

NetLCP Tutorial

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Introduction

Numerous independent networks of regulatory elements including lncRNA, circRNA and pathway have been developed to crucial roles in computational system biology. Crosstalk among those networks as a bridge to construct and decode heterogenous network aids to acquire bio-meaningful information from multidimensional biological knowledge. We introduce NetLCP, an R package with command and shiny-based GUI modes, for customizing prioritization of regulatory elements in heterogenous network with low noisy and detecting variant ‘switch’ in local area of network, which highlights interested regulatory elements or potential dysfunction. The latest version of NetLCP can be downloaded in <https://github.com/mortyran/NetLCP>. Here, we provide the whole example workflow related to cellular senescence.

Installation

```
# Method 1
library(devtools)
devtools::install_github("mortyran/NetLCP")

# Method 2
BiocManager::install('githubinstall')
library(githubinstall)
githubinstall("NetLCP")
```

Example workflow

1. Data preparation

```
cs_url = "C:\\Users\\acer\\Desktop\\NetLCP\\Paper\\Tutorial\\CellularSenescenceData"
cs_data = read.table(cs_url, sep = "\t", header = T)
head(cs_data)
```

1.1 First, we read in the cellular senescence gene set as example transcriptome from github.

	Symbol	Type	Trans_ID
1	AKT1	cellular senescence	207
2	ATF2	cellular senescence	1386
3	BDNF	cellular senescence	627

4	CDK1 cellular senescence	983
5	CDKN2A cellular senescence	1029
6	CLU cellular senescence	1191

1.2 Initialize NetLCP.

1.2.1 (Recommended) Manually download data from internet depository and initialize NetLCP. After acquiring the file named “NetLCPData.tar.gz” from <https://www.dropbox.com/s/4dqtxecpw79iejx/NetLCPData.tar.gz> or <http://hainmu-biobigdata.com/NetLCP/NetLCPData.tar.gz>, please following steps:

Step1: Get the NetLCP package preset data directory.

```
NetLCP_file_path = system.file("extdata", package = "NetLCP")
```

Step2: Copy the downloaded file “NetLCPData.tar.gz” to the directory.

```
# your_file_path is the file path of "NetLCPData.tar.gz" in your computer.
file.copy(your_file_path, NetLCP_file_path)
```

Step3: Run dataInitialize() to initialize the data in R.

```
library(NetLCP)
```

NetLCP,

More information can be found at <https://github.com/rmyhandsome/NetLCP>

If you use NetLCP in you publication, please cite this publication:

NetLCP: An R package for customizing prioritization of regulatory elements in heterogeneous network with

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Please read the tutorial in <https://mortyran.github.io/NetLCP/> for data preparation before you use NetLCP

```
dataInitialize()
```

```
[1] "Data initialization has been finished!"
```

```
library(NetLCP)
dataInitialize()
```

1.2.2 Automatically initialize NetLCP, this will depend on your network connection.

2. Prioritize the biological elements.

Prioritize the biological elements in heterogenous network by input transcriptome (mRNA/miRNA, but mixed miRNA and mRNA is highly recommended). NetLCP supports miRBase ID for miRNA and Entrez ID for mRNA. The parameter **transcriptomeList** is the input transcriptome. The **prioType** represents the biological element type, which currently contains lncRNA, circRNA and pathway(KEGG, Reactome and Wikipathway). **empiricalPvalue** is alternative for an empirical p.value through random disturbance, default value is FALSE(it could take several hours).

```
lncRNA_prio = BioRegElePrioritization( transcriptomeList = cs_data$Trans_ID,
                                       prioType = "lncRNA",
                                       empiricalPvalue = FALSE )
```

2.1 lncRNA prioritization

```
[1] "Filtering the missing elements of transcriptomeList in the input network....."
[1] "Element 10934/7012 have been filtered....."
[1] "Now remain 367"
[1] "Prioritization begins, please wait while we do something....."
[1] "Prioritization finished....."
```

```
head(lncRNA_prio)
```

	NodeName	Ranking	OfficialName	NorRiskScore
1	ENSG00000229807	1	XIST	5.808779
2	ENSG00000247556	2	OIP5-AS1	5.377587
3	ENSG00000251562	3	MALAT1	5.117147
4	ENSG00000225733	4	FGD5-AS1	4.810600
5	ENSG00000230551	5	<NA>	4.662645
6	ENSG00000231074	6	HCG18	4.474082

```
circRNA_prio = BioRegElePrioritization( transcriptomeList = cs_data$Trans_ID,
                                       prioType = "circRNA",
                                       empiricalPvalue = FALSE )
head(circRNA_prio)
```

2.2 circRNA prioritization

```
KEGG_prio = BioRegElePrioritization( transcriptomeList = cs_data$Trans_ID,
                                       prioType = "KEGG",
                                       empiricalPvalue = FALSE )
head(KEGG_prio)
```

2.3 KEGG pathway prioritization

```
Reactome_prio = BioRegElePrioritization( transcriptomeList = cs_data$Trans_ID,
                                       prioType = "Reactome",
                                       empiricalPvalue = FALSE )
```

2.4 Reactome pathway prioritization

```
[1] "Filtering the missing elements of transcriptomeList in the input network....."
[1] "Element 10934/7012 have been filtered....."
[1] "Now remain 367"
[1] "Prioritization begins, please wait while we do something....."
[1] "Prioritization finished....."
```

```
head(Reactome_prio)
```

	NodeName	Ranking	OfficialName
1	R-HSA-2559580	1	Oxidative Stress Induced Senescence
2	R-HSA-8953750	2	Transcriptional Regulation by E2F6
3	R-HSA-69202	3	Cyclin E associated events during G1/S transition
4	R-HSA-2559585	4	Oncogene Induced Senescence
5	R-HSA-8943724	5	Regulation of PTEN gene transcription
6	R-HSA-3108214	6	SUMOylation of DNA damage response and repair proteins

	NorRiskScore
1	5.628055
2	4.593379
3	4.026669
4	3.919940
5	3.813643
6	3.621810

```
Wikipathway_prio = BioRegElePrioritization( transcriptomeList = cs_data$Trans_ID,
                                             prioType = "Wikipathway",
                                             empiricalPvalue = FALSE )
head(Wikipathway_prio)
```

2.5 Wikipathway pathway prioritization

3. Inspect local area of heterogenous network

Current regulation types in local area of heterogenous network include binary elements regulation and multiple elements regulation. NetLCP will map the experimentally verified interactions between input biological elements to the local area of heterogenous network. NetLCP supports Ensembl ID for lncRNA, miRBase ID for miRNA, Entrez ID for mRNA, KEGG ID, Reactome ID, Wikipathway ID for pathway.

Here we explore the regulation among the top 10 of lncRNA and Reactome prioritization results and example transcriptome in local area.

```
# Input data preparation
lncRNA_top10 = lncRNA_prio$NodeName[1:10]
Reactome_top10 = Reactome_prio$NodeName[1:10]
local_elements = c(lncRNA_top10, Reactome_top10, cs_data$Trans_ID)
```

3.1 Binary elements regulation Inspect the binary elements regulation in the local area of heterogenous network. **elementList** represents the biological elements in the interested local area of heterogenous network. **regulationType** represents the regulation type in local area including circRNA-miRNA, lncRNA-miRNA, lncRNA-mRNA, miRNA-mRNA, miRNA-pathway, mRNA-pathway. **allRegulation** is a logical value. If

you set “FALSE”, it will return the regulation in the local area of heterogenous network which only contains biological elements in the input `elementList`, i.e. `local_elements`. If you set “TRUE”, it will search the regulation in the whole depository, which means the results can contain other biological elements. In this case, you can regard as NetLCP as an independent depository to only extract associated regulatory data. “FALSE” (default) is a common setting.

```
# miRNA-mRNA regulation in local area
bi_local_miRNA_mRNA = binaryRegulation( elementList = local_elements,
                                         regulationType = "miRNA-mRNA",
                                         allRegulation = FALSE )

# miRNA-pathway regulation in local area
bi_local_miRNA_pathway = binaryRegulation( elementList = local_elements,
                                           regulationType = "miRNA-pathway",
                                           allRegulation = FALSE )
```

3.2 Multiple elements regulation Inspect the multiple elements regulation in the local area of heterogenous network. `regulationType` represents the regulation type in local area including lncRNA-miRNA-mRNA, circRNA-miRNA-mRNA, lncRNA-miRNA-mRNA-pathway and circRNA-miRNA-mRNA-pathway. Other parameters are the same as `binaryRegulation` function. Here we concentrate on the lncRNA-miRNA-mRNA-pathway regulation in the local area.

```
multi_local = multieleRegulation( elementList = local_elements,
                                  regulationType = "lncRNA-miRNA-mRNA-pathway",
                                  allRegulation = FALSE )
```

```
[1] "Filtering the missing input elements in input network....."
[1] "10934/7012 have been filtered....."
[1] "Now remain 387"
[1] "Multielement regulation extraction begins, please wait while we do something....."
```

```
head(multi_local)
```

	node1	node2	source
1:	MIMAT0000062	ENSG00000255717	LncBaseExperimental
2:	MIMAT0000449	ENSG00000251562	LncBaseExperimental;RAIN
3:	MIMAT0000691	ENSG00000231074	NPInter
4:	MIMAT0000691	ENSG00000270604	NPInter
5:	MIMAT0000681	ENSG00000269821	LncBaseExperimental;NPInter;RAIN
6:	MIMAT0000245	ENSG00000231074	LncBaseExperimental;lncNASNP2;ENCORI

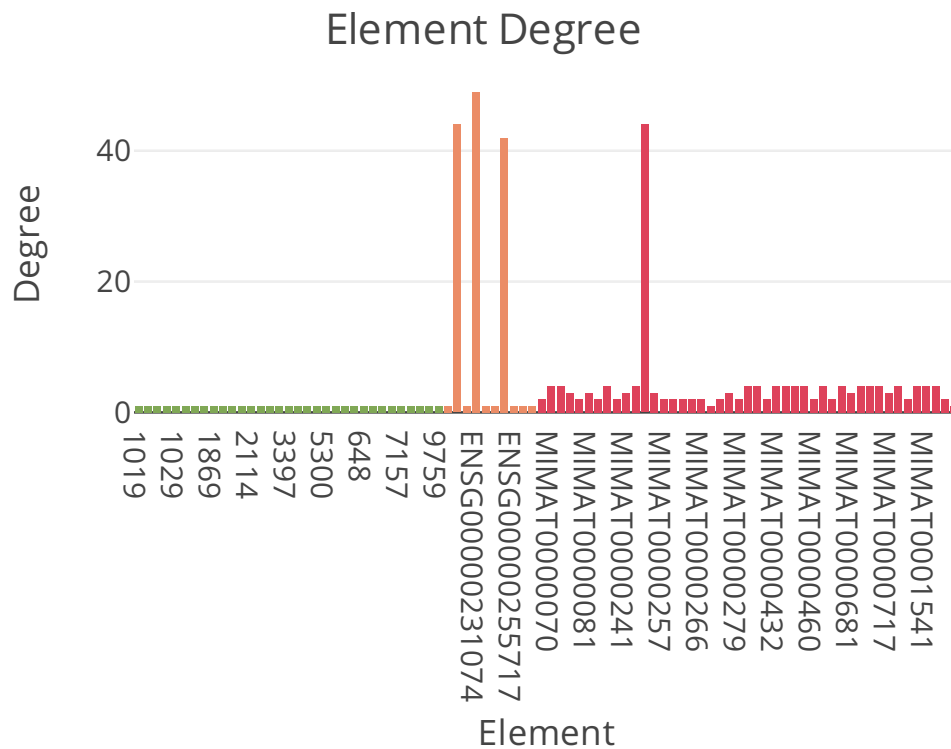
	regType
1:	lncRNA-miRNA
2:	lncRNA-miRNA
3:	lncRNA-miRNA
4:	lncRNA-miRNA
5:	lncRNA-miRNA
6:	lncRNA-miRNA

3.3 Biological elements statistics You can calculate the degree of biological elements in the local heterogenous network and customize the network visualization of results.

`regData` is the standard output of `binaryRegulation` or `multieleRegulation` functions, `filterDegree` means filtering the nodes which are less than it. `selectNode` represents certain or a group of elements

you specify. **netLayout** is the alternative layout of network, “layout_nicely” or “layout_in_circle” (If the network is too large to exhibit, try to use this layout).

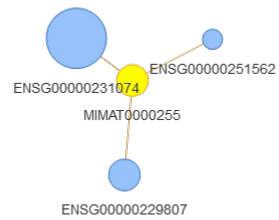
```
# Calculate the degree of biological elements in the local heterogenous network.
regStat( regData = multi_local,
         filterDegree = 40,
         selectNode = NULL )
```



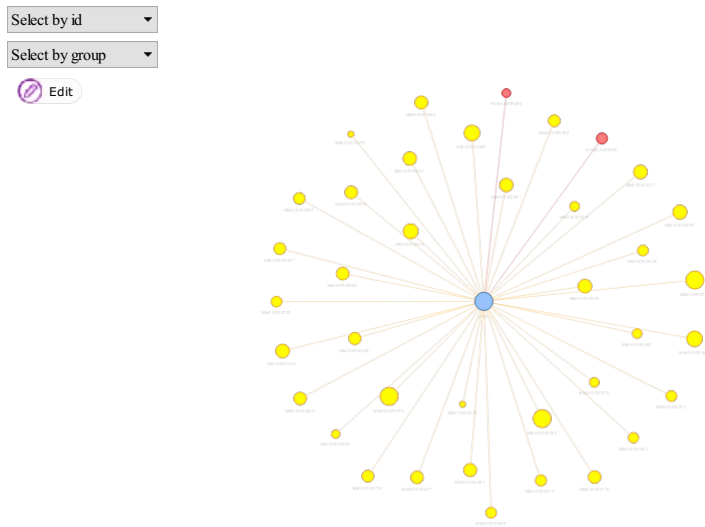
```
# network visualization.
regNetVis( regData = multi_local,
          filterDegree = 40,
          selectNode = NULL,
          netLayout = "layout_nicely" )
```

Select by id ▼

Select by group ▼



```
# if you want to see the associated regulation of the biological  
# element "4193" in local area of heterogeneous network.  
regNetVis( regData = multi_local,  
            filterDegree = 40,  
            selectNode = "4193",  
            netLayout = "layout_nicely" )
```



4. Detecting variant ‘switch’ on local area of heterogenous network.

Variant ‘switch’ includes eQTLs of single biological element and the regulatory variants on its binding site.

4.1 eQTLs of single biological elements and relative statistics.

4.1.1 Detect the eQTLs in the local area of heterogenous network. `regData` is the standard output of `binaryRegulation` or `multieleRegulation` functions.

```
eQTLsData = eQTLsDetection(regData = multi_local)
```

```
[1] "Single biological elements eQTLs extracting extracting begins....."
```

```
head(eQTLsData)
```

	Elements	SNPID	Location	Source	RegType	DataSource
1:	207	rs149007767	chr7:50370254	blood	trans-eQTLs	eQTLGen
2:	1026	rs12589699	chr14:69212885	blood	trans-eQTLs	eQTLGen
3:	1026	rs10512472	chr17:33884804	blood	trans-eQTLs	eQTLGen
4:	1026	rs11653357	chr17:33923607	blood	trans-eQTLs	eQTLGen
5:	1026	rs16971217	chr17:33944055	blood	trans-eQTLs	eQTLGen
6:	1026	rs1465788	chr14:69263599	blood	trans-eQTLs	eQTLGen

4.1.2 eQTLs statistics You can perform statistics on the eQTLs of single elements or regulation and customize the network visualization of results. **regData** is the standard output of **binaryRegulation** or **multieleRegulation** functions, **eQTLsData** is the standard output of “eQTLsDetection”. **regulationType** represent the regulation type of **regData**, which supports circRNA-miRNA, lncRNA-miRNA, lncRNA-mRNA, miRNA-mRNA, miRNA-pathway, mRNA-pathway, circRNA-miRNA-mRNA, lncRNA-miRNA-mRNA, miRNA-mRNA-pathway, lncRNA-miRNA-mRNA-pathway and circRNA-miRNA-mRNA-pathway. **filterDegree** means filtering the nodes which are less than it. **selectNode** represents a group of elements you specify. **netLayout** is the alternative layout of network, “layout_nicely” or “layout_in_circle” (If the network is too large to exhibit, try to use this layout).


```
# count the eQTLs of single elements.
eQTLsSingleEleStat( regData = multi_local,
                    eQTLsData = eQTLsData,
                    filterDegree = 50,
                    selectNode = NULL )
```

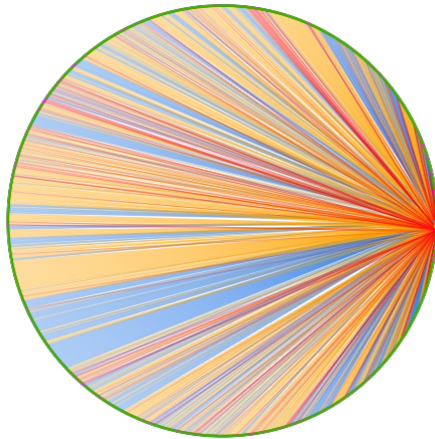
```
# count the eQTLs of multiple elements on regulation.
eQTLsRegStat( regData = multi_local,
              eQTLsData = eQTLsData,
              regulationType = "lncRNA-miRNA-mRNA-pathway",
              filterDegree = 30,
              selectNode = NULL )
```

```
# network visualization.
eQTLsNetVis( regData = multi_local,
             eQTLsData = eQTLsData,
             filterDegree = 30,
             selectNode = NULL,
             netLayout = "layout_in_circle" )
```

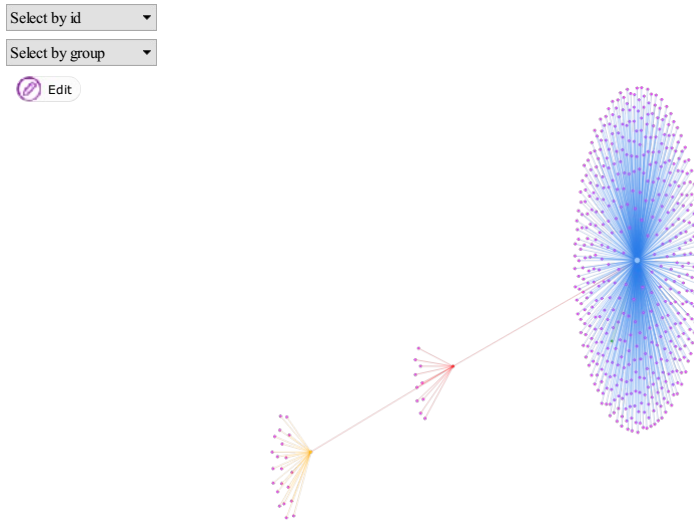
Select by id ▾

Select by group ▾

 Edit



```
# Give that you highlight the regulation "ENSG00000251562 - MIMAT0000081 - 4193 - R-HSA-2559580"
# by statistics, you want to concentrate on the eQTLs of the biological elements in this regulation.
eQTLsNetVis(regData = multi_local,
             eQTLsData = eQTLsData,
             filterDegree = 30, # this parameter will be automatically ignored when setting "selectNode"
             selectNode = c("ENSG00000251562", "MIMAT0000081", "4193", "R-HSA-2559580"),
             netLayout = "layout_nicely")
```



4.2 Regulatory variants on the binding site of biological elements

4.2.1 Detect the regulatory variants on the binding site of biological elements in the local area of heterogenous network `regData` is the standard output of `binaryRegulation` or `multi-eleRegulation` functions. `regulationType` represent the regulation type of `regData`, which supports “miRNA-mRNA”, “miRNA-mRNA-pathway”, “lncRNA-miRNA-mRNA”, “circRNA-miRNA-mRNA”, “lncRNA-miRNA-mRNA-pathway” or “circRNA-miRNA-mRNA-pathway”.

```
regMutData = regVarDetection(regData = multi_local, regulationType = "lncRNA-miRNA-mRNA-pathway")
```

```
[1] "Variants on regulations extracting begins....."
```

```
head(regMutData)
```

	ceRNA	miRNA	mRNA	Location	Population	lncMutType	Source1
1:	ENSG00000231074	MIMAT0000441	9759	6p22.1	KIRC	CNV	LnCeVar
2:	ENSG00000231074	MIMAT0000441	9759	6p22.1	KIRC	CNV	LnCeVar
3:	ENSG00000231074	MIMAT0000441	9759	6p22.1	KIRC	CNV	LnCeVar
4:	ENSG00000231074	MIMAT0000441	9759	6p22.1	LGG	CNV	LnCeVar
5:	ENSG00000231074	MIMAT0000441	9759	6p22.1	LGG	CNV	LnCeVar
6:	ENSG00000231074	MIMAT0000441	9759	6p22.1	LGG	CNV	LnCeVar

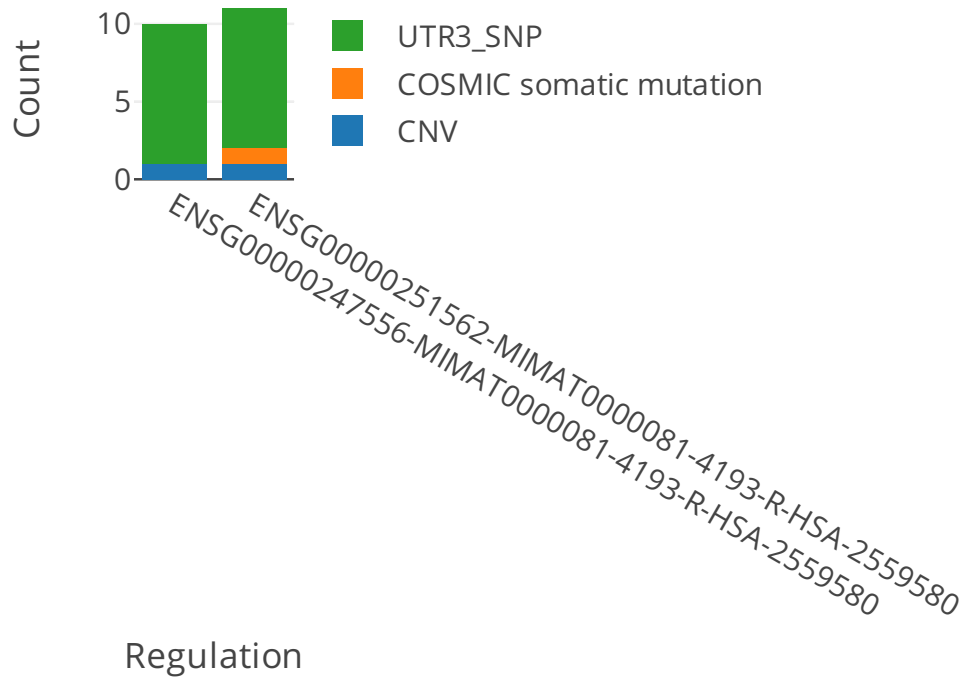
	mim	miRNAID	geneID	SNPID	mimMutType	Source2
1:	MIMAT0000441-9759	MIMAT0000441	9759	rs753530045	UTR3_SNP	miRNASNPv3
2:	MIMAT0000441-9759	MIMAT0000441	9759	rs1314733228	UTR3_SNP	miRNASNPv3
3:	MIMAT0000441-9759	MIMAT0000441	9759	rs999534275	UTR3_SNP	miRNASNPv3
4:	MIMAT0000441-9759	MIMAT0000441	9759	rs753530045	UTR3_SNP	miRNASNPv3

5:	MIMAT0000441-9759	MIMAT0000441	9759	rs1314733228	UTR3_SNP	miRNASNPv3
6:	MIMAT0000441-9759	MIMAT0000441	9759	rs999534275	UTR3_SNP	miRNASNPv3
	pathway	pathwaySource	regType			
1:	R-HSA-4551638	Reactome	mRNA-pathway			
2:	R-HSA-4551638	Reactome	mRNA-pathway			
3:	R-HSA-4551638	Reactome	mRNA-pathway			
4:	R-HSA-4551638	Reactome	mRNA-pathway			
5:	R-HSA-4551638	Reactome	mRNA-pathway			
6:	R-HSA-4551638	Reactome	mRNA-pathway			

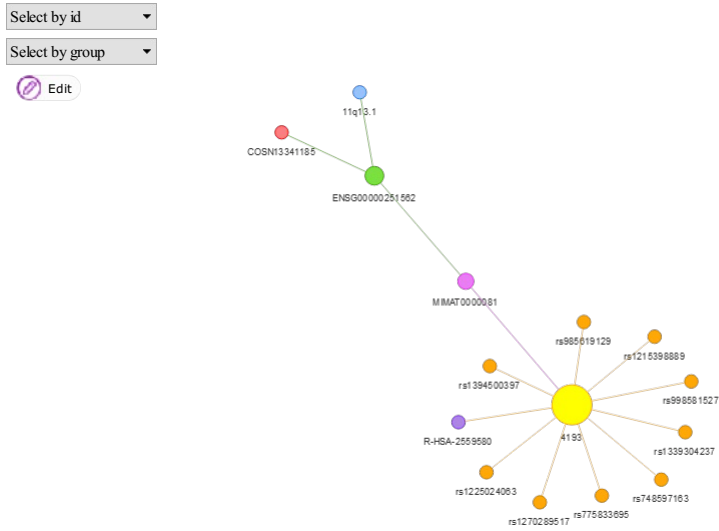
4.2.2 Regulatory variants statistics “regMutData” is the standard output of **regVarDetection** function. **regulationType** is the same as parameter in **regVarDetection** function. **selectNode** is always needed and only accept a group of elements in regulation.

count the regulatory variants on the binding sites of biological elements in regulation.

```
regVarStat(regVar = regMutData,
  regulationType = "lncRNA-miRNA-mRNA-pathway",
  selectNode = c("ENSG00000251562", "MIMAT0000081", "4193", "R-HSA-2559580", "ENSG00000247556"))
```



```
# network visualization.
regVarNetVis(regVar = regMutData,
             regulationType = "lncRNA-miRNA-mRNA-pathway",
             selectNode = c("ENSG00000251562", "MIMAT0000081", "4193", "R-HSA-2559580"))
```



If you have any questions, please contact us without hesitation.

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