

Presentation Project Head and Neck Cancer

Nils Mechtel, Tobias Hub, Niklas Urbanek, Pascal Poc

24. July 2019

Predicting Cancer-Specific Vulnerability via DAISY System

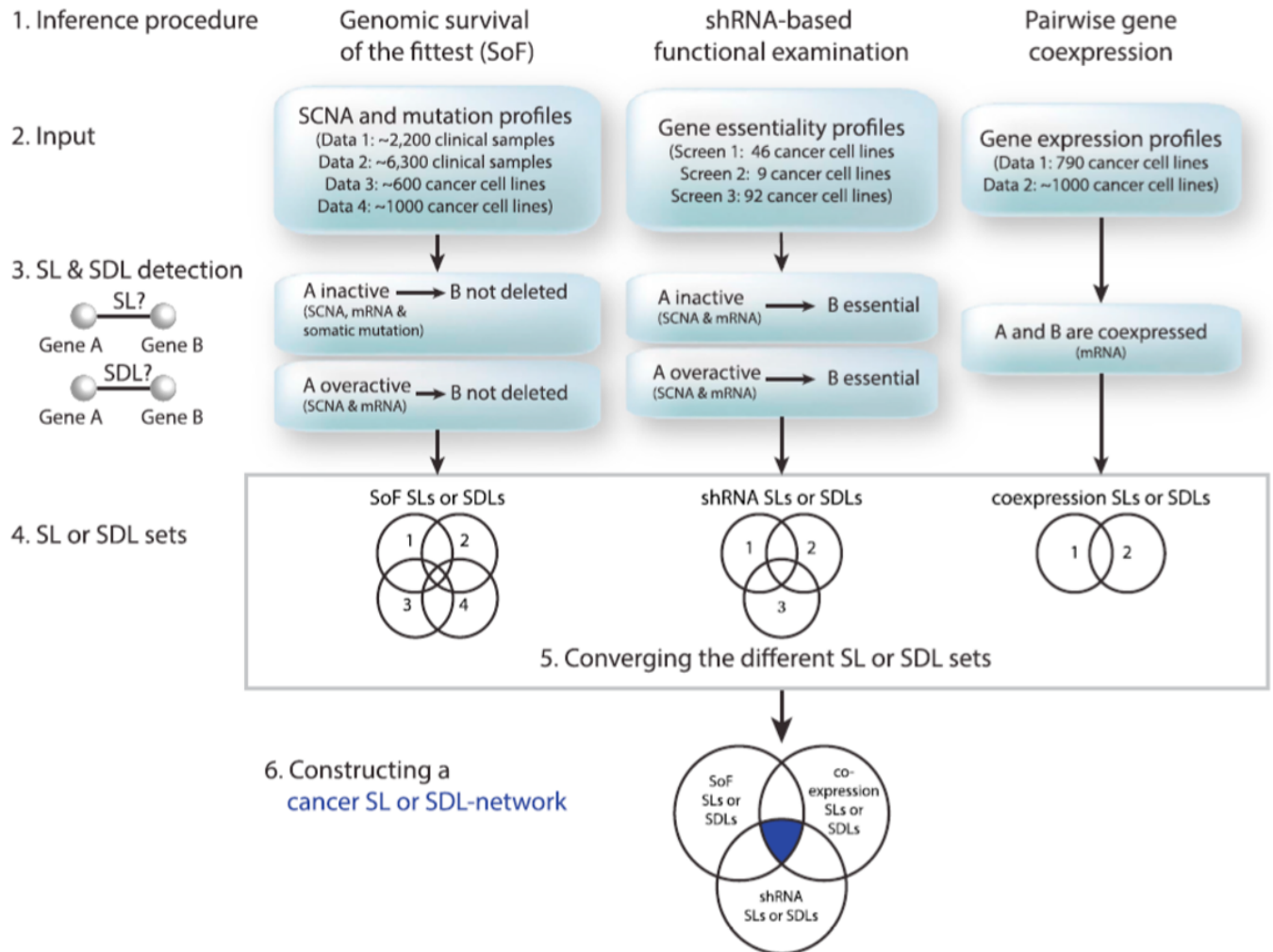


Figure 1: Daisy-Workflow

Milestones

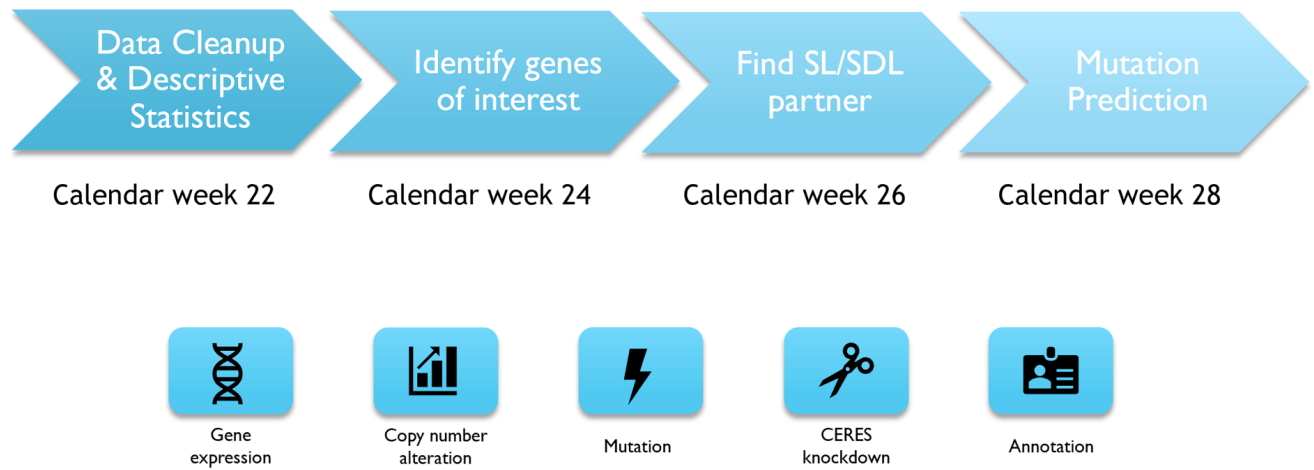


Figure 2: Milestones of our project

Identify genes of interest

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

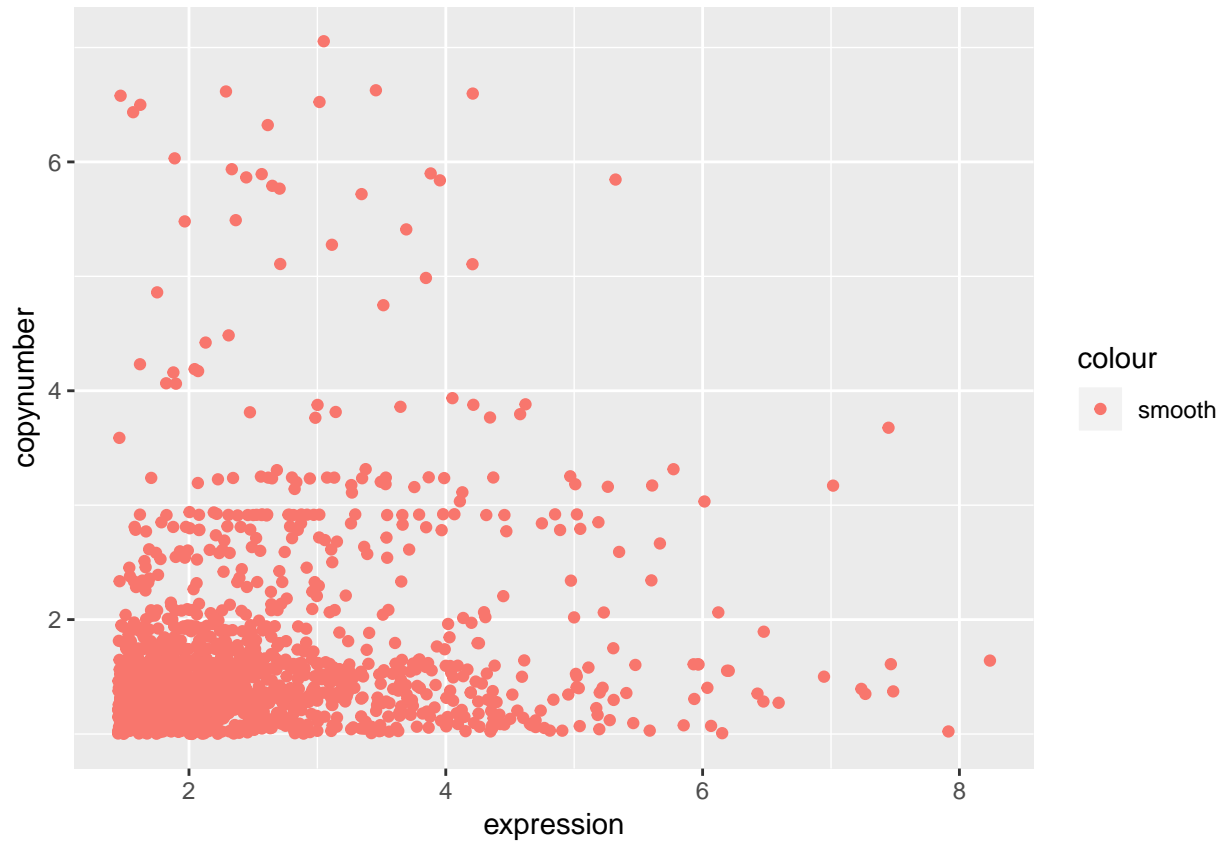
To find potential genes for SDL pair detection, clustering through k-means was executed.

```
# 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()
```



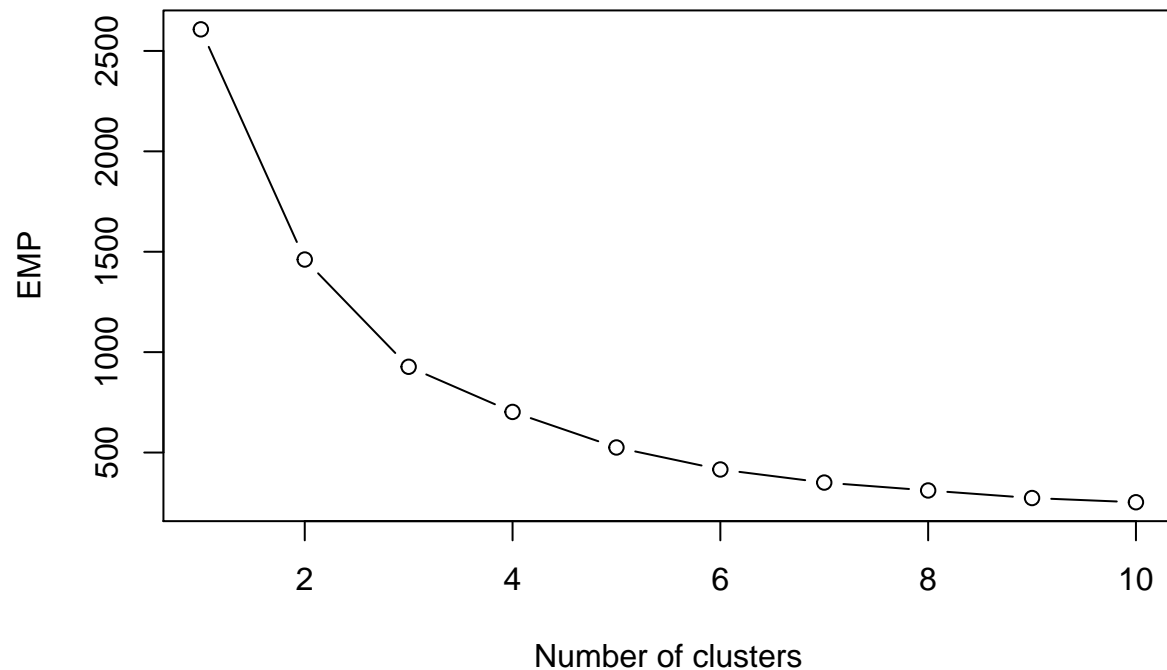
To check the perfect ammount of clusters in k-means, an ellbow test was implemented.

```
# 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 702.1999 525.1874 415.6683 350.4419
[8] 311.1543 273.4154 252.4910
```

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

The Elbow Method

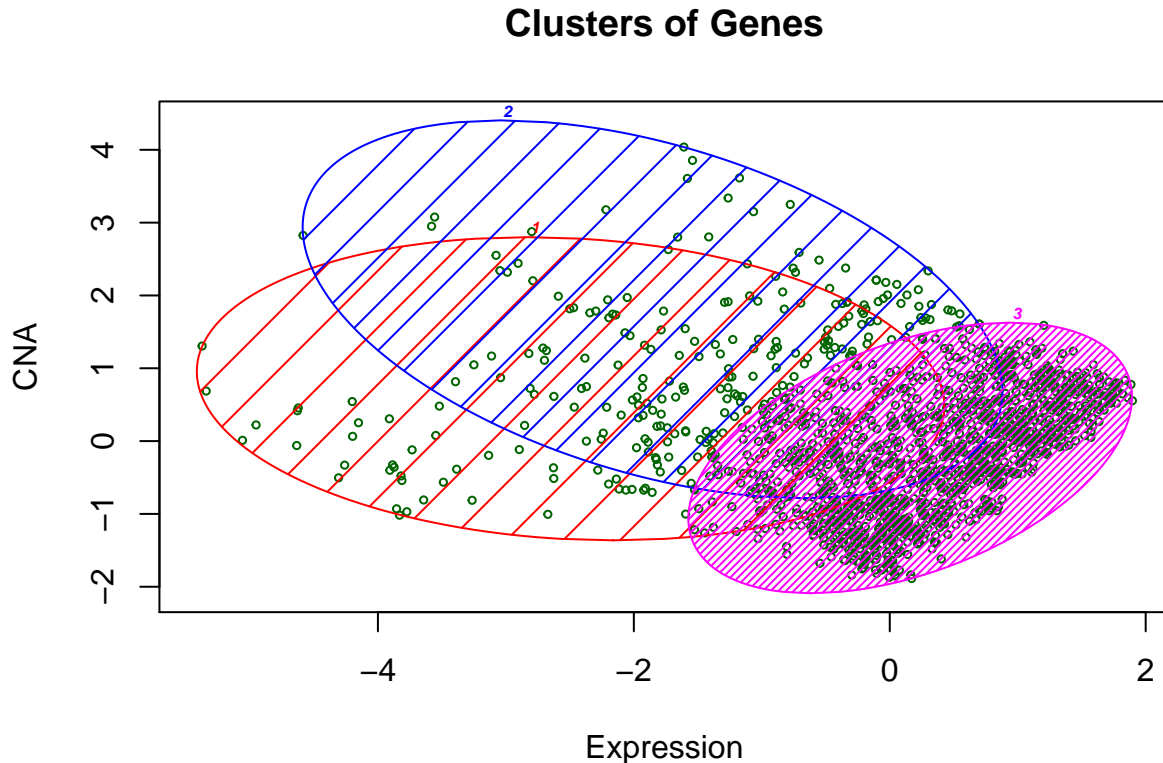


Result: The elbow test shows that 3 clusters are sufficient.

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')
```



These two components explain 72.39 % of the point variability.

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 decreasing 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 altered 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	2
41915	FXYD3	7.914724	1.022479	2
33343	CST6	7.484452	1.372464	2
185157	F3	7.466245	1.610442	2
354894	FOXE1	7.447917	3.676721	2

```

goi.SDL = as.character(goi.sdl$genes[1:5])
# Clean environment
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.

```

# 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 3: 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 almost approximately 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)
```

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 perform 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 expression 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

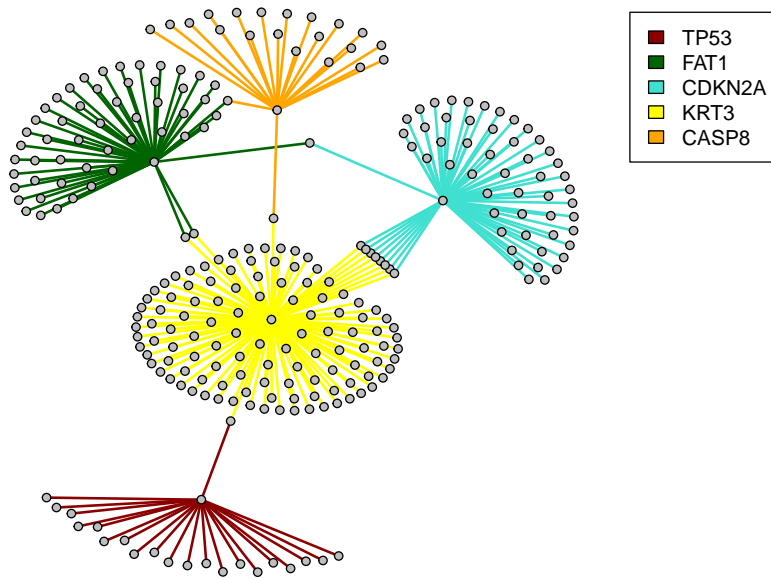
Creation of a function for transforming given data into a format iGraph can work with and plotting of the network.

```
'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 )
```

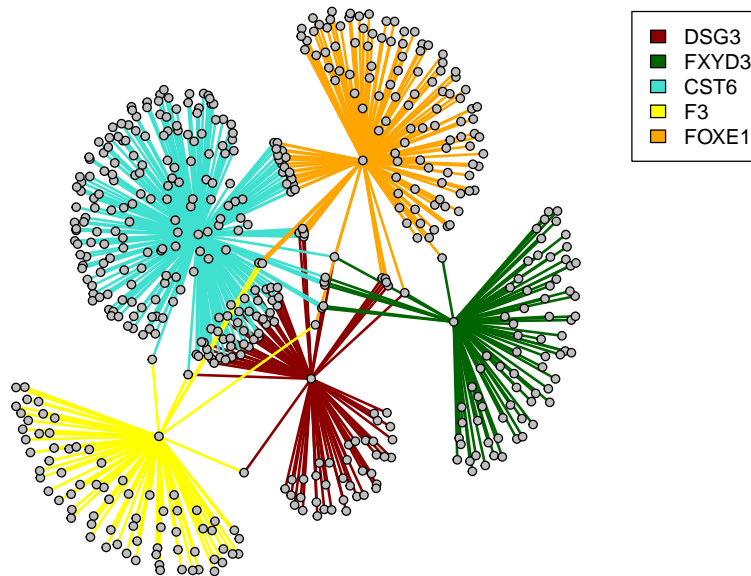
Network of SL interaction



Creating a network for the SDL-partner

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

Network of SDL interaction



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

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.partner/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

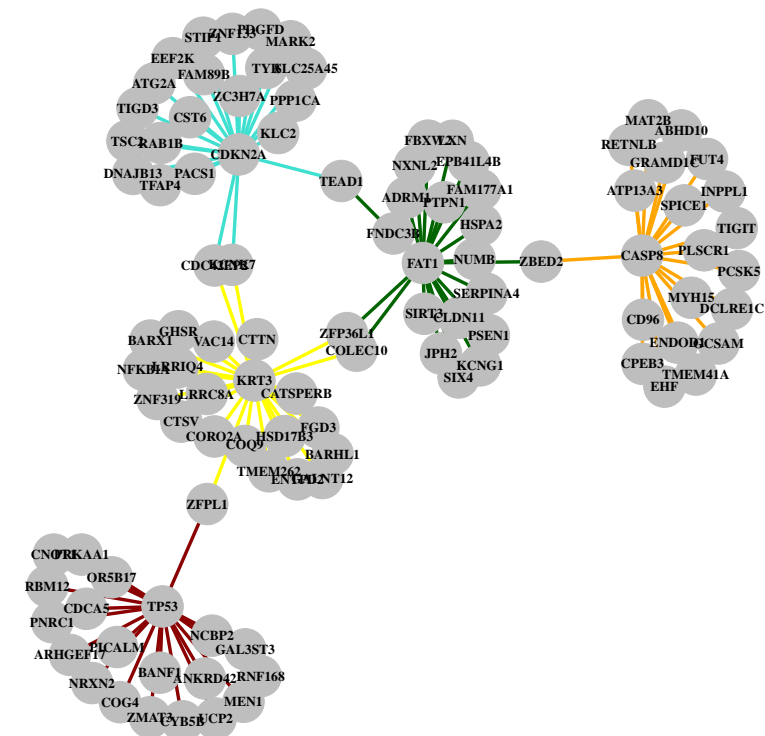
Now the number of SL/SDL partner is much better to plot.

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)
```

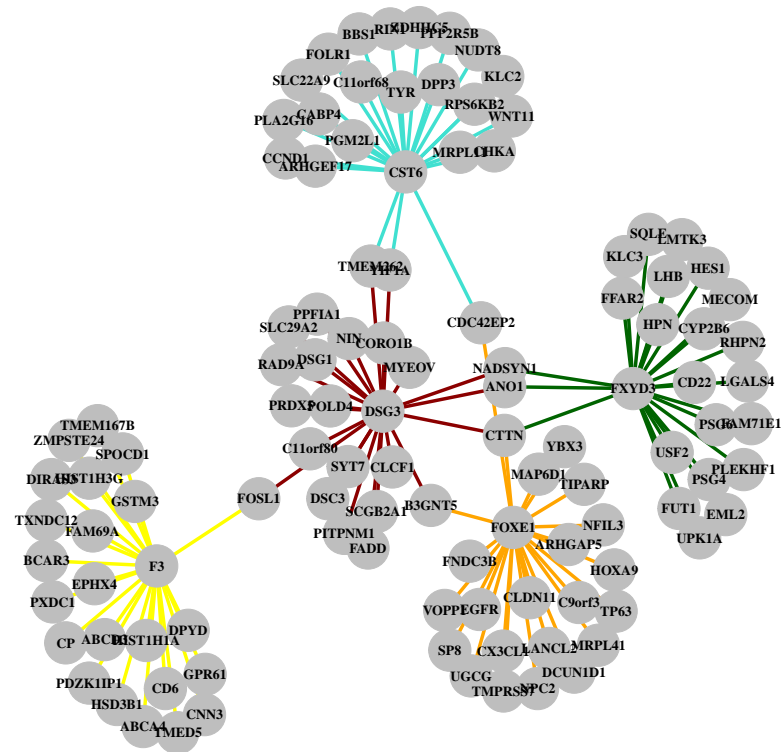
Network of SL interaction (reduced)



SDL interaction plot with reduced partners

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

Network of SDL interaction (reduced)



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

Figure 3 - Leemans, C. R. et al. (2018). The molecular landscape of head and neck cancer. *Nat. Rev. Cancer* 18, 269-282.