    <chr>                                 <dbl>
##  1 JM149_Rep1_L4440_RNAi_L1_1          0.00625
##  2 JM149_Rep2_L4440_RNAi_L1_1          0.00385
##  3 JM149_Rep3_L4440_RNAi_L1_1          0.006
##  4 JM149_Rep2_L4440_RNAi_L1_10         0.006
##  5 JM149_Rep3_L4440_RNAi_L1_10         0.00375
##  6 JM149_Rep1_L4440_RNAi_L1_2          0.0168
##  7 JM149_Rep2_L4440_RNAi_L1_2          0.00525
##  8 JM149_Rep3_L4440_RNAi_L1_2          0.00375
##  9 JM149_Rep1_L4440_RNAi_L1_3          0.00675
## 10 JM149_Rep2_L4440_RNAi_L1_3          0.00375
## # ... with 108 more rows
```

```
# calculate CTCF for each worm
image_ctcf <- image_df %>%
  filter(measurement_type == "GFP") %>%
  dplyr::select(strain, ID, treatment, worm, area, life_stage, experiment, intDen) %>%
  inner_join(background_df, by = "ID") %>%
  rowwise() %>%
  mutate(CTCF = intDen - (area*Mean_Background) )
image_ctcf
```

```
## # A tibble: 118 x 10
## # Rowwise:
##    strain ID                  treatment worm   area life_stage experiment intDen
##    <chr>  <chr>               <fct>     <chr> <dbl> <chr>      <chr>       <dbl>
##  1 JM149  JM149_Rep1_ELT-2_R~ ELT-2     1      8.01 L1         Rep1_ELT-~  20.7
##  2 JM149  JM149_Rep1_ELT-2_R~ ELT-2     2      6.54 L1         Rep1_ELT-~  21.1
##  3 JM149  JM149_Rep1_ELT-2_R~ ELT-2     3      9.72 L1         Rep1_ELT-~  36.2
##  4 JM149  JM149_Rep1_ELT-2_R~ ELT-2     4      6.14 L1         Rep1_ELT-~  22.0
##  5 JM149  JM149_Rep1_ELT-2_R~ ELT-2     5      7.81 L1         Rep1_ELT-~  30.7
##  6 JM149  JM149_Rep1_ELT-2_R~ ELT-2     6     11.5  L1         Rep1_ELT-~  24.5
##  7 JM149  JM149_Rep1_ELT-2_R~ ELT-2     7     11.9  L1         Rep1_ELT-~  29.5
##  8 JM149  JM149_Rep1_ELT-2_R~ ELT-2     8     13.8  L1         Rep1_ELT-~  25.0
##  9 JM149  JM149_Rep1_ELT-2_R~ ELT-2     9      8.39 L1         Rep1_ELT-~  14.6
## 10 JM149  JM149_Rep1_L4440_R~ L4440     1     12.1  L1         Rep1_L444~   8.80
## # ... with 108 more rows, and 2 more variables: Mean_Background <dbl>,
## #   CTCF <dbl>
```

Plot the quantification results

```
ggplot(image_ctcf, aes(treatment, CTCF/area)) +
  geom_boxplot(width = 0.5) +
  geom_jitter(width = 0.25, alpha = 0.5, shape = 16) +
  facet_grid(.~strain) +
  labs(
    title = "Overexpression of ELT-2\npromoter is ELT-7 dependent",
    x = "RNAi Treatment",
    y = "Corrected Total Cell \nFluorescence (A.U.)"
  ) +
  stat_compare_means(comparisons = list(c("L4440", "ELT-2")), method = "t.test") +
  scale_x_discrete(labels = c("L4440", "ELT-2"))+
  theme_classic() +
  theme(
    panel.border = element_rect(colour = "black", fill = NA, size = 1),
    axis.text.x = element_text(size = 15),
    axis.text.y = element_text(size = 15),
    axis.title.x = element_text(size = 18),
    axis.title.y = element_text(size=18),
    plot.title = element_text(hjust = 0.5, size = 18)
    )
```




```
ggsave("../03_output/ELT-2_promoter_analysis_plot_210317.pdf", width = 5, height = 4, useDingbats=FALSE)
```

Measure fold change

To see how much brighter ELT-2 is compared to L4440

```
mean_ctcf <-
  image_ctcf %>%
  group_by(strain, treatment) %>%
  summarise(avgCTCF = mean(CTCF), sdCTCF = sd(CTCF))
```

```
## `summarise()` has grouped output by 'strain'. You can override using the
## `.groups` argument.
```

```
mean_ctcf <-
  mean_ctcf %>% pivot_wider(names_from = treatment,
                            values_from = c(avgCTCF, sdCTCF))
mean_ctcf
```

```
## # A tibble: 2 x 5
## # Groups:   strain [2]
##   strain avgCTCF_L4440 `avgCTCF_ELT-2` sdCTCF_L4440 `sdCTCF_ELT-2`
##   <chr>          <dbl>           <dbl>        <dbl>          <dbl>
## 1 JM149           5.60            28.6         5.26           12.8
## 2 JM259          10.4             11.1         5.19            7.45
```

Calculate fold change and add confidence intervals

```
image_ctcf %>%filter(life_stage=='L1' & treatment == 'ELT-2', strain == "JM149") %>% dplyr::select(CTCF
image_ctcf %>% filter(life_stage=='L1' & treatment == 'L4440', strain == "JM149") %>% dplyr::select(CTCF
```

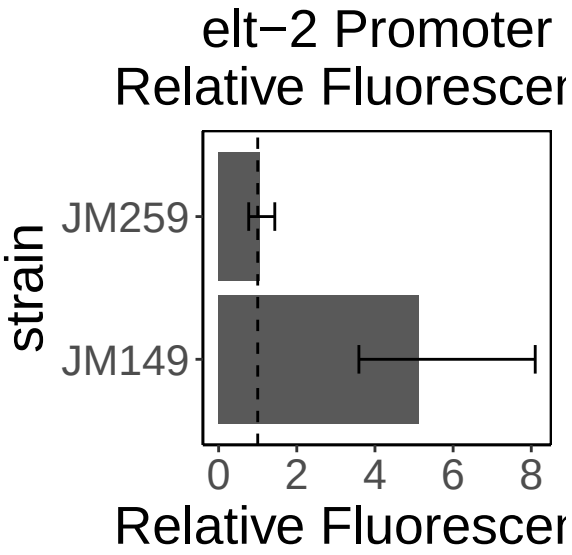
```
ttestratio(JM149_numerator[[1]], JM149_denominator[[1]]) -> L1_ttest_ratio_JM149

image_ctcf %>%filter(life_stage=='L1' & treatment == 'ELT-2', strain == "JM259") %>% dplyr::select(CTCF
image_ctcf %>% filter(life_stage=='L1' & treatment == 'L4440', strain == "JM259") %>% dplyr::select(CTC
ttestratio(JM259_numerator[[1]], JM259_denominator[[1]]) -> L1_ttest_ratio_JM259

mean_ctcf$lower = c(L1_ttest_ratio_JM149$conf.int[1], L1_ttest_ratio_JM259$conf.int[1])
mean_ctcf$upper = c(L1_ttest_ratio_JM149$conf.int[2], L1_ttest_ratio_JM259$conf.int[2])
```

Plot fold change results

```
ggplot(mean_ctcf, aes(x = strain, y = `avgCTCF_ELT-2`/avgCTCF_L4440)) +
  geom_bar(stat = "identity") +
  geom_errorbar(aes(ymin = lower, ymax = upper), width = 0.2) +
  labs(title = "elt-2 Promoter\nRelative Fluorescence") +
  theme_classic()+
  theme(
    panel.border = element_rect(colour = "black", fill = NA, size = 0.5),
    axis.text.x = element_text(size = 16),
    axis.text.y = element_text(size = 16),
    axis.title.x = element_text(size = 20),
    axis.title.y = element_text(size=20),
    plot.title = element_text(hjust = 0.5, size = 20)
    ) +
  geom_hline(yintercept = 1, linetype = "dashed") +
  ylab("Relative Fluorescence") + coord_flip()
```




```
ggsave("../03_output/ELT-2_promoter_analysis_fold_change_210317.pdf", width = 3, height = 3, useDingbats
```