# 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. Crosstalks among those networks as a bridge to build and decode heterogeneous networks from multidimensional biological knowledge, aids to highlight regulatory elements. And combinations of regulatory elements (CREs) in the local area of heterogeneous network have been a hot issue due to its crucial role in biological processes. We introduce NetLCP, an R package with command and shiny-based GUI modes, for prioritizing CREs with variant 'switches'. 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 prioritizing combinations of regulatory elements in the heterogeneous network
Authors: MingYu Ran (rmyhandsome@163.com)
Maintainer: MingYu Ran.
Please read the tutorial in https://mortyran.github.io/NetLCP/ for data preparation before you use NetL
```

```
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 the CREs in local area of heterogenous network

Current CREs types in local area of heterogenous network include binary elements CREs and multiple elements CREs. 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 CREs 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 CREs** Inspect the binary elements CREs in the local area of heterogenous network. **elementList** represents the biological elements in the interested local area of heterogenous network. **regulationType** represents the CREs 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 CREs 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 CREs in the whole depository, which means the results can contain other biological elements. In this case, you can regarded as NetLCP as an independent depository to only extract associated regulatory data. "FALSE" (default) is a common setting.

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

**3.2 Multiple elements CREs** Inspect the multiple elements CREs in the local area of heterogenous network.**regulationType** represents the CREs 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 CREs 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)
```

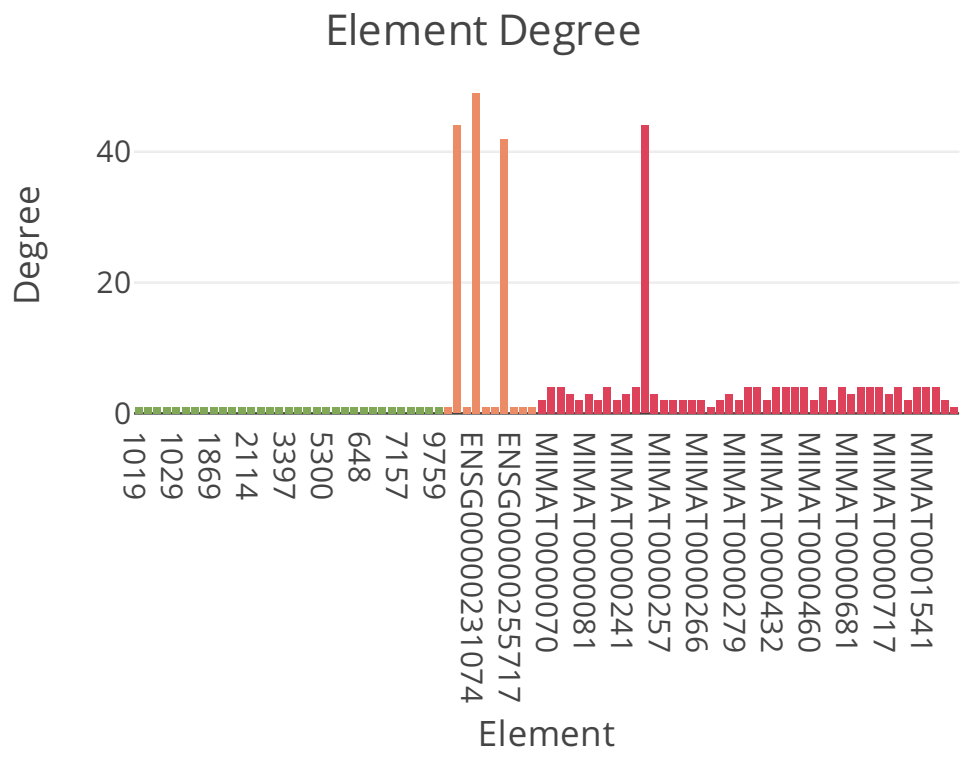
```
          node1           node2                                source
1: MIMAT0000062 ENSG00000255717                  LncBaseExperimental
2: MIMAT0000449 ENSG00000251562              LncBaseExperimental;RAIN
3: MIMAT0000691 ENSG00000231074                               NPInter
4: MIMAT0000691 ENSG00000270604                               NPInter
5: MIMAT0000681 ENSG00000269821      LncBaseExperimental;NPInter;RAIN
6: MIMAT0000245 ENSG00000231074 LncBaseExperimental;lncRNASNP2;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.

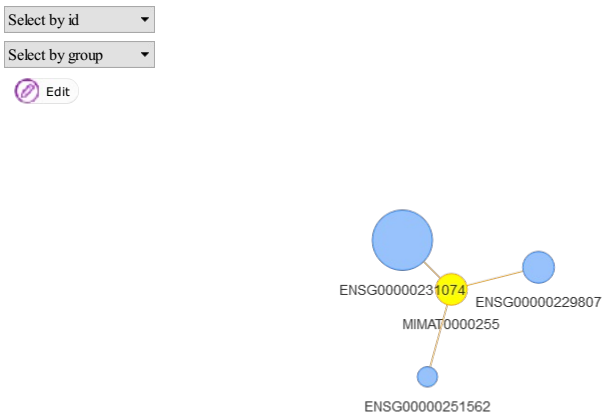
**regData** is the standard output of **binaryRegulation** or **multieleRegulation** functions, **filterDegree** means filtering the nodes which are less than it. **selectNode** represents certain or a group of elements

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).

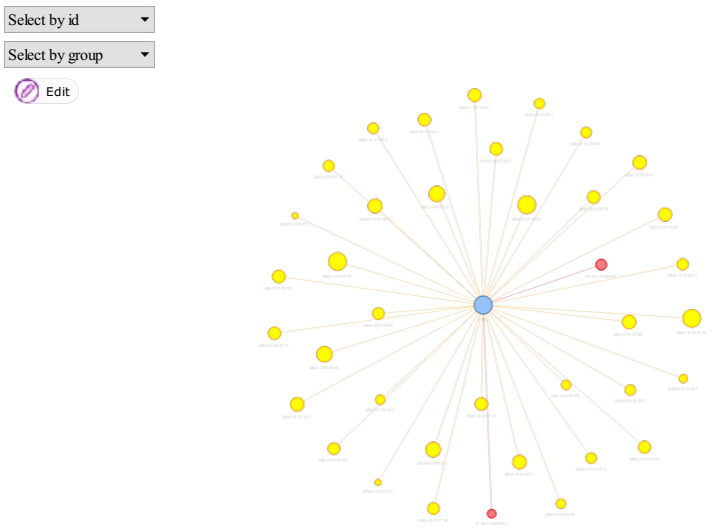
```
# Calculate the degree of biological elements in the local heterogenous network.
regStat( regData = multi_local,
         filterDegree = 40,
         selectNode = NULL )
```




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




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




**4. Prioritizing CREs.**

Prioritize the CREs by their eQTLs numbers.

**4.1 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......"
```

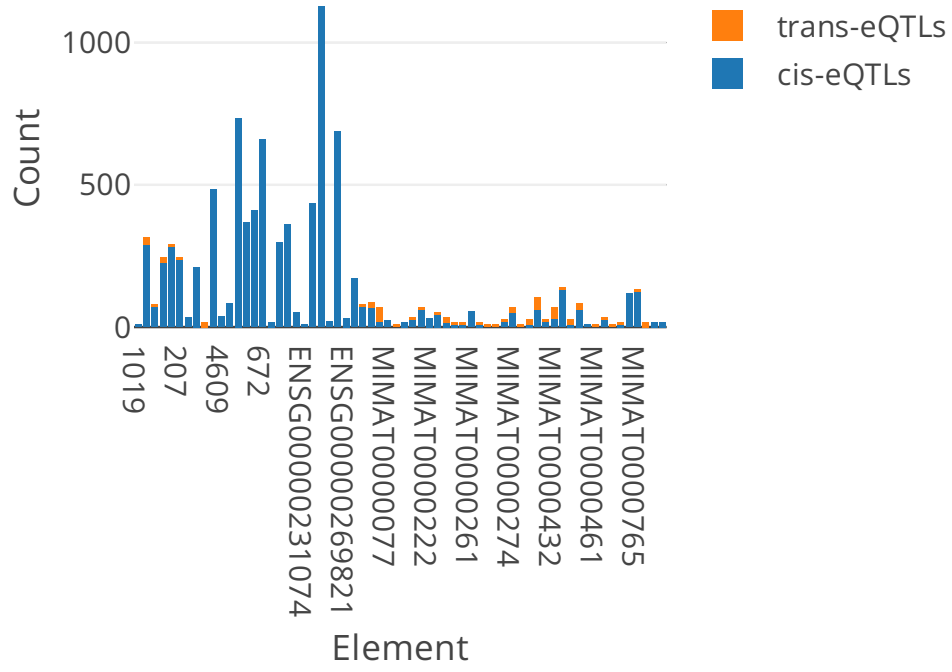
```
head(eQTLsData)
```

```
   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
```

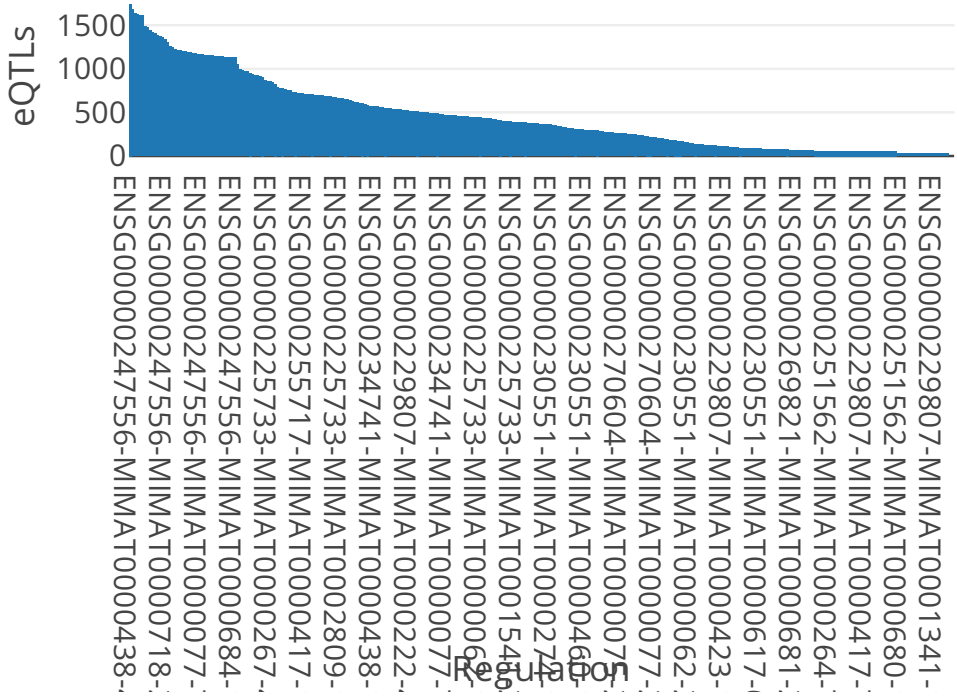
**4.2 eQTLs statistics** You can perform statistics on the eQTLs of single elements or CREs and customize the network visualization of results. **regData** is the standard output of **binaryRegulation** or **multieleRegulation** functions, **eQTLsData** is the standard output of "eQTLsDetection". **regulationType** 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).

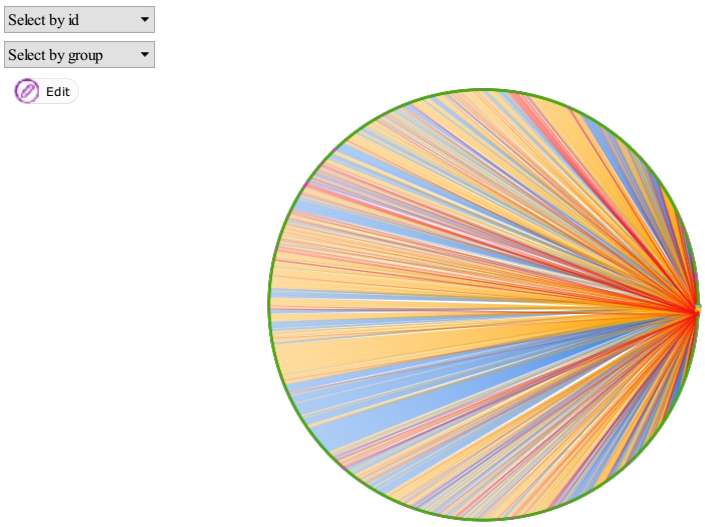
```
# count the eQTLs of single elements.
eQTLsSingleEleStat( regData = multi_local,
                    eQTLsData = eQTLsData,
                    filterDegree = 15,
                    selectNode = NULL )
```



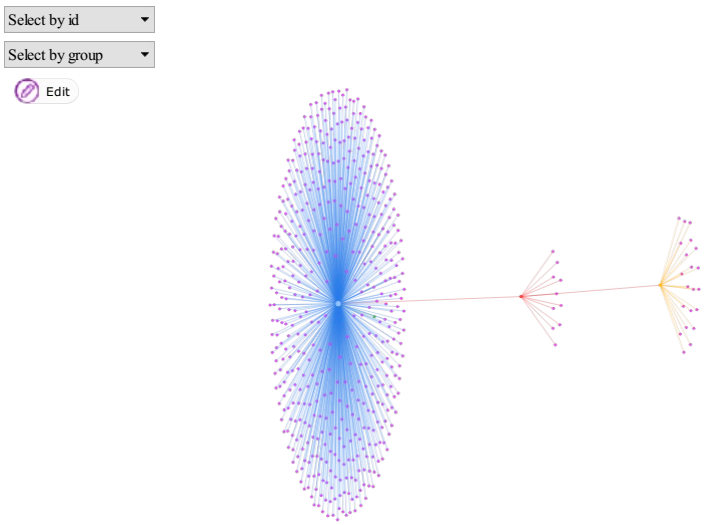

```
# count the eQTLs of CREs.
eQTLsRegStat( regData = multi_local,
              eQTLsData = eQTLsData,
              regulationType = "lncRNA-miRNA-mRNA-pathway",
              filterDegree = 15,
              selectNode = NULL )
```




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




```
# Give that you highlight the CRE "ENSG00000251562 - MIMAT0000081 - 4193 - R-HSA-2559580"
# by statistics, you want to concentrate on the eQTLs of the biological elements in this CRE.
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")
```




**5. Detecting variant 'switches' in CREs.**

Detect variant 'switches' on the binding site in CREs.

**5.1 Detect the variant 'switches' on the binding site of CREs in the local area of heterogenous network** **regData** is the standard output of **binaryRegulation** or **multieleRegulation** 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".

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

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

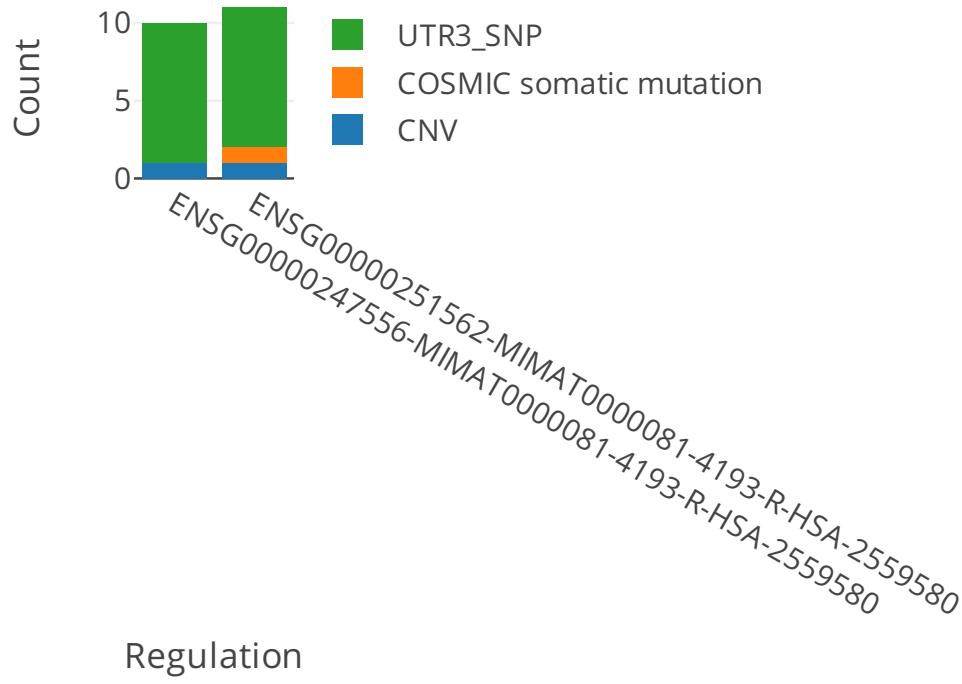
```
head(regVarData)
```

```
             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
```

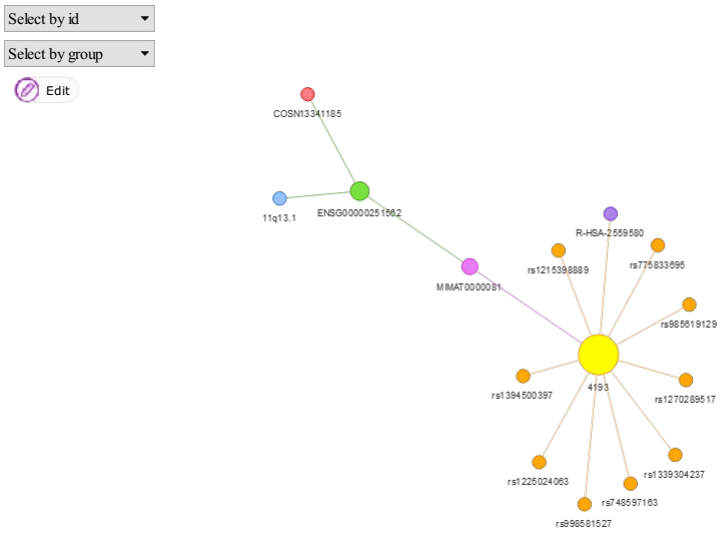
```
3: MIMAT0000441-9759 MIMAT0000441   9759  rs999534275   UTR3_SNP miRNASNPv3
4: MIMAT0000441-9759 MIMAT0000441   9759  rs753530045   UTR3_SNP miRNASNPv3
5: MIMAT0000441-9759 MIMAT0000441   9759 rs1314733228   UTR3_SNP miRNASNPv3
6: MIMAT0000441-9759 MIMAT0000441   9759  rs999534275   UTR3_SNP miRNASNPv3
         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
```

**5.2 Variant 'switches' statistics** "regVarData" 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 CREs.

```
# count the variant 'switches' on the binding sites of CREs.
regVarStat(regVar = regVarData,
           regulationType = "lncRNA-miRNA-mRNA-pathway",
           selectNode = c("ENSG00000251562", "MIMAT0000081", "4193", "R-HSA-2559580", "ENSG00000247556"
```



```
# network visualization.
regVarNetVis(regVar = regVarData,
             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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