

MoTrPAC Animal data: analysis of the phenotypic data

```
# Set the working directory to the folder with the data
dmaqc_data_dir = "/Users/David/Desktop/MoTrPAC/data/pass_1a/dmaqc_pheno/"
all_csvs = list.files(dmaqc_data_dir,full.names = T) # get all files in dir
all_csvs = all_csvs[grepl(".csv$",all_csvs)] # make sure we take csv only
# read all files
csv_data = list()
for(fname in all_csvs){
  csv_data[[fname]] = read.csv(fname,stringsAsFactors = F)
}# supply(csv_data,dim) # check the dimensions of the different datasets
```

Sanity check: Acute tests basic statistics

```
# Get the acute test data
ac_test_data = csv_data[[which(grepl("Acute.Test",names(csv_data))))]
dim(ac_test_data)

## [1] 108 23

# check the time differences between start and end
test_times = as.difftime(ac_test_data$t_complete) - as.difftime(ac_test_data$t_start)
# table of the values: all except for on are 0.5 hours
table(test_times)
```

```
## test_times
## 0.4666666666666667      0.5
##                1      107
```

```
# Get the comment of the sample that is not 0.5h
ac_test_data[test_times!=0.5,"comments"]
```

```
## [1] "Treadmill stopped 28:49 (mm:ss) into the acute bout due to problems with the other rat on the s
ac_test_data$formatted_test_time = test_times
```

Next, we analyze the distances. We illustrate how these are a function of the shocks and sex/weight.

```
# convert the shock lengths to numbers (seconds)
parse_shocktime<-function(x){
  arr = strsplit(x,split=":")[1]
  if(length(arr)<2){return(NA)}
  return(as.numeric(arr[1])*60+as.numeric(arr[2]))
}
tmp_x = ac_test_data$howlongshock
tmp_x = sapply(tmp_x, parse_shocktime)
ac_test_data$howlongshock = tmp_x
rm(tmp_x)

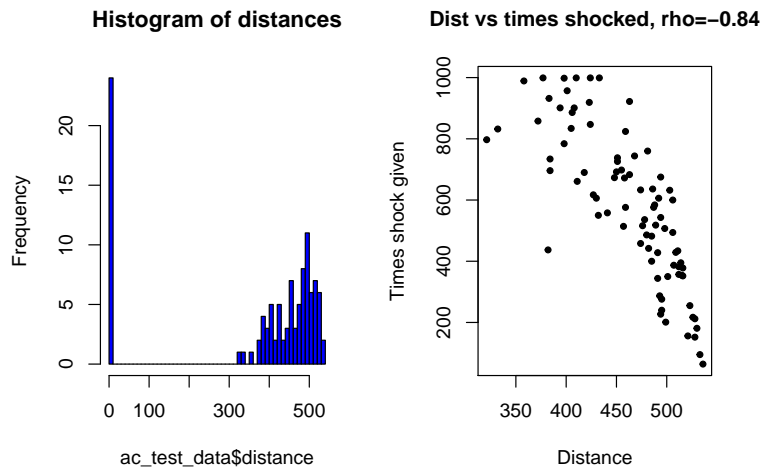
par(mfrow=c(1,2))
# histogram of distances
hist(ac_test_data$distance,col="blue",breaks=50,main = "Histogram of distances")

# Correlation between distance and number of shocks
```

```

# Get the indices of the samples with shock information -
# these the animals that did the acute test
timesshock_inds = !is.na(ac_test_data$timesshock)
# create a new dataframe with the selected animals
trained_animals_data = ac_test_data[timesshock_inds,]
sp_corr = cor(trained_animals_data$distance,
              trained_animals_data$timesshock,method="spearman")
plot(trained_animals_data$distance,trained_animals_data$timesshock,
     main=paste("Dist vs times shocked, rho=",format(sp_corr,digits = 2),sep=""),
     pch=20,ylab="Times shock given",xlab="Distance",cex.main=1.1)

```



```

# A "smarter" analysis: regression of the distance using shock info
dist_lm = lm(distance~timesshock+howlongshock+weight+days_start,
              data=trained_animals_data)
# Summary of the model, points to take: high R^2, significance of
# the features
summary(dist_lm)

```

```

##
## Call:
## lm(formula = distance ~ timesshock + howlongshock + weight +
##     days_start, data = trained_animals_data)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -22.1248  -1.0430   0.6867   2.4416   8.8814
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  562.127804   2.427681  231.549  <2e-16 ***
## timesshock    0.003171   0.003464   0.916   0.363
## howlongshock -0.296415   0.005700 -52.004  <2e-16 ***
## weight       -0.147827   0.006563 -22.526  <2e-16 ***
## days_start    0.032731   0.024534   1.334   0.186
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.623 on 79 degrees of freedom
## Multiple R-squared:  0.9921, Adjusted R-squared:  0.9917

```

```
## F-statistic: 2466 on 4 and 79 DF, p-value: < 2.2e-16
```

```
# We have some clear outliers:
```

```
library(MASS)
par(mfrow=c(1,2))
plot(studres(dist_lm),main="studentized residuals (lm)",ylab="residual")
# Select the top outliers and look at their comments
outliers = abs(studres(dist_lm)) > 2
# how many outliers have we selected?
sum(outliers)
```

```
## [1] 4
```

```
# their comments:
```

```
trained_animals_data[outliers,"comments"]
```

```
## [1] "Increased shock at 20 min."
```

```
## [2] "Treadmill stopped 28:49 (mm:ss) into the acute bout due to problems with the other rat on the s
```

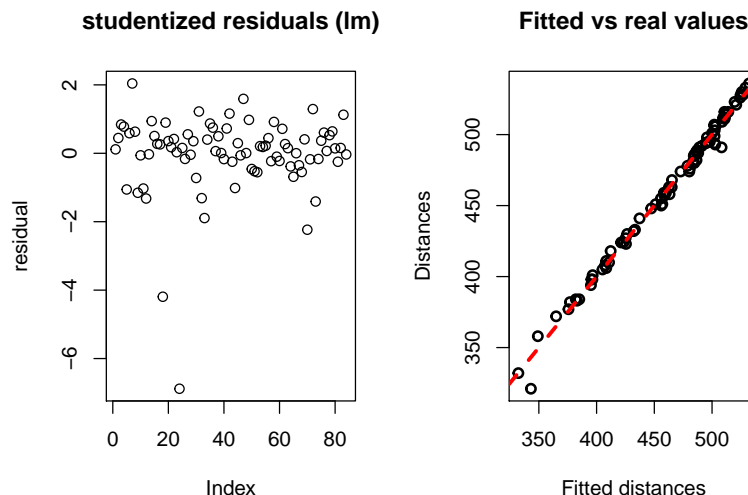
```
## [3] "Shock grid increased to 1.0 mA at 22 minutes. Treadmill bout stopped at 28:49 (mm:ss) due to an
```

```
## [4] ""
```

```
# Plot the fitted values of the linear regression vs.
```

```
# the true distances
```

```
plot(dist_lm$fitted.values,trained_animals_data$distance,lwd=2,
     main="Fitted vs real values",ylab="Distances",xlab="Fitted distances")
abline(0,1,col="red",lty=2,lwd=3)
```



Site comparison

In some versions of the DMAQC data there is a single site. In this case this section will not result in an output.

```
# Load additional information about the animals
```

```
registr_data = csv_data[[which(grepl("Regist",names(csv_data)))]]
```

```
rownames(registr_data) = as.character(registr_data$pid)
```

```
# make the rownames in the test data comparable
```

```
rownames(trained_animals_data) = trained_animals_data$pid
```

```
# add sex to the trained animal data data frame
```

```
sex_key = c("Female","Male")
```

```
trained_animals_data$sex = sex_key[registr_data[rownames(trained_animals_data),"sex"]]
```

```

# Map site Ids to their names
site_names = c("910"="Joslin","930"="Florida")
trained_animals_data$site = site_names[as.character(trained_animals_data$siteID)]

# Sanity check: the numbers should be the same for both sites
table(ac_test_data$siteID)

##
## 910
## 108

table(trained_animals_data$site,trained_animals_data$sex)

##
##           Female Male
##  Joslin         42   42

run_wilcox<-function(x1,x2){
  return(wilcox.test(x1[x2==x2[1]],x1[x2!=x2[1]])$p.value)
}

# Compare the distances, shocks, and weight (if we have multiple site)
if (length(unique(ac_test_data$siteID))>1){
  par(mfrow=c(1,3),mar=c(10,4,4,4))
  # Site only
  p_dist = run_wilcox(trained_animals_data$distance,trained_animals_data$site)
  boxplot(distance~site,data=trained_animals_data,col="cyan",ylab="Distance",
    main=paste("Site vs. distance, p<",format(p_dist,digits = 2)),
    cex.main=1,las=2)
  p_timesshock = run_wilcox(trained_animals_data$timesshock,trained_animals_data$site)
  boxplot(timesshock~site,data=trained_animals_data,col="red",ylab="Times shocked",
    main=paste("Site vs. times shocked, p<",format(p_timesshock,digits = 3)),
    cex.main=1,las=2)
  p_w = run_wilcox(trained_animals_data$weight,trained_animals_data$site)
  boxplot(weight~site,data=trained_animals_data,col="cyan",ylab="Weight",
    main=paste("Site vs. weight, p=",format(p_w,digits = 2)),
    cex.main=1,las=2)
  # Site and sex
  par(mfrow=c(1,3),mar=c(10,4,4,4))
  boxplot(distance~site+sex,data=trained_animals_data,col="cyan",ylab="Distance",
    main="Site vs. distance",cex.main=1,las=2)
  boxplot(timesshock~site+sex,data=trained_animals_data,col="red",ylab="Times shocked",
    main="Site vs. times shocked",cex.main=1,las=2)
  boxplot(weight~site+sex,data=trained_animals_data,col="cyan",ylab="Weight",
    main="Site vs. weight",cex.main=1,las=2)

  # Regress time shocked and distance vs. site and sex
  summary(lm(timesshock~site+sex,data=trained_animals_data))
  summary(lm(distance~site+sex,data=trained_animals_data))
}

```

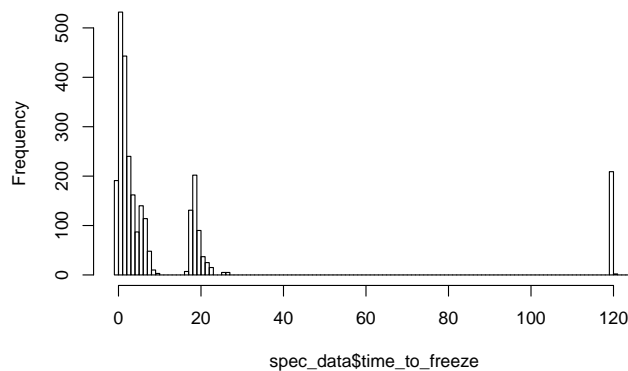
Sanity checks: Biospecimen data

```
# Analysis of biospecimen data
spec_data = csv_data[[which(grepl("Specimen.Processing.csv", names(csv_data))))]]
rownames(spec_data) = spec_data$labelid
# Parse the times and compute the difference between the freeze time and
# the collection time
time_to_freeze1 = as.difftime(spec_data$t_freeze, units = "mins") -
  as.difftime(spec_data$t_collection, units = "mins")
# For some samples we have the edta spin time instead of the collection
# time, use these when there are no other options
time_to_freeze2 = as.difftime(spec_data$t_freeze, units = "mins") -
  as.difftime(spec_data$t_edtaspin, units = "mins")
time_to_freeze = time_to_freeze1
# Fill in the NAs by taking the time between the edta spin and the freeze
table(is.na(time_to_freeze1), is.na(time_to_freeze2))
```

```
##
##          FALSE TRUE
##  FALSE      0 2182
##   TRUE     517    0
```

```
time_to_freeze[is.na(time_to_freeze1)] = time_to_freeze2[is.na(time_to_freeze1)]
spec_data$time_to_freeze = as.numeric(time_to_freeze)
spec_data$time_to_freeze_from_collection = as.numeric(time_to_freeze1)
spec_data$time_to_freeze_from_edta_spin = as.numeric(time_to_freeze2)
hist(spec_data$time_to_freeze, breaks = 100)
```

Histogram of spec_data\$time_to_freeze



```
# Add site by name
site_names = c("910" = "Joslin", "930" = "Florida")
spec_data$site = site_names[as.character(spec_data$siteid)]
table(spec_data$site)
```

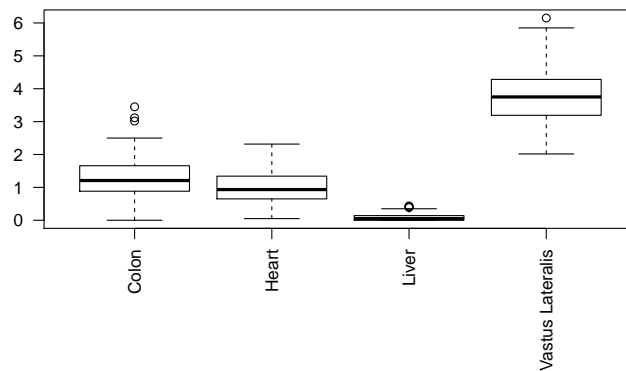
```
##
## Joslin
## 2699
```

```
inds = !is.na(time_to_freeze1)
inds = grepl("adipose", spec_data$sampltypedescription, ignore.case = T)
inds = grepl("heart", spec_data$sampltypedescription, ignore.case = T) |
  grepl("liver", spec_data$sampltypedescription, ignore.case = T) |
```

```

grepl("colon",spec_data$sampletypedescription,ignore.case = T) |
grepl("vastus",spec_data$sampletypedescription,ignore.case = T)
# Using site info:
# Here we use an interaction term and not addition as the R^2 is >2 times
# greater this way
if (length(unique(spec_data$site))>1){
  par(mar=c(10,2,2,2))
  boxplot(time_to_freeze~site:sampletypedescription,data=spec_data[inds,],
    ylab="Time to freeze",las=2)
  summary(lm(time_to_freeze~sampletypedescription:site,data=spec_data[inds,]))
}
# A single site
if (length(unique(spec_data$site))==1){
  par(mar=c(10,2,2,2))
  boxplot(time_to_freeze~sampletypedescription,data=spec_data[inds,],
    ylab="Time to freeze",las=2)
  summary(lm(time_to_freeze~sampletypedescription,data=spec_data[inds,]))
}

```



```

##
## Call:
## lm(formula = time_to_freeze ~ sampletypedescription, data = spec_data[inds,
##   ])
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.78765 -0.33731 -0.05216  0.27994  2.34568
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.32052    0.05478  24.106 < 2e-16
## sampletypedescriptionHeart
##               -0.30046    0.07747  -3.878 0.000122
## sampletypedescriptionLiver
##               -1.23503    0.07747 -15.942 < 2e-16
## sampletypedescriptionVastus Lateralis
##               2.48380    0.07747  32.061 < 2e-16
##
## (Intercept)          ***
## sampletypedescriptionHeart
##                   ***
## sampletypedescriptionLiver
##                   ***
## sampletypedescriptionVastus Lateralis ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##

```

```
## Residual standard error: 0.5693 on 428 degrees of freedom
## Multiple R-squared: 0.8548, Adjusted R-squared: 0.8538
## F-statistic: 839.8 on 3 and 428 DF, p-value: < 2.2e-16
```

Format the metadata table according to vial ids

We now use DMAQC's mapping of label ids to vial ids and use it to generate a single metadata table that we can share with other sites.

```
# Helper function for merging columns from data2 into data1
merge_avoid_col_dup<-function(data1,data2,by_col){
  data2_cols = c(by_col,setdiff(colnames(data2),colnames(data1)))
  return(merge(data1, data2[,data2_cols], by=by_col))
}

# Get the animal data and merge
merged_animal_data = ac_test_data
colnames(merged_animal_data) = paste("Acute.test",colnames(merged_animal_data),sep=";")
colnames(merged_animal_data)[grepl(";pid$",colnames(merged_animal_data))]="pid"
tmp_ac_data = csv_data[[which(grepl("Animal.Familiarization",names(csv_data)))]]
colnames(tmp_ac_data) = paste("Animal.Familiarization",colnames(tmp_ac_data),sep=";")
colnames(tmp_ac_data)[grepl(";pid$",colnames(tmp_ac_data))]="pid"
merged_animal_data = merge_avoid_col_dup(merged_animal_data,tmp_ac_data,by="pid")
tmp_ac_data = csv_data[[which(grepl("Animal.Key",names(csv_data)))]]
colnames(tmp_ac_data) = paste("Animal.Key",colnames(tmp_ac_data),sep=";")
colnames(tmp_ac_data)[grepl(";pid$",colnames(tmp_ac_data))]="pid"
merged_animal_data = merge_avoid_col_dup(merged_animal_data,tmp_ac_data,by="pid")
tmp_ac_data = csv_data[[which(grepl("Animal.Registration",names(csv_data)))]]
colnames(tmp_ac_data) = paste("Animal.Registration",colnames(tmp_ac_data),sep=";")
colnames(tmp_ac_data)[grepl(";pid$",colnames(tmp_ac_data))]="pid"
merged_animal_data = merge_avoid_col_dup(merged_animal_data,tmp_ac_data,by="pid")

# Add the biospecimen data and create a large data frame
merged_dmaqc_data = merge(merged_animal_data,spec_data,by="pid")
```

Compare to the DMAQC computed scores

```
calc_data_file = all_csvs[grepl("Calculated.V",all_csvs)]
calc_data = read.csv(calc_data_file)
rownames(calc_data) = calc_data$labelid

# compare our computed freeze time and the given one above
inds = intersect(rownames(spec_data),rownames(calc_data))
for(tissue in unique(spec_data$sampltypedescription)){
  currinds = inds[spec_data[inds,"sampltypedescription"]==tissue]
  x1 = spec_data[currinds,"time_to_freeze"]
  x2 = calc_data[currinds,"frozetime_after_acute"]
  x2 = x2 - calc_data[currinds,"deathtime_after_acute"]
  if(all(is.na(x2))||all(is.na(x1))){next}
  # plot(x1*60,x2,main=tissue);abline(0,1)
}
```