

Estrogen Receptor Alpha aPCR Timeseries

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Delta-delta ct Calculation

```
#Set variables to make time points easier to spot
time0 <- 1
time45 <- 2
time90 <- 3

#Input raw data
qpcr <- list()
qpcr[[time0]] <- read.table("qpcr-0.txt",
                           header = T,
                           sep = "\t",
                           as.is = T)

qpcr[[time45]] <-
  read.table("qpcr-45.txt",
            header = T,
            sep = "\t",
            as.is = T)

qpcr[[time90]] <-
  read.table("qpcr-90.txt",
            header = T,
            sep = "\t",
            as.is = T)

for (time_point in time0:time90)
{
  qpcr[[time_point]][qpcr[[time_point]] == "No Ct"] <- NA
}

#Prepare lists
ct_values <- list()
ct_values_means <- list()
d_ct_values_means <- list()
input_values <- list()
input_mean <- list()
d_input_mean <- list()
dd_ct_values_means <- list()

for (time_point in time0:time90)
{
  #Create object
  ct_values[[time_point]] <- matrix(NA, 0, 2)

  #Add reps 1:8 to object
```

```

for (rep in 1:8) {
  y_coord <- rep * 12 - 11
  ct_values[[time_point]] <- rbind(ct_values[[time_point]],
                                   cbind(
                                     as.numeric(qpcr[[time_point]]$Ct..dR[y_coord:(y_coord + 4)]
                                     as.numeric(qpcr[[time_point]]$Ct..dR[(y_coord + 5):(y_coord + 9)]
                                   ))
}

#Average technical reps from same isogenic replicate
ct_values_means[[time_point]] <- matrix(NA, 0, 2)
for (rep in 1:8) {
  y_coord <- rep * 5 - 4
  ct_values_means[[time_point]] <-
    rbind(ct_values_means[[time_point]], colMeans(ct_values[[time_point]][y_coord:(y_coord + 4), 1:2])
}

#Import Input from time_point mins

input_values[[time_point]] <- cbind(as.numeric(qpcr[[time_point]]$Ct..dR[12 *
                                                                    (0:4) + 11]),
                                     as.numeric(qpcr[[time_point]]$Ct..dR[12 *
                                                                    (0:4) + 12]))

#Calculate Average of technical reps.
input_mean[[time_point]] <-
  colMeans(input_values[[time_point]], na.rm = T)

if (time_point == 2)
{
  #Plate layout is reversed wrt control and target primers. See lab book.
  d_ct_values_means[[time_point]] <-
    ct_values_means[[time_point]][, 2] - ct_values_means[[time_point]][, 1]
  d_input_mean[[time_point]] <-
    input_mean[[time_point]][2] - input_mean[[time_point]][1]

  dd_ct_values_means[[time_point]] <-
    d_ct_values_means[[time_point]] - d_input_mean[[time_point]]
} else {
  d_ct_values_means[[time_point]] <-
    ct_values_means[[time_point]][, 1] - ct_values_means[[time_point]][, 2]
  d_input_mean[[time_point]] <-
    input_mean[[time_point]][1] - input_mean[[time_point]][2]

  dd_ct_values_means[[time_point]] <-
    d_ct_values_means[[time_point]] - d_input_mean[[time_point]]
}
}

```

```
df_ct <- data.frame(cbind(
  2 ~ dd_ct_values_means[[time0]],
  2 ~ dd_ct_values_means[[time45]],
  2 ~ dd_ct_values_means[[time90]]
))
colnames(df_ct) <- c("0 min", "45 mins", "90 mins")
```

Box plot of Raw Data

```
library("ggpubr")
```

```
## Loading required package: ggplot2
```

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

```
tp1<-cbind(df_ct["0 min"],rep("0 min",8))
tp2<-cbind(df_ct["45 mins"],rep("45 min",8))
tp3<-cbind(df_ct["90 mins"],rep("90 min",8))
```

```
colnames(tp1)<-c("Enrichment", "Time")
colnames(tp2)<-c("Enrichment", "Time")
colnames(tp3)<-c("Enrichment", "Time")
```

```
df<-rbind(tp1,tp2,tp3)
```

```
#Code to remove data points more than 2*IQR from median. P-value are one-sided Wilcoxon rank test. Look
#tp3<-tp3[!(df$Enrichment[df$Time=="90 min"]>median(df$Enrichment[df$Time=="90 min"])+IQR(df$Enrichment
#tp2<-tp2[!(df$Enrichment[df$Time=="45 min"]>median(df$Enrichment[df$Time=="45 min"])+IQR(df$Enrichment
```

```
df<-rbind(tp1,tp2,tp3)
```

```
p <- ggboxplot(df, x = "Time", y = "Enrichment",
  color = "Time", palette =c("#00AFBB", "#E7B800", "#FC4E07"),
  add = "jitter", shape = "Time",
  outlier.shape=NA,
  main = "ER-alpha occupy of the TFF1 promoter",
  ylab = "Enrichment over Input",
  xlab = "Time point",
  show.legend=FALSE) +theme(legend.position="none")
```

```
my_comparisons <- list(c("90 min", "0 min"), c("45 min", "0 min"), c("45 min", "90 min") )
```

```
p + stat_compare_means(comparisons = my_comparisons,
  method="t.test",
  label = "p-value",
  show.legend=FALSE,
  method.args=list(alternative="greater"))
```

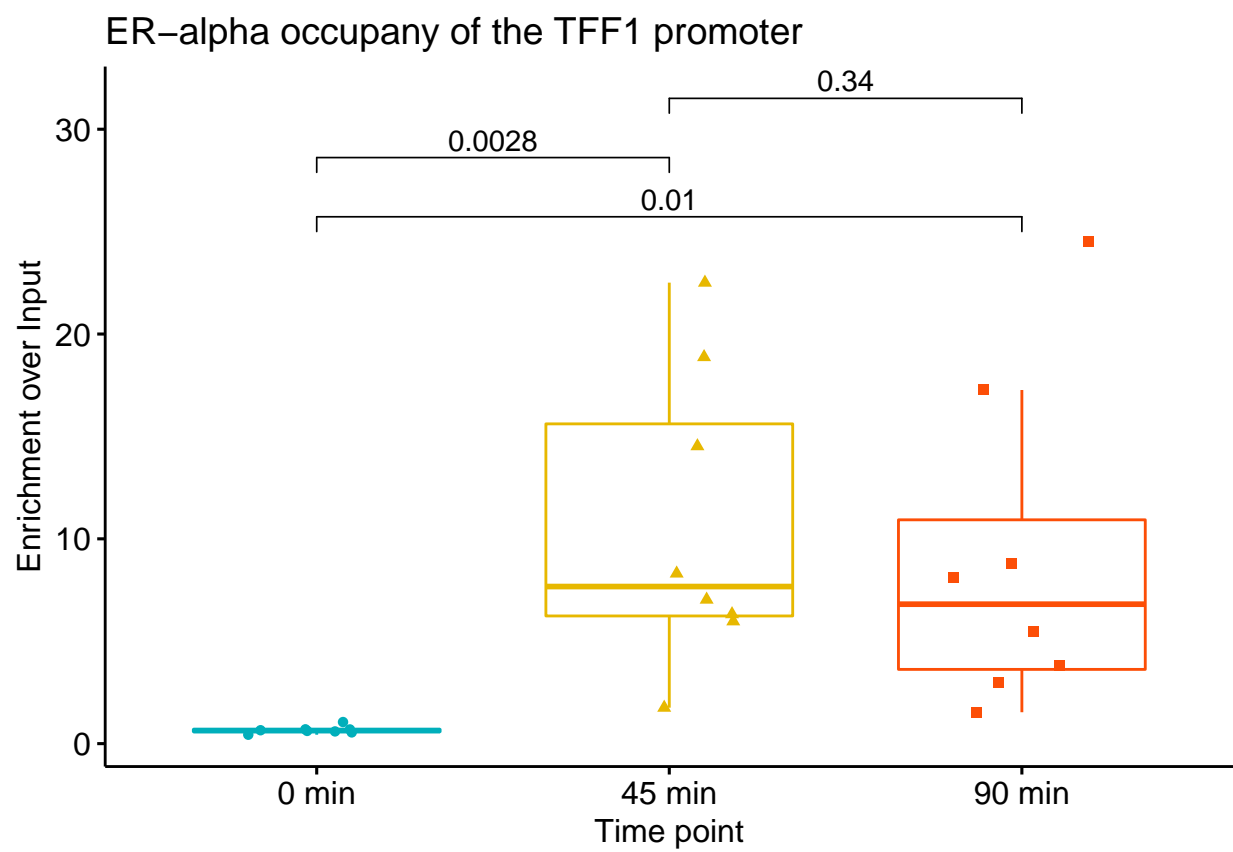


Figure 1: Box Plot showing ChIP enrichment over control loci for ER at 0, 45 and 90 minutes after stimulation with 100nM E2