rpoE graphs

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RpoE fluorescence assay (Fig 5)

Load necessary packages for these graphs:

```
require('pacman')

p_load(dplyr, ggplot2, tidyr, RColorBrewer)
```

Load reformatted plate data

```
##     IPTG_conc rep strain   Tn7 plasmid fluorescence        OD
## 1  background  24   <NA>  <NA>    <NA>     457.0833 0.0414875
## 2           0   1     Ab    WT      WT     884.0000 0.7305000
## 3           0   2     Ab    WT      WT     895.0000 0.6572000
## 4           0   3     Ab    WT      WT     635.0000 0.5337000
## 5           0   4     Ab    WT      WT     644.0000 0.5783000
## 6           0   5     Ab    WT      WT     610.0000 0.7125000
## 7           0   6     Ab    WT      WT     649.0000 0.6001000
## 8           0   1    Eco    WT      WT     680.0000 0.7818000
## 9           0   2    Eco    WT      WT     653.0000 0.7657000
## 10          0   3    Eco    WT      WT     642.0000 0.7960000
## 11          0   4    Eco    WT      WT     820.0000 0.7794000
## 12          0   5    Eco    WT      WT     733.0000 0.7131000
## 13          0   6    Eco    WT      WT     591.0000 0.7687000
## 14      0.025   1     Ab PrpoE      EV     761.0000 0.8360000
## 15      0.025   2     Ab PrpoE      EV     788.0000 0.8884000
## 16      0.025   3     Ab PrpoE      EV     813.0000 0.8925000
## 17      0.025   4     Ab PrpoE      EV     834.0000 0.8615000
## 18      0.025   5     Ab PrpoE      EV     750.0000 0.8957000
## 19      0.025   6     Ab PrpoE      EV     718.0000 0.8578000
## 20      0.025   1    Eco PrpoE      EV    6955.0000 0.8038000
## 21      0.025   2    Eco PrpoE      EV    2567.0000 0.6313000
## 22      0.025   3    Eco PrpoE      EV    5148.0000 0.7754000
## 23      0.025   4    Eco PrpoE      EV    5793.0000 0.7933000
## 24      0.025   5    Eco PrpoE      EV    4767.0000 0.7414000
## 25      0.025   6    Eco PrpoE      EV    5903.0000 0.7996000
## 26      0.025   1     Ab PrpoE    RpoE   36071.0000 0.8195000
## 27      0.025   2     Ab PrpoE    RpoE   13613.0000 0.7320000
## 28      0.025   3     Ab PrpoE    RpoE   23023.0000 0.7165000
## 29      0.025   4     Ab PrpoE    RpoE   11425.0000 0.6831000
## 30      0.025   5     Ab PrpoE    RpoE   36836.0000 0.8049000
```

```
## 31      0.025   6     Ab PrpoE    RpoE   45207.0000 0.8065000
## 32      0.025   1    Eco PrpoE    RpoE   23379.0000 0.6490000
## 33      0.025   2    Eco PrpoE    RpoE   19065.0000 0.5540000
## 34      0.025   3    Eco PrpoE    RpoE   15414.0000 0.3681000
## 35      0.025   4    Eco PrpoE    RpoE   19647.0000 0.6773000
## 36      0.025   5    Eco PrpoE    RpoE   18263.0000 0.5950000
## 37      0.025   6    Eco PrpoE    RpoE   12071.0000 0.3743000
## 38       0.05   1     Ab PrpoE      EV     825.0000 0.8656000
## 39       0.05   2     Ab PrpoE      EV    1026.0000 0.8154000
## 40       0.05   3     Ab PrpoE      EV    1528.0000 0.7938000
## 41       0.05   4     Ab PrpoE      EV     739.0000 0.8646000
## 42       0.05   5     Ab PrpoE      EV     707.0000 0.7465000
## 43       0.05   6     Ab PrpoE      EV     709.0000 0.7821000
## 44       0.05   1     Ab PrpoE    RpoE   15989.0000 0.5636000
## 45       0.05   2     Ab PrpoE    RpoE   10398.0000 0.7613000
## 46       0.05   3     Ab PrpoE    RpoE   26764.0000 0.7690000
## 47       0.05   4     Ab PrpoE    RpoE   22148.0000 0.7336000
## 48       0.05   5     Ab PrpoE    RpoE   20481.0000 0.7229000
## 49       0.05   6     Ab PrpoE    RpoE    8057.0000 0.6070000
```

Subtract off background fluorescence and normalize to OD

```
background_vals <- rpoE_data %>% filter(IPTG_conc == "background")

rpoE_norm <- rpoE_data %>% filter(IPTG_conc != "background") %>%
  mutate(IPTG_conc = as.numeric(IPTG_conc),
         fluorescence = fluorescence - background_vals$fluorescence,
         OD = OD - background_vals$OD)

rpoE_norm$norm_exp <- rpoE_norm$fluorescence/rpoE_norm$OD
```

Center autofluorescence (WT, no expression vectors) around 0 and get stats

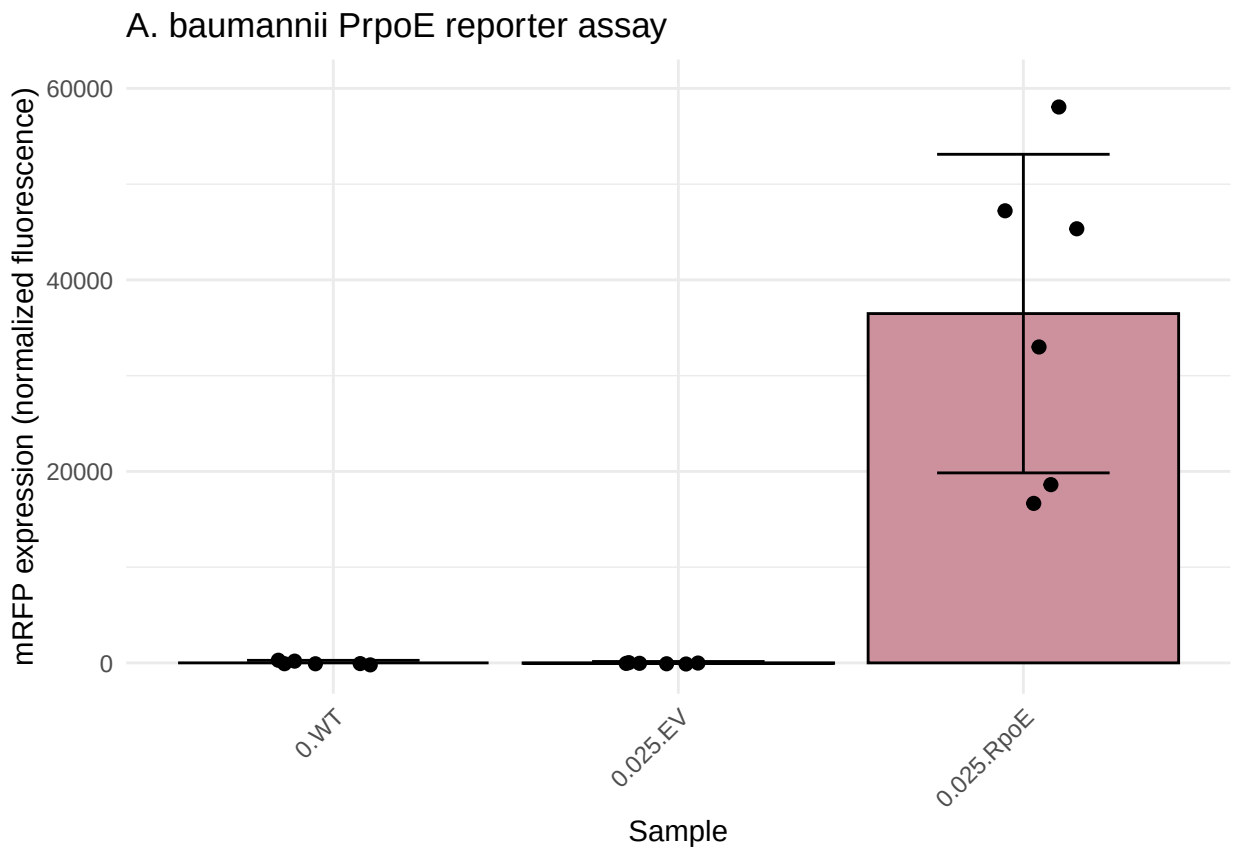
```
WT_stats <- rpoE_norm %>% filter(IPTG_conc == 0) %>%
  group_by(strain) %>%
  summarise(WTmean = mean(norm_exp, na.rm = TRUE),
            WTsd = sd(norm_exp, na.rm = TRUE),
            N = n_distinct(rep))

sample_stats <- rpoE_norm %>%
  group_by(IPTG_conc, strain, plasmid) %>%
  summarise(mean = mean(norm_exp, na.rm = TRUE),
            sd = sd(norm_exp, na.rm = TRUE),
            N = n_distinct(rep))

rpoE_adjusted <- rpoE_norm %>%
  left_join(WT_stats, by = "strain") %>%
  mutate(combination = interaction(IPTG_conc, plasmid),
    norm_exp_adj = norm_exp - WTmean) %>%
  select(-WTmean, -WTsd)

combined_stats <- sample_stats %>%
  left_join(WT_stats, by = "strain") %>%
```

```
  mutate(sd_adj = sqrt(WTsd^2 + sd^2),
         mean_adj = mean - WTmean,
         N_final = ifelse(N.x == N.y, N.x, NA))
```

Plot bar graphs with error-propagated standard deviations

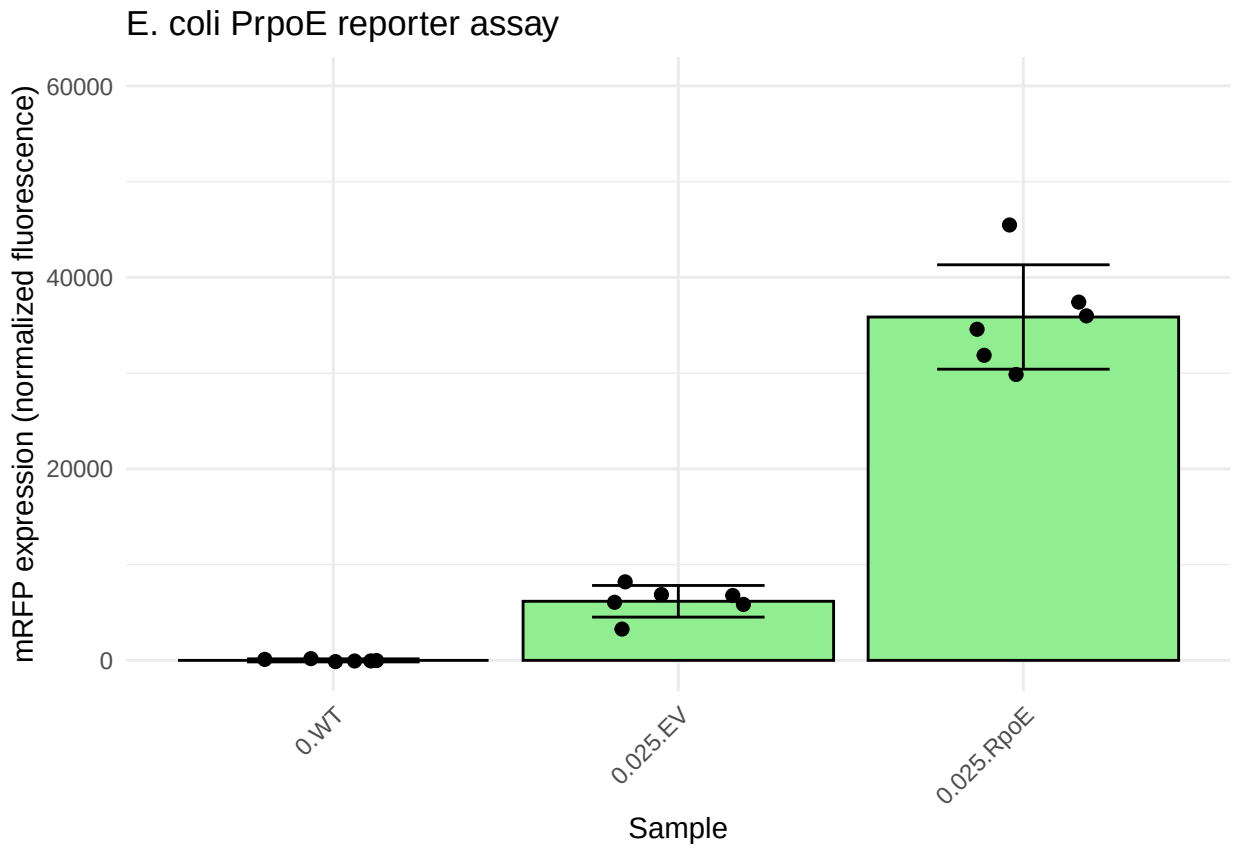
#####for *A. baumannii*

```
ab_points <- rpoE_adjusted %>%
  filter(strain == "Ab") %>% filter(IPTG_conc != 0.050)

combined_stats %>% filter(strain == "Ab") %>% filter(IPTG_conc != 0.05) %>%
  mutate(combination = interaction(IPTG_conc, plasmid)) %>%
  ggplot(aes(x = combination, y = mean_adj)) +
  geom_bar(stat = "identity", fill = "pink3", color = "black") +
  geom_errorbar(aes(ymin = mean_adj - sd_adj,
                    ymax = mean_adj + sd_adj), width = 0.5) +
  geom_point(data = ab_points, aes(y=norm_exp_adj),
             position = position_jitter(width = 0.2), size = 2) +
  ylim(limits = c(-210, 60000)) +
  labs(x = "Sample", y = "mRFP expression (normalized fluorescence)",
       title = "A. baumannii PrpoE reporter assay") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```



#####and for *E. coli*

```r
eco_points <- rpoE_adjusted %>%
  filter(strain == "Eco")

combined_stats %>% filter(strain == "Eco") %>%
  mutate(combination = interaction(IPTG_conc, plasmid)) %>%
  ggplot(aes(x = combination, y = mean_adj)) +
  geom_bar(stat = "identity", fill = "lightgreen", color = "black") +
  geom_errorbar(aes(ymin = mean_adj - sd_adj,
                    ymax = mean_adj + sd_adj), width = 0.5) +
  geom_point(data = eco_points, aes(y=norm_exp_adj),
             position = position_jitter(width = 0.2), size = 2) +
  labs(x = "Sample", y = "mRFP expression (normalized fluorescence)",
       title = "E. coli PrpoE reporter assay") +
  ylim(limits = c(-210, 60000)) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```



And run statistics to determine differences (Welch's t-tests)

```r
# Function to perform pairwise unpaired t-tests
perform_pairwise_t_tests <- function(data) {
  plasmids <- unique(data$plasmid)
  results <- list()
  num_comparisons <- length(plasmids) * (length(plasmids) - 1) / 2

  for (i in 1:(length(plasmids) - 1)) {
```

```
    for (j in (i + 1):length(plasmids)) {
      plasmid1 <- plasmids[i]
      plasmid2 <- plasmids[j]

      data1 <- data %>% filter(plasmid == plasmid1)
      data2 <- data %>% filter(plasmid == plasmid2)

      # Ensure that the number of rows in each group being compared is the same
      if(nrow(data1) == nrow(data2)) {
        # Extract values
        mean1 <- data1$mean_adj
        mean2 <- data2$mean_adj
        sd1 <- data1$sd_adj
        sd2 <- data2$sd_adj
        n1 <- data1$N_final
        n2 <- data2$N_final

        # Calculate standard error of the difference
        se_diff <- sqrt(sd1^2 / n1 + sd2^2 / n2)

        if(all(se_diff > 0)) { # Ensure that no division by zero occurs
          # Calculate Welch's t-statistic
          t_statistic <- (mean1 - mean2) / se_diff

          # Calculate degrees of freedom for Welch's t-test
          num <- (sd1^2 / n1 + sd2^2 / n2)^2
          denom <- ((sd1^2 / n1)^2 / (n1 - 1)) + ((sd2^2 / n2)^2 / (n2 - 1))
          df <- num / denom

          p_value <- 2 * pt(-abs(t_statistic), df)
          bonferroni_adj <- min(p_value * num_comparisons, 1)  # Bonferroni adjustment

          # Store results
          results[[paste(plasmid1, plasmid2, sep = "_vs_")]] <- data.frame(
            Plasmid1 = plasmid1,
            Plasmid2 = plasmid2,
            t_statistic = t_statistic,
            SEM = se_diff,
            degrees_of_freedom = df,
            p_value = p_value,
            Bonferroni_adj = bonferroni_adj
          )
        }
      }
    }
  }

  do.call(rbind, results)
}

# Filter the data for Ab and Eco strains
ab_data <- combined_stats %>% filter(strain == "Ab") %>%
  filter(IPTG_conc != 0.050)
```

```r
eco_data <- filter(combined_stats, strain == "Eco")

# Perform pairwise comparisons within each strain
ab_comparisons <- perform_pairwise_t_tests(ab_data)
eco_comparisons <- perform_pairwise_t_tests(eco_data)
```

Welch's t-test results

```
## [1] "_A. baumannii_ stats"
```

```
##            Plasmid1 Plasmid2 t_statistic       SEM degrees_of_freedom
## WT_vs_EV         WT       EV   0.3717541  133.7297           9.166083
## WT_vs_RpoE       WT     RpoE  -5.3708648 6792.4912           5.002523
## EV_vs_RpoE       EV     RpoE  -5.3784983 6792.0941           5.001354
##                p_value Bonferroni_adj
## WT_vs_EV   0.718524615    1.000000000
## WT_vs_RpoE 0.003007993    0.009023978
## EV_vs_RpoE 0.002991522    0.008974566
```

```
## [1] "_E. coli_ stats"
```

```
##            Plasmid1 Plasmid2 t_statistic       SEM degrees_of_freedom
## WT_vs_EV         WT       EV   -9.104202  677.8571           5.094601
## WT_vs_RpoE       WT     RpoE  -16.104883 2226.8147           5.008692
## EV_vs_RpoE       EV     RpoE  -12.765756 2325.8505           5.911057
##                 p_value Bonferroni_adj
## WT_vs_EV   2.425109e-04   7.275326e-04
## WT_vs_RpoE 1.658713e-05   4.976140e-05
## EV_vs_RpoE 1.587188e-05   4.761563e-05
```

Other RpoE-regulated promoters fluorescence assay (Fig S4)

Load reformatted plate data

```
##        strain rep plasmid    Tn7 fluorescence         OD
## 1  background  14    <NA>   <NA>     206.7857 0.04277143
## 2          Ab   1    none Pempty     295.0000 0.75230002
## 3          Ab   2    none Pempty     319.0000 0.75660002
## 4          Ab   3    none Pempty     318.0000 0.80479997
## 5          Ab   4    none Pempty     316.0000 0.79589999
## 6          Ab   5    none Pempty     274.0000 0.71969998
## 7          Ab   6    none Pempty     270.0000 0.70380002
## 8          Ab   1    RpoE  PmicA   27513.0000 0.59710002
## 9          Ab   2    RpoE  PmicA   43299.0000 0.65160000
## 10         Ab   3    RpoE  PmicA   28501.0000 0.60600001
## 11         Ab   4    RpoE  PmicA   39750.0000 0.57410002
## 12         Ab   5    RpoE  PmicA   40454.0000 0.60979998
## 13         Ab   6    RpoE  PmicA   29606.0000 0.54960000
## 14         Ab   1    RpoE  PrybB    8767.0000 0.44350001
## 15         Ab   2    RpoE  PrybB    6140.0000 0.50459999
```

```
## 16         Ab   3    RpoE  PrybB    7451.0000 0.48870000
## 17         Ab   4    RpoE  PrybB   14022.0000 0.62870002
## 18         Ab   5    RpoE  PrybB    7988.0000 0.51340002
## 19         Ab   6    RpoE  PrybB   13178.0000 0.52010000
## 20         Ab   1    RpoE  PyicJ    1003.0000 0.47999999
## 21         Ab   2    RpoE  PyicJ     791.0000 0.49540001
## 22         Ab   3    RpoE  PyicJ     734.0000 0.48510000
## 23         Ab   4    RpoE  PyicJ     724.0000 0.43759999
## 24         Ab   5    RpoE  PyicJ     878.0000 0.48600000
## 25         Ab   6    RpoE  PyicJ     916.0000 0.44909999
## 26         Ab   1      EV  PmicA     372.0000 0.73830003
## 27         Ab   2      EV  PmicA     299.0000 0.69540000
## 28         Ab   3      EV  PmicA     353.0000 0.77730000
## 29         Ab   4      EV  PmicA     364.0000 0.77039999
## 30         Ab   5      EV  PmicA     332.0000 0.75779998
## 31         Ab   6      EV  PmicA     380.0000 0.77579999
## 32         Ab   1      EV  PrybB     294.0000 0.71749997
## 33         Ab   2      EV  PrybB     284.0000 0.67930001
## 34         Ab   3      EV  PrybB     303.0000 0.70080000
## 35         Ab   4      EV  PrybB     297.0000 0.73180002
## 36         Ab   5      EV  PrybB     282.0000 0.60380000
## 37         Ab   6      EV  PrybB     296.0000 0.69580001
## 38         Ab   1      EV  PyicJ     298.0000 0.55650002
## 39         Ab   2      EV  PyicJ     413.0000 0.63910002
## 40         Ab   3      EV  PyicJ     479.0000 0.66039997
## 41         Ab   4      EV  PyicJ     490.0000 0.65660000
## 42         Ab   5      EV  PyicJ     513.0000 0.63330001
## 43         Ab   6      EV  PyicJ     434.0000 0.67510003
```

Subtract off background fluorescence and OD and normalize

```
background_prom_vals <- prom_data %>% filter(strain == "background")

prom_norm <- prom_data %>% filter(strain != "background") %>%
  mutate(fluorescence = fluorescence - background_prom_vals$fluorescence,
         OD = OD - background_prom_vals$OD)

prom_norm$norm_exp <- prom_norm$fluorescence/prom_norm$OD
```

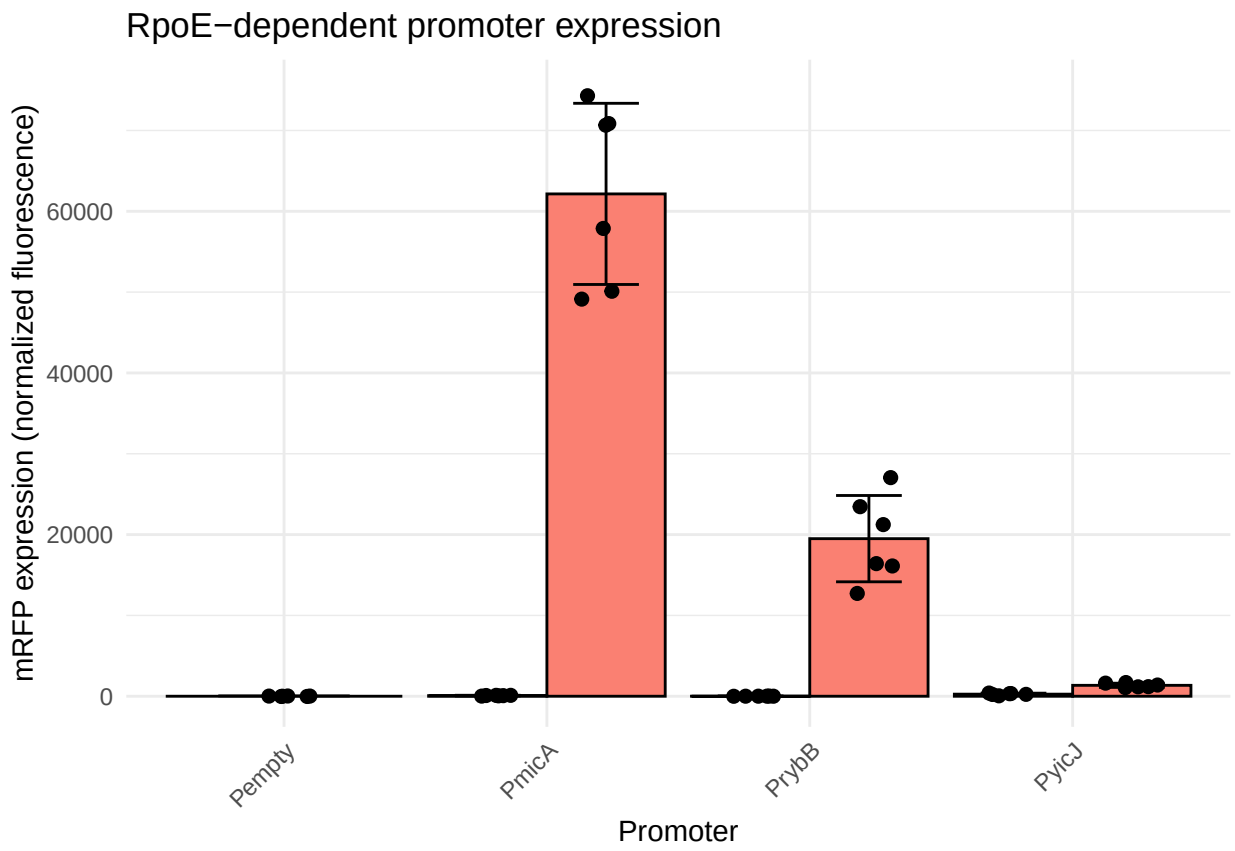
Center background fluorescence (no promoter ahead of mRFP) around 0 and get stats

```
Pempty_stats <- prom_norm %>% filter(Tn7 == "Pempty") %>%
  reframe(strain = 'Ab',
            Pemptymean = mean(norm_exp, na.rm = TRUE),
            Pemptysd = sd(norm_exp, na.rm = TRUE),
            N = n_distinct(rep))

sample_prom_stats <- prom_norm %>%
  group_by(plasmid, Tn7) %>%
  summarise(strain = "Ab",
            mean = mean(norm_exp, na.rm = TRUE),
            sd = sd(norm_exp, na.rm = TRUE),
            N = n_distinct(rep))
```

```
prom_adjusted <- prom_norm %>%
  left_join(Pempty_stats, by = "strain") %>%
  mutate(combination = interaction(plasmid, Tn7),
    norm_exp_adj = norm_exp - Pemptymean) %>%
  select(-Pemptymean, -Pemptysd)

combined_prom_stats <- sample_prom_stats %>%
  left_join(Pempty_stats, by = "strain") %>%
  mutate(sd_adj = sqrt(Pemptysd^2 + sd^2),
         mean_adj = mean - Pemptymean,
         N_final = ifelse(N.x == N.y, N.x, NA))
```

Plot bar graphs with error-propagated standard deviations

```
ggplot(combined_prom_stats, aes(x = Tn7, y = mean_adj, fill = plasmid)) +
  geom_bar(stat = "identity", position = "dodge", color = "black") +
  geom_errorbar(aes(ymin = mean_adj - sd_adj, ymax = mean_adj + sd_adj),
                width = 0.5, position = position_dodge(0.9)) +
  geom_point(data = prom_adjusted, aes(x = Tn7, y = norm_exp_adj, group = plasmid),
             position = position_jitterdodge(jitter.width = 0.2, dodge.width = 0.9), size = 2, color =
  labs(x = "Promoter", y = "mRFP expression (normalized fluorescence)", title = "RpoE-dependent promote
  scale_y_continuous(limits = c(-33, 75000)) +
  scale_fill_manual(values = c("RpoE" = "salmon", "EV" = "grey", "none" = "gray30")) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        legend.position = "none")
```



And run statistics to determine differences (Welch's t-tests)

```
# Filter the data for plasmids
PmicA_data <- filter(combined_prom_stats, Tn7 == "PmicA" | Tn7 == "Pempty")

PrybB_data <- filter(combined_prom_stats, Tn7 == "PrybB"| Tn7 == "Pempty")

PyicJ_data <- filter(combined_prom_stats, Tn7 == "PyicJ"| Tn7 == "Pempty")


# Perform pairwise comparisons within each strain using previously defined function
PmicA_comparisons <- perform_pairwise_t_tests(PmicA_data)
PrybB_comparisons <- perform_pairwise_t_tests(PrybB_data)
PyicJ_comparisons <- perform_pairwise_t_tests(PyicJ_data)
```

Welch's t-test results

```
## [1] "PmicA stats"
```

```
##              Plasmid1 Plasmid2 t_statistic        SEM degrees_of_freedom
## EV_vs_RpoE         EV     RpoE  -13.565967 4576.13191           5.000165
## EV_vs_none         EV     none    3.057865   23.87087           9.553285
## RpoE_vs_none     RpoE     none   13.581958 4576.11845           5.000107
##                   p_value Bonferroni_adj
## EV_vs_RpoE   3.899339e-05   0.0001169802
```

```
## EV_vs_none   1.272323e-02   0.0381697040
## RpoE_vs_none 3.877278e-05   0.0001163183
```

```
## [1] "PrybB stats"
```

```
##              Plasmid1 Plasmid2 t_statistic        SEM degrees_of_freedom
## EV_vs_RpoE         EV     RpoE  -8.9407414 2179.95125           5.000259
## EV_vs_none         EV     none   0.2774118   18.61219           9.228130
## RpoE_vs_none     RpoE     none   8.9430157 2179.97423           5.000470
##                   p_value Bonferroni_adj
## EV_vs_RpoE   0.0002916106   0.0008748317
## EV_vs_none   0.7875785405   1.0000000000
## RpoE_vs_none 0.0002911954   0.0008735862
```

```
## [1] "PyicJ stats"
```

```
##              Plasmid1 Plasmid2 t_statistic       SEM degrees_of_freedom
## EV_vs_RpoE         EV     RpoE   -9.317202 117.52587           7.117880
## EV_vs_none         EV     none    4.895049  52.29611           5.882108
## RpoE_vs_none     RpoE     none   12.585028 107.35012           5.197520
##                   p_value Bonferroni_adj
## EV_vs_RpoE   3.063637e-05   9.190912e-05
## EV_vs_none   2.879341e-03   8.638022e-03
## RpoE_vs_none 4.320655e-05   1.296197e-04
```