

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") %>%
```

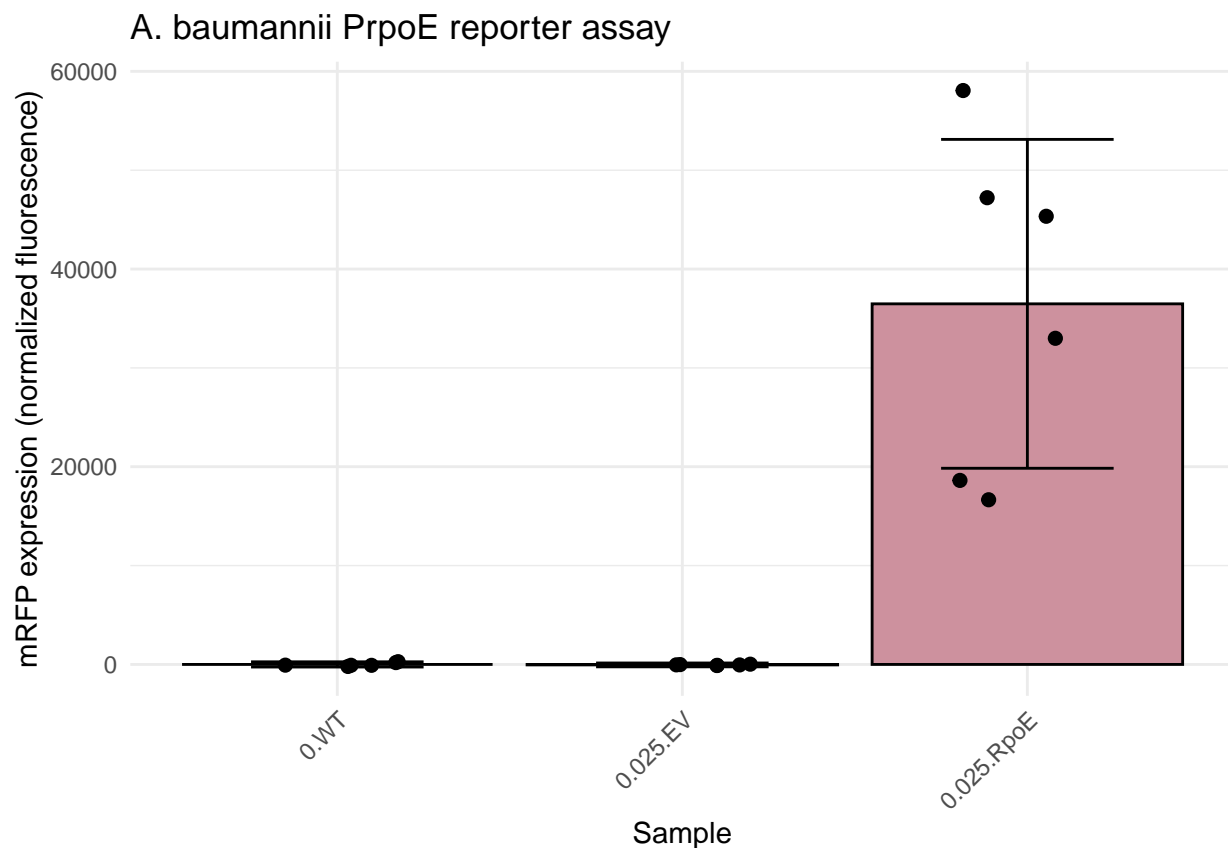
```
mutate(sd_adj = sqrt(WTsd^2 + sd^2),
       mean_adj = mean - WTmean,
       N_final = ifelse(N.x == N.y, N.x, NA))
```

Plot bar graphs with error-propagated standard deviations

#####for *A. baumannii*

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

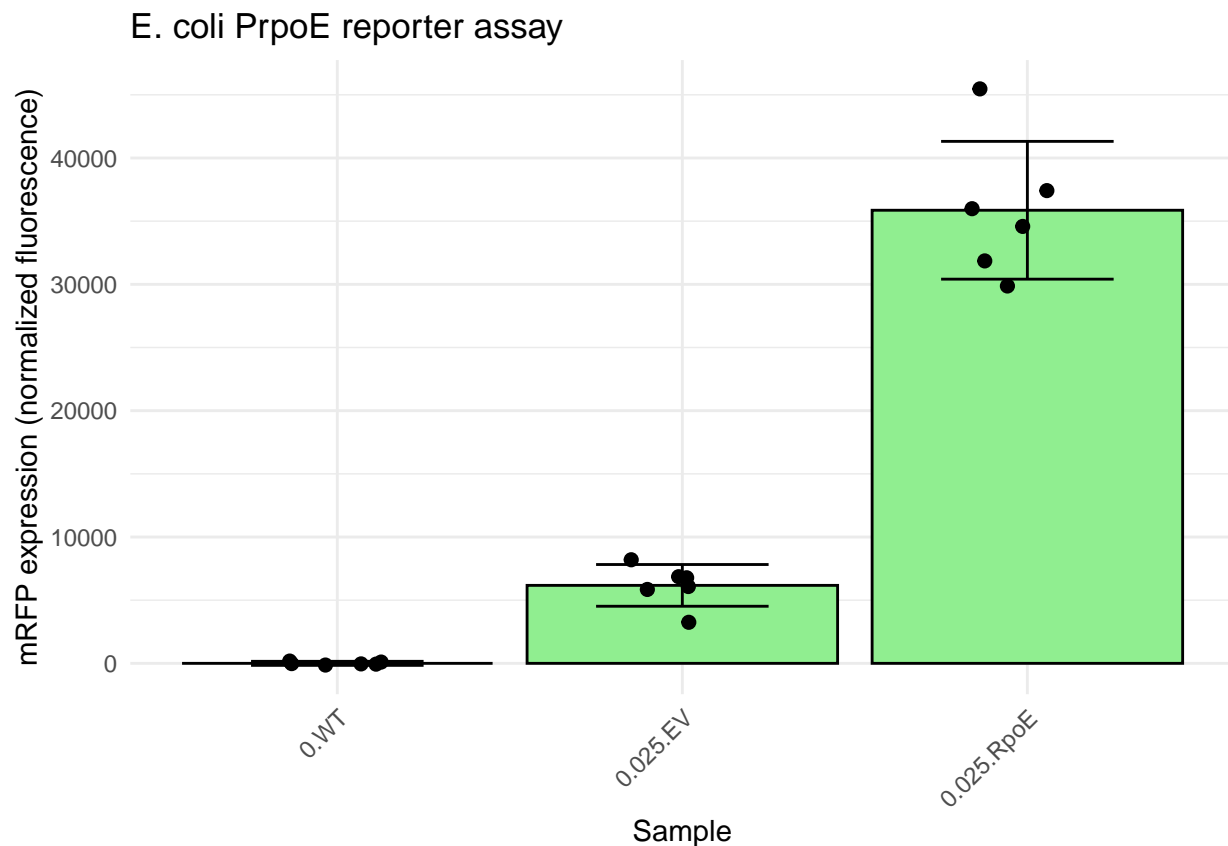
combined_stats %>% filter(strain == "Ab") %>% filter(IPTG_conc != 0.05) %>%
  mutate(combination = interaction(IPTG_conc, plasmid)) %>%
  ggplot(aes(x = combination, y = mean_adj)) +
  geom_bar(stat = "identity", fill = "pink3", color = "black") +
  geom_errorbar(aes(ymin = mean_adj - sd_adj,
                   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) +
  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*

```
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") +
  # scale_y_continuous(limits = c(NA, 60000)) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```



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

```
# 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)

```

```
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))

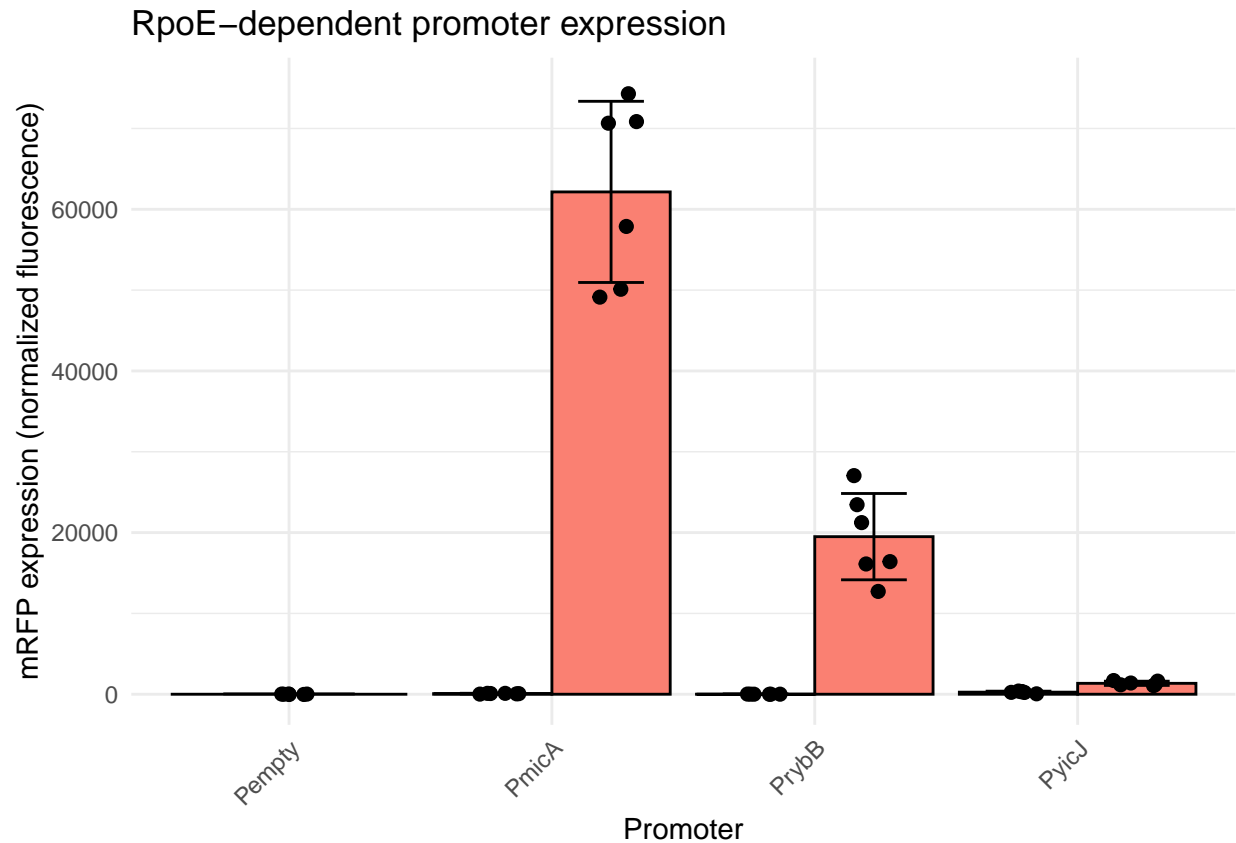
```

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 = "black") +
  labs(x = "Promoter", y = "mRFP expression (normalized fluorescence)", title = "RpoE-dependent promoter activity") +
  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
```