Research Article

Which Gene Combination to Test in Wet Lab? A Pedagogical Walkthrough of R Code Mechanics of ML-Based Search Engine for Biologists/Oncologists

Shriprakash Sinha[1]

1. Independent researcher

Background: In biology/oncology, one is faced with the problem of exploring relevant unknown biological hypotheses in the form of a myriad of combinations of factors that might be affecting the pathway under certain conditions. If discovered, these are potential breakthroughs that could help understand the mechanism of cell biology, leading to scientific discoveries and therapeutic interventions. Currently, a major persisting problem is to cherry-pick the combinations based on expert advice, literature survey, or guesses for investigation. This entails investment in time, energy, and expenses at various levels of research.

Results: To address these issues, a search engine design was recently published, which showed promise by revealing existing confirmatory published wet lab results. Additionally, and of import, an adaptation of the published engine mined up a range of unexplored/untested/unknown combinations of genetic factors in the cell signaling pathways that were affected by ETC-1922159 enantiomer, a PORCN-WNT inhibitor, after the colorectal cancer cells were treated with the drug.

Conclusions: Here, a pedagogical walkthrough of the R code of the machine learning-based search engine is elucidated. This will help biologists/oncologists to locate gene combinations ranked/revealed by using the advanced machine learning-based search engine, instead of wandering in a vast combinatorial forest and later testing the combinations of choice in a wet lab. The article ends with an example of a ranking of a 3^{rd} order combination that has recently been established in another wet lab experiment.

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1. Insight, innovation, and integration

Which gene combination to test in the wet lab? This is a fundamental problem that biologists/oncologists face in their search for potential breakthroughs that could help understand the mechanism of cell biology, leading to scientific discoveries and therapeutic interventions. To address this issue, an elucidation of an adaptation of a machine learning-based search engine is provided. The manuscript explains the R code using an example of static data generated from colorectal cancer (CRC) cells that were treated with ETC-1922159.

2. Why use this code?

In my limited opinion, the following goals can be achieved using the published work -

1. Biologists/oncologists can download the available code in R (and with support from any personnel having R programming experience) and apply it to their data sets to rank/prioritize unknown combinations of genes/proteins which might be working synergistically in a pathway.
2. Because of 1, biologists/oncologists will not have to struggle to search for combinations of interest. The rankings of the combinations shed light on how the combinations can be searched/located. Probably, this work will make life easier.
3. Based on these rankings, the modifications of the work will help in writing grant proposals for testing machine learning-based discoveries. This will also help biologists/oncologists get financial support for their research.
4. Combinations of any order can be generated and ranked. However, computational resources might be required, and the search engine might have to be fine-tuned.
5. Finally, the work might help answer many questions in cell biology. Though experimental tests need to be conducted on the discoveries made by applying the above machine learning-based pipeline.

3. Introduction

I developed a search engine to rank/prioritize unknown/unexplored combinations of genes that might be working synergistically in a signaling pathway[1]. Also, the foundation of this work is based on sensitivity indices. The use of these indices to study when and which genetic factor will have a greater influence on the pathway has been published in[2]. In order to understand the significance of the

solution proposed to the problem of combinatorial search that biologists face in revealing unknown biological search problems, these works are of importance.

This manuscript explains the sequence of the code in the pipeline that constitutes the search engine. Note that the pipeline is generic in nature and can be modified, and here we present a possible solution to the combinatorial problem. This is to resolve the issue of finding potential combinations which might be working synergistically. These combinations are addressed as biological hypotheses. We will also address the issues in the paper as we move through the code and point out openings where the scientific community can work to refine the pipeline. Instead of considering these openings as loopholes, interested parties could tune/refine the pipeline as per their requirement. Currently, the code is broadly divided into three main parts that execute the following - • preprocessing and extraction of data • generation of sensitivity indices on measurements from the data, and • ranking of the sensitivity indices. However, for a more professionalized version, the pipeline can be divided into smaller independent modules. The schematic diagram of the pipeline is represented in figure 1. Also, the code in R is presented, and the coding is explained where necessary. Note that an explanation for the working of any particular package that has been used in the pipeline will not be provided. Instead, references will be provided for these packages or executable files.

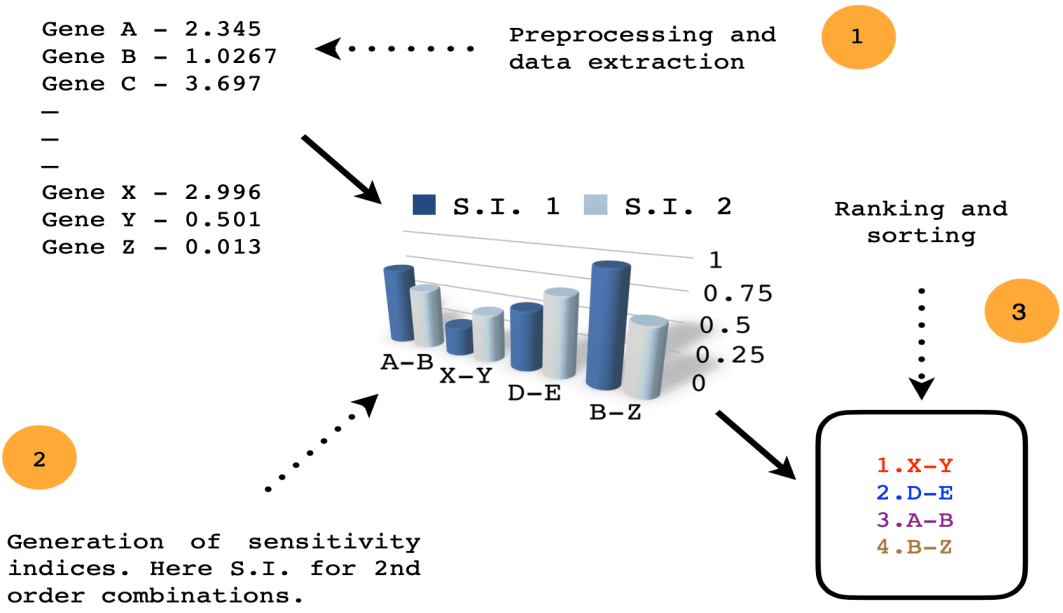

Figure 1. Schematic view of the pipeline. Execution begins with preprocessing and extraction of data, followed by generation of sensitivity indices and culminating in ranking and sorting of the indices and the associated combinations. See steps 1., 2. and 3. Figure from Sinha[1]

4. Source of data

4.1. Description

Data used in this research work was released in a publication by[3]. The ETC-1922159 was released in Singapore in July 2015 under the flagship of the Agency for Science, Technology and Research (A*STAR) and Duke-National University of Singapore Graduate Medical School (Duke-NUS). Note that the ETC-1922159 data show numerical point measurements that is as[3] quote - "List of differentially expressed genes identified at three days after the start of ETC-159 treatment of colorectal tumors. Log2 fold-changes between untreated (vehicle, VEH) and ETC-159 treated (ETC) tumors are reported." The numerical point measurements of differentially expressed genes were recorded using the following formulation of fold changes in equation 1 (see[4][5] and[6]).

$$\log_2 \frac{VEH_{avg}}{ETC_{avg}}$$

4.2. Why choose this data?

The recordings of the gene regulations have been done by[3] in the supplementary table 1. These recordings indicate (up and down) regulation of some 5000 genes which were made available after the drug was tested on colorectal cancer (CRC) cells. Also, each recording is individual. But it is known to everyone in the field of biology/oncology that genes/proteins work in combinations. This work does nothing new or creative or modifies the data, however, it reveals/ranks these gene combinations (whether tested experimentally or yet to be explored/tested) that might be working synergistically, using an adaptation of a published machine learning-based search engine (see[1]). Thus, it solves an important problem of discovering which gene combinations to test in a wet lab, in a vast combinatorial search forest, via the use of real-life data. These point to the efficacy and potential of the search engine. The engine is effective for ranking combinations of any gene of choice and any order of interest.

I tested the modification of the search engine (in[1]) and discovered various 2^{nd} order combinations of genes that might affect various pathways after the drug was administered. **A few of the unpublished results of the work were shared in the first Wnt Signaling: A Pathway Implicated in Animal Development, Stem Cell Control and Cancer (Wnt Gordon Research Conference) in 2017, from 6-11 August, held in Stowe, Vermont 05672, USA**.

5. Steps of execution via code elucidation

Fonts used for different constituents of the code - • variables in file and arguments in *italics*; • file names in sans serif; • functions in **bold**; • R code is in verbatim.

5.1. Preprocessing of ETC-1922159 data

We begin with the first step of the search engine by processing the data that has been provided in[3]. Note that the data had to be manually preprocessed in order to store it in a desired format in a file (here a .txt file). Since the file contained a list of both down and up regulated genes, it was necessary to segregate them into two files. A snapshot of the manually preprocessed file is shown in Figure 2. In this file, the down regulated genes affected by ETC-1922159 have been stored. The orange boundary in Figure 2 contains the file header with different columns separated by a delimiter, here, the "+" symbol (see the magnification in blue). The columns include the titles • *GeneSymbol* or the name of the

abbreviated name of the gene, • *ENSEMBLgeneID* (not used in this code), • *GeneDescription* containing a short detail of the gene, • *log2foldchange(VEH/ETC)* which represents the numerical point measurement, and *BH-adjustedP-value* which represents the changes in gene expression that were considered significant if the Benjamini-Hochberg adjusted P-value <0.0001 (not used in this code). An instance of the tuple in the orange boundary is depicted by specific recorded values for the instance tuple in the red boundary. So, of particular interest would be gene MCM4 and its recorded fold change value of 3.03, from the red boundary; however, before we do that, we need to put the stored information in the .txt file in a particular format for further processing. After manual processing, we stored the list of down and up regulated files in the following two files `onc2015280x2-A.txt` and `onc2015280x2-B.txt`, respectively.

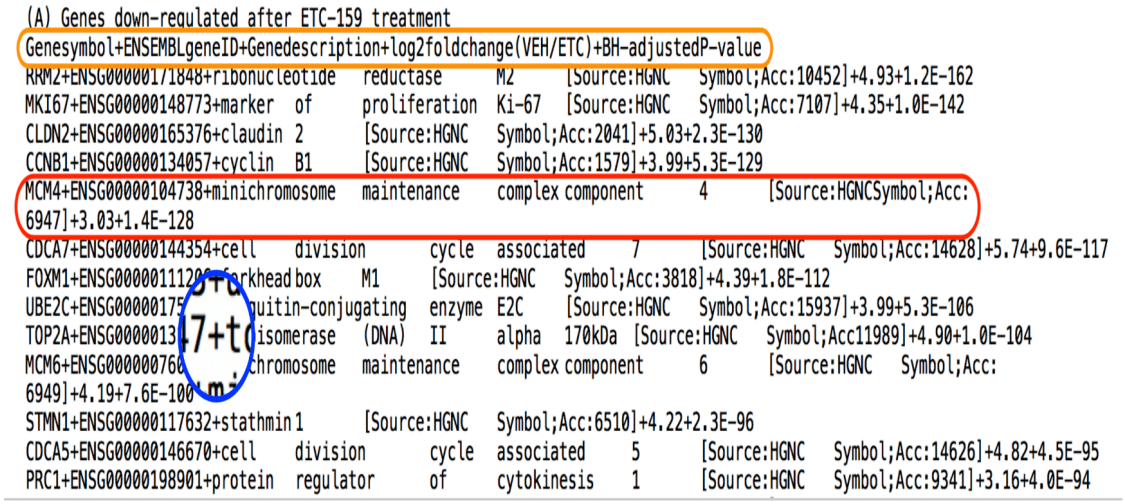

Figure 2. A snapshot of the manually processed file. In this file, the down regulated genes affected by ETC-1922159 have been stored. The orange boundary contains the file header with different columns separated by a delimiter, here, the "+" symbol.

5.2. Extraction of ETC-1922159 data

Once the data has been stored in the required format after manual preprocessing, the next step involves the extraction of data from these files and the storage of the information in a requisite format. This is done using the file `extractETCdata.R` which contains the function **extractETCdata**. We begin with the explanation of the code in a sequential manner below.

5.2.1. Description of extractETCdata.R

The function **extractETCdata** takes in the argument with the name *data.type* that is a numerical value. Here, if the *data type* $= 1(2)$ then the name of the file containing down(up) regulated genes is stored in the *filename*. The **if** condition is used for assigning the file name to *filename* with the condition as argument *data.type* $== 1$ (See lines 1-7 below).

```
1 extractETCdata <- function(data.type){
2   if(data.type == 1){
3    filename <- "../data/onc2015280x2-A.txt"
4   }else{
5    filename <- "../data/onc2015280x2-B.txt"
6   }
7
```

Next, the total number of lines needs to be known once the file to be worked on has been decided. This is required in order to know the number of entries in the file, which gives the list of genes. The processing begins with the **system** command, which executes Unix utilities like **wc** or word count with an option to count lines *l*. We use the option of *intern* to capture the output of the command **wc** in the output (see line 9 below). The output from the command is stored in *lineCnt*. This output is in the form of a string, and the goal is to know the line count from this string. To proceed, the **strsplit** function is used in order to break up the string in *lineCnt* into atomic elements. Further, since the output of the **strsplit** is in the form of a list, to simplify the data structure, **unlist** is used to produce a vector of atomic elements with an argument **strsplit**(*lineCnt*, " "). So here, the output of **strsplit**(*lineCnt*, " ") goes as an argument in the function **unlist**. The output of **unlist**, which is a vector, is stored in *x* (see line 10 below). Next, to extract the numerical value of the number of lines in the file, a **for** command is run, where the iterator *i* takes in one element of the vector in *x* at a time (see line 11 below) and tests if it is a numeric value. If a numeric value is found, the **for** loop breaks; else, it continues with the next element of *x* in the iterator. So, for every value of *x* in *i*, **if** the value in *i* as numeric is not found to be true or is missing/not available, the iterator moves to the next element of *x*. Else, if the value in *i* as numeric is found to be true in the **if** condition, then this value is assigned to *nLines*, and the execution breaks out of the **for** loop (see lines 11-14).

```
8   # find total number of lines in the file
9   lineCnt <- system(command=paste("wc -l ",
            filename,sep=""),intern=TRUE)
10  x <- unlist(strsplit(lineCnt," "))
11  for(i in x){
12   if(is.na(as.numeric(i))){}
13   else{nLines <- as.numeric(i); break;}
14  }
15
```

Once we know the number of lines in the file, we proceed to extract the information in the file, line by line. Lines 20-84 below contain the part of the code that will extract the information from the file. However, due to the length of the code, the explanation is broken up into parts. The entire code for extracting the information is contained in the block within the **while** loop, which starts from line 20. The **while** runs until a condition is no longer true. Before that, the file needs to be opened for processing. This is done using the **file** command, which takes in *filename* as the argument for the variable *description* and *r* to read, as the argument for the variable *open*. This opens the connection for the file of interest, and the connection to the file is stored in a variable *connecTion*. We also set the variable *cnt* to 0 as an iterator for the **while** loop that needs to execute as long as the condition *cnt* $\leq$ *nLines* as an argument to the **while** is met (see line 20). If the condition in line 20 is not met, the execution exits the **while** loop block.

Once inside, the condition is satisfied, and the counter is incremented by 1, stating that the first line is being read. This is indicated by the updated *cnt* value in line 21. Next, the cnt^{th} line is read from the file using the function **readLines**, which takes as arguments the value in *connecTion* and *n* (as 1 to read only one line). The output is the cnt^{th} line that is stored in *sentence*. For processing purposes, the sentence is concatenated with a return character. However, if the cnt^{th} line is the first line, then the loop just skips it and jumps to the next line in the file (see lines 16-24).

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```
16 # extraction information from the file
17  connecTion <- file(description = filename,
              open = "r")
18  cnt <- 0
19  #browser()
20  while(cnt <= nLines){
21   cnt <- cnt + 1
22   sentence <- readLines(con = connecTion,
               n = 1)


23   cat(sentence,"\n")
24   if(cnt == 1){}
```

However, if the cnt^{th} line is the 2^{nd} one in the file, then we know that it contains the information on column names. This needs to be extracted from the *sentence* variable in the second iteration of the **while** loop, which extends from lines 25 to 59. Again, to explain the aspects of the code, we will break this **else if**($cnt = 2$){...} block into parts. This block contains two **for** loop blocks. The first block is used to retrieve the names of the columns that are delimited by a "+" sign (see lines 25-45). The second block is to create corresponding variable names that match the retrieved names, followed by initialization (see lines 46-58).

The **else if** block begins with a few initializations. The cnt^{th} sentence is split and stored in a simple format in *tempSentence*. The length of the sentence in terms of the number of characters is stored in *tempSentenceLength*. The position of the delimiter "+" within the sentence is also stored using the **which** function. Finally, the names of the columns need to be stored in *cnames*, and an index *indx* is used as a start position at a particular location in *tempSentence* for processing purposes. In the first **for** loop, with the iterator *i* taking on values of the position of the delimiter (stored in *delimPlusPos*) one at a time in every loop, a particular piece of code is executed depending on the value of the index position in *indx.* In line 31, if the location of the *indx* is 1, i.e., the starting character of the sentence, then the first name needs to be extracted that lies between position *1* and position (*i-1*). Note that at location *i*, there is a "+" symbol. To execute this, the command **capture.output** is used, which converts the concatenated elements of *tempSentence[1:(i-1)]* via the **cat** command (see line 33). **capture.output** converts the input argument into a string and stores the retrieved name in *tempName*. Next, this column name is stored in *cnames* in line 34. In case the *indx* is the position which contains the last

delimiter, i.e., **length**(*delimPlusPos*), then the last and the penultimate column names need to be retrieved. The penultimate column name can be retrieved from the characters between the penultimate delimiter and the last delimiter, i.e., *delimPlusPos[indx-1]+1):(i-1)* (see line 36). The last column name can be retrieved after the last delimiter and the end of the sentence, i.e., *delimPlusPos[indx]+1):tempSentenceLength* (see line 38). Finally, if the iterator neither points to the 1^{st} nor the last **length**(*delimPlusPos*) delimiter position, then the column name can be retrieved from characters lying between the position of the previous delimiter position and the current delimiter position, i.e., *(delimPlusPos[indx-1]+1):(i-1)* (see line 41). Each of these are ranges that are encased in the vector *tempSentence* and are concatenated via **cat** and captured in *tempName*, and later stored in *cnames*. After the execution of the **for** loop ends, that is, the iterator *i* has covered all the delimiter positions in *delimPlusPos*, *cnames* contains all the column names (see lines 31-45).

```
25   else if(cnt == 2){
26    tempSentence <- unlist(strsplit(
                 x = sentence, split = "+"))
27    tempSentenceLength <- length(tempSentence)
28    delimPlusPos <- which(tempSentence == "+")
29    cnames <- c()
30    indx <- 1
```


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```
31    for(i in delimPlusPos){
32      if(indx == 1){
33      tempName <- capture.output(
           cat(tempSentence[1:(i-1)],sep=""))
34      cnames <- c(cnames, tempName)
35     }else if(indx == length(delimPlusPos)){
36      tempName <- capture.output(cat(
          tempSentence[(delimPlusPos[indx-1]+1):
          (i-1)], sep=""))
37      cnames <- c(cnames, tempName)
38       tempName <- capture.output(cat(
          tempSentence[(delimPlusPos[indx]+1):
          tempSentenceLength], sep=""))
39      cnames <- c(cnames, tempName)
40     }else{
41      tempName <- capture.output(cat(
          tempSentence[(delimPlusPos[indx-1]+1):
          (i-1)], sep=""))
42      cnames <- c(cnames, tempName)
43    }
44    indx <- indx + 1
45   }
```

Once the names of the columns have been stored, the corresponding variables need to be created and initialized. This is done in the second block as described above. The second **for** loop iterates through the list of column names. In each iteration of the **for** loop, a condition is checked which tests whether a particular pattern exists within the column name under consideration. If the condition is satisfied, as above, the corresponding variable is created and initialized. We use the **grep** function to find the *pattern* in the column name *i*, as *i* iterates through *cnames* in the **for** loop. If there is a match and the *pattern* exists in column name *i*, then the length of this match will not be equal to 0, like (**length**(**grep**(*pattern* = "sym", *x* = i)) != 0) on line 47. When this happens, the creation and initialization of a variable follows. In the above example, *Genesymbol* is created and initialized. Finally, the block for **else if** is completed, if one is dealing with the 2^{nd} line of the file (see lines 46-58).

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```
46   for(i in cnames){
47    if(length(grep(pattern = "sym",
          x = i)) != 0){
48      Genesymbol <- c()
49    }else if(length(grep(pattern = "ID",
          x = i)) != 0){
50      ENSEMBLgeneID <- c()
51    }else if(length(grep(pattern = "des",
          x = i)) != 0){
52      Genedescription <- c()
53    }else if(length(grep(pattern = "fold",


          x = i)) != 0){
54      logTwoFC <- c()
55    }else if(length(grep(pattern = "value",
          x = i)) != 0){
56      BHadjustedPvalue <- c()
57    }
58   }
```

Next, for all cnt^{th} lines of the file that are neither 1^{st} nor 2^{nd}, the last part of the **else** block for the **while** loop in line 20 is executed. This block is encoded in lines 59-82. Most of the code is similar to the preceding block with a few changes. Line 60 contains the same command to store the sentence read at cnt^{th} line of the file, and line 61 is used to find the positions where the delimiter is positioned. In the next step, if there is an error in the positioning of the delimiter due to a manual preprocessing error, the command shows that correction needs to be done at line *cnt*. From lines 64 to 83, the coding is similar to the foregoing piece of code, except that if the index *indx* is 1, now the name of the gene is stored in *Genesymbol*; if the index *indx* is **length**(*delimPlusPos*), then $\log_2$ fold change values are stored in *logTwoFC* from the left side of the delimiter and adjusted P-values are stored in *BHadjustedPvalue* from the right side of the delimiter; if *indx* is 2, then the ENSEMBLgeneID value is stored in *ENSEMBLgeneID*, and finally, if *indx* is 3, then the Genedescription is stored in *Genedescription*. Lines starting with # are commented and used only for testing purposes and do not give any value (see lines 59-84). Note that as each line is read, information is stored using the **rbind** function that keeps attaching or binding the current value to the existing vector in a variable. For example, *Genesymbol* $<-$ **rbind**(*Genesymbol*, *tempName*) will append *Genesymbol* with the current *tempName*, thus

increasing the size of the vector in *Genesymbol* by 1. This procedure gets repeated. Thus ends the storage of information from the file in the variables.

```
59   }else{
60     tempSentence <- unlist(strsplit(
            x = sentence, split = "+"))
61     delimPlusPos <- which(tempSentence == "+")
62     if(length(delimPlusPos) != 4){
         cat("Correction needed at - ",cnt);
         break
       }
63    indx <- 1
64    for(i in delimPlusPos){
65     if(indx == 1){
66      tempName <- capture.output(cat(
          tempSentence[1:(i-1)],sep=""))
67      Genesymbol <- rbind(Genesymbol, tempName)
68     }else if(indx == length(delimPlusPos)){
69       tempName <- capture.output(cat(
          tempSentence[(delimPlusPos[indx-1]+1):
          (i-1)], sep=""))
```

```
70       logTwoFC <- rbind(logTwoFC,
            as.numeric(tempName))
71 #     tempName <- capture.output(cat(
   #       tempSentence[(delimPlusPos[indx]+1):
   #       (tempSentenceLength-1)], sep=""))
72       tempName <- capture.output(cat(
           tempSentence[delimPlusPos[indx]+(1:7)],
           sep=""))
73       BHadjustedPvalue <-
           rbind(BHadjustedPvalue, tempName)
74     }else if(indx == 2){
75       tempName <- capture.output(cat(
           tempSentence[(delimPlusPos[indx-1]+1):
           (i-1)], sep=""))
76       ENSEMBLgeneID <- rbind(ENSEMBLgeneID,
           tempName)
77     }else if(indx == 3){
78       tempName <- capture.output(cat(
           tempSentence[(delimPlusPos[indx-1]+1):
           (i-1)], sep=""))
79       Genedescription <-
           rbind(Genedescription, tempName)
80     }
81     indx <- indx + 1
82    }
83   }
84  }
```

Next, we close the open file using the command **close** with an argument *connecTion*. And finally, we combine the variable names in a data frame, a kind of data structure, using **data.frame** and arguments *Genesymbol*, *ENSEMBLgeneID*, *Genedescription*, *logTwoFC*, and *BHadjustedPvalue*. The data frame is stored in the variable *oncETC*. Finally, the return command returns this data frame as output using the function **return** and *oncETC* (see lines 85-89).

```
85  close(connecTion)
86
87  oncETC <- data.frame(Genesymbol,
      ENSEMBLgeneID, Genedescription,
      logTwoFC, BHadjustedPvalue)
88  return(oncETC)
89 }
```

Note that the preprocessing and extraction of data can have different flavours depending on the type of data and experiment one is dealing with. However, the output of the extraction should be a data frame (a kind of variable in R) containing the extracted data that needs to be used in sensitivity analysis. This is explained next.

5.2.2. Exercise

As an exercise, the readers are encouraged to build their own preprocessed file manually, from the data given in[3] and see if they can reproduce the results in the form of a data frame using the function **extractETCdata**.

5.3. Computing the sensitivity indices

We move on to the next stage of the pipeline where the sensitivity indices need to be generated. Why we are generating these indices and how it helps in ranking up a set of factors and its combinations involved in the pathway have been discussed in[7] and[8]. Here we concentrate on the implementation of the pipeline and explanation of the code only. The code has been saved in the file named manuscript-2-2.R, and one of the authors has been lazy enough not to change the name of the code. However, it also points to the fact that the author is not concerned with the show of expertise in the nomenclature of file names, and neither does he wish to earn a PhD in the nomenclature of file names. Moving to the main topic, the code begins with the definitions of some functions and the inclusion of packages from which specific functions can be used during programming.

5.3.1. Description of manuscript-2-2.R

Here, the **library** function is used to call the package "sensitivity," which has been implemented in R and goes as an argument into the function **library**. Details of the package can be found in[9]. Next, we define a function using the function **function** and give this function a name **new.name**. The function takes in as an argument a two-dimensional matrix *X*. *k* is the number of genes under consideration out of a set of genes. The **new.name** implements the g-function (a model) that is used to assign weights to each of the genes, with a random weight. For this, the **runif** function is used. *b* is updated as each gene is considered one at a time in the **for** loop. At the end of the function, the value in **b** is returned implicitly. What happens is as the loop iterates **length**(*a*) times, where the expression shows the number of genes the user has selected. Note that the length of *a* is computed using the value of *k*. This

function is read within lines 3-10. Also, **genSampleComb** is a function that returns a two-dimensional matrix with a specific number of columns defined in *yCol*. More about this function will be talked about at a later stage. Here, definitions of the functions are provided (see lines 1-14).

```
1   library(sensitivity)
2   # Function definitions
3   new.fun <- function(X){
4     a <- runif(k)
5     b <- 1
6     for (j in 1:length(a)) {
7       b <- b * (abs(4 * X[, j] - 2) +
          a[j])/(1 + a[j])
8     }
9     b


10  }
11
12  genSampleComb <- function(yCol){
13    return(disty[,yCol])
14  }
15
```

Since the code can be adapted for different data sets, a query is asked of the user regarding the generation of distribution around point measurements, if they exist in the data under consideration. To query the user, the function **readline** is employed. The user has to type in the option provided in the query for a particular functionality to take effect. Here, the response to the query is stored in the variable *DISTRIBUTION*. Next, if the response in *DISTRIBUTION* is yes, that is, the user typed in "y", then the ensuing block within the **if** command will get executed; else, it will be skipped. Again, the block under consideration defines a new function **gdfetppv** which takes in arguments *n* and *yt*, where *n* contains the number of points which a user might want to generate for a distribution around a numerical point estimate. The measured numerical point estimates for each gene under consideration are assorted in a vector *yt*. The length of *yt* shows the number of genes involved in the study of sensitivity analysis. As the **for** iterates through each gene, for the corresponding numerical point estimate for a particular gene, a distribution around the point estimate is generated with the mean

value being *yt[i]* (*i* is the iterator) and a standard deviation of 0.005. Along with the distribution, a minor jitter or noise is added. Thus, the whole distribution is stored in a vector. Note that the output of the **jitter** function is a vector and R stores vectors in column format. Consequently, as the **for** loop iterates from one gene to another, the columns are bound together using a column binding function **cbind**. Thus, *randyt* keeps on increasing column-wise, till all genes have been covered. Once out of the loop, the row vector *yt* is appended with the distribution matrix *randyt* using the row binding function **rbind**. The output of the function is a two-dimensional matrix containing the point estimate in the first row and the corresponding distribution in the rows below (see lines 20-28).

```
16  # Generation of distributions
17  DISTRIBUTION <- readline("Should i generate
      distribution of data [y/n] - ")
19  if(DISTRIBUTION == "y"){
20    gdfetppv <- function(n,yt){
21      randyt <- c()
22      lenyt <- length(yt)
23      for(i in 1:lenyt){
24        randyt <- cbind(randyt,
            jitter(rnorm(n, mean = yt[i],
            sd = 0.005), factor = 1))
25      }
26      yt <- rbind(yt, randyt)
27      return(yt)
28    }


29  }
```

After the functions have been defined, the main execution begins. The code starts with the extraction of the data using the **readline** argument and provides an option to the user to choose a file that contains data regarding down-regulated genes or up-regulated genes. Once the response is recorded, it is stored in the variable *DATATYPE*. If the user enters a wrong number, then a **while** loop is run which asks to enter the right response. This feedback continues until the user enters the right response (see lines 30-33). After the correct response is recorded, we use the function **extractETCdata** to extract the information from the particular file associated with the response in *DATATYPE*. This is done using the command **extractETCdata**(*DATATYPE*) and the output of the function is stored in *oncETCmain*. We keep

a copy of the stored information in *oncETCmain* and make a second copy in the subsequent line in *oncETC* (see lines 34-35). This manuscript explains the code for retrieving 2^{nd} order combinations, only so as to set a platform for many who would be reading the article. Note, before the use of the function, i.e., the instantiation of the function, the function needs to be defined and initialized. We defined the function and named it. After that, an instance of the function is used to get a certain result. One instance is **extractETCdata**(*1*) and another instance is **extractETCdata**(*2*).

```
30  # Data extraction
31  DATATYPE <- readline("Choose a file to
      process [1/2] \n
      1 - ../data/onc2015280x2-A.txt \n
      Genes down-regulated after ETC-159
      treatment \n
      2 - ../data/onc2015280x2-B.txt \n
      Genes up-regulated after ETC-159
      treatment \n
      File number - ")
32  while(DATATYPE != "1" & DATATYPE != "2"){
      DATATYPE <- readline("Type the kind of
        data to be processed [1/2] - ")
33  }
34  oncETCmain <- extractETCdata(DATATYPE)
35  oncETC <- oncETCmain
```

Of interest is the column containing the $\log_2$ fold change numerical point estimates that are stored in the variable *oncETC*. Since the information under this column is stored in the data frame, it needs to be converted into a matrix for further processing by the sensitivity analysis package. For this, the **as.matrix** function is used, which takes in the object in *x* along with arguments *ncol*, which asks for the number of columns into which the information needs to be divided, and *byrow*, being false, stating that the information will not be lined up row-wise, but column-wise. The result of the transformation is stored in *y*. Next, respective column elements in *y* are allotted their gene names. This is achieved using *oncETC$Genesymbol*. The row names of *y*, which depict the gene names, are thus assigned. We also save the names of the genes in the *factor.names* variable. The dimension or the size of *y* in terms of the number of rows and the number of columns is recorded using the function **dim**, and the measurements are stored in *dim.y*. *dim.y[1]* contains the number of rows, which implicitly defines the

number of genes (stored in *no.genes*). The whole list of genes can be shown on the command prompt during the execution of the code via **cat**(*rownames(y)*) (see lines 35-40).

```
35  y <- as.matrix(x = oncETC$logTwoFC,
      ncol = 1, byrow = FALSE)
36  rownames(y) <- oncETC$Genesymbol
37  factor.names <- oncETC$Genesymbol
38  dim.y <- dim(y)
39  no.genes <- dim.y[1]
40  cat(rownames(y))
```

Next, the user is asked which gene they would like to investigate, and the response is stored in *geneName*. Also, since the pipeline is about investigating combinations, we need to input the number of combinations we are interested in. For this, a similar query is asked, and the value is stored in the variable *k*. Since it is in character format, it needs to be converted into numerical format, and that is done using the **as.numeric** function. The **cat** function helps in displaying messages to ease the user's understanding of what is happening during the execution of the program. The combinations that can be generated from *k* genes, out of the total number of genes *no.genes*, are computed using **combn**. This returns a two-dimensional matrix to **geneComb**, whose number of columns represents the total number of combinations, i.e., **dim**(*geneComb[2]*) (see lines 41-47).

```
41  geneName <- readline("\nEnter name of
gene to be evaluated - ")
42  # Regarding combinations
43  ANSWER <- readline("nCk - choosing k - ")
44  k = as.numeric(ANSWER)
45  cat("choosing ",k," out of the",
      no.genes," genes!\n---\n")
46  geneComb <- combn(no.genes,k)
47  no.geneComb <- dim(geneComb)[2]
```

Next, initialization of the variables needs to be done for processing the data. A series of list data structures is initialized, and names are assigned to each new list as shown from lines 49 to 60. These are the variables where the sensitivity indices will be stored. *siNames* contains the names of the indices which the search engine uses, and the user can pick any one of them for computation. Line 63 prompts

the user to enter the name of the sensitivity index after looking at the displayed list in the command on line 62. This name is stored in the variable *varName*. Next, we search for the pattern (stored in *varName*) in some of the Sobol index names, and if the user has chosen a Sobol sensitivity index, then *ISSOBOL* is assigned to a true value. This will be used and explained later on (see lines 49-66).

```
48  # some initializations
49  sensiFdiv.TV <- list()
50  sensiFdiv.KL <- list()
51  sensiFdiv.Chi2 <- list()


52  sensiFdiv.Hellinger <- list()
53  sensiHSIC.rbf <- list()
54  sensiHSIC.linear <- list()
55  sensiHSIC.laplace <- list()
56  SB.jansen <- list()
57  SB.2002 <- list()
58  SB.2007 <- list()
59  SB.martinez <- list()
60  SBL <- list()
61  siNames <- c("Fdiv.TV", "Fdiv.KL",
      "Fdiv.Chi2", "Fdiv.Hellinger", "HSIC.rbf",
      "HSIC.linear", "HSIC.laplace", "SB.2002",
      "SB.2007", "SB.jansen", "SB.martinez", "SBL")
62  cat("Types of SA - ", siNames, "\n")
63  varName <- readline("Enter a type of SA - ")
64  ISSOBOL <- FALSE
65  if(length(grep(varName, "SB.2002"))!= 0 |
      length(grep(varName, "SB.2007"))!= 0 |
      length(grep(varName, "SB.jansen"))!= 0 |
      length(grep(varName, "SB.martinez"))!= 0
      | length(grep(varName, "SBL"))!= 0){
      ISSOBOL <- TRUE
66  }
```

Regarding the generation of a distribution of numerical point measurements, it is important to specify the number of samples. The user is usually given a choice, as shown in line 67 (here commented). However, for exercise purposes, we set the value of the number of samples to be $n = 10$. Next, the function for generating a distribution of size 9 per gene measurement is used, and the output is converted into a data frame using the function **data.frame**. This data frame is then stored in the

variable *disty* or distribution of y. We again save the number of samples as an extra using the **dim** function, in a variable **no.Samples** (see lines 67-71).

```
67  # n <- as.numeric(readline("Enter number of
    samples for distribution (odd numeric) - "))
68  n <- 9
69  disty <- data.frame(gdfetppv(n,t(y)))
70  no.Samples <- dim(disty)[1]
71  cat("generating sample combinations!\n")
```

The **apply** function is one of the important functions in R language and is widely used for vector programming. It is important here in the sense that we need to compute the indices for combinations of factors. The arguments for **apply** take in a matrix, the indicator for a vector in a matrix over which a function will be applied. Here we see that *geneComb* is the matrix containing the combinations of genes; *MARGIN* with an indicator 2 means the columns of *geneComb* will be worked upon by the function **genSampleComb** in the variable *FUN*. Thus, the function **apply** will apply the function **genSampleComb** to the columns of the matrix *geneComb*. The function **genSampleComb** in line 12 takes in a column of the matrix *geneComb* and returns the *k* distributions that are stored in the matrix *disty*. So, if *k* is 2, then the number of rows in *geneComb* will be 2. These 2 elements associated with a particular column in *geneComb* will contain the gene numbers in a list of genes. During the application of the **apply** function, *yCol* stores a column of *geneComb* and uses **genSampleComb** to generate *disty[,yCol]*, a $n \times k$ matrix, where n is the number of samples and k is the number of elements in the combination. The procedure is applied to all columns of *geneComb* for this (see line 72).

```
72  distyN <- apply(X = geneComb, MARGIN = 2,
    FUN = genSampleComb)
```

Next, the combinatorial distributions stored in *distyN* are processed to segregate the gene combinations that contain the particular gene of interest defined by the user from a list of genes in *geneName*. List variables are defined, and to find combinations containing *geneName*, a **for** loop is executed where the iterator iterates through the total number of C_k^n combinations. For each of the combinations contained in **names**(*distyN[i]*), if *geneName* is found to exist, then the distribution containing *geneName* and another gene in *distyN[i]* is stored in *x.S*, a list. For every such identification,

a counter *cnt* is incremented. Finally, after all combinations have been found which contain *geneName*, the final cnt is assigned to the number of selected gene combinations *no.slgeneComb* (see lines 73-85).

```
73  # Sample with replicates
74  x.S <- list()
75  x.Sfh <- list()
76  x.Ssh <- list()
78  cnt <- 0
79  for(i in 1:no.geneComb){
80    if(geneName %in% names(distyN[[i]])){
81  cnt <- cnt + 1
82  x.S[[cnt]] <- distyN[[i]]
83    }
84  }
85  no.slgeneComb <- cnt
```

Usually, a user will be asked about the total number of iterations for which the sensitivity indices will be generated. This is done to get an average sensitivity index score, which is then used for the ranking of the combinations. For demonstration purposes, we set the iteration number to $itrNo = 50$. The **for** loop iterates the iterator itr for 50 iterations. Every iteration, the number of iterations is displayed at the start of the **for** loop. The samples need to be shuffled every time in order to have variation so that the mean of the sensitivity indices can be generated. This can be done by using the function **sample**, which takes a range of values from 1 to *no.Samples*, and the size of the sample is set to *no.Samples*. The shuffled samples are stored in *sample.index*. *idx.fh* and *idx.sh* are used to divide the sample into two halves in case one is using the Sobol method for generating sensitivity indices. Next, if the method used is Sobol or a variant of the same, as indicated by $ISSOBOL$, then shuffling of the samples in combination happens. This shuffling is done in lines 99-100, for all combinations, i.e., j from 1 to *no.slgeneComb*. For non-Sobol-based methods, the shuffling is simple, as shown in line 104.

```
86  # itrNo <- as.numeric(readline("Number
    of iterations for averaged ranking - "))
87  itrNo <- 50
88  hmean <- list()
89  for(itr in 1:itrNo){
90    # disty <- data.frame(gdfetppv(n,yt))
91    # distyN can be replaced with x.S also

92    cat("generating for sample - ", itr, "\n")
93    sample.index <- sample(x = 1:no.Samples,
        size = no.Samples)
94    idx.fh <- sample.index[1:(no.Samples/2)]
95    idx.sh <- sample.index[((no.Samples/2)+1)
        :no.Samples]

96    # Shuffle the samples
97    if(ISSOBOL){
98      for(j in 1:no.slgeneComb){
99        x.Sfh[[j]] <- x.S[[j]][idx.fh,]
100       x.Ssh[[j]] <- x.S[[j]][idx.sh,]
101     }
102   }else{
103     for(j in 1:no.slgeneComb){
104       x.S[[j]] <- x.S[[j]][sample.index,]
105     }
106   }
```

Once the samples for the combinations have been stored in *x.S*, it is time to generate the sensitivity indices. To generate a sensitivity index of a particular type, the user has to specify the name of the sensitivity index. This has already been done earlier, and the name is stored in *varName*. What follows is a series of condition tests using the **if ... else** to know which type of sensitivity method needs to be taken into account and which necessary method to be initiated to generate the indices. One of the approaches would be to use the **grep** function, which searches for a pattern in *varName*. If the pattern exists, then the length of the finding would not be zero. When this condition holds, then a particular index associated with the pattern is initiated. So, if "TV" is the pattern and it is found to be in the *varName*, then the f-divergence method[10] with a Total variation distance $|t-1|$ needs to be initiated. The short explanation of the theoretical principles of density and variance-based methods has been explained in[2]. Here we concentrate on the flow of the code. In line 109, we define and initialize a new variable *FdivTV*. After some displays on the screen, **lapply** is used on *x.S*. **lapply** returns a list of the

same length as *x.S*, each element of which is the result of applying the function **sensiFdiv** to the corresponding element of *x.S*. Additionally, since the sensitivity method uses a model function, we use extra arguments in the **lapply** function, like *model* = **new.fun**; *nboot* = 0; and *conf* = 0.95. This **new.fun** has earlier been defined at the beginning of the code. So, **lapply** generates sensitivity indices for each of the matrices containing a specific gene combination using the **new.fun** via **sensiFdiv**. The result is stored in a variable *h*. Since we know that **lapply** will generate sensitivity indices for each of the matrices in *x.S*, thus each combination has an associated sensitivity that is stored in *h[[p]]Soriginal*. We bind this to the variable *FdivTV* (see lines 108-113).

Next, since the **for** loop in line 89 works for many iterations, we need to store the sensitivity index computed in this iteration in a certain variable. This is done in *sensiFdiv.TV[[itr]]*, the definition of which was done in line 49. Also, if this is the first iteration, then we define the file name based on the data that is being used and the iteration (see lines 115-117).

```
107   # itr <- 1
108   if(length(grep("TV",varName))!= 0){
109     FdivTV <- c()
110     cat("computing estimate different
          indices ...\n")
111     cat("Fdiv SA - TV\n")
112     h <- lapply(x.S, sensiFdiv,
          model = new.fun, fdiv = "TV",
          nboot = 0, conf = 0.95)
113     for(p in 1:no.slgeneComb){
          FdivTV <- cbind(FdivTV,
            h[[p]]$S$original)
        }
114     sensiFdiv.TV[[itr]] <- FdivTV
115     if(DATATYPE == "1" & itr == 1){
          filename <- paste("order-",k,"-",
            geneName,
            "-DR-A-ETC-T-fdiv-tv-mean.Rdata",
            sep = "")
116     }else if(DATATYPE == "2" & itr == 1){
           filename <- paste("order-",k,"-",
             geneName,
             "-UR-A-ETC-T-fdiv-tv-mean.Rdata",
             sep = "")
117     }
```

```
118   }else if(length(grep("KL",varName))
        != 0){
119     FdivKL <- c()
120     cat("computing estimate different
```

The next series of conditions deals with the different methods in a similar manner as explained for lines 108-117. Lines 118-130 talk about the f-divergence method[10] with a Kullback-Leibler divergence $-\log_e(t)$.

```
118   }else if(length(grep("KL",varName))
        != 0){
119     FdivKL <- c()
120     cat("computing estimate different



          indices ...\n")
121     cat("Fdiv SA - KL\n")
122     h <- lapply(x.S, sensiFdiv,
          model = new.fun, fdiv = "KL",
          nboot = 0, conf = 0.95)
123     for(p in 1:no.slgeneComb){
124       FdivKL <- cbind(FdivKL,
            h[[p]]$S$original)
125     }
125     sensiFdiv.KL[[itr]] <- FdivKL
126     if(DATATYPE == "1" & itr == 1){
127       filename <- paste("order-",k,"-",
            geneName,
            "-DR-A-ETC-T-fdiv-kl-mean.Rdata",
            sep = "")
128     }else if(DATATYPE == "2" & itr == 1){
129       filename <- paste("order-",k,"-",
            geneName,
            "-UR-A-ETC-T-fdiv-kl-mean.Rdata",
            sep = "")
130     }
```

Lines 131-144 talk about the f-divergence method[10] with a χ^2 distance $t^2 - 1$.

```
131     }else if(length(grep("Chi2",varName))
          != 0){
132       FdivChi2 <- c()
133       cat("computing estimate different
            indices ...\n")
134       cat("Fdiv SA - Chi2\n")
135       h <- lapply(x.S, sensiFdiv,
            model = new.fun, fdiv = "Chi2",
            nboot = 0, conf = 0.95)
136       for(p in 1:no.slgeneComb){
137         FdivChi2 <- cbind(FdivChi2,
              h[[p]]$S$original)
138       }
139       sensiFdiv.Chi2[[itr]] <- FdivChi2
140       if(DATATYPE == "1" & itr == 1){
141         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-fdiv-chi2-mean.Rdata",
              sep = "")
142       }else if(DATATYPE == "2" & itr == 1){
143         filename <- paste("order-",k,"-",


              geneName,
              "-UR-A-ETC-T-fdiv-chi2-mean.Rdata",
              sep = "")
144       }
```

Lines 145-158 talk about the f-divergence method[10] with a Hellinger distance $(\sqrt{(}t)-1)^2$.

```
145     }else if(length(grep("Hellinger",varName))
          != 0){
146       FdivHellinger <- c()
147       cat("computing estimate different
            indices ...\n")
148       cat("Fdiv SA - Hellinger\n")
149       h <- lapply(x.S, sensiFdiv,
            model = new.fun, fdiv = "Hellinger",
            nboot = 0, conf = 0.95)
150       for(p in 1:no.slgeneComb){
151         FdivHellinger <- cbind(FdivHellinger,
              h[[p]]$S$original)
152       }
153       sensiFdiv.Hellinger[[itr]]
            <- FdivHellinger
154       if(DATATYPE == "1" & itr == 1){
155         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-fdiv-hellinger-mean.Rdata",
              sep = "")
156       }else if(DATATYPE == "2" & itr == 1){
157         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-fdiv-hellinger-mean.Rdata",
              sep = "")
158       }
```

[11] recently proposed a new set of dependence measures using kernel methods. These have also been implemented in[9]. The following contains variants of different kernels involved in computing the sensitivity indices. Lines 159-172 show similar execution code as above with changes in some of the arguments in the **lapply** function. Here, the "rbf" or radial basis function is used within **sensiHSIC**.

```
159     }else if(length(grep("rbf",varName))
          != 0){
160       HSICrbf <- c()
161       cat("computing estimate different
            indices ...\n")
162       cat("HSIC SA - rbf kernel\n")
163       h <- lapply(x.S, sensiHSIC,
            model = new.fun, kernelX = "rbf",
```

```
            paramX = NA, kernelY = "rbf",
            paramY = NA, conf = 0.95)
164       for(p in 1:no.slgeneComb){
165         HSICrbf <- cbind(HSICrbf,
              h[[p]]$S$original)
166       }
167       sensiHSIC.rbf[[itr]] <- HSICrbf
168       if(DATATYPE == "1" & itr == 1){
169         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-hsic-rbf-mean.Rdata",
              sep = "")
170       }else if(DATATYPE == "2" & itr == 1){
171         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-hsic-rbf-mean.Rdata",
              sep = "")
172       }
```

Lines 173-186 show similar execution code as above with changes in some of the arguments in the **lapply** function. Here, the linear function is used.

```
173     }else if(length(grep("linear",varName))
          != 0){
174    HSIClinear <- c()
175       cat("computing estimate different
            indices ...\n")
176       cat("HSIC SA - linear kernel\n")
177       h <- lapply(x.S, sensiHSIC,
            model = new.fun, kernelX = "linear",
            paramX = NA, kernelY = "linear",
            paramY = NA, conf = 0.95)
178       for(p in 1:no.slgeneComb){
179         HSIClinear <- cbind(HSIClinear,
              h[[p]]$S$original)
180       }
181       sensiHSIC.linear[[itr]] <- HSIClinear
182       if(DATATYPE == "1" & itr == 1){
183         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-hsic-linear-mean.Rdata",
              sep = "")
184       }else if(DATATYPE == "2" & itr == 1){
185         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-hsic-linear-mean.Rdata",
              sep = "")


186        }
```

Lines 187-200 show similar execution code as above with changes in some of the arguments in the **lapply** function. Here, the Laplace function is used.

```
187  }else if(length(grep("laplace",varName))
          != 0){
188       HSIClaplace <- c()
189       cat("computing estimate different
            indices ...\n")
190       cat("HSIC SA - laplace kernel\n")
191       h <- lapply(x.S, sensiHSIC,
            model = new.fun, kernelX = "laplace",
            paramX = NA, kernelY = "laplace",
            paramY = NA, conf = 0.95)
192       for(p in 1:no.slgeneComb){
193         HSIClaplace <- cbind(HSIClaplace,
              h[[p]]$S$original)
194       }
195       sensiHSIC.laplace[[itr]] <- HSIClaplace
196       if(DATATYPE == "1" & itr == 1){
197         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-hsic-laplace-mean.Rdata",
              sep = "")
198       }else if(DATATYPE == "2" & itr == 1){
199         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-hsic-laplace-mean.Rdata",
              sep = "")
200     }
```

Finally, we come to the section where variants of the Sobol function have been encoded. It is here that the use of divided samples *X.Sfh* and *X.Ssh* comes into play. We do not use the **lapply** function. Instead, the[12] variants are encoded using name-specific functions (see below). Lines 201-214 show similar execution code as above, but using **soboljansen**.

```
201     }else if(length(grep("jansen",varName))
          != 0){
202       SBLjansen <- c()
203       cat("computing estimate different
            indices ...\n")
204       cat("Sobol Jansen SA\n")
205       for(p in 1:no.slgeneComb){
206         h <- soboljansen(model = new.fun,
              X1 = t(x.Sfh[[p]]),
              X2 = t(x.Ssh[[p]]), conf = 0.95)
207         SBLjansen <- cbind(SBLjansen,
```

```
            c(h$S$original, h$T$original))
208       }
209       SB.jansen[[itr]] <- SBLjansen
210       if(DATATYPE == "1" & itr == 1){
211         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-sb-jansen-mean.Rdata",
              sep = "")
212       }else if(DATATYPE == "2" & itr == 1){
213         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-sb-jansen-mean.Rdata",
              sep = "")
214       }
```

Lines 215-228 show similar execution code as above, but using **sobol2002**.

```
215     }else if(length(grep("2002",varName))
          != 0){
216       SBL2002 <- c()
217       cat("computing estimate different
            indices ...\n")
218       cat("Sobol 2002 SA\n")
219       for(p in 1:no.slgeneComb){
220         h <- sobol2002(model = new.fun,
              X1 = t(x.Sfh[[p]]),
              X2 = t(x.Ssh[[p]]), conf = 0.95)
221       SBL2002 <- cbind(SBL2002,
            c(h$S$original, h$T$original))
222       }
223       SB.2002[[itr]] <- SBL2002
224       if(DATATYPE == "1" & itr == 1){
225         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-sb-2002-mean.Rdata",
              sep = "")
226       }else if(DATATYPE == "2" & itr == 1){
227         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-sb-2002-mean.Rdata",
              sep = "")
228     }
```

Lines 229-242 show similar execution code as above, but using **sobol2007**.

```
229     }else if(length(grep("2007",varName))
          != 0){
230       SBL2007 <- c()
231       cat("computing estimate different


            indices ...\n")
232       cat("Sobol 2007 SA\n")
233       for(p in 1:no.slgeneComb){
234         h <- sobol2007(model = new.fun,
              X1 = t(x.Sfh[[p]]),
              X2 = t(x.Ssh[[p]]), conf = 0.95)
235         SBL2007 <- cbind(SBL2007,
              c(h$S$original, h$T$original))
236       }
237       SB.2007[[itr]] <- SBL2007
238       if(DATATYPE == "1" & itr == 1){
239         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-sb-2007-mean.Rdata",
              sep = "")
240       }else if(DATATYPE == "2" & itr == 1){
241         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-sb-2007-mean.Rdata",
              sep = "")
242     }
```

Lines 243-256 show similar execution code as above, but using **sobolmartinez**.

```
243     }else if(length(grep("martinez",varName))
          != 0){
244       SBLmartinez <- c()
245       cat("computing estimate different
            indices ...\n")
246       cat("Sobol Martinez SA\n")
247       for(p in 1:no.slgeneComb){
248         h <- sobolmartinez(model = new.fun,
              X1 = t(x.Sfh[[p]]),
              X2 = t(x.Ssh[[p]]), conf = 0.95)
249         SBLmartinez <- cbind(SBLmartinez,
              c(h$S$original, h$T$original))
250       }
251       SB.martinez[[itr]] <- SBLmartinez
252       if(DATATYPE == "1" & itr == 1){
253         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-sb-martinez-mean.Rdata",
              sep = "")
254       }else if(DATATYPE == "1" & itr == 1){
255         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-sb-martinez-mean.Rdata",
              sep = "")
256       }
```

Lines 257-272 show similar execution code as above, but using **sobol**.

```
257     }else if(length(grep("SBL",varName))
          != 0){
258       sbl <- c()
259       cat("computing estimate different
            indices ...\n")
260       cat("SBL SA\n")
261       for(p in 1:no.slgeneComb){
262         h <- sobol(model = new.fun,
              X1 = t(x.Sfh[[p]]),
              X2 = t(x.Ssh[[p]]), order = 1,
              conf = 0.95)
263         sbl <- cbind(SBL, c(h$S$original,
              h$T$original))
264       }
265       SBL[[itr]] <- sbl
266       if(DATATYPE == "1" & itr == 1){
267         filename <- paste("order-",k,"-",
              geneName,
              "-DR-A-ETC-T-sbl-mean.Rdata",
              sep = "")
268       }else if(DATATYPE == "2" & itr == 1){
269         filename <- paste("order-",k,"-",
              geneName,
              "-UR-A-ETC-T-sbl-mean.Rdata",
              sep = "")
270       }
271     }
272   }
```

Once the indices have been generated, they need to be stored in a file for further processing. The following set of lines helps in saving the work, where the function **save** is used and variables *no.slgeneComb*, *x.S*, and the respective sensitivity indices related to *varName* are stored in the *filename*.

```
273   if(length(grep("TV",varName))!= 0){
274     save(no.slgeneComb, x.S,
          sensiFdiv.TV, file = filename)
275   }else if(length(grep("KL",varName))
        != 0){
276     save(no.slgeneComb, x.S,
          sensiFdiv.KL, file = filename)
277   }else if(length(grep("Chi2",varName))
        != 0){
276     save(no.slgeneComb, x.S,
```

```
        sensiFdiv.Chi2, file = filename)
277  }else if(length(grep("Hellinger",varName))
       != 0){
278    save(no.slgeneComb, x.S,
         sensiFdiv.Hellinger, file = filename)
279  }else if(length(grep("rbf",varName))
       != 0){
280    save(no.slgeneComb, x.S,
         sensiHSIC.rbf, file = filename)
281  }else if(length(grep("linear",varName))
       != 0){
282    save(no.slgeneComb, x.S,
         sensiHSIC.linear, file = filename)
283  }else if(length(grep("laplace",varName))
       != 0){
284    save(no.slgeneComb, x.S,
         sensiHSIC.laplace, file = filename)
285  }else if(length(grep("jansen",varName))
       != 0){
286    save(no.slgeneComb, x.S,
         SB.jansen, file = filename)
287  }else if(length(grep("2002",varName))
       != 0){
289    save(no.slgeneComb, x.S,
         SB.2002, file = filename)
290  }else if(length(grep("2007",varName))
       != 0){
291    save(no.slgeneComb, x.S,
         SB.2007, file = filename)
292  }else if(length(grep("martinez",varName))
       != 0){
293    save(no.slgeneComb, x.S,
         SB.martinez, file = filename)
294  }else if(length(grep("SBL",varName))
       != 0){
295    save(no.slgeneComb, x.S,
         SBL, file = filename)
296  }
```

This ends the coding part of estimating sensitivity indices. Once the indices are ready, they can be used in various ways for the evaluation of the combinations. Here, we use one of the ways to rank these scores. However, note that there is no one definite rule to say that one has to rank in this way only. It depends on the research to decide what method one is employing for ranking. We use the SVM^{Rank} algorithm by[13]. Though complex in nature, it does a fair job of ranking the scores. The use of a machine learning approach is also made available to see how the learning algorithms play a

critical role in revealing unknown/untested combinatorial hypotheses. Other reasons for using these will be stated later on.

5.3.2. Exercise

At this stage, it would be great to see how the two codes on extracting data and generating indices work out. The readers are requested to generate the different types of indices based on their choice and see what comparisons can be made using the different indices. Also, try the following exercises -

1. Generate HSIC rbf indices for 2^{nd} order combinations for downregulated AXIN2. What does the combination of AXIN2 with another factor that has the lowest score mean?
2. Generate FDiv KL indices for 2^{nd} order combinations for downregulated MYC. What does the combination of MYC with another factor that has the highest score mean?
3. Generate HSIC laplace indices for 2^{nd} order combinations for downregulated NKD1. What does the combination of NKD1 with another factor that has a score in the middle mean?
4. Generate FDiv TV indices for 2^{nd} order combinations for downregulated CDAN1. What does the combination of CDAN1 with another factor that has a score at the 100 position in ascending order mean?
5. Generate Sobol indices for 2^{nd} order combinations for downregulated MINA. How many kinds of indices can you generate? Compare them for a particular combination!
6. Generate Sobol jansen indices for 2^{nd} order combinations for downregulated MINA. How many kinds of indices can you generate? Compare them for two different combinations!
7. Generate Sobol martinez indices for 2^{nd} order combinations for downregulated MINA. How many kinds of indices can you generate? Compare them for 20 different combinations!
8. Generate Sobol 2002 and 2007 indices for 2^{nd} order combinations for downregulated MINA. How many kinds of indices can you generate? Compare Sobol 2002 vs Sobol 2007.
9. Compare HSIC rbf, HSIC laplace, FDiv TV, FDiv KL, Sobol, Sobol jansen, Sobol martinez, Sobol 2002, and 2007 indices for downregulated LGR5-RNF43.

5.4. Ranking & Sorting

5.4.1. Description of SVM-Results-S-mean.R

This part of the code is the last in the pipeline that works on the generated sensitivity indices. The code is in the file SVMRank-Results-S-mean.R. It ranks the sensitivity indices using a machine learning algorithm. We go through this part of the code and will then come back to the why's and why not's. Lines 1-16 are basic data processing techniques and some formalities that need to be done before we begin on the ranking part. So, by now, it should be expected that the reader is able to work through the lines and understand what is happening if a following line is being executed, at least, theoretically.

```
1     DATATYPE <- readline("Choose a file
        to process [1/2] \n
2       1 - ../data/onc2015280x2-A.txt \n
3       Genes down-regulated after ETC-159
          treatment \n
4       2 - ../data/onc2015280x2-B.txt \n
5       Genes up-regulated after ETC-159
          treatment \n
6       File number - ")
7     while(DATATYPE != "1" & DATATYPE != "2"){
8       DATATYPE <- readline("Type the kind of
          data to be processed - ")
9     }
10
11    CHOOSE <- as.numeric(readline("pick a
        numeric for k in nCk - "))
12    k <- as.numeric(CHOOSE)
13
14    geneName <- readline("Please enter the
        name of the gene to be processed - ")
15    siNames <- c("Fdiv.TV", "Fdiv.KL",
        "Fdiv.Chi2", "Fdiv.Hellinger", "HSIC.rbf",
        "HSIC.linear", "HSIC.laplace", "SB.2002",
        "SB.2007", "SB.jansen", "SB.martinez",
        "SBL")
16    sa.name <- readline("Please enter the
        name of the proposed SA from above
        list - ")
```

We stored the sensitivity indices in different files. These files need to be accessed in order for the procedure of ranking to be initiated. The following lines help retrieve the file names, which can then be loaded into the R workspace from where they can be accessed easily. To retrieve the file name, the function **paste** is employed. Readers are encouraged to find out how the **paste** function works. After the file name is constructed, the contents of the file are loaded using the **load** function, followed by the assignment of the stored data into a variable *h*. Lines 17-113 show the code for various kinds of indices that a user can access after using **paste** and **load**.

```
17    if(length(grep(sa.name,"Fdiv.TV"))
        != 0){
18      if(DATATYPE == "1"){
19        filename <- paste("order-",k,"-",
            geneName,
            "-DR-A-ETC-T-fdiv-tv-mean.Rdata",
            sep = "")
```


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```
20      }else{
21         filename <- paste("order-",k,"-",
             geneName,
             "-UR-A-ETC-T-fdiv-tv-mean.Rdata",
             sep = "")
22      }
23      load(filename)
24      h <- sensiFdiv.TV
25    }else if(length(grep(sa.name,"Fdiv.KL"))
        != 0){
26      if(DATATYPE == "1"){
27        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-fdiv-kl-mean.Rdata",
          sep = "")
28      }else{
29        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-fdiv-kl-mean.Rdata",
          sep = "")
30      }
31      load(filename)
32      h <- sensiFdiv.KL
33    }else if(length(grep(sa.name,"Fdiv.Chi2"))
        != 0){
34      if(DATATYPE == "1"){
35        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-fdiv-chi2-mean.Rdata",
          sep = "")
36      }else{
37        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-fdiv-chi2-mean.Rdata",
          sep = "")
38      }
39      load(filename)
40      h <- sensiFdiv.Chi2
41    }else if(length(grep(sa.name,"Fdiv.Hellinger"))
        != 0){
42      if(DATATYPE == "1"){
43        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-fdiv-hellinger-mean.Rdata",
          sep = "")
```

```
    }else{
      filename <- paste("order-",k,"-",
      geneName,
      "-UR-A-ETC-T-fdiv-hellinger-mean.Rdata",
      sep = "")
    }
    load(filename)
    h <- sensiFdiv.Hellinger
  }else if(length(grep(sa.name,"HSIC.rbf"))
    !=0){
    if(DATATYPE == "1"){
      filename <- paste("order-",k,"-",
      geneName,
      "-DR-A-ETC-T-hsic-rbf-mean.Rdata",
      sep = "")
    }else{
      filename <- paste("order-",k,"-",
      geneName,
      "-UR-A-ETC-T-hsic-rbf-mean.Rdata",
      sep = "")
    }
    load(filename)
    h <- sensiHSIC.rbf
  }else if(length(grep(sa.name,"HSIC.linear"))
    != 0){
    if(DATATYPE == "1"){
      filename <- paste("order-",k,"-",
      geneName,
      "-DR-A-ETC-T-hsic-linear-mean.Rdata",
      sep = "")
    }else{
      filename <- paste("order-",k,"-",
      geneName,
      "-UR-A-ETC-T-hsic-linear-mean.Rdata",
      sep = "")
    }
    load(filename)
    h <- sensiHSIC.linear
  }else if(length(grep(sa.name,"HSIC.laplace"))
    != 0){
    if(DATATYPE == "1"){
      filename <- paste("order-",k,"-",
      geneName,
      "-DR-A-ETC-T-hsic-laplace-mean.Rdata",
      sep = "")
```

```
68      }else{
69        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-hsic-laplace-mean.Rdata",
          sep = "")
70      }
71      load(filename)
72      h <- sensiHSIC.laplace
73    }else if(length(grep(sa.name,"SB.2002"))
        != 0){
74      if(DATATYPE == "1"){
75        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-sb-2002-mean.Rdata",
          sep = "")
76      }else{
77        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-sb-2002-mean.Rdata",
          sep = "")
78      }
79      load(filename)
80      h <- SB.2002
81    }else if(length(grep(sa.name,"SB.2007"))
        != 0){
82      if(DATATYPE == "1"){
83        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-sb-2007-mean.Rdata",
          sep = "")
84      }else{
85        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-sb-2007-mean.Rdata",
          sep = "")
86      }
87      load(filename)
88      h <- SB.2007
89    }else if(length(grep(sa.name,"SB.jansen"))
        != 0){
90      if(DATATYPE == "1"){
91        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-sb-jansen-mean.Rdata",
          sep = "")
```

```
92      }else{
93        filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-sb-jansen-mean.Rdata",
          sep = "")
94      }
95      load(filename)
96      h <- SB.jansen
97    }else if(length(grep(sa.name,"SB.martinez"))
        != 0){
98      if(DATATYPE == "1"){
99        filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-sb-martinez-mean.Rdata",
          sep = "")
100     }else{
101       filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-sb-martinez-mean.Rdata",
          sep = "")
102     }
103     load(filename)
104     h <- SB.martinez
105   }else if(length(grep(sa.name,"SBL"))
        != 0){
106     if(DATATYPE == "1"){
107       filename <- paste("order-",k,"-",
          geneName,
          "-DR-A-ETC-T-sbl-mean.Rdata",
          sep = "")
108     }else{
109       filename <- paste("order-",k,"-",
          geneName,
          "-UR-A-ETC-T-sbl-mean.Rdata",
          sep = "")
110     }
111     load(filename)
112     h <- SBL
113   }
```

Once the indices that have to be worked on have been put in *h*, the indices need to be averaged. For demonstration purposes, we average only 2 iterations and see how things turn out. However, we need to understand how the data is stored in *h*. Figure 3 shows a screenshot of how an element of *h* looks. *h[[25]]* is the 25^{th} element and is a matrix of size 2×2743. 2 is the number of elements in a combination under consideration and 2743 are the total number of distinct 2^{nd} order combinations. We exploit this view of *h* to compute the means for all elements of a combination and over all distinct

combinations. This is done in the **for** loops below in which *p* iterates over elements of the combination and *itr* iterates over the total number of iterations. Thus *h[[itr]][p,]* between the two nested **for** loops considers the p^{th} row of the itr^{th} matrix in *h*. Then, *r* binds all the *h[[1]][p,]*, *h[[2]][p,]*, ..., *h[[itrNo]][p,]*, using **rbind**. After exiting the inner loop, we use the **apply** function to the *r* matrix over the columns (i.e., the distinct combinations) with a function **mean**. Thus we get a vector of mean values of the sensitivity index for each distinct combination over all iterations, for the p^{th} element. This process is again repeated for the next *p* value. Finally, the vector of means is stacked in *SAmean*. Lines 114-124 show this coding below.

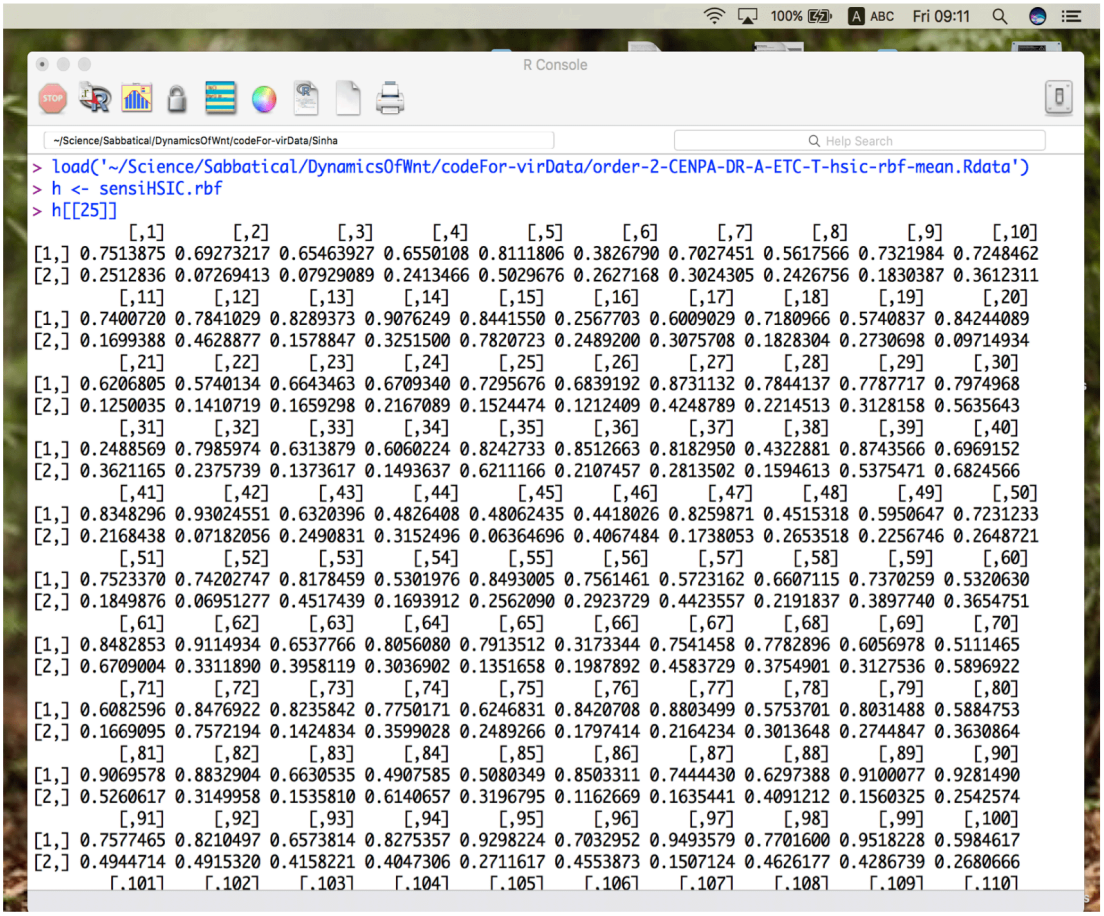

Figure 3. Screenshot of data loaded using the **load** function and the assignment of the stored sensitivity indices to the variable *h*.

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```
114   # Use this for averaged ranking
115   # itrNo <- 50
116   itrNo <- 2
117   SAmean <- c()
118   for(p in 1:k){
119     r <- c()
120     for(itr in 1:itrNo){
121       r <- rbind(h[[itr]][p,])
```

```
1 1:0.626364377309334 2:0.527505676513961 3:0.113762327838243
 2 1:0.115627329301083 2:0.70140375947702 3:0.555882270527448
 3 1:0.544876192378511 2:0.352285442741931 3:0.370635265560798
 4 1:0.192361398371323 2:0.310239010742538 3:0.49204266288273
 5 1:0.58285348514642 2:0.281413460065595 3:0.327493744822505
 6 1:0.351034419102818 2:0.343384617420989 3:0.148505969101492
 7 1:0.782560172370969 2:0.345140938098646 3:0.177258473888461
 8 1:0.630907570829286 2:0.56855192811936 3:0.138584623911373
 9 1:0.216195532101444 2:0.414543916226165 3:0.113980668309361
 10 1:0.283895945186652 2:0.361780765317981 3:0.366435248118148
 11 1:0.16287050503857 2:0.868613911042874 3:0.0989378976820292
 12 1:0.561850003081091 2:0.541890862307686 3:0.23980018742167
 13 1:0.277823319222624 2:0.410498428589253 3:0.149938817070323
 14 1:0.639343729150494 2:0.210620804117997 3:0.318994509227185
 15 1:0.579922798562767 2:0.169151589969098 3:0.505153429556521
 16 1:0.255426206049244 2:0.19861791055204 3:0.603574373800882
 17 1:0.638429174520452 2:0.210968681381445 3:0.31558414172227
 18 1:0.308210498731067 2:0.315067213362584 3:0.369723298974591
 19 1:0.232194144603514 2:0.0899698653586265 3:0.433147306693618
 20 1:0.219438264672644 2:0.237701371516819 3:0.565164770668834
 21 1:0.314375908725559 2:0.0610464637955092 3:0.626864567521388
 22 1:0.714942050999006 2:0.315230485087249 3:0.117059293878145
 23 1:0.181503219052467 2:0.208940762198194 3:0.566116193159678
 24 1:0.344060825165414 2:0.274995877172336 3:0.464454457596415
 25 1:0.531735675357153 2:0.329899209905158 3:0.162742316265039
 26 1:0.403603998882616 2:0.557601792454423 3:0.346263613235206
 27 1:0.249735943401084 2:0.200509610657229 3:0.583767751340706
 28 1:0.473426972917293 2:0.134940754963889 3:0.633285486398736
 29 1:0.400155830984346 2:0.491206008718229 3:0.103962327133218
 30 1:0.22660716984713 2:0.632930663188795 3:0.369777827505766
```

Figure 4. Screenshot of data after transformation in lines 125-136. This is an example of a 3rd order combination.

```
122     }
123     SAmean <- rbind(SAmean, apply(X = r,
          MARGIN = 2, mean))
124   }
```

Next, we format the data in the form that is suitable for the SVM^{rank} machine. The output of the next block of code can be depicted in figure 1. Note that it is a screenshot of how the data is saved for a 3^{rd} order combination. I invite readers to decode the following block by themselves as an exercise.

```
125   # y <- SA
126   y <- SAmean
127   data <- c()
128   for(i in 1:no.slgeneComb){
129     z <- c()
130     z <- cbind(z,i)
131     for(j in 1:k){
132       z <- cbind(z,
            paste(j,":",y[j,i],sep=""))
133     }
134     z <- cbind(z,"\n")
135     data <- rbind(data,z)
136   }
```

Next, the file names for training, testing, model, and prediction are built using paste (see lines 137-140). And finally, data is concatenated to the training and the testing files (see lines 141-142).

```
137   trnfl <- paste("svr-trn-order-",k,
        "-",geneName,".txt", sep = "")
138   tstfl <- paste("svr-tst-order-",k,
        "-",geneName,".txt", sep = "")
139   mdlfl <- paste("svr-mdl-order-",k,
        "-",geneName,".txt", sep = "")
140   predfl <- paste("svr-pred-order-",k,
        "-",geneName,".txt", sep = "")

141   cat(t(data),file=trnfl)
142   cat(t(data),file=tstfl)
```

We now come to the main part of the code, which involves using the support vector ranking algorithm. Since it is a compiled executable file, it needs to be executed using a **system** command. However, before that, the command that needs to be executed must be prepared in the right format. For this, the **paste** command is used (see line 144). In the paste command, svm_rank_learn takes in the C value in the form of $-c20$; $-\#100$ to terminate svm-light QP subproblem optimization if no progress is made after this number of iterations; $-n9$ is the number of new variables entering the working set in each svm-light iteration (default $n = q$) : Set $n < q$ to prevent zig-zagging : We set n to 9 considering the number of samples generated from the distribution; the training file and the model file. Finally, we use the built-up command in the **system** function (see line 145).

```
143   # svm rank learn on C value - 20
144   cmd <- paste(
        "./../svm_rank/svm_rank_learn
        -c 20 -# 100 -n 9",trnfl,mdlfl,sep=" ")
145   system(command=cmd)
```

A similar format is used to classify the test file once the model has been prepared using the *svm_rank_learn*. This is achieved using the *svm_rank_classify*. Note that when there is only one instance of each training data, then after the model is built on it for ranking purposes, we use the same training as the testing file. This might sound strange at first view; however, the model should be able to rank the scores based on the original data. It is not a hard and fast rule to rank through SVMs, but here we show an example of the same. One can use a completely different algorithm also for the same set of training data. Ranking is done in the next lines 146-148.

```
146   # svm rank classify
147   cmd <- paste(
        "./../svm_rank/svm_rank_classify",
        tstfl,mdlfl,predfl,sep=" ")
148   system(command=cmd)
```

Once the ranking is done, the data in the prediction file is stored in a variable via the **read.table** function and later stored in an appropriate file. Again, the file name needs to be constructed. See lines 149-156.

```
149   # read predictions of rankings
150   dataScore <- read.table(predfl)

151   # save it in appropriate file
152   if(DATATYPE == "1"){
153     save(dataScore,file=paste("order-",k,
        "-SA-",sa.name,"-",geneName,
        "-rankingScore-mean-DR.R",sep=""))
154   }else{
155     save(dataScore,file=paste("order-",k,
        "-SA-",sa.name,"-",geneName,
        "-rankingScore-mean-UR.R",sep=""))
156   }
```

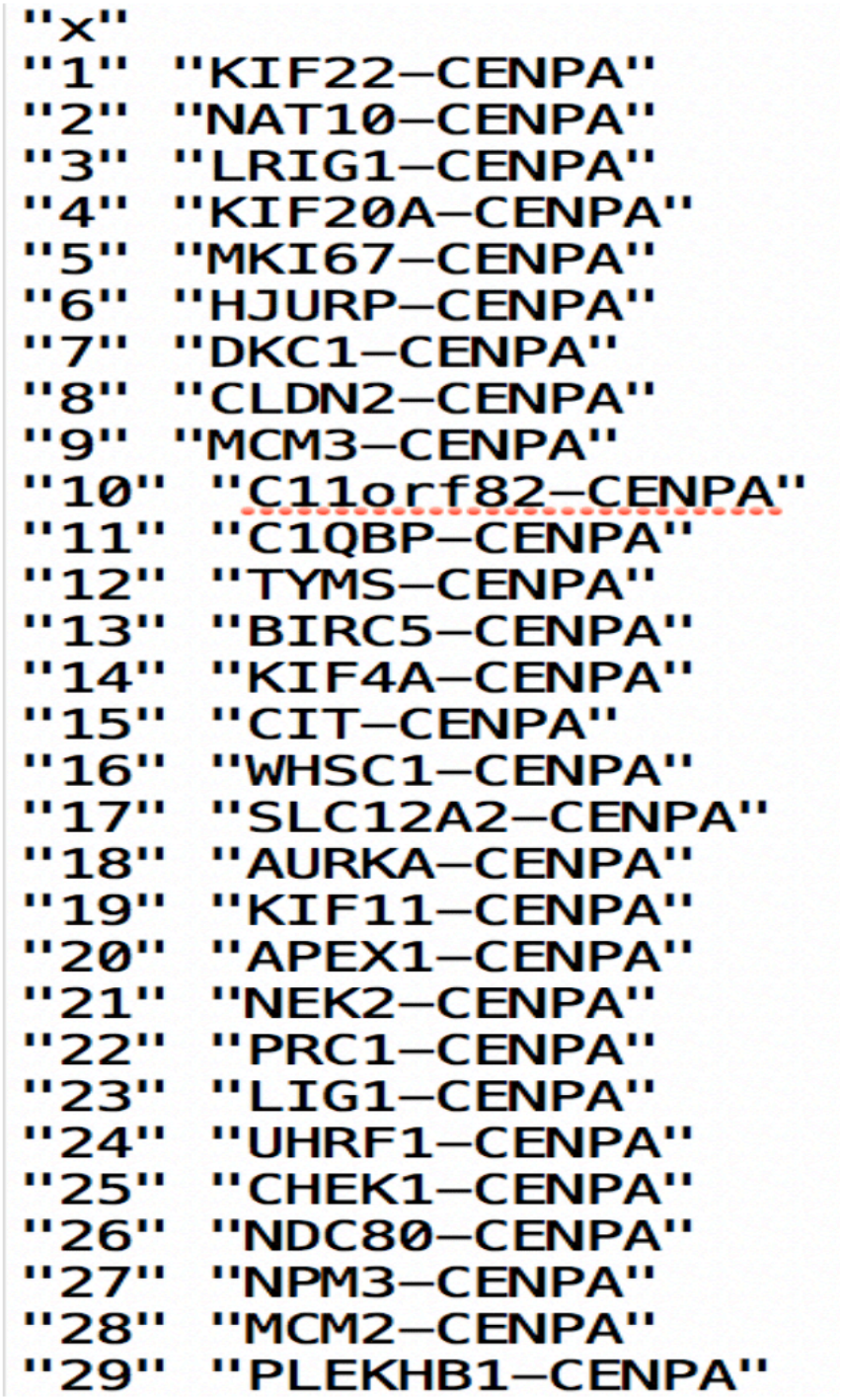
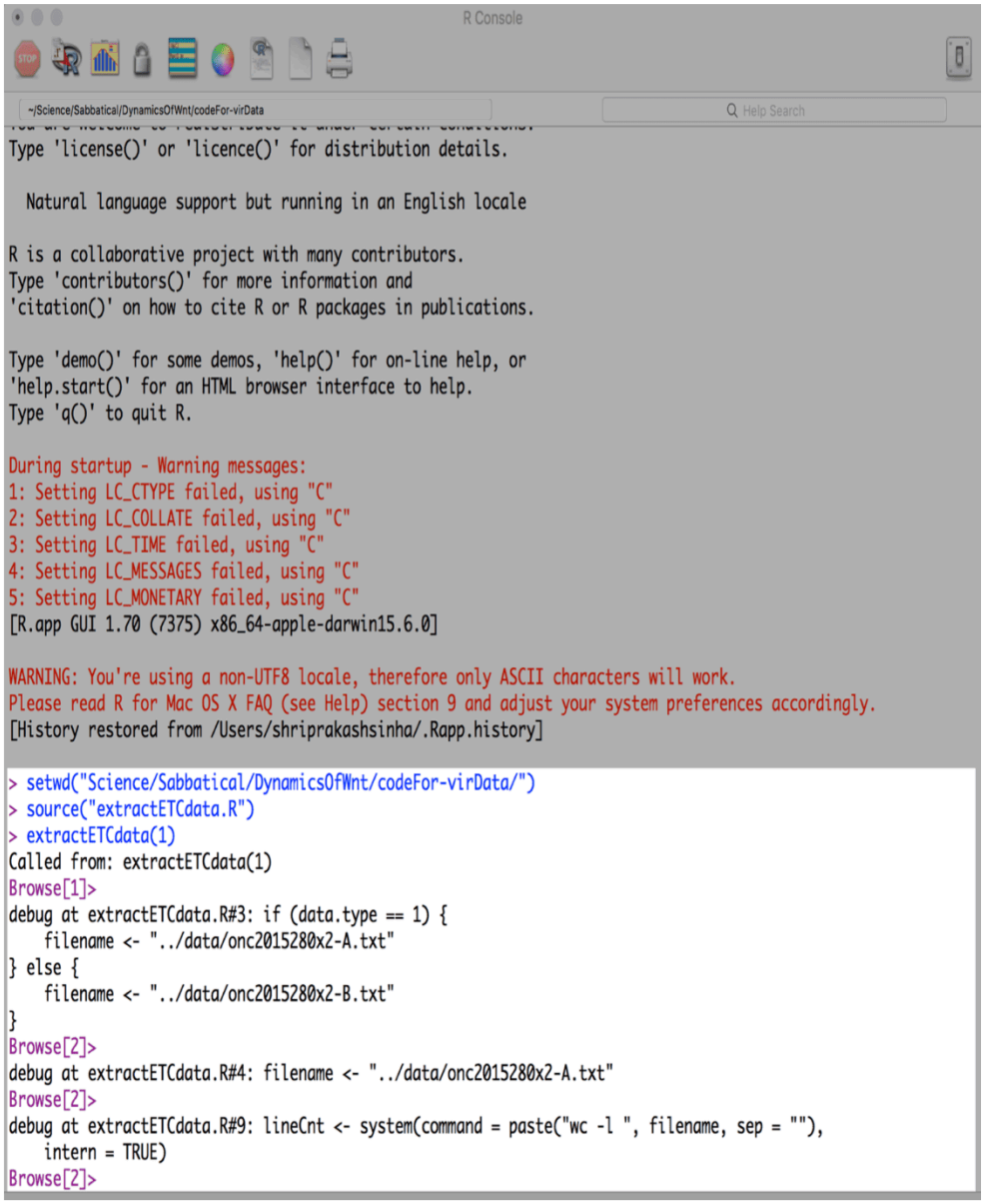


Figure 5. Screenshot of ranked combinations.

5.4.2. *Exercise*

Please download the SVM^{rank} and, as instructed on the website of[13], compile the same to get executable files.



5.4.3. *Sorting*

Finally, we sort the predicted results. These are expressed in the last block from lines 157-172. *dataScore*, which contained the predictions, is sorted using the **sort** function, and the indices of the sorted values are also returned (see line 159). Next, using the **for** loop, we arrange the names of the

combinations in a sorted order using the sorted index from line 159. These sorted combinations are appended to *sortedGenecomb* (see lines 161-167). Finally, we store these results according to the type of data we are dealing with.

```
158   cat("sorting in ascending order
        - top is lowest in ranking")
159   dataScore <- sort(dataScore$V1,
        index.return=TRUE)
160   noCombs <- length(dataScore$ix)

161   # arrange the combinations by ranking
162   sortedGenecomb <- c()
163   for(i in 1:noCombs){
164     idx <- dataScore$ix[i]
165     z <- capture.output(cat(
          names(x.S[[idx]]),sep="-"))
166     sortedGenecomb <- c(sortedGenecomb, z)
167   }
168   if(DATATYPE == "1"){
169     write.table(sortedGenecomb,
          file=paste("order-",k,"-SA-",
          sa.name,"-",geneName,
          "-ranking-mean-DR.txt",sep=""))
170   }else{
171     write.table(sortedGenecomb,
          file=paste("order-",k,"-SA-",
          sa.name,"-",geneName,
          "-ranking-mean-UR.txt",sep=""))
172   }
```

The sorted combinations look like those in figure 2. This finishes the general framework of the pipeline.

6. Code surgery via browser scalpel

R provides a **browser** function which can help one to see the contents of the variables and intermediate outputs once the execution of the code has begun. The function is like a scalpel which helps dissect the entire code as the execution proceeds one step at a time. Various functionalities exist to use the browser function. These can be seen by typing **?browser** at the command prompt. Briefly - • **c** exits the browser and continues execution at the next statement. • **f** finishes execution of the current loop or function and helps print this list of commands. • **n** evaluates the next statement, stepping over

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function calls. For byte-compiled functions interrupted by browser calls, n is equivalent to c. • **s** evaluates the next statement, stepping into function calls. Again, byte-compiled functions make s equivalent to c. • **where** prints a stack trace of all active function calls. • **r** invokes a "resume" restart if one is available; interpreted as an R expression otherwise. Typically, "resume" restarts are established for continuing from user interrupts. • **Q** exits the browser and the current evaluation and returns to the top-level prompt.

As a small example, using the browser function on the **extractETCdata** function is depicted in the snapshot in figure 6. Especially note the output of the browser function in the white patch in the above figure. What we find is that at each press of the "return" or "enter" button on the computer, a following line appears (an instance shown here is where the execution of the browser had reached in the code in extractETCdata.R)-

```
> source("extractETCdata.R")
> extractETCdata(1)
Called from: extractETCdata(1)
Browser[1]>
debug at extractETCdata.R#3: if (data.type == 1){
    filename <- "../data/onc2015280x2-A.txt"
} else {
    filename <- "../data/onc2015280x2-B.txt"
}
Browser[2]>
.
.
```

What is happening is that we inserted a browser function in extractETCdata.R just after *extractETCdata* $<-$ *function(data.type){*. After compiling extractETCdata.R using the **source** function and executing **extractETCdata(1)** at the R prompt $>$, the **browser** function comes into effect. This is shown with an additional *Browser[]*$>$. Evidence of this is provided by the line stating the following - "Called from: extractETCdata(1)". Next, on pressing "return", the execution moves to the next command that it needs to execute. This is denoted by the debug function, and the line reads as "debug at extractETCdata.R#3:", meaning that the execution is waiting at command 3 in the code. Along with it, the whole command that needs to be executed in one go is also presented. Here it is the **if**(*data.type* == 1) {...} **else** {...} command. Since the *data.type == 1* is true, the command *filename* $<-$ *../data/onc201528ox2-A.txt"* is executed. This is shown in the next line that the browser has to execute when the above condition holds true. See figure 6.

6.1. Exercise

Please use the **browser** function to inspect the values in the variables and see how the code executes for extractETCdata.R and manuscript-2-2.R.

Results from the search engine can be found in the recently unpublished preprint in[14].

7. MYC-HOXB8-EZH2

EZH2 encodes enhancer of zeste homolog 2 and is involved in transcriptional repression via epigenetic modifications. It has been found to be either mutated or over-expressed in many forms of cancer. Overexpression of EZH2 leads to the silencing of various tumor suppressor genes, thus implicating it in potential roles in tumorigenesis[15]. EZH2 is a subunit of the highly conserved Polycomb repressive complex 2 (PRC2), which executes the methylation of the histone H3 at lysine-27[16]. Thus, targeting EZH2 has become a major research domain for cancer therapeutics[17]. In colon cancer, it has been shown that depletion of EZH2 has led to the blocking of proliferation of the cancer[18]. This indicates the fact that tumor suppressor genes get activated and lead to the subsequent blocking of the cancer. Also, EZH2 is recruited by PAF to bind with β-catenin transcriptional complex for further Wnt target gene activation, independent of the EZH2 epigenetic modification activities[19].

Consistent with this, ETC-15922159 treatment led to downregulation of EZH2 in colorectal cancer samples[3]. This would have activated a lot of tumor suppressor genes that led to subsequent suppression of regrowth in treated cancer samples. More importantly, MYC directly upregulates core components of PRC2, EZH2 being one of them, in embryonic stem cells[20].[20] This shows that silencing of c-MYC and N-MYC[21] led to a reduction in the expression of PRC2 and thus EZH2. Furthermore, in colorectal cancer cases,[22], it was shown that knockdown of MYC led to a decrease in EZH2 levels. Similar findings have been observed in[23],[24] &[25]. Our in silico findings show consistent results with respect to this downregulation after assigning a low rank of 54 along with MYC-HOXB8.

More specifically, our in silico pipeline is able to approximate the value of the 3^{rd} order combination of MYC-HOXB8-EZH2 by assigning a rank that is consistent with wet lab findings of dual combinatorial behaviour of MYC-EZH2 and MYC-HOXB8. However, since the mechanism of combination of MYC-HOXB8 is not known hitherto, it would be interesting to confirm the behaviour of MYC-HOXB8-EZH2

at 3^{rd} order to reveal a portion of the Wnt pathway's modus operandi in colorectal cancer. Further wet lab tests on these in silico findings will confirm the efficacy of the search engine.

Availability and requirements

- Project name - R Code for Machine learning search engine: Ranks/reveals combination of genes/proteins using ETC-1922159 treated CRC static data
- Project home page - https://zenodo.org/records/14636112
- Operating system(s) - Platform independent
- Programming language - R statistical language[26]
- Other requirements - (1) SVM^{Rank} from https://www.cs.cornell.edu/people/tj/svm_light/svm_rank.html and (2) Sensitivity package in R from https://cran.r-project.org/web/packages/sensitivity/index.html
- License - Creative Commons Attribution 4.0 International

Statements and Declarations

- Funding - No institute/university/NGO/company (private/public)/government organization was involved. The project was carried out on personal funds.
- Conflict of interest/Competing interests - There are no conflicts/competing interests to declare.
- Ethics approval and consent to participate - Not applicable.
- Consent for publication - Not applicable.
- Data availability - Data used in this research work has been released online publicly, in a publication[3]. This data was made available in the form of a supplementary table. Related to this data, on the NCBI Gene Expression Omnibus (GEO) Series GSE69687 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69687, click on the **Download RNA-seq counts** button; it opens a page that contains the **Human gene annotation table** (at the bottom of the page). The file **Human.GRCh38.p13.annot.tsv.gz** contains the range of genes with EnsemblGeneID, all starting with ENSG. This ENSG identifier is used to index the recording of the regulated genes in the data made available in the supplementary table in[3]. The data itself is available as supplementary material in the journal; however, the indexing of the genes used in the supplementary material is available on the NCBI NIH database.

- Code availability - The code of the search engine used to generate the rankings has been made available on CERN-based Zenodo at https://zenodo.org/records/14636112.
- Author contribution - (1) Concept, design, in silico implementation. (2) Analysis and interpretation of results. (3) Manuscript writing/revision/approval.
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Declarations

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