Estrogen Receptor Alpha aPCR Timeseries

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Delta-delta ct Calculation

```r
#Set varibles 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
```

```r
    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_cord
                                                                                                    9)]
                                         ))
    }



    #Average techical 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]))

    #Calucate 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")
```

```
## Warning: package 'ggpubr' was built under R version 3.4.2
```

```
## 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 occupany 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"))
```
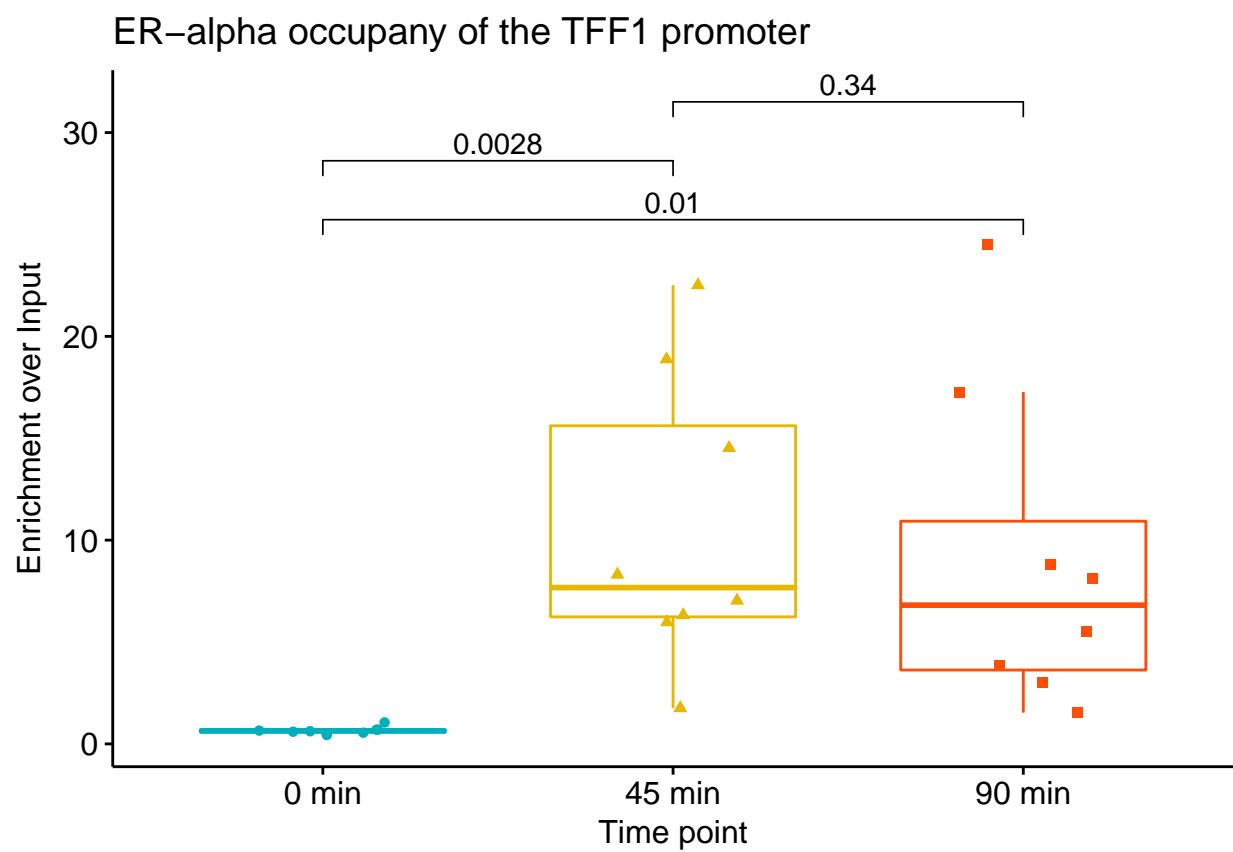
Figure 1: Box Plot showing ChIP enrichment over control loci for ER at 0, 45 and 90 minutes after stimuation with 100nM E2