Project Head and Neck Cancer

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21. July 2019

General Introduction

Head and neck cancer is defined as a group of cancers, concerning the mouth, nose and throat (Figure 1). With over 90%, Squamous cell carcinoma is the most common type in HNC patients. With 5.5 million affected people in 2015 it is the seventh most frequent cancer and the ninth most frequent cause of death. 75% of all HNC cancer types were caused by tobacco or alcohol. Although the cure rate for HNC is really high, around 50 % of concerned patients remain suffering from an advanced disease.

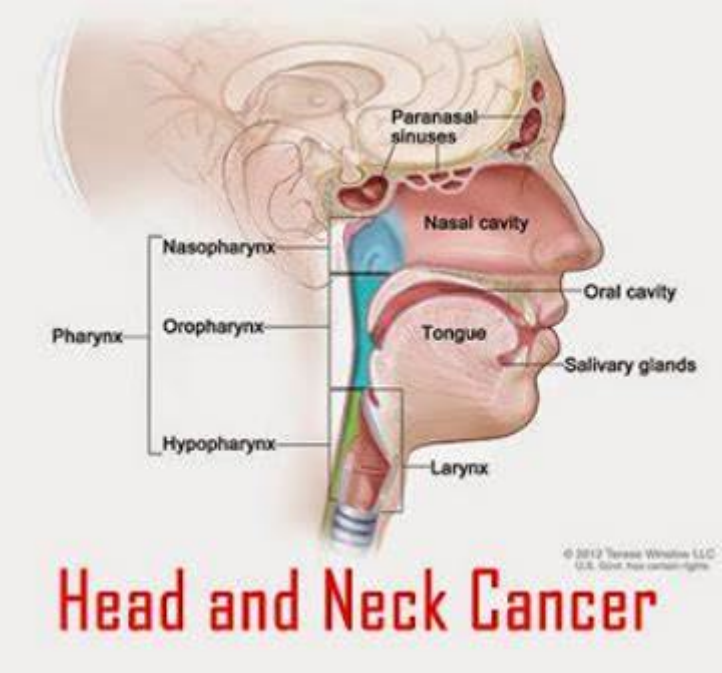


Figure 1: Most affected tissues and organs by head and neck cancer

In the century of next generation sequencing, the genome became an interesting target for scientists. Approaches that directly affect important oncogenes often fail due to the fact that these genes play an important role in healthy cells. The researchers in our paper (Jerby-Arnon, L., et al. (2014). "Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality." Cell 158(5): 1199-1209.) investigated synthetic lethality and synthetic dosage lethality that uses gene interactions to affect tumorous proliferation. Synthetic letahlity describes a gene interaction in which single-gene defects are compatible with cell viability, but the combination of gene effects results in cell death. Synthetic dosage lethality occurs, when the overexpression of one gene is combined with the knockout of another gene.

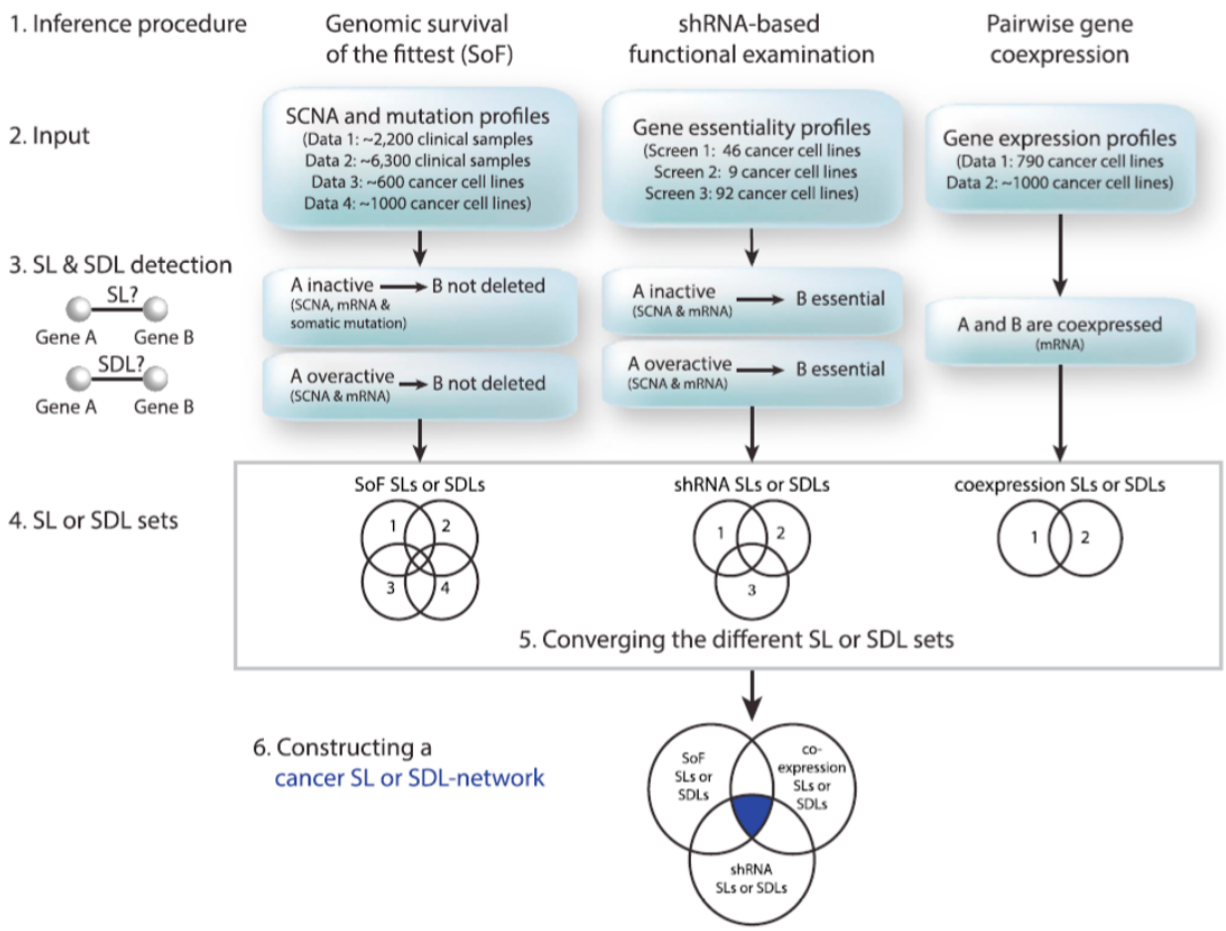


Figure 2: Daisy-Workflow

Daisy consists of three statistical approaches (Figure 2), each processing different sub data from a cancer Dataset. The first strategy is "Survival of the fittest", where just deleted SL-paired genes were selected, that are not in a surviving cancer culture. The second strategy is "functional examination" where SL genes were identified, by knocking out genes, with the additive, that the SL partners are inactive. If the tumor shows decreased proliferation, it accounts for the SL pair group. The third strategy is "pairwise gene co- expression" that is based on the fact that SL pairs are often closely related in biological pathways and therefore exhibit similar occurrence. If a gene pair fulfills every of the three criteria, it might be a potential SL pair according to DAISY. To check for SDL partners the criteria of inactive or active is replaced by under- or overactive, measured through expression numbers and copy number alterations.

Milestones

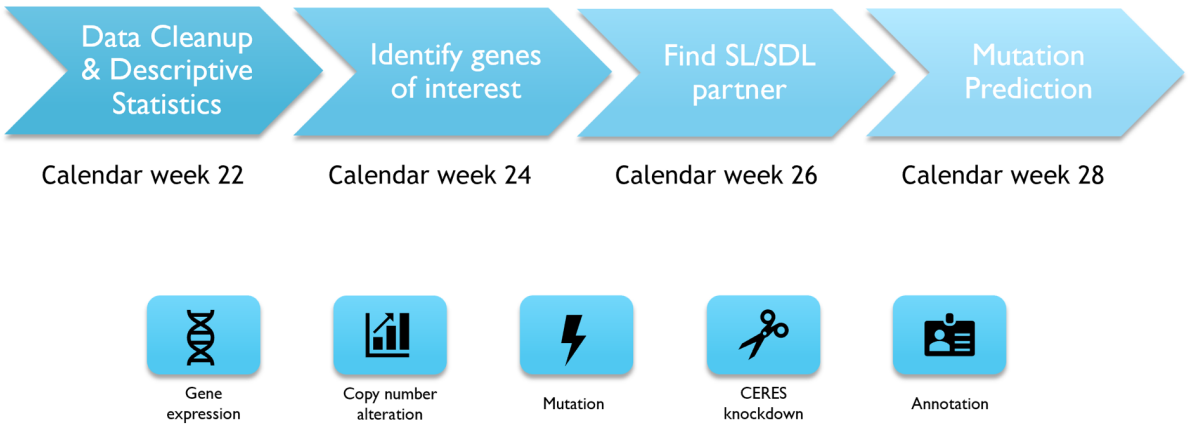


Figure 3: Milestones of our project

The strategy of our project consisted of 4 main milestones (Figure 3). At first we familiarized with the data and did the first descriptive statistics in milestone 1. Secondly, we identified potential genes "of interest" (SL and SDL candidates), according to DAISY-System. In our third milestone, the core task of our project was implemented. The identification of SL and SDL pairs was executed using functional examination, survival of the fittest and pairwise co-expression. Lastly, in our fourth milestone we tested logistic regression to predict mutations.

1. Data Cleanup & Descriptive Statistics

Loading data and defining HNC variables

```
# Load dataset
allDepMapData = readRDS("DepMap19Q1_allData.RDS")

# Creation of sub matrices
expression = allDepMapData[["expression"]]
copynumber = allDepMapData[["copynumber"]]
mutation = allDepMapData[["mutation"]]
kd.ceres = allDepMapData[["kd.ceres"]]
kd.prob = allDepMapData[["kd.prob"]]
annotation = allDepMapData[["annotation"]]
rm(allDepMapData)

# Reducing the samples to head and neck cancer samples
annotation.HNC = annotation[which(annotation$Primary.Disease == "Head and Neck Cancer"), ]
ID = annotation.HNC$DepMap_ID

# Filtering of sub matrices by primary disease "Head and neck cancer"
expression.HNC = expression[ , which(colnames(expression) %in% ID)]
copynumber.HNC = copynumber[ , which(colnames(copynumber) %in% ID)]
kd.ceres.HNC = kd.ceres[ , which(colnames(kd.ceres) %in% ID)]
kd.prob.HNC = kd.prob[ , which(colnames(kd.prob) %in% ID)]
mutation.HNC = mutation[ID]
```

Expression analysis

Checking for NAs

```
sum(is.na(expression) == TRUE)
```

```
[1] 0
```

Result: There are no NAs in the expression data.

Examine expression values

Create a reference group of expression data

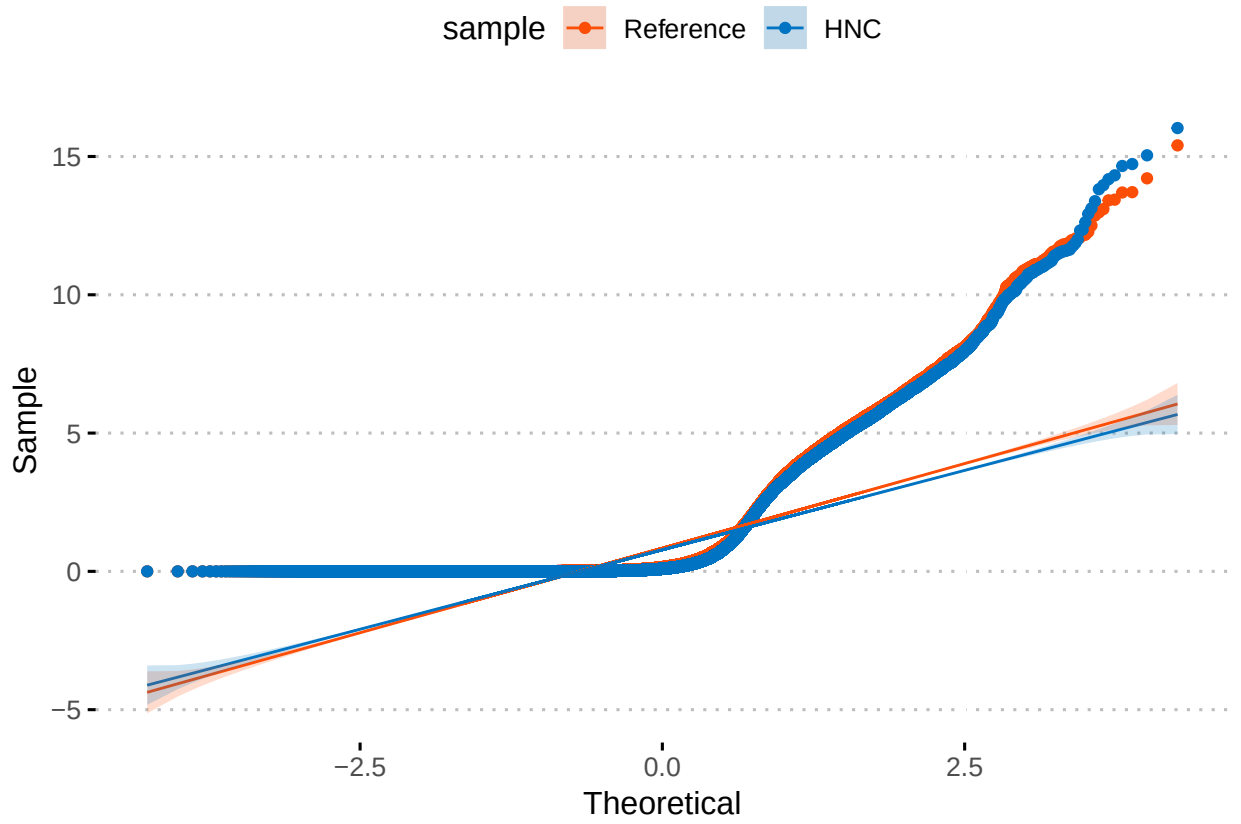
```
'%!in%' = function(x,y)!('%in%'(x,y)) # Define function "is not in"

expression.reference = expression[ , which(colnames(expression) %!in% ID)]
```

and calculate their mean.

```
mean.exp.reference = apply(expression.reference, 1, mean)
mean.exp.HNC = apply(expression.HNC, 1, mean)
```

Firstly, we want to check if the gene expressions of the HNC samples and the reference group are normally distributed.

```
HNC = data.frame(expression_values = mean.exp.HNC, sample = "HNC")
Reference = data.frame(expression_values = mean.exp.reference, sample = "Reference")
mean.expression = rbind(Reference, HNC)

ggqqplot(mean.expression, x = "expression_values",
   color = "sample",
   palette = c("#FC4E07", "#0073C2FF"), # Red and blue
   ggtheme = theme_pubclean())
```



Result: Gene espression is neither in HNC samples nor in reference samples normally distributed.

Then we compare HNC and reference group gene expression using a statistical test. A Wilcoxon rank sum test must be applied because the expression is not normally distributed.

```
wilcox.test(mean.exp.HNC, mean.exp.reference)
```

```
    Wilcoxon rank sum test with continuity correction

data:  mean.exp.HNC and mean.exp.reference
W = 1080737603, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
```

Result: Mean gene expression differs between HNC and reference group samples significantly.

Next we check if the gene expression differs whether a gene has a deleterious mutation or not. AS an example, we compare the mean TP53 gene expression of all samples with and without deleterious TP53 mutation.

```
# Get the ID of samples whith and without deleterious mutation
TP53.mut.ID = lapply(1:length(mutation), function(a) {
  dat_picker = mutation[[a]]
  dat_picker = dat_picker[which(dat_picker$isDeleterious == TRUE),]
  # Filter all IDs which have a deleterious mutation of TP53
  out = ifelse("TP53" %in% dat_picker$Hugo_Symbol, a, NA)
    return(out)
```

```
  })

# ID of samples with no TP53 mutation
TP53.not_mut.ID = grep("NA", TP53.mut.ID)
# ID of samples with TP53 mutation
TP53.mut.ID = as.integer(TP53.mut.ID[is.na(TP53.mut.ID) == FALSE])

# Create two vectors of expression values
# Expression of TP53 deleterious mutated samples
exp.TP53.mut = as.numeric(expression["TP53", TP53.mut.ID])
# Expression of TP53 not deleterious mutated samples
exp.TP53.not_mut = as.numeric(expression["TP53", TP53.not_mut.ID])
```

Compare the expression values by applying Wilcoxon rank sum test.

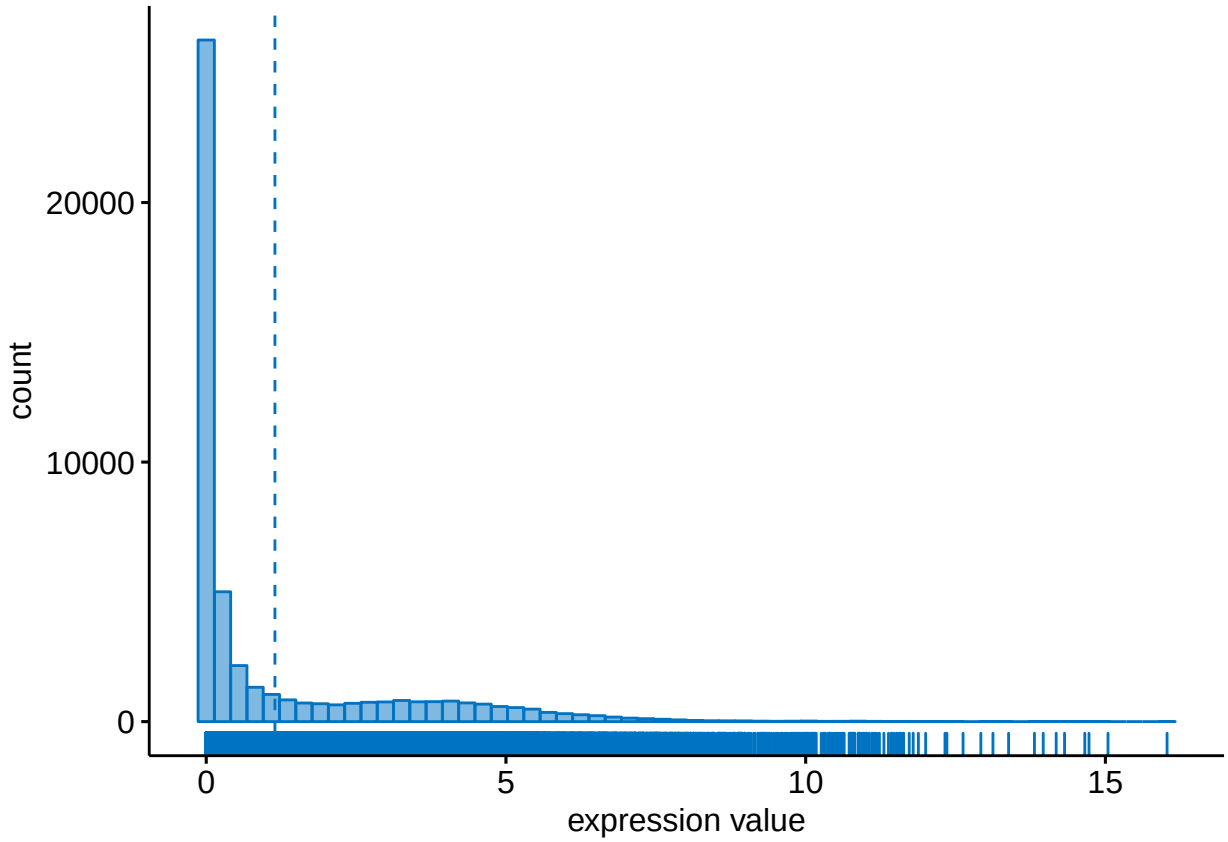
```
wilcox.test(exp.TP53.mut , exp.TP53.not_mut)
```

```
    Wilcoxon rank sum test with continuity correction

data:  exp.TP53.mut and exp.TP53.not_mut
W = 8276.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
```

Result: Gene expression of TP53 as an example gene varies significantly whether the samples has or has no deleterious TP53 mutation.

At last, we plot a histogram to check how the expression values are distributed.

```
gghistogram(as.data.frame(mean.exp.HNC),
            x = "mean.exp.HNC",
            xlab = "expression value",
            bins = 60,
            fill = "#0073C2FF",
            color = "#0073C2FF",
            add = "mean",
            rug = TRUE)
```



Result: The most genes have expression values around 0.

Plot the 20 most under- and overexpressed genes of HNC samples.

For this we need to normalize the expression data from HNC samples

```
expr.HNC.norm = as.data.frame(apply(expression.HNC, 2, function(x) {
  x - mean.exp.reference
}))
```

and calculate the mean of the normalesid expression values.

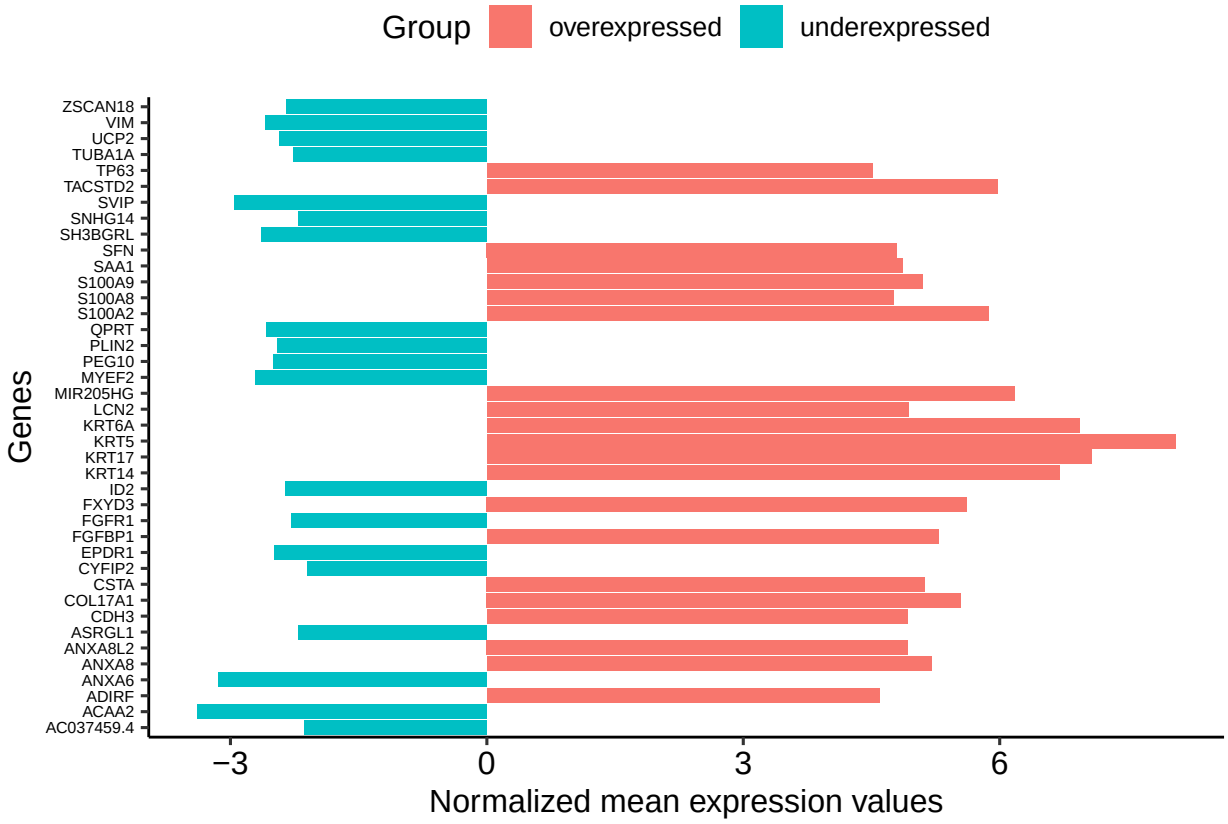
```
mean.expr.HNC.norm = as.data.frame(apply(expr.HNC.norm, 1, mean))
```

Then create a dataframe with needed information for the barplot

```
barplot.data = data.frame(Genes=rownames(expr.HNC.norm),
                          Values=mean.expr.HNC.norm)
names(barplot.data) = c("Genes", "Values")
barplot.data$Group = ifelse(barplot.data$Values>0,"overexpressed","underexpressed")
barplot.data = barplot.data[order(barplot.data$Values),]
barplot.data = barplot.data[c(1:19, (nrow(barplot.data)-20):nrow(barplot.data)),]
```

and plot this dataframe.

```
ggplot(barplot.data,aes(x=Genes,y=Values,fill=Group)) +
  ylab("Normalized mean expression values") +
  geom_bar(stat="identity") +
  coord_flip() +
  theme(axis.text.y = element_text(size=6))
```



Cleanup of the enviroment

```
rm(HNC, Reference, mean.expression, TP53.mut.ID, TP53.not_mut.ID, exp.TP53.mut,
   exp.TP53.not_mut, mean.expr.HNC.norm, barplot.data, mean.exp.HNC,
   mean.exp.reference, expression.reference)
```

Copynumber Alteration

Analysis of NA values

Examination of NA values in the copynumber.HNC data

```
# Sum up all NAs in each row
NA.rows = apply(copynumber.HNC,1, function(x) {
          sum(is.na(x))
})
# Define all rows cotaining missing values
NA.rows = which( NA.rows > 0)
length(NA.rows)
```

```
[1] 107
```

Only 107 out of 23299 gens have NA values and are therefore deleted from the copynumber.HNC dataframe

```
copynumber.HNC = copynumber.HNC[-NA.rows,]
dim(copynumber.HNC)
```

```
[1] 23192    27
```

Creating a reference data and examination of its NA values

```
copynumber.reference = copynumber[,which(colnames(copynumber) %!in% ID)]
dim(copynumber.reference)
```

```
[1] 23299   517
```

```
NA.rows.ref = apply(copynumber.reference,1, function(x) {
          sum(is.na(x))
})
NA.rows.ref = which( NA.rows.ref > 0)
length(NA.rows.ref)
```

```
[1] 107
```

```
summary(NA.rows == NA.rows.ref)
```

```
   Mode    TRUE
logical     107
```

Genes with NA values are the same for the HNC samples and all the other samples. Therefore, they can be deleted in the reference data as well.

```
copynumber.reference = copynumber.reference[-NA.rows.ref,]
copynumber = copynumber[-NA.rows.ref,]
dim(copynumber.reference)
```

```
[1] 23192   517
```

```
dim(copynumber)
```

```
[1] 23192   544
```

Normalisation of the copynumber values

For the HNC normalisation we will use the mean CN value per gen of the reference data.

```
copynumber.ref.mean = apply(copynumber.reference,1, mean)
copynumber.HNC.mean = apply(copynumber.HNC ,1, mean)
```

Creating a dataframe of all copynumber values. The values are normalized to the mean copynumbers of the reference data.

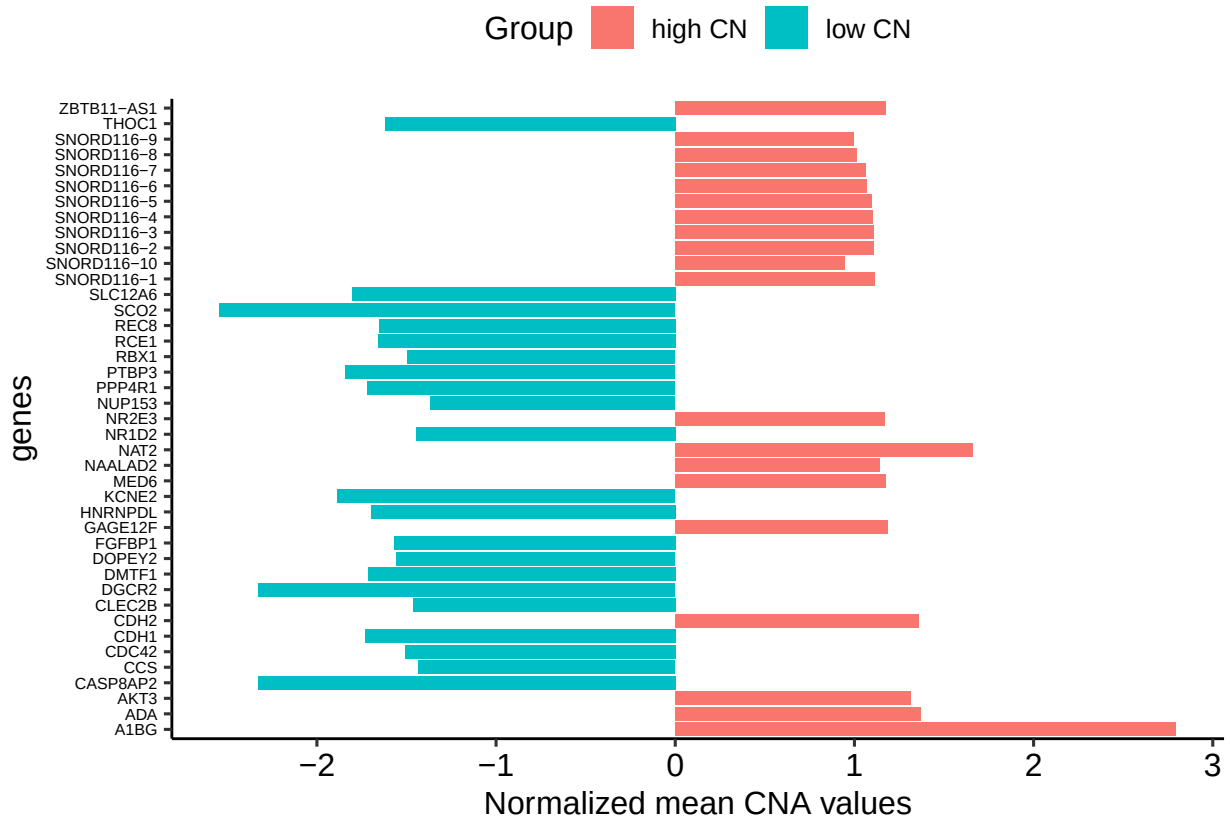
```
cna.HNC.norm = as.data.frame(apply(copynumber.HNC, 2, function(x) {
  x - copynumber.ref.mean
}))
```

Creating a dataframe of HNC copynumber values. The values are normalized to the mean copynumbers of the reference data. For further analysing the dataframe is ordered in a decreasing manner.

```
Values = copynumber.HNC.mean - copynumber.ref.mean
copynumber.HNC.norm = data.frame(values = sort(Values, decreasing = TRUE),
                                 genes = row.names(copynumber.HNC))
copynumber.HNC.norm$Group = ifelse(copynumber.HNC.norm$values > 0,"high CN","low CN")
```

Plotting genes with the highest and lowest copynumbers.

```
ggplot(copynumber.HNC.norm[c(1:20,23172:23192),],aes(x=genes,y=values,fill=Group)) +
  geom_bar(stat="identity") +
  ylab("Normalized mean CNA values") +
  coord_flip() +
  theme(axis.text.y = element_text(size=6))
```



Cleanup of the environment

```
rm(NA.rows, NA.rows.ref, copynumber.HNC.norm, copynumber.HNC.mean,
   copynumber.ref.mean, copynumber.reference, Values)
```

Mutation

Find Mutations with the highest frequencies

Melt all mutation matrices together as data frame and find out most frequent mutations

```
all_mutations = do.call(rbind,mutation)
all_mut_genes = table(all_mutations$Hugo_Symbol)
all_mut_genes_df = as.data.frame(all_mut_genes)
```

Order the found mutations by frequencies and extract 30 most frequent

```
ID_all_highest_frequencies = head(order(all_mut_genes_df[["Freq"]], decreasing = TRUE), 30)
all_mut_genes_df_highest_30 = all_mut_genes_df[
  which(rownames(all_mut_genes_df) %in% ID_all_highest_frequencies), ]
ID_all_highest_30_frequencies = order(all_mut_genes_df_highest_30["Freq"],
                                      decreasing = TRUE)
all_mut_genes_highest_30_ordered = all_mut_genes_df_highest_30[ID_all_highest_30_frequencies, ]
head(all_mut_genes_highest_30_ordered)
```

```
        Var1 Freq
17577    TTN 1567
10296  MUC16  774
17193   TP53  471
10213 MT-ND5  439
11210  OBSCN  368
10304   MUC4  361
```

Name the newly established data frame for the look

```
ID_all_highest_30_frequencies = order(all_mut_genes_df_highest_30["Freq"],
                                      decreasing = TRUE)
all_mut_genes_df_highest_30_ordered = all_mut_genes_df_highest_30[ID_all_highest_30_frequencies,]
rownames(all_mut_genes_df_highest_30_ordered) = 1:30
colnames_ordered = c("Gene", "Freq")
colnames(all_mut_genes_df_highest_30_ordered) = colnames_ordered
```

Melt only HNC sample mutation matrices together and find out most frequent mutations

```
HNC_mutations = do.call(rbind, mutation.HNC)
HNC_mut_genes = table(HNC_mutations$Hugo_Symbol)
HNC_mut_genes_df = as.data.frame(HNC_mut_genes)
```

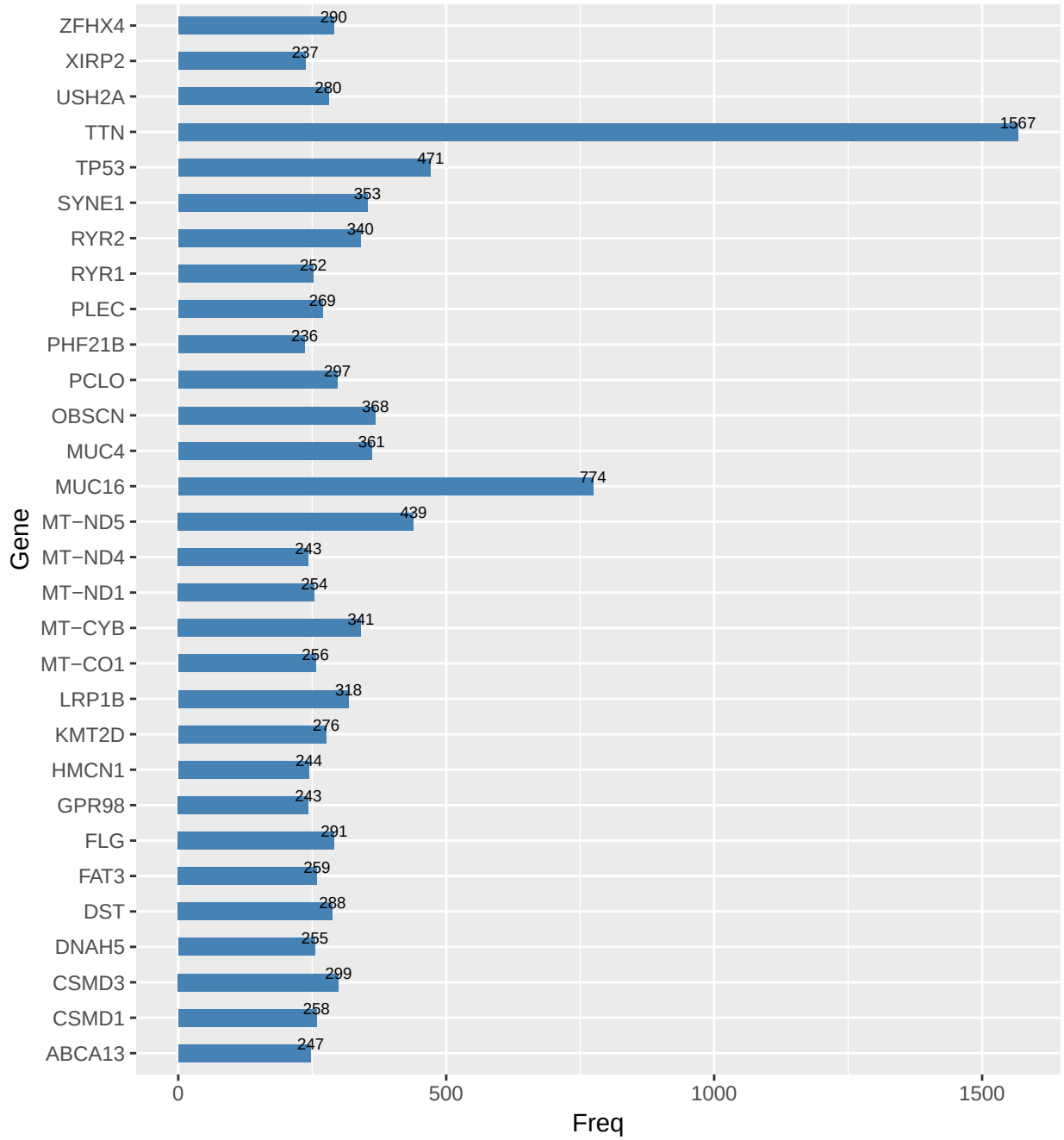
Order the found mutations by frequencies and name the data frame

```
ID_HNC_highest_frequencies = head(order(HNC_mut_genes_df[["Freq"]],
                                        decreasing = TRUE),
                                        length(ID))
HNC_mut_genes_df_highest_ordered = HNC_mut_genes_df[ID_HNC_highest_frequencies, ]
rownames(HNC_mut_genes_df_highest_ordered) = 1:length(ID)
colnames(HNC_mut_genes_df_highest_ordered) = colnames_ordered
```

Plotting mutation frequencies of all samples and of HNC samples

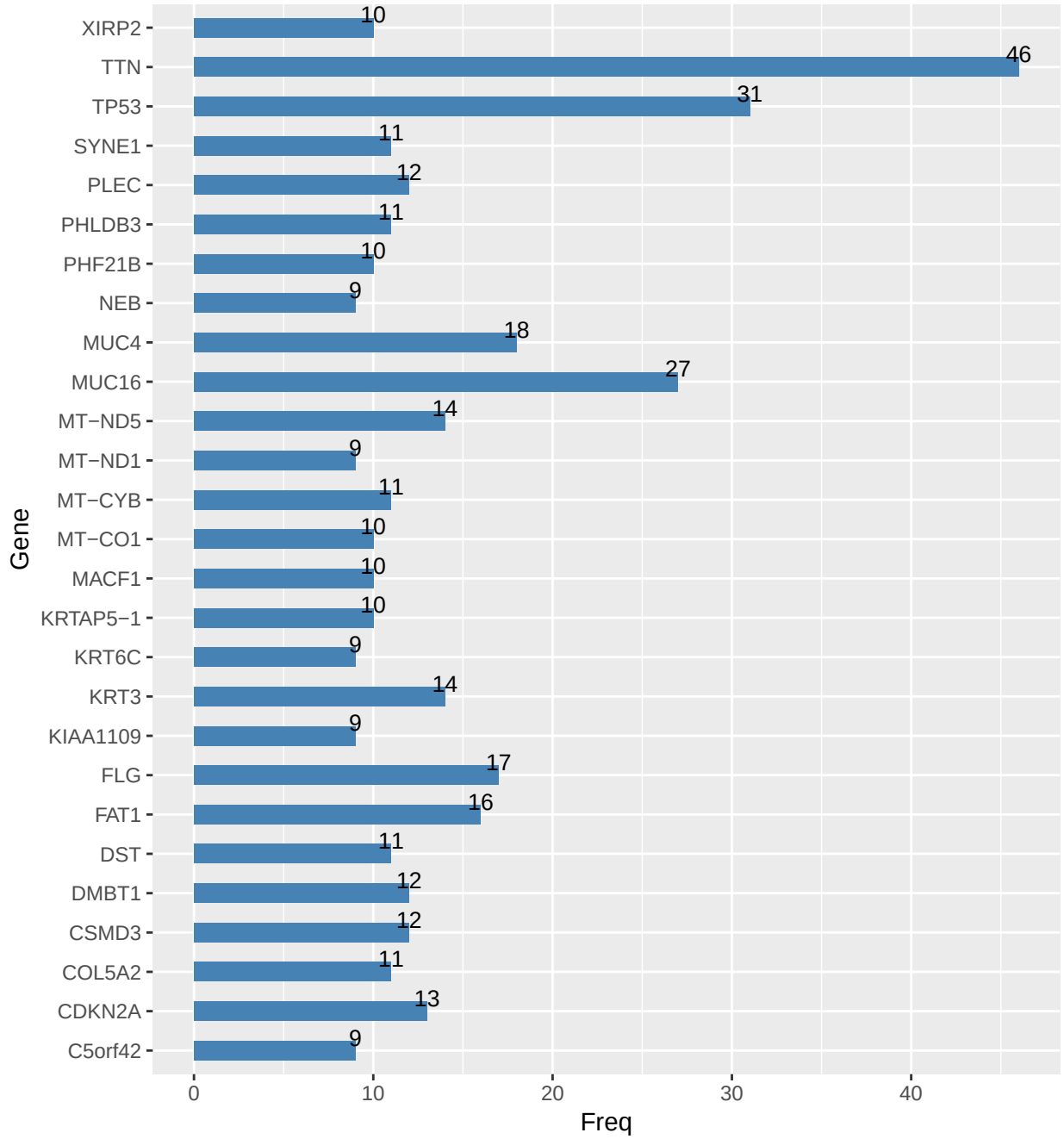
Plot mutations in all samples

```
ggplot(data = all_mut_genes_df_highest_30_ordered, aes(x=Gene, y=Freq)) +
  geom_bar(stat = "identity", width = 0.5, fill="steelblue") +
  geom_text(aes(label=Freq), vjust=-0.3, size=2.5) +
  theme_grey() + coord_flip()
```



Plot mutations in HNC samples

```
ggplot(data = HNC_mut_genes_df_highest_ordered, aes(x=Gene, y=Freq)) +
  geom_bar(stat = "identity", width = 0.5, fill="steelblue") +
  geom_text(aes(label=Freq), vjust=-0.3, size=3.5) +
  theme_grey() +
  coord_flip()
```



Clean mutation from unnecessary data

Due to the needed conditions in the following "Daisy-tests" we now clean the mutation data and extract all "isDeleterious" mutations, since they are essential for the survival or death of a cell. In this way, we later are able to find our genes of interest for the SL more easily.

```
'mutation.clean' = function(data){
  # Data will be mutation (length of 544) or mutation.HNC (length of 27)
  cleaned_data = lapply(1:length(data), function(a) {
    dat_picker = data[[a]] # Pick the element of the list on position 'a'
    # Choose columns with gene name and isDeleterious
    dat_picker = dat_picker[, c("Hugo_Symbol", "isDeleterious")]
    # Choose only deleterious mutations
    dat_picker = dat_picker[which(dat_picker$isDeleterious == TRUE),]
    return(dat_picker)
  })
  names(cleaned_data) = names(data) # Keep the names of the input data
  return(cleaned_data)
}
# Apply the new function on our two mutation data sets to clean them
mutation = mutation.clean(mutation)
mutation.HNC = mutation.clean(mutation.HNC)
head(mutation.HNC[[1]])
```

```
   Hugo_Symbol isDeleterious
1:       NBPF1          TRUE
2:       RBBP4          TRUE
3:       WARS2          TRUE
4:     IGHMBP2          TRUE
5:        KRT3          TRUE
6:        KRT3          TRUE
```

Cleanup of the enviroment

```
rm(all_mutations, all_mut_genes, all_mut_genes_df, ID_all_highest_30_frequencies,
   ID_all_highest_frequencies, all_mut_genes_df_highest_30,
   all_mut_genes_df_highest_30_ordered, colnames_ordered, HNC_mutations,
   HNC_mut_genes, HNC_mut_genes_df, ID_HNC_highest_frequencies,
   HNC_mut_genes_df_highest_ordered)
```

Knockdown

Overview of kd.ceres values in HNC patients

```
sum(is.na(kd.ceres.HNC) == TRUE) # Checking for NAs
```

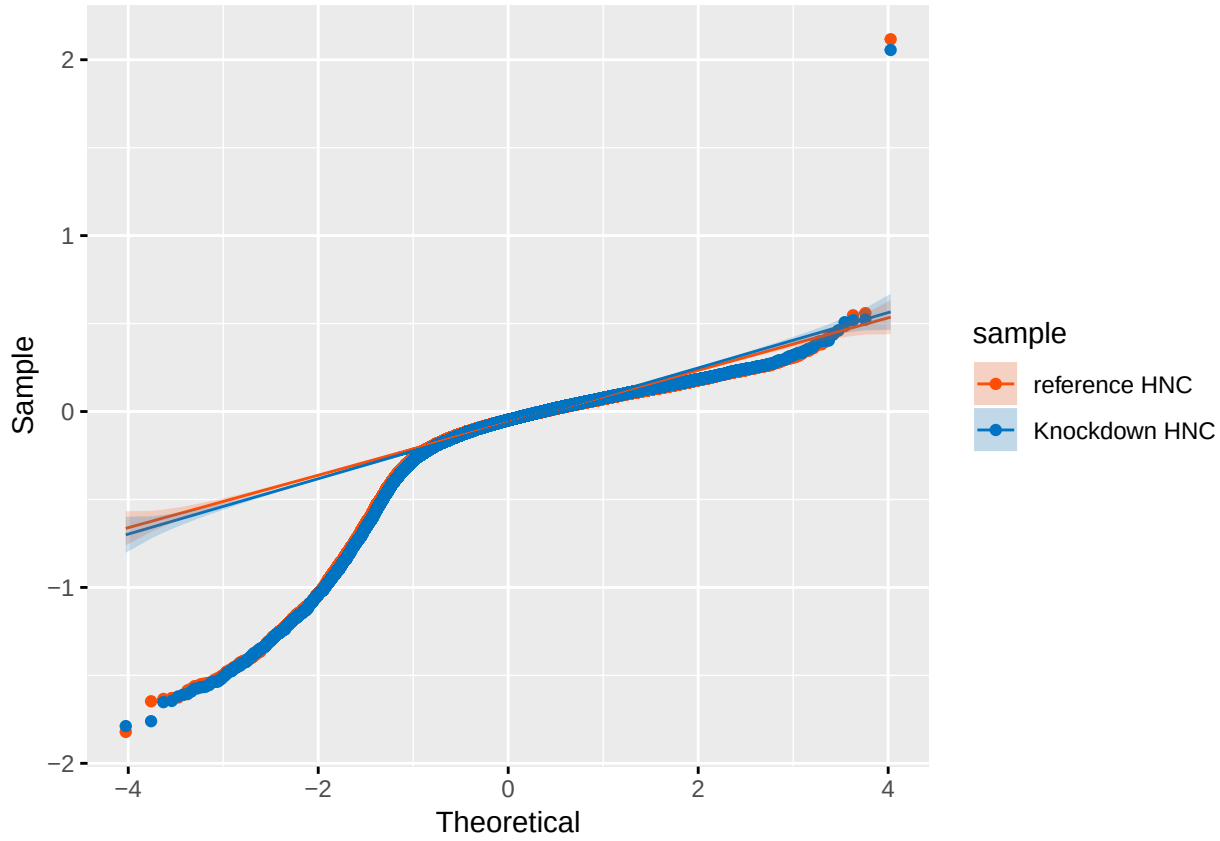
```
[1] 0
```

```
range(kd.ceres.HNC)# Range of data
```

```
[1] -2.615780  2.575905
```

Examine knockdown values

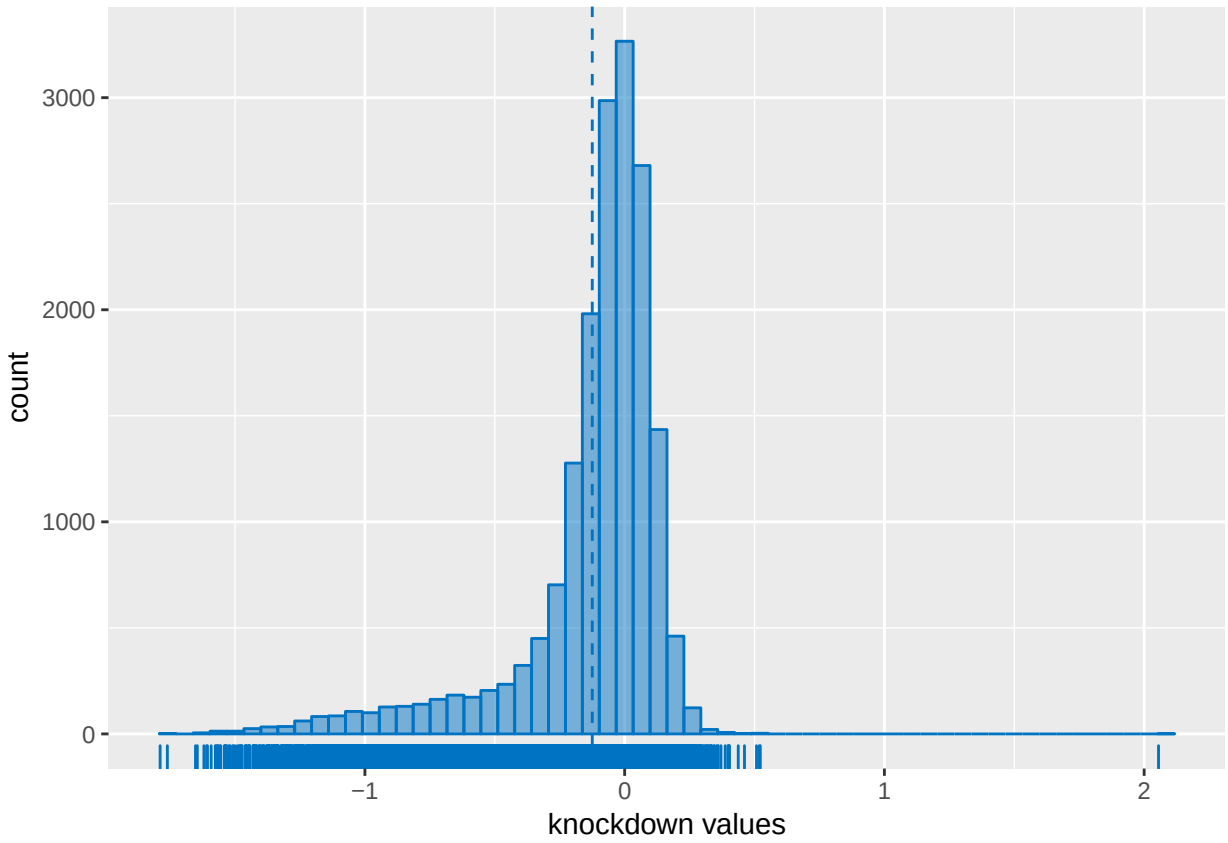
```
# Definition of a compare group
kd.ceres.reference = kd.ceres[ , which(colnames(kd.ceres) %!in% ID)]

# Defining mean of knockdown values from comparegroup and HNC patients
mean.kd.ceres.reference = apply(kd.ceres.reference, 1, mean)
mean.kd.ceres.HNC = apply(kd.ceres.HNC, 1, mean)

# Checking if knockdown values are normally distributed
kd.ceres.HNC.dist = data.frame(kd_values = mean.kd.ceres.HNC, sample = "Knockdown HNC")
kd.ceres.reference.dist = data.frame(kd_values = mean.kd.ceres.reference,
                                     sample = "reference HNC")
mean.kd.ceres.dist = rbind(kd.ceres.reference.dist, kd.ceres.HNC.dist)

ggqqplot(mean.kd.ceres.dist, x = "kd_values",
   color = "sample",
   palette = c("#FC4E07", "#0073C2FF"),
   ggtheme = theme_grey())
```



Result: kd.ceres values are not normal distributed

Checking the distribution for knockdown values

```
gghistogram(kd.ceres.HNC.dist, x = "kd_values", xlab = "knockdown values", bins = 60,
            fill = "#0073C2FF", color = "#0073C2FF",
            add = "mean", rug = TRUE)+theme_grey()
```



To check if there are more essential genes in HNC, the means of HNC patients with the reference group were compared.

```
# Are there genes that tend to be more essential in HNC?
esgene1 = kd.ceres.HNC[which(rowMeans(kd.ceres.HNC)< rowMeans(kd.ceres.reference)),]
# Sort essential genes
esgene1= esgene1[order(rowMeans(esgene1)),]
```

Example EGFR

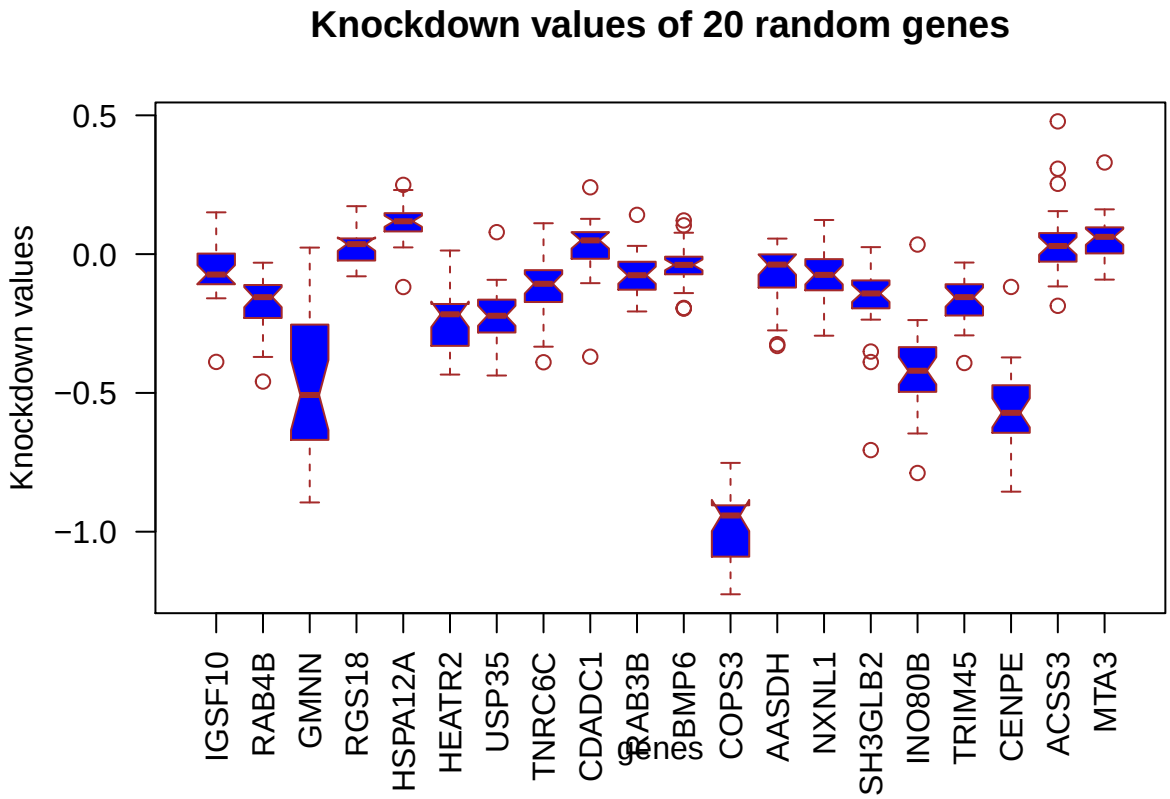
Checking the summary of an example gene

```
# Transposition of dataframe esgene1
esgeneT = as.data.frame(t(esgene1))
summary(esgeneT$EGFR)
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
-0.9636 -0.5200 -0.4221 -0.4097 -0.2866  0.0189
```

Boxplot of 20 random genes

```
# Boxplot of 20 random genes according to knockdown analysis
boxplot(sample(esgeneT,20, replace = FALSE),
        horizontal =F,
        las = 2, #flip gene names
        main="Knockdown values of 20 random genes",
        xlab="genes",
        ylab="Knockdown values",
        col="blue",
        border="brown",
        outline = TRUE,
        notch = TRUE)
abline(col = c("blue", "red", "black", "orange"),
        lty = 2)
```



Applying Wilcoxon rank sum test because knockdown values are not normally distributed

To check if the HNC knockdown values differ from the compare group, a wilcoxon test is executed.

```
wilcox.test(mean.kd.ceres.HNC, mean.kd.ceres.reference)
```

```
    Wilcoxon rank sum test with continuity correction

data:  mean.kd.ceres.HNC and mean.kd.ceres.reference
W = 155160000, p-value = 0.7421
alternative hypothesis: true location shift is not equal to 0
```

The test shows, that ther isn‘t a significiant difference between HNC patients and the reference group. Due to the fact, that knocking down of essential genes has the same effect in healthy cells, than in tumorous cells.

Cleanup of the enviroment

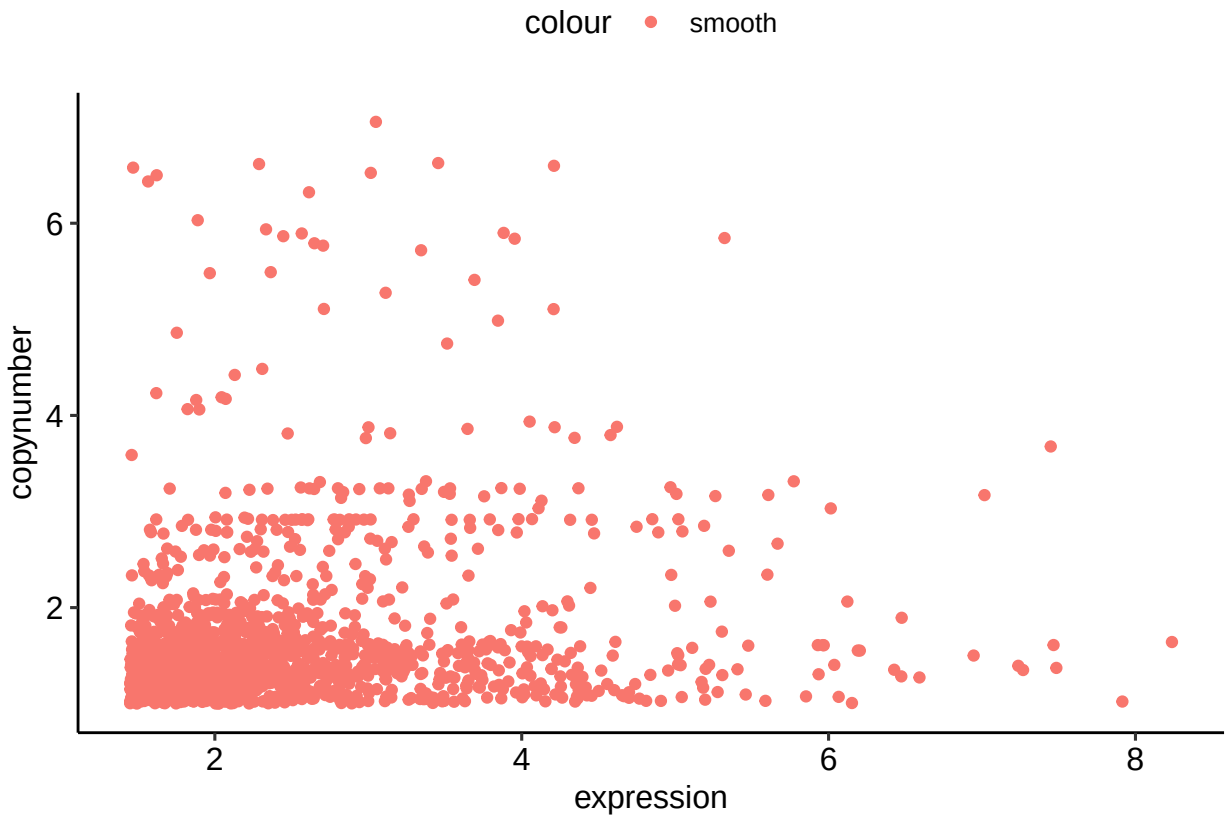
```
rm(kd.ceres.HNC.dist, kd.ceres.reference, kd.ceres.reference.dist, mean.kd.ceres.HNC,
   mean.kd.ceres.reference, mean.kd.ceres.dist, esgene1, esgeneT)
```

2. Identify genes of interest

k-means clustering of CNA and expression data with normalized values (-reference) for SDL GOI

To find potential genes for SDL pair detection, clustering through k-means was executed.

```
# Intersect set of expression and CNA
km.genes = Reduce(intersect, list(rownames(expression.HNC), rownames(copynumber.HNC)))

# Create data rame with expression and CNA values
km.expression = expr.HNC.norm[km.genes,]
km.copynumber = cna.HNC.norm[km.genes,]
km.data = lapply(1:length(ID), function(x) {
  out = data.frame(genes =km.genes,
                   expression = km.expression[,x],
                   copynumber = km.copynumber[,x])
  return (out)
})
# Combine rows for k mean clustering
comb.ex.cna = do.call(rbind, km.data)
comb.ex.cna = comb.ex.cna[which(comb.ex.cna$copynumber >= 1 &
                                  comb.ex.cna$expression >= quantile(comb.ex.cna$expression,0.95)),]

# Plotting of normalized expression valuess against CNA data for a short overview.
ggplot(comb.ex.cna[, 2:3],
       aes(x = expression, y = copynumber, color = "smooth")) +
  geom_point()
```

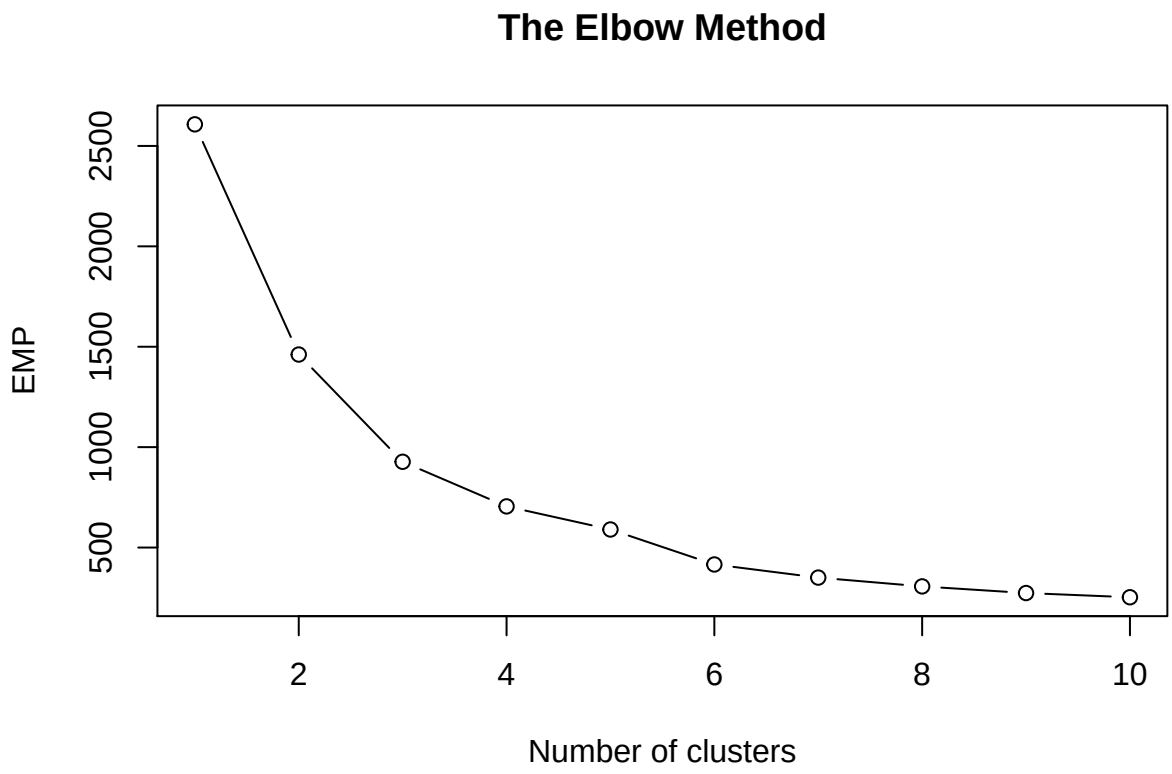


To check the perfect ammount of clusters in k-means, an ellbow test was implemented.

```
# Ellbow method to find optimal ammount of clusters
emp = vector() # Create an empty vector
# Calculate the sum within squared values for 10 clusters
for (i in 1:10) emp[i] = sum(kmeans(comb.ex.cna[2:3], i)$withinss)
emp # Look at the sum within squares for 10 clusters
```

```
 [1] 2607.5505 1461.2845  927.0961  704.8820  589.9574  415.6683  350.3535
 [8]  306.5011  273.3958  252.4910
```

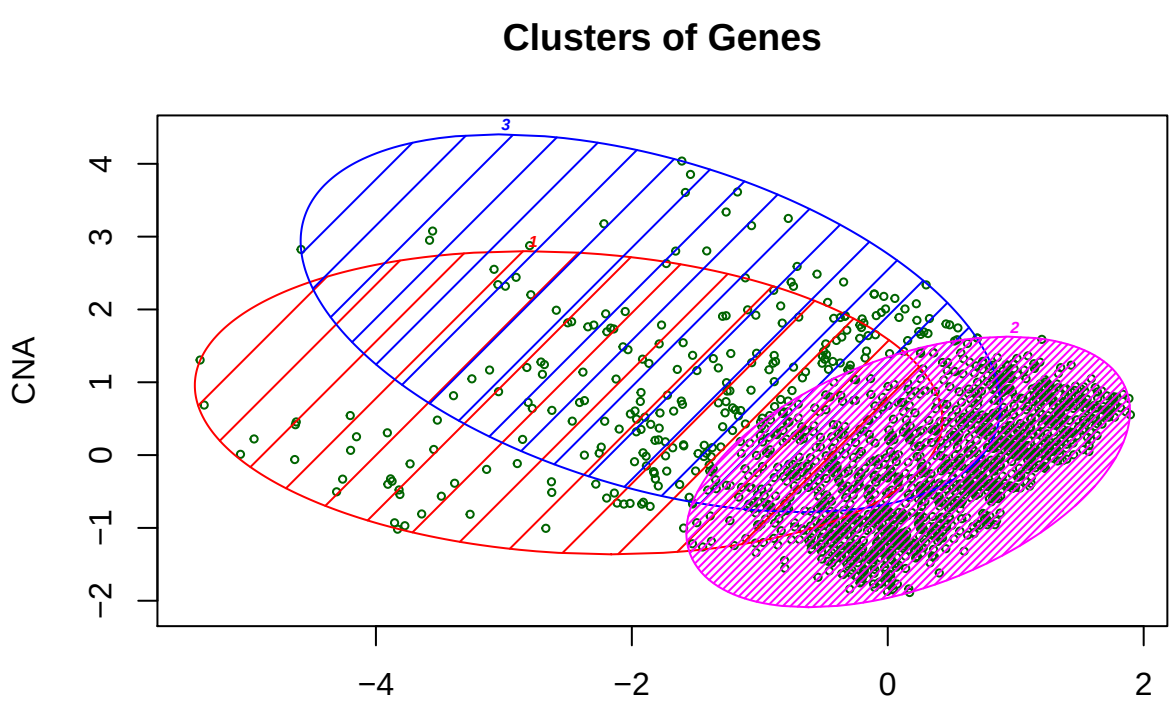
```
# Plotting of Ellbow method
plot(1:10,
     emp,
     type = 'b',
     main = paste('The Elbow Method'),
     xlab = 'Number of clusters',
     ylab = 'EMP')
```



Result: The ellbow test shows that 3 clusters are sufficient.

K-means were executed and plotted. Afterwards the potential SDL candidates were identified through higher expression values.

```
# Fitting k-means to the dataset - elbow method (k = 3)
kmeans = kmeans(x = comb.ex.cna[,2:3], centers = 3,iter.max = 1000, nstart = 1)
y_kmeans = kmeans$cluster

# Visualising the clusters
clusplot(comb.ex.cna,
         y_kmeans,
         lines = 0,
         shade = TRUE,
         color = TRUE,
         labels = 4,
         plotchar = FALSE,
         span = TRUE,
         stand = FALSE,
         cex =0.5,
         main = paste('Clusters of Genes'),
         xlab = 'Expression',
         ylab = 'CNA')
```



The Plot "Cluster of genes" features the 3 clusters of values from expression and CNA data. Due to little variance between the data pairs, the intersection set is comparatively big.

In order to define SDL candidates, the cluster with highest expression values was chosen and filtered again according to expression numbers.

```
comb.ex.cna = cbind(comb.ex.cna, y_kmeans)# Combine kmeans with genes
# Sort comb.ex.cna data decresing for selecting adequate number of cluster
comb.ex.cna = comb.ex.cna[order(comb.ex.cna$expression,decreasing =TRUE),]

# Filtering of the most expressed and alterated genes
goi.sdl = comb.ex.cna[which(comb.ex.cna$y_kmeans == comb.ex.cna[1,4]),]

# Sort dataframe
goi.sdl = goi.sdl[order(goi.sdl$expression,decreasing=TRUE),]
kable(goi.sdl[1:5,])
```

	genes	expression	copynumber	y_kmeans
428012	DSG3	8.237872	1.641760	3
41915	FXYD3	7.914724	1.022479	3
33343	CST6	7.484452	1.372464	3
185157	F3	7.466245	1.610442	3
354894	FOXE1	7.447917	3.676721	3

```r
goi.SDL = as.character(goi.sdl$genes[1:5])
# Clean enviroment
rm(emp, i, km.genes, y_kmeans, kmeans, km.copynumber, km.data, km.expression, comb.ex.cna)
```

Investigate high frequencies of deleterious mutations SL GOI

Create a dataframe with data for the heatmap.

```r
# All mutation data of HNC samples in one dataframe
mutation.HNC.df = do.call(rbind, mutation.HNC)
# Dataframe with all mutated genes in HNC samples just once
heatmap.genes = as.data.frame(table(mutation.HNC.df$Hugo_Symbol))

heatmap.data = as.data.frame(sapply(1:(length(ID)), function(a) {
  # Per HNC sample: every gene which is mutated gets a 1, otherwise a 0
  out = ifelse(heatmap.genes$Var1 %in% mutation.HNC[[a]]$Hugo_Symbol, 1, 0)
  return(out)
}))

# Define row and column names
rownames(heatmap.data) = heatmap.genes$Var1
colnames(heatmap.data) = ID

# Order from high to low frequency
heatmap.data$sum = apply(heatmap.data, 1, sum)
heatmap.data = heatmap.data[order(-heatmap.data$sum), 1:27]

# Heatmap of the most frequently deleterious mutated genes in HNC samples
heatmap(data.matrix(heatmap.data)[1:40,],
        Rowv=NA,
        Colv=NA,
        col = c("lightgrey", "tomato2"),
        scale="column",
        cexRow = 0.8,
        cexCol = 0.8,
        main = "Most frequently deleterious mutated genes in HNC samples")
```

Most frequently deleterious mutated genes in HNC samples

Result: TP53, FAT1, CDKN2A, KRT3 and CASP8 are the 5 most frequently deleterious mutated genes in our HNC samples.

According to Leemans et al. (2018) these are frequent mutations in HNC:

Cellular process	Gene	Protein	Type of gene	Mutation frequency (%)	CNA frequency (%)
Cell cycle	*CDKN2A*	$p16^{INK4A}$	Tumour suppressor	22	32
	TP53	p53	Tumour suppressor	72	1.4
	CCND1	G1–S-specific cyclin D1	Oncogene	0.6	25
Growth signals	*EGFR*	EGFR	Oncogene	4	11
Survival	*PIK3CA*	Catalytic p110α subunit of class 1 PI3Ks	Oncogene	18	21
	PTEN	PTEN	Tumour suppressor	3	4
WNT signalling	*FAT1*	Protocadherin FAT1	Tumour suppressor	23	8
	AJUBA	LIM domain-containing protein AJUBA	Tumour suppressor	7*	1
	NOTCH1	NOTCH1	Tumour suppressor	18	4
Epigenetic regulation	*KMT2D*	Histone-lysine *N*-methyltransferase KMT2D	Tumour suppressor	16	0.4
	NSD1	Histone-lysine *N*-methyltransferase NSD1	Tumour suppressor	12*	0.8

Data from REF. 3. Mutation data were taken from The Cancer Genome Atlas (TCGA) ($n = 504$) using the cBioPortal. CNA, copy number alteration; EGFR, epidermal growth factor receptor. *Putative passenger mutation that requires further functional studies.

Figure 4: Genes with frequent and highly significant somatic genetic changes in HNSCC

Compare our SL GOI with genes from literature.

```
# create dataframe with goi from literature
goi.lit = data.frame(Gene = c("CDKN2A", "TP53", "CCND1", "EGFR", "PIK3CA", "PTEN", "FAT1",
                              "AJUBA", "NOTCH1", "KMT2D", "NSD1"),
                     "Mutation.frequency" = c(22, 72, 0.6, 4, 18, 3, 23, 7, 18, 16, 12),
                     "CNA.frequency" = c(32, 1.4, 25, 11, 21, 4, 8, 1, 4, 0.4, 0.8))

# Search for the position of goi.lit in mutated genes of HNC samples
heatmap_position = sapply(1:nrow(goi.lit), function(a) {
  position = which(rownames(heatmap.data) == goi.lit$Gene[a])
  # If gene is not mutated in HNC samples its position is replaced with NA
  if(length(position) == 0) {position = NA}
  return(position)
})

goi.lit$"Heatmap.position" = heatmap_position # Add heatmap position to goi.lit
rownames(goi.lit) = goi.lit$Gene
goi.lit$Gene = NULL
goi.lit = goi.lit[order(goi.lit$"Heatmap.position"),]
kable(goi.lit) # Show a table of goi.lit
```

	Mutation.frequency	CNA.frequency	Heatmap.position
TP53	72.0	1.4	1
FAT1	23.0	8.0	2
CDKN2A	22.0	32.0	3
AJUBA	7.0	1.0	28
KMT2D	16.0	0.4	66
NOTCH1	18.0	4.0	82
NSD1	12.0	0.8	715
CCND1	0.6	25.0	NA
EGFR	4.0	11.0	NA
PIK3CA	18.0	21.0	NA

	Mutation.frequency	CNA.frequency	Heatmap.position
PTEN	3.0	4.0	NA

Because our most frequent mutated genes aproximately match with frequent mutations from literature we choose the 5 genes with most frequent mutations in HNC samples.

```
goi.SL = rownames(heatmap.data)[1:5]
goi.SL
```

```
[1] "TP53"   "FAT1"   "CDKN2A" "KRT3"   "CASP8"
```

Clean up of the enviroment

```
rm(mutation.HNC.df, heatmap.genes, heatmap.data, heatmap_position)
```

3. Find SL/SDL partner

SL/SDL partner search following the daisy model

To examine gene pairs A and B which fulfill the criteria of SoF and functional examination we performe a Wilcoxon rank sum test on copynumber or CERES knockdown data. We have 5 inactive genes (goi.SL) and 5 overactive genes (goi.SDL) which are defined as genes A. The Wilcoxon rank sum test now returns all genes B which pass the test in a significant manner ($p<0.05$).

```
'Daisy.Wilcox' = function(input_genes,input_data, inactive_overactive, less_greater){
  Daisy_Wilcox = lapply(1:length(input_genes), function(m) {
  goi = input_genes[m] # Set 1 of 5 goi
  # Depending on SL or SDL partner search
  # (1) gene B is overactive (SDL)
  if(inactive_overactive == 1) { # inactive_overactive == 1 chooses overactive
    mean.exp.goi = mean(t(expression[goi,]))
    exp.goi.norm = expression[goi,] - mean.exp.goi # Normalised expression values of goi
    mut_overa.ID = lapply(1:ncol(expression), function(a) {
      # Filter all IDs which are overactive: overexpressed and CNA > 0,3
      out = if(exp.goi.norm[goi, a] > 0 & copynumber[goi,a] > 0.3) {a} else {NA}
      return(out)
    })
  # (2) gene B is inactive (SL)
  } else { # inactive_overactive == 0 chooses inactive
    mut_overa.ID = lapply(1:length(mutation), function(a) {
      # Filter all IDs which have a mutation of goi
      out = ifelse(goi %in% mutation[[a]]$Hugo_Symbol, a, NA)
      return(out)
    })
  }

  # ID of samples which have NO inactivity/overactivity of goi
  not_mut_overa.ID = grep("NA", mut_overa.ID)
  # ID of samples which HAVE inactivity/overactivity of goi
  mut_overa.ID = as.integer(mut_overa.ID[is.na(mut_overa.ID) == FALSE])

  p.value = sapply(1:nrow(input_data), function(b) {
    mut_overa.data = t(input_data[b, mut_overa.ID])
    not_mut_overa.data = t(input_data[b, not_mut_overa.ID])
    if (less_greater == 1) {
      # One sided (greater) Wilcoxon Sum Rank Test
      p = wilcox.test(mut_overa.data, not_mut_overa.data, alternative = "greater")$p.value
    } else {
      # One sided (greater) Wilcoxon Sum Rank Test
      p = wilcox.test(mut_overa.data, not_mut_overa.data, alternative = "less")$p.value
    }

  })

  out = data.frame(genes = rownames(input_data), p_value = p.value)
  out = out[which(out$p_value < 0.05),]
  return(out)
  })
```

```
  names(Daisy_Wilcox) = input_genes
  return(Daisy_Wilcox)
}
```

To examine gene pairs A and B which fulfill the criteria of gene co expresssion we performe a Spearman correlation test on the expression data. We have 5 inactive genes (goi.SL) and 5 overactive genes (goi.SDL) which are defined as genes A The correlation test now returns all genes B that correlate to gene A in a significant manner ($p<0.05$).

```
'Daisy.Spearman' = function(input_genes){
  # Build up a list with the 5 goi; lapply: goes through the code for each gene A
  Daisy_Spearman = lapply(1:length(input_genes), function(m) {
  goi = input_genes[m]

  # Creation of a list with the length of the expression data
  # Go through every gene and calculate cor.test
  p.value = sapply(1:nrow(expression), function(a){
    expression.goi =t(expression[goi,]) # Expressiondata of the goi (gene A)
    expression.GeneB =t(expression[a,]) # Expressiondata of all other genes (gene B)
    # One sided spearman correlation test
    p = cor.test(expression.goi,expression.GeneB, alternative = "greater",
                 method = "spearman", exact = FALSE)$p.value
  })

  # Return a dataframe with the columns genes(HugoSymbols) and p_values (p.values)
  out = data.frame(genes = rownames(expression), p_value = p.value)
  out = out[which(out$p_value < 0.05),] # Filter for significant p.values
  # Only keep genes wich are not goi (would be a corr of 1)
  out = out[which(out$genes != goi),]
  return(out)
  })
  names(Daisy_Spearman) = input_genes
  return(Daisy_Spearman)
}
```

Both functions return a list, which contains the five genes of interest, each containing a dataframe with all genes examined as potential SL/SDL partners plus their calculated p-values.

SL-parter

SoF

```
# SoF, inactive, one sided Wilcoxon rank test, alternative = "greater"
SL.SoF = Daisy.Wilcox(goi.SL, copynumber,
                      inactive_overactive = 0,
                      less_greater = 1)
# Example of SL SoF for first GOI (TP53)
head(SL.SoF[[1]])
```

```
        genes    p_value
10    NAALAD2 0.01270171
88   NAALADL1 0.01787355
```

```
105    MCTS2P 0.01801226
109  SNORD119 0.03415298
120 SNORD111B 0.04370297
148    MIR875 0.02447163
```

Functional examination

```
# Functional examination, inactive, one sided Wilcoxon rank test, alternative = "less"
SL.functional.examination = Daisy.Wilcox(goi.SL, kd.ceres,
                                         inactive_overactive = 0,
                                         less_greater = 0)
# Example of SL functional examination for first GOI (TP53)
head(SL.functional.examination[[1]])
```

```
     genes     p_value
10   AADAC 0.008677123
21    AAR2 0.016419751
70   ABCG1 0.010374932
100   ABL2 0.042951952
149   ACN9 0.006751253
211 ACTR1B 0.010514096
```

Gene coexpression

```
SL.coexpression = Daisy.Spearman(goi.SL)
# Example of SL coexpression for first GOI (TP53)
head(SL.coexpression[[1]])
```

```
      genes      p_value
4     SCYL3 7.196232e-06
5  C1orf112 2.516883e-04
10     NFYA 2.641665e-09
13    LAS1L 3.844908e-02
17   ANKIB1 1.345544e-02
24     CD99 8.535496e-03
```

SDL-parter

SoF

```
# SoF, overactive, one sided Wilcoxon rank test, alternative = "greater"
SDL.SoF = Daisy.Wilcox(goi.SDL, copynumber,
                       inactive_overactive = 1,
                       less_greater = 1)
```

Functional examination

```
# Functional examination, overactive, one sided Wilcoxon rank test, alternative = "less"
SDL.functional.examination = Daisy.Wilcox(goi.SDL, kd.ceres,
                                          inactive_overactive = 1,
                                          less_greater = 0)
```

Gene coexpression

```
SDL.coexpression = Daisy.Spearman(goi.SDL)
```

Converging the different SL and SDL sets

Define function to converge SL/SDL sets.

```
'partner' = function(input_genes, SoF, functional.examination, coexpression){
  partner = lapply(1:length(input_genes), function(a) {
    dat_picker.SoF = SoF[[a]]
    dat_picker.func.exam = functional.examination[[a]]
    dat_picker.coexpression = coexpression[[a]]
    # Intersect of genes from all three tests
    partnergenes = Reduce(intersect, list(dat_picker.SoF$genes,
                                          dat_picker.func.exam$genes,
                                          dat_picker.coexpression$genes))
    out = data.frame(
      genes = partnergenes,
      # Column with reduced genes
      SoF = dat_picker.SoF[which(dat_picker.SoF$genes %in% partnergenes), "p_value"],
      functional_examination = dat_picker.func.exam[which(dat_picker.func.exam$genes
                                                          %in% partnergenes), "p_value"],
      coexpression = dat_picker.coexpression[which(dat_picker.coexpression$genes
                                                   %in% partnergenes), "p_value"]
    ) # Column with p value from coexpression
    return(out)
  })
  names(partner) = input_genes
  return(partner)
}
```

Converge different sets.

```
SL.partner = partner(goi.SL, SL.SoF, SL.functional.examination, SL.coexpression)
SDL.partner = partner(goi.SDL, SDL.SoF, SDL.functional.examination, SDL.coexpression)
kable(data.frame("SL genes" = names(SL.partner),
           "SL number of partner" = sapply(1:length(SL.partner), function(a){
             nrow(SL.partner[[a]])
             }),
           "SDL genes" = names(SDL.partner),
           "SDL number of partner" = sapply(1:length(SDL.partner), function(a){
             nrow(SDL.partner[[a]])
             })
           ))
```

SL.genes	SL.number.of.partner	SDL.genes	SDL.number.of.partner
TP53	21	DSG3	96
FAT1	54	FXYD3	78
CDKN2A	60	CST6	232
KRT3	124	F3	78
CASP8	22	FOXE1	136

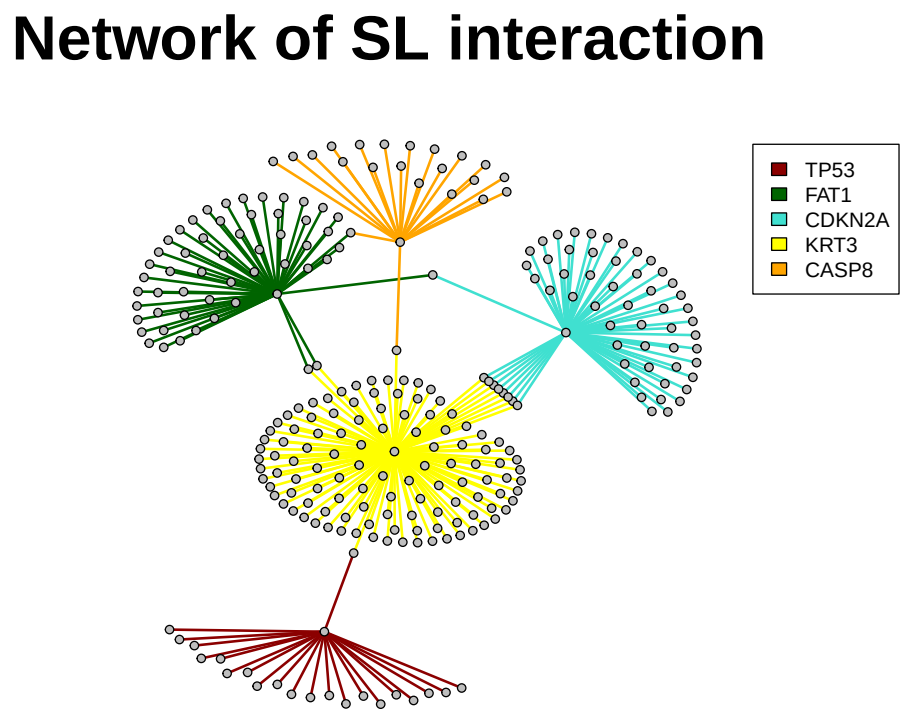
Plotting networks of the Interactions

Creation of a function for transforming given data into a format iGraph can work with and plotting of the network.

```
'network' = function(X.partner,width,node.color,frame.color,node.size,Titel,label,GOI){
  # Input: SL/SDL.partner, number, string, string, number, string, NA or names(plot)

  # Define colours
  color.vector = c("darkred", "darkgreen", "turquoise", "yellow", "orange")
  # Transform data into a dataframe with 3 colums for each GOI
  edgelist = lapply(1:length(X.partner), function(a){
    dat_picker = X.partner[[a]]
    edge.list = data.frame(Gene.A = names(X.partner)[a],
                           Gene.B = dat_picker$genes,
                           colours = color.vector[a])
  })
  edge.list = do.call(rbind, edgelist) # Combine all goi-dataframes to one.

  # Change column colours from factor to chracter for plotting function
  edge.list$colours = as.character(edge.list$colours)

  # Tranform the dataframe into an iGraph object
  net = graph_from_data_frame(edge.list, directed = T)
  layout1 = layout_with_kk(net) # Given layout

  # Plotting parameters are defined. vertex= Genes edges = interactions
  plot(net, layout = layout1, rescale = T,
       ylim = c(-0.9,1),
       xlim = c(-0.1,-0.1),
       edge.color = edge.list$colours,
       edge.width = width,
       edge.arrow.mode = 0,
       vertex.color = node.color,
       vertex.frame.color = frame.color,
       vertex.size = node.size,
       vertex.label.font = 2,
       vertex.label.color = "black",
       vertex.label = label,
       margin = 0.1)

  # Add legend to the graph
  legend (x = 1.2,
          y = 1, GOI,
          fill = c("darkred", "darkgreen", "turquoise", "yellow", "orange"))

  # Add titel to the graph
  title(Titel,cex.main=3,col.main="black ")

}
```

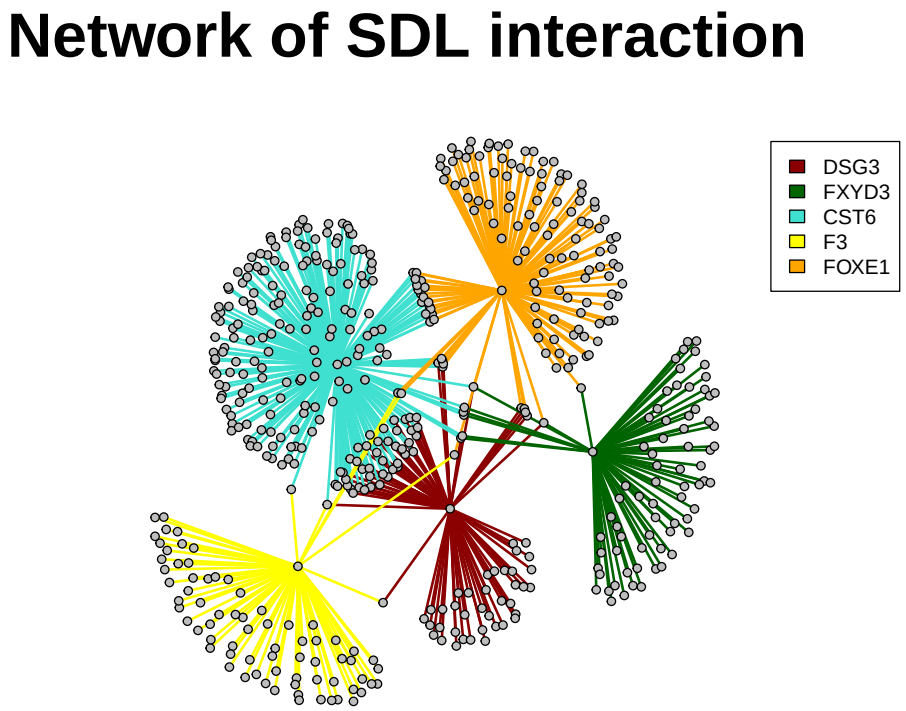
Creating a networkplot for the SL-partner

```
network(SL.partner,2,"grey","black",3,"Network of SL interaction", NA, goi.SL )
```



Creating a network for the SDL-partner

```
network(SDL.partner,2,"grey","black",3,"Network of SDL interaction", NA, goi.SDL )
```



Because there are to many SL/SDL partner to plot a network we reduce SL/SDL partner to the most significants.

To do so, we reduce the number of partner genes for better plotting without removing genes which are connected to more than one GOI.

```
'reduce.partner' = function(list){
  # Go through every element of SL/SDL partner
  reduced_partner = lapply(1:length(list), function(a){
    dat_picker = list[[a]]

    # Create an extra column with the mean p value of all 3 tests
    dat_picker$order = apply(dat_picker[, 2:4], 1, mean)
    # Order the partner genes after this mean value from most to least significant p value
    ordered_data = dat_picker[order(dat_picker$order), ][, 1:4]

    gene_intersect = lapply(1:length(list), function(b) {
      # Search for genes that are partner for more than one GOI
      as.list(Reduce(intersect, list(dat_picker$genes), list[[b]]$genes))
    })
    gene_intersect[a] = NULL # Remove intersect of the GOI partner genes with itself
    keep_genes = c(gene_intersect[[1]],
```

```
                    gene_intersect[[2]],
                    gene_intersect[[3]],
                    gene_intersect[[4]]) # Combine all the genes in a list
    position = sapply(1:length(keep_genes), function(c) {
      which(as.character(ordered_data$genes) == keep_genes[c])
    })
    # Partner genes that are already in the first 20 genes don´t need to be chosen
    position = position[which(position > 20)]

    if (length(position) > 3) {
      # If there are still more than 3 extra genes only choose the first 3
      position = position[1:3]
    }
    out = ordered_data[c(1:20, position), ]
    return(out) # Return reduced data
  })
  names(reduced_partner) = names(list)
  return(reduced_partner)
}
```

Reduce SL.parter/SDL.partner to the most significant

```
SL.partner.reduced = reduce.partner(SL.partner)
SDL.partner.reduced = reduce.partner(SDL.partner)

kable(data.frame("SL genes" = names(SL.partner),
            "SL partner" = sapply(1:length(SL.partner.reduced), function(a){
              nrow(SL.partner.reduced[[a]])
              }),
            "SDL genes" = names(SDL.partner),
            "SDL partner" = sapply(1:length(SDL.partner.reduced), function(a){
              nrow(SDL.partner.reduced[[a]])
              })
            ))
```

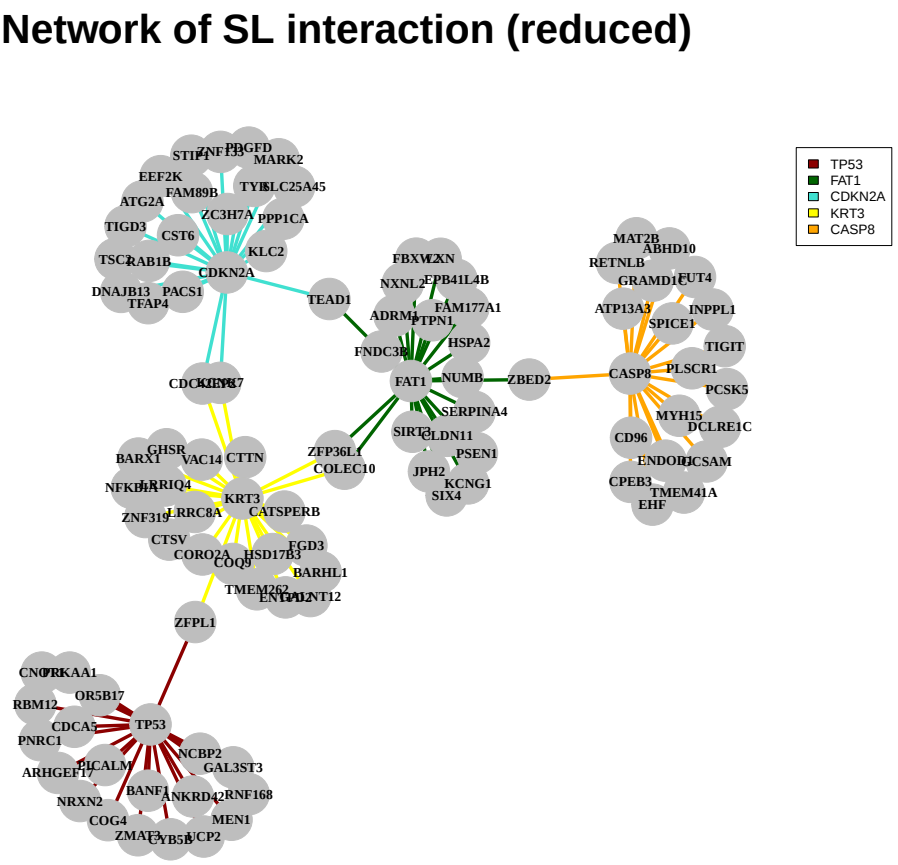
SL.genes	SL.partner	SDL.genes	SDL.partner
TP53	20	DSG3	23
FAT1	21	FXYD3	23
CDKN2A	22	CST6	23
KRT3	23	F3	23
CASP8	20	FOXE1	23

Now the number of SL/SDL partner is much better to plot.

Plotting SL/SDL partner network with reduced number of partner

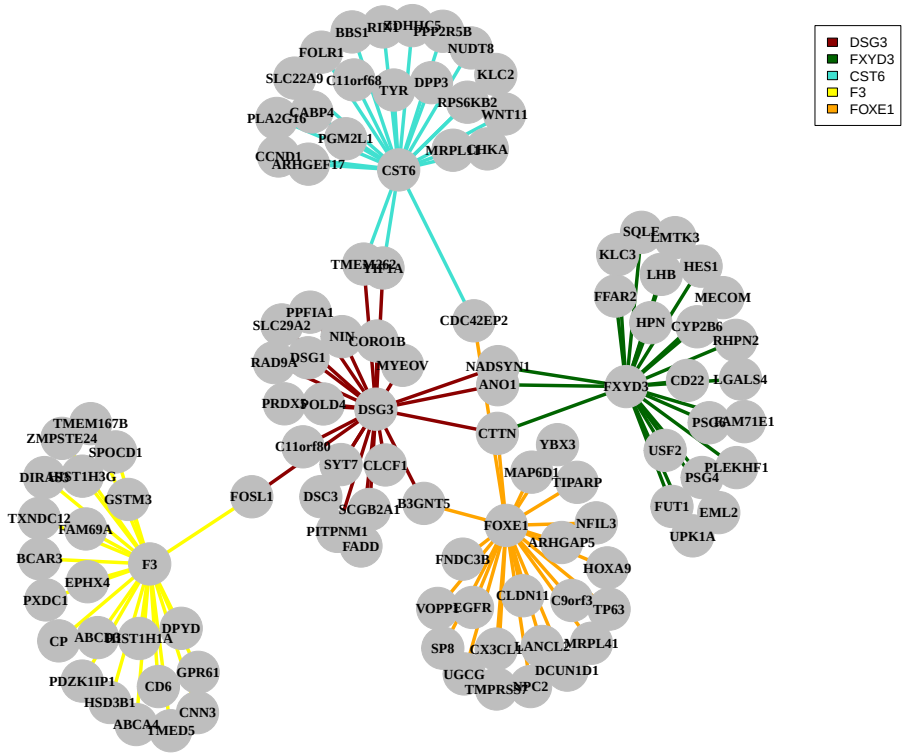
SL interaction plot with reduced partners

```
network(SL.partner.reduced,4,"grey","grey",12,"Network of SL interaction (reduced)",
        names(plot),
        goi.SL)
```



SDL interaction plot with reduced partners

```
network(SDL.partner.reduced,4,"grey","grey",12,"Network of SDL interaction (reduced)",
        names(plot),
        goi.SDL)
```



In Conclusion, the search for potential SL and SDL partner genes led us to our two main networks depicted above. Most of the found SL/SDL partners play crucial roles in key steps of cell development, for example like fostering transcription and translation or directly influencing the cell organization. This explains why the tested tumor cells died after a knockdown/knockout of those genes and why they became essential for the cells.

4. Mutation prediction via logistic regression

Prepare data for regression model

```
# Reduce all genes to those of which we have expression, copynumber and knockdown data.
regression.genes = Reduce(intersect,
                          list(rownames(expression.HNC),
                               rownames(copynumber.HNC),
                               rownames(kd.ceres.HNC)
                          ))

# Create a dataframe with expression, CNA, knockdown and mutation data from a HNC sample.
regression.data = lapply(1:length(ID), function(a){

  regression.mutation = sapply(1:length(regression.genes), function(b){
    ifelse(regression.genes[b] %in% mutation.HNC[[a]]$Hugo_Symbol, TRUE, FALSE)
  })

  regression.data = data.frame("genes" = paste(regression.genes, ID[a], sep = "_"),
                               "expression" = expression.HNC[regression.genes, a],
                               "copynumber" = copynumber.HNC[regression.genes, a],
                               "kockdown" = kd.ceres.HNC[regression.genes, a],
                               "mutation" = regression.mutation)
})

# Bind these 27 dataframes to one dataframe
regression.data = do.call(rbind, regression.data)

dim(regression.data)
```

```
[1] 458190      5
```

```
head(regression.data)
```

```
                genes expression copynumber    kockdown mutation
1     DPM1_ACH-000188  5.3593103     0.1574 -0.42737583    FALSE
2    SCYL3_ACH-000188  1.2570106     0.1441 -0.01577348    FALSE
3 C1orf112_ACH-000188  3.1093606     0.1441 -0.03149540    FALSE
4      FGR_ACH-000188  0.1634987     0.2019 -0.10972076    FALSE
5      CFH_ACH-000188  4.0285692    -0.0637  0.03189585    FALSE
6    FUCA2_ACH-000188  4.6536333     0.1872  0.02271554    FALSE
```

Reformat the data because strings can not be an input for a machine learning model

```
regression.data$mutation = factor(regression.data$mutation, levels = c("FALSE", "TRUE"))
# Reformat the rownames
rownames(regression.data) = regression.data$genes
# Get rid of the gene names becaue they are now the rownames
regression.data = regression.data[,2:ncol(regression.data)]
head(regression.data)
```

```
                    expression copynumber    kockdown mutation
DPM1_ACH-000188      5.3593103     0.1574 -0.42737583    FALSE
SCYL3_ACH-000188     1.2570106     0.1441 -0.01577348    FALSE
C1orf112_ACH-000188  3.1093606     0.1441 -0.03149540    FALSE
FGR_ACH-000188       0.1634987     0.2019 -0.10972076    FALSE
CFH_ACH-000188       4.0285692    -0.0637  0.03189585    FALSE
FUCA2_ACH-000188     4.6536333     0.1872  0.02271554    FALSE
```

Split the data into train and test-data

```
# 75% of data is used to train, 25% to test the regression model
inTrain = createDataPartition(y = regression.data$mutation, p = .75, list = FALSE)
# Only get training data
train.set = regression.data[inTrain,]
head(train.set)
```

```
                  expression copynumber    kockdown mutation
DPM1_ACH-000188   5.3593103     0.1574 -0.42737583    FALSE
SCYL3_ACH-000188  1.2570106     0.1441 -0.01577348    FALSE
FGR_ACH-000188    0.1634987     0.2019 -0.10972076    FALSE
CFH_ACH-000188    4.0285692    -0.0637  0.03189585    FALSE
FUCA2_ACH-000188  4.6536333     0.1872  0.02271554    FALSE
GCLC_ACH-000188   4.1497471     0.1549  0.06840387    FALSE
```

```
# Only get testing data
test.set = regression.data[-inTrain,]
head(test.set)
```

```
                    expression copynumber    kockdown mutation
C1orf112_ACH-000188   3.109361     0.1441 -0.03149540    FALSE
KRIT1_ACH-000188      3.524816    -0.2043  0.02438860    FALSE
LAP3_ACH-000188       5.096346    -0.7520 -0.18606702    FALSE
HS3ST1_ACH-000188     2.217231    -0.7520  0.01858038    FALSE
HECW1_ACH-000188      1.673556     0.5658  0.06333549    FALSE
MAD1L1_ACH-000188     4.816600     0.6233  0.06533135    FALSE
```

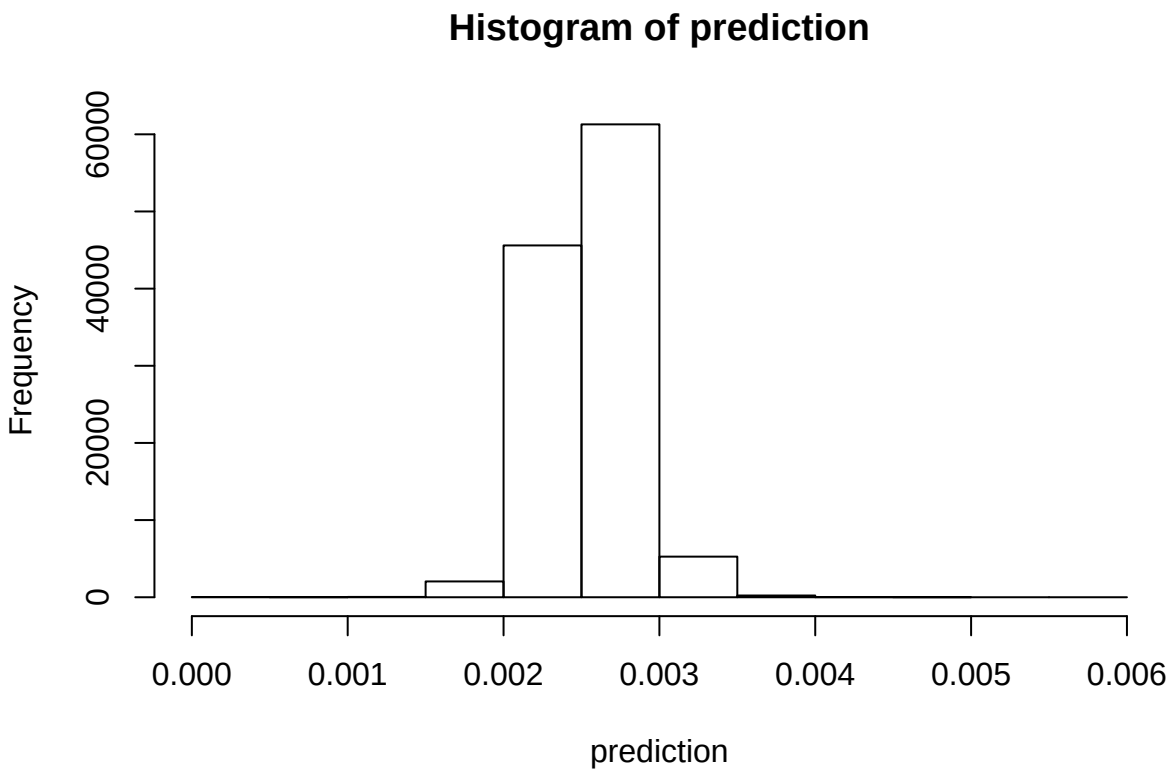
Train the model

```
regression.model = glm(mutation ~ ., data = train.set, family = "binomial")
summary(regression.model)
```

```
Call:
glm(formula = mutation ~ ., family = "binomial", data = train.set)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.1018  -0.0737  -0.0714  -0.0693   3.5951

Coefficients:
            Estimate Std. Error  z value Pr(>|z|)
(Intercept) -6.00532    0.05068 -118.501   <2e-16 ***
expression   0.02607    0.01488    1.752   0.0797 .
copynumber   0.13156    0.08002    1.644   0.1002
kockdown     0.28073    0.12449    2.255   0.0241 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 12249  on 343642  degrees of freedom
Residual deviance: 12239  on 343639  degrees of freedom
AIC: 12247

Number of Fisher Scoring iterations: 9
```

Evaluate the precision and the recall of the model

```
prediction = predict(regression.model, newdata = test.set, type="response")
hist(prediction) # Look at the predictions
```



Devide prediction in threshholds

```
threshhold_ls = seq(min(prediction), max(prediction), by = 0.001) # Make a thresshold list
length(threshhold_ls)
```

```
[1] 6
```

```
threshold_list = lapply(1:length(threshhold_ls), function(a){
  # Check for every threshhold:
  table(prediction > threshhold_ls[a], # Returns TRUE or FALSE
        test.set$mutation) # Comparing if return fits actual bimodal value
  # Counting True Negative, False Negative, False Positive, True Positive
  })

threshold_list[[1]]
```

```
       FALSE   TRUE
  FALSE     1      0
  TRUE  114253    293
```

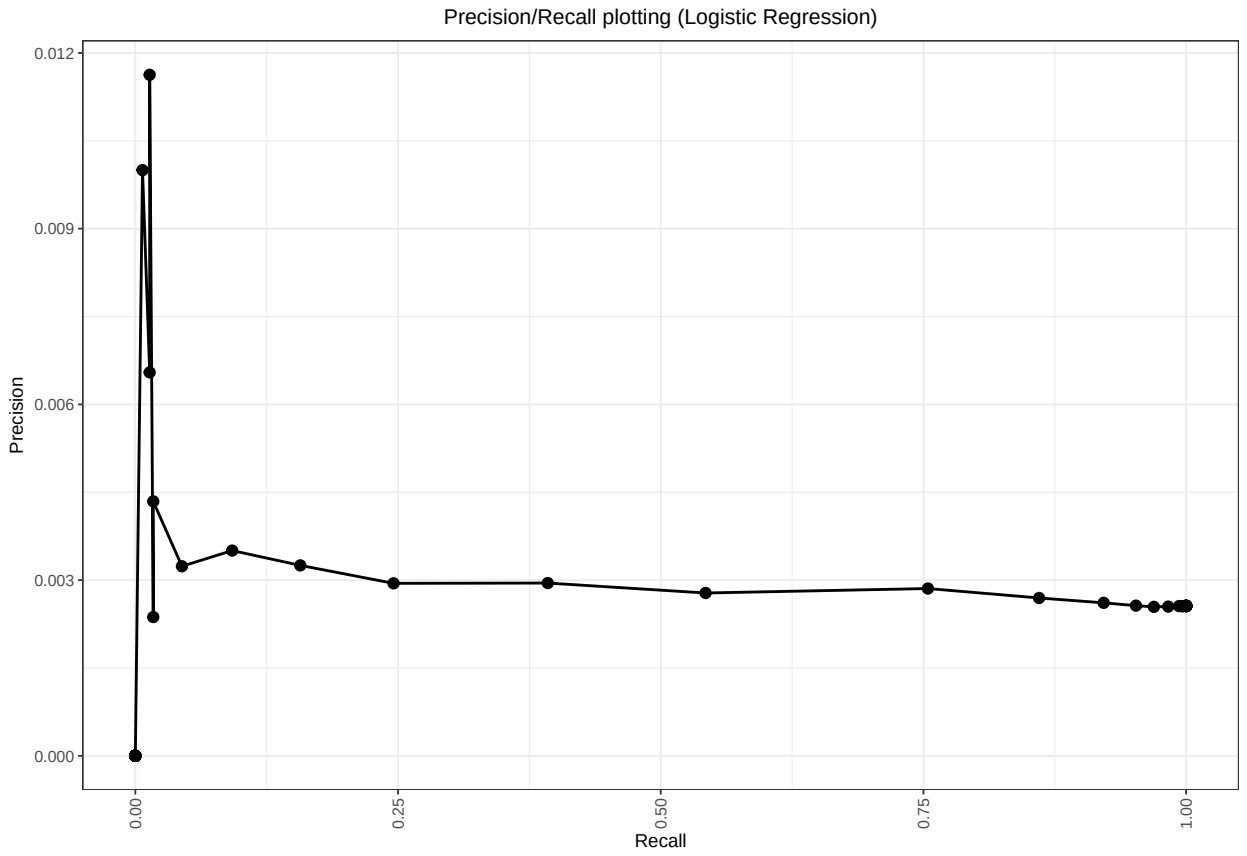
Calculate the recall and precision values

```
# Make a dataframe out of the list of lists
results_df = lapply(1:length(threshold_list), function(a) {
  threshold = threshold_list[[a]]
  threshold_value = threshhold_ls[[a]]

  df_precision_recall = as.data.frame(list(threshold["FALSE", "FALSE"],
                                           threshold["FALSE", "TRUE"],
                                           threshold["TRUE", "FALSE"],
                                           threshold["TRUE", "TRUE"]))
  df_precision_recall$threshold = threshold_value
  colnames(df_precision_recall) = c("TN", "FN", "FP", "TP", "Thresh")
  return(df_precision_recall)
})

# Bind all the lists together
results_df = do.call(rbind, results_df)

# Calculate the recall; recall = True positive / all positive events
recall = apply(results_df, 1, function(x){ x[["TP"]] / (x[["TP"]]+x[["FN"]]) })

# Calculate the precision; precision = True positive / all positive detected events
precision = apply(results_df, 1, function(x){ x[["TP"]] / (x[["TP"]]+x[["FP"]]) })

results_df$recall = recall # Bind recall to the data
results_df$precision = precision # Bind precision to the data
head(results_df) # Look at the data
```

```
  TN FN     FP  TP       Thresh recall   precision
1  1  0 114253 293 4.752206e-05      1 0.002557924
2 19  0 114235 293 1.475221e-04      1 0.002558326
3 22  0 114232 293 2.475221e-04      1 0.002558393
4 28  0 114226 293 3.475221e-04      1 0.002558527
5 30  0 114224 293 4.475221e-04      1 0.002558572
6 31  0 114223 293 5.475221e-04      1 0.002558594
```

Plot the data

```
ggplot(results_df, aes(recall,precision)) +
  geom_point() +
  geom_line() +
  ggtitle("Precision/Recall plotting (Logistic Regression)") +
  ylab("Precision") +
  xlab("Recall") +
  theme_bw(base_size = 7) +
  theme(legend.position="bottom",
        legend.direction="horizontal",
        plot.title = element_text(hjust = 0.5),
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1),
        legend.title= element_blank(),
        strip.text.y = element_text(angle = 0))
```



Looking on the precision it can be said that our logistic regression model for prediction of deleterious mutation in head and neck cancer scores very poorly. To be just in about 0,3% of all cases precise with the prediction is an unusuable model and has plenty of room for improvement. Especially regarding to declining precision towards a recall of 1.00, which is a true mutation and a result on which basis further decisions would be drawn.

Clean the enviroment

```
rm(precision, prediction, recall, regression.genes, threshhold_ls, inTrain,
   regression.model, results_df, test.set, threshold_list, train.set)
```

References

Articles

1.) Jerby-Arnon, L., et al. (2014). "Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality." Cell 158(5): 1199-1209.

2.) Ashworth, A., et al. (2011). "Genetic Interactions in Cancer Progression and Treatment." Cell 145(1): 30-38.

3.) O'Neil, N. J., et al. (2017). "Synthetic lethality and cancer." Nature Reviews Genetics 18: 613.

Figures

Figure 1 - https://crukcambridgecentre.org.uk/patient-care/clinical-research/head-and-neck

Figure 2 - Jerby-Arnon, L., et al. (2014). "Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality." Cell 158(5): 1199-1209.

Figure 4 - Leemans, C. R. et al. (2018). The molecular landscape of head and neck cancer. Nat. Rev. Cancer 18, 269-282.