# Photographic_Bleaching

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## Loading in required libraries.

## Reading in quality controlled color data.

Protocol for ImageJ Bleaching Score Analysis found here.

```
Data <- read.csv("RAnalysis/data/color_score/color_score_colonies.csv", header=T, sep=",", na.string="N
Data <-na.omit(Data) # removing all rows with NA values
Data$SP.Time.ID <- paste0(Data$ColonyID, "_",Data$Photo_date, "_", Data$treatment)# generating a groupi

#make photo.date a factor
Data$Photo_date = as.factor(Data$Photo_date)
```

Normalize coral color to color standards.

```
Data$Red.Norm.Coral <- Data$Red_coral/Data$Red_standard #normalize to standard
Data$Green.Norm.Coral <- Data$Green_coral/Data$Green_standard #normalize to standard
Data$Blue.Norm.Coral <- Data$Blue_coral/Data$Blue_standard #normalize to standard
```

## Principal components analysis to reduce color score to single value representing the majority of the variance

```
score <- Data %>% select("ColonyID", "treatment", "genotype", "tank.num", "Photo_date", "Period","SP.Tim
blch.scor <- score  %>% select("Red.Norm.Coral","Green.Norm.Coral", "Blue.Norm.Coral") #subset datafram
blch.scor <- as.matrix(blch.scor) #create matrix
rownames(blch.scor) <- score$SP.Time.ID #name columns in matrix

dist <- vegdist(blch.scor, method="euclidean") #calculate distance matrix of color scores

PCA.color <- princomp(dist) #run principal components analysis
PCA.color # view variance explained by PCs

## Call:
## princomp(x = dist)
##
## Standard deviations:
##        Comp.1       Comp.2       Comp.3       Comp.4       Comp.5       Comp.6
## 5.040370e+00 3.306822e+00 6.296869e-01 5.337312e-01 3.758244e-01 2.117522e-01
##        Comp.7       Comp.8       Comp.9       Comp.10       Comp.11       Comp.12
```

```
## 1.812661e-01 1.544898e-01 1.362027e-01 1.070146e-01 9.772188e-02 8.181071e-02
##       Comp.13      Comp.14      Comp.15      Comp.16      Comp.17      Comp.18
## 6.827680e-02 6.508467e-02 5.400698e-02 5.220087e-02 4.725283e-02 4.153029e-02
##       Comp.19      Comp.20      Comp.21      Comp.22      Comp.23      Comp.24
## 3.932913e-02 3.553545e-02 3.306034e-02 3.219140e-02 3.067941e-02 2.818712e-02
##       Comp.25      Comp.26      Comp.27      Comp.28      Comp.29      Comp.30
## 2.698527e-02 2.634908e-02 2.330911e-02 2.203884e-02 2.137489e-02 2.000187e-02
##       Comp.31      Comp.32      Comp.33      Comp.34      Comp.35      Comp.36
## 1.883881e-02 1.852883e-02 1.703958e-02 1.699736e-02 1.642819e-02 1.575175e-02
##       Comp.37      Comp.38      Comp.39      Comp.40      Comp.41      Comp.42
## 1.537211e-02 1.488427e-02 1.454332e-02 1.396422e-02 1.306331e-02 1.302957e-02
##       Comp.43      Comp.44      Comp.45      Comp.46      Comp.47      Comp.48
## 1.228816e-02 1.220449e-02 1.198772e-02 1.139009e-02 1.099507e-02 1.057572e-02
##       Comp.49      Comp.50      Comp.51      Comp.52      Comp.53      Comp.54
## 1.049092e-02 1.008653e-02 9.945285e-03 9.297230e-03 9.081710e-03 8.973942e-03
##       Comp.55      Comp.56      Comp.57      Comp.58      Comp.59      Comp.60
## 8.768489e-03 8.591405e-03 8.531593e-03 8.458304e-03 8.166522e-03 8.037005e-03
##       Comp.61      Comp.62      Comp.63      Comp.64      Comp.65      Comp.66
## 7.979337e-03 7.599793e-03 7.446989e-03 7.361667e-03 7.280099e-03 6.939759e-03
##       Comp.67      Comp.68      Comp.69      Comp.70      Comp.71      Comp.72
## 6.800597e-03 6.745095e-03 6.720598e-03 6.506867e-03 6.367152e-03 6.292722e-03
##       Comp.73      Comp.74      Comp.75      Comp.76      Comp.77      Comp.78
## 6.129784e-03 5.991344e-03 5.855249e-03 5.724118e-03 5.613223e-03 5.475701e-03
##       Comp.79      Comp.80      Comp.81      Comp.82      Comp.83      Comp.84
## 5.444411e-03 5.388912e-03 5.247951e-03 5.126000e-03 5.075080e-03 4.998606e-03
##       Comp.85      Comp.86      Comp.87      Comp.88      Comp.89      Comp.90
## 4.967835e-03 4.925400e-03 4.852873e-03 4.727579e-03 4.661009e-03 4.600950e-03
##       Comp.91      Comp.92      Comp.93      Comp.94      Comp.95      Comp.96
## 4.532901e-03 4.474108e-03 4.396819e-03 4.370409e-03 4.319429e-03 4.269467e-03
##       Comp.97      Comp.98      Comp.99     Comp.100     Comp.101     Comp.102
## 4.193292e-03 4.121796e-03 4.042199e-03 3.998163e-03 3.983063e-03 3.921581e-03
##      Comp.103     Comp.104     Comp.105     Comp.106     Comp.107     Comp.108
## 3.764474e-03 3.725028e-03 3.703014e-03 3.643476e-03 3.630929e-03 3.599729e-03
##      Comp.109     Comp.110     Comp.111     Comp.112     Comp.113     Comp.114
## 3.566891e-03 3.542023e-03 3.499211e-03 3.459244e-03 3.430081e-03 3.406180e-03
##      Comp.115     Comp.116     Comp.117     Comp.118     Comp.119     Comp.120
## 3.361524e-03 3.311071e-03 3.267090e-03 3.217164e-03 3.182113e-03 3.153394e-03
##      Comp.121     Comp.122     Comp.123     Comp.124     Comp.125     Comp.126
## 3.146543e-03 3.112188e-03 3.089537e-03 3.080890e-03 3.040322e-03 3.030106e-03
##      Comp.127     Comp.128     Comp.129     Comp.130     Comp.131     Comp.132
## 2.997647e-03 2.942041e-03 2.936099e-03 2.921836e-03 2.889546e-03 2.880024e-03
##      Comp.133     Comp.134     Comp.135     Comp.136     Comp.137     Comp.138
## 2.850648e-03 2.840361e-03 2.820965e-03 2.796074e-03 2.781437e-03 2.767353e-03
##      Comp.139     Comp.140     Comp.141     Comp.142     Comp.143     Comp.144
## 2.735785e-03 2.727107e-03 2.687316e-03 2.667828e-03 2.613706e-03 2.610851e-03
##      Comp.145     Comp.146     Comp.147     Comp.148     Comp.149     Comp.150
## 2.577644e-03 2.572855e-03 2.563944e-03 2.529950e-03 2.522256e-03 2.466497e-03
##      Comp.151     Comp.152     Comp.153     Comp.154     Comp.155     Comp.156
## 2.458810e-03 2.446376e-03 2.438397e-03 2.432264e-03 2.379843e-03 2.355003e-03
##      Comp.157     Comp.158     Comp.159     Comp.160     Comp.161     Comp.162
## 2.351984e-03 2.321596e-03 2.313964e-03 2.267328e-03 2.248315e-03 2.236039e-03
##      Comp.163     Comp.164     Comp.165     Comp.166     Comp.167     Comp.168
## 2.232273e-03 2.218878e-03 2.210994e-03 2.192774e-03 2.188296e-03 2.170932e-03
##      Comp.169     Comp.170     Comp.171     Comp.172     Comp.173     Comp.174
```

```
##  2.164422e-03 2.153464e-03 2.147608e-03 2.138137e-03 2.119747e-03 2.086855e-03
##       Comp.175      Comp.176      Comp.177      Comp.178      Comp.179      Comp.180
##  2.078267e-03 2.055368e-03 2.052496e-03 2.037647e-03 2.027434e-03 2.016227e-03
##       Comp.181      Comp.182      Comp.183      Comp.184      Comp.185      Comp.186
##  2.004572e-03 1.996323e-03 1.987405e-03 1.975862e-03 1.968571e-03 1.942219e-03
##       Comp.187      Comp.188      Comp.189      Comp.190      Comp.191      Comp.192
##  1.934869e-03 1.928502e-03 1.903305e-03 1.892318e-03 1.890959e-03 1.867471e-03
##       Comp.193      Comp.194      Comp.195      Comp.196      Comp.197      Comp.198
##  1.861338e-03 1.843773e-03 1.831365e-03 1.805497e-03 1.802637e-03 1.796445e-03
##       Comp.199      Comp.200      Comp.201      Comp.202      Comp.203      Comp.204
##  1.795585e-03 1.790345e-03 1.780135e-03 1.778613e-03 1.769049e-03 1.745935e-03
##       Comp.205      Comp.206      Comp.207      Comp.208      Comp.209      Comp.210
##  1.739519e-03 1.738177e-03 1.733474e-03 1.696171e-03 1.692757e-03 1.689824e-03
##       Comp.211      Comp.212      Comp.213      Comp.214      Comp.215      Comp.216
##  1.679662e-03 1.647476e-03 1.645162e-03 1.638211e-03 1.634599e-03 1.632511e-03
##       Comp.217      Comp.218      Comp.219      Comp.220      Comp.221      Comp.222
##  1.605194e-03 1.590512e-03 1.587403e-03 1.579092e-03 1.561203e-03 1.560652e-03
##       Comp.223      Comp.224      Comp.225      Comp.226      Comp.227      Comp.228
##  1.552243e-03 1.548076e-03 1.534876e-03 1.511510e-03 1.510421e-03 1.504090e-03
##       Comp.229      Comp.230      Comp.231      Comp.232      Comp.233      Comp.234
##  1.497023e-03 1.471733e-03 1.460790e-03 1.459040e-03 1.438332e-03 1.434750e-03
##       Comp.235      Comp.236      Comp.237      Comp.238      Comp.239      Comp.240
##  1.431662e-03 1.426657e-03 1.416576e-03 1.400149e-03 1.385721e-03 1.379393e-03
##       Comp.241      Comp.242      Comp.243      Comp.244      Comp.245      Comp.246
##  1.378433e-03 1.364944e-03 1.356076e-03 1.350963e-03 1.348725e-03 1.346166e-03
##       Comp.247      Comp.248      Comp.249      Comp.250      Comp.251      Comp.252
##  1.335511e-03 1.325887e-03 1.322489e-03 1.306292e-03 1.299885e-03 1.285714e-03
##       Comp.253      Comp.254      Comp.255      Comp.256      Comp.257      Comp.258
##  1.274988e-03 1.274121e-03 1.268601e-03 1.260367e-03 1.258837e-03 1.240127e-03
##       Comp.259      Comp.260      Comp.261      Comp.262      Comp.263      Comp.264
##  1.229763e-03 1.219684e-03 1.216119e-03 1.207773e-03 1.206569e-03 1.200264e-03
##       Comp.265      Comp.266      Comp.267      Comp.268      Comp.269      Comp.270
##  1.199622e-03 1.181125e-03 1.180401e-03 1.177765e-03 1.165386e-03 1.159399e-03
##       Comp.271      Comp.272      Comp.273      Comp.274      Comp.275      Comp.276
##  1.147381e-03 1.142688e-03 1.138843e-03 1.129466e-03 1.121793e-03 1.111056e-03
##       Comp.277      Comp.278      Comp.279      Comp.280      Comp.281      Comp.282
##  1.101618e-03 1.095006e-03 1.094192e-03 1.090142e-03 1.085116e-03 1.083873e-03
##       Comp.283      Comp.284      Comp.285      Comp.286      Comp.287      Comp.288
##  1.080091e-03 1.069979e-03 1.063533e-03 1.055453e-03 1.051244e-03 1.045130e-03
##       Comp.289      Comp.290      Comp.291      Comp.292      Comp.293      Comp.294
##  1.037905e-03 1.019941e-03 1.009762e-03 1.008179e-03 1.006411e-03 9.872284e-04
##       Comp.295      Comp.296      Comp.297      Comp.298      Comp.299      Comp.300
##  9.831612e-04 9.816839e-04 9.792276e-04 9.720337e-04 9.578819e-04 9.517944e-04
##       Comp.301      Comp.302      Comp.303      Comp.304      Comp.305      Comp.306
##  9.484399e-04 9.445668e-04 9.389836e-04 9.273165e-04 9.268288e-04 9.264108e-04
##       Comp.307      Comp.308      Comp.309      Comp.310      Comp.311      Comp.312
##  8.940274e-04 8.926646e-04 8.893836e-04 8.713891e-04 8.672677e-04 8.527217e-04
##       Comp.313      Comp.314      Comp.315      Comp.316      Comp.317      Comp.318
##  8.467582e-04 8.326001e-04 8.182166e-04 8.068463e-04 7.886184e-04 7.827531e-04
##       Comp.319      Comp.320      Comp.321      Comp.322      Comp.323      Comp.324
##  7.785282e-04 7.619267e-04 7.569728e-04 7.478286e-04 7.431940e-04 7.342221e-04
##       Comp.325      Comp.326      Comp.327      Comp.328      Comp.329      Comp.330
##  7.030908e-04 6.963579e-04 6.761963e-04 6.754651e-04 6.693085e-04 6.644328e-04
##       Comp.331      Comp.332      Comp.333      Comp.334      Comp.335      Comp.336
```

```
## 6.606586e-04 6.578559e-04 6.304369e-04 6.232563e-04 6.058417e-04 5.855124e-04
##      Comp.337     Comp.338      Comp.339      Comp.340      Comp.341      Comp.342
## 5.768542e-04 5.700406e-04 5.510442e-04 5.172550e-04 4.893665e-04 4.883332e-04
##      Comp.343     Comp.344      Comp.345      Comp.346      Comp.347      Comp.348
## 4.864131e-04 4.811739e-04 3.905283e-04 3.267504e-04 3.186541e-04 5.277737e-08
##      Comp.349     Comp.350      Comp.351      Comp.352      Comp.353      Comp.354
## 2.861649e-08 2.100271e-08 1.005172e-08 7.346569e-09 3.453043e-09 0.000000e+00
##      Comp.355     Comp.356      Comp.357      Comp.358      Comp.359      Comp.360
## 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00
##
## 360  variables and  360 observations.
```

```r
Blch <- as.data.frame(-PCA.color$scores[,1]) #extract PC1
Blch$SP.Time.ID <- rownames(blch.scor) # add the sample names to the bleahcing score

Blch  <- left_join(Blch, Data, by="SP.Time.ID") #make a dataframe of PC1 and experiment factors
colnames(Blch) #view column names
```

```
##  [1] "-PCA.color$scores[, 1]" "SP.Time.ID"           "ColonyID"
##  [4] "treatment"              "genotype"             "tank.num"
##  [7] "ExperimentalDay"        "Period"               "Photo_date"
## [10] "Photo_ID"               "Red_standard"         "Green_standard"
## [13] "Blue_standard"          "Red_coral"            "Green_coral"
## [16] "Blue_coral"             "Tech"                 "Notes"
## [19] "Red.Norm.Coral"         "Green.Norm.Coral"     "Blue.Norm.Coral"
```

```r
names(Blch)[1] <- "Bleaching.Score" #name column

hist(Blch$Bleaching.Score)
```

**Histogram of Blch$Bleaching.Score**
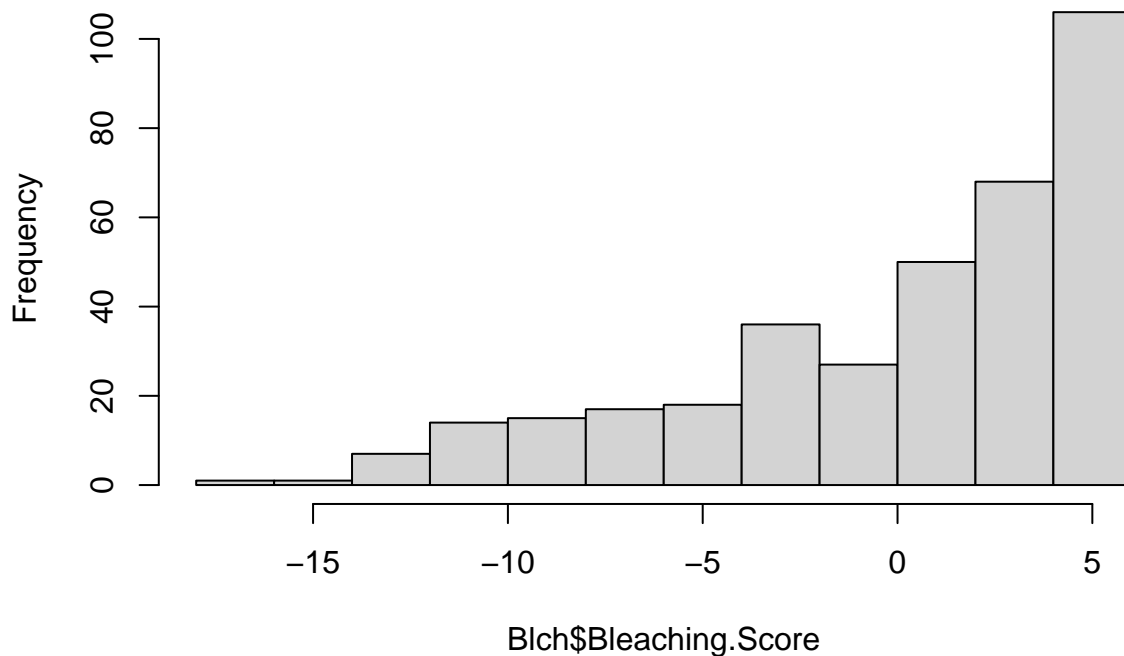
# Plot data for outliers and Write out Color Score dataframe

```
Blch.meanse <- Blch %>% group_by(treatment, Photo_date) %>% #group data by factors
  summarise(mean = mean(Bleaching.Score), #calculate mean by group
            sem = std.error(Bleaching.Score)) #calculate sem by group
```

```
## `summarise()` has grouped output by 'treatment'. You can override using the `.groups` argument.
# Assign labels to use for figure
#species.names <- list(
#   'Mcapitata'="Montipora capitata",
#   'Pacuta'="Pocillopora acuta")
#species_labeller <- function(variable,value){
#   return(species.names[value])}

cols <- c( "blue", "red3") #set colors

pdf("RAnalysis/output/color_score/colorscore_with_outliers_colonies.pdf")
Blch_plot1 <- ggplot(Blch.meanse, aes(x=Photo_date, y = mean, group = treatment, color = treatment)) +
  geom_line() + geom_point() + #plot points and lines
  #facet_grid(~Species, scales = "free", labeller = species_labeller) + #facet by Species
  geom_errorbar(aes(ymin=mean-sem, ymax=mean+sem), width=.2) + #plot sem error bars
  scale_color_manual(values = cols) + #color points
  xlab("Timepoint") + #label x axis
  ylab("Color Score") + # label y axis
  theme_classic() + #set theme
  theme(legend.position = c(0.1,0.3)) + #set legend position
  theme(legend.text = element_text(size = 7)) + #set legend text size
  theme(legend.title = element_text(size = 9)) + #set legend text title size
  geom_vline(xintercept = c(7.8), colour="black", linetype="dotted") + #add vertical line
  theme(plot.title = element_text(face = 'bold.italic', size = 12, hjust = 0)) +  #set font
  #scale_x_discrete(breaks = c("Week1", "Week2", "Week4", "Week6", "Week8", "Week12", "Week16"), labels
  theme(axis.text.x = element_text(angle = 60, vjust = 1.2, hjust = 1.3)) + #Set the text angle
  theme(panel.border = element_blank(), #set theme
  panel.grid.major = element_blank(),  #set theme
  panel.grid.minor = element_blank(),  #set theme
  # Change axis line
  axis.line = element_line(colour = "black"))
Blch_plot1 #view plot
dev.off()
```

```
## pdf
##   2
Blch_plot1
```
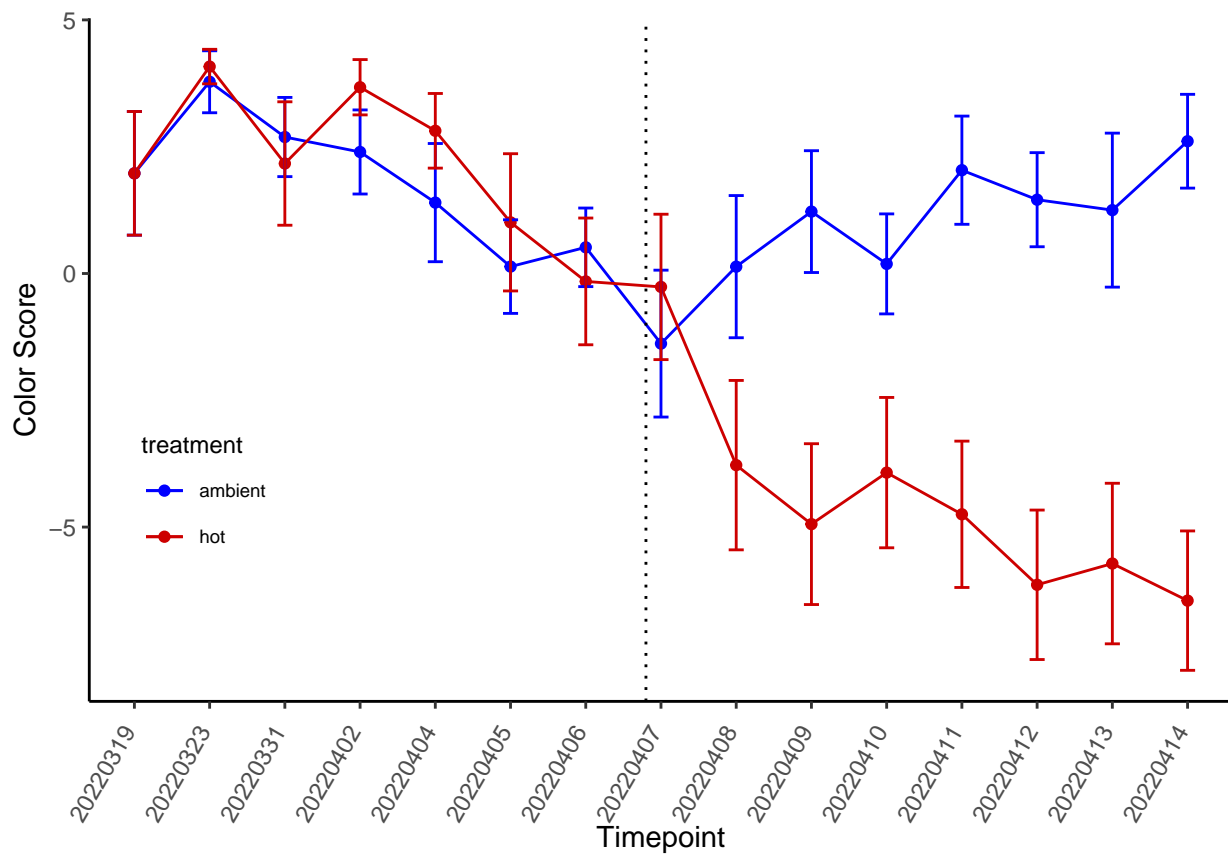
```
write.table(Blch, file = "RAnalysis/output/color_score/color_score_colony_output.csv", append = FALSE, d
```

## Detect and Remove Outliers

```
hist(Blch$Bleaching.Score) #view data distribution
```

## Histogram of Blch$Bleaching.Score
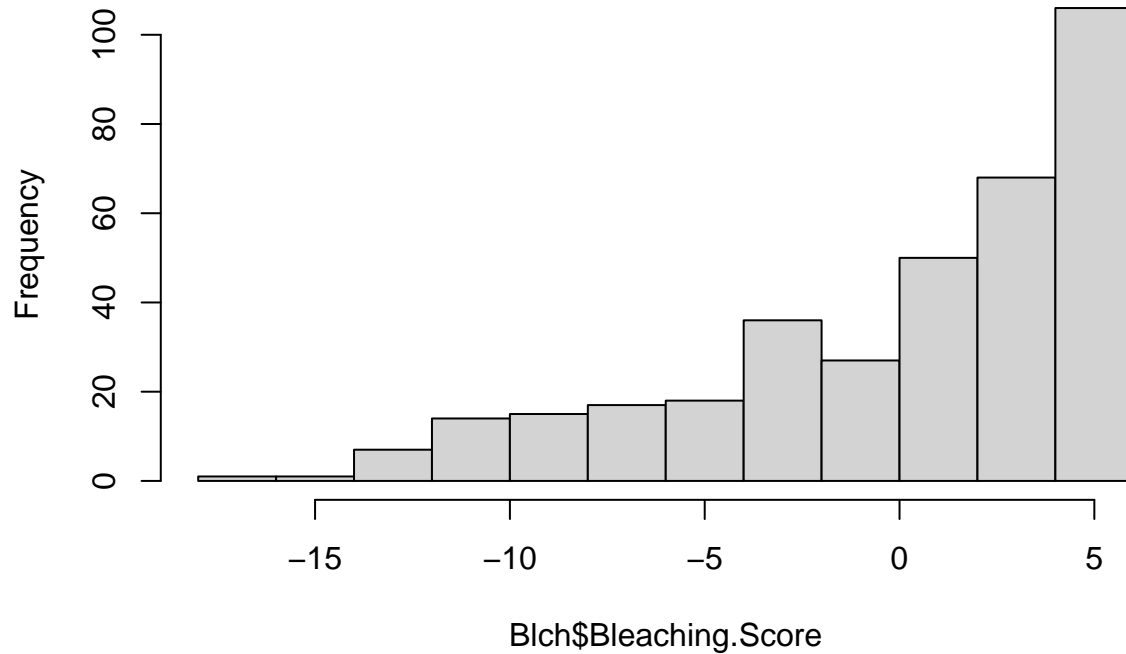
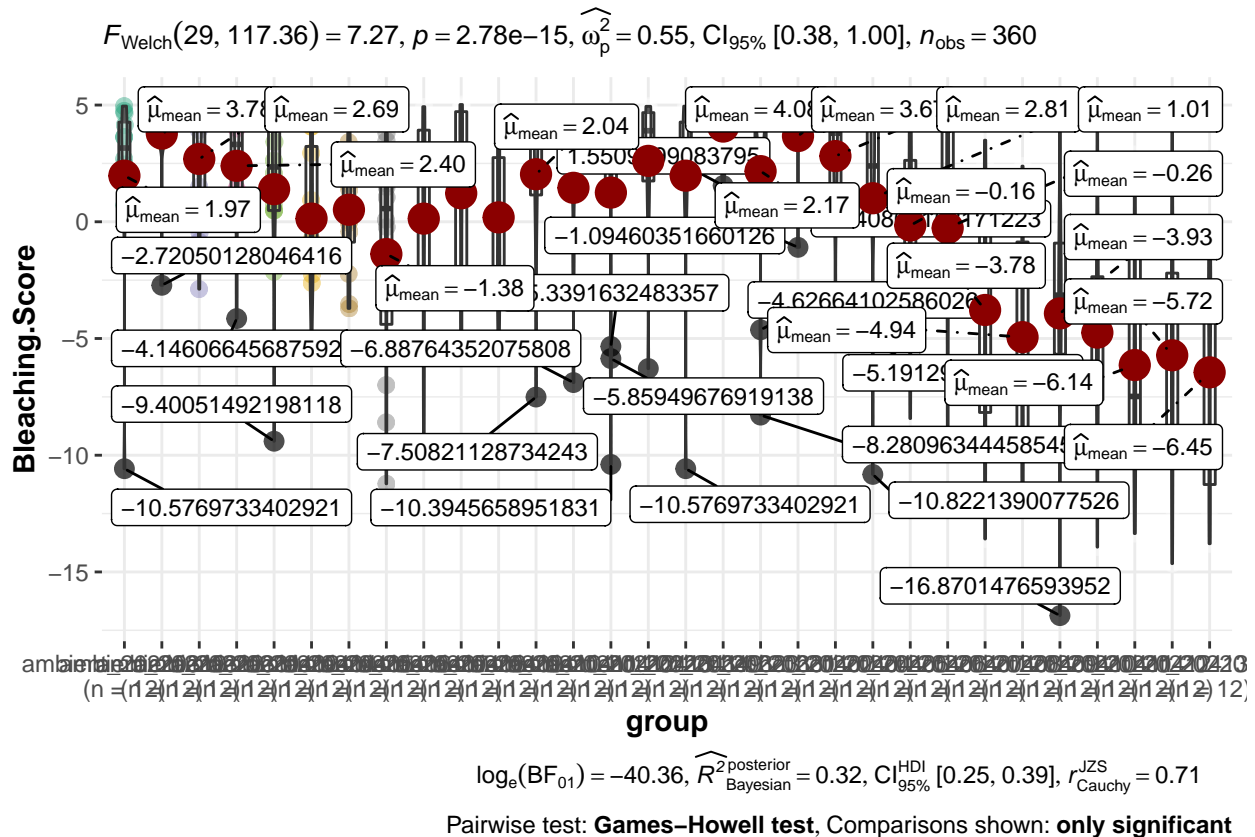

```
Blch$group <- paste0(Blch$treatment,"_", Blch$Photo_date)

#identify outliers
outlier.plot <- ggbetweenstats(Blch,group, Bleaching.Score, outlier.tagging = TRUE)
```

```
## Warning: Number of labels is greater than default palette color count.
##  Try using another color `palette` (and/or `package`).
```

```
##
```

```
outlier.plot
```

```
## Warning: ggrepel: 1 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```

```
## Warning: ggrepel: 11 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```

$F_{\text{Welch}}(29, 117.36) = 7.27, p = 2.78\text{e}{-}15, \widehat{\omega_p^2} = 0.55, \text{CI}_{95\%} [0.38, 1.00], n_{\text{obs}} = 360$

$\log_e(\text{BF}_{01}) = -40.36, \widehat{R^2}_{\text{Bayesian}}^{\text{posterior}} = 0.32, \text{CI}_{95\%}^{\text{HDI}} [0.25, 0.39], r_{\text{Cauchy}}^{\text{JZS}} = 0.71$

Pairwise test: **Games–Howell test**, Comparisons shown: **only significant**

```r
#set quantile values
q <- c(0.25, 0.75)

# calculate quantile values by time and Treatment groups
Quants <- Blch %>%
  group_by(treatment, Photo_date) %>%
  summarize(quant25 = quantile(Bleaching.Score, probs = q[1]),
            quant75 = quantile(Bleaching.Score, probs = q[2]),
            IQRbyGroup=IQR(Bleaching.Score))
```

## `summarise()` has grouped output by 'treatment'. You can override using the `.groups` argument.

```r
# add a group name by pasting Time and Treatment
Quants$group <-paste0(Quants$treatment,"_", Quants$Photo_date)

#Calculate Quantile upper and lower ranges
Quants$upper <-  Quants$quant75+1.5*Quants$IQRbyGroup # Upper Range
Quants$lower <- Quants$quant25-1.5*Quants$IQRbyGroup # Lower Range

#join outlier cutoffs with rate data
data <- left_join(Blch, Quants)
```

## Joining, by = c("treatment", "Photo_date", "group")

```r
#remove outliers from rates
data <- data %>%
  filter(Bleaching.Score < upper) %>%
  filter(Bleaching.Score > lower)
```

```
# Visualize data following outlier removal
r_plot<-data %>%
    ggplot(., aes(x = Photo_date, y = Bleaching.Score, colour=treatment)) +
    geom_point(aes(fill=treatment, group=interaction(treatment, Photo_date)), pch = 21, size=2, alpha=0
    xlab("Time Point") +
    scale_fill_manual(name="treatment", values=c("#3450A3","#E9A33D"))+
    scale_color_manual(name="treatment", values=c("#3450A3","#E9A33D"))+
    ylab("Color Score") +
    theme_classic() +
    theme(legend.position="none",
      axis.title=element_text(face="bold", size=16),
      axis.text=element_text(size=12, color="black"),
      legend.title=element_text(face="bold", size=14),
      legend.text=element_text(size=12)); r_plot
```
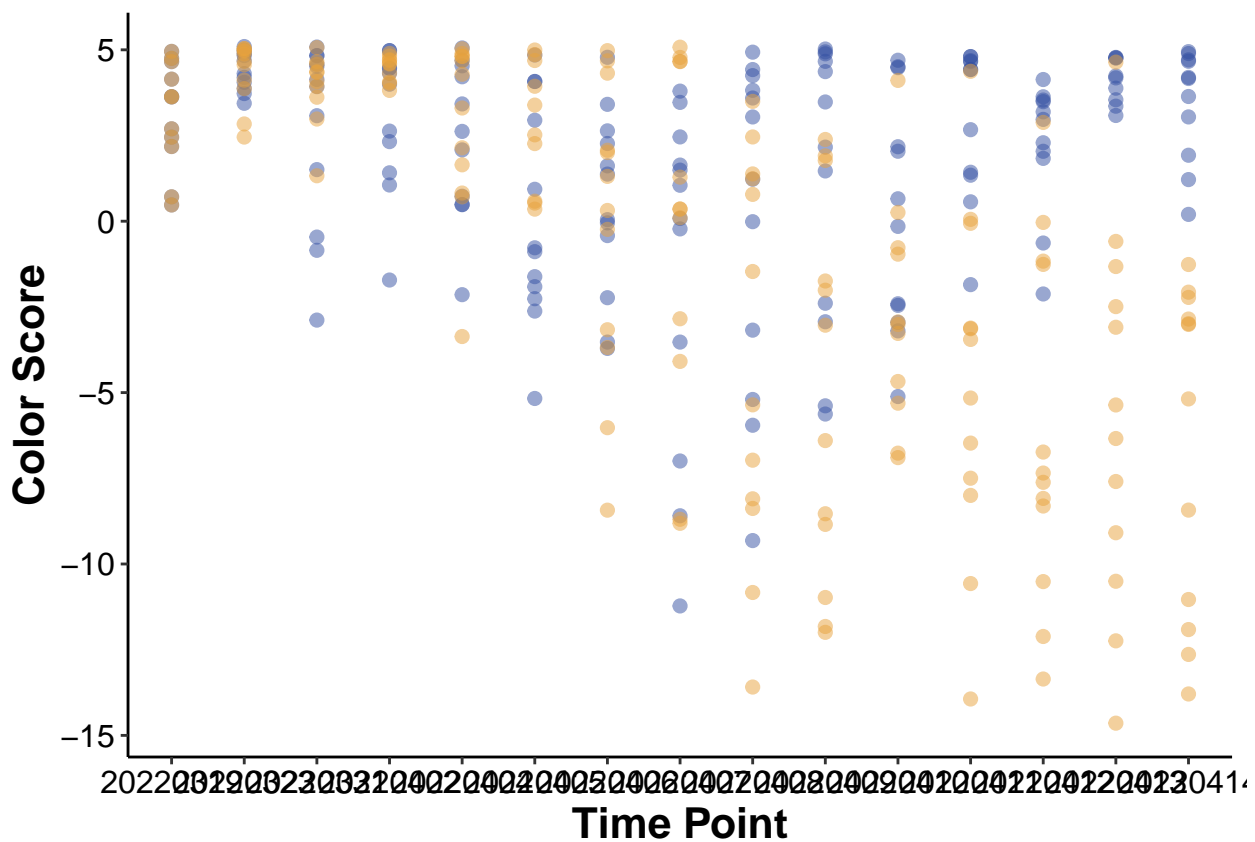


## Testing Transformations

```
#Our random factor is `(1|PLUG.ID)` because fragments were repeatedly measured
Color_sqrt <- lmer(((Bleaching.Score+500)^(1/2)) ~ treatment*Photo_date + (1|ColonyID), na.action=na.om
par(mfrow = c(2, 2))
hist(residuals(Color_sqrt)) #view distribution
boxplot(residuals(Color_sqrt)) #view distribution
qqPlot(residuals(Color_sqrt)) #view qq
```

```
## [1] 161 185
```

```
plot(fitted(Color_sqrt), residuals(Color_sqrt)) #view variance
```
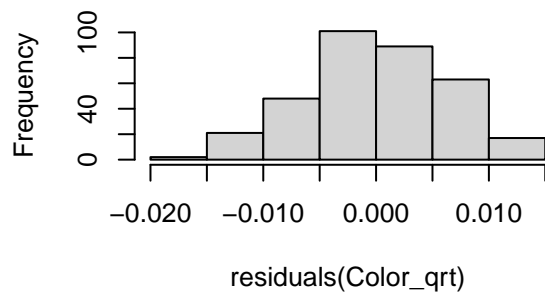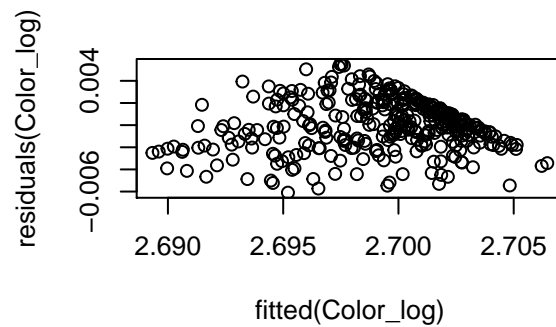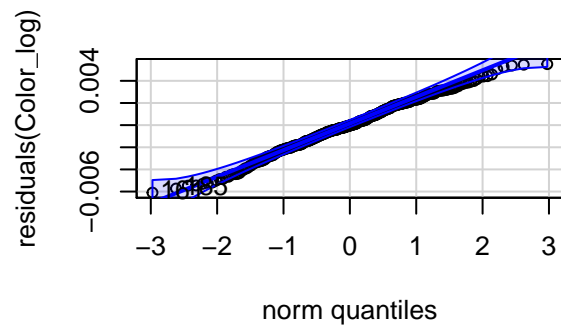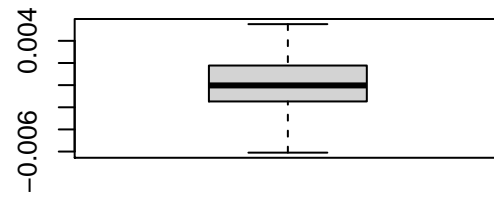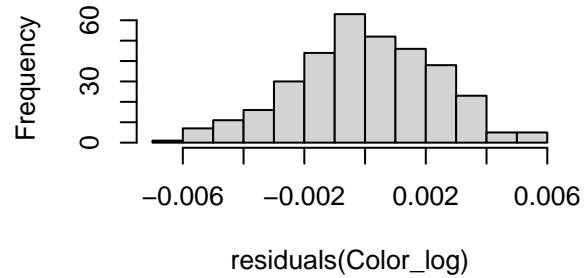
**Histogram of residuals(Color_sqrt)**
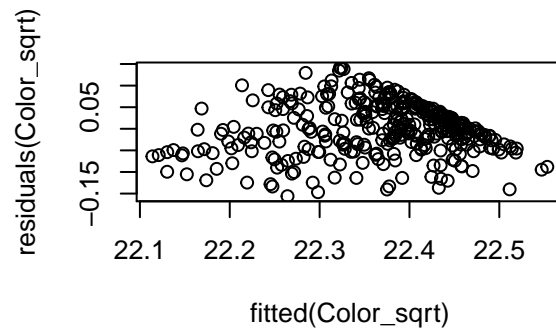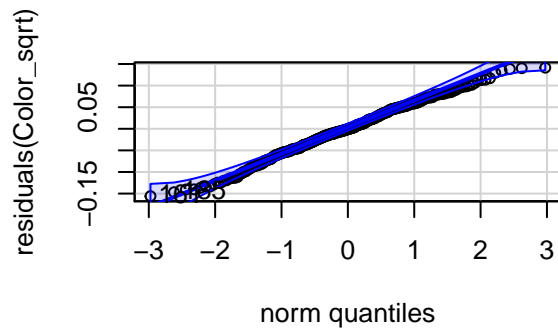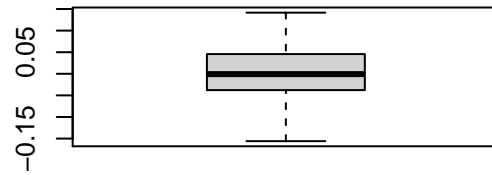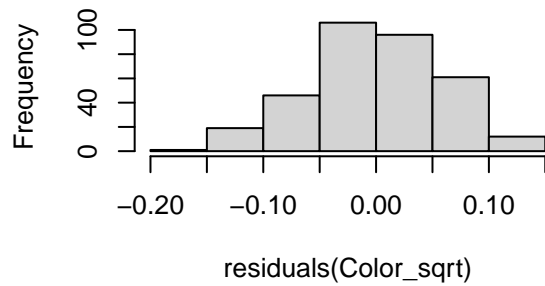


```
Color_qrt <- lmer(((Bleaching.Score+500)^(1/4)) ~ treatment*Photo_date + (1|ColonyID), na.action=na.omi
par(mfrow = c(2, 2))
hist(residuals(Color_qrt))#view distribution
boxplot(residuals(Color_qrt))#view distribution
qqPlot(residuals(Color_qrt))#view qq
```

```
## [1] 161 185
```

```
plot(fitted(Color_qrt), residuals(Color_qrt))#view variance
```

**Histogram of residuals(Color_qrt)**
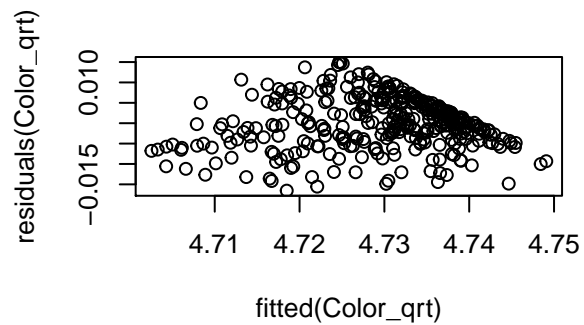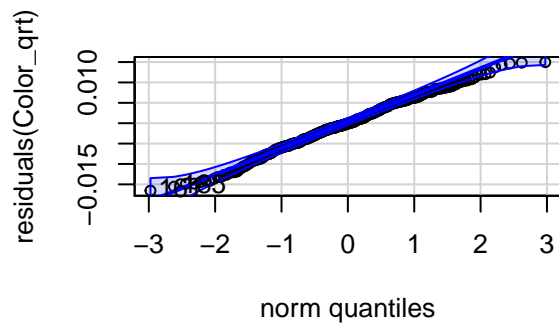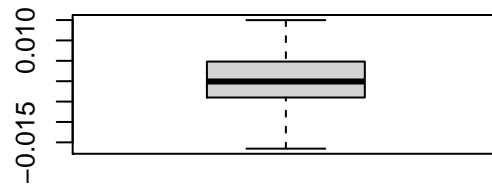


```
Color_log <- lmer(log10((Bleaching.Score+500)) ~ treatment*Photo_date + (1|ColonyID), na.action=na.omit
par(mfrow = c(2, 2))
hist(residuals(Color_log))#view distribution
boxplot(residuals(Color_log))#view distribution
qqPlot(residuals(Color_log))#view qq
```

```
## [1] 161 185
```

```
plot(fitted(Color_log), residuals(Color_log))#view variance
```
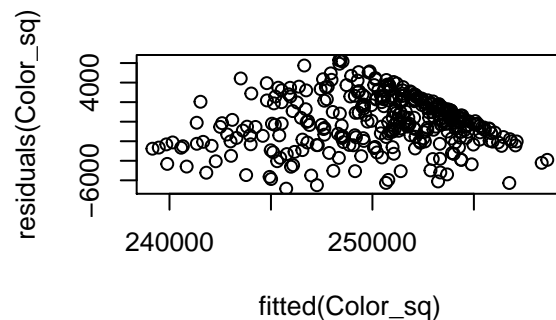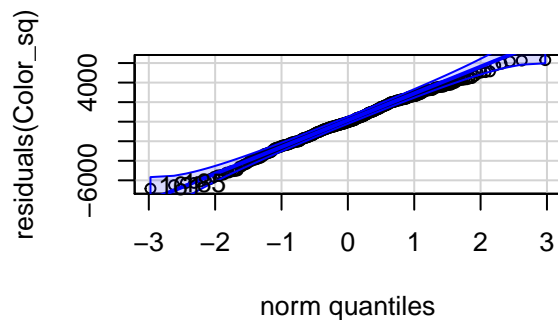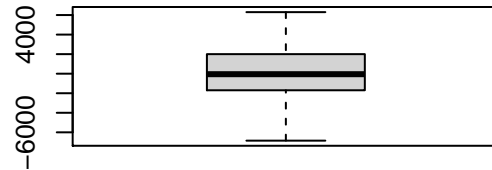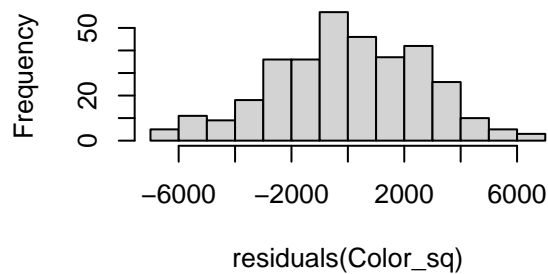
## Histogram of residuals(Color_log)



```
Color_sq <- lmer(((Bleaching.Score+500)^2) ~ treatment*Photo_date + (1|ColonyID), na.action=na.omit, da
par(mfrow = c(2, 2))
hist(residuals(Color_sq))#view distribution
boxplot(residuals(Color_sq))#view distribution
qqPlot(residuals(Color_sq))#view qq
```

```
## [1] 161 185
```

```
plot(fitted(Color_sq), residuals(Color_sq))#view variance
```

**Histogram of residuals(Color_sq)**







*#The data transformation chosen was*

# Analyze Color Score using Mixed effect modeling and Plot data

```
Color_sq <- lmer(((Bleaching.Score+500)^2) ~ treatment*Photo_date + (1|ColonyID), na.action=na.omit, da

## significance of model
Anova(Color_sq, ddf="lme4", type='III') # Type III Wald chisquare tests

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: ((Bleaching.Score + 500)^2)
##                       Chisq Df Pr(>Chisq)
## (Intercept)        57019.0892  1  < 2.2e-16 ***
## treatment             0.0555  1     0.8137
## Photo_date           52.9417 14  1.946e-06 ***
## treatment:Photo_date 172.3781 14  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Blch.meanse <- data %>% group_by(treatment, Photo_date) %>% #group data by factors
  summarise(mean = mean(Bleaching.Score), #calculate mean by group
            sem = std.error(Bleaching.Score)) #calculate sem by group
```

```
## `summarise()` has grouped output by 'treatment'. You can override using the `.groups` argument.
```

```
pdf("RAnalysis/output/color_score/colorscore_without_outliers_colonies.pdf")
Blch_plot2 <- ggplot(Blch.meanse, aes(x=Photo_date, y = mean, group = treatment, color = treatment)) +
```

```
  geom_line() + geom_point() + #plot points and lines
  #facet_grid(~Species, scales = "free", labeller = species_labeller) + #facet by Species
  geom_errorbar(aes(ymin=mean-sem, ymax=mean+sem), width=.2) + #plot sem error bars
  scale_color_manual(values = cols) + #color points
  xlab("Timepoint") + #label x axis
  ylab("Color Score") + # label y axis
  theme_classic() + #set theme
  theme(legend.position = c(0.1,0.3)) + #set legend position
  theme(legend.text = element_text(size = 7)) + #set legend text size
  theme(legend.title = element_text(size = 9)) + #set legend text title size
  geom_vline(xintercept = c(7.8), colour="black", linetype="dotted") + #add vertical line
  theme(plot.title = element_text(face = 'bold.italic', size = 12, hjust = 0)) +  #set font
  #scale_x_discrete(breaks = c("Week1", "Week2", "Week4", "Week6", "Week8", "Week12", "Week16"), labels
  theme(axis.text.x = element_text(angle = 60, vjust = 1.2, hjust = 1.3)) + #Set the text angle
  theme(panel.border = element_blank(), #set theme
  panel.grid.major = element_blank(),  #set theme
  panel.grid.minor = element_blank(),  #set theme
  # Change axis line
  axis.line = element_line(colour = "black"))
Blch_plot2 #view plot
dev.off()

## pdf
##   2

Blch_plot2
```