Resuscitation Promoting Factor

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OVERVIEW

Initial experiments to assess activity of new recombinant Rpf. Using EnzChek Lysozyme Assay Kit (https://tools.lifetechnologies.com/content/sfs/manuals/mp22013.pdf). EnzcChek detects the fluorescence of labeled peptidoglycan uppon hydrolysis. Also testing effects of histidine removal via thrombin digest.

Brent did two experiments on January 21, 2015. Similar treatments in both experiments; just different repcliation (exp 1 vs. exp 2). Negative control was no Rpf added. First treatment was Rpf with histidine tag intact. Second treatment was Rpf with histidine tag removed via thrombin digest. There was also a postive control where lysozyme was used, but fluorescence values were all maxed out.

SETUP

```
rm(list=ls())
getwd()
setwd("~/GitHub/Rpf/enzyme")
```

LOAD DATA

```
exp1 <- read.table("20150121a_Rpf_Enz.txt", sep = "\t", header = TRUE)
exp2 <- read.table("20150121b_Rpf_Enz.txt", sep = "\t", header = TRUE)
```

SETTING UP ANOVA TREATMENTS

```
treat1 <- factor(exp1$treat, levels = c('neg', 'his_plus', 'his_rem'))
treat2 <- factor(exp2$treat, levels = c('neg', 'his_plus', 'his_rem'))
```

CALCUATING MEANS OF TREATMENTS

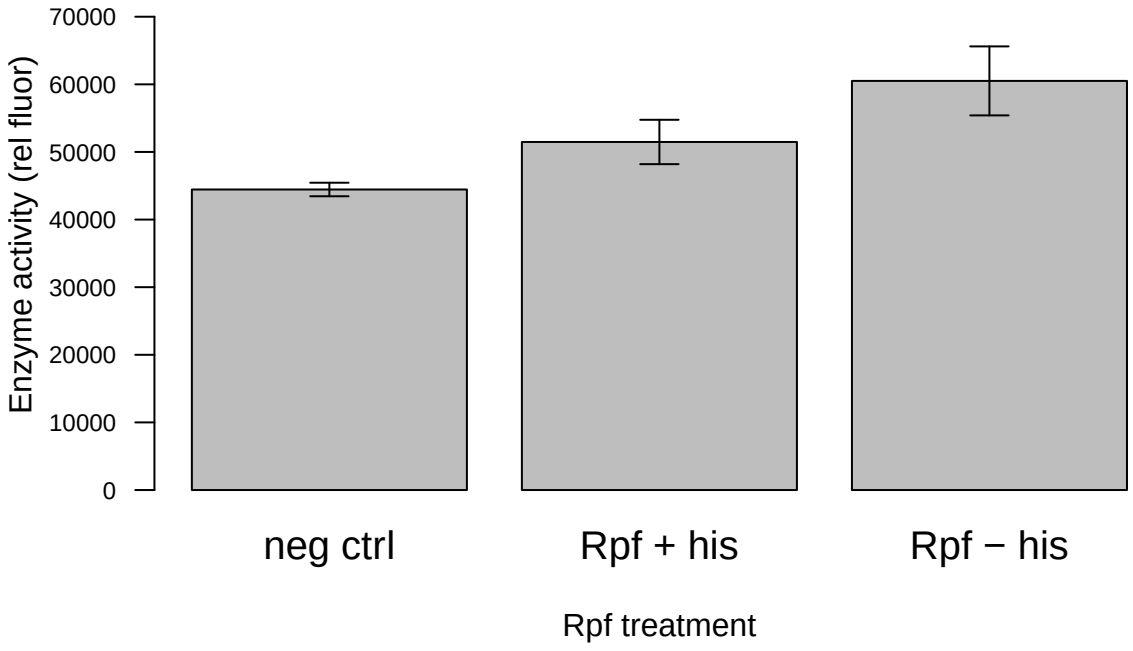
```
exp1.means <- tapply(exp1$fluor, treat1, mean)
exp2.means <- tapply(exp2$fluor, treat2, mean)
```

CALCUATING SEM OF TREATMENTS

```
sem <- function(x){
  sd(na.omit(x))/sqrt(length(na.omit(x)))
  }

exp1.sem <- tapply(exp1$fluor, treat1, sem)

exp2.sem <- tapply(exp2$fluor, treat2, sem)
```

BARPLOT - EXPERIMENT 1: JANUARY 12, 2015

```
exp1_bp <- barplot(exp1.means, ylim =c(0, round(max(exp1$fluor), digits = 0)),
              pch = 15, cex = 1.25, las = 1, cex.lab = 1.0, cex.axis = 0.75,
              xlab = "Rpf treatment",
              ylab = "Enzyme activity (rel fluor)",
              names.arg = c("neg ctrl", "Rpf + his", "Rpf - his"))

arrows(x0 = exp1_bp, y0 = exp1.means, y1 = exp1.means - exp1.sem, angle = 90,
       length=0.1, lwd = 1)
arrows(x0 = exp1_bp, y0 = exp1.means, y1 = exp1.means + exp1.sem, angle = 90,
       length=0.1, lwd = 1)
```



ANOVA - EXPERIMENT 1: JANUARY 12, 2015

```
anova_exp1 <- aov(exp1$fluor ~ exp1$treat, data = exp1)

summary(anova_exp1)
```

```
##             Df   Sum Sq  Mean Sq F value Pr(>F)
## exp1$treat   2 5.19e+08 2.59e+08    5.15  0.032 *
## Residuals    9 4.53e+08 5.04e+07
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(anova_exp1)
```

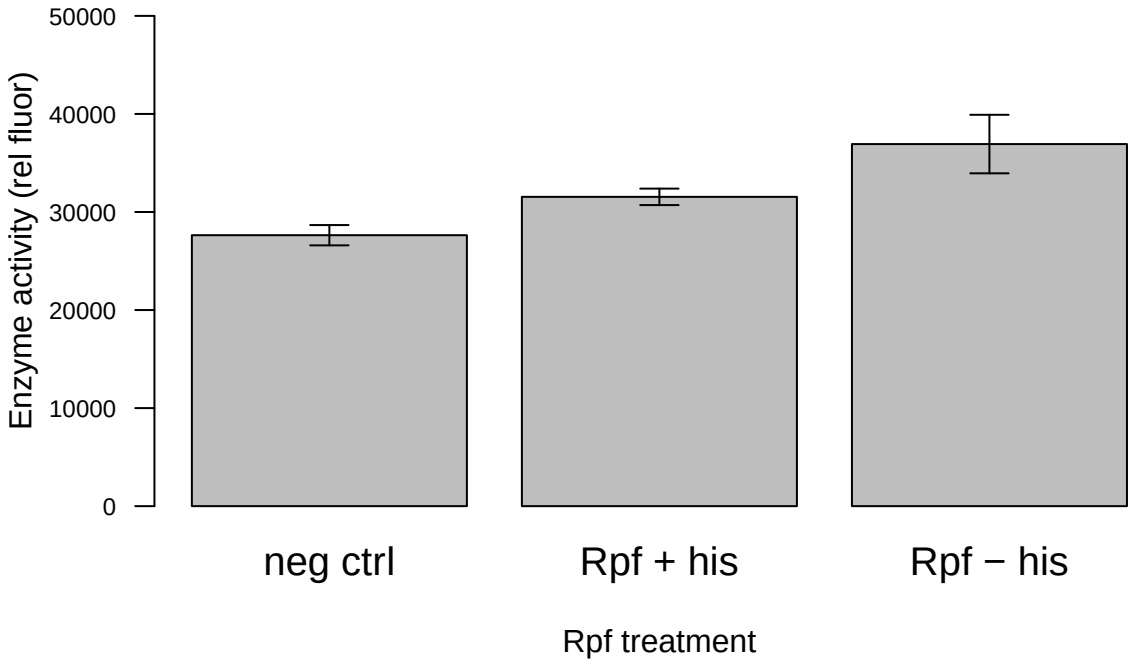
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = exp1$fluor ~ exp1$treat, data = exp1)
##
## $`exp1$treat`
##                     diff    lwr   upr  p adj
## his_rem-his_plus   9033  -4980 23046 0.2239
## neg-his_plus      -7028 -21041  6985 0.3808
## neg-his_rem      -16061 -30074 -2049 0.0265
```

INTERPRETATION OF EXPERIMENT 1

Overall ANOVA model is significant. Tukey HSD indicates that fluorescence is higher when comparing histdine removed Rpf versus negative control. No difference, however, when comparing fluorescence of treatments with histidine intact vs. removed.

BARPLOT - EXPERIMENT 2: JANUARY 12, 2015

```
exp2_bp <- barplot(exp2.means, ylim =c(0, round(max(exp2$fluor), digits = 0)),
              pch = 15, cex = 1.25, las = 1, cex.lab = 1.0, cex.axis = 0.75,
              xlab = "Rpf treatment",
              ylab = "Enzyme activity (rel fluor)",
              names.arg = c("neg ctrl", "Rpf + his", "Rpf - his"))

arrows(x0 = exp2_bp, y0 = exp2.means, y1 = exp2.means - exp2.sem, angle = 90,
       length = 0.1, lwd = 1)
arrows(x0 = exp2_bp, y0 = exp2.means, y1 = exp2.means + exp2.sem, angle = 90,
       length= 0.1, lwd = 1)
```



ANOVA - EXPERIMENT 2: JANUARY 12, 2015

```
anova_exp2 <- aov(exp2$fluor ~ exp2$treat, data = exp2)

summary(anova_exp2)
```

```
##             Df   Sum Sq  Mean Sq F value Pr(>F)
## exp2$treat   2 3.28e+08 1.64e+08    5.63  0.012 *
## Residuals   20 5.83e+08 2.92e+07
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(anova_exp2)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = exp2$fluor ~ exp2$treat, data = exp2)
##
## $`exp2$treat`
##                   diff    lwr   upr  p adj
## his_rem-his_plus  5382  -1450 12214 0.1399
## neg-his_plus     -3912 -10984  3160 0.3600
## neg-his_rem      -9294 -16366 -2222 0.0091
```

INTERPRETATION OF EXPERIMENT 2: JANUARY 12, 2015

Overall ANOVA model is significant. Tukey HSD indicates that fluorescence is higher when comparing histdine removed Rpf versus negative control. No difference, however, when comparing fluorescence of treatments with histidine intact vs. removed.

EXPERIMENT 3: FEBRUARY 6, 2015

Compared Rpf from pBAD (old expression system) with pET15b (new expression systems). Motivation was that we had observed better muralytic activit with pBAD. Rpf concentration from PET15b and pBAD was 520 ng/ul

Calcuating Molarity of Recombinant Rpf

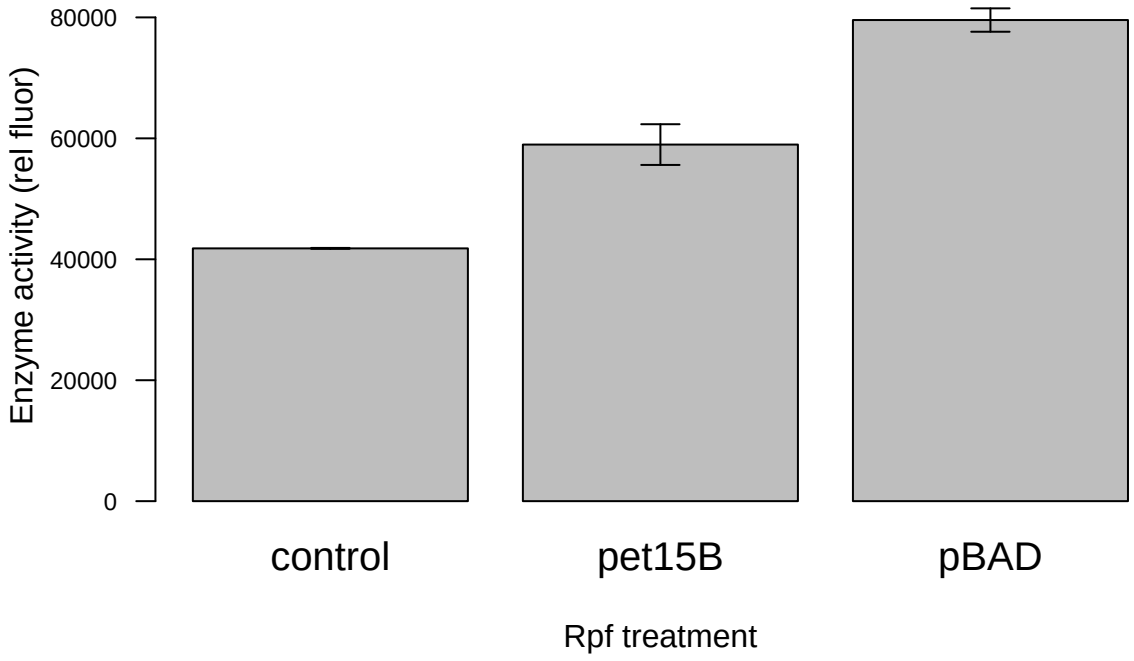
```
elements <- c("C", "H", "N", "O", "S")
atoms.mass <- c(12, 1, 14, 16, 32)
atoms.rpf <- c(1243, 1950, 362, 424, 3)
product <- atoms.mass * atoms.rpf
mol.rpf <- sum(product)
rpf.conc.mass <- 520 # ng/uL
rpf.conc.mol <- (rpf.conc.mass * 1000) / mol.rpf # umol/L
```

SETTING UP DATA AND TREATMENTS

```
exp3 <- read.table("20150206_Rpf_Enz.txt", sep = "\t", header = TRUE)
treat3 <- factor(exp3$treat, levels = c('control', 'pet15B', 'pBAD'))
exp3.means <- tapply(exp3$fluor, treat3, mean)
exp3.sem <- tapply(exp3$fluor, treat3, sem)
```

BARPLOT - EXPERIMENT 3: FEBRUARY 6, 2015

```
exp3_bp <- barplot(exp3.means, ylim =c(0, round(max(exp3$fluor), digits = 0)),
              pch = 15, cex = 1.25, las = 1, cex.lab = 1.0, cex.axis = 0.75,
              xlab = "Rpf treatment",
              ylab = "Enzyme activity (rel fluor)",
              names.arg = c("control", "pet15B", "pBAD"))

arrows(x0 = exp3_bp, y0 = exp3.means, y1 = exp3.means - exp3.sem, angle = 90,
       length = 0.1, lwd = 1)
arrows(x0 = exp3_bp, y0 = exp3.means, y1 = exp3.means + exp3.sem, angle = 90,
       length= 0.1, lwd = 1)
```



ANOVA - EXPERIMENT 3: FEBRUARY 6, 2015

```
anova_exp3 <- aov(exp3$fluor ~ exp3$treat, data = exp3)

summary(anova_exp3)
```

```
##             Df   Sum Sq  Mean Sq F value  Pr(>F)
## exp3$treat   2 2.06e+09 1.03e+09    39.8 0.00015 ***
## Residuals    7 1.81e+08 2.58e+07
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(anova_exp3)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = exp3$fluor ~ exp3$treat, data = exp3)
##
## $`exp3$treat`
##                   diff    lwr    upr  p adj
## pBAD-control     37762  24797  50727 0.0001
## pet15B-control   17168   4203  30133 0.0142
## pet15B-pBAD     -20594 -31180 -10008 0.0018
```

INTERPRETATION OF EXPERIMENT 3: FEBRUARY 6, 2015

Overall ANOVA model is significant (P = 0.000151) Tukey HSD indicates that fluorescence is different among all treatments. That is, pet15B fluor and pBAD fluor are both individually higher than the control (P = 0.0142482 and P = 0.0001479, respectively). Futhermore, pBAD fluor is 34% higher than pet15B fluor (P = 0.0017805).