

R Data AI190322

Date: 190327 Sampled: 15 plants 5 days post infection (dpi), one leaf disc per plant for treatment w/ EC36309, coinfiltration treatments 9-18, 9-19, 9-20 (EC36309 w/ EC36318/19/20). Co-infiltration data unusable due to strong hypersensitive response in leaf spots.

Two separate technical replicates of 36309 endpoint fluorescence measurement, data from AI190320 and AI190322.

Analysis of EC36309 using ggplot2:

```
merged9_df <- read.table("mergedEC36309.csv", header=TRUE,
                        sep=",")
```

#merged dataset of AI190320 (n = 15) and AI190322 (n=15), only analyzing EC36309 treatment (no observab

```
attach(merged9_df)
summary(merged9_df)
```

##	ID	GFP	RFP	GFP.RFP
##	EC36309:30	Min. : 3.400	Min. : 6.20	Min. :0.2700
##		1st Qu.: 6.600	1st Qu.:12.62	1st Qu.:0.3725
##		Median : 8.250	Median :19.05	Median :0.4600
##		Mean : 9.967	Mean :23.23	Mean :0.4777
##		3rd Qu.:12.650	3rd Qu.:28.23	3rd Qu.:0.5675
##		Max. :22.500	Max. :65.00	Max. :0.7600

```
library(ggplot2)
library(reshape2)
```

changing the df to long format

```
merged9_df_long <- melt(merged9_df,
                        id.vars=c("ID"),
                        measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                        variable.name="Channel",
                        value.name="RFU")
```

```
attach(merged9_df_long)
```

The following object is masked from merged9_df:

##

ID

taking out GFP/RFP ratios for GFP to RFP comparisons

```
merged9_df_long_red <- merged9_df_long[-c(61:90), ]
merged9_df_long_red
```

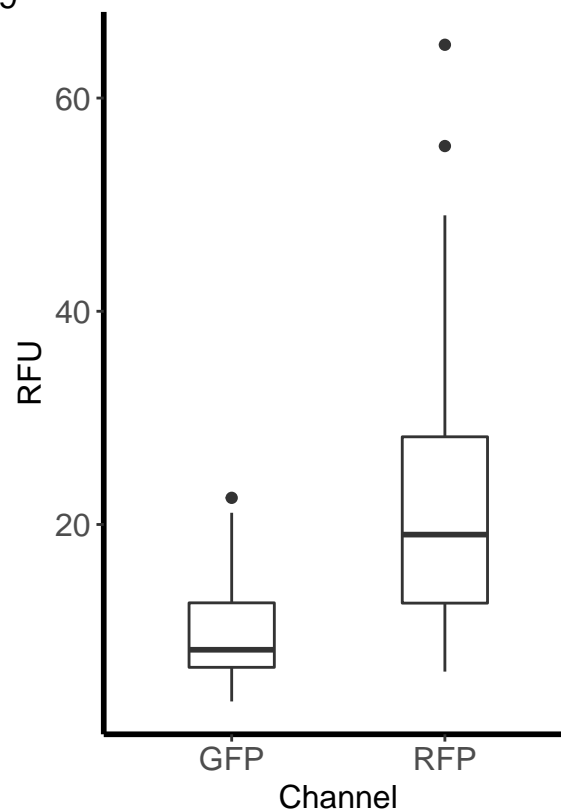
##	ID	Channel	RFU
## 1	EC36309	GFP	16.5
## 2	EC36309	GFP	8.8
## 3	EC36309	GFP	4.7
## 4	EC36309	GFP	7.4
## 5	EC36309	GFP	3.8

## 6	EC36309	GFP	8.2
## 7	EC36309	GFP	6.3
## 8	EC36309	GFP	5.1
## 9	EC36309	GFP	4.7
## 10	EC36309	GFP	5.3
## 11	EC36309	GFP	7.4
## 12	EC36309	GFP	6.5
## 13	EC36309	GFP	9.1
## 14	EC36309	GFP	3.4
## 15	EC36309	GFP	6.9
## 16	EC36309	GFP	12.5
## 17	EC36309	GFP	17.6
## 18	EC36309	GFP	7.1
## 19	EC36309	GFP	15.0
## 20	EC36309	GFP	10.7
## 21	EC36309	GFP	7.9
## 22	EC36309	GFP	22.5
## 23	EC36309	GFP	15.9
## 24	EC36309	GFP	10.7
## 25	EC36309	GFP	21.1
## 26	EC36309	GFP	8.3
## 27	EC36309	GFP	12.7
## 28	EC36309	GFP	17.4
## 29	EC36309	GFP	8.4
## 30	EC36309	GFP	7.1
## 31	EC36309	RFP	41.1
## 32	EC36309	RFP	12.5
## 33	EC36309	RFP	6.2
## 34	EC36309	RFP	14.5
## 35	EC36309	RFP	7.1
## 36	EC36309	RFP	17.6
## 37	EC36309	RFP	12.2
## 38	EC36309	RFP	8.1
## 39	EC36309	RFP	7.7
## 40	EC36309	RFP	13.9
## 41	EC36309	RFP	13.0
## 42	EC36309	RFP	10.0
## 43	EC36309	RFP	16.8
## 44	EC36309	RFP	8.6
## 45	EC36309	RFP	16.7
## 46	EC36309	RFP	40.8
## 47	EC36309	RFP	39.4
## 48	EC36309	RFP	20.9
## 49	EC36309	RFP	25.6
## 50	EC36309	RFP	19.1
## 51	EC36309	RFP	19.2
## 52	EC36309	RFP	65.0
## 53	EC36309	RFP	29.1
## 54	EC36309	RFP	39.3
## 55	EC36309	RFP	49.0
## 56	EC36309	RFP	25.2
## 57	EC36309	RFP	21.3
## 58	EC36309	RFP	55.5
## 59	EC36309	RFP	22.6

```
## 60 EC36309      RFP 19.0
```

```
ggplot(data=merged9_df_long_red, aes(x=Channel,y=RFU)) +
  geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  labs(tag = "EC36309") +
  scale_color_grey() +
  theme_classic() +
  coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )
```

EC36309



```
summary(merged9_df_long_red)
```

```
##      ID      Channel      RFU
## EC36309:60  GFP      :30  Min.   : 3.400
##              RFP      :30  1st Qu.: 7.625
##              GFP.RFP: 0   Median :12.600
##              Mean    :16.600
##              3rd Qu.:19.625
##              Max.    :65.000
```

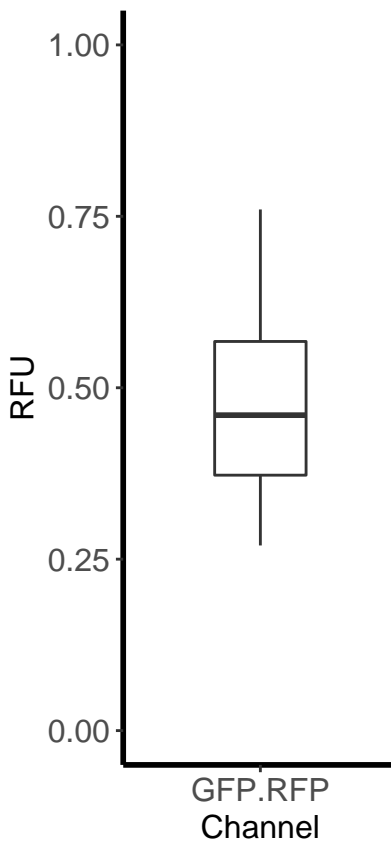
```
# df with only GFP/RFP ratios:
```

```
merged9_df_long_red2 <- merged9_df_long[-c(1:60), ]
```

```
merged9_df_long_red2
```

```
##      ID Channel  RFU
## 61 EC36309 GFP.RFP 0.40
## 62 EC36309 GFP.RFP 0.70
## 63 EC36309 GFP.RFP 0.76
## 64 EC36309 GFP.RFP 0.51
## 65 EC36309 GFP.RFP 0.54
## 66 EC36309 GFP.RFP 0.47
## 67 EC36309 GFP.RFP 0.52
## 68 EC36309 GFP.RFP 0.63
## 69 EC36309 GFP.RFP 0.61
## 70 EC36309 GFP.RFP 0.38
## 71 EC36309 GFP.RFP 0.57
## 72 EC36309 GFP.RFP 0.65
## 73 EC36309 GFP.RFP 0.54
## 74 EC36309 GFP.RFP 0.40
## 75 EC36309 GFP.RFP 0.41
## 76 EC36309 GFP.RFP 0.31
## 77 EC36309 GFP.RFP 0.45
## 78 EC36309 GFP.RFP 0.34
## 79 EC36309 GFP.RFP 0.59
## 80 EC36309 GFP.RFP 0.56
## 81 EC36309 GFP.RFP 0.41
## 82 EC36309 GFP.RFP 0.35
## 83 EC36309 GFP.RFP 0.55
## 84 EC36309 GFP.RFP 0.27
## 85 EC36309 GFP.RFP 0.43
## 86 EC36309 GFP.RFP 0.33
## 87 EC36309 GFP.RFP 0.60
## 88 EC36309 GFP.RFP 0.31
## 89 EC36309 GFP.RFP 0.37
## 90 EC36309 GFP.RFP 0.37
```

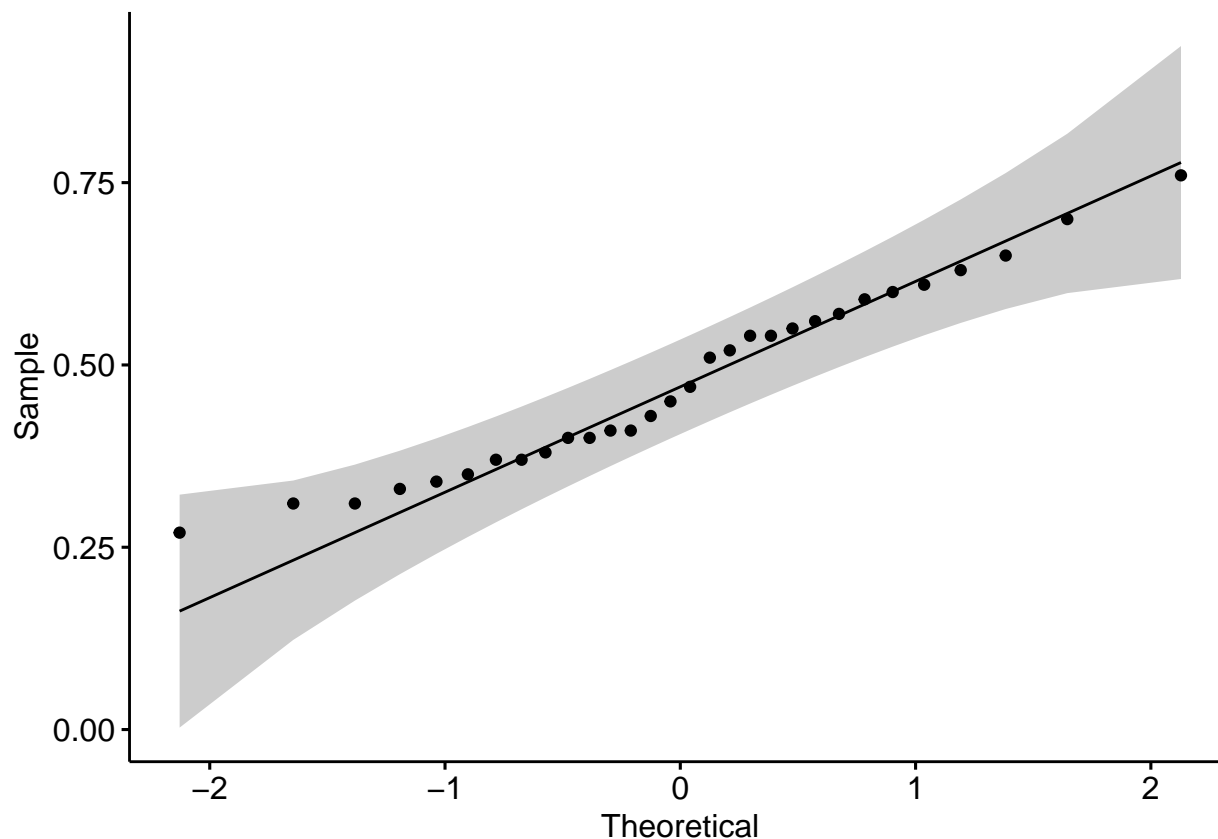
```
ggplot(data=merged9_df_long_red2, aes(x=Channel,y=RFU)) +
  geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  #scale_y_continuous(breaks = 1) +
  scale_color_grey() +
  theme_classic() +
  coord_fixed(ratio = 3, xlim = NULL, ylim = c(0,1), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )
```



```
# Testing normality, qq-plot  
library(ggpubr)
```

```
## Loading required package: magrittr
```

```
qq9 <- ggqqplot(data=merged9_df_long_red2$RFU)  
qq9
```



```
shapiro.test(merged9_df_long_red2$RFU)
```

```
##
##  Shapiro-Wilk normality test
##
```

```
## data:  merged9_df_long_red2$RFU
## W = 0.96422, p-value = 0.3951
```

```
# p-value = 0.4, sample distribution is likely to be normal.
```

```
#The null hypothesis of these tests is that "sample distribution is normal". If the test is significant
```

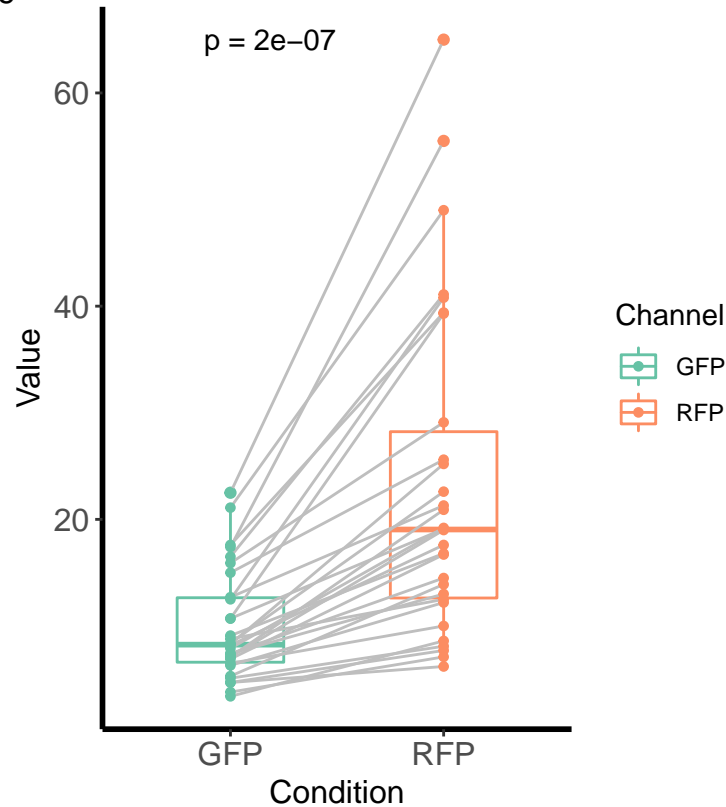
Quite stark differences in absolute values could be normalized by the GFP/RFP ratio between both replicates. Shapiro-Wilk normality test returns p-value of 0.39, large probability that the dataset is normally distributed. qqplot further underscores this.

Paired box plot:

```
library(ggpubr)
ggpaired(merged9_df_long_red, x = "Channel", y = "RFU", color = "Channel", width = 0.5, line.color = "grey",
  #geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  labs(tag = "EC36309") +
  scale_colour_brewer(palette = "Set2") +
  theme_classic() +
  coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
    axis.title.x = element_text(size = 12),
    axis.title.y = element_text(size = 12),
    axis.text.x = element_text(size=12),
```

```
axis.text.y = element_text(size=12)
) +
stat_compare_means(method = "t.test", label = "p.format", paired = TRUE)
```

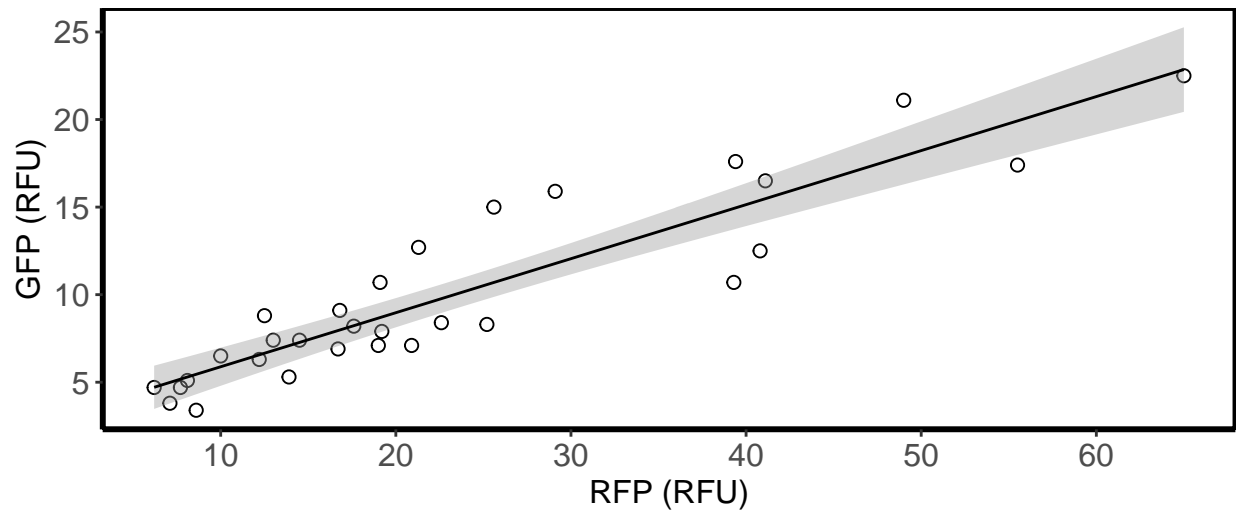
EC36309



Linear regression in ggplot2:

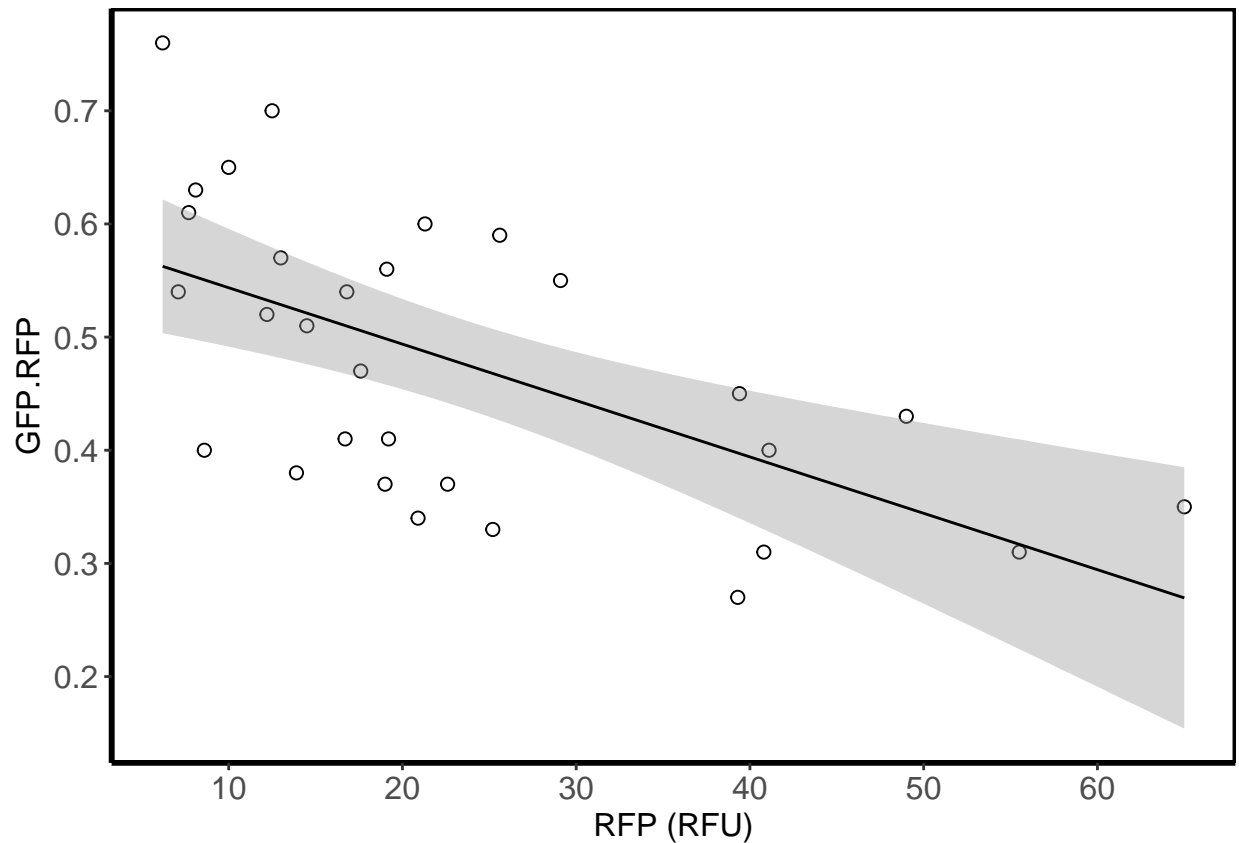
```
library(ggplot2)
reg2 <- ggplot(merged9_df, aes(x=RFP, y=GFP)) +
  labs(x="RFP (RFU)", y="GFP (RFU)") +
  scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  scale_y_continuous(breaks = pretty(GFP, n = 5)) +
  geom_point(shape=1, size=2) +
  geom_smooth(method=lm, colour="black", size=0.5) +
  scale_color_grey() +
  theme_classic() +
  coord_fixed(ratio = 1, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size=12),
        axis.text.y = element_text(size=12),
        panel.border = element_rect(size = 1, colour = "black", fill = NA)
  )
  #theme(panel.background = element_rect(fill = "white", colour = "black"))

reg2
```



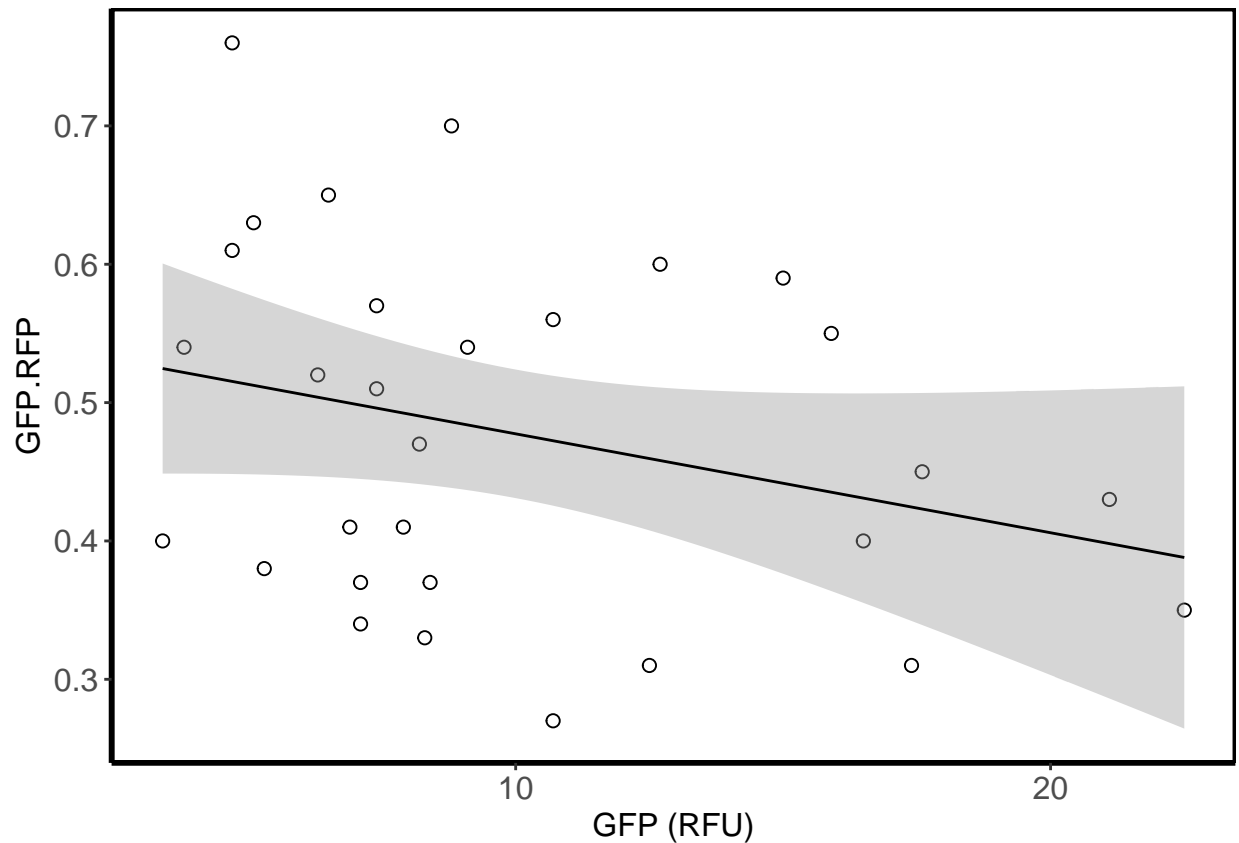
```
reg3 <- ggplot(merged9_df, aes(x=RFP, y=GFP.RFP)) +
  labs(x="RFP (RFU)", y="GFP.RFP") +
  scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  scale_y_continuous(breaks = pretty(GFP.RFP, n = 5)) +
  geom_point(shape=1, size=2) +
  geom_smooth(method=lm, colour="black", size=0.5) +
  scale_color_grey() +
  theme_classic() +
  #coord_fixed(ratio = 30, xlim = c(0:65), ylim = c(0:1)) +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12),
        panel.border = element_rect(size = 1, colour = "black", fill = NA)
  )
  #theme(panel.background = element_rect(fill = "white", colour = "black"))

reg3
```

```
reg4 <- ggplot(merged9_df, aes(x=GFP, y=GFP.RFP), ylim = c(0:1)) +
  labs(x="GFP (RFU)", y="GFP.RFP") +
  scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  scale_y_continuous(breaks = pretty(GFP.RFP, n = 5)) +
  geom_point(shape=1, size=2) +
  geom_smooth(method=lm, colour="black", size=0.5) +
  scale_color_grey() +
  theme_classic() +
  #coord_fixed(ratio = 15, xlim = c(1:25), ylim = c(0:1), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size=12),
        axis.text.y = element_text(size=12),
        panel.border = element_rect(size = 1, colour = "black", fill = NA)
  )
  #theme(panel.background = element_rect(fill = "white", colour = "black"))

reg4
```

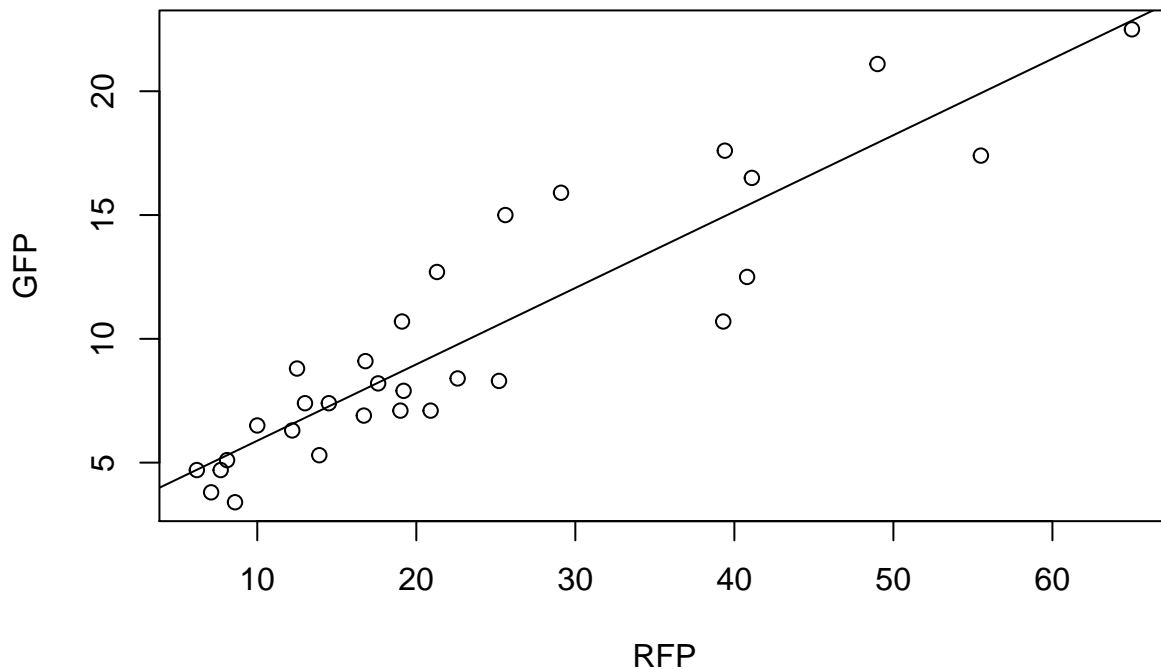


Correlating GFP to RFP in reference measurement EC36309, $n = 30$. GFP and RFP signals correlate very well. Reporter system seems usable for our hypothesis testing.

```
reg1 <- lm(GFP~RFP,data = merged9_df)
summary(reg1)
```

```
##
## Call:
## lm(formula = GFP ~ RFP, data = merged9_df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -4.2241 -1.5132 -0.2298  1.0939  4.3031
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.79797    0.73454   3.809 0.000699 ***
## RFP          0.30855    0.02657  11.612 3.22e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.18 on 28 degrees of freedom
## Multiple R-squared:  0.8281, Adjusted R-squared:  0.8219
## F-statistic: 134.8 on 1 and 28 DF,  p-value: 3.221e-12

with(merged9_df,plot(RFP, GFP))
abline(reg1)
```



```
reg2 <- lm(GFP.RFP~RFP, data = merged9_df)
summary(reg2)
```

```
##
## Call:
## lm(formula = GFP.RFP ~ RFP, data = merged9_df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.150591 -0.097111  0.002252  0.079586  0.197448
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.593449   0.035137  16.890 3.24e-16 ***
## RFP         -0.004983   0.001271  -3.921 0.000519 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1043 on 28 degrees of freedom
## Multiple R-squared:  0.3544, Adjusted R-squared:  0.3314
## F-statistic: 15.37 on 1 and 28 DF,  p-value: 0.0005194
```

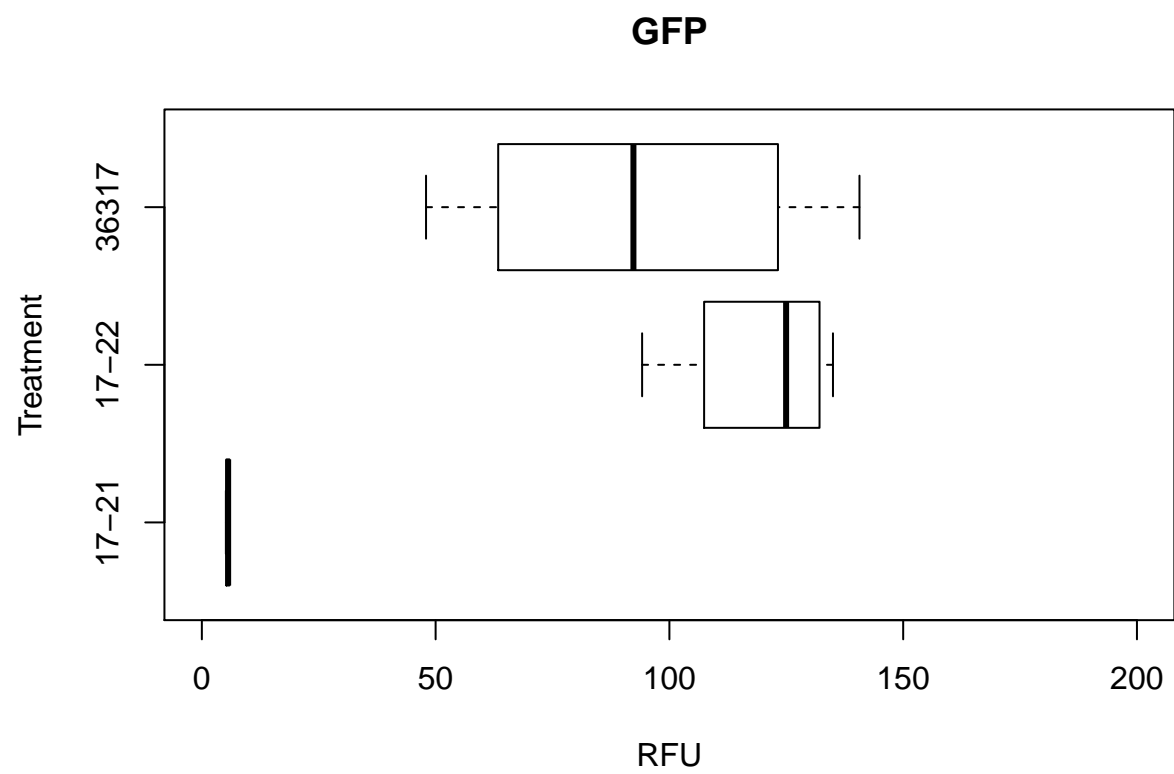
```
reg3 <- lm(GFP.RFP~GFP, data = merged9_df)
summary(reg3)
```

```
##
## Call:
```

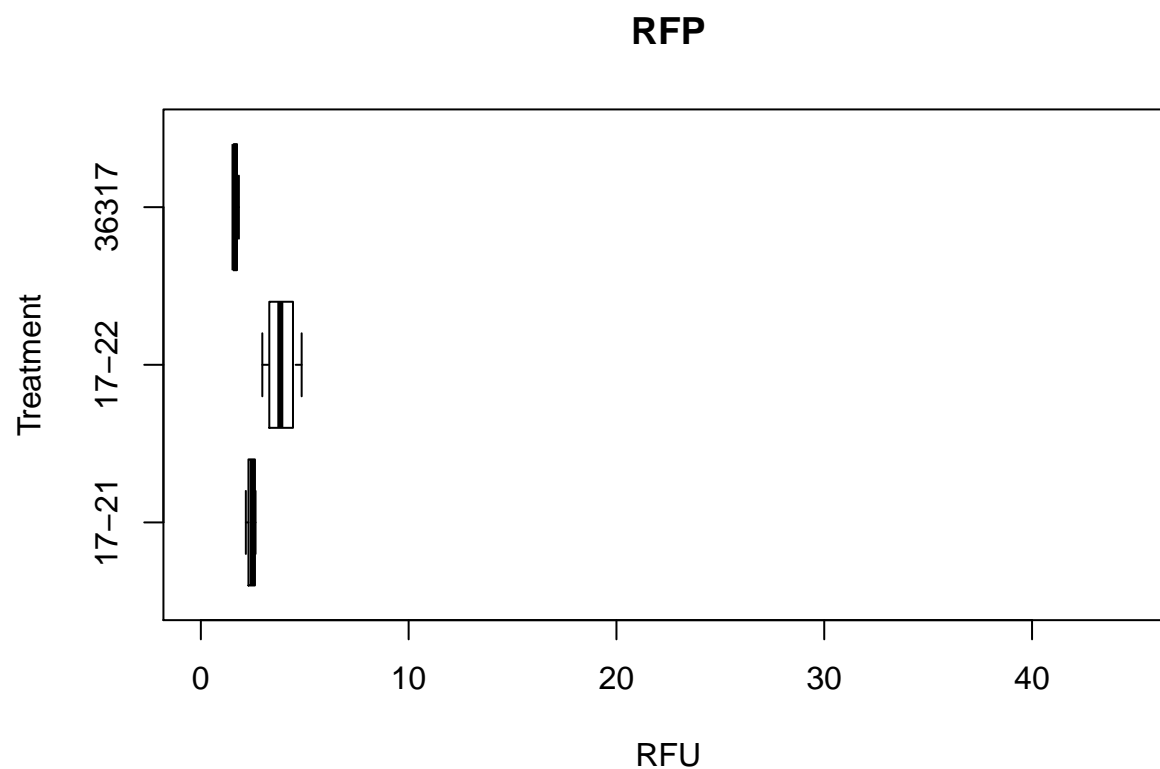
```
## lm(formula = GFP.RFP ~ GFP, data = merged9_df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.20242 -0.11778  0.01505  0.09290  0.24468
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.548927   0.049952  10.989 1.15e-11 ***
## GFP         -0.007150   0.004465  -1.601   0.121
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1242 on 28 degrees of freedom
## Multiple R-squared:  0.08388,    Adjusted R-squared:  0.05116
## F-statistic: 2.564 on 1 and 28 DF,  p-value: 0.1206
# newx = seq(min(RFP),max(RFP), by = 0.05) #,by = 1.12
# conf_interval <- predict(reg1, newdata=data.frame(x=newx), interval="confidence",
#                               level = 0.95)
# plot(RFP, GFP, xlab="RFP", ylab="GFP", main="Regression")
# abline(reg1, col="black")
# matlines(newx, conf_interval[,2:3], lty=2, col = "blue")
```

CRISPR validation with EC36317: Treatment: EC36317.2, 4 plants (n = 4), three spots per leaf, one per treatment. No RFP signal, therefore data inconclusive.

```
EC36317dat1 <- read.table("AI190322EC36317.csv", header=TRUE,
                        sep=",") #data from 36317 treatment from AI190322
boxplot(GFP~ID, data=EC36317dat1, main="GFP", horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatment")
```



```
boxplot(RFP-ID, data=EC36317dat1, main="RFP", horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatment")
```



EC36317.2 showed again only GFP expression and no RFP signal, the construct and glycerol stocks were discarded. Co-infiltration with CRISPR constructs EC36321 and 36322 targeting mNeonGreen and tdTomato CDS respectively. 17-21 showed no GFP expression, treatment with construct targeting mNeonGreen. Without tdTomato reference, this could still be just an artifact from improper overall expression of the construct.